Determination the best concentration of antimicrobial ingredients with a mixture of paper to create active paper packaging

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Abstract. Perishable food really needs proper handling. Therefore, various industries and researchers continue to innovate, especially in the development of packaging. The development of active packaging is a new innovation in the field of packaging technology. This packaging innovation was designed to maintain the quality of packaged food. This study aimed to obtain the best concentration of antimicrobial garlic ingredients with a mixture of paper as an active packaging material. The concentration of garlic extract used in this study was 0%, 5%, 10%, and 15%. Based on the result obtained, it could be concluded that the active paper with the addition of garlic extract concentration of 15% had the best effectiveness then followed by concentrations of 10%, 5%, and 0%. The effectiveness could be seen from the diameter of inhibition zone formation resulted.

1. Introduction
Indonesia has huge potential in the fisheries sector. This is supported by abundant natural resources. The fisheries commodity is quickly damaged. That is because fish have high nutritional value but are easily damaged because they contain high levels of protein with free amino acids that are used for the metabolism of microorganisms [1]. A second problem that is often being a matter of public debate is still widespread use of banned substances (formaldehyde) to preserve fish. The use of formaldehyde is as a result of the increased cost of preserving fish, including the cost of purchasing ice to preserve [2]. Another problem that often occurs is that the packaged comestible is not suitable for consumption again but is still being traded. This case can disserve consumers in terms of economics and health. Therefore, packaging technology is needed that is able to maintain the quality of fish, one of which is active packaging.

Lately, the use of active packaging had become a Hot Issue in various countries, especially in developed countries. Active packaging innovation is intended to inhibit the growth of microbes and replace the use of preservatives that are not safe for food, especially in fisheries commodities. Several active packaging materials have been made and marketed, such as oxygen absorber, moisture absorber, carbon dioxide release, and antimicrobial packaging. Antimicrobial elements added to fisheries commodities can be obtained from natural ingredients such as cinnamon powder, rosemary, oregano, and garlic [1,3]. Making active and smart packaging can be done in various ways, including through the process of extrusion, dissolving active ingredients into solvents, added to edible coatings, or mixed into fillers in paper or cardboard making [4–10].
This study aimed to determine the best concentration of antimicrobial ingredients with a mixture of paper as active paper material. Antimicrobial material used was garlic extract because this material contains allicin compound which is volatile and has a high permeability in penetrating bacterial cell walls by destroying sulfihydryl groups that make up bacterial cell membranes so that the structure of bacterial cell walls is damaged and growth is inhibited [11]. The addition of active ingredients can improve the ability of packaging in maintaining aspects of product quality and safety from microbial growth, causing decay.

2. Materials and methods

2.1 Materials
The equipment used in this research were blenders, knife, jars, filters, evaporators, beakers, oven gutters, ovens, petri dishes, incubators, and calipers. While, the materials used in this study were garlic, alcohol 96%, filter paper, aquadest, tapioca starch, chitosan powder, 1% acetic acid, tween 80, Styrofoam 38x29x30 cm, fish, LDPE plastic, NA agar media, and Staphylococcus sp bacterial suspension.

2.2 Procedure

2.2.1 Garlic extract procedure [12]
The manufacture of garlic extract used the maceration method. The procedure for making garlic extract began with the process of sorting garlic, then stripping the skin. After that, 50 g of garlic was blended until smooth, then put into a jar containing 250 ml of 96% alcohol with a ratio (1: 5) for 3 x 24 hours. Stirring was done 12 times for 15 minutes. Furthermore, filtering was done to separate the garlic pulp from the filtrate. The filtrate was then evaporated at 45°C until a thick reddish-yellow garlic extract was obtained.

2.2.2 Active paper procedure [13]
Filter paper cut (2 x 2 mm) 10 g soaked in 170 ml of distilled water with a ratio (1:17) for 24 hours. Soaking paper pieces added with 170 ml of distilled water and blended for 5 minutes until it became pulp. Next, the paper pulp was squeezed until the water run out. After that, tapioca starch as much as 6 g or 60% (w / w) was dissolved into 30 ml of distilled water in a ratio (1: 5) and mixed with pulp and then homogenized. Chitosan powder 0.6 g or 6% (w / w) was added to the beaker containing 30 ml of 1% acetic acid by comparison (1:50). The solution was then mixed with pulp and then blended for 5 minutes. After that, the addition of garlic extract with a concentration of 0%, 5%, 10%, and 15% (w / v) and each paper dough was added by tween 80 6% (w / v) into the paper mixture. Lastly, the paper dough was then printed manually on an oven chamfer with a size of 8 x 15 cm² according to the size of the styrofoam. After that, the sheet of wet paper was dried using an oven at 40°C for ± 15 hours.

2.2.3 Application of active paper on fish fillets packaging
Fresh fish was sterile prepared, then cut to a size of 100 g / pack. The fish fillet was then placed in a Styrofoam that was coated with active paper measuring 15 x 8 cm². After that, the fish fillet was packed using LDPE plastic.

2.3 Observation parameter

2.3.1 Antibacterial activity testing agar diffusion method [14]
Testing the antibacterial activity of active paper aimed to determine whether there was any inhibition of active paper against the growth of Staphylococcus sp bacteria, which were spoilage bacteria in fish. A sheet of active paper with a size of 5 mm was placed on top of the NA media, which surface had been spread by Staphylococcus sp as much as 0.1 ml evenly. Subsequently, the petri dish was incubated at 37°C for 24 hours. Inhibition was determined based on the clear zone formed around the
active paper. The formed resistance zone was then measured using a caliper. Classification of responses to bacterial growth inhibition could be seen in Table 1.

| Diameter (mm) | Response to Growth Obstacles |
|---------------|-----------------------------|
| 0 - 3 mm      | Weak                        |
| 3-6 mm        | Medium                      |
| > 6 mm        | Strong                      |

Table 1. Classification of response to bacterial growth obstacles

Sources: Pan, Chen, Wu, Tang, and Zhao (2009) in Upa et al., (2017) [15,16].

2.4 Data analysis

The data in this study were calculated in the 2007 Microsoft Excel software and were analyzed using analysis of variance (ANOVA) with twice repeated. If the results of the analysis of variance showed a real effect, then further tests would be conducted with Duncan's method. Data analysis results were displayed in graphical form. Data were analyzed using SPSS 22 software.

3. Result and discussion

3.1 Analysis of active paper antibacterial activity

The analysis of the antibacterial activity of the active paper aimed to determine the effect of adding garlic extract with different concentrations to the formation of the zone of bacterial growth inhibition. Figure 1 showed the diameter of the active paper antibacterial inhibition zone against the growth of fish spoilage bacteria. Figure 2 showed the analysis of variance, while Figure 3 showed Further Tests with the Duncan method.

![Graph of an antibacterial inhibitory zone on active paper](image)

Figure 1. Graph of an antibacterial inhibitory zone on active paper

Based on quantitative data, the diameter of the active paper antibacterial inhibition zone against the growth of Staphylococcus sp. showed the results that the addition of garlic extract with different concentrations affected the diameter of the inhibition zone formation. The inhibition zone diameter in the treatment without the addition of garlic extract (0%) was smaller than the inhibition zone diameter in the addition of garlic extract concentration of 5%, 10%, and 15%. Based on the classification of bacterial growth inhibition responses proposed by Pan et al. 2009; Upa et al., 2007, inhibition zone diameter in active control paper (0%) and the addition of garlic extract 5% and 10% included in the category of weak in inhibiting bacterial growth while for the addition of garlic extract 15% included in the medium category in inhibiting growth bacteria. However, to find out further whether there was or not a real difference in the effect of adding garlic extract with different concentrations to the diameter
of the inhibition zone formation, a variance test was performed. After testing, the variance showed a real difference, with a significance value of 0.005 (P <0.05). Therefore, the analysis continued to the Duncan test phase at a level of 5%. Based on Duncan's test results, it was known that there were differences in the effect of the addition of garlic extract with different concentrations on the diameter of the inhibition zone formation.

The difference in diameter of inhibition zone formation that occurs was closely related to the administration of varying concentrations of garlic extract. This was because garlic extract added to active paper had allicin compounds that play a role in inhibiting microbial growth. The ability of garlic as an antimicrobial in inhibiting the growth of microorganisms was supported by research Ankri (1999), which stated that allicin was an active substance in garlic, which was effective in killing microbes. Allicin had varied antimicrobial activity. Allicin, in its pure form, had a broad spectrum antimicrobial power, including strains of \textit{Escherichia coli}, \textit{Candida albicans} (fungi), and protozoan parasites [17]. In addition, based on Sivam, 2001; Cutler and Wilson, 2004; Ichsan, 2009 stated that allicin had a broad spectrum of antibiotics against gram-positive and gram-negative bacteria, such as penicillin [18–20]. Muslim, Holty, and Widjajanti (2009) also asserted that garlic could be antibacterial because garlic contained allicin, which had high permeability in penetrating bacterial cell walls by destroying sulfhydryl groups that made up bacterial cell membranes so that the structure of bacterial cell walls was damaged and its growth was inhibited [11]. This was also supported by Wiryawan (2005), which stated that the higher of garlic extract concentration also had a higher antibacterial activity [21]. This was what causes the active paper with the addition of garlic extract with a higher concentration (15%) had a bacterial inhibition zone diameter that was greater than the addition of garlic extract concentration of 10%, 5%, and control (0%). These results indicated that the administration of garlic extract with a concentration of 15% on active paper was more effective in inhibiting bacterial growth, which was characterized by the large diameter of the inhibitory zone formed.

The formation of inhibitory zones was also suspected because there was an effect of adding chitosan to active paper, making as much as 6% (w / w) of the paperweight. Chitosan is also known to have antimicrobial activity. The ability of chitosan as an antimicrobial in inhibiting the growth of microorganisms is due to chitosan having the form of a porous membrane so that when applied to food, it will absorb free water contained in the food that can be utilized by microorganisms to grow so that it can inhibit microbial growth. Sarwono (2010) argued that chitosan could also act as an antibacterial because chitosan had a positively charged functional amine group (-NH\textsubscript{2}) that was very strong and could attract negatively charged amino acid molecules which were protein-forming in bacteria. These positive and negative charges interact electrostatically, which caused the membrane to undergo permeable pressure, which caused osmotic pressure inside the cell to be unbalanced so that it could inhibit the growth of bacteria. The functional group of amines also had free electron pairs so that those could attract minerals (Mg\textsuperscript{2+}) contained in ribosomes and minerals (Ca\textsuperscript{2+}) found in bacterial cell walls form coordinating covalent bonds that caused leakage of intracellular constituents. The event of the leakage of the cell wall caused the release of cell electrolytes so that the bacteria would die [22]. Based on research conducted by Wulandari (2008), it was concluded that chitosan chin skin (\textit{Penaeus monodon}) 1% was very effective as an antibacterial against \textit{Staphylococcus aureus}, \textit{Bacillus substilis}, \textit{Pseudomonas aeruginosa}, and \textit{Escherichia coli} [23]. Also, based on research Killay A, (2013), that chitosan with a concentration of 0.5% and 1% could inhibit the growth of bacteria in dried salted fish [24].
3.2 Design of active paper packaging

![Figure 2. The appearance of active paper](image1)

![Figure 3. Design of active paper packaging](image2)

4. Conclusion
Based on the result obtained, it could be concluded that the active paper with the addition of garlic extract concentration of 15% had the best effectiveness then followed by concentrations of 10%, 5%, and 0%. The effectiveness could be seen from the diameter of inhibition zone formation resulted.

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