

Review Article

Study of Nanochitosan (Definition, Analysis of Characteristics, Manufacture and Utilization) : Review

ABSTRACT

Nanochitosan is a nanoparticle of chitosan with a size between 100-400 nm. Nanochitosan has a higher antibacterial ability than ordinary chitosan. The morphology of nanochitosan is in the form of a whole sphere and has typical chitosan functional groups such as amine, hydroxyl and carboxyl groups. The manufacture of chitosan into nanochitosan can be done by ionic gelation method, namely the complexation of polyelectrolyte between positively charged of chitosan and negatively charged of sodium tripolyphosphate. Nanochitosan as an antibacterial can be used as a natural preservative to maintain food products. Based on several studies, nanochitosan which is used as a natural preservative affects the physiochemical characteristics of food products.

Comment [U1]: *The abstract did not clearly state the objectives*

Keywords :Antibacterial, functional group, morphology, nanochitosan, natural preservative, physiochemical

1. INTRODUCTION

Fishery waste is residual material obtained from fishing activities, both catching, handling and processing fishery products. The fishing industry is the largest solid waste contributor Nugroho et al. [1]. Fishery industry waste includes filet, skin, head, bone, shell, fin and tail. Shells from crustaceans are waste produced from fishery products from crustaceans, shells from crustaceans such as shrimp, crabs and small crabs can be processed into chitosan and used as a natural preservative because of its antibacterial properties. Chitosan contains lysozyme enzymes that can digest cell walls in the body which causes the loss of the ability of bacteria to cause disease. Chitosan can inhibit the growth of gram-positive and gram-negative bacteria isolated from fishery products. Baharuddin and Isnaeni [2]

Chitosan derived from crustacean shell waste can be used as a natural preservative in other fishery products so that fishery products can be utilized optimally. Chitosan can be modified into nanochitosan through the ionic gelation method, because nanochitosan can provide higher antibacterial abilities than ordinary chitosan. In maintaining the quality of fishery products during storage, nanochitosan can be processed as an edible coating, because it can coat fishery products during storage and provide an antibacterial effect so that stored fishery products can be maintained and their shelf life is longer. Nanochitosan which is processed into edible coating and used as a natural preservative will affect the physiochemical characteristics of fishery products.

Comment [U2]: *The introduction section should be more focused on the previous works done on this topic*

Comment [U3]: *The methods section did not clearly explain the research methods which is used*

2. Nanochitosan

Nanochitosan is chitosan that has been modified into a smaller size, the nanochitosan size ranges from 100-400nm. Magani et al [3]. The

antibacterial properties of nanochitosan are higher than ordinary chitosan, nanochitosan is able to provide better penetration because it has

a large surface area, nanochitosan is obtained from chitosan with appropriate quality standards,

some characteristics of chitosan are presented in Table 1.

Table 1.Chitosan Quality Standard

Parameter	Standard	
	Korean Dalwoo	Labs. Japanese Protan
Sightings	White or yellow powder	Clear solution
Particle size	25-200 mesh	Flakes to powder
Water content	10%	10%
Ash content	0.5%	2%
Protein content	0.3%	-
Degree of Deacetylation (DD)	70%	70%
Viscosity	50-500 cps	200-2000 cps
Isolation	1%	-
Heavy metal content : As, Pb	10 ppm	-
pH	7-9	7-8
Smell	No smell	No smell

Source : Rochima [4]

Modification of chitosan into nanochitosan can be done by ionic gelation method, namely the complexation of polyelectrolyte between negatively charged chitosan and negatively charged sodium tripolyphosphate. Suptijah et al. [5], according to Chandrasekaran [6] modification of nanochitosan using the ionic gelation method is the best method because the cross-linking process does not use toxic chemicals. Chitosan which has a positive charge will bind with negatively charged sodium tripolyphosphate, then break the size of the chitosan into smaller ones without changing the functional group of the chitosan itself. Chitosan

which is broken into smaller sizes will stick and form agglomerations, so a surfactant is needed to spread the chitosan so it doesn't stick and agglomerate, tween 80 can be used as a surfactant in the nanochitosan modification process. The formed nanochitosan still has a unique functional group from chitosan itself and a phosphate group appears because the process has cross-linked between sodium tripolyphosphate through ionic bonds which indicates that nanoparticles have been formed Rosyada et al. [7]. The modification mechanism of chitosan with ionic gelation method can be seen in Fig1.

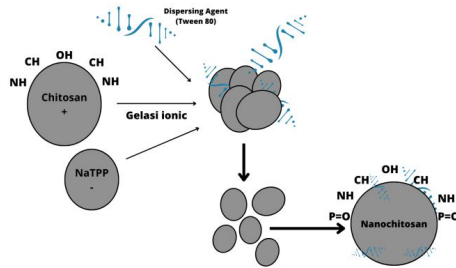


Fig 1. Mechanism of Chitosan Modification Using Ionic Gelation Method

Nanochitosan has a positively charged amine group that is able to bind to the negatively charged bacterial wall, the small nanochitosan particle size makes it easier for nanochitosan particles to enter the bacterial cell wall and then change electron transport and damage the main structure of microbial cells. Modification of chitosan into nanochitosan by ionic gelation method causes the formation of phosphate functional groups derived from cross-linking between chitosan and sodium tripolyphosphate

3. Characterization of Nanochitosan

3.1 Nanochitosan Particle Size

Nanochitosan has a size between 100-400 nm, the size of nanochitosan is formed due to the complexation of polyelectrolytes between tripolyphosphate and chitosan solution, is also influenced by the pH of the solution, the concentration of chitosan and tripolyphosphate, molecular weight, and stirring conditions. Size

can be analyzed using the Particle Size Analyzer test which has the working principle of scattering laser light by sample particles, then the laser will cause light to be emitted through a small needle and then sent to the particles in the sample, the particles in the sample will scatter the light back and enter the detector, the identified signal will be converted into a digital signal, then processed into an arithmetic series. Nuraeni et al. [8]. The small size of nanochitosan will facilitate the penetration process in carrying out the antibacterial properties in it. Nanochitosan will enter the bacterial cell wall and then change electron transport, then damage the main structure of microbial cells such as cell walls, cytoplasm, ribosomes, and also the cytoplasmic membrane, microbial metabolism will be disrupted which causes disruption of the physiological activity of bacteria and bacterial cells will die Chandrasekaran [6]. Several studies regarding the size comparison of nanochitosan are presented in Table 2.

Table 2. Comparison of Nanochitosan Particle Size

Size of Nanochitosan	Method Making	Source
85.3 nm	Ionic Gelation	Taurina et al. [9]
100 nm	Ionic Gelation	Alboghbeish and Khodanazary [10]
100 nm	Ionic Gelation	Elkassas et al. [11]
228.74 nm	Ionic Gelation	Nadia et al. [12]
232.5 nm	Beads Milling	Rochima et al. [13]

3.2 Nanochitosan Functional Group

Nanochitosan has several functional group absorption bands that are the same as typical chitosan functional groups such as Carboxyl (CH), Hydroxyl (OH) and Amine (NH) with the addition of a phosphate functional group absorption band (P=O), the formation of a phosphate functional group due to cross-linking between the sodium tripolyphosphate group and Chitosan through cross-linking and the formation of nanoparticles. The amine group contained in nanochitosan is a function that shows the degree of deacetication which will

affect the performance of the chemical properties present in nanochitosan at the time of application. Rochima et al. [13]. The functional groups in nanochitosan can be analyzed using Fourier Transform Infrared with the working principle of placing the sample in the infrared path, then it will transmit and absorb light, then the signal will penetrate the sample detector, the intensity of radiation that moves into the sample and is transmitted through the sample by the detector and shows a schematic diagram. Munajad et al. [14]. The working principle of the Infrared Fourier Transform can be seen in Fig 2.

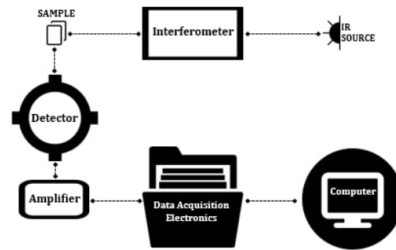


Fig 2. Working Principle of the Fourier Transform Infrared
Source: Munajad et al. [14]

3.3 Nanochitosan Morphology

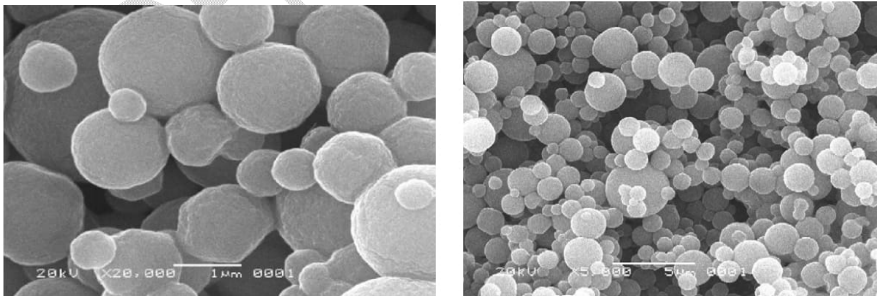


Fig 3. Magnification of Nanochitosan Morphology Using SEM
Source: Suptijah et al. [5]

Nanochitosan has a morphology in the form of a whole sphere resembling a ball and

wrinkled. Suptijah et al. [5], The morphology of nanochitosan can be seen in Fig 3. The

morphology of nanochitosan can be carried out using a Scanning Electron Microscopy test with a certain magnification, the working principle is to produce an electron beam by an electron piston, then the electron beam will be accelerated towards the sample which will then

be scanned, then the sample will release new electrons which are then emitted. received by the detector which is then sent to the monitor . Pambudi et al. [15]. The working principle of the Scanning Electron Mycroscopy test can be seen from Fig 4.

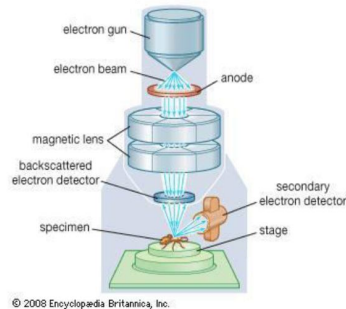


Fig 4. Scanning Electron Microscopy (SEM)
Source: Pambudi et al. [15]

4. Nanochitosan as Edible Coating

Utilization of nanochitosan as a natural preservative because it has higher antibacterial properties than ordinary chitosan can be applied as an edible coating, edible coating can be applied directly to food ingredients to coat the surface of food in order to improve quality, extend shelf life and prevent bacterial contamination from outside. In the nanochitosan book. Rumengan et al, [16] the application of edible coatings to food ingredients consists of 4 ways , namely dipping, spraying, casting and brushing. The dipping method can be used on foods that have an uneven surface, such as meat, vegetables, fish and livestock products, the spraying method is commonly used on food products that have two sides, such as pizza. The spraying method can produce a product with a thinner and more uniform layer than the dipping method. The casting method is a method used to make a film layer that can stand alone or separate from the product. Brushing method is a coating method by applying an edible solution to the product to be coated.

Manufacture a solution of nanochitosan edible coating can be done by adding a concentration of nanochitosan into distilled water,

homogenized at a speed of 350 rpm at a temperature of 60°C for 15 minutes using a magnetic stirrer, then adding glycerol as a plasticizer, the edible coating solution is left to cool and can be directly applied to the material. the food to be coated. Rasulu et al. [17].

5. Characteristics of Edible Coating

The use of nanochitosan that is applied as an edible coating on food needs to pay attention to the characteristics of the edible coating material that is formed, so that when applying the edible coating it can coat the food material and provide good antibacterial properties during storage. Some characteristics of edible coatings that need to be considered such as transparency and viscosity. It is necessary to pay attention to the transparency of edible coatings so that when applied to edible coated foodstuffs it does not change the original appearance of the food, the value of edible coating transparency can be measured using a UV-Vis Spectrophotometer at a wavelength of 200-800 nm, by inserting an edible sample into a cuvette, then The cuvette is inserted into the spectrophotometer, the absorbance value will be automatically recorded after the wavelength is set . Ulyarti et al. [18] .

The application of edible coatings also needs to pay attention to the viscosity of the edible solution used, the higher the concentration of

nanochitosan used in the manufacture of edibles will higher the viscosity value, and more difficult it will be for the edible solution to diffuse to the coated food material. The edible structure will soften with the addition of glycerol as a plasticizer, because glycerol is able to reduce internal hydrogen bonds in the solution. Based on the Commercial Quality Standards and Protan, the viscosity value of chitosan is > 200 mPas. In the application of edible coatings to materials, it is necessary to pay attention to the optimal viscosity value, not too high and not too low, because if the viscosity value is low, the edible coating solution evaporates easily, and does not coat the surface of the food material optimally, as well as the viscosity value is too high, the higher the viscosity value, will longer the diffusion process of nanochitosan as an edible coating, then the longer the drying time and coating on the surface of the food material. Vatria et al. [19]. Measurement of the viscosity value can be done using a Brookfield

Viscometer, by inserting the sample into the viscometer tube, the tool is turned on and the viscometer speed is set on a scale of 0.3 to 30 rpm, so that the rotating speed is not too slow and not too fast. Nugroho [20].

6. Utilization of Edible Coating Nanochitosan on Fish Fillets

Utilization of nanochitosan as an edible coating on fish filets can be done by dipping, which is dipping the fillets into edible suspension for 20 minutes, then drained and packaged, fish filets that have been packaged can be stored at room temperature or cold temperatures. In cold storage, it is generally stored in the refrigerator at a temperature of 5-10°C, the dipping method is used because of the uneven surface of the filet. During storage, the quality of fish fillets decreases through their physiochemical characters such as weight loss, pH and Total Volatile Base during storage. Several studies regarding the use of nanochitosan edible coatings on fish can be seen in Table 3.

Table 3. Utilization of Nanochitosan as Edible Coating on Fish Filet

Fish Filet	Best Concentration	Temperature	Saving Time	Source
Trevally	2%	4°C	12 days	Alboghbeish and Khodanazary [10]
Tilapia	2%	4°C	10 days	Elkassas et al [11]
Fresh Tuna	2%	27°C	12 hours	Rasulu et al. [17]
Smoked Tuna	3%	27°C	72 Hours	Rasulu et al. [17]
Silver Carp	2%	4 °C	12 Days	Ramezani et al. [21]

6.1 Weight Loss

Weight loss is one of the indicators in testing the freshness level of fish fillets during the storage period, the freshness of the quality of fish fillets can be seen by the higher weight loss value.

Weight loss in fish fillets occurs due to water dripping out of the fish meat, due to the inability of the fish muscles to hold the water in the meat. Afrianto et al. [22]. The value of weight loss can be measured by calculating the initial weight using an analytical balance, then the final weight

is measured at the time of storage and calculated using the following formula

$$\text{Weight Loss} = \frac{\text{Initial Weight} - \text{Final Weight}}{\text{Initial Weight}} \times 100\%$$

6.2 Potential of Hydrogen (pH)

The potential of hydrogen (pH) is a parameter used to measure the freshness of fish, the higher the pH value will facilitate the growth of harmful bacteria and will damage the fish flesh, and the contrary, the lower the pH value, the spoilage bacteria will be difficult to grow. Damayanti et al. [23]. Measurement of the pH value can be done using a pH meter that has been calibrated using a buffer solution of pH 4 and pH 7, after calibration, the fish filet sample to be measured is homogenized first in distilled water for 1 minute, and the pH value can be measured. Rasulu et al. [16]. The pH value of fish filets treated with nanochitosan edible coating, at the beginning of storage will tend to decrease because the constituent ingredients of the edible coating contain acid, and the pH of fresh fish decreases at the beginning of the shelf life then continues to rise towards alkaline, due to the growth of spoilage bacteria.

6.3 Total Volatile Base

Total volatile base (TVB) is volatile nitrogen formed in meat muscle tissue, including ammonia, trimethylamine, and dimethylamine obtained from the decomposition of protein and other nitrogen compounds in meat, either by autolysis or microbiologically. Fish filets stored at cold temperatures without treatment can last up to 5-6. Sitakar et al. [24], according to Natsir et al. [25] the limit of TVB value for fishery products is 30 mgN/100 g, fish freshness is based on TVB levels as follows:

- a) Very fresh fish (TVB < 10 mgN/100g)
- b) Fresh Fish (10 ≤ TVB ≤ 20 mgN/100g)
- c) Fish is still fit for consumption (20 ≤ TVB ≤ 30 mgN/100g)
- d) Fish not fit for consumption (> 30 mgN/100g)

CONCLUSION

Nanochitosan is one of the processed products from fishery waste, which can be used as a natural preservative because nanochitosan has antibacterial properties. Utilization of crustacean shell waste to be used as nanochitosan is a good way to maximize the utilization of fishery products. Modification of chitosan into nanochitosan can be done using ionic gelation method, namely polyelectrolyte complexation between chitosan and sodium tripolyphosphate. Nanochitosan has a characteristic particle size of 100-400 nm, with functional groups such as hydroxyl, carboxyl, amine and phosphate. The use of nanochitosan as a natural ingredient in fishery products can be applied as an edible coating, because the edible coating is able to coat the product so that it is not contaminated with microbes from outside and maintains the quality of fishery products. In filet products, the decrease in quality of filet can be seen from its physiochemical characteristics such as weight loss, pH and total volatile base. Weight loss during storage will decrease due to drip on the meat, the pH value of fish filets will decrease and then increase towards alkaline due to the growth of bacteria, the total volatile base value will increase with the length of storage time, the threshold value for the total volatile base of fishery products is 30 mgN/100 g.

DISCLAIMER

The products used in this study are generally and mostly used products in the research area and our country. There is absolutely no conflict of interest between the author and the manufacturer of the product as we do not intend to use this product as an avenue for any litigation but for the advancement of knowledge. Also, this research was not funded by the producing company but rather was funded by the author's personal efforts.

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