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Edible Film from Jack Bean Flour for Use as an Antioxidative Packaging Incorporating Extract of Green Tea

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***Abstract*— Addition of green tea's extract in edible film's matrix will improve film's functionality. The film will has antioxidant properties and can protect food from rancidity and discoloration. In this research film based component was jack bean flour which has high content of carbohydrate and protein. The film had good mechanical and physical characters. The aim of this research was studying effect of green tea's extract addition on phenolic, tannin concentration and antioxidant activity of *edible film*. Extract of green tea was prepared with variation of temperature (28°C, 50°C and 100°C). The result showed that increasing of extraction temperature would increase phenolic content, tannin concentration and antioxidant activity. When green tea extracted at 100°C phenolic concentration was 144.179 mg/g, tannin was 50.345 mg/g and antioksidant activity was 58.8% (DPPH inhibition). After the extract was incorporated in to *edible film*, total phenolic concentration of film was 113.544 mg/g, tannin was 41.842 mg/g and antioxidant activity was 45.22%. Edible film with green tea addition, showed ability in inhibiting rancidity of peanut oil whereas peroxide value and TBA (Thio Barbituric Acid) number of peanut oil was 11.5 meq O_2/kg and 0.13 ml/g after edible film immersion in 5 days. Peroxide value and TBA number of peanut oil without edible film immersion was 20.3 meq O_2/kg and 0.32 ml/g.**

***Keywords*— Phenolic, tanin, DPPH inhibition, peanut oil, peroxide value, TBA number**

I. INTRODUCTION

Consumers need in healthy and high quality of food intensified interest on edible film researchs. Edible films may offer an alternative to commercial packaging material especially for food products and would likely reduce packaging waste. Edible film can be produced from hydrocolloids include protein and carbohydrate.

Jack bean (Canavalia ensiformis) are non oilseed legume which has high content of carbohydrate and protein. Our previous research showed [1] edible film from jack beans protein concentrate had 6,92 x 10^{-3} Mpa of tensile strength, 2,22 mm of elongation, 0,11 mm of thickness, 14,79 % of soluble protein and 1,87 gr/mm^2/hr of water vapor transmition rate. Reference [2] shows edible film from jack bean flour had better physical and mechanical characters than film from protein concentrate.

Edible films are a viable means for incorporating food additives and other substances to enhance packaging performance. Antioxidants can be added to edible film to protect food from oxidative rancidity, degradation and discoloration. The two types of food antioxidants are acids (as well as their salts and esters) and phenolic compounds [3]. Antioxidant packaging can retard oxidative changes in packaged foods containing fatty components. Incorporation of synthetic antioxidant compounds, such as butylated hydroxytoluene and butylated hydroxyanisole, in high-density polyethylene has been shown to protect cereals from oxidation [4], [5]. However, because of a growing concern in food safety, there is interest in using natural antioxidant in the fabrication of the active packaging materials.

Among natural resources for phenolic compounds, green tea has the longest history in the world and used in about 160 countries everyday for drinking. Green tea is one of the the most popular beverages, besides coffee and cocoa [6]. Green tea is composed of about 30% of phenolic compounds (dry basis). Phenolic compounds have been well-known to have various excellent biological activities such as inhibition of oxidation. The inhibition effect of green tea phenolic on lipid oxidation was higher than that of the synthetic antioxidant, butylated hydroxytoluene (BHT) [7].

The main objective of this work was to study the effect of green tea extract addition on antioxidant properties of edible film from jack beans flour. This research would evaluate phenolic and tannin concentration, antioxidant activity and lipid rancidity inhibition. Edible film produce from this research was aimed as active packaging with antioxidant

properties in other can retard oxidative changes in packaged foods containing fatty components.

II. Materials And Methods

A. Materials

Jack beans (dry beans 8 – 9%), green tea optain from local market, glycerol (technical grade). Reagen and solvent obtain from Merck, Wako, Sigma and Riedel Dehaen.

B. Jack Bean Flour Preparation

Jack bean was immersed in water at room temperature for 24 h. The bean's pericrap was peeling before slice 2 mm thickness. Drying in cabinet drier at 60oC for 24 h was done after. The dry beans was crushed in dry blender and screened therefore 60 mesh particle size was obtained. Table 1 showed composition of jack beans flour that was analyzed according AOAC method [8].

C. Green Tea Extract Preparations

Green tea extract was made in 3 variation of extraction temperature for 10 minute. That were room temperature, 50oC and 100oC. 12 g of green tea was extracted in 500 ml aquadest. Filtered cloth was used to filter the extract in other clear solution was obtained. The extract would be used as solvent in edible film making.

TABLE I
Composition of Jack Beans Flour

Substances	Composition (%)
Water	9.20
Protein	27.24
Fat	4.32
Carbohydrate	56.74
Ash	2.50

D. Edible Film Preparation

10 g of jack beans flour was dispersed in 100 ml green tea extract whereas 2.5 g glycerol already added. Solution was stirred for 10 min and heated for 15 min in water bath (100^{o}C) until gel was formed. 10 g gel was casted on 10 x 10 cm2 porcelain plate and tempered in room temperature for 10 min. Edible film gel was dried in cabinet drier at 50^{o}C for 20 h than peeled from porcelain plate and stored. All films were conditioned in desiccators that already controlled with silica gels for 2 days prior to analysis. For preparation of edible film extract, 12 g (dry basis) of edible film was extracted in 500 ml aquadest. The film was crushed by stirrer and centrifuge to obtain clear filtrate.

E. Measurement of Phenolic Concentration

Phenolic concentration was analysed according to reference [9]. 0.1 ml extract was mixed with 1 ml ethanol, 5 ml aquadest and 0.5 ml follin-ciocalteu (50%) in borosilicate tubes, followed by vortex mixing and allowed to react for 5 min before adding 1 ml 5% Na_2CO_3 and allowed to stand 60 min at dark room temperature. Absorbance was measured at 725 nm. A standard curve for the measurements was prepared using galic acid.

F. Measurement of Tannin Concentration

Tanin concentration was assayed by protein precipitation method according to reference [10]. 1.6 ml extract was mixed with 2 ml Bovine serum albumin in centrifuge tube, followed by vortex mixing and allowed to react for 15 min to allow precipitation. The sediment was dilute with 4 ml sodium dedoxy sulfate and 1 ml 5 % $FeCl_3.6H_2O$ followed by vortex and allowed to stand 30 min at room temperature. Absorbance was measured at 510 nm. A standard curve for the measurements was prepared using tannic acid

G. Measurement of Antioxidant Activity as percentage DPPH (α,α, Diphenyl-β-picryl-hydrazyl) inhibition

Antioxidant activity was estimated according to reference [11]. An 0.01 ml of extract was mixed with 0.5 ml and 400 µM DPPH reagent in ethanol (final volume of 5 ml). The mixture was shaken vigorously and left in the dark at room temperature for 20 min. The absorbance of the resulting solution was measured at 517 nm. The capability to scavenge DPPH radicals was calculated by the following equation (blank is sample without extract addition)

$$\% \text{ inhibition} = \frac{\text{absorbance of blank} - \text{absorbance of sample}}{\text{absorbance of blank}} \quad (1)$$

H. Measurement of Rancidity Inhibition

Rancidity was tested using peanut oil. 10 x 10 cm^2 of edible film was immersed in peanut oil and storage for five days at 50^{o}C. Peroxide and tiobarbituric acid number of peanut oil was measured everyday to evaluate the degree of rancidity.

Measurement of peroxide number was evaluated according to reference [12]. A sample of 5 g was taken in to Erlenmeyer and added with 30 ml of acetic acid-chloroform (3:2) solution, 0.1 ml calcium iodine saturated solution. The mixed solution was allowed to react for 1 minute and 30 ml aquadest was added. Titrated the solution with 0.1 N $Na_2S_2O_3$ until yellow colour was disappeared then added with 0.5 ml of 1% starch solution followed by titration with 0.1 N $Na_2S_2O_3$ until blue colour disappeared. Peroxide number was presented in ml-equivalent to oxygen in 1000 g sample (meq O_2/kg).

Thiobarbituric acids (TBA) were measured during the storage of peanut oil according to the method of reference [13]. A sample of 0.05 – 0.1 g was taken from the cup, and added to 1 ml of TBA, 3 ml of ethanol and 1 ml isobutanol. The mixed solution was heated in boiling water for 15 min, and then centrifuged at 12,000 rpm for 5 min. The absorbance of the separated oil phase was determined at 532 nm using a UV-visible spectrophotometer to give the malondialdehyde (MDA) equivalent. A standard curve for the measurements was prepared using 1,1,3,3-tetraethoxypropane .

III. Results and Discussions

A. Phenolic Concentration

Green tea was extracted in three temperature variation. Table 2 shows phenolic concentration of green tea extract increase concomitant with extraction temperature. Extraction at 100^{o}C produces highest phenolic concentration.

According to reference [14] polyphenol is polar compound that easy soluble in water. The solubility will increase as extraction temperature increase.

The increase of phenolic compound in the extract results in increase of edible film phenolic concentration. Edible film without green tea extract addition has phenolic contains. According to [15] and [16] beans contain considerable amounts of phenolic compounds that possess varying degrees of antioxidant activity. Addition of green tea extract could increase phenolic concentration of edible film. There were decreases in levels of phenolic concentration after incorporated in edible film matrix. The degradation was 15.792%, 50.983% and 21.422% whereas green tea extracted at room temperature, 50°C and 100°C respectively.

B. Tannin Concentration

Tannins are a special group of phenolics, with high molecular weight, that occur in green tea. Tannin present in some food can react with proteins. Table 3 shows tannin concentration of green tea extract increase concomitant with extraction temperature. Extraction at 100°C produces highest tannin concentration. Tannin concentration in edible film increased as tannin concentration in the extract. Edible film without green tea extract had 1.315 mg/g of tannin concentration. This indicates that jack bean flour has tannin content.

There was degradation in tannin concentration in edible film than in green tea extract. The degradation decreased as tannin concentration in the extract increased. The degradation of tannin was greater than phenolic compounds. This was probably because tannin reacts with protein to form insoluble macromolecule [17].

TABLE II
CONCENTRATION OF PHENOLIC COMPOUND IN GREEN TEA EXTRACT AND EDIBLE FILM

Treatment	Green tea extract (mg/g)	Edible Film (mg/g)	% degradation
Control		15.418 ± 0.259	
Extraction at room temperature	35.903 ± 1.552	30.233 ± 0.776	15.792
Extraction at 50°C	94.888 ± 1.661	46.511 ± 0.259	50.983
Extraction at 100°C	144.499 ± 0.321	113,544 ± 1.423	21.422

Control ; edible film without green extract

TABLE III
CONCENTRATION OF TANNINS IN GREEN TEA EXTRACT AND EDIBLE FILM

Treatment	Green tea extract (mg/g)	Edible Film (mg/g)	% degradation
Control		1.315 ± 0.155	
Extraction at room temperature	34.917 ± 0.278	4.760 ± 0.031	86.367
Extraction at 50°C	39.747 ± 0.309	8.518 ± 0.031	78.569
Extraction at 100°C	50.346 ± 0.154	41.845 ± 1.113	16.885

Control ; edible film without green extract

C. Antioxidant Activity

The proton-radical scavenging action is known as an important mechanism of antioxidation. DPPH was used to determine the proton-radical scavenging action of the extract because it possesses a proton free radical and shows a characteristic absorption at 517 nm. The purple colour of the DPPH solution fades rapidly when it encounters proton-radical scavengers [18]. In this research, antioxidant activity was presented as percentage of DPPH inhibition.

Table 4 shows, antioxidant activity of green tea extract and edible film increase with extraction temperature. The highest DPPH inhibition was obtained at 100°C. There was degradation in edible film antioxidant activity than in green tea extract. This phenomenon could be attributed to decrease of phenolic concentration. Phenolic compound in green tea has been shown to be potent antioxidants in many chemical and biochemical studies [19]. Edible film without green tea extract had antioxidant activity due to jack bean flour perhaps, contain phenolic compound.

TABLE IV
ANTIOXIDANT ACTIVITY OF GREEN TEA EXTRACT AND EDIBLE FILM

Treatment	Green tea extract (mg/g)	Edible Film (mg/g)	% degradation
Control		16.400 ± 2.900	
Extraction at room temperature	26.900 ± 3.200	18.200 ± 2.500	32.343
Extraction at 50°C	44.900 ± 1.800	25.700 ± 2.400	42.762
Extraction at 100°C	58.800 ± 1.600	40.700 ± 2.700	30.782

Control ; edible film without green extract

D. Inhibition of Lipid Oxydation

The inhibition effect of edible film on lipid oxidation was measured using peanut oil. According to reference [20] phenolic compounds from green tea has an antioxidative properties when tested using fish oil. Peanut has 50% – 55% oil whereas 30% - 35% linoleic acid and 45% - 50% oleic acid it can lead to oxidative rancidity [21]. In this research rancidity was evaluated with peroxide number and Tiobarbituric acid (TBA) presence. Peroxide is intermediate product of rancidity reaction and TBA is final product that cause rancid odor.

There was decrease in peroxide value after edible film immersed in peanut oil. Levelling of peroxide number increased as increase of extraction temperature. This was due to increase of phenolic concentration that has antioxidant activity. After storage at 50°C for five days the highest peroxide number was possessed by peanut oil without edible film immersion whereas the lowest was immersion of edible film with green tea extracted at 100°C (Table 5).

The peroxide value of peanut oil prior storage was 3 meq /1000g. Table 5 shows there are no increase of peroxide value after peanut oil immersed with edible film that already added with green tea extract that extracted at 100°C. These results are somewhat same with a report by reference [22] where the use of catechins from green tea extract could maintain peroxide value until the first day of storage. According to reference [23] maximum peroxide value that indicates the oil does not deteriorate is 10 meq O_2/kg. Edible film that already added with green tea which had been extracted at 100°C could enhance peanut oil from rancidity until four days storage.

TABLE V.
CHANGE OF PEROXIDE VALUE OF PEANUT OIL DURING STORAGE FOR FIVE DAYS AT 50OC

Treatment	Peroxide value (meq O_2/kg)			
	Second day	Third day	Fourth day	Fifth day
Control	10.5 ± 0.7	14.8 ± 3.2	15.5 ± 3.5	20.3 ± 5.3
Edible film without green tea extract	10 ± 0,1	14 ± 2.8	15 ± 1.4	17 ± 0,1
Edible film with green tea extract addition, extracted at room temperature	5.5 ± 2.1	12.3 ± 3.9	15 ± 1.4	15.8 ± 0.4
Edible film with green tea extract addition, extracted at 50°C	5 ± 1.4	11 ± 2.8	13.5 ± 0.7	15 ± 1.4
Edible film with green tea extract addition, extracted at 100°C	3 ± 0.1	7.5 ± 2.1	9.5 ± 3.5	11.5 ± 2.1

Note: Control: peanut oil without edible film immertion. Peroxide number of peanut oil prior to storage was 3 (meg/1000g)

TABLE VI
CHANGE OF TBA NUMBER OF PEANUT OIL DURING STORAGE FOR FIVE DAYS AT 50OC

Treatment	TBA number (ml/gr)			
	Second day	Third day	Fourth day	Fifth day
Control	0.09 ± 0.01	0.23 ± 0.02	0.27 ± 0.03	0.32 ± 0.03
Edible film without green tea extract	0.07 ± 0.02	0.18 ± 0.04	0.23 ± 0.04	0.30 ± 0.02
Edible film with green tea extract addition, extracted at room temperature	0.04 ± 0.01	0.12 ± 0.03	0.17 ± 0.01	0.24 ± 0.03
Edible film with green tea extract addition, extracted at 50°C	0.04 ± 0.01	0.11 ± 0.03	0.14 ± 0.01	0.16 ± 0.06
Edible film with green tea extract addition, extracted at 100°C	0.03 ± 0.01	0.10 ± 0.02	0.11 ± 0.01	0.13 ± 0.08

Note: Control: peanut oil without edible film immertion

Malonaldehyde is aldehyde ($C_3H_4O_2$) compounds that is formed during oxidation of unsaturated fatty acids and can be used to evaluate oxidative rancidity. Malonaldehyde concentration during rancidity can be measured using TBA. Malonaldehyde and TBA react to form a complex with red colour [24]. According to reference [23], TBA number threshold that shows the peanut oil has been rancid is 0.76 (ml/gr).

Table 6 shows that during five days storage all treatment indicated TBA number under 0.76 (ml/g) in other words the peanut oil had not been rancid. Probably, this is due to observation was conduct only in four days storage and malonaldehyde produce was limit. In rancidity reaction peroxide degrade to hydroperoxide and short chain hydrocarbon compound include aldehyde and keton that will produce rancid flavour. Increasing of TBA number usually follow with decrease of peroxide value [23].

Immersion of edible film that had been added with green tea had levelling off TBA number which increasing of green tea extraction temperature would decrease TBA number. This was due to increase of phenolic concentration that has antioxidant activity. The highest TBA number was possessed by peanut oil without edible film immersion after storage for five days. The result of this research shows that incorporation of green tea extract in edible film provide positive effect in retardation of lipid oxidation

IV. CONCLUSIONS

Antioxidant edible film was fabricated from jack bean flour with green tea extract addition whereas the extraction temperature was varied. Increasing of extraction temperature increased phenolic and tannin concentration resulting increased antioxidant activity. When green tea extracted at 100°C total phenolic concentration was 144.179 mg/g, tannin was 50.345 mg/g and antioksidant activity was 58.8% (DPPH inhibition). After the extract was incorporated in to edible film total phenolic concentration of film was 113.544 mg/g, tannin was 41.842 mg/g and antioxidant activity was 45.22%. During five days storage of peanut oil that immersed with edible film showed rancidity inhibition that evaluated with peroxide and TBA number. The inhibition increased as extraction temperature increased. Peroxide value and TBA (Thio Barbituric Acid) number of peanut oil was 11.5 meq O_2/kg and 0.13 ml/gr after immersion of edible film in 5 days. Peroxide value and TBA number of peanut oil without edible film immersion was 20.3 meq O_2/kg and 0,32 ml/gr. Edible film containing green tea extract shows potential as active antioxidant packaging that can extend shelf life of fatty foods

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