THE INFLUENCE OF SOAKING DURATION OF ARTIFICIAL CHITOSAN FROM BY
PRODUCT FROZENING SHRIMP ON THE EFFECTIVENESS OF FRESH QUALITY
TILAPIA (*Oreochromis niloticus*)

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ABSTRACT

One of the problems that often arise in the fisheries sector is to maintain quality. Chitosan as a natural preservative is an alternative because it is synthesized from shrimp shells and from invertebrate animal shells. Shrimp shell containing chemical compounds chitin and chitosan is a waste that is easily obtained and available in large quantities, which has not been utilized optimally, therefore it is necessary to utilize shrimp waste into chitosan as a preservation of freshness quality in tilapia. Realizing healthy food ingredients and types of preservation that are safe for the human body is the first step to improve a better quality of life. This study aims to determine the benefits of artificial chitosan as a preservative to improve the quality of freshness in tilapia (*Oreochromis niloticus*). The method in this research is an experimental method by preserving tilapia (*Oreochromis niloticus*) with different immersion times of artificial chitosan. The design carried out was a completely randomized non-factorial design. The observed parameters were the organoleptic test, the TVB test, and the test of the peroxide results from all tests showing that three hours of submersion was better than 1 and 2 hours of substation, all in relation to 7, 9 and 11 hours of observation. A seventh-hour observation is picking up no difference between 1, 2 and 3 hours of submersion.

**Keywords:** Artificial Chitosan, Freshness Quality, Tilapia (*Oreochromis niloticus*)

INTRODUCTION

The export value of Indonesian shrimp reaches 142,000 tons, without heads and shells, with a total waste of shrimp shells and heads that are not utilized reaching 60,000 tons. The abundant shrimp shell waste can be used for raw materials and industrial products. Shrimp shell waste contains chitin and chitosan compounds which have high economic value and the processed products can be used for various purposes. Chitosan has more uses and benefits than chitin, so chitosan is dubbed the magic of nature. Chitosan can be used in food processing, medicine, biotechnology and is an attractive material in biomedical and...
pharmaceutical applications. Chitosan is non-toxic, has biological activity, biocompatibility, biodegradability and can be modified chemically and physically (Lee, 2004).

At room temperature, fish enter the rigor mortis phase faster and last for a shorter time. If the rigor phase cannot be maintained longer then the decay by the activity of enzymes and bacteria will take place more quickly. The activity of these enzymes and bacteria causes very rapid changes so that the fish enter the post rigor phase. This phase indicates that the quality of the fish is low and unfit for consumption (FAO, 1995). Fish quality can be maintained if the fish is handled carefully (carefully), clean (clean), stored in a room with a cold temperature (cold), and quickly (quick). Preservation methods can be broadly divided into three groups, namely natural preservation, biological preservation, and chemical preservation. The chemical preservation process uses chemicals that can prevent the growth of microorganisms.

Preservatives that are often used are chemicals, but if used in excess it can endanger health, so the preservative we use is chitosan as a natural food preservative and is safe to use. Borax as a thickener and formalin as a meatball preservative are still found in the community, so it is necessary to find a safer and healthier alternative. Chitosan as a natural preservative is one alternative (Estiasih and Ahmadi, 2009).

Another alternative material as a natural antimicrobial so that it is not harmful to health is the use of chitosan to inhibit microbial activity. Shrimp shell contains protein (25% - 40%), calcium carbonate (45% - 50%), and Chitin (15% - 20%), but the amount of these components depends on the type of shrimp. There are many uses of chitosan, including for food preservatives (substitutes for formalin and borax), waste treatment, slimming drugs, cosmetics, and so on. Chitosan has an active group that will bind to microbes so that chitosan is also able to inhibit microbial growth. Chitosan is a material whose sources are abundant and renewable, so in a situation of reducing sustainable natural resources and the rapid development of biotechnology, the use of alternative natural resources such as shrimp shell waste is indispensable, therefore it is necessary to use waste shrimp into chitosan as a preservation of freshness quality in tilapia. Realizing healthy food ingredients and types of preservation that are safe for the human body is the first step to improve a better quality of life.

RESEARCH METHODS

Materials

The materials used in this study were shrimp shell waste, NaOH, Acetic Acid (CH3COOH), Aquades, Tilapia (Oreochromis niloticus), Commercial Citosan, Artificial Citosan, TCA 7%, TCA 5%, Boric Acid 2%, K2CO3, HCl 0.02 N, Cloroform Acetic Acid, KI, 1% Starch Indicator, Na2S2O3, PCA (Plate Count Agar), BFP (Butterfield Phosphate Buffered) Solution.

Research methods

The method in this research is an experimental method by preserving tilapia (Oreochromis niloticus) with different types of chitosan. The design used was a non-factorial completely randomized design.
(Gaspersz, 1991). The use of chitosan as a preservative to improve the quality of freshness in tilapia through immersion time of 1 hour, 2 hours, and 3 hours using three replications, so that there were 9 experimental and control units as indicators. Parameters observed were organoleptic test, TVB test, and Peroxide Number test.

The recommended mathematical model according to Gaspersz's (1991) design is as follows:

\[
Y_{ij} = \mu + \tau_i + \sum ij
\]

Where:
- \(Y_{ij}\) = Observation value from the -j test that received the -i treatment
- \(\mu\) = General mean
- \(\tau_i\) = Effect of treatment i
- \(\sum ij\) = The effect of the -j error that gets the -i treatment

Procedure for Preserving Tilapia (*Oreochromis niloticus*)

The manufacture of chitosan solution according to Wardaniati (2009) is chitosan powder as much as 2 grams per 100 ml of 1% acetic acid solution. Each fish sample was immersed in a chitosan solution with a concentration of 1 kg of fish per 1 liter of chitosan solution (Silvia et al, 2014). Fish were soaked with 3 variations of time, namely 1 hour, 2 hours and 3 hours with three replications and using control.

RESULTS AND DISCUSSION

Characteristics of Artificial Chitosan

| Specification | Description of Chitosan (Fatimah, 2012) | Description of Artificial Chitosan |
|---------------|--------------------------------------|-----------------------------------|
| appearance    | Brownish white                       | Brownish white                    |
| Smell         | No smell                             | No smell                          |
| texture       | powder                               | powder                            |
| yield         | 22.9 %                               | 24 %                              |

The use of chitosan as a preservation of tilapia (*Oreochromis niloticus*) on the duration of immersion, to determine the effect of chitosan in maintaining the freshness quality of tilapia can be viewed from the results of organoleptic tests, peroxide tests, TVB, and TPC.
ORGANOLEPTIC TEST

APPEARANCE

Appearance assessment on sensory testing can be viewed from the overall assessment of the eyes, gills, mucus and flesh. The use of chitosan as fish preservation value also affects the length of the rigormortis phase. Observations were made in the post rigormortis phase (the fish were rotting), for this reason, observations were made at the 7th hour, 9th hour, and 11th hour. The freshness quality of tilapia can be seen from the results of the appearance test. Observation of the quality value of the appearance at the 7th hour, the lowest value was on A11, which was 7.15 and the highest value was found in A33, which was 8.18, at the 9th hour the lowest was on A11 and A13, which was 5.45 and the highest was on A33, which was 6.35, and at the 11th hour the lowest value was on A13, which was 3.84 and the highest was on A21, which was 5.58. The difference between treatments A1, A2, A3 was not very significant, which means that the results obtained were correct and the results showed that between 1, 2 and 3 immersion, 3 hours of immersion was better than 1 and 2 hours of immersion. The effect on observations 7, 9 and 11 shows the results that at the 7th hour the value is still on average 7 and at the 9th hour it decreases by an average of 5 and at the 11th hour the results also decrease, namely getting an average value of 3 - 5, but still soaking for 3 hours is better than soaking 1 and 2 hours.

![Figure.1. Artificial Chitosan](image)

**Figure.1. Artificial Chitosan**

**Figure.2. The effect of immersion time on the quality value of the appearance of tilapia in the post rigormortis phase**

The average results obtained, the appearance test was carried out on tilapia (*Oreochromis niloticus*) that at 9 hours the fish were still experiencing the rigor mortis phase. Organooleptic value data for the
appearance of tilapia (*Oreochromis niloticus*). Based on the organoleptic value, the appearance at 7 hours had an average of 7.35 for 1 hour immersion, 7.51 for 2 hours and 7.75 for 3 hours organoleptic for 1 and 2 hours of immersion. -gray, slightly cloudy cornea, gills, slightly dull red color, without mucus, the mucus layer begins to cloud slightly, the color is slightly white, less transparent, the cut is slightly less bright, species specific, there is no milking along the spine, the abdominal wall flesh is intact, while at 3 hours the eyes are bright, the eyeballs are flat, the cornea is clear, the gills are not bright red, without mucus, the mucus layer is clear, transparent, bright, there is no color change and the flesh incision is bright specific to the type, there is no milking along the spine, walls whole stomach.

Based on Murniyati and Sunarman (2000), the process of fish spoilage occurs in the Hyperaemia stage, namely the fish mucus is released from the glands in the skin, forming a thick clear layer around the fish body. In addition, if the ambient temperature rises, the activity of bacteria becomes faster, thus making the mucus release from the glands thick and cloudy. According to Septiarni (2008), fish gills are the organs of the body that are most susceptible to rot and rot faster than other organs due to the accumulation of bacteria in high numbers in the gills. Based on Pia (2008)'s research, which looked at the organoleptic quality of tilapia given carbonic acid, that the texture properties of fresh fish muscles were much influenced by aggregation (collection) and protein denaturation due to the acidic nature of ascorbic acid.

**SMELL**

The odor assessment in the organoleptic test is a quality indicator to determine damage to tilapia. If the odor produced emits a strong ammonia H2S and a sour smell accompanied by rot, the fish is categorized as rotten fish that has experienced a deterioration in quality. Also obtained the results of F arithmetic A (6.40367) > from F table (0.05) which means H0 is rejected at the 95% confidence level, also obtained differences, namely between A1 is different from A2, A1 is different from A3 and A2 is the same as A3, and also obtained results of F arithmetic B (7.31102) > from F table (0.05) which means H0 is rejected at the 95% confidence level, also obtained differences, namely between B1 is different from B3, B2 is different from B3 and B1 is the same as B2.

The average results obtained, odor tests were carried out on tilapia (*Oreochromis niloticus*) that at 9 hours the fish were still experiencing the rigor mortis phase. odor at 7 hours has an average for soaking of 1 hour 7.32, and 2 hours 7.62, and 3 hours 7.67 organoleptically, the fresh smell has started to disappear, there is no sour smell and it is still acceptable. The 9th hour has an average for immersion of 1 hour 5.60, and 2 hours 6.17, and 3 hours 6.27 organoleptically at a neutral and acceptable odor. The 11th hour has an average for immersion of 1 hour 3.63, and 2 hours 4.77, and 3 hours 5.00 organoleptically at 1 and 2 hours immersion the smell of ammonia was strong, there was a H2S odor, the sour smell was clear and rotten, while at 3 hours immersion organoleptic the smell of ammonia started to smell, slightly sour smell. According to Ilyas (1983) that spoilage in fish is more oxidative rancidity. These changes occur due to fat oxidation, causing an unwanted rancid odor.
Texture assessment on organoleptic test is a quality indicator to determine damage to tilapia. If the texture of tilapia is very soft, finger marks do not disappear when pressed, it is easy to tear the meat from the spine, the fish is categorized as rotten fish that has experienced a decline in quality.

The texture value at the 7th hour, the lowest value was on A11, which was 7.50 and the highest value was on A33, which was 8.40, at the 9th hour the lowest was on A11, which was 5.30 and the highest was on A33, which was 6.75, and at the 11th hour the lowest value is on A11, which is 3.00 and the highest is on A33, which is 5.30. The difference between treatments A1, A2, A3 was not very significant, which means that the results obtained were correct and the results showed that between 1, 2 and 3 immersion, 3 hours of immersion was better than 1 and 2 hours of immersion. The effect on observations 7, 9 and 11 shows the results that at the 7th hour the value is still on average 7 and at the 9th hour it decreases by an average of 5 - 6 and at the 11th hour the results also decrease, namely getting an average value of 3 – 4, but still soaking for 3 hours is better than soaking 1 and 2 hours.
The average results obtained, odor tests were carried out on tilapia (*Oreochromis niloticus*) that at 9 hours the fish were still experiencing the rigor mortis phase. The average for soaking 1 hour is 7.72, and 2 hours is 7.78, and 3 hours is 7.98 organoleptically rather dense, elastic when pressed with fingers, difficult to tear the meat from the spine and still acceptable. The 9th hour has the average for soaking 1 hour 5.57, and 2 hours 6.35, and 3 hours 6.42 organoleptically slightly soft, less elastic when pressed with fingers, rather easy to tear meat from the spine. The 11th hour had an average average for immersion 1 hour 3.47, 2 hours 4.67 and 3 hours 4.70 organoleptically at 1 hour immersion soft, finger marks visible when pressed, easy to tear meat from the spine at 2 and 3 hours immersion organoleptic slightly soft, less elastic when pressed with your fingers, it is rather easy to tear the flesh from the spine. According to Berhimpon (1993) that changes in texture where the meat becomes softer occurs when the fish has started to experience a decline in quality. This is caused by the start of the overhaul of the muscle tissue of the meat by an enzymatic process.

**PEROXIDE NUMBER TEST**

One of the parameters for decreasing the freshness quality of tilapia is the peroxide value. Peroxide testing is needed to determine the level of spoilage in fresh fish because peroxide testing is carried out to determine the level of rancidity in fresh fish. Peroxide number is used to measure the levels of peroxides and hydroperoxides formed in the early stages of fat oxidation reactions. If the peroxide number is high, it indicates that it has been oxidized so that the freshness of the fish will be low.

The value of the peroxide number at the 7th hour is the lowest value is found in A31 and A32 which is 6.00 and the highest value is found in A11 and A12 which is 8.00, at the 9th hour the lowest is on A31 which is 17.00 and the highest is on A11 and A12, namely 21.00, and at the 11th hour the lowest values are on A31 and A32, namely 19.00 and the highest is on A12, which is 22.00. The difference between treatments A1, A2, A3 was not very significant, which means that the results obtained were correct and the results showed that between 1, 2 and 3 immersion, 3 hours of immersion was better than 1 and 2 hours of immersion. The effect on observations 7, 9 and 11 shows the results that at the 7th hour the value is still on average 6-7 and at the 9th hour there is an increase that is an average of 18 - 20 and at the 11th hour the results also increase, namely getting an average value an average of 19-21, but still at immersion for 3 hours the value was better than immersion for 1 and 2 hours.
Figure 5. Effect of immersion time on the value of peroxide value in tilapia in the post rigormortis phase

The average results obtained, the peroxide test carried out on tilapia (*Oreochromis niloticus*) showed that at 9 hours the fish still did not smell rancid. The standard allowable peroxide value in food is 20-40 mEq/kg. Peroxide number provides a measure of the level of primary oxidation that has occurred in the oil/fat (Chakrabarty, 2003). Odors and tastes associated with oxidative rancidity are mostly produced by carbonyl-type components. These components are formed in low concentrations in the initial oxidation process (Shahidi, 2005). The peroxide number is the amount of oxygen peroxide per kilogram of oil or fat. With continued oxidation, peroxides are degraded to aldehydes or bind to proteins (Woyewoda et al, 1986).

TOTAL BASE VOLATILE TEST

TVB testing is also very important in preserving fresh fish because by doing a TVB test we can find out the level of rottenness of fresh fish. TVB testing is one indicator of the freshness quality of Tilapia. The value from the observation of the TVB quality value at the 7th hour, the lowest value was found in A32, A33 and A22, namely 15.69 and the highest value was found in A11, which was 17.37, at the 9th hour the lowest was in A31, namely 17.93 and the highest was in A11, which is 21.85, and at the 11th hour the lowest value is on A31 which is 19.05 and the highest is on A11 and A12 which is 23.53. The difference between treatments A1, A2, A3 was not very significant, which means that the results obtained were correct and the results showed that between 1, 2 and 3 immersion, 3 hours of immersion was better than 1 and 2 hours of immersion. The effect on observations 7, 9 and 11 shows the results that at the 7th hour the value is still on average 15 - 16 and at the 9th hour there is an increase that is an average of 18 - 20 and at the 11th hour the results also increase, namely getting an average value the average is 20 – 23, but still soaking for 3 hours is better than soaking 1 and 2 hours.
Figure 6. Effect of immersion time on TVB test values in tilapia in the post rigormortis phase

The highest TVB content at 9 hours was found in A1 and B1 (20.92 and 20.54 mgN/100g) and the lowest was at A3 and B3 (18.49 and 18.68 mgN/100g). Based on the TVB content, all fish samples still met the requirements for consumption because the TVB content was still lower than the maximum allowable value, which was > 30 mgN/100g. The results of research by Nurjanah et al (2004) stated that the gain of TVB at each stage was 18.67 – 20 mgN/100g (pre rigor) and 20 – 24 mgN/100g (rigor mortis). Fish freshness based on TVB levels according to Farber (1965) as follows: 1. Very fresh fish (TVB < 10 mgN/100g), 2. Fresh fish (10 TVB 20 mgN/100g), 3. Fish is still fit for consumption (20 TVB 30 mgN/100g), 4. Fish is not fit for consumption (> 30 mgN/100g).

Kirk and Sawyer (1991) stated that the TVB value of 30-40 mgN/100g was the maximum limit. Likewise, Connell (1995) stated that the acceptance limit for fish was 30 mgN/100g. TVB concentrations of 25 to 35 mgN/100g of meat are suggested as acceptable limits for fresh whole commercial fish and processed fish products (Connell, 1995; Dalgaard, 2000). Rejection of fresh fish based on TVB concentration was determined based on sensory acceptance. It is important to note that TVB can only be used as an indicator of suitability for consumption and not as an index of fish freshness. TVB is also a good indicator of spoilage.

CONCLUSION

Chitosan can be useful for maintaining the quality of fresh fish and prolonging the rotten period of fish. The results of all tests showed that immersion for 3 hours was better than immersion for 1 and 2 hours, all of which were reviewed from the observation hours for 7, 9 and 11 hours. At the 7th hour observation, the results showed that there was no difference between immersion for 1, 2 and 3 hours, while at the 9th and 11th hour observations, different results were obtained between 1, 2 and 3 hours of immersion, and 3 hours of immersion. best. From the results of the study, it was concluded that the results of soaking using Chitosan solution, soaking 3 hours was better to preserve the quality of fresh fish than soaking 1 hour and 2 hours.
This is due to the nature of Chitosan which protects and binds fish in order to survive the proliferation of bacteria because at 3 hours of soaking Chitosan can perfectly bind and protect fish from microbes.

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REFERENCES

Berhimpon, S. 1993. Mikrobiologi Perikanan Ikan. Bagian 1. Ekologi dan Pertumbuhan Mikroba Serta Pertumbuhan. Biokimia Pangan. Laboratorium Pengolahan dan Pembinaan Mutu Hasil Perikanan. Fakultas Perikanan dan Ilmu Kelautan. Universitas Sam Ratulangi. Manado.

Chakrabarty, MM., 2003. Chemistry and Technology of Oils & Fats. Allied Publishers, November ISBN : 8177644955, 9788177644951.

Connell J.J., Control of fish quality. Fourth edition published by fishing Books, a division of Blackwell Scientific Ltd. 1995.

Dalgaard P. Fresh and lightly preserved seafood. In: Man CMD, Jones AA, editors. Shelf-life evaluation of foods. second ed. Gaithersburg, MD, USA: Aspen Publishing Inc; 2000. pp. 110–139.

Estiasih, T., dan Ahmad, 2009. Teknologi Pengolahan Pangan. Bumi Aksara. Malang. ISBN 979-010-5673

FAO. 1995. Quantity and Quality Changes in Fresh Fish, by Huss, ed. Rome: Fisheries Technical Paper No.384. 95 pp.

Farber, L. 1965. Freshness Test. In: Fish as Food Vol IV. Borgstormg (ed). New York, Academic Press

Gaspersz, V. 1991. Metode Perancangan Percobaan. CV.ARMICO. Bandung.

Ilyas, S. 1983. Teknologi Refrigerasi Hasil Perikanan 1, Teknik Pendinginan Ikan. Paripurna. Jakarta.

Kirk R, Sawyer R. 1991. Pearson’s Composition and Analysis of Foods, 9th ed., pp 642-643. Longman Scientific and Technical, Singapore, Singapore.

Lee, D.W. 2004. Engineered chitosans for drug detoxification preparation, characterization and drug uptake studies. Dissertation: University of Florida.

Murniayati, A.S. dan Sunarman, 2000. Pendinginan, Pembekuan dan Pengawetan Ikan. Kanisius. Yogyakarta. 220 hal.

Nurjanah, Setyaningsih, Sukarno, dan Muldani, M. 2004. Kemunduran Mutu Ikan Nila Merah (Oreochromis sp.) Selama Penyimpanan Pada Suhu Ruang. Buletin Teknologi Hasil Perikanan 7(1): 37-42.

Pia, S. 2008. Aplikasi Minuman Ringan Berkarbonasi Dalam Menghambat Laju Mutu Ikan Nila (Oreochromis niloticus). Fakultas Perikanan dan Ilmu Kelautan. Institut Pertanian Bogor. Bogor.

Shahidi, F.; Ying Zhong, 2005. Citrus oils and essences. In: Bailey’s Industrial Oil and Fat Products, Sixth Edition, John Wiley & Sons, Inc.
Septiarni, T. 2008. Karakteristik Mutu Ikan Tenggiri (*Scomberomorus commersonii*) Di Kecamatan Manggar, Kabupaten Belitung Timur. [Skripsi]. Program Studi Teknologi Hasil Perikanan Fakultas Perikanan dan Ilmu Kelautan Institut Pertanian Bogor.

Silvia, R., Waryani, S.W., dan Hanum, F. 2014. Pemanfaatan Kitosan dari Cangkang Rajungan (*Portonus sanginolentus*. I) sebagai Pengawet Ikan Kembung (*Rastrelliger* sp) dan Ikan Lele (*Clarias batrachus*). Jurnal Teknik Kimia USU. Vol. 3 No. 4: 18-24.

Wardaniati, Setyaningsih. 2009. Pembuatan Chitosan dari kulit Udang dan Aplikasinya untuk Pengawetan Bakso. http://repository.usu.ac.id/bitstream/123456789/21006/5/Chapter%20I.pdf Diakses tanggal 5 Mei 2014.

Woyewoda, A.D., Shaw, S.J., Ke, P.J. and Burns, B.G. (1986) Quality Indices-Lipid Related. In: Recommended Laboratory Methods for Assessment of Fish Quality: Canadian Technical Report of Fisheries and Aquatic Science, Department of Fisheries and Oceans, Halifax.