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# Characterization and Application of Chitosan and Nanochitosan for the Durability of the Humpback Grouper Fillets During Cold Storing

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**ABSTRACT**

The present work examined the effect of different chitosan sizes (bulk and nano) on the shelf life of fillets made of *Cromileptes altivelis* flesh in cold temperatures. Research procedures involved the characterization of chitosan and nanochitosan from the prawn skin (the study used the *Litopenaeus vannamei* species) and the preservation of *C. altivelis* fillet in chitosan and nanochitosan solution during the cooling process. Fresh *C. altivelis* flesh weighing 800-950 gram were sliced into skin-off fillets before being immersed in chitosan and nanochitosan solution for 15 minutes. The flesh was then covered and stored at a cold temperature (± $5^0$C) for nine days with an observation interval of three days. Observations were made on the values of TVB-N, TMA-N, pH, TPC, FFA, peroxide value, and the sensory values of humpback grouper fillets. The present work employed a randomized block group with a factorial pattern. All parametric data were analyzed using the ANOVA and Duncan tests. The experiment was divided into three chitosan and nanochitosan treatments with a duration of 0 day, 3 days, 6 days, and 9 days. The results showed that chitosan and nanochitosan caused significant differences ($P< 0.05$) in the values of TVB-N and TPC. Similarly, a significant difference was recorded in all test parameters' storage time ($P< 0.05$). Using nanochitosan solution can suppress the degradation of fish fillets in terms of the TVB-N and TPC values. Moreover, the best treatment was the fillet soaked in nanochitosan solution that was stored for six days. The present work concluded that nanochitosan affects the shelf life of *C. altivelis* fillet during the cooling process.

## INTRODUCTION

Humpback grouper, taxonomically specified as *Cromileptesaltiveils*, Valenciences, is a species living in the tropical ocean of the Indo-Pacific. This fish is protogynous hermaphrodite by nature **(BSN, 2011)**. Humpback grouper has a high economic value as it contains protein and unsaturated fat, which is essential for the human body. Many people consume this fish due to its taste and nutritional content, increasing the market price **(Suprayudi *et al.*, 2016; Pamungkas & Sari, 2021)**. Humpback grouper is sold as a fresh product and frozen fillet.

Fish fillet products experiences a rise in the market share since they are easy serving food; many business people consider fish fillet to increase their income **(Fauzi *et al.*, 2016)**. However, it should be noted that fish fillet has a poor shelf life, as it only lasts for five to six days, considering the detrimental effect of fillet processing on the natural preservation capabilities of fish. Such an issue urges natural solutions to preserve and maintain the freshness of the fillet. Natural preservatives, such as chitosan from prawn skin, are biodegradable and non-toxic, which make them the best way to preserve fish fillet **(Novinyuk *et al.*, 2018)**. One of the common materials for such preservatives is the shell of *L. vannamei* prawn **( Miteluț *et al.*, 2015; Elfaig *et al.*, 2020)**.

Chitosan - poli-β-(1,4)-d-glucosamine is a product of deacetylation of chitin - poli-β-(1,4)-N acetyl-d-glucosamine, natural polysaccharide that is abundant in nature

commonly found in the crustacean shells and cell walls of the fungi **(Miteluț *et al*., 2015; Philibert *et al*., 2017; Elfaig *et al*., 2020)**. Chitosan is a potential antimicrobe materials. Its capacity to suppress bacterial growth is due to the positive polycation contents **(Riski & Sami, 2015).**

Furthermore, the antimicrobe characteristics of chitosan are caused by the permeable membrane of chitosan, which is capable of absorbing water in food and hindering microbe growth. Strong positive contents of amine ($-NH_2$), the functional group in chitosan, also draws the negative amino acid molecule that forms protein in microbe **(Divya *et al*., 2014; Nagarajan *et al*., 2021)**. **Alishahi and Aïder (2012)** argued that chitosan is a potential antimicrobe agent due to its antibacterial and antioxidant properties, the ability to form edible films and coatings, the capability to process seafood industry waste, increased gelling properties, micro- and nanocarriers, as the bioactive compound, functional food and medicine compounds from aquaculture and seafood.

However, one should consider the drawback of chitosan, i.e., poorly soluble in water and organic solvents. Physical modification is, thereby, needed by reducing the size of the chitosan particles. Such is because the smaller the particle size, the greater the surface area of a material which increases the speed of dissolving of the material **(Lukiyono *et al*., 2020)**. A study by **Ramezani *et al*. (2015)** reported that the nanochitosan is able to inhibit the increase in TVB-N and is capable of hindering microbe growth in the cold-storing silver carp (*Hypophthalmichthysmolitrix*) compared to bulk-sized chitosan. Such a condition is due to the larger surface area of nanochitosan and higher affinity with the bacterial cell.

Nano-sized materials are claimed to have tremendous energy because they have a large area to increase contact with other particles, resulting in more optimal mixing performance **(Naiu *et al*, 2020)**. Atoms on the surface determine material reactivity as they are in direct contact with other materials **(Rai & Bai, 2011; Suwarda & Maarif, 2013)**.

Previous studies show that chitosan's capacity as a natural preservative requires improvement to prolong the freshness of fish meat. The present work aimed to fill this gap by modifying the chitosan size (into nano-sized) to preserve the fillet of the humpback grouper (*C. altivelis*) during cold storage. Its goal was to explore the effect of different chitosan sizes (bulk and nano) on the fish fillets' shelf life at low temperatures.

## MATERIALS AND METHODS

### Material

The tools of the present work involved: digital scales, filter paper, plastic, knives, cutting boards, basins, label paper, rubber binders, sprayers, analytical tools including pH meters, pipettes, Petri dishes, vortexes, spatulas, incubators, test tubes, distillate tubes, Erlenmeyer, Conway cup, homogenizer model D-500 (D-Lab), incubator, oven, refrigerator, measuring cup, beaker, and score sheet. Meanwhile, the materials included bulk ice, *L. vannamei* shrimp shells from shrimp ponds in Pohuwato Regency, Gorontalo Province, and the Humpback grouper fish (*C.altivelis*) obtained from fishermen in Pentadu Barat Village, Boalemo Regency, Gorontalo Province. Other materials include acetic acid, distilled water, tween 80, STTP, perchloric acid, phenolphthalein indicator, silicone anti-foaming, NaOH, $H_3BO_4$, Tashiro indicator, 0.02 N HCl, $K_2CO_3$, $H_3BO_3$, PP indicator, 0.1 N NaOH, PCA media, Butterfield's Phosphate Buffered solution, Thricloroaceric acid (Merck), Thioglycolate agar, glacial acetic acid (Merck), chloroform, saturated KI, starch solution, 0.1 N $Na_2S_2O_3$, and 95% ethanol.

### Research procedures

The present work consisted of two phases: the initial and the main phases. The production of chitosan consists of three stages: deproteination, demineralization, and deacetylation, based on the method by **Setijawati *et al*. (2021)**. Chitosan from the prawn species mentioned earlier was then processed into nanochitosan based on the guidelines seen in a study by **Suptijah *et al*. (2011)**.The initial phase functioned to characterize the chitosan

and nanochitosan that will be used as the preservative. Meanwhile, the main phase aimed to examine the effect of the two preservatives on the freshness of the fish fillet. Humpback groupers weighing 950 to 1,000 grams were prepared into skin-off fillets. The fillets were then distributed into two groups: the chitosan (FK) treatment and the nanochitosan treatment (FNK). Each group was further divided into different categories depending on the cold storage duration. The fillets were immersed in chitosan and nanochitosan solution for 15 minutes **(Sipayung *et al.*, 2015)** and stored at a cold temperature (± $5^0$C) for nine days, with an interval of three days of observation. Observations were made on the values of TVB-N, TMA-N, pH, TPC, FFA, peroxide value, and sensory values of the grouper fish fillets. The treatment comprised three replications. All primary research data were then analyzed statistically using the factorial, randomized block design. If a treatment yields specific results, the Duncan test would be performed to identify the treatments' differences.

## RESULTS

### Chitosan and nanochitosan characteristics

The yield of the chitosan was 18.52 % ± 0.26. Furthermore, the chitosan was characterized by several parameters, i.e degree of deacetylation, viscosity, and color. Chitosan produced from *L. vannamei* shrimp shells was analyzed using Fourier Transform Infra Red Spectroscopy (henceforth, FTIR).

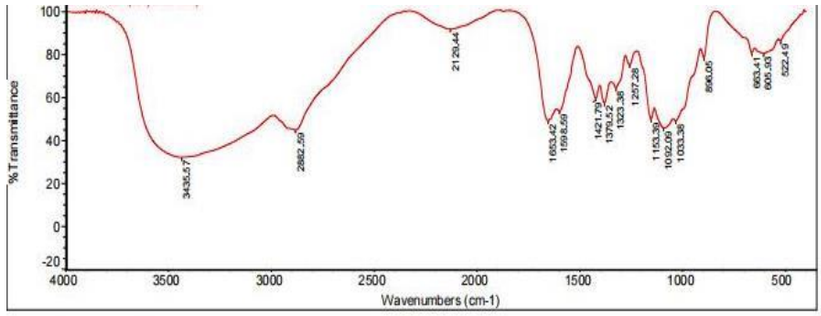


**Fig. 1.** FTIR chitosan analysis

Based on Fig. (1), there is an absorption band at wave number 3435.57 $cm^{-1}$, which shows the OH and NH stretch. The absorption band at wave number 2882.59 $cm^1$ indicates the $CH_2$ bend, while the band at wave 1653.42 $cm^{-1}$ indicates the presence of C=O amide stretch. Meanwhile, the absorption band at wave 1153.39 – 1033.38 $cm^{-1}$ indicates the presence of the C-O-C stretch, and the absorption band at wave 896.05 $cm^1$ indicates the presence of silica minerals.

A spectroscope device FTIR was used to determine the degree of deacetylation. The degree was calculated using the baseline method, which Domszy and Roberts define as a method performed by noting the highest peak and measuring the basic band. The calculated deacetylation degree was 86.03 ± 3.17 %. Table (1) provides information regarding the physical characteristic of chitosan derived from the *L. vaname* shell. The distribution of nanochitosan size is illustrated in Fig. (2).

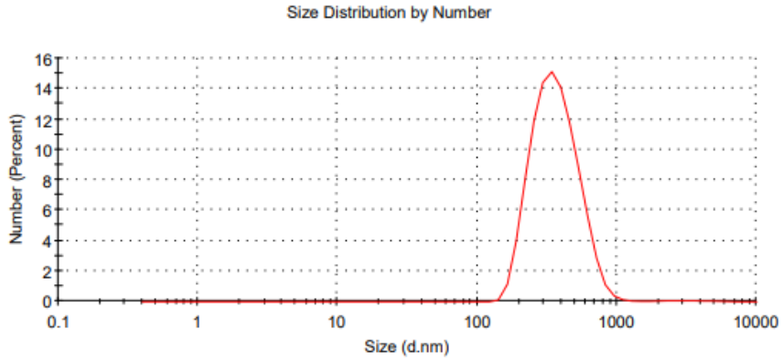

**Fig. 2. The distribution of the nanochitin size**

The characteristics of nanochitosan produced from *L. vannamei* shrimp shell chitosan can be seen in Table (1).

**Table 1.** Characteristics of nanochitosan from *L. vannamei* shrimp flour

| Parameter | Results |
|---|---|
| Z-average (nm) | 481. 88 ± 19.79 |
| PdI | 0.52 ± 0.03 |
| Zeta potential (mV) | 40.3 ± 36.85 |
| Viscosity (cPs) | 1.267 ± 0.31 |

Considering this notion, the nanoparticles produced in this study have met the recommended size (481. 88 ± 19.79 nm).

The polydispersity index (PdI) test showed that the resulting chitosan nanoparticles exhibited homogeneous dispersion (0.52). If the PdI value is close to 0, the dispersity of the particle size is homogenous. On the other hand, if the PdI value is greater than 0.5, the heterogeneity is considered high. Based on Table (2), the viscosity value is 767 cPs. After changing the chitosan particles to nanoparticle size, the viscosity value decreased to 1.267 cPs. This increase in viscosity is probably due to changes in particle size and the chitosan polymer chains used in producing nanochitosan.

**TVB-N value**

The results of the TVB-N test shown in Table (2) reveal that, on average, there was an increase in TVB-N values during cold storage (0, 3, 6, and 9 days) using chitosan and nanochitosan solutions. The TVB-N value of the humpback grouper fillet treated with chitosan solution during storage ranged from 7.98 to 75.76mgN per 100g sample. Meanwhile, the TVB-N value of humpback grouper fillet treated with nanochitosan solution during storage ranged from 7.98 to 59.99mgN per 100g sample.

**Table 2.** TVB-N value of the humpback grouper (*C. altivelis*) during cold storing

| Sample | Cooling duration | | | |
|---|---|---|---|---|
| | 0 Day | 3 Days | 6 Days | 9 Days |
| FK | $7.98 \pm 0.03^{a}$ | $14.03 \pm 0.04^{a}$ | $67.21 \pm 0.18^{c}$ | $75.76 \pm 0.51^{c}$ |
| FNK | $7.98 \pm 0.03^{a}$ | $7.92 \pm 0.06^{a}$ | $26.75 \pm 0.03^{b}$ | $59.99 \pm 0.12^{c}$ |

Description: Different superscript letters indicate differences ($P< 0.05$) determined by Duncan's test; FK (chitosan fillet); FNK (nanochitosan fillet).

Based on the results of the ANOVA analysis, fish fillets receiving treatment (chitosan, nanochitosan) and the storing duration contribute to the value of TVB-N ($P< 0.05$). Table (2) shows that using chitosan solution as a natural preservative in the grouper fillets during cooling can suppress the increase in the amount of TVB-N until the third day of cooling (14.03mgN/ 100g). Meanwhile, the nanochitosan solution suppresses the increase in TVB-N value until the sixth day of cold storing (26.75mgN/ 100g).

Based on the research results, the TVB-N value of the fillets with nanochitosan solution after the sixth day of preservation was categorized as fresh fish (TVB-N≤ 30mgN/ 100g).

**TMA-N**

The results of the TMA-N test shown in Table (2) reveal an average increase in TMA-N values during cold storage (0, 3, 6, and 9 days) using chitosan and nanochitosan solutions. The TMA-N value of the humpback grouper fillet stored in cold temperature and treated with chitosan solution ranged from 0.23 to 6.73mgN per 100g sample. On the other hand, the TMA-N value of the humpback grouper fillet treated with nanochitosan solution during storage ranged from 0.23 to 6.67mgN per 100g sample.

**Table 3.** TMA-N value of the humpback grouper (*C. altivelis*) during cold storing

| Sample | Cooling duration | | | |
|---|---|---|---|---|
| | 0 Day | 3 Days | 6 Days | 9 Days |
| FK | $0.23 \pm 0.03^{a}$ | $0.92 \pm 0.26^{a}$ | $5.42 \pm 0.02^{b}$ | $6.73 \pm 0.07^{c}$ |
| FNK | $0.23 \pm 0.03^{a}$ | $0.58 \pm 0.003^{a}$ | $4.33 \pm 0.03^{b}$ | $6.67 \pm 0.06^{c}$ |

Description: Different superscript letters indicate differences ($P< 0.05$) determined by Duncan's test; FK (chitosan fillet); FNK (nanochitosan fillet).

Based on the results of the ANOVA, the treatment groups (chitosan, nanochitosan) did not contribute to the TMA-N value ($P> 0.05$). On the one hand, the cold storage time affected the TMA-N value ($P <0.05$). Table (3) shows that using chitosan and nanochitosan solutions as a natural preservative in grouper fillets during cooling can suppress the increase in the TMA-N until day nine of cold storage. Overall, the TMA-N value of the humpback grouper preserved with chitosan and nanochitosan solution for nine days at cold temperatures was still at the maximum acceptable threshold.

**The pH value**

The pH test results for the humpback grouper (*C. altivelis*) are shown in Table (4).

**Table 4.** Results of pH test for *C. altivelis* under study during cold storage

| Sample | Cooling duration | | | |
|---|---|---|---|---|
| | 0 Day | 3 Days | 6 Days | 9 Days |
| FK | $5.80 \pm 0.68^{a}$ | $7.31 \pm 0.03^{a}$ | $7.92 \pm 0.07^{b}$ | $8.07\pm 0.01^{b}$ |
| FNK | $5.80 \pm 0.68^{a}$ | $6.96 \pm 0.09^{a}$ | $7.49 \pm 0.04^{a}$ | $7.65 \pm 0.02^{b}$ |

Description: Different superscript letters indicate differences ($P< 0.05$) determined by Duncan's test; FK (chitosan fillet); FNK (nanochitosan fillet).

Based on the results of the ANOVA, fish fillets receiving treatment (chitosan, nanochitosan) stored for several days affect the pH values ($P< 0.05$). The test results for the pH value (acidity degree) of the cold-stored humpback grouper (*C. altivelis*) showed an alkaline pH value (above 7) after three to nine days of storage. In addition, the pH value is more likely to be related to low levels of TVB-N in muscle tissue due to chitosan or nanochitosan treatment during cold storage (Table 2).

**TPC**

**Table 5.** TPC value of the humpback grouper (*C. altivelis*) fillet during cold storing

| Sample | Cooling duration | | | |
|---|---|---|---|---|
| | 0 Day | 3 Days | 6 Days | 9 Days |
| FK | $3.93 \pm 0.13^{a}$ | $5.55 \pm 0.10^{b}$ | $7.07 \pm 0.01^{c}$ | $7.09 \pm 0.06^{c}$ |
| FNK | $3.93 \pm 0.13^{a}$ | $3.98 \pm 0.01^{a}$ | $5.50 \pm 0.37^{b}$ | $6.11 \pm 0.54^{c}$ |

Description: Different superscript letters indicate differences ($P< 0.05$) determined by Duncan's test; FK (chitosan fillet); FNK (nanochitosan fillet).

Based on the results of the ANOVA analysis, fish fillets receiving treatment (chitosan, nanochitosan) stored for several days affect the TPC counts ($P< 0.05$). Table (5) shows the capability of chitosan solution as a natural preservative in suppressing the increase in TPC until the third day of cooling (log 5.55). Meanwhile, nanochitosan solution can suppress the increase in TPC value until the sixth day of cold storage (log 5.50).

**FFA**

The results of the FFA test shown in Table (6) reveal an average increase under cold storing (0, 3, 6, and 9 days) using chitosan and nanochitosan solutions. Furthermore, the FFA value of the humpback grouper fillet stored in chitosan solution ranged from 0.17 to 1.11%. Meanwhile, the FFA value of humpback grouper fillet stored in nanochitosan solution ranged from 0.17 to 0.94%.

**Table 6.** FFA value of the humpback grouper (*C. altivelis*) fillet during cold storing

| Sample | Cooling duration | | | |
|---|---|---|---|---|
| | 0 Day | 3 Days | 6 Days | 9 Days |
| FK | $0.17 \pm 0.09^{a}$ | $0.20 \pm 0.05^{a}$ | $0.40 \pm 0.05^{a}$ | $1.11 \pm 0.09^{b}$ |
| FNK | $0.17 \pm 0.09^{a}$ | $0.17 \pm 0.09^{a}$ | $0.34 \pm 0.09^{a}$ | $0.94 \pm 0.09^{b}$ |

Description: Different superscript letters indicate differences ($P< 0.05$) determined by Duncan's test; FK (chitosan fillet); FNK (nanochitosan fillet).

Based on the results of the ANOVA, the treatment groups (chitosan, nanochitosan) were not impactful to the FFA value ($P> 0.05$). On the other hand, the extended storage in cold temperatures contributed to the FFA value ($P<0.05$).

Chitosan and nanochitosan also play a part in preventing the FFA value from soaring during storage. Nevertheless, nanochitosan solution has better capacities in suppressing FFA value more than chitosan solution until day nine of storing.

**Peroxide value**

Table (7) reveals that the peroxide value of the fish fillet increases on average during cold storage (0, 3, 6 and 9 days) in both experimental solutions, chitosan and nanochitosan. The peroxide value of humpback grouper fillet stored in chitosan solution ranged from 1.53 to 6.73 meq/kg. Meanwhile, the peroxide value of the humpback grouper fillet stored in nanochitosan solution ranged from 1.53 to 6.60 meq/kg.

**Table 7.** Peroxide value of the humpback grouper (*C. altivelis*) during cold storing

| Sample | Cooling duration | | | |
|---|---|---|---|---|
| | 0 Day | 3 Days | 6 Days | 9 Days |
| FK | $1.53 \pm 0.12^{a}$ | $2.2 \pm 0.20^{b}$ | $4.26 \pm 0.11^{c}$ | $6.73 \pm 0.11^{d}$ |
| FNK | $1.53 \pm 0.12^{a}$ | $2.0 \pm 0.20^{b}$ | $4.20 \pm 0.20^{c}$ | $6.60 \pm 0.21^{d}$ |

Description: Different superscript letters indicate differences ($P< 0.05$) determined by Duncan's test; FK (chitosan fillet); FNK (nanochitosan fillet).

Based on the results of the ANOVA, the treatment groups (chitosan, nanochitosan) were not impactful to the PV value ($P> 0.05$). On the other hand, the treatment groups with a long storage in cold temperatures were impactful to the PV value ($P< 0.05$). In the present work, there was an average increase in peroxide value (PV) of the humpback grouper fillets until day 9 of cold storage using chitosan and nano-chitosan solutions. The increase, however, was slight. This is caused by chitosan, which functions as an antioxidant due to its $NH_2$ content. $NH_2$ plays a role in capturing unstable free radicals, suppressing the oxidation process of the fish fillets during cooling.

In the present work context, the increase in peroxide value (PV) is more likely due to the shape of the humpback grouper fillets, which resulted in the fat oxidation considering the direct contact of the fish flesh with oxygen. The breakdown of fats and oils reduces the nutritional value, distorting the taste and smell of the fat. The results of PV test show that after day nine, the fish fillets were still below the standard PV value threshold.

**Sensory value**

During cold storage, the sensory value of the humpback grouper fillets was measured on days 0, 3, 6, and 9. Table (8) reveals that the sensory values declined during cold storage using chitosan and nanochitosan solutions. The sensory value of the humpback grouper fillet stored in chitosan solution ranged from 3 to 9. Meanwhile, the sensory value of the humpback grouper fillets stored in nanochitosan solution ranged from 5 to 9.

**Table 8.** Sensory value of the humpback grouper (*C. altivelis*) fillet during cold storing

| Sample | Sensory value | Cooling duration | | | |
|---|---|---|---|---|---|
| | | 0 Day | 3 Days | 6 Days | 9 Days |

| | | | | | |
|---|---|---|---|---|---|
| FK | Appearance | $9 \pm 2.86^{a}$ | $8 \pm 1.92^{b}$ | $5 \pm 1.28^{b}$ | $5 \pm 0.99^{c}$ |
| | Odor | $9 \pm 1.92^{a}$ | $7 \pm 1.19^{b}$ | $5 \pm 1.41^{b}$ | $3 \pm 1.05^{c}$ |
| | Texture | $9 \pm 1.92^{a}$ | $7 \pm 1.28^{b}$ | $5 \pm 0.92^{b}$ | $3 \pm 1.40^{c}$ |
| FNK | Appearance | $9 \pm 3.86^{a}$ | $8 \pm 1.03^{b}$ | $7 \pm 1.08^{b}$ | $5 \pm 1.56^{c}$ |
| | Odor | $9 \pm 1.92^{a}$ | $8 \pm 1.61^{b}$ | $7 \pm 0.99^{b}$ | $6 \pm 0.92^{c}$ |
| | Texture | $9 \pm 1.92^{a}$ | $8 \pm 0.92^{b}$ | $7 \pm 0.75^{b}$ | $5 \pm 0.70^{c}$ |

Description: Different superscript letters indicate differences ($P< 0.05$) determined by Duncan's test; FK (chitosan fillet); FNK (nanochitosan fillet).

Based on the results of the ANOVA, the treatment groups (chitosan, nanochitosan) did not affect the sensory value ($P> 0.05$). Whereas, the long storage in cold temperatures contributed to the sensory value ($P<0.05$). Table (8) shows that the average organoleptic value of the humpback grouper fillets during the cooling time on day 0 was measured at 9. The criteria involve shining color and fresh smell with a solid, compact, and elastic texture. Beginning from day 9 of the cold storage, a decline was detected in the fish fillets.

The level of quality acceptance based on Table (8) for the use of nanochitosan solution was still acceptable by the panelists until the sixth day of cooling with a sensory value of 7 (less shining, neutral odor; dense, less compact, and less elastic in texture). The chitosan solution on day 6 resulted in an average sensory value of 5 days (changes in color, dull; slightly rancid, musty smell; rather soft and less elastic in texture, and a little watery).

Soaking the fillets in nanochitosan solution can maintain sensory values until the 6th day of cooling. Meanwhile, chitosan solution can only maintain the sensory values of the grouper fish fillets until day 3.

## DISCUSSION

To prove the reading of the absorption band of the wave number on the chitosan functional group, the results of FTIR analysis refer to the results seen in **Nandiyanto *et al*. (2019)**. Based on Fig. (1) of the reading of the chitosan functional groups, the wavenumbers in the functional groups correspond with a study by **Bahri *et al*. (2015)**. There was a widening of the absorption band of the OH functional group in chitosan with the wavenumber of 3435.57 $cm^{-1}$. This is due to the functional group -H that overlaps with O-H. The functional group C=O has a medium absorption band at a wavenumber of 1653.42 cm-1. The weaker the C=O absorption band at wavenumbers around 1640, the more C=O functional groups will be deacetylated **(Wiyarsi & Priyambodo, 2011)**. N-H bending is found in the wavenumber of 1629.70 $cm^{-1}$ **(Nandiyanto *et al*., 2019)**. This aligns with the study of **Bahri *et al*. (2015)**, who found no indication of N-H bending at the wavenumber of 1629.70 $cm^{-1}$. Such a condition is caused by the functional group -H that overlaps with C=O amide, signified by the widening of the absorption band at a wavenumber of 1653.42 $cm^{-1}$.

This study's findings validate the efficacy of the approach provided by **Setijawati *et al*. (2021)** in converting chitin into chitosan. Chitosan from the prawn species mentioned earlier was then processed into nanochitosan based on the guideline seen in a study by **Suptijah *et al*. (2011)** employing the ionic gelation method with a homogenizer device D-500 model (D-Lab). A nanoparticle solution is said to have a nanoparticle size if the diameter ranges from 10 to 1000nm **(Nagavarma *et al*., 2012; Iswandana *et al*., 2013; Napsah & Wahyuningsih, 2014)**. This is in line with the results of **Juliantoni *et al*., (2020)**, who stated that the polydispersity index is a sum calculated from two simple parameters for correlation data. Nanoparticles with a PdI value of 1 have an extensive size distribution and contain large particles or aggregates, which can undergo sedimentation, and monodisperse systems usually have PdI values below 0.05.

The higher the zeta potential value, the greater the electrostatic repulsion between nanoparticles in the solution. This condition enables the production of more stable

nanoparticles for a long time without any settling. Furthermore, the particles can maintain their homogeneity. The expected standard zeta potential is (+/-) 30 mV **(Mohanraj & Chen, 2006)**. Zeta potential indicates surface charge that can affect the stability of particles in suspension through electrostatic repulsion between particles. Higher zeta potential values imply more stable microparticles, while lower values indicate colloidal instability **(Al-Shdefata *et al*., 2019)**. Dispersion systems with low zeta potential values are more likely to form aggregates along with Van der Waals forces in particle interactions **(Juliantoni *et al*., 2020)**. If the size of the chitosan polymer chain is smaller, the rate of translational movement becomes faster. According to **Thariq *et al.*(2016)** chitosan is insoluble in water but soluble in acids, and has a relatively high viscosity when dissolved.

Bacterial activity is the leading cause of fish TVB-N accumulation during postmortem storage **(Hong *et al*., 2012)**. According to **Ramezani *et al*. (2015)**, the nanochitosan has capabilities in hindering TVB-N and microbe growth of the cold-storing silver carp (*Hypophthalmichthys molitrix*) compared to the bulk-sized chitosan. This situation is due to a larger surface area of nanochitosan and higher affinity with the bacterial cell **(Ramezani *et al*., 2015)**. This finding correlates with the result seen in **Amegovu *et al*. (2012)** that the TVB-N value of highly fresh fish is < 25mg/ 100g; fresh fish is ≤ 30mg/ 100g; edible fish is ≤ 35mg/ 100g, and; inedible fish is > 35mg/ 100g).

A rise in the TVB-N value on day six (for chitosan treatment) and day nine (for nanochitosan treatment) is more likely due to psychrophilic microorganisms responsible for decomposing the fish fillets**. Mile (2013)** claimed that a rise in the number of psychrophilic bacteria is thought to be caused by the survivability of the bacteria at low temperatures, enabling the microorganisms to decomposition fish meat.

The use of chitosan and nanochitosan inhibits the increase in TMA-N value of the humpback grouper (*C. altivelis*) fillets during cold storage. This finding is in accordance with the statement by **Mohan *et al*. (2012)** postulating an elevated number of microbes can play a role in the breakdown of compounds such as trimethylamine oxide (TMAO), peptides, and amino acids. Consequently, this study's addition of nanochitosan inhibits the growth of bacteria. Furthermore, the reduction in TMA-N levels may be attributed to the limited microbial activity in the decomposition of TMAO compounds at low temperatures **(Sedana *et al*., 2015).**

The pH value is one of the indicators used to determine the freshness of fish. In the process of fish decomposition, changes in the pH of fish meat play a significant role because of their effect on the process of autolysis and bacterial attacking. The pH measurement was carried out using a pH meter. This increase in pH value is more likely caused by enzymes derived from fish meat and microbes decomposing proteins and fats to produce alkaline compounds **(Wally *et al*., 2015)**. According to **Suprayitno (2020)**, the pH of fish in the rigor mortis and post rigor ranges from 6.2 to 6.6 and 7.5 - 8.0, respectively. The present work on the humpback grouper (*C. altivelis*) falls under the post-rigor category starting from day three of cold storage (pH 7.31 and 6.96). Fish that are not fresh have a high flesh pH (if) compared to fresh fish **(Suprayitno, 2020)**. This is due to the emergence of alkaline compounds, e.g., ammonia, trimethylamine, and other volatile compounds.

The acceptable number of colonies is 25-250 per plate **(BSN, 2015)**. Based on the TPC value, the grouper fish fillets preserved with nano-chitosan solution until the sixth day are still edible compared to fish fillets preserved with chitosan solution due to their threshold that is still below the standard of fish according to SNI 2729.2013, which is log 5.69 (5 x $10^5$ colony/g) **(BSN, 2013)**. Chitosan is antimicrobial since it has a permeable membrane capable of absorbing water in food and hindering microbe growth. Furthermore, chitosan has strong, positive charge of amine (-$NH_2$) that can draw the negative amino acid molecule capable of forming protein in microbe **(Divya *et al*., 2014; Nagarajan *et al*.,**

**2021)**. According to **Cauerhff *et al.* (2013)**, using nanochitosan can increase its antibacterial ability because the size change allows it to become more reactive toward bacteria, resulting in bacterial growth inhibition. Such a notion corresponds to the result that nanoparticles are superior to similar materials in bulk since nanoparticles have a greater ratio between surface area and volume, signifying greater reactivity. Similarly, the ability of chitosan in the form of nanoparticles also greatly inhibits bacterial growth because it can directly enter the bacterial cell **(Cauerhff *et al.*, 2013)**.

Based on Table (6), the results of the FFA test for the humpback grouper (*C. altivelis*) fillets during cold storage showed increases of FFA.The higher the temperature, the faster free radicals and free fatty acids are formed, and vice versa **(Jiang *et al.*, 2021)**. In this study, the fillet was stored at a temperature ranging from 4 to $5^0$C (refrigerator temperature) to reduce the increase in FFA values. This is due to the bioactive compounds in nanochitosan that are more reactive in counteracting free radicals causing rancidity (free fatty acids). Free fatty acids are unbound free fatty acids, such as triglycerides. Free fatty acids produced by hydrolysis and oxidation processes usually combine with neutral fats. This reaction will be accelerated by factors including temperature (heat), water, acidity, and catalysts (enzymes). The longer this reaction lasts, the more free fatty acids are formed, resulting in an unpleasant taste and smell **(Nurhasnawati *et al.*, 2015)**. Based on the standards of **IFOMA (1998)**, the free fatty acid content in good crude fish oil is 1-7%. In specific industries, however, the standard free fatty acid content used is 2-5%.

The increase in peroxide value of the humpback grouper (*C. altivelis*) fillets during cold storage is likely due to the fact that the samples were stored in fillet form so that the fish meat is in direct contact with air. **Ketaren (1996)** asserts that allowing fatty materials to coming into contact with air spontaneously initiates the oxidation of fat by oxygen. Meanwhile, the use of chitosan and nanochitosan can slow down the increase in peroxide value during cold storage. This finding is in accordance with the results of research conducted by **Sari *et al.* (2013)**, who reported that chitosan solution has antioxidant activity which results in the binding of free radicals by chitosan. In addition, the $OH^+$ radical group from the lipid oxidation process reacts with hydrogen ions from the ammonium ion group ($NH^{3+}$) in chitosan to produce a more stable molecule and produce antioxidant compounds. According to the International Fishmeal and Oil Manufactures Association **(IFOMA, 1998)** the standard peroxide value (PV) is 3-25 meq/kg sample.

The decrease in the sensory quality of the humpback grouper fillets was in line with the increase in the TVB-N value and the number of bacteria (ALT). Such a finding is in line with the argument of **Çetinkaya *et al.* (2021)** elucidating that the decrease in the sensory quality of the catfish meat was probably caused by the formation of several volatile low molecular weight compounds, the occurrence of lipid oxidation, and protein degradation during cold storage.

Based on SNI No 2696:2013 **(BSN, 2013)**, the minimum organoleptic value is 7 (1 - 9 score). The use of nanochitosan solution can suppress sensory loss due to the size of the nanoparticles, which causes the solution to be more reactive in suppressing bacterial growth, oxidation processes, and the formation of volatile compounds. The nanostructure, i.e., nanoemulsions, nanoparticles, and nanofibers, can be used to limit rapid sensory impairment **(Chellaram *et al.*, 2014; Ozogul *et al.*, 2017)**. For example, essential oil nanoemulsions can suppress fishy odors and positively affect the sensory quality of fish (the rainbow trout) stored at $4^0$C **(Durmus, 2020)**. In another study, the skin brightness and mucus, meat texture, odor, and sea bass color (*Dicentrarchus labrax*) yield better sensory values than those without nanoemulsion **(Yazgan *et al.*, 2017)**.

## CONCLUSION

Based on the study's results, the characteristics of the resulting nanochitosan have a DD value of 86.03, a viscosity of 767 cPs, and a pale yellow color. Meanwhile, the nanochitosan has a Z-average value of 481.88 nM, a PdI 0.52, a zeta potential of 40.3 mV,

and a viscosity of 1.267 cPs. Using nanochitosan solution derived from *L. vannamei* shrimp shells can prevent the decline in the quality of the humpback grouper (*C. altivelis*) fillets in terms of several parameters: TVBN-N, pH, and TPC, until the 6th day of storage at cold temperatures (± 5°C).

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