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Edible coating application of Kecombrang leaves to reduce gourami sausage damage

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Abstract. Gourami sausage is a processed fish product which have short shelf life. To extend shelf life, sodium nitrate as artificial preservative (synthetic) was used. However, if it consumed continuously, the synthetic preservative induced negative effects to human health. Another alternative to avoid the use of synthetic preservative is using antimicrobial edible coatings. In this regard, Kecombrang (*Nicolaiaspeciosa*) leaves contain bioactive compounds that able to act as natural anti-microbial for edible coating production. This research aimed to know the effect of different level of Kecombrang leave concentrate in edible coating to the microbial growth inhibition in fish sausage gourami during storage at low-temperature storage ($\pm 4^{\circ}\text{C}$). The result shows that edible coating contained natural anti-microbial have inhibitory effect to total mold and yeast, bacteria, and microbial. The addition of 4% concentrate of Kecombrang leaves shows better in suppressing the growth of mold and yeast, bacteria, and microbe.

1. Introduction

The use of preservatives, mostly synthetic, occurs in almost all food products. In Indonesia, the use of synthetic preservatives in food products is regulated by Indonesian National Agency Drug and Food Control. In the animal processed products, synthetic preservatives such as sodium nitrate is use for preservatives in sausages for example. According to the regulation of Indonesian National Agency Drug and Food Control the maximum amount of sodium nitrite in meat products is 30 mg/kg [1]. Above limit, the use sodium nitrate would affect to human health. This due to nitrite can bind to amino or amide resulting made nitrosamine derivatives which are toxic and carcinogenic [2]. Therefore, safe preservatives were needed for human health.

One promising natural preservative is antimicrobial edible coatings, a thin layer made by natural ingredients and contains bioactive compounds function as antimicrobial substances and as a protective layer. Natural preservatives can be obtained from organic ingredients that have a potency to be antimicrobial agents. The previous study [3], showed that parts of the Kecombrang plant such as leaves contain bioactive compound such as alkaloids, saponins, phenolics, flavonoids, triterpenoids, steroids, and glycosides.



Antimicrobial edible coatings able to preserve frozen food products such as gourami sausages. According to [4], coated food products with edible coatings have been carried out and proven to extend shelf life as well as to improve product quality. The use of antimicrobial edible coatings as ingredients in fish sausage coatings is expected to reduce the use of synthetic preservatives and maintain the quality the product damage, lead to extend the shelf life of the product.

The purpose of this study was to determine the effect of of Kecombrang leaf concentrate addition in edible coating to the inhibition of microbes' growth of edible coating gourami sausage during low-temperature storage ($\pm 4^{\circ}\text{C}$).

2. Research methodology

2.1. Materials and treatment design

The materials used are Kecombrang leaves obtained from Kotayasa-Baturaden Village, gourami fillets, tapioca flour, wheat flour, and palm oil, ice water, cellulose sausage casings, technical ethanol 96%, Nitrogen gas (N_2), aquadest, *carboxymethyl cellulose* (CMC), glycerol, Natrium Chloride (NaCl) 0,85% "Merck", *Plate Count Agar* (PCA) "Oxoid", *Nutrient Agar* (NA) "Merck", *Potato Dextrose Agar* (PDA) "Merck", culture *Escherichia coli* (FNCC 0091), *Bacillus cereus* (FNCC 0057), *Staphylococcus aureus* (FNCC 0047), and *Pseudomonas aeruginosa* (FNCC 0063). The factor studied was the addition of concentrations of Kecombrang leaf concentrate consisting of four levels, 1%, 2%, 3%, and 4% of the observed storage time at low temperatures ($\pm 4^{\circ}\text{C}$) for 8 days of storage. As controls treatment, sausages without edible coating, edible coating sausages without the addition of Kecombrang concentrate, and commercial sausages with synthetic sodium nitrate preservatives "X brand chicken sausages".

2.2. Gourami fish sausage

The initial stage of making gourami sausage, which is weeding fish to obtain gourami fillets. Then wash with streaming water. Grinding 100 g of gourami fillet obtained was milled with a meat grinder twice. Ground meat weighed 100 g, added 22% tapioca, 23% wheat flour, 8% ice water and palm oil 3% (b/b) of the total gourami meat. Mixing gourami meat with additional ingredients using chopper until smooth for ± 3 minutes at medium speed. Filling in the emulsion sleeve is done carefully so that no air bubbles are formed. After the sleeve is filled with a solid mixture weighing ± 25 g / sleeve and a length of ± 7 cm, the ends are tied with ropes. Fish sausage cooking by steaming at 100°C for 20 minutes. Once cooked, the sausage is cooled at room temperature for ± 30 minutes [5].

2.3. Concentrate of Kecombrang leaf

The concentrate was obtained from extraction of Kecombrang leaf powder by maceration method, Kecombrang leaf powder extracted with 96% technical ethanol (1:4 b/v). The residue is extracted again with 96% technical ethanol (1:4 b/v). The extraction process was carried out maceration at 37°C with a rotational speed of 150 rpm for 2-4 hours at each level. After that filtering is done using Whatman disc paper No. 1 until the filtrate is obtained (extract). The extract is separated from the solvent by evaporation in the evaporator. The solvent is evaporated at a maximum temperature of 50°C and the remaining solvent is removed by nitrogen gas. The obtained concentrate was used as a sample to be added to the making of edible coating which was analyzed.

2.4. Edible coating Kecombrang concentrate

100 ml of distilled water was added with a CMC stabilizer with a concentration of 0.5% and then added 1% glycerol then homogenized using a hand blender for ± 1 minute. Then the edible coating solution was transferred to a beaker glass to be heated on a magnetic stirrer hot plate until the temperature reached 70°C . After that, an edible coating added Kecombrang concentrate according to treatment and homogenized again using a hand blender for ± 2 minutes [6].

2.5. Preparation of microbial test

The tested bacteria were *Escherichia coli* (FNCC 0091), *Bacillus cereus* (FNCC 0057), *Staphylococcus aureus* (FNCC 0047), and *Pseudomonas aeruginosa* (FNCC 0063) obtained from Gadjah Mada University. The culture of bacteria is kept on nutrient slants and stored at 4°C. The bacterial strain was cultured in Broth Nutrient at 37°C for 24 hours before being used for the analysis of antibacterial activity.

2.6. Antibacterial activity analysis

Antibacterial activity of edible coating of Kecombrang leaf concentrate on test bacteria was carried out using the diffusion-agar method. Antibacterial activity is expressed as the diameter of the inhibition zone (mm) formed by bacteria [7] and edible coating without concentrates is used as a negative control.

2.7. Microbial analysis

Microbiological analysis carried out measurements of total mold and yeast, total bacteria, and total microbes (TPC) [8] on sausage products edible coating of Kecombrang leaf concentrate.

2.8. Statistical analysis

Data were analyzed using analysis of variance (F test) at the level of 5%, followed with Duncan's Multiple Range Test at the level of 5%.

3. Result and discussion

3.1. Antibacterial activity

Edible coating added with Kecombrang leaf concentrate with concentrated addition concentration of 1%; 2%; 3%; and 4% (b/v) tested for antibacterial activity by well diffusion method. Antibacterial activity testing was carried out on all four test bacteria. The diameter of the inhibitory zone produced by testing the antibacterial activity of edible coating of Kecombrang leaf concentrate with well diffusion method is presented in Table 1.

Table 1. The diameter of the edible coating inhibition zone of Kecombrang leaf concentrate produced from testing the antibacterial activity by the well diffusion method

Material	Diameter Inhibitory zone on tested bacteria (mm)			
	<i>E. coli</i>	<i>B. cereus</i>	<i>S. aureus</i>	<i>P. aeruginosa</i>
Control edible	0	0	0	0
<i>Edible leaf concentrate:</i>				
1%	4,5±0,11	6,2±0,13	5,5±0,14	5,5±0,08
2%	5,3±0,04	7,1±0,08	5,8±0,11	11,4±0,08
3%	6,7±0,06	7,8±0,04	5,7±0,08	12,4±0,07
4%	11,9±0,11	9,7±0,10	8,5±0,14	13,0±0,08

Description: The diameter of the inhibition zone does not include the diameter of the well (6 mm)

The results of this study indicate that the inhibition zone diameter of the Kecombrang leaf concentrate added to the edible coating as a whole can inhibit the activity of the test bacteria. The presence of antimicrobial activity is indicated by the presence or absence of a barrier zone (clear zone) that is formed in the agar medium. The diameter of inhibitory zones of 10-20 mm has strong inhibitory strength, the diameter of the 5-10 mm inhibition zone has a moderate inhibition and the inhibition zone diameter <5 mm has a weak inhibitory power [9].

Edible coating with the addition of Kecombrang leaf concentrate 4% (b/v) resulted in the largest to the smallest inhibition zone in *P. aeruginosa*, *E. coli*, *B. cereus*, and *S. aureus* bacteria respectively 13 mm; 11.9 mm; 9.7 mm; and 8.5 mm. Judging from Davis and Stout (1971), the antibacterial activity

of edible coating of 4% Kecombrang leaf concentrate on *P. aeruginosa* and *E. coli* bacteria had a strong inhibitory ability, whereas in *B. cereus* and *S. aureus* bacteria had a moderate inhibitory ability.

The average value of inhibitory zone diameter of edible coating with various variations in the concentration of addition of Kecombrang concentrate on the test bacteria can be seen in Figure 1.

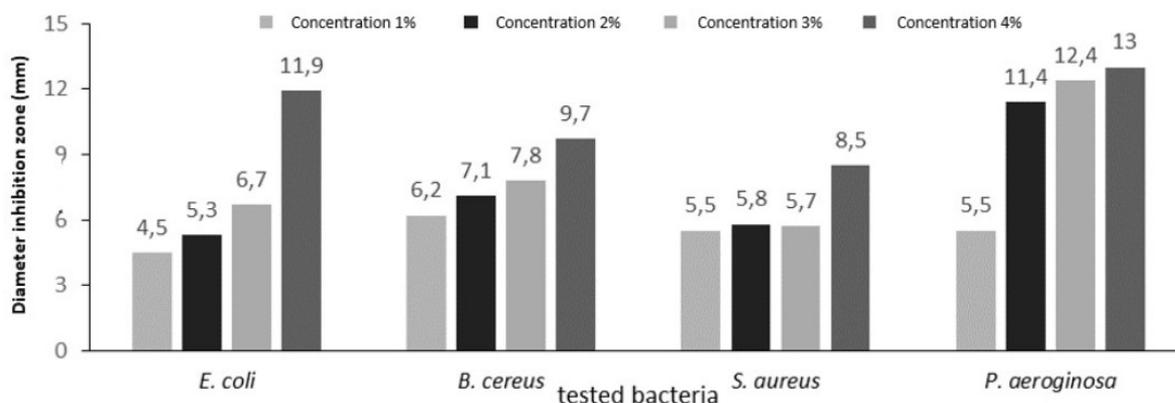


Figure 1. Average value of inhibitory zone diameter of edible coating with various variations of concentration of addition of Kecombrang leaf concentrate on test bacteria

Based on Figure 1, the addition of Kecombrang leaf concentrate on edible coating can inhibit the activity of Gram-negative bacteria *P. aeruginosa* and *E. coli* greater than Gram positive bacteria. Inhibition of Gram-negative bacteria *P. aeruginosa* and *E. coli* is greater due to differences in the structure of the bacterial cell wall. The cell wall of Gram-positive bacteria consists of several layers of peptidoglycan which form a thick and rigid structure and contain a cell wall substance called teichoic acid, while the cell wall of Gram-negative bacteria consists of one or more thin layers of peptidoglycan which do not contain teichoic acid. Therefore, cell walls of Gram-negative bacteria are more susceptible to antibiotics or other antibacterial ingredients [10]. The results of research by [11] also showed that chloramphenicol has a broad spectrum or has a greater inhibitory ability of Gram-negative bacteria than Gram positive on chloramphenicol 10 µg. Chloramphenicol inhibition zone against *S. aureus* and *E. coli* was 17.5 mm and 22.66 mm.

The effectiveness of bioactive components in the manufacture of Kecombrang leaf concentrate with ethanol solvent also affected the inhibition of Gram-negative bacteria. In *E. coli* bacteria, there was a tendency that the polar (ethanol) fraction produced a higher inhibition than the semi-polar (ethyl acetate) fraction. Polar fraction (ethanol) is thought to have optimal polarity so it is easier to diffuse and can inhibit *E. coli* growth [12]. Previous study [13] reporting the activity of ethanol extract of Mayana leaf (*Coleus atropurpureus* [L] Benth) in inhibiting the growth of *E. coli* Gram-negative bacteria was more sensitive when compared to Gram-positive bacteria *S. aureus*.

Figure 1 shows the higher concentration of the addition of Kecombrang leaf concentrate on edible coating, the higher inhibition formed. In general, the average inhibition zone diameter has increased along with the increase in concentration given [14]. The effectiveness of an antimicrobial agent in inhibiting growth depends on the microbial properties of the test, concentration and length of contact time [11]. Bacteriostatic properties can increase with increasing concentration added.

3.2. Microbial analysis of edible coating fish sausage

3.2.1. Total mold and yeast. Total mold and yeast were analyzed using the pour plate method with Potato Dextrose Agar (PDA) media. Media to add 1% chloramphenicol as much as 1% into the media so that before pour plate. The purpose of adding 1% chloramphenicol to the agar medium is to prevent

bacterial growth [15]. The effect of the addition of Kecombrang leaf concentrate to edible coating sausages on the total value of total mold and yeast is presented in Figure 2.

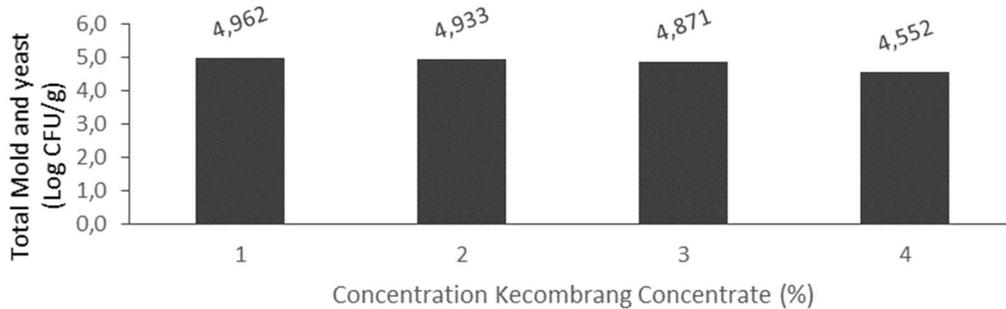


Figure 2. The total average value of mold and yeast of gourami sausages edible coating on the treatment of the effect of the addition concentration of Kecombrang leaf concentrate

Figure 2 shows that the higher the concentration of Kecombrang concentrate added to the edible coating, the higher inhibitory ability carried out on the growth of mold and yeast in fish sausages. This is characterized by a decrease in the total average value of mold and yeast. The biggest inhibitory ability occurred at 4% concentration, which was 4,552 log CFU / g, while the smallest inhibitory ability occurred at 1% concentration, which was 4,962 log CFU / g. According to [16], the higher the concentration of Kecombrang flower extract, the higher the inhibition of the fungus *Chalaropsis* sp. [17] reported that Kecombrang flower extract can inhibit the growth of food-destroying fungi such as *Aspergillus flavus*, *Penicillium funiculosum* and *Rhizopus oligoporus*. Growth inhibition of *Aspergillus flavus* and *Penicillium funiculosum* occurs at a concentration of 15% and *Rhizopus oligoporus* at a concentration of 30%.

3.2.2. *Total bacteria.* The influence of the concentration of addition of Kecombrang leaf concentrate on edible coating sausages on the total value of total bacteria is presented in Figure 3.

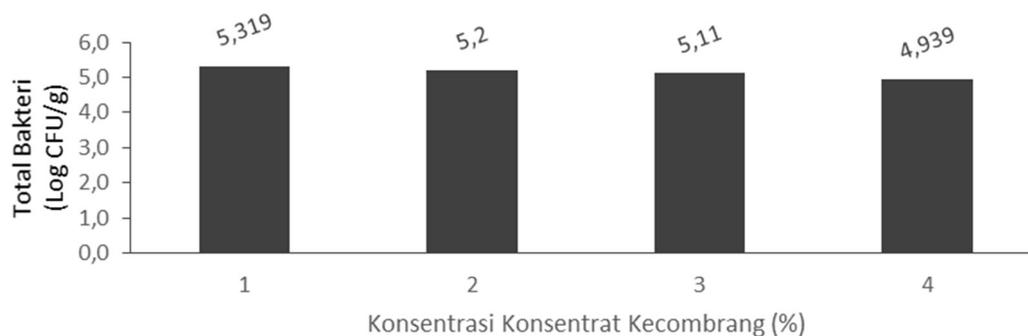


Figure 3. The average total value of edible coating gourami sausage bacteria on the treatment effect of the concentration of Kecombrang leaf concentrate addition

Figure 3 shows that along with the increase in concentration of Kecombrang leaf concentrate added to edible coating, the more inhibitory ability carried out on bacterial growth. This is characterized by a decrease in the average total value of bacteria in fish sausages. The biggest inhibitory ability occurred at 4% concentration which was 4,939 log CFU/g, while the smallest inhibitory ability occurred at 1% concentration 5,319 log CFU/g. This means that the bioactive components in the concentration of Kecombrang leaf concentration of 4% can inhibit the work activity of bacterial cell membranes [18]. Reaction with cell membranes occurs because bioactive components can disrupt and affect the integration of the cytoplasmic membrane which results in intracellular leakage, causing cell lysis, protein denaturation and inhibiting ATP-ase bonds in cell membranes [19].

3.2.3. *Total Microbes (TPC)*. The effect of the addition of Kecombrang leaf concentrate on edible coating sausages to the total microbial average value is presented in Figure 4.

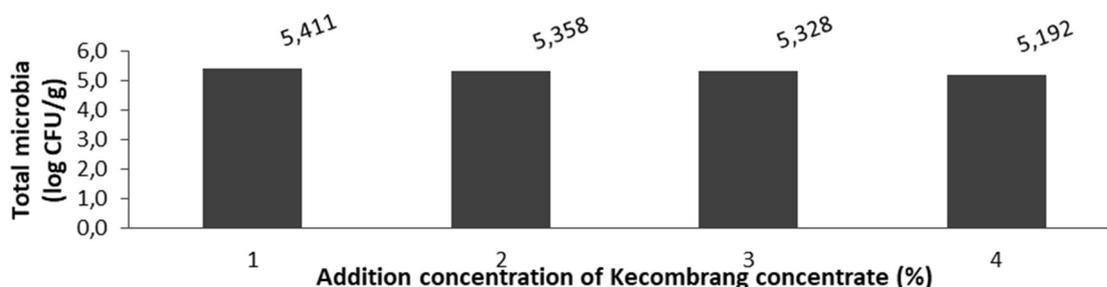


Figure 4. The average total value of microbial gourami sausage edible coating on the treatment effect of the concentration of Kecombrang leaf concentrate addition

Figure 4 shows that the higher the concentration of Kecombrang concentrate added, the more the inhibitory ability carried out on microbial growth. This is characterized by a decrease in the total average value of microbes. The biggest inhibitory ability occurs at a concentration of 4%, which is 5,192 log CFU/g. The effectiveness of the preservative is determined by the concentration, type of preservative, and conditions when the preservative is added [20]. Generally, higher concentration of preservatives given, the effectiveness will increase [11].

4. Conclusions

Kecombrang leaf concentrate added to the edible coating effectively inhibits microbial growth based on microbiological properties of gourami sausages. The concentration of addition of 4% Kecombrang concentrate on edible coating is more effective in inhibiting the growth of gourami sausage microbes compared to the addition of concentrations of 1%, 2%, and 3%. Microbiological properties of gourami sausage during low temperature storage ($\pm 4^{\circ}\text{C}$) in Kecombrang leaf concentrate concentrated 4% as follows: total mold and yeast 4,552 log CFU/g; total bacteria 4,939 log CFU/g; and total microbes 5,192 log CFU/g.

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