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Review article: Application of Chitosan for Fish Preservation and Processed Products

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ABSTRACT

The purpose of this review article is to examine chitosan and its manufacture, the quality of chitosan, the mechanism of chitosan as a preservative, the application of chitosan as a natural preservative. Based on a review of various literature both journals and other literature, it can be concluded that chitosan is a deacetylation compound of chitin, consisting of N-acetyl glucosamine and N glucosamine units. The production of chitosan consists of deproteination - demineralasation - deacetylation steps. The quality of chitosan can be assessed from the aspects of particle shape, color, degree of deacetylation, pH, moisture content and ash content. The mechanism of chitosan as a preservative is to interact with rotting bacterial cell membranes to disrupt membrane permeability and chitosan also has a very strong affinity with microbial DNA so that it can interfere with mRNA and protein synthesis.

Keywords: Mechanism, quality, manufacture, deacetylation, color.

INTRODUCTION

Fish is a nutritious food commodity and is widely consumed by Indonesians. Fish is a source of animal protein for humans because one-fifth of the fish's body is a protein component composed of amino acids that are needed by the human body (Irianto 2014). Fresh fish and fish that have been processed into a certain product cannot be separated from quality deterioration. Irianto (2014) explained that if the quality of the fish has decreased, the quality will be low so that it is no longer suitable for consumption.

Damage and deterioration of quality in fresh and processed fish include rot, rancidity, red spots and a sour and mildew odor (Adawyah 2008).. This can occur because of the large content

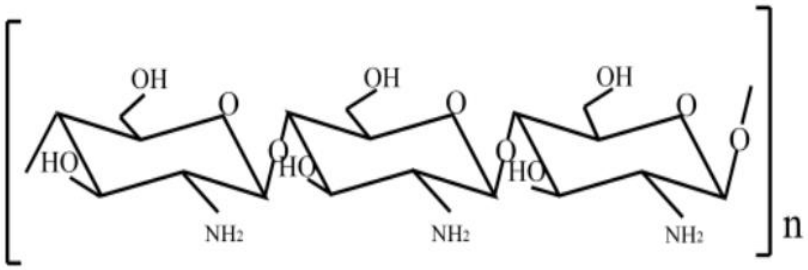
of organic compounds in the fish. The main characteristic of fish is that they are easily damaged and rot. In addition, fish are a good living substrate for the growth of spoilage microbes, especially bacteria. Therefore, special treatment is needed to inhibit the activity of these bacteria, one of which is preservation.

The use of hazardous preservatives can have a major impact on health, so an alternative preservative that is safer is needed. One of the efforts to extend the safe shelf life is the addition of natural preservatives such as chitosan.

Chitosan can be used as a preservative because it has the property of inhibiting the growth of destructive microorganisms as well as coating the preserved products so that there is minimal interaction between the product and the environment (Hardjito 2006). According to Wardaniati and Setyaningsih (2009), chitosan has the potential to be used as a preservative because it contains the enzyme lysosim and aminopolysacharide groups which can inhibit bacterial growth and the efficiency of chitosan's inhibitory power against bacteria. Shrimp shells or crab shells are widely processed to produce chitosan which is then used as a food preservative. Chitosan is a natural material that is more environmentally friendly, easily degraded biologically, is non-toxic and does not leave harmful residues in the human body (Aider 2010). The purpose of this review article is to examine chitosan and its manufacture, the quality of chitosan, the mechanism of chitosan as a preservative, the application of chitosan as a natural preservative.

DEFINITION OF CHITOSAN

Chitosan is a compound resulting from chitin deacetylation, consisting of N-acetyl glucosamine and N glucosamine units. The presence of amino reactive groups on C-2 atoms and hydroxyl groups on C-3 and C-6 atoms in chitosan is useful in its wide application, namely as a preservative for fishery products and a color stabilizer for food products and additives for agrochemical products. Chitosan with the molecular formula $(C_6H_{11}NO_4)_n$ has the structure shown in Figure 1 below.



Picture 1. Structure of Chitosan (Taufan & Zufahmi 2010)

Chitosan is one type of polysaccharide which can act as a good barrier because polysaccharide coatings can form a strong and compact matrix. Chitosan is a derivative of chitin which is obtained by removing the acetyl group from chitin using a concentrated solution of lye with a certain temperature and time treatment and a certain ratio. Then proceed with the washing process until neutral, drying, milling, grading and sorting and packing chitosan (Bastaman 1989).

Chitosan is a linear polymer composed of 2000-3000 N-acetyl-D-glucosamine monomers in β- (1-4) glycoside bonds, non-toxic and has a molecular weight of 800 kDa. This molecular weight depends on the degree of deacetylation produced at the time of extraction. According to Tang *et al* (2007), the more acetyl groups that are lost from the chitosan biopolymer, the stronger the interaction between ions and hydrogen bonds from chitosan. Chitosan is a yellowish white amorphous solid with a specific rotation of [α] D 11 -3 to -10o (at a concentration of 2% acetic acid). Chitosan dissolves in most organic acid solutions at a pH of about 4.0, but it is insoluble at a pH greater than 6.5, nor is it soluble in water, alcohol and acetone solvents. In concentrated mineral acids such as HCl and HNO_3, chitosan dissolves at a concentration of 0.15-1.1%. The solvent commonly used to dissolve chitosan is acetic acid or vinegar with a concentration of 1-2%. Acid-soluble chitosan is unique in that it forms a stable gel and has two poles, namely a negative charge on the carboxylic group and a positive charge on the NH_2 group. Characterization of chitosan can be determined from its solubility in weak acids such as acetic acid. Chitosan is more soluble in 1-2% acetic acid and will form an ammonium acetate salt (Tang *et al* 2007). Tang *et al* (2007) explained that chitosan has high chemical reactivity properties that can bind water and oil. Because of its ability, chitosan can be used as an excellent thickening or gelling agent, as a binder, stabilizer and texturizer.

EXTRACTION PROCEDURE

1. Deproteination

   Deproteination is carried out to remove protein from the shell. Protein removal in the deproteination process aims to remove proteins bound in the skin matrix (Sugita *et al* 2009). In the outer skeleton of animal shells contain chitin which binds directly to calcium carbonate ($CaCO_3$) and protein. The protein bound in the shell can reach between 30-40% of the total organic compound, depending on the species. Deproteination is a hydrolysis reaction of chitin in alkaline conditions using 5% NaOH solution at room temperature for 1 hour or 90°C for 1 hour and the results of deproteination are then neutralized using distilled water (Shaji *et al* 2010).

2. Demineralization

   Demineralization, namely removing minerals or inorganic compounds present in shell waste. The demineralization process was carried out by adding 1N HCl with a ratio of material weight and extracting volume 1: 7 (w / v) and heated at 90°C for 1 hour (Suptijah 2004).

3. Deacetylation

   The process of making chitosan from chitin is called the deacetylation stage where at this stage, the acetyl group in the chitin is removed through a hydrolysis reaction using a strong base of 50% NaOH at 120°C for 5 hours then the formed precipitate is washed using distilled water until it is neutral (Muzzarelli and Rochetti 1985). The longest deacetylation time with high temperature will cause a decrease in yield (Sugita *et al* 2009).

QUALITY OF CHITOSAN

The quality standard of chitosan needs to be done to determine the quality of the chitosan that will be used. The purity of chitosan can be seen from the value of its deacetylation degree. The higher the degree of deacetylation, the higher the number of amine groups (NH_2) in the chitosan molecular chain so that the chitosan is purer.

According to the National Standardization Agency for Indonesia (2013), the quality standards for chitosan are as follows.

Table 1. Chitosan Quality Standards

No.	Type of test	Unit	Requirements
1.	Particle shape	-	Flakes until powder
2.	Color	-	Light brown to white
3.	Physics		

	- Foreign object	-	Negative
4.	Chemistry		
	- Degree of deacetylation	%	Min. 75
	- pH	-	7-8
	- Ash content	%	Max. 5
	- Water content	%	Max. 12

Source: National Standardization Agency for Indonesia (2013)

THE MECHANISM OF CHITOSAN AS A PRESERVATIVE

Chitosan is an organic compound which is a derivative product of chitin polymer. This chitosan product comes from waste byproducts in the processing of the fisheries industry, especially shrimp, crab and crab, has a shape similar to cellulose but there is a difference that lies in the C-2 group, is crystalline and has a white color, can dissolve in a solution of organic acids such as acids. acetic, propionic acid, lactic acid and formic acid but not soluble in other organic solvents (Cahyaningrum *et al* 2011).

The process of obtaining chitin from shrimp shells involves processes of mineral separation (demineralization) and protein separation (deproteination). The deproteination process is to remove the protein content in the raw material, which initially covalently bonds with chitin, using hot NaOH alkaline solution for a relatively long time. The demineralization process to remove inorganic salts or mineral content present in chitin, especially $CaCO_3$, uses a dilute HCl acid solution at room temperature. Chitin can produce chitosan by removing the acetyl group (CH_3 - CO) so that the molecule can dissolve in an acidic solution, this process is called deacetylation, which produces a free amine group (-NH) so that chitosan has the characteristics as a cation whereas if chitin is dissolved in an alkaline solution, The chitosan will settle. In general, the degree of deacetylation for chitosan is around 60%, and around 90-100% for chitosan which is fully deacetylated. Because chitin in the deacetylation process that occurs is almost never finished, chitosan still has acetyl groups attached to several N groups (Kusumawati 2009).

Chitosan as a natural preservative has been used to extend the shelf life of whole fish, fish fillets, shrimp, and chicken meat which are used as a coating and antibacterial agent. Chitosan is polyelectrolytic, non-toxic, can react with other organic substances such as protein and is easily biodegradable. The properties possessed by chitosan so that it can be used as a preservative, namely that it can inhibit the growth of destructive microorganisms, chitosan also coats preserved products or coatings, so that there is minimal interaction between the product and the environment (Hardjito 2006).

Protein food ingredients are generally decomposed by anaerobic bacteria that cause "putrefaction" or putrefaction. This putrefaction is caused by the breakdown of proteins by proteolytic enzymes. Proteins are broken down into amino acids and subsequently into compounds containing sulfur and nitrogen with low molecular weight such as mercaptans, hydrogen sulfide, ammonia, and amines which cause foul odors (Sri 2007).

The compounds in chitosan that play a role in its application as preservatives and color stabilizers are the presence of amino reactive groups and hydroxyl groups. Chitosan has an active group that binds to microbes, so chitosan is able to inhibit microbial growth. a preservative because it contains the enzyme lysosim and aminopolysacharida groups. According to Wardaniati and Setyaningsih (2009), chitosan has the potential to be used as a preservative because it can inhibit bacterial growth. The ability of chitosan in suppressing the growth of bacteria and molds is due to the fact that chitosan has a positively charged polycation.

Chitosan can interact directly with the cell membrane so that it disrupts membrane permeability and causes leakage of cell protein material. In addition, chitosan also has a very strong affinity with microbial DNA so that it binds to DNA which then interferes with mRNA and protein synthesis. The positive charge of the NH_3^+ group on chitosan can interact with the negative charge on the surface of the bacterial cell. Any damage to the cell wall results in weakening of the cell wall strength, the shape of the cell wall becomes abnormal, and the pores of the cell wall become enlarged. This results in the cell wall being unable to regulate the exchange of substances from and into the cell, then the cell membrane becomes damaged and undergoes lysis so that metabolic activity will be inhibited and in the end it will die. With these properties chitosan can inhibit bacterial growth so that it can be used as an antimicrobial. Chitosan also functions as a chelating agent that can bind trace elements and essential nutrients for microbial growth. In addition, chitosan is also thought to have a function as a cryoprotectant. Cryophotectants are used to slow protein denaturation and are also used to protect cells on slow cooling in the event of a solution effect that can damage cell structure (Warinangin *et al* 1999).

CHITOSAN APPLICATION FOR PRESERVATION

Chitosan can be used as a preservative because of its properties, namely that it can inhibit the growth of destructive microorganisms and simultaneously coat the preserved products so that there is minimal interaction between the product and the environment. According to Pratiwi (2014) in their use in human life, chitin and chitosan have a very broad term, for example as adsorbents

of heavy metal waste and dyes, preservatives, anti-garnishes, cosmetics, pharmaceuticals, flocculants, anti-cancer, and anti-bacterial. Chitosan has antimicrobial properties, because it can inhibit pathogenic bacteria and rotting microorganisms, including fungi, gram-positive bacteria, and gram-negative bacteria (Helander 2001 in Nurainy *et al* 2008).

One of the most perishable food products is fish products. After the fish is caught or dies, the process of spoilage of the fish will immediately occur. Depending on the species, gear or fishing method, fish can start to rot within 12-20 hours (Mahatmanti *et al* 2010 in Arifin 2006). The process of putrefaction in fish is caused by the activity of enzymes, microorganisms, and oxidation in the body of the fish itself, with changes such as a foul odor, stiffening of the flesh, fading of the eyes, and the presence of mucus on the gills and the outside of the body. Fish bodies that contain high water content (80%) and body pH are close to neutral, making it easier for the growth of putrefactive bacteria. The appearance of a rancid odor in fish meat is caused by the oxidation process of high levels of unsaturated fatty acids in fish meat (Adawyah 2008).

Sari (2008) explained that the advantages of chitosan natural preservatives compared to synthetic preservatives include organoleptic aspects, durability, food safety and economic value. Apart from being a preservative, the advantage of chitosan is that it can inhibit the growth of microbes that cause typhoid disease that have experienced resistance to ampicillin chloramphenicol, tetracyclin such as Salmonella enterica, S. enterica var. Paratyphi-A and S. enterica var Paratyphi-B (Yadaf and Bhise 2004). Another advantage of using chitosan as a preservative for fish is that it can extend the life of the fish to less than 5 hours. The advantages of using chitosan from tiger shrimp shells can also be used as an anti-bacterial.

Arifin (2006) stated in his research that the best time to preserve catfish using 2.5% chitosan solution was obtained at the 14th hour and was still able to withstand bacterial growth at the 17th hour to below 5x105 cabbage / g. Meanwhile, the antibacterial ability of chitosan 2 (CS 2) which has a deacetylation degree of 81.04% is better than chitosan 1 (CS 1) (deacetylation degree of 80.51%). CS 2 was able to suppress bacterial growth up to 0.07x105 cabbage / g. This can be explained that the higher the deacetylation degree of chitosan, the more cations (NH_3^+) chitosan has. So that more negative bacterial cells are bound by chitosan. The positive charge of the NH_3^+ group on chitosan can interact with the negative charge on the surface of the bacterial cell. The damage to the cell wall results in a weakening of the cell wall strength, the shape of the cell wall becomes abnormal, and the pores of the cell wall become enlarged. This results in the

cell wall being unable to regulate the exchange of substances from and into the cell, then the cell membrane becomes damaged and undergoes lysis so that metabolic activity will be inhibited and in the end it will die. With these properties chitosan can inhibit the growth of bacteria in catfish so that it can be used as an antimicrobial. The results of this study have not been able to predict a more specific type of decomposition bacteria. Pseudomonas (32-60%) especially Pseudomonas aeruginosa and Bacilllus (<18%) are the most dominant decomposition bacteria in fresh fish (Jay 2005 in Arifin 2006). Meanwhile, the organoleptic test results of catfish were not significantly different. This results in the cell wall being unable to regulate the exchange of substances from and into the cell, then the cell membrane becomes damaged and undergoes lysis so that metabolic activity will be inhibited and in the end it will die. With these properties chitosan can inhibit the growth of bacteria in catfish so that it can be used as an antimicrobial. The results of this study have not been able to predict a more specific type of decomposition bacteria. Pseudomonas (32-60%) especially Pseudomonas aeruginosa and Bacilllus (<18%) are the most dominant decomposition bacteria in fresh fish (Jay 2005 in Arifin 2006). Meanwhile, the organoleptic test results of catfish were not significantly different. This results in the cell wall being unable to regulate the exchange of substances from and into the cell, then the cell membrane becomes damaged and undergoes lysis so that metabolic activity will be inhibited and in the end it will die. With these properties chitosan can inhibit the growth of bacteria in catfish so that it can be used as an antimicrobial. The results of this study have not been able to predict a more specific type of decomposition bacteria. Pseudomonas (32-60%) especially Pseudomonas aeruginosa and Bacilllus (<18%) are the most dominant decomposition bacteria in fresh fish (Jay 2005 in Arifin 2006). Meanwhile, the organoleptic test results of catfish were not significantly different. then the cell membrane becomes damaged and undergoes lysis so that metabolic activity will be inhibited and in the end it will experience death. With these properties chitosan can inhibit the growth of bacteria in catfish so that it can be used as an antimicrobial. The results of this study have not been able to predict a more specific type of decomposition bacteria. Pseudomonas (32-60%) especially Pseudomonas aeruginosa and Bacilllus (<18%) are the most dominant decomposition bacteria in fresh fish (Jay 2005 in Arifin 2006). Meanwhile, the organoleptic test results of catfish were not significantly different. then the cell membrane becomes damaged and undergoes lysis so that metabolic activity will be inhibited and in the end it will experience death. With these properties chitosan can inhibit the growth of bacteria in catfish so that it can be used as an antimicrobial. The

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According to the results of silvia's research (2014), it shows that fish preservation treatment by immersion can increase the shelf life of fish for more than 5 hours, namely by adding 1.5% chitosan, while fish preservation by spraying is the addition of 2.5% chitosan and can extend shelf life. fish for less than 5 hours. In his research, the material used as a source of raw material for making chitosan is crab, its use is based on high levels of chitin, which ranges from 20-30% and the material is easy to obtain because it is widely consumed by the surrounding community. The concentration of chitosan used was varied by dissolving chitosan (w / v) into 1% acetic acid, this was intended to find the optimal value of chitosan as a preservative (Ahmad M et al. 2003 in Silvia 2014).

In Toynbe's (2015) study, the results of testing the snakehead fish meat coated with chitosan showed that the ground beef fish treated with chitosan (1.5% and 3%) had higher protein content than the control (0%). The addition of chitosan causes fish meat to avoid hydrolysis, this occurs because chitosan is antibacterial which causes bacterial activity to be inhibited. According to Tranggono (2002), the occurrence of inhibition of changes in protein molecular structure causes changes in physical, chemical and biological properties, proving that the presence of chitosan solution by coating (coating) the ground beef of snakehead fish has an effect on the preservation of snakehead fish meat.

Based on the results of research, Nirmala (2016) explains that the effect of chitosan treatment on total bacteria testing (TPC) in this study which can inhibit the growth of the best

bacteria is seen in 1.5% chitosan solution on the 12th day. The main causes of food damage are microbial growth, enzyme activity and chemical changes. In general, an increase in the number of bacterial colonies that have occurred during storage, because the growth of these microorganisms is affected by time. Regarding the effect of chitosan on kamaboko fish curisi products, the highest protein content test results were found in chitosan 1.5% at 17.12% and on the 12th day the highest was also the same at 1.5% chitosan at 16, 95%.

Research by Ariyani (2008) regarding the preservation of pindang naya fish using chitosan states that there is a potential to extend the longevity of pindang fish by using a solution of chitosan in acetic acid. The results of his research explained that at room temperature storage, the shelf life soaked in 0.25% chitosan solution (in 0.04% acetic acid solution) and 0.5% chitosan solution (in 0.08% acetate solution) was 3 days, meanwhile control tenacity 1 day. Selection of pindang naya. because the salt content is relatively small and the water content is still high enough, the durability of the "naya" is very short, ranging from 1–3 days or 2–7 days depending on the type of fish. This condition often causes pindang damage due to stale or fungal growth.

One of the uses of chitosan made from shrimp skin is as a natural food package in the form of an edible coating. Edible coating is a thin layer made from edible materials. Edible coatings can be made from a variety of materials including polysaccharides, proteins and lipids. Coatings can be applied directly to food ingredients or made into edible films which are then used to coat food surfaces. The advantage of using edible coating made from chitosan in food or processed fish is that it improves quality and extends shelf life which acts as a barrier to oxygen and water, thereby slowing down bacterial growth (Ouattara et al 2007). The advantages of chitosan coating can also increase the ability to inhibit bacterial growth so that processed fish can last up to 2 days. In addition, processed fish with chitosan coating can reduce the growth rate of bacteria compared to processed fish without chitosan coating. The ability to suppress bacterial growth is due to chitosan having a positively charged polycation which can inhibit the growth of bacteria and molds (Wardaniati 2009).

Coating the chitosan in Wulandari's research (2015) describes an increase in the inhibitory ability of bacterial growth in tuna fish balls that have been treated with chitosan coating so that they can last up to 2 days. In addition, chitosan-coated tuna meatballs can reduce the growth rate of bacteria compared to tuna fish balls without chitosan coating. Fish balls spoil quickly because they contain high levels of protein and water content. Based on research on protein content and

water content, tuna fish balls contain 17.25% protein and a water content of 67.36%. With a high protein content and water content, fish balls are susceptible to damage so they have a maximum shelf life of only one day at room temperature.

Based on the experimental results of making chitosan from the skin and head of sea shrimp and its application as a preservative for fresh anchovy conducted by Mardyaningsih (2014), the characteristics of shrimp chitosan waste from the sea waters of Kupang, East Nusa Tenggara, have the form of flake particles, water content of 2.81%. ; ash content 0.75%; nitrogen content of 7.26%; and deacetylation degree 79.11%. These characteristics have met the standards of the Laboratory Staff. Analysis of the quality of fresh anchovy with 1.5% chitosan treatment, both microbiological and proximate tests, is the value of yeast fungi in the range 13.04 - 0.05 Cfu / g, water content ranges from 40-46%, fat content ranges from 1-1, 5%, protein content 24.59 - 40.12%. At room temperature storage, the durability of anchovy treated with 1.5% chitosan solution (in 1% acetic acid solution) is 3 days,

In Sedjati's (2010) study, the concentration of chitosan which was effective in suppressing bacterial growth significantly in dried anchovies during room temperature storage was 0.5%. However, the results showed that the chitosan concentration variable had a very significant effect ($p < 0.01$) only on the total bacteria variable. While the storage time variable had a significant effect ($p < 0.05$) on the variable water content and the total bacteria of dried anchovies. The interaction between chitosan concentration and storage time only had a very significant effect ($p < 0.01$) on total bacteria. Chitosan is hydrophobic, but due to the relatively small concentration of chitosan, it does not statistically have a significant effect on the water content of dried anchovies. The use of 0.5% and 1% chitosan did not produce air content that was significantly different from the control treatment (0%).

CONCLUSION

Based on a review of various literature both journals and other literature, it can be concluded that chitosan is a deacetylation compound of chitin, consisting of N-acetyl glucosamine and N glucosamine units. The production of chitosan consists of deproteination - demineralasation - deacetylation steps. The quality of chitosan can be assessed from the aspects of particle shape, color, degree of deacetylation, pH, moisture content and ash content. The mechanism of chitosan as a preservative is to interact with rotting bacterial cell membranes to disrupt membrane permeability and chitosan also has a very strong affinity with microbial DNA so that it can interfere

with mRNA and protein synthesis. Chitosan as a preservative has been applied to pindang fish, salted anchovies, catfish fillets and others.

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