Antimicrobial edible coating application of Kecombrang flower concentrate to reduce microbial growth on gourami fish sausage

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Abstract. Kecombrang (Nicolaia speciosa) is a plant having bioactive compound which can be utilized as natural preservative for food products, by applying as edible coating to perishable food such as gourami sausage. For that, this study aims to know the level of Kecombrang flower concentrate addition to the microbiological variable of gourami sausage during storage time. The experimental design used in this study was Completely Randomized Design. The observed factors were the level of Kecombrang flower concentrate consisted of 4 levels (1, 2, 3, 4%). The observations were conducted for 8 days in 4°C storage time. The results showed that the application of edible coating with concentrate of Kecombrang flower give higher inhibitory effect on total microbial, total mold and yeast of gourami. The addition of 4% concentrate indicates a higher ability to inhibit microbial growth. The mean of total microbes, bacteria and mold, and yeasts with the addition of 4% concentrates were 5.13, 5.18, 4.29 log CFU/g, respectively. The largest zone of inhibition was obtained in 4% concentration in four types of bacteria namely Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus and Bacillus cereus were 9.80; 9.07; 7.06; 1.33 mm, respectively.

1. Introduction
Fresh fish is belonging to short shelf life foodstuffs. There are efforts being made to reduce the damage of fish product by processing fish into sausage. Considering that Indonesia's fisheries resources are quite large, it is necessary to think about the business of diversifying processed sausage products by utilizing fish as raw material.

Fish sausage is a processed product of fishery products with raw material for fish meat or surimi meat, at least 50%, mixed with flour and other ingredients, filling into sausage casings and boiling or steaming [1]. Gourami is chosen as the raw material for making fish sausages because of its high nutrition content, delicious taste, thick meat and easy to obtain. gourami has limited processing which is generally only consumed in fresh. One step to diversifying food and utilizing gourami can be done by processing the gourami into fish sausage.

Currently the role of edible coatings is being widely used for coating processed meat products such as sausages [2]. Edible coatings provide a selective barrier to gas, water vapor and dissolved material displacement, as well as protection against mechanical damage [3]. Sausage is a food that is classified as perishable food due to its high water and protein content, and environmental conditions that are very suitable for microbial decay growth.
Edible coating with the addition of Kecombrang flower concentrate will have bioactive compounds that act as antimicrobials on gourami sausage products. Kecombrang (Nicolaia speciosa) can be used as food preservative because it contains various bioactive compounds. According to previous study, Kecombrang flower chemical compounds including alkaloids, flavonoids, polyphenols, steroids, saponins, and essential oils [4]. Phenol is a substance that influences bacterial inhibition, so that the Kecombrang flower is antimicrobial. Therefore, this application is expected to be able to inhibit and reduce the growth of damaging microbes thus it can increase the shelf life of fish sausage products and nutritional value, therefore it can be accepted both physically and sensory by consumers.

Based on these descriptions, this study aims to determine the level of Kecombrang flower concentrate addition to the microbiological variables of gourami sausages during storage time.

2. Research methodology

2.1. Material and treatment design

The ingredients used were Kecombrang flowers obtained from Kotayasa-Baturaden Village, gourami fillets, tapioca flour, wheat flour, and palm oil, ice water, cellulose sausage casings, 96% technical ethanol, Nitrogen gas (N2), distilled water, 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) from PAU UGM. The experimental design used was a Completely Randomized Design (CRD) with the factors observed, namely level of Kecombrang flower concentrate addition consisting of four levels, 1, 2, 3, and 4% during observation of storage time at low temperatures (± 4°C) for 8 days of storage. The sample used as a control is sausage without edible coating, edible coating sausage without Kecombrang concentrate addition, and commercial sausage “chicken sausage merk X”.

2.2. Gourami fish sausage

Sausages are made from meatless gourami fillet meat. The procedure for making sausages begins with washing the fish, mixing the ingredients and making in the sleeve. Fish fillets as much as 100 g are ground with 8% ice water (w/w) added to keep the meat temperature low during grinding. The ground meat was mixed with tapioca flour 22% (b/b) and wheat flour 23% (b/b) and the addition of palm oil 3% (b/b), stirred until well blended. The sausage dough is inserted into the sleeve diameter 18.45 mm with a sausage length of ± 7 cm. Sausage is steamed at 100 °C for 30 minutes or until cooked [5].

2.3. Concentrate processing

The concentrate was obtained from extraction of Kecombrang flower powder carried out by maceration method. The flower powder was extracted twice with 96% Ethanol (1: 4 b/v). The extraction process was carried out by maceration at 37° C, with a rotational speed of 150 rpm for 2 hours at each level. The filtrate is separated from the solvent by evaporation in a rotary evaporator until no solvent drips again. The first solvent was evaporated at 40°C, the second solvent was evaporated at 50°C. The remaining solvent was removed with nitrogen gas so that a concentrate was produced [6]. The obtained concentrate was used as a sample to be added to the making of edible coating which was then analyzed.

2.4. Edible coating concentrate of Kecombrang

The process of making edible coating using distilled water as a solvent, 0.5 g / 100 ml CMC and 1 g glycerol / 100 ml distilled water. The three ingredients were homogenized using hand blender for 2 minutes then heated on a magnetic stirrer to a temperature of 70°C. If the temperature has decreased, added Kecombrang flower concentrate according to treatment and homogenized with the hand blender for 1 minute. Edible coating solution with the addition of Kecombrang flower concentrate is regulated with pH using sodium bicarbonate (NaHCO3) 5% to pH 6.
2.5. Preparation of microbial test
The tested bacteria are *Escherichia coli* (FNCC 0091), *Bacillus cereus* (FNCC 0057), *Staphylococcus aureus* (FNCC 0047), and *Pseudomonas aeroginosa* (FNCC 0063) from PAU, 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 the Kecombrang flower edible coating on the test bacteria was carried out using the agar diffusion method. Antibacterial activity is determined 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. Microbiological analysis
Microbiological Analysis is measuring on mold and yeast, total bacteria, and total microbes (TPC) [8] on edible coating sausage of Kecombrang flower concentrate.

2.8. Statistical analysis
The data obtained will be analyzed for variance (Test F) at a 95% significance level, if there is a difference then it will be followed by a significant difference test using Duncan Multiple Range Test (DMRT) analysis at a significance level of 95.

3. Results and discussions
3.1. Antibacterial activity
Antimicrobial compounds have ability to inhibit the growth of microorganisms. The activity test of antimicrobial compounds was carried out on edible coatings which had been added with Kecombrang flower concentrate at various addition concentrations (1%, 2%, 3% and 4%). The diameter of the zone of inhibitory edible coating of the Kecombrang flower concentrate obtained can be seen in Table 1.

| Treatment                     | Diameter inhibition zone (mm) |  |  |  |
|-------------------------------|-------------------------------|---|---|---|
|                               | *E. coli*                     | *P. aeruginosa* | *S. aureus* | *B. cereus* |
| Control                       | 0,00                          | 0,00          | 0,00         | 0,00         |
| Edible coating concentrate of |                               |              |              |              |
| Kecombrang flower             | 1%                            | 5,00±0,13     | 4,93±0,09    | 1,47±0,03    | 1,08±0,03    |
|                               | 2%                            | 6,93±0,02     | 5,73±0,06    | 3,07±0,09    | 1,11±0,02    |
|                               | 3%                            | 9,57±0,05     | 7,77±0,04    | 5,80±0,03    | 1,13±0,02    |
|                               | 4%                            | 9,80±0,02     | 9,07±0,01    | 7,60±0,04    | 1,33±0,02    |

Description: The diameter of the inhibition zone does not include the diameter of the well.

The test results showed that edible coating with the addition of Kecombrang flower concentrate was able to inhibit the activity of the four types of test bacteria compared to the control treatment. Pervious report [9] showed that Kecombrang flower extract has broad inhibitory spectrum including Gram positive, negative and spore-forming bacteria. Based on Table 1, the results of the largest inhibitory zone area were obtained in the treatment of addition of Kecombrang flower concentrate with a concentration of 4% of the four-test bacteria *Escherichia coli*, *P. aeruginosa*, *S. aureus* and *B. cereus*, namely 9.80, 9.07, 7.60, 1.33 mm, respectively.

The diameter of the inhibited edible coating zone of the Kecombrang flower concentrate can be seen in Figure 1.
Figure 1. The diameter of inhibition zone of edible coating Kecombrang flower on concentration to tested bacteria.

Figure 1 shows that the inhibition zone diameter produced in the four types of microbes varies, the more concentration added to the edible coating, the more diameter of the inhibitory zone was formed. When compared to the standard value limit for the grouping of activity barriers proposed by [10], the inhibition zone of the Kecombrang flower concentrate edible coating was included in the category of moderate (5-10 mm) inhibitory activity against *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* test bacteria.

Bacteria tested on *Escherichia coli*, *Pseudomonas aeruginosa* on *Staphylococcus aureus* is giving high inhibition zone, whether *Bacillus cereus* have a small inhibition zone. Extraction process on processing Kecombrang flower and fruits in this research using ethanol solvent. Earlier study [11] stated that the extract of Kecombrang flower on ethanol fraction is effective to inhibit the growth of *Escherichia coli*. The inhibition zone of *Escherichia coli* is higher than *Bacillus cereus*.

According to [9], Phytochemical compounds of Kecombrang flower extracted with ethanol solvent is phenolic compound. Phenolic act as antibacterial by poisoning protoplasm, damaging and penetrating cell walls, and precipitating bacterial cell proteins [12]. In addition, according to [13] the formation of the inhibitory zone produced was influenced by the determination of the concentration of Kecombrang extract used. The inhibitory mechanism of action on *Pseudomonas aeruginosa* bacteria by Kecombrang flower extract is to damage the cell membrane and interfere with the function of genetic material [14].

3.2. Microbiological analysis of edible coating fish sausage

3.2.1. Total microbes (Total Plate Count). One mechanism of decay that damages food is microbial growth. The total number of plates or total microbial testing is done to determine the number of microbes in a product. The results of total average value of gourami sausage microbe’s edible coated with Kecombrang flower concentrate during storage in low temperature (± 4°C) were obtained in Figure 2.
Figure 2. The average total value of microbes gourami sausage with an edible coating of Kecombrang flower concentrate on the treatment of the addition of concentrates during storage.

The total average value of microbes shows that higher concentration of the addition of concentrates in edible coatings for the application of gourami sausages can provide a higher inhibitory power of microbial growth. In Figure 2, the average total value of microbes continues to decrease until the addition of concentration of Kecombrang flower concentrate is 4%.

At concentration of 4% the average total microbial value was 5.13 log CFU / gram. Based on this, it shows that the addition of Kecombrang flower concentrate by 4% into edible coating has a considered able to inhibit microbial growth compared to other treatments. Smaller concentration dose, the less the amount of active substance contained in it so that the lower the ability to inhibit the growth of a bacterium [15, 16].

According to [17], the limit of microbial contamination in processed fishery materials that are boiled or steamed, the total plate value (TPC) is a maximum of 5 x 10^5 or 5.70 log CFU / g. While the total value of microbes found in fish sausages with the Kecombrang flower concentrate edible coating treatment is 5.26 log CFU / g. This value has met with the requirements of minimal amount of microbial contamination according to Indonesian National Standard (SNI). Phytochemical content from Kecombrang flowers include alkaloids, saponins, tannins, phenols, flavonoids, triterpenoids, steroids, and glycosides which have active role as antibacterial [18].

3.2.2. Total bacteria. The effect of Kecombrang flower concentrate addition to the total average value of gourami sausage bacteria during storage in low temperatures (± 4°C) can be seen in Figure 3.

Figure 3. Average value of total bacteria (log CFU / g) gourami sausage edible coating of Kecombrang flower concentrate at concentration treatment during storage.
According to [19], antimicrobial substances in spices can be bactericidal, namely killing bacteria and bactericatics, which inhibits bacterial growth. Kecombrang flowers contain various compounds that work as antibacterials according to what is stated by [4] that Kecombrang flowers also contain several phenolic components [20].

Based on Figure 3, it is shown that the average value of total bacteria decreases as the concentration of Kecombrang flower concentrate addition increases in the edible coating applied to gourami sausages. These results can be concluded that the addition of concentrates which are up to 4% able to provide stronger inhibitory activity of bacterial growth. This is in line with [21] who explained that the ability of antibacterial compounds to inhibit bacterial growth is influenced by the concentration of antibacterial substances.

Based on Figure 3, the average total value of gourami sausage bacteria with the lowest Kecombrang flower edible coating treatment was obtained at a concentration of 4%, namely 5.18 log CFU / g. These results indicate that the use of high concentrations of up to 4%, is more able to provide inhibitory growth of bacterial power seen from the average value of total low bacteria. This is because the number of bioactive components in the Kecombrang flower concentrate is 4% more than the concentration of 1%, 2% and 3%. Kecombrang flower concentrate contains several phytochemical components such as phenolic, steroids, terpenoids, alkaloids, and glycosides capable of inhibiting total bacterial growth during storage [22].

3.2.3. Total Mold and Yeast. Mold is a microbial consisting of more than one cell in the form of fine threads called hyphae, the collection of hyphae is called mycelium, multiplying with spores, while yeast is a single-oval single-celled microbe and multiplies itself through bud formation or ascospora, but does not form mycelium (SNI 7388-2009). The total average of gourami sausage and yeast mold with Kecombrang flower concentrate edible coating on the treatment of the concentration of added concentrate is shown in Figure 4.

**Figure 4.** The average total value of mold and yeast (log CFU / g) of gourami sausages with Kecombrang flower edible coating on the treatment of the concentration of addition concentrate during storage.

Based on these results (Figure 4) obtained the total average value of mold and yeast which is quite low in fish sausages with the application of edible coating of Kecombrang flower concentrate at a concentration of 1%, 2%, 3% and 4%, respectively in consecutive 4.68, 4.62, 4.46 and 4.29 log CFU / g. The active compound found in the Kecombrang flower is a phenolic component. [23] states that tannins are phenolic polymers which are usually used as a freshener, have antimicrobial properties and are toxic to yeast, bacteria, and mold. Triterpenoids according to [24] are terpenoids which have antifungal, insecticidal, antibacterial and antiviral properties.
The total mean value of mold and yeast in Figure 4 shows that the value is lower along with an increase in the concentration of the addition of concentrate which is a concentration of up to 4%. The lowest total mold and yeast values were obtained at 4% concentrate concentration of 4.29 log CFU / gram, while the highest average total fungi and yeast values were obtained at 1% concentrate. Based on this, it is shown that the use of higher concentrations of concentrate addition into edible coatings for gourami sausage applications provides stronger inhibitory activity compared to the addition of fewer concentrates to the growth of mold and yeast.

The results in Figure 4 can be concluded that the concentration of concentrate addition of 4% gives a higher inhibitory activity in suppressing the total growth of mold and yeast so that the average total value of mold and yeast is lower during low-temperature storage.

4. Conclusion

The edible coating application of Kecombrang flower concentrate with a concentration of 4% on edible coating showed considerable inhibitory power on microbial growth in gourami sausages. This result was indicated by the average total values of microbes, bacteria and molds and yeast respectively 5.13, 5.18, 4.29 log CFU / g. In addition, the use of concentrated addition concentration of 4% gave a higher inhibition zone diameter in the four-test bacteria *Escherichia coli*, *P. aeruginosa*, *S. aureus*, and *B. cereus* which were 9.80; 9.07; 7.06; 1.33 mm, respectively.

References

[1] BSN 2013 SNI Fish Sausage (Jakarta :BSN) SNI 7755:2013
[2] Krochta J M, Baldwin E A and Nisperos-Carriero M O 1994 *Edible Coatings and Films to Improve Food Quality* (Lancaster : Technomic Publishing)
[3] Santoso B, Saputra D dan Pambayun R 2004 *Journal of Technology and Food Industry* 15 239–244
[4] Naufal R and Rukmini H S 2013 *Animal Production* 15 8–14
[5] Pahlevi Y R 2011 *Application of Edible Coating Chitosan-Teak Leaf Extract in Beef Sausage to Inhibit Microbiological and Oxidative Damage* (Surakarta : Essay Sebelas Maret University)
[6] Naufal R 2013 *PATPI National Seminar Paper 2013* (Purwokerto : Jenderal Sudirman University) ISBN : 978-602-9030-49-5
[7] Hechard Y, Chantal J, Francois L, Raymond J, Yves C and Marie H R 1992 *App Environment Mich.* 41 71–76
[8] Fardiaz S 1993 *Food Microbiology Analysis* (Jakarta : PT. Raja Grafindo Persada)
[9] Naufal R and Rukmini H S 2012 *Natural Preservatives in Food Products*. (Purwokerto : UPT. Printing and Publishing Jenderal Sudirman University)
[10] Morales G, Sierra P, Mancilla, Paredes A, Loyola L A, Gallardo O and Borquez J 2003 *J. Chile Chem.* 48
[11] Naufal R and Rukmini H S 2018 *IOP Conference Series: Earth and Environmental Science* 102 012035
[12] Pelczar M J and Chan E C S 2005 *Fundamentals of Microbiology 2* (Jakarta : University of Indonesia Press)
[13] Hudaya A 2010 *Antioxidant and antibacterial tests of Kecombrang flower water extract (Etingera elatior) as functional food for Staphylococcus aureus and Escherichia coli* (Jakarta : Essay Syarif Hidayatullah State Islamic University)
[14] Naufal R 2017 *Kecombrang: Antimicrobials and their Utilization as Food Preservatives* (Purwokerto : Jenderal Sudirman University)
[15] Ajizah A 2004 *Bioscientiae* 1 8–31
[16] Naufal R, Jenie B S L, Kusnadhar F and Rukmini H S 2005 *Journal of Food Technology & Industry* 12 119–125
[17] BSN 2009 Maximum Limit of Microbial Contamination in Food Materials (Jakarta : BSN) SNI 7388:2009
[18] Naufalin R 2005 *Study of the antimicrobial properties of Kecombrang (Nicolaia speciosa Horan) flower extracts against various pathogenic microbes and food destroyers* (Bogor : Disertasi Institut Pertanian Bogor)

[19] Fardiaz S 1989 *Food Microbiology* (Bogor : Center for Intermediary Food and Nutrition Agricultural Bogor University)

[20] Naufalin R, Rukmini H S and Erminawati 2010 *Proceedings of the 2010 National Seminar The Role of Regional Superior Products Food Safety in Supporting Food Security and Pressing the Rate of Inflation* ISBN 978-602-98156-0-3 pp 86-100

[21] Naufalin R and Rukmini H S 2017 *International Food Research Journal* 24 379–385

[22] Fardiaz S 1992 *Food Microbiology I* (Jakarta : PT Gramedia Pustaka Utama)

[23] Assani S 1994 *Medical Microbiology Revised Edition* (Jakarta : Binarupa Aksara)

[24] Robinson 1995 *High content of organic plant compounds* Translated by Prof. Dr. Kosasih Padmawinata (Bandung)