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## The effect of edible coating contained Kecombrang leaves concentrate on gourami fish fillet quality

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**Abstract.** Gourami fillet is fishery product that perishable and has short shelf life. To prolongs its shelf life, the application of edible coating could be performed. Edible coating is a thin layer that covers food products and serves as a protector against mass transfer (such as moisture, oxygen, light, lipids, and solutes) and as an additive carrier. Kecombrang contains bioactive compounds that act as antimicrobials. This study was aimed to know the effect of concentration addition of Kecombrang leaves concentrate to gourami fillet quality during storage at  $\pm 4^{\circ}\text{C}$ . The experimental design used was Randomized Complete Design. The treatments were concentration of added concentrate of Kecombrang leaves (K) at amount of 1% (K1), 2% (K2), 3% (K3), and 4% (K4). The results showed that the application of edible coating contained Kecombrang leaves concentrates at concentration of 4% on gourami fillets showed best results according to color intensity, pH, free fatty acid content, and the total plate count of bacteria.

### 1. Introduction

Gourami is freshwater fish which consumed by many Indonesian people. According to previous report [1], Gourami production in 2015 reached 22,635 tons. Gourami is one of commodities widely developed by farmers due to its high demand market, easy maintenance and relatively stable in prices. Some regions in Indonesia become a center of gourami production, such as Purwokerto Central Java [2].

Gourami is processed into fillet, aimed to make it easy to the consumer Previously stated [3], fish fillets is a processed product of fish from fresh/raw fish that are subjected to weeding, slicing, with or without skin disposal, tidying, washing, with or without freezing, fresh or frozen storage.

The shelf life of gourami fillets is short and nutritional content will decrease physically, chemically and microbiologically. Thus, it is needed to minimize the occurrence of nutritional decline which will reduce the shelf life of gourami fillets. The shelf life of gourami fillets can be prolonged by adding preservatives. One material that can be used as a preservative is Kecombrang. According to previous study [4], Kecombrang is known as a natural preservative because it has bioactive components such as triterpenoids, steroids, phenolics, flavonoids, alkaloids, and glycosides which act as antimicrobial.

Other preservation methods are using edible coating. According to [5], edible coatings include biodegradable packaging which is a new technology introduced in food processing which plays a role in obtaining products with a longer shelf life. Edible coatings produced with a combination of



hydrocolloid and lipids such as *Carboxyl Methyl Cellulose* (CMC) and glycerol. Thus, the application of edible coating using combination between edible coating and Kecombrang leaf concentrate is expected to prolong shelf life of gourami fillets. For that, this study was to determine the effect of the addition of Kecombrang leaf concentrate to the quality of gourami fillets during storage.

## 2. Research methodology

### 2.1. Materials and experimental design

The materials used were gourami from Purwokerto, Kecombrang leaf obtained from Kotayasa-Baturaden Village and additional ingredients such as ethanol 70% and 96%, *carboxyl methyl cellulose* (CMC), glycerol, vaseline, nitrogen, baking soda, NaOH 0,1 N, phenolphthalein indicator (PP), formaldehyd 37%, aquades, kalium oksalat, *buffer* pH 7 and *plate count agar* (PCA). The experimental design used was a Completely Randomized Design (CRD), the concentration of Kecombrang leaf concentrate addition consist four levels that are 1%, 2%, 3%, and 4%. All treatment level was store at  $\pm 4^{\circ}\text{C}$  for 6 days.

### 2.2. Preparation gourami fish fillet

The first step of making fish fillet preparation is that fish with an average weight of  $\pm 550\text{-}650$  grams are selected. Knives and cutting boards are cleaned with distilled water then separate the head and bones of fish [6].

### 2.3. Kecombrang leaf concentrate

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  $37^{\circ}\text{C}$  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 (extract) is obtained. The extract is separated from the solvent by evaporation in the evaporator. The solvent is evaporated at a maximum temperature of  $50^{\circ}\text{C}$  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 then analyzed.

### 2.4. Edible coating concentrate Kecombrang

A total of 100 ml of distilled water was added to the CMC stabilizer with a concentration of 0.5% and then added 1% glycerol then homogenized using a hand blender for  $\pm 1.5$  minutes. Then the edible coating solution was transferred to a beaker glass to be heated on a magnetic stirrer hot plate until the temperature reached  $70^{\circ}\text{C}$ . After that, edible coating added Kecombrang concentrate according to treatment and homogenized again using hand blender for  $\pm 2$  minutes [7].

### 2.5. Edible coating with spraying fillet of gourami

Spraying with *hand sprayer*, amount 50-100ml *edible coating* solvent into *sprayer*. So, *edible coating* sprayed to *fillet* gourami. So, *fillet* guramy is air dried [8].

### 2.6. Storage of gourami fillet

Fish *fillets* weighing 30g are packed using PP plastic and then stored in cold temperatures ( $4\pm 1^{\circ}\text{C}$ ) for 6 days [9] and [10].

### 2.7. Hardness analysis

Hardness test of gourami fillets using a penetrometer, the test was carried out twice as many replications at one midpoint to obtain the value of hardness ( $\text{kg} / \text{cm}^2$ ) [11].

### 2.8. Color intensity analysis

The color of fish fillets was tested using the Color Reader CR-10 tool. The color reading system used to evaluate the color of fish fillets is lightness ( $L^*$ ), redness / redness level ( $a^*$ ), and yellowness / yellowish level ( $b^*$ ) [12]. There are three areas measured, namely the upper, middle and lower areas. Fish fillet samples were tested three times and then averaged. Color intensity ( $C$ ) is calculated by the following formula:  $C = \sqrt{a^2 + b^2}$

### 2.9. pH analysis

PH measurements are carried out using a digital pH meter which has been calibrated first with pH 4 (acetate buffer) and pH 7 (phosphate buffer). A total of 5 grams of sample were mashed, plus 50 mL of distilled water, then stirred using a magnetic stirrer for 10 minutes, then filtered. Then the electrode is dipped in the sample filtrate for a while to obtain a measured pH. Electrodes are removed and rinsed with aquades [13].

### 2.10. Free fatty acid analysis

The sample is weighed as much as  $7 \pm 0.2$  grams in Erlenmeyer, the ingredients must be stirred evenly and are in a liquid state at the time the sample is taken. Then add 12.5 mL of hot neutral alcohol and 0.5 mL of PP indicator. The sample was titrated with a standardized 0.1 N NaOH solution until the pink color was reached and did not disappear for 30 seconds. Free fatty acids (FFA) are expressed as% [14]. Free fatty acid levels are calculated by the formula:

$$\%FFA = \frac{ml\ NaOH \times N \times MW\ fatty\ acid}{sample\ weight \times 1000} \times 100\%$$

### 2.11. Formol value analysis

Soluble protein levels using formol titration method. The first step is to weigh as much as 2 grams of sample that has been mashed with porcelain plates and dissolved in 20 mL of distilled water and filtered the filtrate. The filtrate was put into a 125 mL erlenmeyer, then 20 mL of distilled water were added; 0.4 mL of saturated K-oxalate (K-oxalate: aquades = 1: 3); and 1 mL of phenolphthalein 1%, then the mixture was allowed to stand for 2 minutes. The sample solution was titrated with 0.1 N NaOH solution to form a pink color. Then 2 mL of 40% formaldehyde solution was added and titrated again with NaOH until the standard color was reached again (the titration was recorded as titration II).

20 mL of aquades put into 125 mL Erlenmeyer, then 0.4 mL of K-oxalate, 1 mL of phenolphthalein 1%, and 2 mL of formaldehyde 40% were added. Then it was titrated using NaOH solution until it was pink (the titration was recorded as blank titration) [14]. Formol values are calculated by the formula:

$$\%N = \frac{formol\ titration \times N\ NaOH \times 14,008 \times FP}{sample\ weight\ (g) \times FP}$$

### 2.12. TPC analysis (Total Plate Count)

A total of 1 g of the sample was diluted with 9 ml of physiological saline solution (0.85% NaCl) which had been sterilized. This dilution is calculated as a 10-1 dilution. The dilution is then carried out by dissolving 1 ml of the 10-1 dilution solution with 9 ml of physiological saline solution and counting as a 10-2 dilution and so on. As much as 1 ml of sample from dilution, pipette and put into sterile petri dishes, then poured  $\pm$  15 ml of sterile PCA media and evenly shaken or like number 8 on the table. After the media solidified, the cup is wrapped in paper and then incubated in a reverse position at 36 °C – 37 °C for 48 hours. The total number of microbes is calculated and expressed in the CFU / g log [15]. microbial calculation :

$$Colony\ number\ (log\ CFU/g) = log\ colony\ number \times \frac{1}{FP}$$

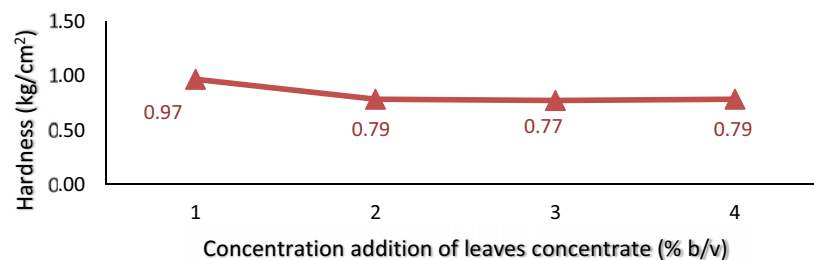
### 2.13. Statistical analysis

Data were analysed using analysis of variance (F test) at the level of 5%, if significant effect were found then Duncan's Multiple Range Test at the level of 5% was performed.

## 3. Result and discussion

### 3.1. Hardness of edible coating gourami fish fillet

The average value of hardness of gourami fillets with edible coating application adding Kecombrang leaf concentrate during storage can be seen in Figure 1.

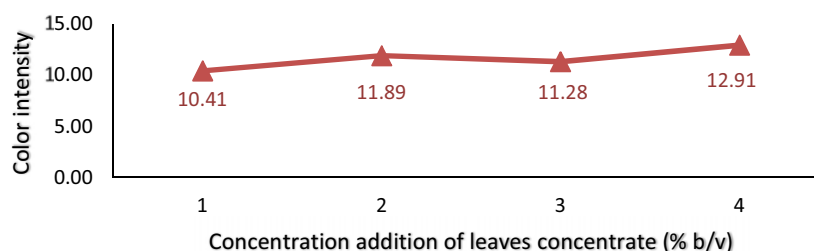


**Figure 1.** Average value of hardness of gourami fillets with edible coating application adding Kecombrang leaf concentrate during storage

Figure 1 shows that the average hardness value of gourami fillets with the addition of Kecombrang leaf concentrate was relatively stable. The leaf concentrate can reduce the decrease of hardness in gourami fillets. The content of bioactive compounds in the leaf concentrate is thought to inhibit the protease action which is thought to be optimal in its inhibition. This is agree with [16] that polyphenol compounds can bind to other proteins or polymers such as cellulose, hemicellulose and pectin to form a stable complex bond. Thus the action of the protease enzyme (trypsin and khimotripsin) and cellulase can be inhibited.

### 3.2. Analysis of color intensity edible coating gourami fish

The color intensity value is calculated by the formula, the value of a is the red degree while the value of b is yellow. The average value of the color intensity of carp fillets with the addition of edible coating application Kecombrang leaf concentrate during storage can be seen in Figure 2.



**Figure 2.** Average value of the color intensity of gourami fillets with edible coating application adding Kecombrang leaf concentrate during storage

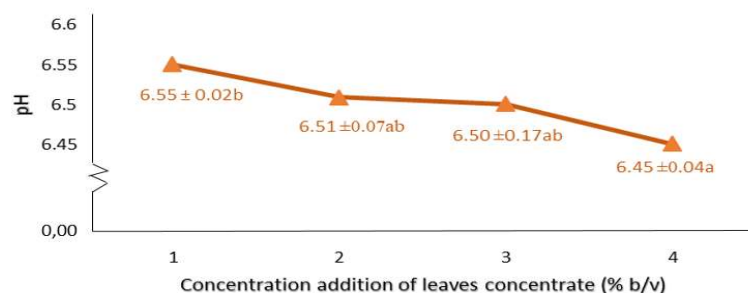
Based on Figure 2, the concentration of the addition of Kecombrang leaf concentrate 4% (12.91) has the highest color intensity value on gourami fillets. This shows that the higher the concentration of the addition of Kecombrang leaf concentrate used, the higher the intensity value of the color on the fish fillet. The high-intensity value shows that Kecombrang concentrate can reduce color changes in

gourami fillets. It is suspected that Kecombrang leaf concentrate has compounds that can inhibit the color change of gourami fillets.

Color changes can occur due to oxidation of pigments contained in fish. Quality degradation due to oxidation factors can be accelerated in the presence of oxygen, water, light, and temperature [17]. Kecombrang has antioxidant content which is thought to be able to inhibit the rate of oxidation in gourami fillets, namely flavonoids. Kecombrang leaves contain alkaloids, saponins, phenolics, flavonoids, triterpenoids, steroids, tannins [4] and essential oils [18] which are thought to have the potential for natural antioxidants.

### 3.3. PH analysis of gourami fillets with edible coating

The average pH value of gourami fillets with edible coating application addition of Kecombrang leaf concentrate during storage can be seen in Figure 3. During storage, fish fillets can change pH. The change in pH occurs due to chemical and microbiological processes. According to [19] and [20], changes in pH in fish fillets indicate changes that occur during storage, both chemical changes and the activity of microorganisms contained in them.



**Figure 3.** Average value of gourami fillet pH with edible coating application addition of Kecombrang leaf concentrate during storage

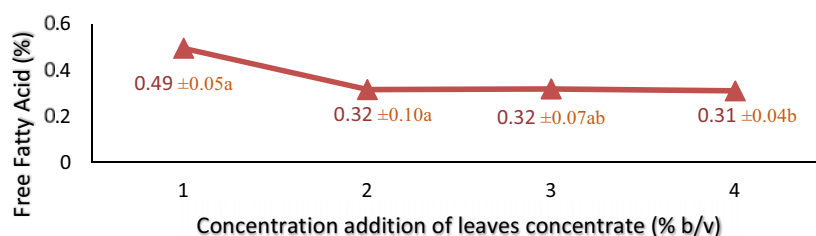
Based on Figure 3, the higher the concentration of the addition of Kecombrang leaves, the lower the pH value of the gourami fillets. It is suspected that the higher the concentration of the addition of Kecombrang leaf concentrate can reduce the pH increase in fish fillets. Inhibition of increasing pH is expected to maintain the quality of gourami fillets.

According to [19] and [20], changes in pH in fish fillets indicate changes that occur during storage, both chemical changes and the activity of microorganisms contained in them. Fish that are not fresh usually have a high pH (base) because of the emergence of alkaline compounds such as ammonia, trimethylamine, and other volatile compounds by spoilage bacteria.

Decreasing the pH value of fish fillets along with an increase in the concentration of addition of Kecombrang leaf concentrate is thought to be influenced by the activity of glycogen-breaking enzymes that produce lactic acid, thereby reducing the acidity of fish fillets [21]. This event is likely to occur in the presence of lactic acid bacteria that come from food and the environment. [22] and [23] added that the decrease in pH was also caused by the accumulation of lactic acid resulting from the glycolysis process of fish that have died naturally. The content of glycogen in the fish's body also affects the pH reduction.

### 3.4. Free fatty acid (FFA) analysis guramy fish fillet with edible coating

The average value of free fatty acid levels of edible coating application with the addition of Kecombrang leaf concentrate to gourami fillets during storage can be seen in Figure 4.



**Figure 4.** Average value of free fatty acids Gourami fillets with edible coating application adding Kecombrang leaf concentrate during storage

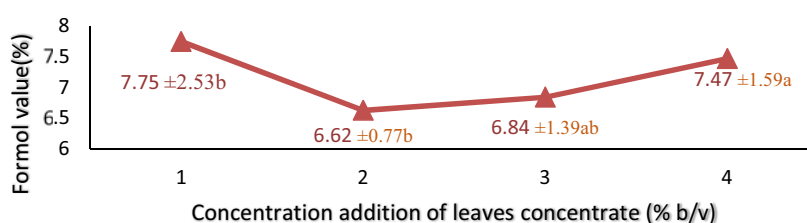
Gourami fillets can experience chemical changes, one of them is free fatty acid levels. Figure 4 shows the higher concentration of the addition of Kecombrang leaf concentrate, the lower the level of free fatty acids in gourami fillets. It is suspected that the addition of Kecombrang leaf concentrate can reduce the increase of free fatty acid levels in Gourami fillets during storage.

Addition of Kecombrang leaf concentrate with a concentration of 4% showed the lowest free fatty acid level. This shows that the concentration of 4% is better able to inhibit oxidation reactions in fat of gourami fillet because of its higher antioxidant content. The use of antioxidants appears as an effective method for controlling damage in oil and food [24].

Meanwhile, the highest levels of fatty acids were the addition of 1% concentration of Kecombrang leaf at 0.49%. Even so, the level of free fatty acids is still acceptable because it does not exceed the standard limit. This is agreed with [25] in [26] that the quality of raw fish fat can still be accepted with a limit of 5% of the FFA value.

### 3.5. Analysis of formol value of gourami fillets with edible coating

The average value of formol edible coating application with the addition of Kecombrang leaf concentrate to the gourami fillets during storage can be seen in Figure 5.



**Figure 5.** Average value of gourami fillet formol with edible coating application adding Kecombrang leaf concentrate during storage

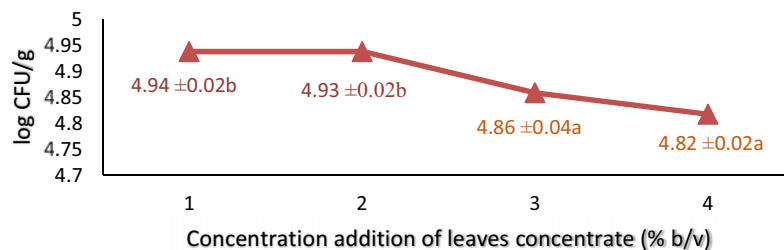
Based on Figure 5, the addition of Kecombrang leaf concentrate can reduce the increase in formol value in fish fillets. Antioxidant levels in the leaf can inhibit the action of protease enzymes from gourami and microbial fillets. According to [27], Kecombrang ethanol and methanol extracts contained antioxidant activity, where extracts from leaves showed the highest activity. In addition, Kecombrang leaves contain tannin compounds [4]. The presence of tannin able to inhibit the action of the protease enzyme (trypsin and chymotrypsin) and cellulase [16].

Figure 5 shows that the higher the concentration of addition of Kecombrang leaf concentrate, the lower the formol value in fish fillets. It is suspected that the higher the concentration of the Kecombrang leaf concentrate will be more optimal in inhibiting the protein overhaul of fish fillets. According to [21], protein overhaul by enzymes in fillets will affect the function of proteins as the

binding of body fluids decreases and fluid will come out of the tissues of [11] adding that protein changes in fish meat can occur due to enzymes and bacteria.

### 3.6. Total Plate Count (TPC) gourami fish fillet with edible coating

The average value of TPC edible coating application with the addition of Kecombrang leaf concentrate to the gourami fillets during storage can be seen in Figure 6.



**Figure 6.** average value of gourami fish TPC *fillet* with *edible coating* addition Kecombrang leaf concentrate during storage

Based on Figure 6, the total microbes in gourami fillets were lower along with the high concentration of Kecombrang leaf concentrate used. It is suspected that the higher the concentration of addition of concentrated stems and leaves of Kecombrang, the greater the effectiveness of antimicrobial compounds. This is in line with [28] which states that the higher the concentration, the more content of antibacterial active ingredients. According to [29], the effectiveness of an antimicrobial agent in inhibiting growth depends on the microbial properties of the test, concentration and length of contact time.

Addition of Kecombrang leaf concentrate with a concentration of 4% has the lowest total microbial value in gourami fillets. While the highest total microbes are the addition of Kecombrang leaf concentrate with a concentration of 1%. Even so, the total microbes in gourami fillets during 6 days of storage are still below standard. Based on [30], the limit on the number of microbes that can still be permitted in fishery products is a maximum of  $5.0 \times 10^5$  CFU / g or 5.699 log CFU / g.

## 4. Conclusion

Edible coating application with the addition of Kecombrang leaf concentrate on the gourami fillets showed the best results, they are concentration of 4% in terms of color intensity 12.91; pH 6.45; free fatty acid levels of 0.31%; and TPC 4.82 log CFU / g.

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