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## Application of Concentrates Flower Kecombrang on Edible Coating as Antioxidant to Suppress Damage on Gourami Sausage

To cite this article: R Naufalin *et al* 2019 *IOP Conf. Ser.: Earth Environ. Sci.* **255** 012040

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# Application of Concentrates Flower Kecombrang on Edible Coating as Antioxidant to Suppress Damage on Gourami Sausage

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**Abstract.** Gourami is popular in the community and can develop to gourami sausage to increase economic value. However, foods based on fish are easily damage because microorganism and oxidative damage that was caused by fatty acids content in fish is high. Preservation fatty foods are by giving an antioxidant compound. Kecombrang is contained bioactive compounds as antioxidant and antimicrobial; it can maintain the quality and extend the shelf life of various foods product. This research purpose is to know the effect of concentration of concentrates addition on the edible coating to oxidative damage of gourami sausage during storage. The design used Completely Randomized Design. The factors were concentration concentrates addition on the edible coating, consist of 1%, 2%, 3%, and 4%. The variables are chemical characteristic of edible coating (total phenolic content, total flavonoids content, antioxidant activity), and the quality of gourami sausage during storage (free fatty acid levels (FFA) and malondialdehyd levels (MDA)). The results showed that addition of the concentrates of flower kecombrang was able to prevent oxidative damage on gourami sausage during storage. Concentrate on flower kecombrang causes the FFA and MDA levels are relatively stable. Concentration 4% of concentrate in edible coating had total phenolic content, total flavonoid content, and antioxidant activity higher than concentration of 1%, 2%, and 3%. Concentration 2% of concentrate in edible coating is already able to suppress oxidative damage on gourami sausage by lowering FFA and MDA levels, that scores are 0,812% FFA and 0,155  $\mu\text{mol}$  MDA/g sample with the addition concentrate of fruit kecombrang, and 0,792% FFA and 0,143  $\mu\text{mol}$  MDA/g sample with the addition of flower kecombrang.

## 1. INTRODUCTION

Sausages are food products obtained from pulverized meat mixed with spices and wrapped with sausage casings [1]. Gurami is one type of fish that is very popular in society, so that it has the potential to be further developed into various processed food products, such as gourami sausages, and can increase its economic value. However, fish-based food processing is easily damaged, such as decay caused by microorganisms and rancidity due to oxidative damage to fat. This is because there are more types of fatty acids in fish meat than fattyacids found in land animal meat [2]. The way to preserve fatty foods that are commonly used is by giving antioxidant compounds.

According to [3], antioxidants are compounds that can inhibit oxidation reactions, by binding to free radicals and highly reactive molecules. Antioxidant compounds that are often used in the food processing industry are synthetic antioxidant compounds, such as butylated hydroxyanisole (BHA), butylated hydroxyl toluene (BHT), propyl galate (PG), and tert-butyl hydroquinone (TBHQ). The use of synthetic antioxidant compounds is increasingly feared, because it can be a carcinogenic agent that causes cancer [4] and has a toxic effect [5]. This condition encourages the use of natural antioxidants that can extend the shelf life of processed foods but are safe for human



consumption.

Kecombrang (*Nicolaia speciosa* Horan) is a spice plant that has the potential to be used as a natural food preservative because it is known to contain antimicrobial compounds and antioxidants that can extend the shelf life of various processed foods [6]; [7]. According to [8]; [9], Kecombrang contains active ingredient components consisting of alkaloids, flavonoids, polyphenols, steroids, saponins, and essential oils. Kecombrang application as a natural food preservative can be in the form of powder / flour or extract / concentrate. In this study, kecombrang flowers will be processed in the form of concentrates then applied in edible coating formulas as gourami sausage coatings. The addition of bioactive compounds in edible coatings / films will create packaging that is antimicrobial and antioxidant, thus slowing the occurrence of microbiological damage and oxidative damage to fat. The antimicrobial combination with film packaging to control microbial growth in food can extend shelf life and improve food quality [10].

The purpose of this study was 1) to determine the effect of adding kecombrang flower concentrate in edible coating on oxidative damage to gourami sausage products during storage; 2) comparing the concentration of addition of kecombrang flower concentrate in edible coating to the quality of gourami sausages during storage.

## **2. RESEARCH METHODOLOGY**

### **2.1. Material and Design Trial**

The materials used in this study consisted of kecombrang flower concentrate, gourami fillets, tapioca flour, flour, ice, vegetable oil, carboxymethyl cellulose (CMC), glycerin, sausage sleeves, aquades, tannic acid, Folin-Ciocalteu reagent, sodium bicarbonate (NaHCO<sub>3</sub>), 96% ethanol, phenolphthalein (PP), 2,2-diphenyl-1-picrylhydrazyl (DPPH) indicator "Sigma Aldrich", "Merck" tiobarbituric acid (TBA), "Merck" trichloroacetic acid (TCA), acid chloride (HCl), isobutanol, 0.1N sodium hydroxide (NaOH) solution, quercetin, glacial acetic acid, aluminum chloride (AlCl<sub>3</sub>), and nitrogen gas (N<sub>2</sub>).

The experimental design used was a completely randomized design (CRD) with 4 treatment combinations and repeated 3 times. The factors studied were the concentration of the addition of kecombrang flower concentrate in edible coating consisting of four levels, namely 1%, 2%, 3%, and 4%. Observation of the quality of gourami sausages was carried out for 12 days of storage and carried out measurements on each storage period of 0, 4, 8, and 12 days. The variables observed were: 1) chemical characteristics of edible coating, consisting of analysis of total phenol levels, levels total flavonoids, and antioxidant activity; and 2) the quality of gourami sausages, consisting of analysis of free fatty acid (FFA) and malondialdehyde (MDA) levels.

### **2.2. Gourami Fish Sausage**

The first step of making gourami sausages, namely weeding fish to obtain gourami fillets. Then wash with clean running water. Grinding 100 g of gourami fillets which are obtained are ground with a meat grinder (chopper) twice. Milled meat weighed as much as 100 g, added 22% tapioca, 23% flour, 8% ice water and 3% (b / b) vegetable oil from the total meat of carp. 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 as not to form air bubbles. After the sleeve is filled with solid dough weighing ± 25 g / sleeve and ± 7 cm long, the ends are tied with a rope. Cooking fish sausages by steaming at 100 ° C for 20 minutes. Once cooked, the sausage is cooled at room temperature for ± 30 minutes [11].

### **2.3. Kecombrang Flower Concentrate**

The concentrate obtained from the extraction of kecombrang flower powder [12] was carried out by maceration method at 37°C, with a rotation speed of 150 rpm for 2 hours each level. Kecombrang flower powder is extracted with technical ethanol 96% (1: 4 b / v), then the residue is extracted again with technical ethanol 96% (1: 4 b / v). After that filtering is done using Whatman No. disc paper. 1 to obtain the filtrate (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 with nitrogen gas. The concentrate obtained was used as a sample to be added to the manufacture of edible coating which was then analysed [13];[14].

## **2.4. Edible Coating**

Edible coating solution was made by adding 100 ml of distilled water with 0.5% CMC and 1% glycerol, then homogenized using a hand blender for  $\pm$  1 minute. Then the edible coating solution was transferred into a beaker glass to be heated on top of the hot plate magnetic stirrer until the temperature reached 70 ° C. After that, edible coating was added to the kecombrang flower concentrate and homogenized again using a hand blender for  $\pm$  2 minutes[15].

## **2.5. Analysis of Chemical Characteristics of Edible Coating**

### **2.5.1. Analysis of total phenol levels**

Analysis of total phenol content was carried out on edible coating solution samples which had added kecombrang flower concentrate to several concentrations of concentrated additions, namely 1%, 2%, 3%, and 4%. A total of 100 mg of sample was added 4 ml of 70% ethanol and then in the shaker for 2 hours at a speed of 200 rpm. 0.4 ml of supernatant was added 1.5 ml of Folin-Ciocalteu reagent and 1.5 ml of sodium bicarbonate (NaHCO<sub>3</sub>) 0.556 M, then vortexed and incubated for 90 minutes in the dark room. Then absorbance was measured at a wavelength of 725 nm. The standard curve is made by using tannic acid as a stock solution in the concentration range of 0.02–0.1 mg / ml then the same treatment is carried out as in the edible coating sample. The results of the analysis are expressed as equivalent to the standard tannic acid (Tannic Acid Equivalent / TAE). [16];[17].

### **2.5.2. Analysis of total flavonoid levels**

Analysis of total flavonoid levels was carried out on edible coating samples which had added kecombrang flower concentrates to several concentrations of adding concentrates, namely 1%, 2%, 3%, and 4%. One ml of edible coating sample was dissolved with 96% ethanol to 10 ml. The sample was transferred into a 25 ml volumetric flask and then added with 1 ml of AlCl<sub>3</sub> solution (2 g of AlCl<sub>3</sub> was dissolved with 100 ml of 5% glacial acetic acid) and a 5% glacial acetic acid solution was added to the flask. The sample was vortexed and incubated for 60 minutes, then measured the absorbance at a wavelength of 370 nm. The standard curve is made by using quercetin as a stock solution in the concentration range of 4-20  $\mu$ g / ml then the same treatment is done as in the edible coating sample. The analysis results are expressed as the equivalent of standard quercetin (Quercetin Equivalent / QE)[18].

### 2.5.3. Analysis of antioxidant activity

Analysis of antioxidant activity was carried out on edible coating samples which had added kecombrang flower concentrates to several concentrations of adding concentrates (1%, 2%, 3%, and 4%). One ml of sample was dissolved with distilled water up to 10 ml then filtered using 500 mesh Monyl cloth. A total of 0.5 ml of the diluted sample was added with 3.5 ml of 0.1 mM DPPH solution then vortexed and incubated for 60 minutes in the dark room. Then absorbance was measured at a wavelength of 515 nm. The blank is made by replacing the sample using distilled water at the same volume. The percentage of DPPH free radical capture is expressed by the percentage of inhibition (inhibition). [19];[20].

$$\% \text{ Inhibition} = \frac{Ab - As}{Ab} \times 100 \%$$

Note. : Ab = Absorbing blank

As = Absorbing sample

## 2.6. Quality Analysis of Gouramy Sausages

### 2.6.1. Analysis of free fatty acid levels (FFA)

Analysis of levels of free fatty acids (FFA) was carried out by titration method in samples of gouramy sausages that had been applied with edible coating concentrated kecombrang at several storage times (0, 4, 8, and 12 days). The mashed sausage sample was taken as much as  $\pm 7$  g, added with 12.5 ml of hot neutral alcohol and 0.5 ml of PP indicator. Then titrate with a standardized 0.1 N NaOH solution until a pink color is formed that does not disappear for 30 seconds. The free fatty acids contained in the sample are expressed as% FFA.[21].

$$\% \text{ FFA} = \frac{\text{ml NaOH} \times \text{N NaOH} \times \text{BM Asam Lemak}}{\text{Berat sampel} \times 1000} \times 100 \%$$

### 2.6.2. Analysis of malondialdehyde levels(MDA)

Analysis of the levels of malondialdehyde (MDA) was carried out on samples in the form of gouramy sausages that have been applied to edible coating concentrated kecombrang at several storage times (0, 4, 8, and 12 days). TBA reagent was made beforehand by adding 0.375 g of tiobarbituric acid (TBA) with 15 ml trichloroacetic acid (TCA) and 25 ml of 37% hydrochloric acid (HCl) and then dissolving it with distilled water up to 100 ml. Then as much as  $\pm 0.1$  g of the mashed sausage sample was added with 1 ml of the TBA reagent that had been made. The sample is heated in boiling water for 15 minutes and immediately cooled to running water. Then the sample was added with 1 ml of isobutanol and 3 ml of 96% ethanol and then vortexed and centrifuged at 4000 rpm for 5 minutes. The supernatant obtained was measured for its absorbance at a wavelength of 535 nm. The MDA value is expressed in  $\mu\text{mol MDA} / \text{g sample}$ [22].

## 2.7. Statistical analysis

Data were analyzed using variance analysis (F test) at the level of 5%, and if it was significant, continued with Duncan's Multiple Range Test at the level of 5%.

## 3. RESULT AND DISCUSSION

### 3.1. Chemical Characteristics of Edible Coating

#### 3.1.1. Analysis of total phenol levels

Edible coating which has been added kecombrang flower concentrate in several concentrations of adding concentrates (1%, 2%, 3%, and 4% (b / v)) was carried out to test the total levels of phenol compounds contained in each sample. The standard solution used in the analysis is tannic acid, so the results obtained are expressed as the equivalent standard of tannic acid (Tannic Acid Equivalent / TAE). The choice of standard depends on the majority form of phenol compounds contained in the test material. Tannic acid is a standard for measuring samples of food and beverages that are thought to contain phenol compounds [10].

The results of the variance analysis on the treatment of variations in concentrations of adding concentrates (K) in this study showed a very significant effect on the total phenol level in edible coating. The average value of the total phenol content of edible coating with the treatment of the concentration concentration addition concentration is presented in Figure 1.

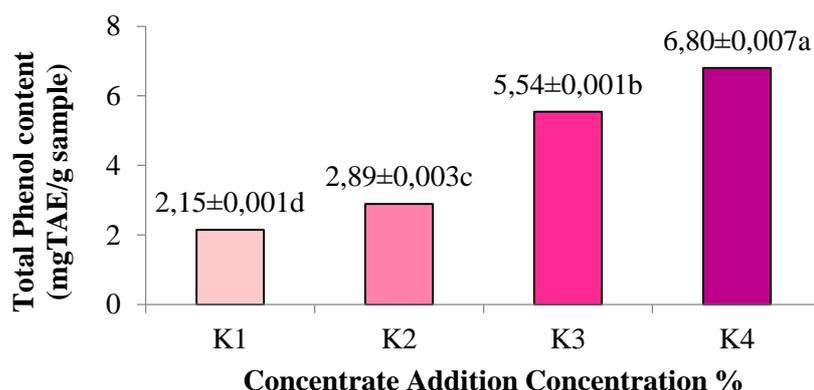


Figure 1. Total phenol content of edible coating with variations in the concentration of addition of kecombrang flower concentrate.

The results of the analysis of the total phenol content of edible coating samples with the addition of kecombrang flower concentrates showed that there was a significant effect on each concentration of concentrate addition. The results of the analysis showed the highest average value of total phenol in the edible coating sample with the addition of the kecombrang flower concentrate at a concentration of 4% (K4), which was equal to 6.8 mgTAE / g sample.

Research on kecombrang plants has been done before and it is known that in the kecombrang there are bioactive compounds, including alkaloids, flavonoids, polyphenols, triterpenoids, steroids, saponins, and essential oils that are spread throughout the plant from the rhizome to the fruit. Phenol compounds are substances that have aromatic rings with one or more hydroxyl groups so that they are soluble in polar solvents [6]. A combination of several phenol groups is called polyphenols. The content of phenol compounds allows the ingredients they contain to act as antioxidants. This is because phenol can act as a reducing agent by donating H<sup>+</sup> ions from its hydroxyl group. The ability to donate H<sup>+</sup> ions is what makes phenol compounds can act

as antioxidants.

The results of the analysis in this study showed an increase in the average value of total phenol levels along with the increased concentration of concentrated additions in edible coating. This is because the higher the concentration of concentrated addition, the higher the amount of phenol compounds accumulated. The red color of the kecombrang flower concentrate can also be an indicator of the content of phenol compounds. Increasing the concentration of the addition of concentrates in edible coating produces an increasingly thick red color, thus proving the higher the phenol content.

According to [23], in kecombrang plants contain chemical components which are phenol compounds in the form of alkaloids, flavonoids, polyphenols, steroids, saponins, and essential oils. The amount of content of phenol compounds in soybean is the cause of high levels of total phenol in edible coating samples which are added kecombrang flower concentrates.

### 3.1.2. Analysis of total flavonoid levels

Edible coating samples that have been added to the kecombrang flower concentrate at several concentrations (1%, 2%, 3%, and 4%) were analyzed for the total levels of flavonoids. Determination of total levels of flavonoids using quercetin as a standard solution. The results of measurements of total flavonoid levels were expressed as the equivalent of standard quercetin (Quercetin Equivalent / QE).

The results of the variance analysis on the treatment of variations in concentrations of adding concentrates (K) in this study showed a very significant effect on the total levels of flavonoids in edible coating. The average value of the total level of edible coating flavonoids with variations in the concentration of concentrated addition is presented in Figure 2.

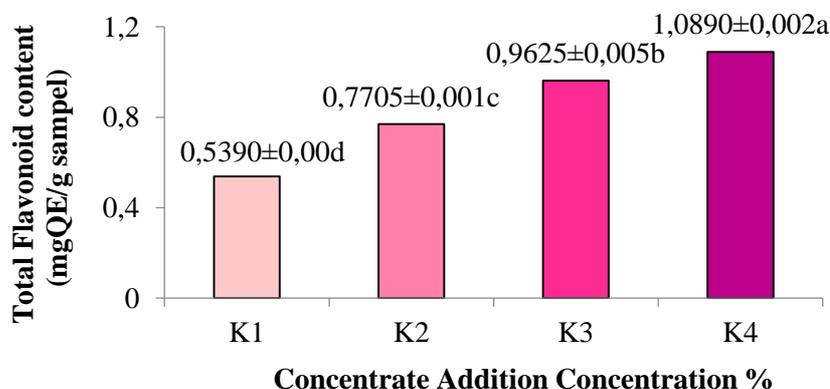


Figure 2. Total levels of edible coating flavonoids with variations in the concentration of addition of kecombrang flower concentrate.

The results of the analysis of edible coating samples with the addition of kecombrang flower concentrates showed a significantly different effect on total flavonoid levels. The highest average value of total flavonoid content was in edible coating samples with the addition of kecombrang flower concentrate at a concentration of 4% (K4), which is equal to 1,089 mgQE / g sample.

Figure 2 shows an increase in total levels of flavonoids as the concentration of concentrates increases in edible coating. Increasing the concentration of the addition of kecombrang flower concentrates in the edible coating sample caused a thick red color. According to [24], flavonoids are a group of phenolic compounds, where these compounds are red, purple, blue, and some yellow substances found in plants. Thus, the red color of the kecombrang flower concentrate can be an indicator of the presence of flavonoid compounds. The more concentrated red is, the more molecules will absorb light at a certain wavelength, resulting in higher absorbance values. A high absorbance value will produce a high total flavonoid value.

Flavonoids are one of the largest natural phenol groups found in all green plants. Flavonoids are polyphenolic compounds that have 15 carbon atoms, consisting of 2 benzene rings which are connected into 1 linear chain consisting of 3 carbon atoms. There are several types of flavonoids including flavones, flavonols, flavonoids, bilavonil, chalcone, auron, anthocyanidin and the most commonly found is in the form of flavonol. A number of medicinal plants containing flavonoids have been reported to have antioxidant activity, antibacterial, antiviral, anti-inflammatory, hypo-allergenic, and anticancer. The antioxidant effect of this compound is caused by free radical capture through a donor hydrogen atom from the hydroxyl group of flavonoids [18].

### 3.1.3. Analysis of antioxidant activity

The measurement of the antioxidant activity of the edible coating sample in this study was carried out using the DPPH (2,2-diphenyl-1-picrylhydrazil) method. The DPPH method is often used to test compounds that act as free radical scavengers or hydrogen donors, evaluate their antioxidant activity and quantify the amount of radical antioxidant complexes formed [25].

The main analysis of the variety of edible coatings with the treatment of variations in concentrations of adding concentrates (K) has a significant effect on antioxidant activity. The average value of the antioxidant activity of edible coating with variations in concentrations of adding concentrates is presented in Figure 3.

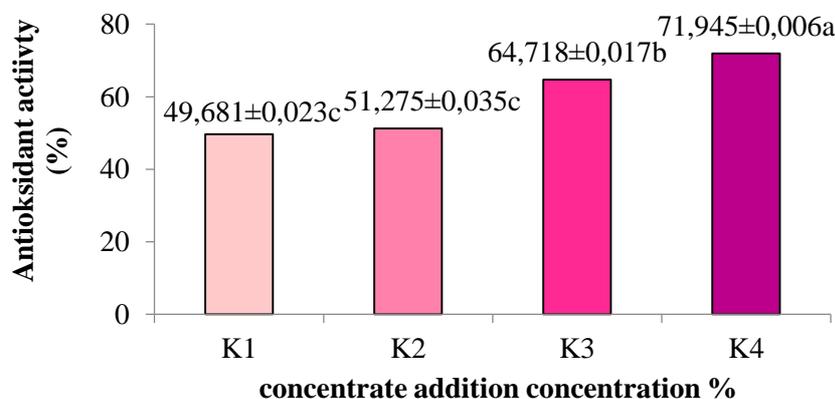


Figure 3. The average value of edible coating antioxidant activity with variations in the concentration of addition of kecombrang flower concentrate.

The results of the analysis of the variation in concentrations of concentrated additions in edible coating produced the highest average antioxidant activity in the edible coating sample with the addition of kecombrang flower concentrate at a concentration of 4% (K4), which was 71.945%.

Figure 3 shows an increase in antioxidant activity along with an increase in the concentration of adding concentrates in edible coating. This proves that kecombrang flowers have antioxidant activity, where the greater the concentration in the sample, the greater the ability to reduce radical DPPH. In line with the research of [26], the percentage of DPPH reduction increased with increasing concentrations of the ethanol extract of the kecombrang flower, thus indicating that the kecombrang flower extract had free anti-radical activity.

The results of the analysis of the edible coating antioxidant activity added with the kecombrang flower concentrate in reducing DPPH radicals showed a positive correlation with the results of the analysis of the total levels of phenol compounds and their flavonoids. The correlation relationship explained that phenol and flavonoid compounds play an important role in their ability as antioxidants. It can be seen that the higher the levels of phenol and flavonoids compounds, the higher the ability of concentrates to reduce free radicals.

The strong or weak antioxidant activity of essential oils of various plants is influenced by the components of compounds which are antioxidants [27]. [28] further stated that of the many flavonoids that have antioxidant properties, quercetin is one of the most active flavonols (class of flavonoids) and has strong antioxidant abilities. This means that among several types of phenol compounds contained in kecombrang flowers, flavonoids are one of the groups of phenol compounds that determine the amount of ability as an antioxidant in reducing free radicals.

The antioxidant effects of flavonoids are caused by free radical capture through donor hydrogen atoms from the flavonoid hydroxyl group [18]. Phenol compounds can act as antioxidants by acting as a reducing agent that donates H<sup>+</sup> ions from their hydroxyl groups. The ability to donate H<sup>+</sup> ions makes phenol compounds act as antioxidants.

## **3.2. Quality Analysis of Gouramy Sausages**

### **3.2.1. Analysis of free fatty acid levels (FFA)**

Measuring the level of damage to fatty food can be done by measuring the levels of free fatty acids (FFA) contained in these foods. Processed fish meat is known to contain fatty acids which can lead to rancidity due to oxidation reactions. The occurrence of an oxidation reaction causes the levels of FFA in food to increase. Free fatty acids are fatty acids in an unesterified form so they can cause oxidative stress and as a result produce lipid peroxidation products, namely malondialdehyde (MDA) [29].

In this study, an analysis of the extent of damage to gouramy sausage products was applied with the edible coating concentrating kecombrang for 12 days of storage by measuring the level of free fatty acids contained in the sausage samples. Gouramy sausages are stored in the refrigerator at 4°C. Generally, the longer the storage time, the higher the level of FFA contained, indicating the greater the level of food damage that occurs. Measuring the level of food damage can illustrate the effectiveness of kecombrang as an antioxidant compound that can prevent or inhibit the occurrence of such food damage.

The results of the analysis of the average value of free fatty acid sausage levels of gourami during storage with concentrations of adding concentrates (K) in this study are presented in Figure 4.

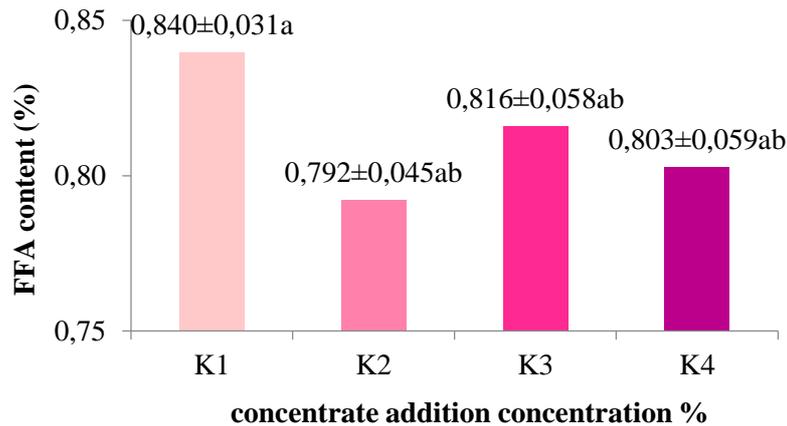


Figure 4. The average value of FFA of gourami sausages during storage with varying concentrations of adding concentrates in ediblecoating.

The results of the analysis of free fatty acid levels (FFA) showed that the addition of the kecombrang flower concentrate in edible coating was able to inhibit the formation of free fatty acids in gourami sausages during storage. The higher the concentration of addition of kecombrang flower concentrate in edible coating, the lower the FFA level in gourami sausage products during storage. This is due to the content of bioactive compounds, namely phenol and flavonoid compounds contained in kecombrang flower concentrates.

Phenol and flavonoids are known to be able to act as antioxidants in reducing free radicals. According to [30], antioxidants can work by reducing the formation of free radicals, or turning free radicals into stable molecules, or cutting the elongation of the peroxidation chain. In this study, kecombrang flower concentrate was thought to have antioxidant activity by inhibiting the occurrence of oxidation reactions and converting free radicals into stable compounds. Therefore, the formation of free fatty acids can be prevented and there is a decrease in the levels of free fatty acids as the concentration of concentrates increases.

Figure 4 shows that the concentration of the addition of a concentrate of 2% has been able to reduce the level of FFA of gourami sausage products during storage. This shows that kecombrang flower concentrate has strong antioxidant activity and is able to prevent and slow down the occurrence of fatty food oxidative damage at low concentrations.

Overall, the results of the analysis of the average value of free fatty acid gourami sausages during storage in this study are fairly low. According to [31], the maximum value of free fatty acids in foodstuffs does not exceed 5%. The highest free fatty acid content in this study was gourami sausage samples with the addition of kecombrang flower concentrate of 1%, which is 0.840%. Thus, gourami sausage products in this study have fulfilled the food quality requirements based on the levels of free fatty acids.

### 3.2.2. Analysis of malondialdehyde levels(MDA)

According to [32], malondialdehyde (MDA) is a secondary product of oxidation of unsaturated fatty acids as a result of decomposition of peroxide. MDA is widely used as an indicator of oxidative stress in a measurement using tiobarbituric acid (TBA). The more MDA content in food, the higher the oxidative stress that occurs, thus illustrating the greater the level of damage to the food.

The results of the analysis of the average value of malondialdehyde (MDA) of gourami sausages during storage with the treatment of variations in concentrations of adding concentrates (K) in this study are presented in Figure 5.

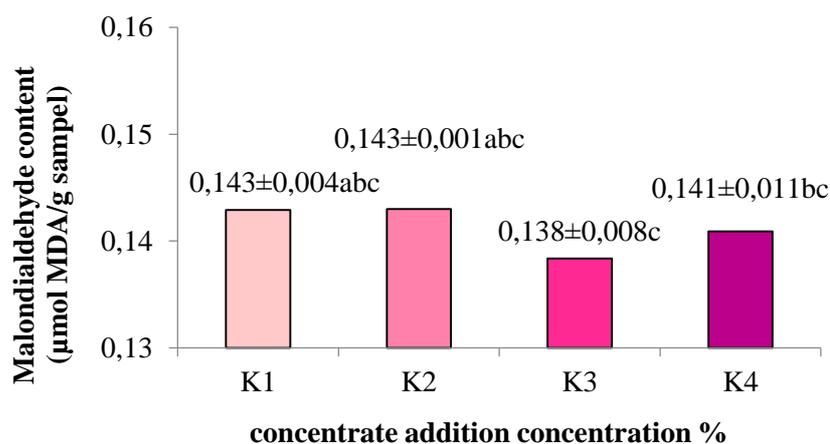


Figure 5. The average value of MDA levels of gourami sausages during storage with varying concentrations of adding concentrates in edible coating.

The results of the MDA level analysis that the addition of the kecombrang flower concentrate caused the MDA level of gourami sausage products to be relatively stable at each concentration of concentrate addition, in line with the results of the analysis of the levels of free fatty acids. According to [33], the increase in FFA value will cause the MDA value to increase, this is caused by a series of oxidation reactions and hydrolysis processes. This condition proves that antioxidant activity in kecombrang flower concentrates is by preventing oxidation reactions that can cause oxidative stress. Stable free fatty acid levels in the sausage sample produced a stable average MDA level.

Figure 5 shows that the MDA levels of gourami sausages during storage are relatively stable and begin to experience a decrease in the addition of concentrated concentrations by 3%. Meanwhile, the highest value of MDA level for gourami sausage was 0.143 µmol MDA / g of sample during storage. This proves that kecombrang flower concentrate has high

antioxidant activity and is able to prevent and slow down the occurrence of fatty food oxidative damage at low concentrations.

The tolerance limit of foodstuffs which can still be consumed to a maximum number of TBAs is 18  $\mu\text{mol MDA / kg sample}$  [34]. Thus, gourami sausage products during storage in this study still meet food quality requirements based on their malondialdehyde levels.

What needs to be considered in the use of kecombrang flower concentrate is the possibility of the formation of prooxid compounds. This is because strong antioxidant activity can trigger the appearance of prooxid compounds. Several studies have found that high concentrations of antioxidant compounds can cause increased oxidative damage due to the appearance of prooxid compounds, including research on antioxidants from seagrass extracts [35], the antioxidant and antioxidant properties of vitamin C and green tea [36], and antioxidants extract of mangosteen peel on biodiesel [37].

The antioxidants are derived from antioxidants which accumulate in high concentrations. According to [38], at high concentrations, antioxidant activity often disappears and even becomes a prooxidant. Antioxidants will only function when there are prooxid compounds (triggers the oxidation process). When the dose of antioxidants and prooxins is not balanced or high levels of antioxidants while prooxides are low, it will trigger the formation of prooxid compounds in order to balance the levels of antioxidants. This condition will actually cause the level of oxidative damage that occurs to be higher, indicated by an increase in the level of malondialdehyde (MDA). Besides, the bioactive component is able to activate microbes in sausage products [39]. Not only on the flowers, but also kecombrang leaves can minimize damage to gourami products and their processed products [40] [41].

Thus, it can be concluded that the addition of kecombrang flower concentrate in this study is still safe because it can reduce the levels of FFA and MDA in gourami sausage products during storage and does not cause prooxid compounds.

#### **4. CONCLUSION**

The addition of the kecombrang flower concentrate in edible coating is able to prevent and slow down the oxidative damage of gourami sausage products during storage. The concentration of adding concentrates in edible coating by 4% resulted in levels of total phenol, total flavonoids, and the value of antioxidant activity greater than the concentrations of 1%, 2%, and 3%. The concentration of adding 2% concentrate in edible coating has been able to suppress oxidative damage to gourami sausages, ie with FFA values of 0.792% and 0.143  $\mu\text{mol MDA / g samples}$ .

#### **Acknowledgements**

Authors wish to thank to the DRPM Kemenristek Republic of Indonesia which has provided research funding through Strategis Nasional Grant Scheme 2018 also to Institute for Research and Community Service of Jenderal Soedirman University for the support.

## REFERENCES

- [1] Anggraini, D.R., Tejasari, and Y. Praptiningsih. 2016. Karakteristik fisik, nilai gizi, dan mutu sensori sosis lele dumbo (*Clarias gariepinus*) dengan variasi jenis dan konsentrasi bahan pengisi. *Jurnal Agroteknologi* 10 (1):25-35.
- [2] Adawyah, R. 2007. *Pengolahan dan Pengawetan Ikan*. Bumi Aksara, Jakarta.
- [3] Winarsi, H. 2007. *Antioksi dan Alami dan Radikal Bebas*. Kanisius, Yogyakarta.
- [4] Hernani and M. Raharjo. 2005. *Tanaman Berkhasiat Antioksidan*. Swadaya, Jakarta.
- [5] Kikuzaki, H. and K. Nakatani. 1993. Antioxidant effect of some ginger constituents. *Journal Food Science* 58: 1407-1410.
- [6] Naufalin, R. 2017. *Kecombrang: Antimikroba dan Pemanfaatannya sebagai Pengawet Pangan*. Universitas Jenderal Soedirman.
- [7] Naufalin, R. 2018. *Mikrobiologi Pangan*. Plantaxia, Yogyakarta.
- [8] Naufalin, R., and S. R. Herastuti. 2017. Antibacterial activity of *Nicolaia speciosa* fruit extract. *International Food Research Journal* 24.1:379-385.
- [9] Naufalin, R., and S. R. Herastuti. 2018. [Antibacterial activity of kecombrang flower extract \(\*Nicolaia speciosa\*\) microencapsulation with food additive materials formulation](https://doi.org/10.1051/e3sconf/20184701002). IOP Conf. Series: Earth and Environmental Science. 102012035. doi :10.1088/1755-1315/102/1/012035.
- [10] Naufalin, R., Wicaksono, R., Arsil, P. And M.F. Salman. 2018. Antimicrobial coating on quality attributes of sausage during refrigerated storage. E3S Web of Conferences 47, 01002 (2018) <https://doi.org/10.1051/e3sconf/20184701002>
- [11] Pahlevi, Y. R. 2011. Aplikasi *edible coating* chitosan-ekstrak daun jati pada sosis daging sapi untuk menghambat kerusakan mikrobiologis dan oksidatif. *Skripsi*. Universitas Sebelas Maret, Surakarta.
- [12] Naufalin, R dan S.R. Herastuti. 2012. *Bubuk Kecombrang (*Nicolaia Speciosa*) Sebagai Pengawet Alami Pada Bakso Ikan Tenggiri*. *Agricola*(2):124-147.
- [13] Naufalin, R., dan S.R. Herastuti. 2013 Microcapsule application of kecombrang flower extract: effect of concentration, type of fraction, pH of medium and NaCl on microbiological properties of minced beef. *Journal Animal Production* 15 (1):8- 14.
- [14] Naufalin, R., S.R, Herastutidan R. Wicaksono. 2013. Encapsulation Of Natural Antimicrobia Extract From Kecombrang Flower (*Nicolaias peciosa*) Using Maltodextrin-Gelatin As Filler Ingredient. *International Food Safety Conference. Kuala Lumpur 2-3Desember*.

- [15] Raeisi, M., H. Tajik, J. Aliakbarlu, and S. Valipour. 2014. Effect of carboxymethyl cellulose edible coating containing *Zataria multiflora* essential oil and grape seed extract on chemical attributes of rainbow trout meat. In *Veterinary research forum: an international quarterly journal*, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran 5(2):89-93.
- [16] Othoman, A., Ismail, A., Ghani, N.A. and I. Adenan. 2005. Antioksidant capacity and phenolic content of cocoa beans. *Journal of Food Chemistry* 100:1523-1530.
- [17] Naufalin, R. dan S.R, Herastuti. 2014. Natural Nanoencapsulant Antioxidants Based on Kecombrang Fruit (*Nicolaia speciosa*). *International Proceedings of Chemical, Biological and Environmental Engineering* (71):15-18.
- [18] Neldawati, Ratnawulan, and Gusnedi. 2013. Analisis nilai absorbansi dalam penentuan kadar flavonoid untuk berbagai jenis daun tanaman obat. *Journal Pillar of Physics* 2:76-83.
- [19] Suhartatik, N., Muhammad, N.C., Sri, R. and S.R. Endang. 2013. Aktivitas antioksidan antosianin beras ketan hitam selama fermentasi. *Jurnal Teknologi dan Industri Pangan* 24 (1):115-119.
- [20] Naufalin, R. and S.R, Herastuti. 2016. Antioxidant Activity and Physicochemical Properties of *Nicolaia speciosa* Flower Extract. *Agriculture and Agricultural Science Procedia* 9 : 297 –303.
- [21] Sudarmadji, S. B., Haryono and Suhardi. 1997. *Prosedur Analisa Bahan Makanan dan Pertanian*. Liberty, Yogyakarta.
- [22] Yuwanti, S. 2005. Potensi asap cair sebagai antioksi dan pada bandeng presto. *Jurnal Teknologi Pertanian* 6 (2): 81 –85.
- [23] Tampubolon, O.T., S. Suhatsyah, and S. Sastrapradja. 1983. Penelitian Pendahuluan Kimia Kecombrang (*Nicolaia speciosa* Horan). *Risalah Simposium Penelitian Tumbuhan Obat III*. Fakultas Farmasi, UGM, Yogyakarta.
- [24] Gafur, M.A. 2013. Isolasi dan Identifikasi Senyawa Flavonoid dari Daun Jamblang (*Syzygium cumini*). *Skripsi*. Fakultas MIPA, Universitas Negeri Gorontalo.
- [25] Prakash, A., F. Rigelhof, and E. Miller. 2001. Antioxidant activity. *Medallion Laboratories Analytical Progress* 19 (2):1-4.
- [26] Suwarni, E. and K.D. Cahyadi. 2016. Aktivitas anti radikal bebas ekstrak etanol bunga kecombrang (*Etlingera elatior*) dengan metode DPPH. *Jurnal Medicamento* 2 (2): 39-46.
- [27] Politeo, O., M. Jukic, and M. Milos. 2006. Chemical composition and antioxidant activity of essential oils of twelve spice plants. *CroaticaChemicaActa* 79:545-552.

- [28] Fuhrman, B. and M. Aviram. 2002. Polyphenols and flavonoids protect LDL against atherogenic modification. *Dalam: Cadenas, E., Packer, L., (Eds). Handbook of Antioxidant 2<sup>nd</sup> ed: Revised and Expanded.* Marcel Dekker Inc, NewYork.
- [29] Wahjuni, S. 2012. *Malondialdehid Prekursor Stress Oksidatif.* Fakultas Matematika dan Ilmu Pengetahuan Alam, Universitas Udayana, Denpasar.
- [30] Wijaya, A. 1996. Radikal bebas dan parameter status antioksidan. *Forum Diagnosticum.* Lab KlinikProdia1:1-12.
- [31] Jacob, L.A., Uktolseja, and J. Wakrewa. 2014. Salut dari campuran daun biji (*Psidium guajava*), rimpang jahe (*Zingiber officinalis*) dan daun teh (*Camellia sinensis*) meningkatkan kestabilan oksidatif pakan induk udang air tawar (*Macrobrachium idea*). *Pros Nas Entrepreneurship.* 228-237.
- [32] Bangol, E., L.I. Momuat, and J. Abidjulu. 2014. Aktivitas antioksidan ekstrak etanol dan n-heksana dari daun rumput santamaria (*Artemisia vulgaris* L.) pada minyak ikan. *Jurnal Ilmiah Sains* 14: 129-135.
- [33] Fauzi, A., T. Surti, and L. Rianingsih. 2016. Efektivitas daun teh (*Camellia sinensis*) sebagai antioksidan pada fillet ikan bandeng (*Chano schanos* Forsk.) selama penyimpanan dingin. *Jurnal Pengolahan dan Bioteknologi Hasil Perikanan* 5 (4).
- [34] Sukandar, D., A. Muawanah, T. Rudiana, and K.F. Aryani. 2017. Pemanfaatan minyak atsiri kulit buah honje sebagai antioksidan produk sosis ayam. *Jurnal Teknologi dan Industri Pangan* 28 (1):20-26.
- [35] Puspasari, A.R., E.N. Dewi, and L. Rianingsih. 2017. Aplikasi antioksidan dari ekstrak lamun (*Cymodocea rotundata*) pada minyak ikan tongkol (*Euthynnus affinis*). *Jurnal Agritech* 32 (2):115-120.
- [36] Yomes, A.T. 2006. Sifat Prooksi dan Antioksidan Vitamin C dan The Hijau Pada Sel Khamir *Candida* sp. Berdasarkan Peroksidasi Lipid. *Skripsi.* Fakultas Matematika dan Ilmu Pengetahuan Alam, Institut Pertanian Bogor.
- [37] Suaniti, N.M., M. Manurung, and N.M.M. Utari. 2017. Efek penambahan antioksidan ekstrak methanol kulit buah manggis (*Garcinia mangostana* L.) terhadap perubahan kadar FFA, bilangan asam, dan bilangan peroksida biodiesel. *Jurnal Kimia* 11 (1): 49-55.
- [38] Gordon, M.H. 1990. The mechanism of antioxidants action in vitro. *Dalam: B.J.F. Hudson. Food Antioxidant.* Elsevier Applied Science, London.
- [39] Putri, F.A., R. Naufalin, R. Wicaksono. 2019. Antimicrobial edible coating application of Kecombrang flower concentrate to reduce microbial growth on gourami fish sausage. IOP Conf. Series: Earth and Environmental Science 250 (2019) 012056.
- [40] Latifasari, N., R. Naufalin, R. Wicaksono. 2019. Edible coating application of kecombrang leaves to reduce gourami sausage damage. IOP Conf. Series: Earth and Environmental Science 250 (2019) 012055.
- [41] Hanifah, M.R., R. Naufalin, R. Wicaksono. 2019. The effect of edible coating contained Kecombrang leaves concentrate on gourami fish fillet quality. IOP Conf. Series: Earth and Environmental Science 250 (2019) 012057.