The Effect of Chitosan Solutions with Different Molecular Weights on the Shelf Life of Tilapia Fillets (*Oreochromis niloticus*)

ABSTRACT

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| Tilapia fillets are a food ingredient that is very easily damaged and has a very short shelf life due to the quality degradation process that occurs due to autolysis, enzymatic activity, fat oxidation, and microorganism activity. Chitosan is known to have good antibacterial properties because it contains lysozyme enzymes and aminopolysaccharide groups that can limit bacterial growth and inhibit bacterial efficiency. Chitosan has the potential to protect tilapia fillets as an edible coating. The antibacterial properties of chitosan depend on its characteristics, chitosan with a low molecular weight has better antibacterial properties because in its application as an edible coating, chitosan with a low molecular weight is able to penetrate meat tissue and has high antibacterial properties. This article aims to produce good chitosan characteristics (including: molecular weight, viscosity, degree of deacetylation, yield, and solubility) to improve its antibacterial ability, as well as to analyze the effect of adding chitosan with different characteristics as an edible coating in inhibiting the decline in the quality of tilapia fillets during storage based on pH value and weight loss, as well as total plate count (TPC) according to the Indonesian National Standard, namely 5 x 105 cfu/g. |

*Keywords: Tilapia fillet, chitosan, edible coating, molecular weight, shell life*

1. INTRODUCTION

Tilapia (Oreochromis niloticus) is one of the freshwater fish commodities consumed in Indonesia that has high economic value (Solihah 2023). Tilapia as a food ingredient has a high nutritional content, namely protein (43.76%), fat (7.01%), and ash (6.80%) (Souhoka et al. 2019). One way to process tilapia currently is by making it into fillets. Fish fillets have the characteristics of being perishable food (Masengi et al. 2021). Fish meat has a very high water content (±80%) and is easily digested by autolysis enzymes, causing fish meat to be susceptible to rapid quality deterioration. (Lestari et al. 2020). The process of quality deterioration that occurs is caused by the activity of microorganisms, enzymes, and oxidation that occurs in the fish body (Adawyah 2007).

Efforts to extend the shelf life of fish fillets include proper processing, storage, and packaging techniques. Packaging is one way to inhibit the process of protein denaturation in fish fillets and can prevent rancidity (Hakim et al. 2016). Edible coating is a commonly used and safe packaging method. Edible coating can extend the shelf life of food by preventing contamination from water and oxygen, and inhibiting the growth of microorganisms (Vatria et al. 2021). Chitosan is a natural polysaccharide that is non-toxic, biodegradable, and biocompatible. Chitosan is polycationic, thus offering potential and benefits in the food industry (One et al. 2025).

The antimicrobial properties of chitosan against bacteria or microorganisms depend on its molecular weight and degree of deacetylation. A lower molecular weight and a higher degree of deacetylation indicate that microbial activity is increasingly inhibited (Killay 2013). Chitosan effectively kills Gram-negative bacteria by blocking the flow of nutrients, O₂, and water (Alhuur et al. 2020). Research by Lee et al. (2019) reported that tilapia fillets coated with low molecular weight chitosan (50 kDa) can maintain the fillet's pH value during storage. The antimicrobial properties of chitosan with a molecular weight of 55 kDa-155 kDa (low molecular weight) show antibacterial activity of more than 200 ppm against E. coli strains (Liu et al. 2006).

The process of shortening the molecular chain and reducing the molecular weight of chitosan can be carried out using the depolymerization method. The depolymerization method can reduce the molecular weight and shorten the molecular chain of chitosan by breaking the glycosidic bonds (Tanasale et al. 2016). Depolymerization produces chitosan with a shorter molecular chain, thus having potential as an antibacterial and antimicrobial agent (Nuryadin et al. 2020).

**2. TILAPIA FILLET**

Tilapia has excellent nutritional value, including being rich in omega-6 fatty acids and omega-3 fatty acids, higher than chicken or beef, and high levels of vitamins B3, B12, minerals (phosphorus, selenium, potassium), and niacin (Dailami et al. 2021). Fillets are a semi-finished product that can be used to fortify various processed products (Suryaningrum et al. 2012).

Fish fillets quickly experience a decline in quality because fish have a high protein and fat content, so bacteria and enzymes are quickly activated in the fillets (Ridwan et al. 2015). This decline in quality is generally caused by autolysis, enzymatic activity, fat oxidation, and microorganism activity (Ramezani et al. 2014).

**3. CHITOSAN**

Chitosan is a polysaccharide derived from chitin, found in the shells of crustaceans. Chitosan's properties make it useful in various industrial fields, such as pharmaceuticals, health, and food (Damayanti et al. 2016). Chitosan has a β (1-4)-2-amino-2-deoxy-D-glucose polysaccharide chain (Hambali et al. 2017).

According to Yahya et al. (2015), chitosan has the ability to be used as an antibacterial agent, because it contains the enzyme lysozyme and aminopolysaccharide groups that are able to limit the development of bacteria and the efficiency of chitosan's inhibitory power against bacteria, because chitosan is bacteriostatic which is able to prevent bacterial cell metabolism and is able to prevent its growth. (Subuhi et al. 2023) Chitosan antimicrobial works by killing microorganisms or inhibiting their growth which can be applied to a food product in the form of an edible coating that is able to coat the outermost part of the food so that it is protected from microbes that might enter the food (Yusfiani et al. 2019)

**4.CHITOSAN DEPOLYMERIZATION**

Depolymerization of chitosan through the cleavage of glycosidic bonds produces oligochitosan, which is soluble in water (neutral pH) and has various applications (Sanria et al. 2017). The depolymerization process occurs after the cleavage of the β-glycosidic bond, resulting in a lower molecular weight than the chitosan before depolymerization. The reduced molecular weight of chitosan results in greater solubility (Agusnar and Ilyas 2022). The use of oligochitosan has increased significantly in various fields, especially in the pharmaceutical, health, and food industries.

According to Ardean et al. (2021), low-molecular-weight chitosan, ranging from 5 to 10 kDa, has stronger antibacterial, antifungal, and hypopolyemic properties. There are several methods for depolymerizing a polymer, including chemical, physical, and enzymatic methods.

**4.1CHEMICAL DEPOLYMERIZATION**

Chemical depolymerization can use a strong acid catalyst (H+) until a hydrolysis process occurs that will cut the chitosan polymer chain (Yulina et al. 2014). Chemical depolymerization of chitosan can be carried out using acids such as HCl, H2SO4, and CH3COOH, with the use of oxidants such as O3, NaNO2, and H2O2 (Tanasale et al. 2016). Chemical methods are commonly used and require a short time to produce oligochitosan. Chemical depolymerization causes depolymerization that is difficult to control and produces too many monomers and produces oligosaccharides with a low degree of polymerization (DP 2-5) which is due to low efficiency and cutting (Hasri 2010).

**4.2PHYSICAL DEPOLYMERIZATION**

Physical depolymerization of chitosan can be achieved using radiation (UV, γ), microwave ultrasound, and heat treatment. One physical method that has been tried is using microwave radiation with a microwave oven, which produces chitosan oligomers. Physical depolymerization requires specialized equipment and the resulting molecule size cannot be controlled (Lin et al. 2009). This physical method reduces the impact of pollution on the environment because it does not produce chemical residues.

**4.3ENZYME DEPOLYMERIZATION**

Enzymatic depolymerization can be performed using enzymes, such as chitinase, chitosanase, glucanase, lipase, and several proteases. Some non-specific enzymes include lysozyme, cellulase, amylase, papain, and pectinase (Liu et al. 2006). Enzyme concentration and incubation time can affect the results of the depolymerization process (Fawzya et al. 2009). Enzymatic depolymerization of chitosan is effective because enzymes act specifically on reactive sites, breaking molecules into oligomers, or working from one end by sequentially releasing monomers or dimers (Almanza et al. 2019).

Enzymatic methods have the ability to control reactions and product formation through enzyme concentration, pH, temperature, and reaction time (Sinha et al. 2016). Research by Laokuldilok et al. (2017) used the papain enzyme depolymerization method with an enzyme concentration of 0.003% and a temperature of 40°C, and the best hydrolysis time of 16 hours to produce oligochitosan with a molecular weight of 4.3 kDa.

**5. CHITOSAN AS AN EDIBLE COATING**

*Edible coating is an edible coating made from natural ingredients. Edible coating can replace plastic packaging because it is biodegradable and acts as a barrier to control moisture, oxygen, and fat. The material used must be able to retain oxygen and water vapor, be colorless, tasteless, not alter the properties of the food, and be safe for consumption (Ridwan et al. 2015). Environmentally friendly edible coatings are made from biodegradable materials that are abundant in nature (Ridwan et al. 2015). Chitosan is one material that can be used for edible coating (Swastawati et al. 2008).*

**5.** **CHARACTERISTICS OF CHITOSAN**

**5.1 Chitosan Viscosity**

Viscosity can be measured using an Ostwald viscometer. Measurements are made to measure the flow time of the chitosan solution (Suryani et al. 2023). Viscosity that is too high will affect the thickness of the chitosan produced (Siregar et al. 2016). The optimal viscosity value for edible coating applications is in the range of 113-225 cP, low viscosity facilitates dipping and drying speed during application (Anggraini et al. 2016)

**5.2 Molecular Weight of Chitosan**

The molecular weight of chitosan is the sum of the atomic masses that make up its molecule (Hadi et al. 2024). This weight is expressed in Daltons (Da). Molecular weight (MW) can affect the antibacterial properties of chitosan. Chitosan with a lower molecular weight produces better antibacterial properties against E. Coli (gram-negative) than chitosan with a higher molecular weight (Liu et al. 2006). Other studies also stated that the antibacterial activity produced by chitosan against gram-negative bacteria was proven to be higher with decreasing molecular weight, degree of acetylation, and pH of chitosan (Younes et al. 2014). The molecular weight of chitosan also depends on the degradation that occurs during the deacetylation process (Rumengan et al. 2018).

Based on its molecular weight, chitosan can be divided into three categories (Roman-Doval et al. 2023):

1. Low molecular weight chitosan with a molecular weight <150 kDa
2. Medium molecular weight chitosan, with a molecular weight between 150 kDa and 700 kDa
3. High molecular weight chitosan, with a molecular weight >700 kDa

**5.3 Degree of Deacetylation**

The degree of deacetylation is the percentage of acetyl groups that can be eliminated from chitin compounds to produce chitosan. A higher degree of deacetylation of chitosan results in a lower acetyl group content, resulting in stronger hydrogen bonding and ion interactions. Chitosan is positively charged due to the release of acetyl groups, allowing it to bind negatively charged compounds, such as proteins and polysaccharide anions, to form neutral ions (Setha et al. 2019). Mursida et al. (2018) stated that the deacetylation process aims to remove the acetyl group by breaking the covalent bond between the acetyl group and nitrogen in the acetamide group of chitin, resulting in a deacetylated amine group (-NH2).

**5.4 Yield**

Yield is one of the parameters used as a quality indicator in a process. Yield is calculated based on the percentage of chitosan weight that has undergone a molecular chain shortening process divided by the weight of the initial raw chitosan material (Chamidah et al. 2019). A low yield content indicates that the deproteination, demineralization, and deacetylation processes are running optimally. Based on research by Mursida et al. (2018), a lower chitosan yield will produce better quality, because it is suspected that the demineralization, deproteination, and deacetylation processes are able to dissolve or remove minerals, proteins, and acetyl groups.

The percentage of chitosan yield will produce different yield calculation results due to particle size, reagents used, too high temperatures, and the type of raw material used is different species. Research by Ma'mun et al. (2016) reported that the yield of chitosan obtained from crab shells was less than the yield of chitosan derived from shrimp raw materials. This is because the mineral content contained in crab shells is 53.70% - 78.40%, this value is greater than the mineral content in shrimp shells as much as 45-50%.

**5.5 Solubility**

Solubility is the maximum amount of a solute that can be dissolved in a particular solvent to form a homogeneous solution (Pari et al. 2022). Chitosan is insoluble in water with a neutral pH, but can dissolve in acidic solvents (Lodhi et al. 2014). Solubility can be used as a standard for assessing the quality of chitosan because solubility is the maximum quantity of a dissolved chemical substance that can dissolve in a particular solvent to form a homogeneous solution. The solubility of chitosan can be affected by the length of immersion in the NaOH solution and the concentration of the NaOH solvent used (Rochima 2007).

The solubility of chitosan is closely related to the degree of deacetylation, because the higher the degree of deacetylation, the more acetyl groups are converted into amine groups, thereby reducing the hydrogen bonds between the acetyl and hydroxyl groups (Trimulyadi 2013). The solubility of chitosan depends on the degree of deacetylation (DD) and molecular weight (BM). Chitosan with a DD value of >70% can be partially dissolved in neutral pH, while chitosan that can dissolve well in water has a degree of deacetylation of >85% (PachecoTorgal 2016).

**6. APPLICATION OF CHITOSAN SOLUTION ON NILE FISH FILLETS**

**6.1 Total Plate Count (TPC)**

*Total plate count (TPC) is a method of calculating the total number of microbes present in meat using Plate Count Agar (PCA) media (Samudra et.al. 2016). The microbiological quality of a food ingredient is determined by the number of bacteria present in the food ingredient (Sufyan et al. 2014).*

The Total Plate Count (TPC) test is intended to indicate the number of microbes contained in a product by counting bacterial colonies grown on agar media. The principle of this method is that if living microbial cells are grown on agar media, the cells will reproduce and form colonies that can be seen directly without using a microscope. The method of planting the culture in a dish is done using the pour plate method. The results of the number of colonies counted are adjusted to the Standard Plate Count (SPC) (Fardiaz 2004). The total microbial (TPC) test uses the standard limit set by the Indonesian National Standard, namely 5 x 105 cfu/g (National Standardization Agency (BSN) 2015).

**6.2 pH Value**

The pH value is one indicator used to determine the freshness of fish. Changes in the pH of the meat play a significant role because they influence the autolysis process and bacterial attack. The use of low temperatures affects pH fluctuations in tilapia. Storing tilapia at low temperatures inhibits the activity of enzymes in the meat, resulting in slower quality deterioration. The lower the temperature, the slower the enzyme activity. Enzymes play a crucial role in the glycolysis process leading to the formation of lactic acid. This slows down the accumulation of lactic acid, thus slowing the decrease in the fish's pH. The process of protein breakdown into alkaline compounds by bacteria is also inhibited, resulting in a slower increase in the fish's pH (Munandar 2009).

pH testing on fillets is performed to determine the pH accumulation used to determine the extent of fillet quality during storage. The pH value is used to support other quality parameters (Baygar et al. 2008). Generally, the first chemical change in fish meat is a change in pH, but changes in fish pH values depend on the species, so pH values are not a definitive criterion for detecting the freshness and quality of fish meat and its processed products (Zega et al. 2017).

**6.3 Weight Loss**

Weight loss is an important indicator for evaluating fish quality because it can affect the sensory and economic value of the fish (Duan et al. 2010). Weight loss is caused by the water capacity of the meat being evaporated during storage, altering the nutritional and structural composition of the fish. Higher weight loss indicates that the fish fillet has lost essential components (Mohan et al. 2012).

Weight loss occurs after undergoing a specific process, such as cooling. Weight loss in fillets is generally caused by the loss of water content in the fish fillet itself during the cooling process. Weight loss during cooling can be caused by moisture in the material leaving the surface of the material and entering the surrounding air through the process of water vapor condensation (Zega et al. 2017). Weight loss is related to the water content in the material; the longer the storage period, the greater the weight loss of a material, resulting in the material's weight shrinking.

**7. Conclusion**

Tilapia is a freshwater fish commodity consumed in Indonesia that has economic value due to its high nutritional content. One way to process and market tilapia is by making it into fillets. Fish fillets have a weakness because they are easily spoiled. The high water content in fish makes them susceptible to rapid quality deterioration. The use of chitosan as an edible coating has the potential to protect fish fillets because chitosan has antibacterial properties. Chitosan's antibacterial properties work by killing or inhibiting the growth of microorganisms. Chitosan's antibacterial properties depend on its characteristics. Chitosan characteristics include viscosity, molecular weight, degree of deacetylation, yield, and solubility. The abilities that influence chitosan's ability are molecular weight and degree of deacetylation. Chitosan has a long chain so that it has relatively low solubility. Depolymerization is carried out to cut the chitosan chain with the aim of improving chitosan's performance and expanding its applications. Based on several studies, chitosan with a low molecular weight (<150 kDa) and a high degree of deacetylation has better inhibitory properties against microbial growth. Low molecular weight chitosan has higher antibacterial properties than high molecular weight chitosan. The application of chitosan to tilapia fillets can prevent quality deterioration that occurs in tilapia fillets during storage. Tilapia fillets will also be protected from deterioration in quality characteristics such as pH, weight loss, and total microbial count.

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