Singlet Oxygen Quenching Effect of Andaliman (*Zanthoxylum acanthopodium* DC.) Extracts in Light-Induced Lipid Oxidation

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ABSTRACT

Addition of andaliman extract has been shown to provide increased protection from lipid oxidation during cooking. Although andaliman extract has reported as an effective antioxidant in autoxidized system, no study have been published on its effects on light-induced oxidation or photosensitized oxidation. The objective of this research is to determine singlet oxygen quenching effects of andaliman extract on erythrosine-sensitized photooxidation of linoleic acid and in palm oil. Freeze dried ground andaliman fruit was sequentially extracted first using hexane, followed by acetone, and finally the residue was extracted by ethanol. Each of these three extracts were added to light-induced lipid peroxidation in a reaction system containing linoleic acid or palm oil with the presence of erythrosine as a photo sensitizer. Upon the exposure of fluorescent light at 4000 lux singlet oxygen is presumably formed from triplet oxygen which initiate the lipid peroxidation process. The sequential extraction of andaliman resulted in ethanolic extract containing phenolic compounds consistently show antioxidative activity presumably through singlet oxygen quenching in light-induced lipid peroxidation in either linoleic acid or RBD palm oil reaction mixture containing erythrosine as a photosensitizer. The andaliman hexane and acetone extracts may contain not only phenolic compounds, but also trace amount of plant pigments which resulted in stronger lipid peroxidation effect than the antioxidative effect of phenolic substances, especially at higher level of addition.

Keywords : Andaliman extracts, lipid peroxidation, singlet oxygen quenching

INTRODUCTION

The oxidation reaction of food components leads to nutritional losses, produces undesirable flavor, and other food quality deterioration which make the food not acceptable to consumers. This oxidation reaction can be initiated by either diradical triple oxygen or non-radical singlet oxygen. The formation of singlet oxygen in food can resulted from triplet oxygen by photosensitized reactions. The formation of singlet oxygen in foods ant its quenching mechanisms have been the focus of many researches in the last twenty years (Min and Boff, 2002). This is due the fact that the rate of singlet oxygen oxidation is at least a thousand times faster than the atmospheric triplet oxygen oxidation. Singlet oxygen is produced by photosensitizers in the presence of light and triplet oxygen. Chlorophylls, pheophytin, myoglobin derivatives, riboflavin, erythrosine and methylene blue are reportedly efficient photochemical sensitizers for the formation of singlet oxygen (Whang and Peng, 1988; Yang, *et*

al., 2002). Rawls and Van Santen (1970) reported that singlet oxygen participated in the initiation step of oil oxidation and the reaction rate of singlet oxygen with linoleic acid is about 1450 times greater than that of triplet oxygen.

The undesirable photosensitized lipid oxidation can be reduce by quenching singlet oxygen. Tocopherols, carotenoids, and ascorbic acid can be used for the practical reduction of singlet oxygen oxidation of oils and other oil-soluble components (Lee *et al.*, 1997). Tocopherols can be also act antioxidant in autocatalytic condition but, their singlet oxygen-quenching abilities are not effective as the carotenoid. b-carotene is an active $^1O_2$ quencher in soybean oil (Lee and Min, 1990). The bleached and unbleached rosemary oleoresins had quenching effect in the soybean oil on light-sensitised oxidation (Hall and Cupppett, 1993).

The nature antioxidant compounds can be obtained at part of rich plant phenolics was generally found in spices and aromatic plant in tropical. The using of natural product had been long used ancestor of generation to generation as additive for food industry, besides the natural product was easy to obtained, safety consumtion, and abudant. One of the potensial sources of natural product from Indonesia did not most developed, that was wild plant that used as spices, for example andaliman fruit. This fruits is less information about fungtion, characteristic and merit of a plant as spices.

Recently, the use of spice as antioxidant in processed food is a promising alternative to the use of synthetic antioxidant. One of the potential source of traditional spice from North Sumatera as antioxidant is andaliman fruit (*Zanthoxylum acanthopodium DC.*). It has a strong specific odor and pungent taste like fresh citrus and bitter taste that stimulate saliva production. As a spices, andaliman gives flavour such as *umami,* also it is used to preserve cooked meat for few days against rancidity and spoilage. In addition, andaliman fruit could remove odor of fish or meat.

Wijaya (1999) found that andaliman extracts is effective in stabilizing system of linoleic acid emulsi againts oxidation. Edi Suryanto and Rorong (2001) have been reported that andaliman oleoresin exhibits antioxidant activity greater than a-tocoferol in peanut oil. Addition of andaliman extract has been shown scavenging activity in DPPH radikal system (Edi Suryanto *et al.*, 2004). Than andaliman extract has been shown to provide increased protection from lipid oxidation during cooking. Althought andaliman extract has proven to be an effective antioxidant in autoxidized system, no study have been published on its effects on light-induced oxidation photosensitized oxidation. In addition, the specific compounds responsible for antioxidant active components in andaliman extracts having singlet oxygen quenching effect has not been published.

The objective of this research was determine singlet oxigen quenching effects of andaliman extract on chlorophyll-sensitized photooxidation of linoleic acid and in RBD palm oil.

MATERIALS AND METHODS

Materials

Andaliman fruit obtained from a local market at North Sumatra will be cleaned sample, freeze dried and ground to 40 mesh. Palm oil (refined, bleached, and deodorized, RBD) were obtained Bitung, North Sulawesi. Erythrosine were purchased from local market. Hexane, acetone, ethanol, chloroform, sodium thiosulphate, acetic acid, potassium iodide, amylum, sodium carbonate, Folin-Ciocalteu were purchased from Merck (Darmstadt, Germany).

Sample preparation

Andaliman fruit were cleaned, freeze dried and ground to a fine powder (100 g) were extracted sequentially with 500 mL of hexane, acetone and ethanol for 24 hours After filtration, residue was extracted once more with an additional 500 mL hexane. The hexane extracts were then pooled and saved. Similary, the acetone extract and ethanol were obtained by extracting the residue remaining after twice extraction. The solvents in the three extracts were then evaporated using a vacum evaporator. The resulting three extratcs were then weighed and store at –20°C until use.

Determination of total phenol content of andaliman extracts

The content of total phenol in andaliman extracts was measured by Folin-Ciocalteu assay (Hung and Yen, 2002). The absorbance of extracts was read at 750 nm in a Shimadzu UV 1601 UV-Vis Spectrophotometer. Results were expressed as mg of gallic acid/100 g extract.

Effects of andaliman extract on erythrosine-sensitized photooxidation

Procedure is method according to Lee *et al.* (1997) with minor modification. To study the effects of andaliman extract on photosensitized oxidation of linoleic acid, sample of 0, 500, 1000, and 1500 ppm (wt/vol) andaliman extract in 0,05 M of linoleic acid (for palm oil 10.0 %) were prepared in methanol that also contained 100 ppm (wt/vol) erythrosine as a photosensitizer. Samples containing 500 ppm (wt/vol) a-tocopherol were used as a positive control in the system. For palm oil were prepared in a solvent mixture (acetone/methanol, 4:1, vol/vol). Ten mL of sample was transferred into a 30 mL serum bottle. The bottles were sealed aie-tight with Teflon septa and aluminium caps and then were placed in the light box. The light intensity at the sample level was 4,000 lux, and temperature was room temperature. The experiment was carried out in duplicate.

Light storage conditions for the study

The method of Lee and Min (1988) was used. The light storage box consisted of two rectangular chambers : a glass chamber (60 cm x 30 cm x 50 cm) for sample storage and the wooden box (70 cm x 50 cm x 60 cm) for light sources to the glass chamber was 12 cm. Samples were placed on the wire netting which was 10 cm above the bottom of glass chamber. The light sources, four Sylvania 15 watt cool white fluorescence lamps, were placed on the 4,000 lux. The temperature of the light storage box was kept constant at room temperature.

Determination of oxidation of oil samples

Oxidation stability of linoleic acid and palm oil was determined by measuring the peroxide value every 5 hours according to the AOCS (1990) method. Peroxide value will be mesuared every time interval of 1hours. This activity was planed to be completed in one year. Extraction of andaliman fruit and quenching effects determination will be done at Chemistry and Biochemistry Laboratory of Food and Agricultural Product Technology, Faculty of Agricultural Technology Gadjah Mada University, Yogyakarta

Statistical analysis

Experimental data were analyzed by the analysis of variance (ANOVA) and the significant differences among means were determined by Duncan's multiple range test (DMRT) using SPSS version 10 for windows and $p<0.05$ was considered to be statistically significant. Analysis were performed for the response variables of peroxide value.

RESULTS AND DISCUSSION

Effect of solvent on yield

The extraction was done sequentially with some solvent that posseses different polarity to separate compounds in andaliman fruit. Extraction using hexane could dissolve less polar compounds, whereas acetone could dissolve semi polar compounds and the use of ethanol would recover the more polar compounds. The yield of hexane extract (HE) gave higher yields from sequential extraction than ethanol extract (EE) and acetone extract (AE). Hexane extract had the highest yield, due to the essential oil and lipid were probably extracted.

Table 1. Extraction yield and total phenol content

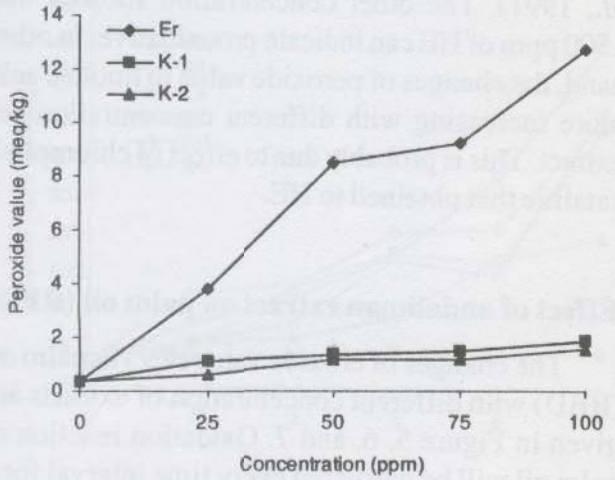
Andaliman extract	Yield (mg/g of andaliman extract)	Total phenol content (g/100 g of extract)
Hexane extract (HE)	78,06 ± 2,48	2,77 ± 0,58
Acetone extract (AE)	31,75 ± 5,56	9,10 ± 0,03
Ethanol extract (EE)	69,98 ± 3,36	12,53 ± 0,59

Mean value of three measurements ± SD

Choice of solvent and extraction procedures were also considered. Presence of chlorophyll in hexane and acetone extracts was responsible for the deep-green coloration. Chlorophyll and their derivatives had been reported effective as sensitizer for production of singlet oxygen under light illumination (Rawls and Van Santen, 1970). Hexane had been considered the solvent of choice for further extraction, due to the essential oil, oleoresin and a posible presence of fatty acids of andaliman fruit can be removed by this solvent.

Effect of erythrosine on linoleic acid

Effect of erythrosine at different concentrations on linoleic acid on peroxide value for 0.03 M of linoleic acid under light (4000 lux) exposure for 5 hours in room temperature are shown in Figure 1. Effect of concentrations at 25, 50, 75, and 100 ppm showed increased changes in peroxide value during expose to light for 5 hours ($p<0,05$). However, changes in peroxide value of without erythrosine not showed increasing significantly. This result proved that without erythrosine couldn't produced oxygen singlet of triplet oxygen, althought the sample under light exposure for 5 hours.



Figur 1. Changes in peroxide value of linoleic acid (0,03 M) with different concentration erythrosine during exposure to light (4000 lux) for 5 hours (Er: erythrosine, K-1: light without erythrosine and K-2: dark with erythrosine).

Min and Bob (2002) had reported that singlet oxygen could be produced triplet oxygen with precence sensitizer and light. There is sensitizer as erythrosine can increase oxidation reaction, due to sensitizer possess ability for absoption of light energy. The sensitizer can transfer its energy to the most stable triplet oxygen and converting it to a higher energy level, singlet oxygen. It would then attack the double bonds in unsaturated fatty acids (Khan and Shahidi, 2002). The primary products of photooxidation is hidroperoxide, for example photooxidation on linoleic acid can produce hidroperoxide of position of conjugated double bond at 9-OOH and 13-OOH and non conjugated at 10-OOH and 12-OOH. The contrary, the autooxidation of triplet oxygen only produce conjugated hidroperoxide at 9-OOH and 13-OOH (Neff and Frangkel, 1980). This hidroperoxides are subsequently decomposed to off-flavours volatiles (Warner and Frankel, 1987; Jung *et al.*, 1991).

Effect of andaliman extract on linoleic acid

Effect of variation concentration from hexane extract (HE), acetone extract (AE), and ethanol extract (EE) on peroxide value for 0.05 M of linoleic acid in methanol for expose to light in room temperature (Figure 2, 3, and 4). Effect of EE concentration from extracts showed significant on sample peroxide value (PV) for 5 hours exposure to light in room temperature, for example EE concentration of 500, 1000, and 1500 ppm, sample peroxide is the similar wherease at concentrations of 500, 1000, and 1500 ppm HE can enhance peroxide value. However, AE at concentration of 1500 ppm indicate increasing peroxide value. A probably explanation, photosensitizer of erythrosine may product singlet oxygen from triplet oxygen when there is exposed to light. Data of peroxide value in linoleic acid is relative similar for 5 hours to expose light without sensitizer. The formation of singhlet oxygen product by photosensitizer that accelarated lipid oxidation. Therefore, erythrosine may act as photosensitizer for accelarate of linoleic acid oxidation in model system to expose light (Yang *et al.*, 2002).

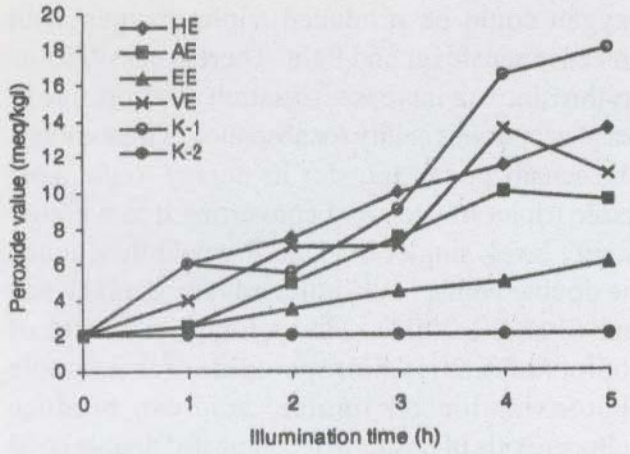


Figure 2. Effect of 500 ppm andaliman extract on oxidation of singlet oxygen in linoleic acid for 5 hours (VE: a-tocopherol; K-1: light with erythrosine and K-2: light without erythrosine).

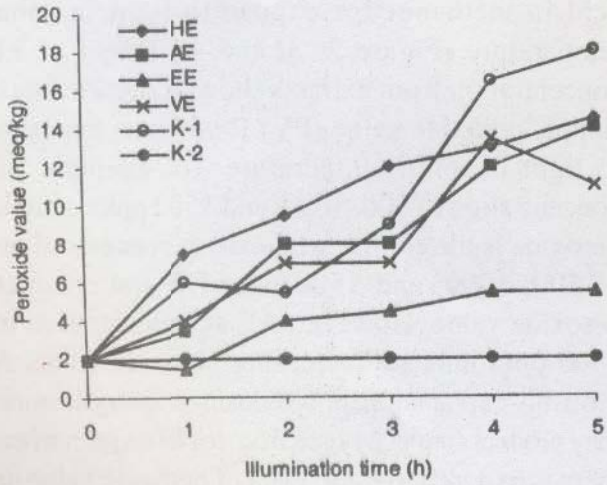


Figure 3. Effect of 1000 ppm andaliman extract on oxidation of singlet oxygen in linoleic acid for 5 hours (VE: a-tocopherol; K-1: light with erythrosine and K-2: light without erythrosine).

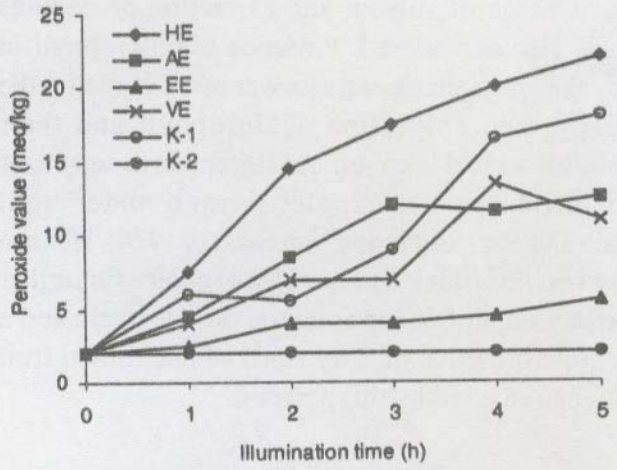


Figure 4. Effect of 1500 ppm andaliman extract on oxidation of singlet oxygen in linoleic acid for 5 hours (VE: a-tocopherol; K-1: light with erythrosine and K-2: light without erythrosine).

Effect of concentrations present of 500, 1000, and 1500 ppm AE in model system showed that different significantly with 1000 ppm a-tocopherol. Although the differences in concentrations of 500 ppm and 1000 ppm AE is not significantly with 1000 ppm of a-tocopherol. Vitamine E (VE) is nature compound to used inhibitor in lipid oxidation for food. Therefore, a-tocopherol had reported as quencher of singlet oxygen in soy been oil (Jung *et al.*, 1991). The other concentration showed that 1500 ppm of HE can indicate prooxidative. In other hand, the changes of peroxide value in linoleic acid more increasing with different concentrations of extract. This is probably due to effect of chlorophyll catalitic that obtained to HE.

Effect of andaliman extract on palm oil (RBD)

The changes of eroxide value (PV) in palm oil (RBD) with different concentration of extracts are given in Figure 5, 6, and 7. Oxidation reaction of palm oil will be mesuared every time interval for 5 hours at room temperature and stored exposed to light of 4000 lux. Peroxide value increase in palm oil that addition erythrosine as photosensitizer of 45 meq/kg, whereas palm oil without sensitizer was 4.5 meq/kg.

The curve in Figure 5, 6, and 7 showed that EE at concentrations of 500, 1000, 1500 and 500 ppm of AE could be increased rate of oxidation for peroxide. However, fourth the extracts is not different with tocopherol ($p<0.05$) as the control positive in this study. At concentrations of 500, 1000, and 1500 ppm showed that increasing peroxide value and as prooxidative. This could be seem from peroxide value of HE is not different with palm oil that addition erythrosine ($p<0.05$). This is as well as for 1000 ppm and 1500 ppm of AE. This explanation caused when to extract with hexane and acetone, perhaps chlorophyll extracted in both the solvent, whereas at EE was not extracted. In this study has not done determination of chlorophyll content for every the extracts. The chlorophyll is sensitizer which further probably can act synergistically with erythrosine to generate singlet oxygen so that oxidation rete of peroxide formation is increase. Therefore, dechlorophyll of extracts as nature antioxidant resource in protect of unsaturated fatty acid could be prolong storage.

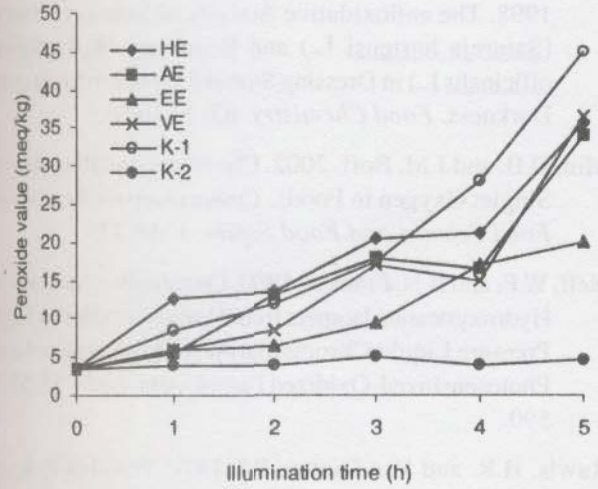


Figure 5. Effect of 500 ppm andaliman extract on oxidation of singlet oxygen in RBD palm oil for 5 hours (VE: a-tocopherol; K-1: light with erythrosine and K-2: light without erythrosine).

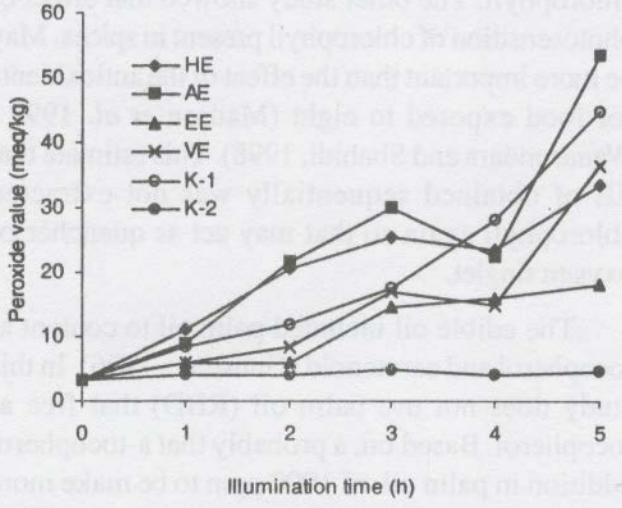


Figure 6. Effect of 1000 ppm andaliman extract on oxidation of singlet oxygen in RBD palm oil for 5 hours (VE: a-tocopherol; K-1: light with erythrosine and K-2: light without erythrosine).

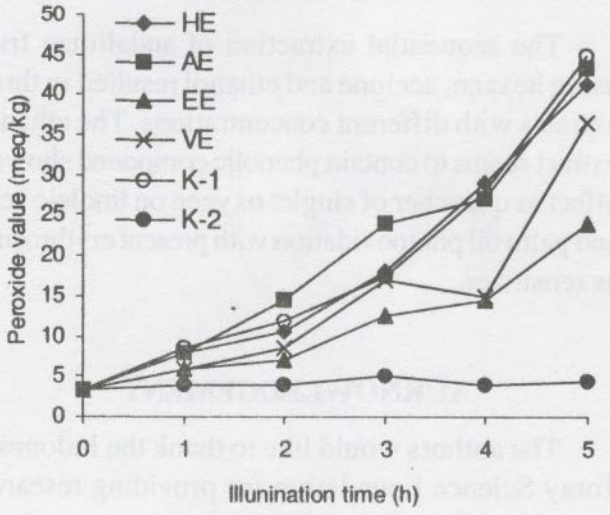


Figure 7. Effect of 1500 ppm andaliman extract on oxidation of singlet oxygen in RBD palm oil for 5 hours (VE: a-tocopherol; K-1: light with erythrosine and K-2: light without erythrosine).

Effect of prooxidative from HE and AE could be caused to present chlorophyll. Endo *et al.* (1985) had reported that chlorophyll and its derivate can promote lipid oxidation for storage. Wanasundara and Shahidi (1998), in its study showed that etanol-aqueous extract possesed effect of prooxidant in edible oil, perhaps caused effect of catalitic from

chlorophyll. The other study showed that effect of photosensition of chlorophyll present in spices. May be more important than the effect of the antioxidants for food exposed to eight (Madsen *et al.* 1998 ; Wanasundara and Shahidi, 1998). This estimate that EE of obtaincd sequentially was not extracted chlorophyll again so that may act as quencher of oxygen singlet.

The edible oil included palm oil to content a-tocopherol and carotenoid (Gunstone, 1996). In this study does not use palm oil (RBD) that free a-tocopherol. Based on, a probably that a-tocopherol addition in palm oil of 1000 ppm to be make more effective as quencher of oxygen singlet. Addition for, it may be phenolics compound to obtain in EE and a-tocopherol are naturally in palm oil (RBD) possesse effect of synergismcally.

CONCLUSION

The sequential extraction of andaliman fruit using hexane, acetone and ethanol resulted in three extracts with different concentrations. The ethanol extract seems to contain phenolic compound showed effect as quencher of singlet oxygen on linoleic acid and palm oil photooxidation with present erythrosine as sensitizer.

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