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# Feasibility Test of Osteichthyes Wet Preparations through the Utilization of Chitosan on Storage Quality as Practical Media for Vertebrate Zoology

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**Abstract:** This study aims to evaluate the use of chitosan as a substitute for formalin in making wet preparations of true bony fish (Osteichthyes) and to assess the feasibility of storing them as practicum media in the Vertebrate Zoology course. Osteichthyes body size varies from 1 cm to 6 meters. The main characteristic of Osteichthyes is the presence of a skeleton consisting of native bone and a hard calcium phosphate matrix. The skin is protected by various types of scales, including ganoid, cycloid, or stenoid scales. The research method used is Experimental Research Method with descriptive approach, which includes hedonic test and shelf life test (organoleptic) through panelist participation. Tilapia (Oreochromis niloticus) was used as the specimen in this experiment with variations in chitosan concentration of 0.50, 1.50, 2.50, and 3.50%. The study included evaluation of Osteichthyes wet preparations by considering aroma and odor, texture, color, as well as monitoring storage stability for one week. The results showed that the wet preparation using chitosan concentration of 3.5% gave satisfactory results for the observation of Osteichthyes structure. In addition, the storage evaluation showed that the quality of the preparations could be well maintained over a certain period. This indicates that chitosan can be an effective alternative in making Osteichthyes wet preparations for Zoology practicum.

**Keywords:** Chitosan; Hedonic test; Oreochormis niloticus; Organoleptic; Vertebrate zoology; Wet preparations

## Introduction

Vertebrate Zoology is a scientific discipline that focuses on understanding the developmental patterns, anatomical characteristics, and morphology of vertebrate animals. Teaching Vertebrate Zoology involves a variety of methods, including lectures, presentations, discussions, practical activities, and field explorations. The concept of the lecture discusses the phylum Chordata which includes classification and explanation of the anatomical and morphological characteristics of the classes: Pisces, Amphibians, Reptiles, Aves, and Mammals (Aryanti & Suhaerah, 2019).

Osteichthyes comes from a combination of Greek words, where "osten" means bone and "ichthys" means fish, and belongs to the Pisces class. Osteichthyes can be found in the sea, freshwater, and swamp. Osteichthyes body size range from 1 cm to 6 m. The main characteristics of Osteichthyes is the presence of a skeleton containing native bone and a hard calcium phosphate matrix. The skins are covered by ganoid, cycloid, or stenoid scales, although there are some that lack scales. Its body consists of segmented muscles, is equipped with jaws, teeth, and tongue, which is collectively known as Gnathostoma (Maya & Nur, 2021).

Vertebrate Zoology lectures on the Osteichthyes superclass demand students to understand in depth

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about the anatomical structure and morphological characteristics, which can be achieved through the use of wet preparations as a practicum tool. Fish wet preparations generally use formaldehyde or known as 40% formalin, which is a chemical that can be used for fish preservation (Aini et al., 2016). This fact is supported by recent news and research stating that the use of formaldehyde in fish preservation is very popular, especially in Asian countries (Marzuki et al., 2012; Andrews, 2013). An alternative preservative to formaldehyde can be used in the process of making chitosan fish preserves. This is because chitosan has antimicrobial and antioxidant properties that can slow down the damage process, maintain product quality, secure preparations, and extend the storage period of fish (Hussain et al., 2021). The use of formaldehyde often causes unsafe effects and is very dangerous for humans, while Chitosan is considered safer (Iswadi, 2018).

Chitosan can inhibit the growth of bacteria and fungi, and its antibacterial activity is often affected by molecular weight and deacetylation process (Wang & Xuea, 2020). Mohan et al. (2012) reported that chitosan with a deacetylation level (DD) of 83% was effective in inhibiting bacterial growth significantly. Chitosan has also been the focus of research and proven successful in maintaining the quality of various types of fishes and fish products (Soares et al., 2015). The raw materials of chitosan are shrimp and crab shells, through the process of decalcification, deproteinization, and decolorization to produce chitin. Subsequently, the mixture is mixed with 40-50% NaOH to remove acetyl and produce chitosan (Wang & Xuea, 2020; Gildberg & Stenberg, 2001).

Tilapia *(Oreochromis niloticus*), which has been cultivated in more than 100 countries worldwide, is one of the most common types of aquaculture in the world (FAO, 2018). Tilapia is highly demanded by consumers in Asia, North Africa, Central Africa, and South America due to its savory taste, affordable price, and valuable content of essential amino acids, unsaturated fatty acids, vitamins, and minerals (Shi et al., 2019; Yang et al., 2019). This study aims to test the feasibility of wet preparations of Osteichthyes, tilapia (*Oreochromis niloticus*) using chitosan solution with the concentrations of 0.5, 1.5, 2.5, and 3.5 on the preservation quality as practicum media in Vertebrate Zoology courses. The difference in the concentration, according to Sombo et al. (2020) and Cahyono (2018) is in order to compare which solution is very effective.

## Method

The research method used was experimental with a descriptive approach, involving hedonic tests (favorability testing) and shelf life tests (organoleptic testing) (Qamariah et al., 2022) on Osteichthyes (*Oreochromis niloticus*) wet preparations. The chitosan solution used various concentrations, which are 0.5, 1.5, 2.5, and 3.5%. This study involved assessment of Osteichthyes wet preparation by evaluating aroma and odor, texture, color, as well as observing storage stability over a 1-week storage period.

*Tools and Materials*

The tools used to support this research are digital laboratory scales, petri dishes, glass jars (100 and 500 ml), digital pH meters, and 600 ml glass jars for storing preserves. Meanwhile, the materials include chitosan powder made from 98% purity of crab or shrimp shell extract, 4 tilapia (*Oreochromis niloticus*) with an average mass of 56 grams, 1% acetic acid ($CH_3COOH$), and distilled water.

*Sample Preparation*

The creation of chitosan solution with various concentrations of 0.5, 1.5, 2.5, and 3.5%. Then, chitosan is diluted with 1% acetic acid solution (Cahyono, 2018; Mulatsari et al., 2022). After dissolving in acetate, pour it into a glass jar that has been filled with distilled water until it fills 550ml. The next stage is putting tilapia into each different chitosan solution and measuring the pH.

*Hedonic Test*

The hedonic test (liking and disliking) uses an organoleptic test, which includes assessment of aroma and smell, texture, and color (Sulaiman et al., 2022). The higher the score, the higher the panelists' preference for the wet-prepared osteichthyes samples. Panelists were given a questionnaire with a scale or score of 1 (most dislike) to 5 (most like). This hedonic test is a comparison of descriptive tests to identify product quality (Agustina et al., 2021). The hedonic test scale is presented in the following table.

**Table 1.** Hedonic Test Scale (Agustina et al., 2021)

| Hedonic Scale | Score |
|---|---|
| Most dislike | 1 |
| Dislike | 2 |
| Like | 3 |
| Most like | 4 |

*Shelf Life Test*

This observation was carried out by storing osteichthyes wet preparation samples that had been treated with various concentrations of chitosan solution in glass jars. Over a one week period, those samples are observed for changes in terms of aroma as well as smell, texture, and color.

## Result and Discussion

*Hedonic Test*

The test involved 15 panelists from the students of Biology Education Study Program, FKIP, Universitas Majalengka. These observations were made after a storage period of 1 week. The average hedonic test scores of the panelists on the osteichthyes wet preparation samples, as shown in the illustration on Figure 1.

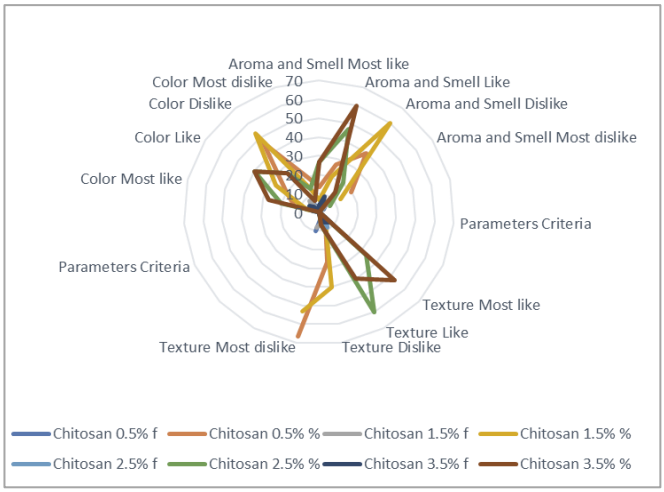


**Figure 1.** Average panelist hedonic test score on osteichthyes wet preparations

In accordance of Figure 1, it can be concluded that the panelists liked the aroma and smell, texture, and color of the fish specimen preparations using a 3.5% concentration of chitosan solution the most, because it has an aroma and smell that is not pungent, and it has a fresh texture though the color of the solution is slightly dark, which does not change too much from the initial color before being preserved. So that fish specimens that are used as wet preparations on day 7 provide excellent storage quality. The specific presentage of hedonic test results can be seen in Figure 2, Figure 3, and Figure 4 below.

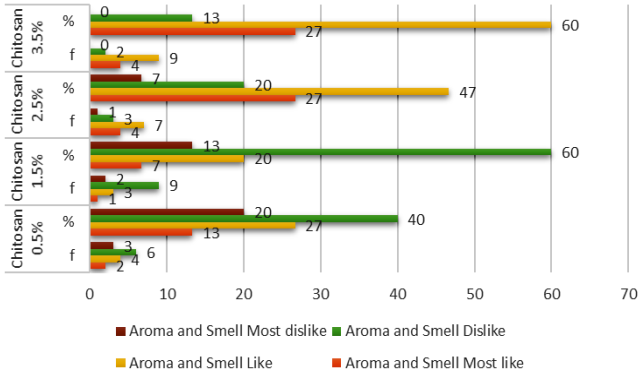


**Figure 2.** Hedonic test results for aroma and smell indicators

Figure 2 shows that the percentage of chitosan solution with a concentration of 0.5 and 1.5%, produces an aroma and odor that is not liked, with a percentage achievement of 40-60%. This is because at the concentration of the solution, the aroma and odor produced is very strong, especially from the fishy smell of the fish specimens used as preservation samples. Osteichthyes wet preparations that have a smell and odor favored by panelists are at a concentration of 2.5 and 3.5% with the percentage of liking are at 47 and 60%. Based on the percentage obtained, it can be concluded that the aroma and odor of osteichthyes wet preparations are most effective at 3.5% chitosan concentration, because the aroma and odor produced are weaker (Sombo et al., 2020), in terms of wet preparation products for this practicum media which are considered good.

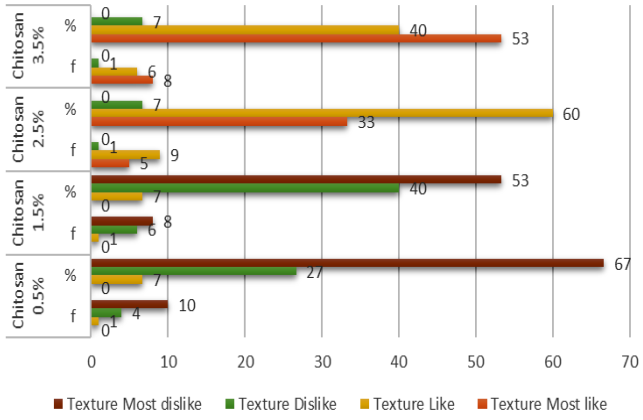


**Figure 3.** Hedonic test results for texture indicators

The percentage of hedonic test on texture at an 0.5% concentration are: 0% 'very like'; 7% 'like'; 27% 'dislike'; and 67% 'most dislike'. Chitosan in 0.5% concentration can be concluded that the texture is considered poor, because the fish specimen is no longer intact. The use of chitosan concentrations of 0.5 and 1.5% as a preservative, no longer reaches the level of optimality, thus creating opportunities for destructive microbes (Gita et al., 2021). The most favored texture of the fish by panelists were at concentrations of 2.5 and 3.5%.

Based on Figure 4 of hedonic test of color indicators, at a concentration of 0.5%, the average panelists chose 'dislike' by 47%. Concentration of 1.5%, panelists chose 'dislike' reaching 53%. Concentrations of 2.5 and 3.5% have a color that is considered good, because it reaches the level of preference with 60 and 67%. Fish specimens that are used as preparations at these concentrations still look good in color.

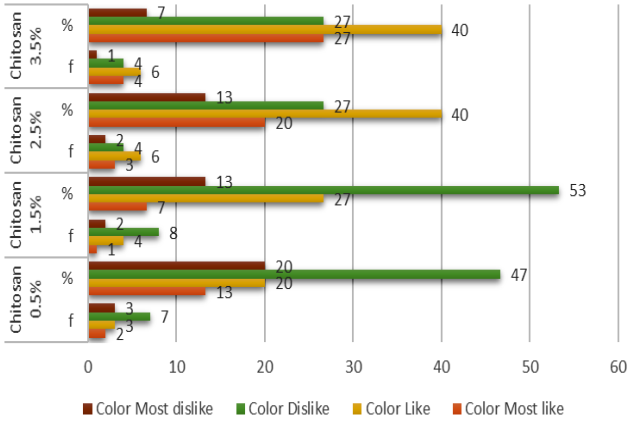


**Figure 4.** Hedonic test results on color indicators

*Shelf Life Test*

Testing the storage durability of Osteichthyes wet preparations using tilapia (*Oreochromis niloticus*) samples was carried out immediately after the preparation of chitosan solutions with varying concentrations of 0.5, 1.5, 2.5, and 3.5%, involving organoleptic (hedonic) test. In the context of this study, the storage period was set at 1 week (7 days). Stability is a crucial factor in a formulation, as it affects the consistency of the product during storage (Qamariah et al., 2022). The purpose of this shelf life test is to determine the quality of the preparation in the storage process from the first day to the seventh. The following are the results of observation of the stability of wet preparations for 1 week.

**Table 2.** Organoleptic (Hedonic) Test Results of Osteichthyes Wet Preparations Using Chitosan Solution

| Chitosan concentration (%) | Day | Aroma and Smell | | | | | Texture | | | | | Color | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | 1 | 2 | 3 | 4 | 5 | 1 | 2 | 3 | 4 | 5 |
| 0.5 | 0 | | | | √ | | | | | | √ | | | | √ | |
| | 1 | | | | √ | | | | | | √ | | | | √ | |
| | 2 | | | | √ | | | | | √ | | | | | √ | |
| | 3 | | | √ | | | | | | √ | | | | √ | | |
| | 4 | | | √ | | | | | √ | | | | √ | | | |
| | 5 | | | √ | | | | | √ | | | | √ | | | |
| | 6 | | √ | | | | | √ | | | | √ | | | | |
| | 7 | | √ | | | | √ | | | | | √ | | | | |
| 1.5 | 0 | | | | | √ | | | | | √ | | | | √ | |
| | 1 | | | | | √ | | | | | √ | | | | √ | |
| | 2 | | | | √ | | | | | | √ | | | | √ | |
| | 3 | | | | √ | | | | | √ | | | | | √ | |
| | 4 | | | | √ | | | | | √ | | | | √ | | |
| | 5 | | | √ | | | | | √ | | | | √ | | | |
| | 6 | | | √ | | | | | √ | | | | √ | | | |
| | 7 | | | √ | | | | √ | | | | √ | | | | |
| 2.5 | 0 | | | | | √ | | | | | √ | | | | | √ |
| | 1 | | | | | √ | | | | | √ | | | | | √ |
| | 2 | | | | | √ | | | | | √ | | | | | √ |
| | 3 | | | | | √ | | | | | √ | | | | | √ |
| | 4 | | | | √ | | | | | | √ | | | | | √ |
| | 5 | | | | √ | | | | | √ | | | | | √ | |
| | 6 | | | | √ | | | | | √ | | | | | √ | |
| | 7 | | | √ | | | | | √ | | | | | √ | | |
| 3.5 | 0 | | | | | √ | | | | | √ | | | | | √ |
| | 1 | | | | | √ | | | | | √ | | | | | √ |
| | 2 | | | | | √ | | | | | √ | | | | | √ |
| | 3 | | | | | √ | | | | | √ | | | | | √ |
| | 4 | | | | | √ | | | | | √ | | | | | √ |
| | 5 | | | | √ | | | | | | √ | | | | | √ |
| | 6 | | | | √ | | | | | √ | | | | | | √ |
| | 7 | | | | √ | | | | | √ | | | | | √ | |

Based on Table 2 and Figure 5, shortly after preparation, organoleptic (hedonic) tests were started which included aroma and odor, texture, color of specimens with chitosan solution in concentrations of 0.5, 1.5, 2.5, and 3.5%. The results show that specimens of various concentrations and indicators of organoleptic (hedonic) test observations are still in a good condition. Day 1-3, the condition of the prepared specimens still has not changed, but the color of the chitosan solution of 0.5 and 1.5% concentration looks a little cloudy and the

smell on the 0.5% concentration is slightly smelly. The result of panelist observation on the 4th day of the 0.5% chitosan solution, the aroma began to smell fishy, the texture was damaged, marked by peeling of epidermal and muscle tissue, and the color looked faded. Specimens using 1.5% chitosan solution had all measurement indicators rated as slightly good. While those using 2.5 and 3.5% chitosan are still in very good condition.

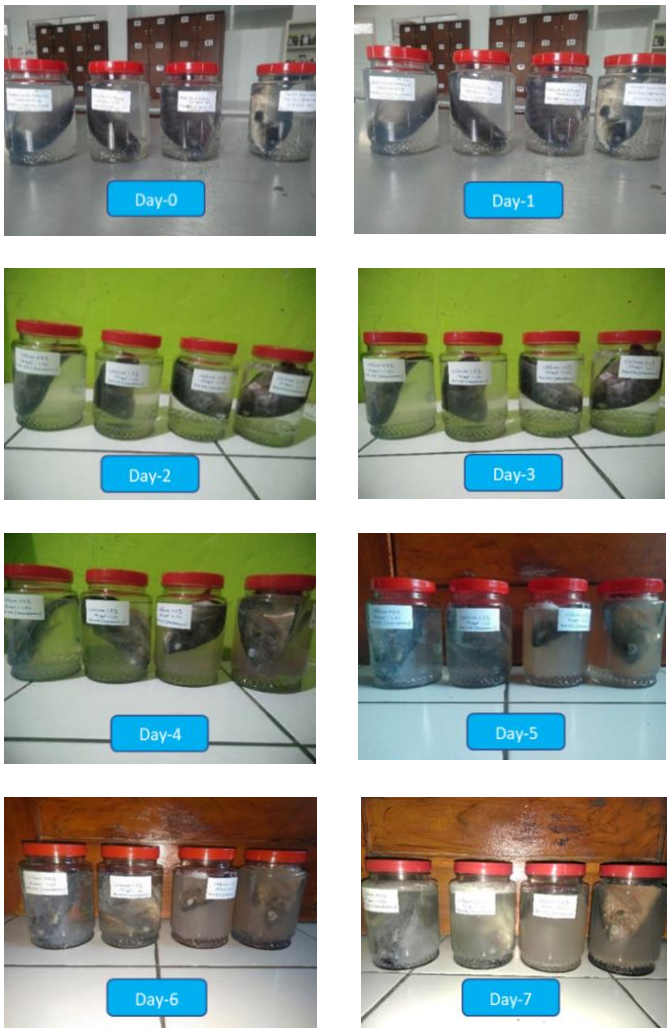


**Figure 5.** Documentation of organoleptic (hedonic) tests of osteichthyes wet preparations

Day 5-6, preparations with a concentration of 0.5% smell more pungent. This is because the fish specimens that are used as preparations begin to decay, the morphological shape of the fish tissue is destroyed, and the color fades. At a concentration of 1.5%, the smell is almost the same as that using a concentration of 0.5%. It's only the texture and color are slightly better. Meanwhile, at the concentration of 2.5 and 3.5%, the results of panelist observations of odor, texture, and color, are still in a state of 'like'. The color of the specimen using a 3.5% chitosan solution, the panelists rated it 'most like'. In conclusion, that on day 5-6, the condition of the specimens in preparations using 2.5 and 3.5% chitosan, is still in a good condition. On the seventh day, osteichthyes preparations with chitosan concentration of 0.5 and 1.5% were rated 'dislike' to 'most dislike' by the panelists on average. This is because the quality of the preparation specimens is no longer shaped, the smell of odor is more pungent even though the storage medium is tightly closed. The 2.5% concentration on seventh day, received an assessment in the 'rather good' category on all indicators of the organoleptic (hedonic) test. Moreover, the 3.5% concentration was categorized as 'good' in terms of smell, texture, and color. Based on the results of the shelf life test for one week that the Osteichthyes wet preparation, with a concentration of 3.5% chitosan solution, has the best shelf life quality.

## Conclusion

The use of chitosan powder with the right concentration in making Osteichthyes wet preparations with tilapia (*Oreochromis niloticus*) specimen samples can be used as an effective and sustainable alternative practicum media in Vertebrate Zoology courses. The concentration of chitosan that is considered good based on the organoleptic (hedonic) test is at a level of 3.5%, because it does not produce a strong odor, and the texture and color are in the good category.

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**Author Contribution**
This research group played a crucial role in crafting this scientific paper, including generating ideas, designing the study, gathering and analyzing data, interpreting results, drafting the manuscript, writing the article, undergoing revisions, and securing funding for the research.

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**Conflicts of Interest**
The authors clarify that there is no conflict of interest.

## References

Agustina, R., Fadhil, R., & Mustaqimah, M. (2021). Organoleptic Test Using the Hedonic and Descriptive Methods to Determine the Quality of Pliek U. *IOP Conference Series: Earth and*

*Environmental Science*, *644*(1). https://doi.org/10.1088/1755-1315/644/1/012006

Aini, B. N., Siddiquee, S., & Ampon, K. (2016). Development of Formaldehyde Biosensor for Determination of Formalin in Fish Samples; Malabar Red Snapper (Lutjanus malabaricus) and Longtail Tuna (Thunnus tonggol). *Biosensors*, *6*(3). https://doi.org/10.3390/bios6030032

Andrews, J. (2013). Formaldehyde Detected in Supermarket Fish Imported from Asia. *Food Safety News*, 9–11.

Aryanti, F., & Suhaerah, L. (2019). The Effectiveness of a Field Study on Vertebrate Zoology to Improve the Mastery of Student Concept. *AIP Conference Proceedings*, *2120*. https://doi.org/10.1063/1.5115715

Cahyono, E. (2018). Karakteristik Kitosan dari Limbah Cangkang Udang Windu (Panaeus monodon). *Akuatika Indonesia*, *3*(2), 96. https://doi.org/10.24198/jaki.v3i2.23395

FAO. (2018). Fishery and Aquaculture Statistics 2016. In *FAO yearbook*.

Gildberg, A., & Stenberg, E. (2001). A New Process for Advanced Utilisation of Shrimp Waste. *Process Biochemistry*, *36*(8–9), 809–812. https://doi.org/10.1016/S0032-9592(00)00278-8

Gita, R. S. D., & Afandi, A. (2021). Uji Efektivitas Khitosan terhadap Daya Awet Ikan Nila (Oreochromis niloticus). *BIOEDUSAINS: Jurnal Pendidikan Biologi dan Sains*, *4*(2). https://doi.org/https://doi.org/10.31539/bioedusains.v4i2.2987

Hussain, M. A., Sumon, T. A., Mazumder, S. K., Ali, M. M., Jang, W. J., Abualreesh, M. H., Sharifuzzaman, S. M., Brown, C. L., Lee, H. T., Lee, E. W., & Hasan, M. T. (2021). Essential Oils and Chitosan as Alternatives to Chemical Preservatives for Fish and Fisheries Products: A Review. *Food Control*, *129*(108244). https://doi.org/10.1016/j.foodcont.2021.108244

Iswadi, D. (2018). Penggunaan Khitosan sebagai Pengganti Formalin untuk Pengawetan Ikan Teri The Utilization of Chitosan for Anchovy Preservation. *Jurnal Ilmiah Teknik Kimia*, *2*(1). https://doi.org/10.32493/jitk.v2i1.1086

Marzuki, N., Bakar, F., Salleh, A., Heng, L., Yusof, N., & Siddiquee, S. (2012). Electrochemical Biosensor Immobilization of Formaldehyde Dehydrogenase with Nafion for Determination of Formaldehyde from Indian Mackerel (Rastrelliger kanagurta) Fish. *Current Analytical Chemistry*, *8*(4), 534–542. https://doi.org/10.2174/157341112803216843

Maya, S., & Nur, R. A. (2021). Zoologi Vertebrata. *Angewandte Chemie International Edition*, *6*(11), 951–952.

Mohan, C. O., Ravishankar, C. N., Lalitha, K. V., & Gopal, T. K. S. (2012). Effect of Chitosan Edible Coating on the Quality of Double Filleted Indian Oil Sardine (Sardinella longiceps) During Chilled Storage. *Food Hydrocolloids*, *26*(1), 167–174. https://doi.org/10.1016/j.foodhyd.2011.05.005

Mulatsari, E., Mumpuni, E., Qodriah, R., Budiati, A., Gunawan, J., Soeroso, M. H., & Serlahwaty, D. (2022). Synthesis and Characterization of Chitosan from Crab Shell Waste and Its Applications as Edible Coating. *International Journal of Applied Pharmaceutics*, *14*(Special Issue 3), 13–17. https://doi.org/10.22159/ijap.2022.v14s3.02

Qamariah, N., Handayani, R., & Mahendra, A. I. (2022). Uji Hedonik dan Daya Simpan Sediaan Salep Ekstrak Etanol Umbi Hati Tanah. *Jurnal Surya Medika*, *7*(2), 124–131. https://doi.org/10.33084/jsm.v7i2.3213

Shi, C., Qian, J., Zhu, W., Liu, H., Han, S., & Yang, X. (2019). Nondestructive Determination of Freshness Indicators for Tilapia Fillets Stored at Various Temperatures by Hyperspectral Imaging Coupled with RBF Neural Networks. *Food Chemistry*, *275*, 497–503. https://doi.org/10.1016/j.foodchem.2018.09.092

Soares, N. M. F., Oliveira, M. S. G., & Vicente, A. A. (2015). Effects of Glazing and Chitosan-Based Coating Application on Frozen Salmon Preservation During Six-Month Storage in Industrial Freezing Chambers. *Lwt*, *61*(2), 524–531. https://doi.org/10.1016/j.lwt.2014.12.009

Sombo, D. E., Turambi, S. I. M. W. A., & Cahyono, E. (2020). Efektivitas Kitosan sebagai Bahan Pengawet Alami pada Produk Tradisional Ikan Layang (Decapterus russeli) Asap Pinekuhe. *Jurnal Kemaritiman: Indonesian Journal of Maritime*, *1*(2), 54–67. https://doi.org/https://doi.org/10.17509/ijom.v1i2.29290

Sulaiman, I., Irfan, I., & Syahputra, R. (2022). The Influence of Temperature and Duration of Brewing on the Sensorial Value of Gayo Arabica Wine Coffee, a Comparison of Hedonic and Cupping Test Methods. *Coffee Science*, *17*. https://doi.org/10.25186/.v17i.2032

Wang, W., & Xuea, C. X. Z. M. (2020). Chitosan: Structural Modification, Biological Activity and Application. *International Journal of Biological Macromolecules*, *164*, 4532–4546. https://doi.org/10.1016/j.ijbiomac.2020.09.042

Yang, X., Zhou, Y., Wang, B., Wang, F., Han, P., & Li, L. (2019). Tartary Buckwheat Extract and Chitosan Coated Tilapia (Oreochromis niloticus) Fillets Determine Their Shelf Life. *Journal of Food Science*, *84*(6), 1288–1296. https://doi.org/10.1111/1750-3841.14649