Antibacterial and Antioxidant of Uwi (Dioscorea Alata L) Starch Edible Film Incorporated with Ginger Essential Oil

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Antibacterial and Antioxidant of Uwi (*Dioscorea Alata L*) Starch Edible Film Incorporated with Ginger Essential Oil

Miksusanti, Herlina, and K. I. Masril

Abstract—**It has been done the testing of antibacterial and antioxidant activity of edible film which incorporated with ginger essential oil. Antibacterial activity testing has been done by disk method. An Antioxidant activity test has performed with a spectrophotometric method using DPPH as a radical source. The result of the research showed that ginger essential oil has antibacterial activity against *Escherichia coli*. The best antibacterial activity of edible film was at 3% essential oil with inhibitor zone 0.5 cm. The best antioxidant occurs in 3% essential oil with 31.50 percent reduction of DPPH. An Edible films containing 3% essential oil has the tensile strength is 24.96 kPa and elongation percent is 20% and 0.3 mm of film thickness. The edible film can produce antibacterial and antioxidant properties of the optimum by the addition of 3% essential oil.**

Index Terms—**Edible film, uwi starch, antibacterial, antioxidant, DPPH, escherichia coli.**

I. Introduction

Foodstuffs are sensitive and prone to degradation due to environmental factors, such as chemistry, biochemistry, and microbiology. Loss of quality can be accelerated by the presence of oxygen, water, light, and temperature. Edible antibacterial films and coatings have shown to be an efficient alternative in controlling food contamination. It has been reported that the growth of deteriorating and pathogenic microorganisms may be prevented through the incorporation of antimicrobial agents into edible films. In the last years, research has been performed concerning the uses of edible films that has been incorporated with organic acid, wood extract and preservative (salt) [1]-[3].

Ginger essential oil also has antibacterial and antioxidant activity. The *Escherichia coli* that always contaminated channel of humankind could be inhibited with the ginger essential oil.

The objective of this research was to produce antibacterial and antioxidants edible film by incorporation ginger essential oil in uwi starch (*Dioscorea alata L*) edible film. It has been seen that uwi starch is used much lesser extent than other starches for food. Anyhow this uwi flour has potential as a source of starch. In this research, uwi starch was used as a matrix for development of edible films.

To minimize the use of synthetic additives it is necessary added essential oils of ginger, which has antibacterial properties and antioxidants so that the side effects from the use of synthetic additives can be minimized and also see how the influence of the addition of ginger essential oil toward the physical characters of the film edible. Aspect examined in this study is the antibacterial activity as measured by the diameter inhibitory and antioxidant activities of antibacterial film. Testing of antibacterial activity tested against gram-negative bacteria *Escherichia coli* using discs as well as tested against antioxidant activity by spectrophotometer method using the DPPH (1,1-diphenyl-2-picrylehydrazyl) as a source of free radicals.

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II. Procedure

A. Plant Material and Isolation of Essential Oil

Uwi tuber was purchased from Indralaya local market and the ginger tuber were obtained from 16 ilir market in Palembang city South Sumatra. Extraction of ginger essential oil by Steam distillation [5]. Thin slices of ginger Rhizome by as much as 1 kg of dry smashed into a little delicate then distilled using steam distillation apparatus with steam pressure setting to 5 Kbar. Distillation is carried out by vapor pressure (100-115°C) for 6 hours. Water that still was brought with volatile oil was separated with sodium hermetically sealed glass containers with rubber lids, covered with aluminum foil to protect the contents from light and kept under refrigeration at 8°C until used.

B. Bacterial Strains

Typical meat product bacterial contaminants used in this study were *Escherichia coli* . It obtained from the culture collection of PAU Gajah Mada University. The bacterial cultures were grown on nutrient agar slants and kept at 4°C. Sub culturing was carried out every month to maintain bacterial viability.

C. Bacterial Strains

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D. Preparation of Film

Uwi starch was provided from uwi tuber by previous research [6]. To make edible film, 7 gram of the uwi starch was dissolved in 115 mL aquadest, and heated at 1.6^{o}C/min on magnetic stirrer with hot plate, Carboxymetil cellulose (CMC) 0,7 g and glycerol 1.75 mL were added slowly while continuing to be stirred. The heating was carried out until

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the system entered in the gelatinization. After entering the gelatinization, ginger essential oil were added in to the system at different concentration (0.5 %, 1%, 1.5%, 2%, 2.5%, 3% v/w). After gelatinization, films were casted over glass plates and dried at 50° C (R.H 22%, for 2 h). Drying was completed in a controlled temperature chamber at 25°C and RH, 80-90% during a week.

E. Anti Bacterial Activity Test

Preparation Bacterial Culture Assay undiluted bacteria obtained from the laboratory of centre university of Gajah Mada University. Before use, the bacteria were grown on media NA slant.

Antibacterial activity test on films was carried out using the agar diffusion method [7]. The zone of inhibition assay on solid media was used for determination of the antibacterial effects of films against *Escherichia coli* . The edible films were cut into 6-mm diameter discs and then placed on nutrient agar plates, which had been previously seeded with 0.2 ml of inoculums containing approximately 10^5 - 10^6 CFU/ml of tested bacteria. The plates were then incubated at 37°C for 24 h. After that, the plates were examined for 'zone of inhibition' on the film discs.

F. Assessment of the Antioxidant Properties of Edible Film Uwi Starch Incorporated with Ginger Essential Oil

The antioxidant activity of the film samples was evaluated using DPPH (2, 2-diphenyl-1-picrylhydrazyl) free radical scavenging assay [8]. Briefly, 3 mL of film solution were mixed with 1mL of 1 mM methanol solution of DPPH (Merck, Darmstadt, Germany). The mixture was homogenized using vortex and incubated in the dark at ambient temperature for 30 min. When the DPPH solution was mixed with the sample mixture acting as a hydrogen atom donor, a stable non radical form of DPPH is obtained with simultaneous change of the violet color to pale yellow. The absorbance was then measured at 517 nm. The percentage of DPPH free radical quenching activity was determined using the following equation:

DPPH scavenging effect %=

$$\frac{Abs\ \mathrm{DPPH} - Abs\ Extract}{Abs\ \mathrm{DPPH}} \times 100$$

where *Abs DPPH* is the absorbance value at 517 nm of the methanol solution of DPPH and Abs extract is the absorbance value at 517 nm for the sample extracts. Each sample was assayed at least three times.

G. Film Characterization

Test filmstrips (8 × 2.5 cm) were cut from preconditioned samples (23°C; 75% RH) and mounted between the grips of the probe A/TGT of the TA.XT2i texturometer (Stable Micro Systems, UK). The tests was conducted according to the ASTM D882-00 (2001) method [9]. Ten specimens was tested for the best antibacterial and antioxidant formulation. Average film thicknesses of the preconditioned samples (75% RH, 23°C) were obtained using a flat parallel surface micrometer with 1p.m resolution. Five measurements was taken at three different randomly selected positions.

III. Results and Discussion

From 6 kg ginger tuber gave 3.3 mL essential oil by using steam distillation method. Mayor component in ginger essential oil were gingerol, geraniol, zingiberene, zingerone paradol and shogaol Volatile oil that was consisted in ginger contain 1,2-dimetilbenzen, alpha-pinen, 1 methylene-4-isopropyl and 1-alpha-terpineol [4]. These molecules are belong to low and medium chain hydroxyl cyclic hydrocarbon compound.

The influence of the concentration of essential oil on the growth of bacteria towards the antibacterial activity was carried out using the disc method with various concentration of volatile oil from 0.5%; 1%; 1.5%; 2%; 2.5% and 3% (v/b). Incorporation ginger essential oil less than 0.5% in the film could not turn the uwi starch film in to antibacterial edible film. In this concentration the molecule which was trapped in the film was not enough to inhibit the growth of *Escherichia coli*. At more than 0.5% essential oil, the edible film start to exhibit a clear inhibitory zone indicated by the absence of bacterial growth around the film strips. The average area of the fully formed zones increased by increasing the concentration of incorporated essential oil in the film.

Antibacterial properties of low weight hydroxyl cyclic hydrocarbon compound is affected by it's capability to expansion the bacterial membrane. Result in a destabilization of the membrane and consequently the leakage the membrane and kill the bacteria [10]. The compounds consisted in ginger essential oil were belonged to group of low and medium hydroxyl cyclic hydrocarbon compound.

The maximum ginger essential oil (see Table I and Table II) can be incorporated into uwi starch edible film was 3%. At concentration more than 3%, the film lost it's mechanical properties, the edible film become wet and could not be peeled out from the plates even after oven drying for 20-24 h.

TABLE I: ANTIBACTERIAL ACTIVITY OF UWI STARCH EDIBLE FILM INCORPORATED WITH GINGER ESSENTIAL OIL

Bacteria	Ginger Essential oil (%v/w)	Observation at 24 h	
		Inhibitory zone (mm diameter±SD)	Contact area
Escherichia coli	0 (Control)	0	-
	0.5	0	+
	1.0	1.00±3.4	+
	1.5	2.73±4.2	+
	2.0	4.33±5.1	+
	2.5	7.90±4.8	+
	3.0	9.73±6.2	+

* Contact area is the part of agar on petri dish directly underneath film pieces.

DPPH scavenging assay was used to indicate antioxidant activity of the film. As the concentration of essential oil was increased, DPPH scavenging activity of the films increased significantly. In the films containing 3% essential oil, the antioxidant activity was increased 6.1 folds more than the control samples. Mayor compound of ginger extracts have antioxidant activity higher than vitamin E [4].

The uwi starch films with no essential oil showed some scavenging activity on DPPH (0.20%). It is assumed associated with the fact that free radicals can react with the

residual hydroxyl (-OH) groups of uwi starch to form stable macromolecule radicals, and the -OH groups can form (OH^{2+}) groups by absorbing a hydrogen ion from the solution [7]. However, results of this study indicated that incorporation of ginger essential oil in uwi starch films improved antioxidant activity of the film.

TABLE II: ANTIOXIDANT ACTIVITY OF UWI STARCH EDIBLE FILM INCORPORATED WITH GINGER ESSENTIAL OIL

Concentration of ginger essential oil in uwi starch film (%v/w)	% Inhibition of DPPH ±SD	
	30 minute incubation	90 minute incubation
0 (control)	0.2±5.3	0.5±4.6
0.5	5.68±3.4	5.8±5.3
1.0	11.8±4.7	13.2±3.6
1.5	15.2±6.2	15.8±5.4
2.0	19.3±4.2	21± 4.7
2.5	23.9±3.8	24.6±5.9
3.0	31.5±6.3	31.7± 4.6

Tensile strength, percent elongation and the thickness of uwi starch film containing 3% essential oil are 24.96 kPa, 20% and 0.3 mm respectively.

IV. CONCLUSION

For the first time, the association of uwi starch film and ginger essential oil allowed the films has antibacterial and radical scavenging activities. Incorporation of ginger essential oil into uwi starch film at level more than 0,5% led to a significant inhibitory effect to *Escherichia coli*. As the concentration increased, the zone of inhibition also increased. The greatest zone of inhibition with good mechanical properties was observed at 3% level against *Escherichia coli*. Incorporation ginger essential oil in to uwi starch can make uwi starch film to be radical scavenging edible film in range concentration 5%-3% v/w.

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