Modification of nanochitosan with NaCl and surfactant for wood preservative

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Abstract. Chitosan is a natural preservative which is really potential termicide to resist attack termites. Chitosan in size nanometers (nanochitosan) is expected to improve the effectiveness of the nature of anti-termite properties. The smaller size of particles has advantage to easily penetrate the wood pores. Nanochitosan was modified with surfactants (polysorbate-20 and polysorbate-80) and NaCl to increase retention and penetration of preservatives and its anti-termite properties. The purpose of this study was to evaluate the effect of surfactant and NaCl on the parameters of preservation tests which included absorption, retention and termite mortality, and particle size data as supporting data. The average value of absorption and retention of preservatives ranged from 105.79-124.43 kg/m$^3$ and 9.18-18.26 kg/m$^3$. The termite mortality was 33.11-46.89 % for 4 weeks of observation. Data were analyzed using variance analysis (ANOVA) to determine the significance of each treatment. It showed that polysorbate had significant effect to the absorption of preservatives and termite mortality while the retention was influenced by the type and concentration of surfactants. The treatments with the various single compounds showed that both surfactants had a significant effect on absorption, whereas polysorbate-20 had a significant effect on all treatment interactions, and NaCl treatment significantly affected the termite mortality. It was thought that the surfactant treatments increased the retention of nanochitosan by hydrophobic interactions whereas the addition of NaCl increased anti-termite properties and made particles smaller and stable which caused in higher retention. In this study, nanochitosan had an average size of 233 - 798 nm.

1. Introduction

Wood preservatives from chemical synthetic compounds (chemical termicide) are preservatives which are generally used because it will make wood resistant to insect and fungal attacks [10]. If chemical termicides are always used to prevent termite attacks, its active chemical ingredients will accumulate in environment so that endanger ecosystems and humans [8]. However, the use of these chemicals has bad effects on the environment because it cannot be decomposed completely (non-biodegradable). Therefore friendly environmentally preservatives are needed which are effective enough to prevent the attack of dry wood termites.

The types of wood preservatives are pasta, emulsion, solution, aqueous systems and dispersion in liquid hydrocarbons. For the application, the aqueous system is better than liquid hydrocarbons because it is easy to spread to the whole wood and can reduce the risk of fire. However, a disadvantage is that it is difficult to remove from the environment due to adsorption with soil or sediment. Therefore, it is necessary to develop environmentally friendly environmentally preservatives which are effective enough to prevent the attack of dry wood termites.

Chitosan is a natural preservative which is really potential termicide to resist attack termites. Chitosan is a natural polyelectrolyte obtained from the deacetylation of chitin that is a natural product of crustaceans and arthropods. Chitosan is a linear polysaccharide consisting of glucosamine and N-acetyl glucosamine. Due to the hydroxyl group on C2 and C3, chitosan is soluble in alkaline solution. The low solubility of the molecule in water is the main limiting factor of the use of chitosan as a preservative. This is due to the strong interactions between the hydroxyl group present on the molecule and the hydroxyl group of water molecules, which reduces the solubility of the molecule in water. Therefore, various modification methods have been developed to improve the solubility of chitosan in water and to increase the effectiveness of the termicide properties.

Nanochitosan is a modification of chitosan in size nanometers (nanochitosan). The smaller size of particles has advantage to easily penetrate the wood pores. Nanochitosan was modified with surfactants (polysorbate-20 and polysorbate-80) and NaCl to increase retention and penetration of preservatives and its anti-termite properties. The purpose of this study was to evaluate the effect of surfactant and NaCl on the parameters of preservation tests which included absorption, retention and termite mortality, and particle size data as supporting data. The average value of absorption and retention of preservatives ranged from 105.79-124.43 kg/m$^3$ and 9.18-18.26 kg/m$^3$. The termite mortality was 33.11-46.89 % for 4 weeks of observation. Data were analyzed using variance analysis (ANOVA) to determine the significance of each treatment. It showed that polysorbate had significant effect to the absorption of preservatives and termite mortality while the retention was influenced by the type and concentration of surfactants. The treatments with the various single compounds showed that both surfactants had a significant effect on absorption, whereas polysorbate-20 had a significant effect on all treatment interactions, and NaCl treatment significantly affected the termite mortality. It was thought that the surfactant treatments increased the retention of nanochitosan by hydrophobic interactions whereas the addition of NaCl increased anti-termite properties and made particles smaller and stable which caused in higher retention. In this study, nanochitosan had an average size of 233 - 798 nm.
because of its odor, flammability, and toxicity. The composition of wood preservatives in the aqueous consists of surfactants which are resistant to fungal and insect attacks [24].

One of compromising method for wood preservation is contacting wood with a preservative composition consisting of an aqueous polymer dispersion which has an average size of particles less than 1000 nm with temperatures less than 75 °C, non-ionic or anionic surfactants, wood compounds preservatives which can consist of halogenated isothiazolone biocides, halogenated carbamate fungicides and azole fungicides [5]. In this study, the composition of the preservatives used were chitosan as a polymer, polysorbate-20 and polysorbate-80 as non-ionic surfactants, and NaCl as natural biocides.

Chitosan is a natural biopolymer of chitin derivative which is a constituent compound of crustacean animal shells. The use of chitosan as a natural preservative is one of the efforts to prevent environmental damage from the negative effects of using chemicals because it is biodegradable so it is safe for the environment [22]. It can also be used as bio-insecticides on environmentally friendly wood [4]. This compound is not soluble in water but can dissolve in acidic solutions, such as acetic acid, with a pH <6.3, which is below its pKa value. At this pH, the glucosamine (-NH$_2$) group protonates to (-NH$_3^+$) so it can be dissolved in water. The application of chitosan to wood can be done by pressing, soaking, or feeding on wood as termite bait.

Several studies on the effectiveness of chitosan to prevent termite attacks have been done before. Arinana and Rismayadi [1] stated that chitosan with a concentration of 0.5% in acetic acid solvents proved to have a very significant effect on mortality of termites. Wulandari [25] and Zaida et al. [26] conducted a wood preservation using a spraying method that succeeded in killing termites with chitosan concentrations of 0.1% - 2%.

Modification of chitosan particles into nanometer size was conducted so that compounds can more easily penetrate wood pores [13] so that it becomes more effective and efficient. Research by Nowrouzi et al. [15] proved that the dispersion of nanochitosan in methacrylic acid by the addition of K$_2$S$_2$O$_8$ with heat treatment could improve the physical properties of wood and also increased the shock resistance of wood. However, the research on nanocitosan as a wood preservative for the prevention of termite attacks is still very limited. Khademi Bami et al. [12] made nanochitosan as wood preservatives using the impregnation method from polymerization of chitosan with nitric acid then it was modified to form N, N, N-trimethylchitosan and sodium tripolyphosphate (TPP) as crosslinker.

The synthesis of chitosan nanoparticles (nanochitosan) was conducted out by ionic gelation method with crosslinker sodium tripolyphosphate (TPP) as crosslinker. The application of preservatives to wood samples was done by cold soaking method. In this dispersions, the addition of surfactants as penetrating agents in wood [9] for increasing the penetration and retention of preservatives into wood whereas the addition of NaCl to stabilize and increase the anti-termite properties of nanochitosan dispersions [19]. The purpose of this study was to evaluate nanochitosan modified by NaCl and surfactant as *Falcataria moluccana* preservative. The level of chitosan and surfactant concentrations were observed in this research.

2. Materials and methods

2.1. Nanochitosan dispersions

Chitosan 0.4% (w/v) was dissolved in acetic acid (Sigma Aldrich, USA) used 1% (v/v) to form chitosan-acetic acid dispersion. The various concentrations of polysorbate-20 and polysorbate-80 were 10, 20, and 30% (w/v). The sodium tripolyphosphate (Sigma Aldrich, USA) concentration used was 0.84 g/L to form nanochitosan dispersion. The volume ratio of chitosan solution: TPP was 5: 2. The NaCl concentration added to the nanochitosan dispersion was 3% (w/v).
2.2. Preparation of wood samples

*Falcataria moluccana* wood was from Sleman, Yogyakarta, Indonesia. For each treatment, 39 miniblock samples of 5 x 5 x 5 cm [1] were taken from the center of the stem. The samples consisted of 36 of treatment and 3 samples without treatment. The size of the test sample used in this study refers to the Protocol for Assessment of Wood Preservatives, with the minimum test sample dimension for preservation is 15 (radial) x 25 (tangential) x 50 mm (longitudinal) [1]. The samples were coated by resin on both transversal and longitudinal sides to avoid double infiltrations. Water content and specific gravity measurements used samples of 2 x 2 x 2 cm according to British Standard 373 as supporting data.

2.3. Preservation process of wood samples

The preservation method was the cold soaking method [10] for 1-4 days. The nanochitosan dispersions were stained with the coloring agent then prepared into immersion containers. The amount of preservative that can be absorbed into the wood was recorded as absorption.

The control samples were three samples without preservatives. After the preservation, the test samples were fed to dry wood termites. The glass tube (diameter 2.5 cm, height 4 cm) was placed on the side of cube samples without coating resin for termite bait.

Fifty wood termites (*Cryptotermes cynocephalus*) at the nymph stage were placed in the glass tube and stored for 30 days at room temperature in a carton box and placed in a dark room. The test samples were guarded from various predators of termites, such as ants, lizards, and spiders. Termite mortality observations were carried out every day for 30 days. Supporting data in the form of particle size were analyzed by Particle Size Analyzer (PSA).

Analysis of variance (two-way ANOVA) with SPSS 16 software was conducted to determine the factors that significantly affected the parameters including absorption, retention, and mortality of termites. The analysis was continued with the Honestly Significant Difference (Tukey HSD) to investigate the level of factors that had a significant effect on this experiment.

The surfaces of preservated samples were studied in the Scanning Electron Microscope (SEM) JEOL JSM-6510LA. The thin layers of surface were sputter-coated with platinum using JEOL JEC-300FC. The layers were observed at 20 kV acceleration voltage.

3. Results and discussion

3.1. Absorption and retention

Absorption is one of the parameters that showed the effectiveness of preservative. It is the amount of preservative that absorbed into the wood. Figure 1a showed that nanochitosan with a surfactant concentration of 30% indicating the greatest absorption with a value of 132.50 kg/m$^3$ while a concentration of 20% produced the least absorption of 105.79 kg/m$^3$. Data trend showed fluctuating results at the concentration of 10 to 30%.
Figure 1. Average absorption of nanochitosan dispersion (a) surfactant concentrations (b) surfactants. The same alphabet showed no significant difference at the 5% test level in Tukey HSD.

It was suspected that phenomenon occurred to be influenced by the conductivity of each surfactant. The number of micelle structures which increased by the conductivity of preservative dispersion were affected the hydrophobic interaction [22]. The hydrophobic interaction played an important role in the adsorption of nonionic surfactants on solid surfaces. At a concentration of 20 to 30% the increase in the number of micelle would cause the distance of the micelle and particles became nearer and restricted electrical conductivity of nanochitosan dispersion [17]. This was suspected to affect the hydrophobic interaction resulted in reducing absorption on the wood surface. In this research, that phenomenon could be seen at concentration of 10 to 20% where absorption decreased to 105.79 kg/m$^3$ and achieved maximum value with 30% of surfactant.

From Figure 1b, it could be seen that nanochitosan with polysorbate-20 surfactant produced higher average preservative absorption. Nanochitosan with polysorbate-20 had 26.65% greater absorption than nanochitosan with polysorbate-80. ANOVA was performed to analyze the significance of the treatment of the absorption of preservatives. The results of analysis of variance showed that the type of surfactant had very significant effects on absorption ($p < 0.01$). It indicated that surfactant had a significant influence on the absorbed preservative. The addition of surfactant substances made the viscosity of the nanochitosan dispersion increase. This causes the impregnation of preservatives to be inhibited. The addition of polysorbate-80 would cause the dispersion to be thicker than polysorbate-20 because it has longer hydrophobic tail that form a complex structure which results in thicker dispersion [17].

Retention is the amount of preservative left in the wood. Retention values in this experiment ranges from 8.12 to 22.11 kg/m$^3$. The retention of preservatives recommended by National Standardization Agency of Indonesia [11] has a minimum value of 8 kg/m$^3$ so the retention value in this study had reached the standard.
The preservative retention data were presented in Figures 2 (a) and (b). The results showed that the retention value of preservatives tend to increase with increasing concentration of surfactants. The highest retention was samples with the highest surfactant concentration of 30% resulting mean retention value of 18.26 kg/m$^3$ and 10% of surfactant concentration showed the smallest retention with a value of 9.18 kg/m$^3$ while a concentration of 20% had a retention of 11.04 kg/m$^3$.

The results of the analysis of variance showed that the concentration factor and type of surfactant had a very significant effect on the retention results of the study results (p <0.01). Further analysis using Tukey HSD for concentration factors obtained a very significant effect on the concentration level of 30%.

The difference in retention values were seen between the two types of surfactants. Polysorbate-20 had a higher average retention of 14.63 kg/m$^3$, while nanochitosan with modification of polysorbate-80 had an average retention value of 11.02 kg/m$^3$. This phenomenon was related to Rinaldi et al. [20] which stated that high absorption resulted in high retention. Increasing concentration caused the value of absorption and retention to increase because of the increasing number of preservatives entering the wood. The data trend of absorption and retention at various concentration was different. It was presumably during the immersion process more solvents were absorbed into the wood than the preservative. It was thought to be caused by different anatomical structures of wood, so the absorption ability of preservatives was different.
Figure 3 showed that average retention from various types of treatment was 5.08-9.97 kg/m$^3$ with the highest retention was obtained by the addition of polysorbate-20. The average retention that reached the SNI requirements was the treatment of polysorbate-20 and polysorbate-80 with values of 9.97 kg/m$^3$ and 8.95 kg/m$^3$ respectively.

One-way ANOVA results showed the treatment of acetic acid (AA) and chitosan-acetate (KA) was significantly different from the treatment of both types of surfactants while the treatment of TPP and salt (NaCl) had a very significant effect on the treatment of polysorbate-20 (P-20). It indicated that surfactants played a role in the retention of preservatives. However, chitosan has properties that are easily degraded [3]. Therefore, leaching in wood might be prevented by fixation of chitosan. It could be achieved by interaction of the electrostatic charge between chitosan and the negatively charged cellulose surface.

In this study wood observation retention was also carried out using both surfactants without any nanochitosan. Polysorbate-20 retention was higher than polysorbate-80 (P-80) retention because polysorbate-20 had a more stable molecular configuration with low compressibility and a high hydration number. It indicated a stronger intermolecular interaction [21] so that it is thought to cause greater retention of preservatives in wood.

In the other hand, the addition of polysorbate-80 would cause nanochitosan dispersion to be more viscous than the addition of polysorbate-20 because it had a longer hydrophobic tail which causes the distance of the formed micelles nearer so electrical conductivity becomes lower [17]. It was tought to make retention lower.

3.2. Anti-termite properties
Termite mortality is one of the parameters for measuring the effectiveness and toxicity of a preservative against termites. The treatment of wood preservation was considered effective if the termite mortality rate was at least 70% [7]. From the observations, at the beginning of feeding there were not many termites that died for all concentrations and types of surfactants and controls used. It was caused by the influence and adaptation of the environment from the location of the nest into the glass tube in the sample. Termite deaths were also thought to be influenced by wood condition factors as food source, such as surface hardness and the presence of ingredients that could stimulate termite feeding activities. The difference of wood condition caused differences in termite feeding activities which cause different levels of damage due to differences in the physical and chemical properties of the wood [13].

![Figure 4. Mortality of termites using (a) polysorbate-20 (b) polysorbate-80](image-url)
Figure 5. Mortality percentages of nanochitosan dispersion (a) surfactant concentrations (b) surfactants and control. The same alphabet showed no significant difference at the 5% test level in Tukey HSD.

It can be seen from Figure 4 that the mortality of termites for concentrations of 10, 20, and 30% for both types of surfactant per day was higher than the control, especially after observation of the 10th day. After 10 days of feeding to termites, samples with preservative treatment showed differences with control samples. Samples with preservative treatment are not preferred by termites than control samples. This showed that chitosan was effective enough to work to kill termites in preserved samples rather than control samples.

Figure 5 showed that samples preserved with nanochitosan produced the average of termite mortality percentage of 33.11 – 46.89%. It was greater than the results for control samples which had average termite mortality of 13.33%. Based on the observation, it could be seen that the polysorbate-20 surfactant produced the average termite mortality percentage of 46.89%. It was higher than the treatment with polysorbate-80 which had 33.11% of mortality percentage.

ANOVA showed that type of surfactant had significant difference to the termite mortality. It related to the retention parameter that retention of Polysorbate-20 (P-20) modified nanochitosan was higher than polysorbate-80 (P-80) because of the stable molecular configuration by P-20. The greater retention means the more nanochitosan left in to the wood. It resulted in the greater termite mortality.
The death of termites increased in the second week of observation. This was caused by the characteristic of chitosan which was slow in killing termites. This result was the same as previous research by Prasetiyo and Yusuf [18]. It stated that chitosan was slow action in killing termites by carrying out the performance of protozoa that play a role in the digestive system of termites. It caused termites could not be able to get food digested by protozoa.

Research on preserving chitosan in termite Coptotermes curvignathus Holmgren [25] had termite mortality of 63.05% with a concentration of 2% chitosan. In this study the best mortality was 46.89% with 0.4% (w/v) of chitosan. The mortality of termite Coptotermes curvignathus Holmgren by 40% was achieved with a concentration of chitosan of 0.5% with observations for 4 days on rubber plants.

From the results, on the first to the fifth day the number of dead termites was relatively low. It was caused by attached nanochitosan on the surface of the wood. Nanochitosan could still be detected by termites. Termite deaths increased on the fifth day because it began to starve and eat the wood bait. The mechanism of chitosan in controlling termites is the interaction of the positive charge of chitosan with a negative charge on the microbial cell membrane causing damage to protein elements and other elements of microbial intracellular [14] constituents. Chitosan is a polysaccharide that can function as a coating which is a good barrier. Chitosan coatings can form a strong and compact matrix. It is permeable to oxygen and carbon dioxide so that it becomes a barrier to the entry of protozoa that play a role in the digestive system of termites.

In a separate experiment, observations were made on the effect of the components in preservatives on the death of termites. The results of the observation showed that the highest NaCl solution caused the death of termites which was 46.67%. This phenomenon occurred because NaCl are termite resistant. It could inhibit cellulose enzymes that function to digest wood in the stomach of termites [6]. As a result, termites in samples with NaCl treatment cannot digest cellulose causing death. Samples with soaking Polysorbate-20 solution caused the death of termites by 28.67%, greater than Polysorbate-80 by 18%. It was related to observations which showed the average percentage of deaths of 46.89% for nanochitosan with a modification of Polysorbate-20 higher than Polysorbate-80 modified chitosan of 33.11% (Figure 6 b).

The results of the analysis of variance stated that the type of surfactant had a very significant effect on the mortality of termites. It was caused by the interaction between different surfactants and polyions because the tail length of the surfactant was different [17]. It affected the form of chitosan conformation in the dispersion and the effectiveness of chitosan as an anti-termite.
3.3. Impression of touch
There was a difference in impression between the conditions before and after the application of preservation with nanocitosan. The test sample with soaking preservative produced a finer and more transparent texture. Ornum [16] explained that the application of chitosan coated the wood surface so it covered uneven pores and fibers. This smooth wood texture was caused by a slippery and transparent layer of chitosan film. It showed that chitosan had the potential as a finishing material that could improve the smoothness of wood surfaces. Chitosan bonds with cellulose polymers were suspected to be able to fill cavities in the cell wall.

| no | sample | size, nm | treatment |
|----|--------|---------|-----------|
| 1  | 10N3A  | 244.3   | 10% Polysorbate-20 |
| 2  | 10N3B  | 292.1   | 10% Polysorbate-80 |
| 3  | 20N3A  | 309.7   | 20% Polysorbate-20 |
| 4  | 20N3B  | 233.0   | 20% Polysorbate-80 |
| 5  | 30N3A  | 798.8   | 30% Polysorbate-20 |
| 6  | 30N3B  | 346.7   | 30% Polysorbate-80 |

Based on the PSA analysis, it was found that nanochitosan with modification of NaCl and surfactant produced particle size with a range of 233.0-798.8 nm. The graphs of particle size distribution were presented in Figure 7.

![Figure 7. Particle size distribution of nanochitosan with NaCl and (a) polysorbate-20 (b) polysorbate-80](image_url)

The figures showed that nanochitosan with a concentration of 30% with both types of surfactants had a narrow and uniform particle size distribution in the range of 279.04 - 1741.10 nm although it produced the highest average size of 798.8 nm and 346.7 nm for polysorbate-20 and polysorbate-80 respectively. The small and uniform particle size were thought to help chitosan to penetrate wood pores.

The addition of NaCl into nanochitosan dispersion will reduce the formation of inter-particle coagulation. The presence of monovalent salts chitosan-TPP nanoparticles are more stable and have a smaller particle size and narrow particle size distribution [19].
Figure 8. SEM image of nanochitosan particles in tangential side of *Falcataria moluccana* with 10% of (a) polysorbate-20 1000x magnification (b) polysorbate-80 5000x magnification

Figure 9. SEM image of nanochitosan particles in transversal side of *Falcataria moluccana* with 10% of (a) polysorbate-20 1500x magnification (b) polysorbate-80 650x magnification

Figure 10. SEM image of nanochitosan particles in tangential side of *Falcataria moluccana* with 20% of (a) polysorbate-20 1500x magnification (b) polysorbate-80 230x magnification
Figure 11. SEM image of nanochitosan particles in tangential side of *Falcataria moluccana* with 30% of (a) polysorbate-20 2500x magnification (b) polysorbate-80 900x magnification

SEM analysis were performed to observe further the morphology of nanochitosan that covered the wood surface. Figure 8 to 11 were SEM of wood samples treated with nanochitosan with various concentrations and surfactants. The results showed that nanochitosan was attached to the surface of the wood vessel cells at the tangential surfaces while the transversal side of the nanochitosan penetrated the pits of wood (Figures 8 and 9).

According to Hunt and Garrat [10], the anatomical structure of wood was the difficult factor to control on the impregnation of preservatives into wood. The anatomical structure of *Falcataria moluccana* was thought to have a wide pore so as to accelerate the absorption of preservatives into wood. The difference in absorption in other studies was influenced by wood preparation before preservation, preservative materials, and the concentration of preservatives.

4. Conclusions

The modification of NaCl and surfactant had a significant effect on wood durability parameters. The type of surfactant had a significant effect on absorption, nanochitosan retention, and termite mortality while the surfactant concentration only affected retention. The results from independent component treatment showed that the types of surfactants had a significant effect on the absorption value while the interaction between TPP, NaCl and polysorbate-20 had a significant effect on retention. The surfactant treatments affected the hydrophobic interaction resulted in increasing absorption and retention on the wood surface. In the other hand, the addition of NaCl increased anti-termite properties and made particles smaller and stable which caused in higher retention.

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