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Antimicrobial coating on quality attributes of sausage during refrigerated storage

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Abstract. Edible coating based on carboxymethyl cellulose (CMC) environmentally friendly. Addition on Kecombrang (*Nicolaia speciosa*) extract used to be antimicrobial and antioxidants coating. CMC-based edible coating added with antimicrobial of kecombrang was used to reduce the oxidative and microbial degradation sausages stored at refrigerator at 10°C for 12 days. The cmc coating reduced malonaldehyde substances and peroxide value by 0.88 mg.kg and 92.29%, respectively, compared with the controls. The moisture barrier effect was significantly better for the CMC coating compared to the control. The CMC coating of sausages inhibits the growth of either the total plate counts of *Bacillus cereus*, *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeroginosa*. Data show that cmc can effectively be used as a natural antioxidative and moisture barrier coating to extend the quality and shelf life of sausages.

1 Introduction

Gourami fish has the potential to be utilized to be one of the processed food products namely sausage, which is generally made from beef. The high-fat content in sausage cause rancidity due to oxidative damage in the form of lipid oxidation process. Synthetic preservatives such as nitrate/nitrites and / or salts have been widely used to inhibit oxidative damage and inhibit microbial growth in preserving sausages. Consumption of synthetic preservatives continuously causes negative impacts on the health of consumers either directly or indirectly (carcinogenic) [3].

The edible coating is a technology that can minimize the damage and extend the shelf life of food products. Edible coatings enriched with natural ingredients that have antioxidant and antimicrobial compounds can enhance the edible coating ability to extend the shelf life of food products. The results showed that edible coating of teak leaf extract could inhibit microbiological and oxidative damage to the sausage [20], another study also showed that edible coating with the addition of green tea extract could decrease the lipid oxidation rate in the sausage [24].

Several studies have been conducted on bioactive compounds in kecombrang that are able to act as antioxidants and antimicrobials. Phytochemical content contained in flowers, stems, rhizomes and leaves kecombrang on the results of studies of alkaloids, saponins, tannins, phenolic, flavonoids, triterpenoids, steroids, and glycosides that use an active role as antioxidants. The stem and leaves of kecombrang have higher phytochemical compounds than other kecombrang [10].

Edible coating used in this research made of CMC (Carboxy Methyl Cellulose) and glycerol and the addition of concentration of leaf kecombrang at certain concentration. Edible coating base materials of CMC and glycerol are selected to produce stable and plastic edible coatings for easy application to food products. CMC acts as emulsifier and stabilizer, while glycerol acts as a plasticizer which enhances flexibility. The method of edible coating application on gouramy sausage used in this research is spraying because this method has the advantage of producing coating products with thinner and more uniform layers than dyeing techniques. This study aims to determine the effect of

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addition of kecombrang leaf concentrate to the edible coating on oxidative damage of gouramy sausage during low temperature storage

2 Material and methods

2.1 Place and Time of Research

The research was conducted at the Laboratory of Agricultural Technology, Faculty of Agriculture UNSOED. The research held from January until Juny 2018.

2.2 *Materials*

Materials needed research consisting of: *Nicolaia speciosa* leaf. Chemicals used among other technical solvents, namely ethanol 96 %; coating (Carboxy Methyl Cellulose), ethanol 99.8 % (PA), linoleic acid, distilled water, ammonium thiocyanate, 0.02 M FeCl2 tetrahydrate, concentrated HCl, N_2 gas. Other materials needed include a coarse filter paper, Whatman No. 41 paper, plastic, paper label, paper towels and aluminum foil.

2.3 Equipment

The tools used in this research that cabinet dryer dryers, blenders, analytical scales, rotary evaporator (Bibby RE 200), shaker, oven (Memmert, Japan), desiccators, pipettes (Pyrex, Germany), pH meter. Tool for the analysis of total phenol and antioxidant activity consists of spectrophotometers, centrifuges, glass tools (Pyrex, Germany), vortex, Fial tubes, micropipette (Gilson), and bath tobacco leaf waste, i.e. shaker, rotary, tube N_2. Tool to extract formulation, a set of tools for testing antimicrobial activity glasses, vortex, micro pipettes, incubator 37°C, desiccator, refrigerator and vacuum pump.

2.4 Undertaking experiment

N. speciosa leaf powder sample preparation, N. *speciosa* flower are selected. Material selection results cleaned with water, then dried in a dryer at a temperature of 50°C until the moisture content of 8-10%. Furthermore simplicia dry milled to obtain a homogeneous powder.

The *N. speciosa* leaf powder was extracted twice with ethanol (1:4 w / v) [16]. The extraction process is done by maceration at 37°C, with a rotation speed of 150 rpm for 24 hours every level. Filtrat separated from the solvent by evaporation in a rotary evaporator until no solvent dripping again. The solvent was evaporated at a temperature of 50°C. Residual solvent is removed with nitrogen gas to produce an essential oil [16].

Separated *N. speciosa* leaf extract had weighed and added by sterilizing distilled water with a ratio of 1:1 (w/v) respectively. Then blended for 3 min and filtered using a filter clothe to separate the extract and precipitate (residue) to obtain *N. speciosa* leaf extracts with concentration of 50%. The process of *N. speciosa* encapsulation is by adding the CMC with the different concentration. Using hotplate stirrer in 50°C temperature, the filtrate heated and add the coating and stirred on 15 minutes [17].

2.5 Determination procedure

Measurement of Total Phenol was modified. Activity of antioxidant formulas was analyzed using modified iron thiocyanate method. This determination is based on the formation of peroxide as a product of oxidation of linoleic acid. Percent oxidation was calculated by comparing the absorbance value of the test sample (As) with control absorbance value (A ctrl) multiplied by 100%.

2.6 Statistical analysis

The observed variables in this research are antioxidant activity of edible coating pada sosis ikan gurami involved total phenol measurement,. Quantitative data was analyzed used variance test (F test) in 5%, continued in Duncan's Multiple Range Test (DMRT) if it finds the significant difference.

3 Results And Discussions

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3.1 Total Phenol

The result of variance analysis showed that the kecombrang plant part has no significant effect on the total content of phenol edible coating. The mean total phenol edible coating of kecombrang leaves was 17.97 mg TAE / g samples. The total value of phenol edible coating in this study was higher than 0.13 mg TAE / g sample for edible coating kecombrang and 1.44 mg TAE / g samples for edible coating of kecombrang leaves (with the same concentrate) [4]. This difference in total phenol content can be due to environmental factors for different plants such as plant growth, soil composition, temperature, rainfall, and ultraviolet radiation [2].

The result of variance analysis showed that concentration concentration of carbohydrate have significant effect to total content of phenol edible coating. The average value of total fenol edible coating of leaf of kecombrang on concentration treatment of concentrate kecombrang can be seen in Figure 1.

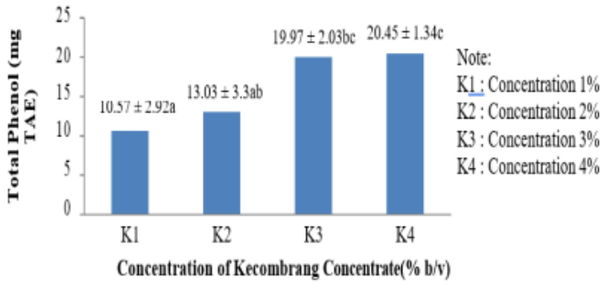

Fig. 1. The mean of total *phenol* edible *coating* on treatment concentration of kecombrang concentrate

Figure 1 shows that the mean total phenol edible coating of leaves of kecombrang increases from addition consentrate. The increase in the mean total phenol edible coatings along with the increased concentration of concentrate due to the total phenol detected in the edible coating derived from phenol compounds contained in the concentrate of leaves kecombrang, so the more concentrated kecombrang will be higher value of total phenol. The phytochemical content of flowers, stems, rhizomes and leaves kecombrang that is composed of alkaloids, saponins, tannins, phenolics, flavonoids, triterpenoids, steroids, and glycosides that use an active role as antioxidants [10].

3.2 Antioxidant activities

Generally, the antioxidant activity is directly proportional to the total phenol because the total phenol is quantitative of all antioxidant compounds of phenol group in a plant, including flavonoids (Widyastuti, 2010). This study showed the opposite result was suspected because the flavonoid compound on the leaves kecombrang is a class of phenol compounds that play the most antioxidant role, so in this study the value of flavonoids is directly proportional to the antioxidant activity. The flavonoids act as antioxidants by giving their hydrogen atoms or through their ability to metal clatting [23].

The result of variance analysis showed that kecombrang concentration very significant effect on edible coating antioxidant activity. The average value of antioxidant activity of edible coating of leaves of kecombrang on concentration treatment of kecombrang concentrate can be seen in Figure 2.

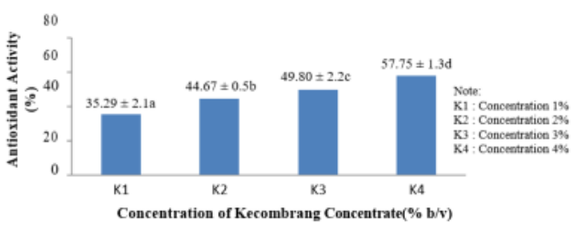

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Fig. 2. The mean of antioxidant activity *edible coating* on treatment concentration of kecombrang concentrate

Figure 2 shows the antioxidant activity of leaves increased as the concentration of concentrate increased. This is because the mount of bioactive compounds in the edible coatings increases as the concentration of added ingredients increases. Several studies have shown that the concentration of extracts and plant concentrates has a linear correlation with the result of antioxidant activity. This is reinforced by the results of research Cahyaningtiyas [4] that the more concentrated kecombrang added to the edible coating can increase antioxidant activity.

The results of analysis of antioxidant activity in this study indicate a linkage with the total value of flavonoids is the higher the value of flavonoids then the value of antioxidant activity produced. The correlation coefficient between the total flavonoid with IC50 cape fruit with a variety of solvent extract is equal to 0.9993 [2]. That is, increased antioxidant activity is closely related to the increase in the total value of flavonoids. There are several factors that influence the correlation between antioxidant activity and the type of antioxidant compound in a material. The first factor is the type of plant. The antioxidant compound content is scattered throughout the plant with types and concentrations according to the type of plant [5]. This type of antioxidant compound can cause synergistic and antagonistic effects of these antioxidant compounds. The antioxidant activity is not always correlated with both phenol and flavonoid levels [6]. This is due to the difference of active components in plants that cause synergistic and antagonistic effects that affect the antioxidant activity in plants.

3.3 Oxidative Damage

Malondialdehyde levels are one indicator of oxidative damage levels in foodstuffs. Malondialdehyde is a lipid peroxidation end product, usually used as biological biomarker of lipid peroxidation and represents the degree of oxidative stress. The value of Malondialdehyde levels in gouramy sausage during storage at plant part treatment can be seen in Figure 3.

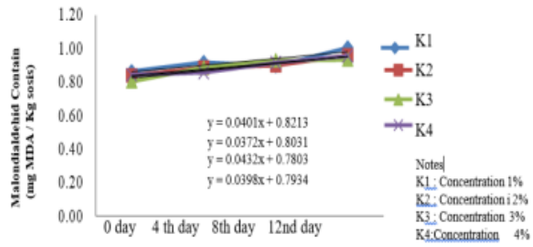

Fig. 3. Malondialdehyde levels have increased during storage

Figure 3 shows that malondialdehyde levels have increased during storage. This is because the free fatty acids present in the sausage undergo further oxidation so that it can produce malondialdehyde. The malondialdehyde is an end product of lipid peroxidation, which levels are influenced by the high number of free radicals [29]. The presence of oxidation rates in foodstuffs may lead to increased levels of malondialdehyde [25].

Based on the result of the analysis of the variety, the treatment of plant part on the application of edible coating kecombrang significantly to the malondialdehyde content of gouramy sausage in this study. Based on Figure 7, malondialdehyde content of gouramy sausage with edible coating application of stems and leaves of kecombrang were 0.039 and 0.0408 respectively. The slope value of the edible coating of the kecombrang rod is lower than the slope value of the edible coating of the leaves. This suggests that edible coating sticks kecombrang better able to suppress malondialdehyde levels in gouramy sausage due to oxidative damage. This is due to the antioxidant activity of edible coating sticks kecombrang higher than the antioxidant activity of edible coating leaves kecombrang in this study. The consumption of antioxidant supplements cause the presence of antioxidant activity can suppress levels of malondialdehyde in the body [22].

Based on the analysis of variance, malondialdehyde content of gouramy sausage with edible coating application of kecombrang on concentration treatment did not have significant effect. It can also be seen from the slope value of the average regression equation of malondialdehyde content of gouramy sausage during storage at each concentrate which is fluctuating. Slope value of malondialdehyde content of gouramy sausage on edible coating application with concentration of 1%, 2%, 3% and 4% respectively, that is 0,0401; 0.0372; 0.0432; and 0.0398. The higher concentrations of antioxidants are proportional to the ability to inhibit the formation of malondialdehyde [26]. This

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mismatch is caused by changes in oxidative stress levels that occur in gouramy sausages. This indicates that the levels of malondialdehyde are unstable in a material. The situation with high levels of oxidative stress can significantly increase malondialdehyde levels, but if the oxidative stress condition is resolved, the malondialdehyde level will decrease again [21].

4. Conclusion

Leaves part of kecombrang effect on flavonoid and edible coating antioxidant activity, while concentration variation effect to total phenol, flavonoid, and edible coating antioxidant activity. Application of edible coating kecombrang can be effect on free fatty acid content and malondialdehyde gouramy sausage during storage.

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