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Research Article

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Utilization of the phenolic compounds from the fruit of *Toona Sureni* (blume) Merr as an antioxidant in coconut oil

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ABSTRACT

This research has been conducted on the use of phenolic compounds from the fruit extracts of Toona Sureni as an antioxidant bioactive materials in preventing the oxidation of coconut oil. The study was done in three path ways, i.e. extraction of phenolic compound from the fruits of Toona Sureni, coconut oil extraction, and oil quality measuring by means of determining peroxide value (PV) and free fatty acids (FFA). In oil quality testing, sample was heated at 125 ºC for 3 hours. That sample were analyzed by determining peroxide value (PV) and free fatty acids (FFA). The results showed that the addition of extract to the oil that was heated for 3 hours at temperature of 125 ºC could inhibit the increase of peroxide value and free fatty acids. The inhibiratory of Toona Sureni's extract to peroxide value was effective at 200 ppm of concentration, 0.96 meq/kg. Antioxidant activity of the extract was expressed effectively by inhibihatory the increase of free fatty acids in addition 150 ppm of extract, 0.19%.

Keywords: antioxidant, free fatty acids, peroxide value, phenolic compounds.

INTRODUCTION

Antioxidants are needed to prevent some of the damage of vegetable oils by interact with and stabilize free radical that can be oxidated to produce flavor and an unpleasant smell, known as rancidity. Antioxidants commonly added to food products are synthetic antioxidants such as butylatedhydroxyanisole (BHA), butylatedhydroxytoluene (BHT), tert-butyl hydroquinone (TBHQ), and propyl gallic (PG). The antioxidants are more often used as an antioxidant cooking oil because it is cheap and quite effective. Recently, using synthetic antioxidants began to get a negative response because of it's toxic potential in the body [1-2], and the toxicity effect that can lead sweelingof the heart and affect the activity of liver enzymes [3-4].

The tendencyto replace the synthetic antioxidants with natural antioxidants hasincreased[5]. Therefore, using of natural antioxidants as substituent the synthetic antioxidant preferably because it is believed more healthy. Some of researches indicatedthat natural antioxidants like phenolic compounds also showed the effect as an antioxidant in edible oil [6-9].

Toonasureni (Blume) Merr is one of species in the *Meliaceae* family. This plants are traditionally used for medicinal herb e.g.diarrhea, dysentery, fever, and swelling of the spleen [10]. Research on*Toonasureni*revealed that plants contain phenolic compounds that act as antioxidants [11], and antibacterial activities [12].

In this research, we studied on the effectiveness of the use of phenolic compounds extracts from the fruit of *Toona sureni* as an antioxidant in coconut oil.

EXPERIMENTAL SECTION

Plant Material
The fruit of *Toona sureni* (Blume) Merr that were used in this research were collected in Padang and were identified in Andalas University Herbarium, Padang, with specimen M.Taufik Ekaprasada, 0107 (ANDA. Fr). The coconut oil was fermentation extracted in Microbiology Laboratory.

Chemicals
Chemicals that were used are: TLC plats (silica gel 60 F254 Merck), silica gel E.Merck 7733, *n*-hexane, acetone, methanol, alcohol, HCl 0.1 N in alcohol, KOH 0.1 N in alcohol, ethyl acetate, TBHQ, BHT, chloroform, KI, $Na_2S_2O_3$, phenolphtalein, starch, $K_2Cr_2O_7$, and aquadest.

Figure 1a: ***Toonasureni*** **fruit**

Figure 1b: ***Toonasureni*** **plant**

Procedure:
Extraction and Purifying Phenolic Compounds*:*
One point five kilograms (1.5 kg) air-dried and powdered fruits were macerated with 2 x 6 L of *n*-hexane for 2 days at room temperature. After filtration, the residu was macerated with 3 x 4 L of acetone for 3 days and 2 x 4 L of methanol for 5 days. Each fractions was concentrated with rotary evaporator.

Methanol extract (15 g) was eluted in column chromatography (silica gel) by increasing percentage of ethyl acetate in n-hexane (100:0 up to 0:100 with 300 mL of each of them) and ethyl acetate:methanol (90:10 (300 mL)). Fraction with the same Rf on TLC were combined and rechromatographed on the silica gel column by increasing percentage of ethyl acetate in n-hexane, and then recrystalized from n-hexane to produce white needle crystals.

Detection Method:
Preparation sampel for detection the quality of coconut oil (PV and FFA) was done by using three kind of samples. Sample without antioxidant as a control, 2 samples with addition synthetic antioxidant (100 ppm of BHT and 180 ppm of TBHQ), and the last, sample that was added extract of *Toona sureni* (100 ppm, 150 ppm, 200 ppm) as the natural antioxidant. 25 mL of coconut oil with synthetic antioxidant or natural antioxidant or none at all was heated at 125 ºC temperature for 3 hours. For each sample, Free Fatty Acids (FFA) content was assayed, alongside Peroxide Value (PV).

Peroxide Value*:*
Oil sample (2.5 g) was put into 250 mL of erlenmeyer flask and diluted with 15 mL acetic acid : chloroform (3:2) while shaking. Saturated potassium iodida (0.5 mL) was added into the solution. The solution was placed for one minutes then 15 mL aquadest was poured into it. The mixing was titrated with 0.05 N $Na_2S_2O_3$ until yellow colour almost gone. Starch solution (1%, 0.5 mL) was added immediately then the titration was being continued until the blue colour just dissapears. Peroxide value was ditermined by using this equation:

$$PV = \frac{\text{ml Na2S2O3 x N Na2S2O3 x 1000}}{\text{samples weight (g)}}$$

Free Fatty Acids*:*
Preparing a neutralizing alcohol by boiling 100 mL of alcohol in erlenmeyer flask, was then added 0.5 mL of phenolphtalein. After cooling until 70 ºC temperature, neutralized with 0.1 N potassium hydroxide in alcohol.

Five grams of oil samples was diluted in neutralizing alcohol, then boiled for 30 minutes. If the solution wasn't alkalis, it was cooled until 70 ^{o}C temperature was then titrated with 0.1 N KOH in alcohol until the pink colour was made.
Equation:

$$FFA = \frac{VxNx\ 0.2}{W} x\ 100\%$$

Where,V is the volume in mililitres, of 0.1 N KOH. N is normality of KOH. W is the weight in grams, of oil samples. Then, 0.2 is accorded by molecular weight of lauric acid

RESULTS AND DISCUSSION

Extraction and Purifying Phenolic Compounds:
Isolation of chemical compound from methanol fraction were obtained 289.4 mg of white needle crystals. Identification of the compound by using $FeCl_3$ produced dark greenish solution that showed it is phenolic compound. Detection with thin layer chromatography (TLC) using n-hexane:ethyl acetate = 3:7 (v/v) as an eluent produced one spot at Rf = 0.625. This spot has the same Rf with methyl gallate that had been reported earlier [11].

Peroxide Value:
The result of peroxide value test on 6 samples were obtained in Table 1. and Figure 1.Table 1. showed that peroxide value of coconut oil that heated without addition of antioxidant was 92.91 meq/Kg. This value is exceed of the limited peroxide value that allowed by SNI (2 meq/Kg). Addition of antioxidant to coconut oil that had been heated for 3 hours inhibited the increasing of peroxide value. Inhibitatory of extract more effective than synthetic antioxidant (BHT and TBHQ) and 200 ppm of *Toona sureni* is optimum concentration to inhibite the increasing of peroxide value. According to Figure 1, only 200 ppm of extract of *Toona sureni* that has peroxide value lower than maximal limited of SNI. It cause the higher of peroxide value in oil sample that was used. Peroxide is produced by oxidation of unsaturated fatty acid in coconut oil, both of trigliserides and free fatty acids. It cause by many factor, heated process is one of them.

Another important parameter used to assess the quality of coconut oil is the peroxide value which is an indicator of the level of lipid peroxidation or oxidative degradation. In this process involving unsaturated fatty acids, specially reactive hydrogen atoms from methylene ($-CH_2-$) groups adjacent to double bonds undergo a chain reaction mechanism involving free radicals as intermediates and generating lipid peroxides as end products. These lipid peroxides later undergo additional chain cleavage at the level of the hydroperoxide group to form secondary oxidation products such as short chain aldehydes and products bearing ketone, epoxy or alcohol groups responsible for the rancid smell and taste of the oil[13]. Peroxide value is used to assess the stability or rancidity of fats by measuring the amount of lipid peroxides and hydroperoxides formed during the initial stages of oxidation and thus, estimate to which extent spoilage of a dietary oil (expressed by the level of rancidity) has advanced. Beside these visible harmful effects on the sensory quality of the oil, peroxidation also makes the oil dangerous for human health, as the free radicals generated by this process are proven to be carcinogenic.

Table 1. The influence of addition synthetic antioxidant and extract of *Toona sureni* to peroxide value of coconut oil after 3 hours heating at 125 ^{o}C temperature.

Antioxidant	Concentration	Peroxide Value (meq/Kg)^{a}
BHT	100 ppm	52.44
TBHQ	180 ppm	21.25
Extract	100 ppm	52.44
Extract	150 ppm	46.25
Extract	200 ppm	0.96
Without antioxidant	-	92.91

a: SNI specification for maximal peroxide value that allowed in coconut oil is 2 meq/Kg

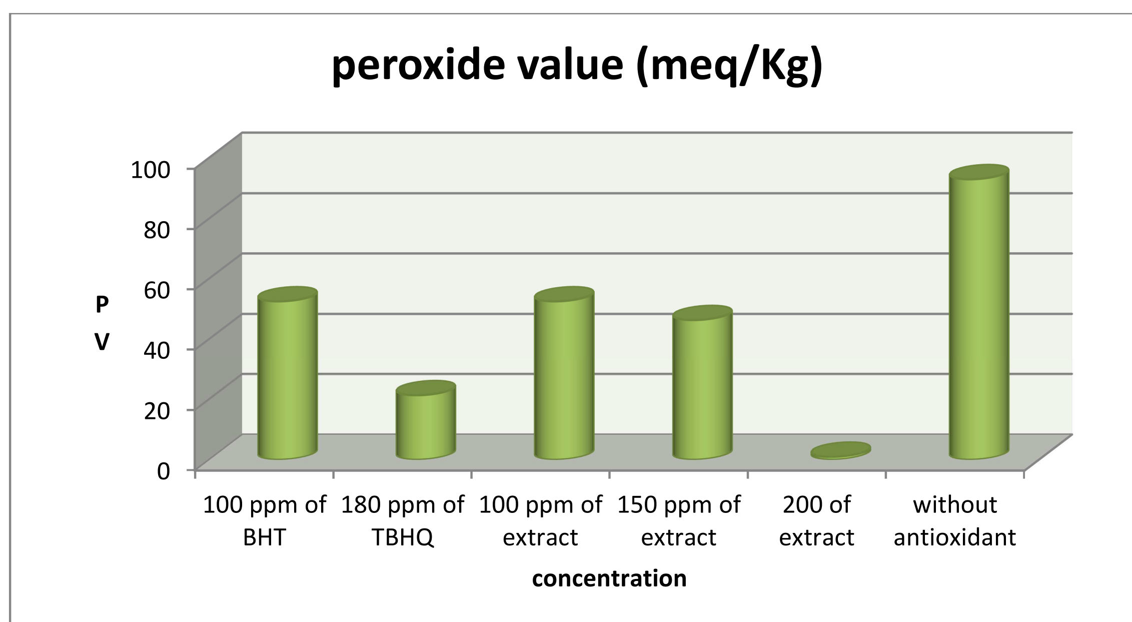

Figure 1. The influence of additionsynthetic antioxidant and extract of *Toona sureni* toperoxide value of coconut oil after 3 hours heating at 125 °C temperature

Free Fatty Acids:

Free fatty acids content incoconut oil is used to determine how worst the oil is. If it more than 0.3%, can caused rancidity and damage in human health. The FFA content was then expressed as a percent of Lauric Acid, the major fatty acid in coconut oil

Table 2. showed that coconut oil that was used had 0.34% of free fatty acids, higher than SNI standard (0.3%). Heated of oil by addition of antioxidant (synthetic and phenolic compound) for 3 hours can decrease free fatty acids content. Decreasing of free fatty acid content by added 150 ppm of extract was lower than 100 ppm and 200 ppm, they were 0.19%. Addition 150 ppm of extract also more effective than synthetic antioxidant, BHT and TBHQ, although 180 ppm of TBHQ was lower than 100 ppm and 200 ppm of extract. According to Figure 2, all of the antioxidant (sythetic and phenolic compound) are effective as an antioxidant in coconut oil through decreasing free radical that can make hydrolysis and oxidation in oil.

Table 2. The influence of additionsynthetic antioxidant and extract of *Toona sureni* tofree fatty acid content in coconut oil after 3 hours heating at 125 °C temperature

Antioxidant	Concentration	Free Fatty Acids (%)[b]
BHT	100 ppm	0.27
TBHQ	180 ppm	0.22
Extract	100 ppm	0.24
Extract	150 ppm	0.19
Extract	200 ppm	0.24
Without antioxidant	-	0.34

b: SNI specification for maximal free fatty acidscontent that allowed in coconut oil is 0.3 %

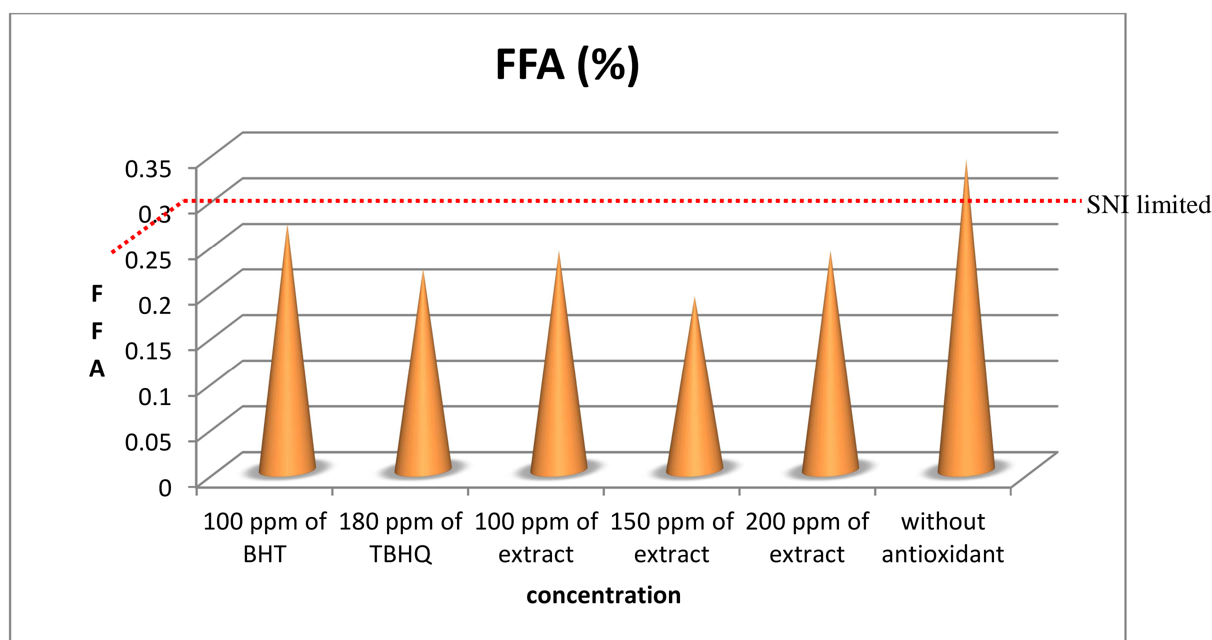

Figure 2. The influence of additionsynthetic antioxidant and extract of *Toona sureni* tofree fatty acid content in coconut oil after 3 hours heating at 125 °C temperature

CONCLUSION

This research can be concluded that the addition of the phenolic compounds to the coconut oil heated for 3 hours at a temperature of 125 °C can inhibit the increasing the peroxide value and free fatty acid value.

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