

Utilization of catechin as an antioxidant in vegetable oils

M. Taufik Eka Prasada*, Dwimaryam Suciati, Dartini

Department of Chemical Analysis Polytechnic ATI Padang, West Sumatera, Indonesia

Abstract

The research about utilization of catechin derived from gambier as an antioxidant in vegetable oils have been done. It aimed to learn the effectiveness of catechin as natural antioxidant in coconut and palm oils. This research was carried out in three stages, i.e purification of catechin from gambier; vegetable oils treatment by adding catechin then heated to five repetitions at 180 °C for 3 hours; and quality measurement on two parameters, free fatty acids (FFA) and peroxide value (PV). The result showed that the addition of catechin at 200 ppm was effectively used to reduce the increase of FFA and PV in coconut and palm oils up to five cycles.

Keywords: antioxidant, gambier, catechin, vegetable oils, rancidity

INTRODUCTION

Natural compound that was extracted from some plants have to be widely used antioxidant, because synthetic antioxidant that used in food product have long received negative responses from consumer. It was caused of their toxicity potential [1] and tumour promoters [2].

Recently, hundreds of both natural and synthetic antioxidant compounds have been evaluated for their effectiveness and safety for humans. Its about twenty compounds are known to be safe for human health, and allowed to use as food additive. Among of them, only five product are widely used in various country, e.g. BHA (butylated hydroxyanisole), BHT (butylated hydroxytoluene), propyl gallate, TBHQ (tertiarybutyl hydroquinone), dan tocopherol. However, attention to the side effect of synthetic antioxidant continues to increase of. In some developed country, such as Japan and Canada, the used of BHA, BHT and TBHQ have been banned. Some research to replace synthetic antioxidant with natural antioxidant are going on, because of their safety in human health [3].

Catechin is one of natural antioxidants contained in Gambier, *Uncaria gambir*, Roxb (Fig.1). The content of catechin in solid extract of gambier varies from 35% up to 95% [4]. Catechin is also found in *Toona sureni* [5] and tea leaves [6]. This is one of flavonoids, colorless, slightly soluble in cold water but easily soluble in hot water, ethyl acetate and alcohol. Some literatures showed that catechin has bioactivity like was antioxidant [7,8], and antifungal activity [9]. In this study, catechin that was purified from gambier in West Sumatera are used as an antioxidant in vegetable oils.



Fig.1-Uncaria gambir, Roxb

MATERIALS AND METHODS

The material used in this research are solid extract of gambier (Fig.2) from Pesisir Selatan of West Sumatera, coconut oil, and palm oil. While the chemicals used are

ethanol, ethyl acetate, 2-propanol, acetic acid, chloroform, 0.1 N KOH in alcohol, sodium thiosulfate, and aquadest.

The equipment used are rotary evaporator, vacuum pump, oven, hotplate, analytical balance, microburet, and glass equipment for extraction.



Fig.2 - solid extract of gambier

Purifying catechin from solid extract of gambier

500 gram of solid extract of gambier were finely ground then sifted with 100 mesh of shifter. Diluted in 2 L of ethyl acetate, then strained and evaporated to dryness. The filtrate was washed by 15% alcohol at 60 °C temperature. Cooled that solution in freezer until white needle solid of catechin was formed. Separated catechin by way of filtration. Repeated the washing work up to 10 times. Dried catechin at 40 °C for 24 hours.

Vegetable oil treatment

Preparation samples for detection the quality of coconut oil (PV and FFA) were done by using three kind of samples. Sample without antioxidant as a control, 3 samples that were added of various catechin (100 ppm, 150 ppm, 200 ppm) as the natural antioxidant, and the last, sample with addition of synthetic antioxidant (180 ppm of TBHQ). 100 gram of coconut oil with synthetic antioxidant or natural antioxidant or none at all was heated at 180 °C temperature for 3 hours. Warmed up for 5 repetitions. For each samples, Free Fatty Acids (FFA) content were assayed, alongside Peroxide Value (PV). The same procedures was done on palm oil also.

Peroxide Value (PV)

Oil sample (2.5 g) was putted into 250 mL of erlenmeyer flask and diluted with 15 mL acetic acid : chloroform (3:2) while shaking until dissolved. 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 Na₂S₂O₃ 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 disappears. Peroxide value is expressed in milli equivalents of peroxide in 1000 g of samples, and was determined by using this equation:

$$PV = \frac{\text{ml Na}_2\text{S}_2\text{O}_3 \times N \text{ Na}_2\text{S}_2\text{O}_3 \times 1000}{\text{sample weight (g)}}$$

Free Fatty Acids (FFA)

Free fatty acid (FFA) is the result of the hydrolysis of triglyceride which are easily oxidized causing rancidity in the oil. The determination of FFA can be done by acid base titration and used phenolphthalein as indicator. FFA measurement of coconut oil is based on lauric acid content, and palmitic acid in palm oil.

FFA of coconut oil

Preparing a neutralizing alcohol by boiling 100 mL of alcohol in erlenmeyer flask, was then added 0.5 mL phenolphthalein. After cooling until 70 °C temperature, neutralized with 0.1 N potassium hydroxide in alcohol. Five grams of oil samples was diluted in neutralizing alcohol, was then boiled for 30 minutes. If the solution wasn't alkaline, cooled until 70 °C temperature was then titrated with 0.1 N KOH in alcohol until the violet reddish was made. The FFA content is determined as the following equation:

$$\%FFA = \frac{\text{ml KOH} \times N \text{ KOH} \times 0.2}{\text{sample weight (g)}} \times 100\%$$

FFA of palm oil

Three grams of oil sample diluted with 50 mL of neutralized isopropyl alcohol. Homogenized the solution by heating over until there is a bubble. Added 3 drops of phenolphthalein indicator, then titrated with 0.1N NaOH until the violet reddish can last for at least 30 seconds. The FFA content is determined as palmitic acid with the following equation:

$$\%FFA = \frac{\text{ml NaOH} \times N \text{ NaOH} \times 0.256}{\text{sample weight (g)}} \times 100\%$$

RESULTS AND DISCUSSION

Purifying catechin from solid extract of gambier

37,5 gram (7.5%) of white solid needles of catechin have been isolated from solid extract of gambier as shown in Fig.3. the solid testing with thin layer chromatography yielded a single spot with Rf 0.325 in ethyl acetate : n-hexane (8:2 v/v) and 94.3 – 94.9 °C of melting point ranged.



Fig.3 - Catechin

Table 1. FFA in coconut oil with antioxidant containing after heated at 180 °C for 3 hours

| No | Antioxidant | Concentration (ppm) | FFA (%) | | | | | |
|----|-------------|---------------------|--------------------|------|------|------|------|------|
| | | | Heated repetitions | | | | | |
| | | | 0 | 1 | 2 | 3 | 4 | 5 |
| 1 | Catechin | 0 | 0.65 | 0.73 | 0.55 | 0.52 | 0.32 | 0.77 |
| 2 | Catechin | 100 | 0.65 | 0.69 | 0.52 | 0.49 | 0.46 | 0.77 |
| 3 | Catechin | 150 | 0.65 | 0.67 | 0.56 | 0.45 | 0.44 | 0.57 |
| 4 | Catechin | 200 | 0.65 | 0.64 | 0.52 | 0.41 | 0.48 | 0.75 |
| 5 | TBHQ | 180 | 0.65 | 0.59 | 0.49 | 0.43 | 0.42 | 0.65 |

Table 2. FFA in palm oil with antioxidant containing after heated at 180 °C for 3 hours

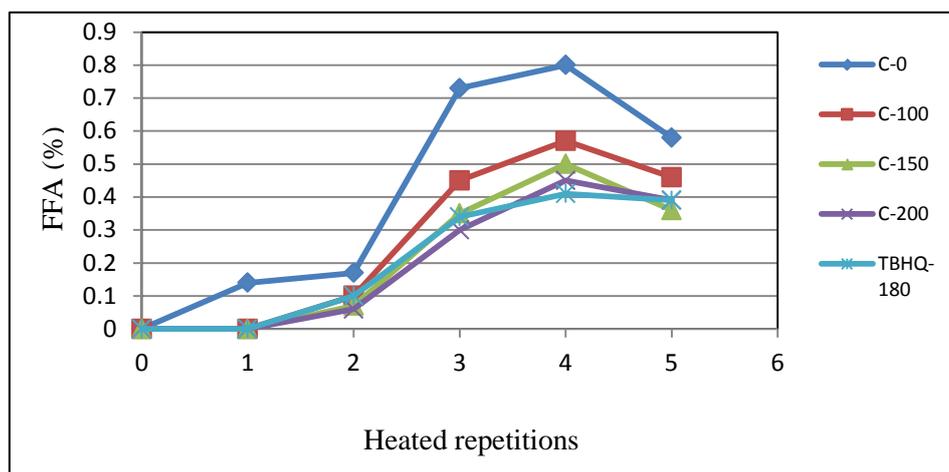
| No | Antioxidant | Concentration (ppm) | FFA (%) | | | | | |
|----|-------------|---------------------|--------------------|------|------|------|------|------|
| | | | Heated repetitions | | | | | |
| | | | 0 | 1 | 2 | 3 | 4 | 5 |
| 1 | Catechin | 0 | 0 | 0.14 | 0.17 | 0.73 | 0.80 | 0.58 |
| 2 | Catechin | 100 | 0 | 0 | 0.10 | 0.45 | 0.57 | 0.46 |
| 3 | Catechin | 150 | 0 | 0 | 0.07 | 0.35 | 0.50 | 0.36 |
| 4 | Catechin | 200 | 0 | 0 | 0.06 | 0.30 | 0.45 | 0.39 |
| 5 | TBHQ | 180 | 0 | 0 | 0.10 | 0.34 | 0.41 | 0.39 |

Table 3. PV in coconut oil with antioxidant containing after heated at 180 °C for 3 hours

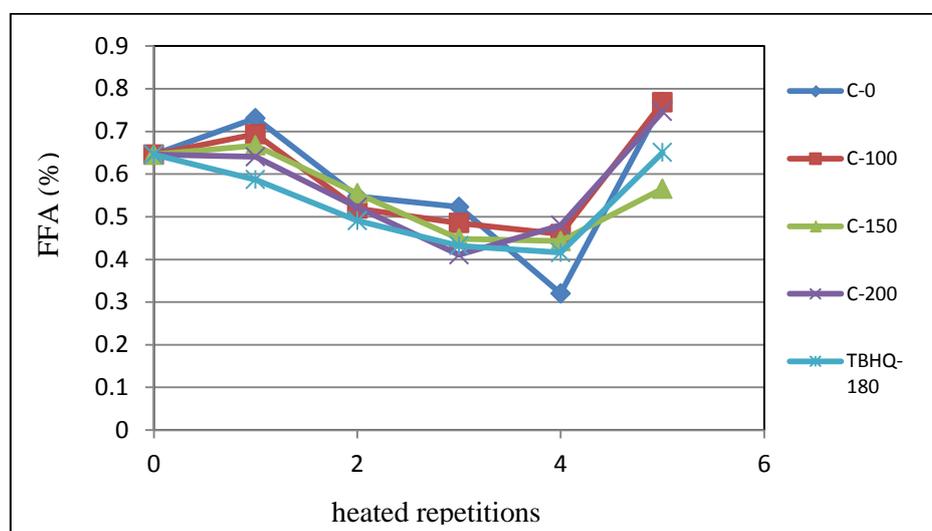
| No | Antioxidant | Concentration (ppm) | FFA (%) | | | | | |
|----|-------------|---------------------|--------------------|------|------|------|-------|-------|
| | | | Heated repetitions | | | | | |
| | | | 0 | 1 | 2 | 3 | 4 | 5 |
| 1 | Catechin | 0 | 3.31 | 4.99 | 6.67 | 8.31 | 12.05 | 13.31 |
| 2 | Catechin | 100 | 3.31 | 3.33 | 4.99 | 6.65 | 10.40 | 11.63 |
| 3 | Catechin | 150 | 3.31 | 3.32 | 4.15 | 4.99 | 6.65 | 8.32 |
| 4 | Catechin | 200 | 3.31 | 1.66 | 2.50 | 3.33 | 5.20 | 6.66 |
| 5 | TBHQ | 180 | 3.31 | 1.66 | 1.67 | 3.33 | 4.99 | 6.66 |

Table 4. PV in palm oil with antioxidant containing after heated at 180 °C for 3 hours

| No | Antioxidant | Concentration (ppm) | FFA (%) | | | | | |
|----|-------------|---------------------|--------------------|------|------|------|-------|-------|
| | | | Heated repetitions | | | | | |
| | | | 0 | 1 | 2 | 3 | 4 | 5 |
| 1 | Catechin | 0 | 0 | 5.94 | 7.98 | 9.95 | 12.94 | 19.86 |
| 2 | Catechin | 100 | 0 | 3.93 | 5.98 | 5.99 | 6.96 | 7.96 |
| 3 | Catechin | 150 | 0 | 2.95 | 3.96 | 3.97 | 5.98 | 5.98 |
| 4 | Catechin | 200 | 0 | 1.98 | 1.98 | 3.96 | 3.99 | 3.98 |
| 5 | TBHQ | 180 | 0 | 1.47 | 1.98 | 2.98 | 3.99 | 4.01 |



Note: C-0 = without catechin; C-100 = 100 ppm catechin; C-150 = 150 ppm catechin, C-200 = 200 ppm catechin and TBHQ-180 = 180 ppm TBHQ

Fig. 4. The effect of antioxidant concentration to FFA in palm oil after heated at 180 °C for 3 hours.

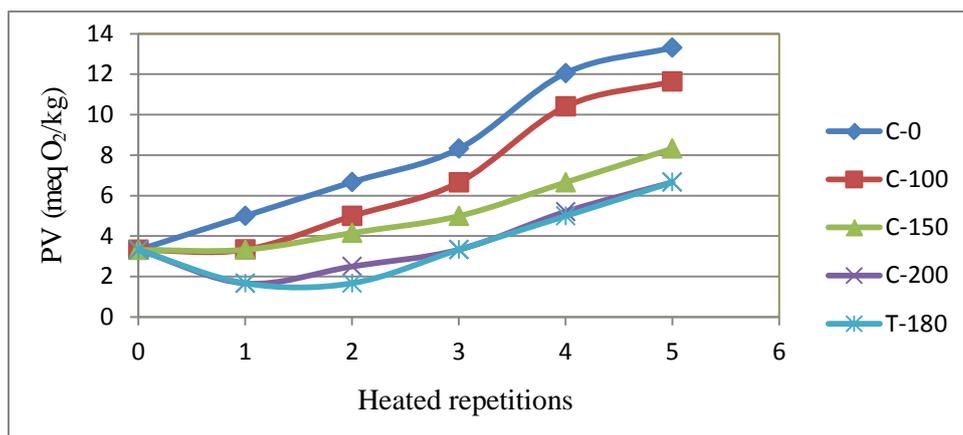
Note: C-0 = without catechin; C-100 = 100 ppm catechin; C-150 = 150 ppm catechin, C-200 = 200 ppm catechin and TBHQ-180 = 180 ppm TBHQ

Fig. 5. The effect of antioxidant concentration to FFA in coconut oil after heated at 180 °C for 3 hours.

Determining of Free Fatty Acids (FFA) and Peroxide Value (PV)

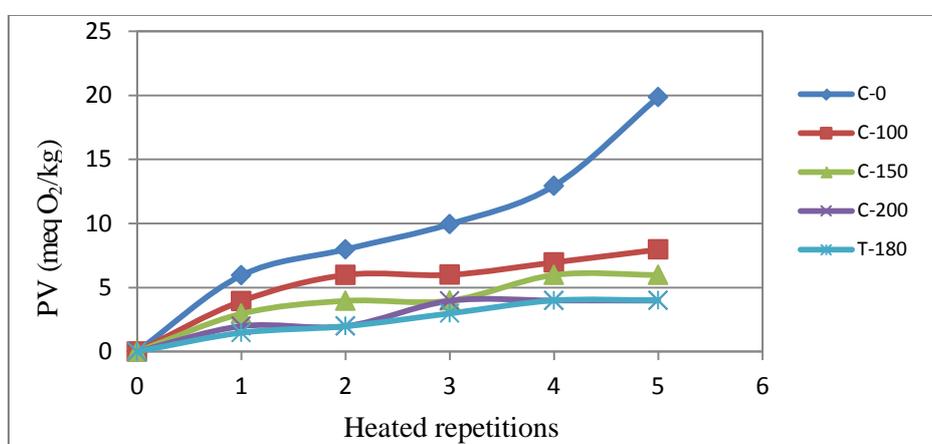
The content of FFA and PV of both of coconut oil and palm oil with several concentration of catechin are shown in **Table 1** and **Table 2**. The addition of 100 – 200 ppm of catechin in palm oil that heated at 180 °C for 3 hours can reduce the increase of FFA degree as shown in **Fig.4**. The higher concentration of catechin in palm oil can increase the inhibitory of hydrolysis reaction to form FFA.

Catechin in 200 ppm concentration had the same power to inhibit production of FFA with 180 ppm of TBHQ that always used as antioxidant in palm oil. After five times heating, the FFA of palm oil increased to 0.58% while palm oil that addition of 200 ppm of catechin increased to 0.39%. Otherwise, addition of catechin in coconut oil did not show any effect on the increase of FFA as shown in **Fig.5**



Note: C-0 = without catechin; C-100 = 100 ppm catechin; C-150 = 150 ppm catechin, C-200 = 200 ppm catechin and TBHQ-180 = 180 ppm TBHQ

Fig.6 - The effect of antioxidant concentration to PV in coconut oil after heated at 180 °C for 3 hours



Note: C-0 = without catechin; C-100 = 100 ppm catechin; C-150 = 150 ppm catechin, C-200 = 200 ppm catechin and TBHQ-180 = 180 ppm TBHQ

Fig.7 - The effect of antioxidant concentration to PV in palm oil after heated at 180 °C for 3 hours

The effect of addition of catechin in coconut oil and palm oil in several concentration are shown in **Table 3** dan **Table 4**. Data show that catechin at concentration of 100 – 200 ppm can inhibit the increase of peroxide value both in coconut oil and palm oil that heated at 180 °C for 3 hours (**Fig. 6** and **Fig.7**).

After heating five repetitions at 180 °C for 3 hours, peroxide value of coconut oil and palm oil increased to 13,31 meq O₂/kg and 19,86 meq O₂/kg respectively. The addition of 200 ppm of catechin in coconut oil heated for five times repetition at 180 °C for 3 hours can inhibit the increase of peroxide value to 6,66 meq O₂/kg. It is the same inhibitory power as addition of 180 ppm of TBHQ. In other side, palm oil with concentration of catechin at 200 ppm can inhibit the increase of PV to 3,98 meq O₂/kg. While the use of 180 ppm of TBHQ can inhibit to 4,01 meq O₂/kg.

CONCLUSION

This research can be concluded that the addition of 200 ppm of catechin to both of coconut oil and palm oil that heated for 3 hours at temperature of 180 °C are effectively used to inhibit the increase of free fatty acids and peroxide value up to five times of heating.

Acknowledgements

We would like to thanks to the Centre of Industry Training and Education, Ministry of Industry and Director of Polytechnic ATI Padang who have provided financial support and facilities in this research.

REFERENCES

- Jeong, S.M., Kuo, S.Y. D., Kim, R.S., Jo, C., Nam, K.C., Ahn D U, and Lee, S.C., *J. Agric. Food Chem.*, 2004, **52**, 3389–3393.
- Kahl, R., Kappus,H., *Z Lebensm Unters Forsch*,1993, **196**(4), 329-38.
- Si, W., Liang, Y., Ma, K. Y., Chung, H .Y, and Chen, Z. Y., *J. Agric. Food Chem.*, 2012, **60**, 6230–6234.
- Amos, *Jurnal Standardisasi* , 2010, **12**(3), 149 – 155.
- Dartini, Nurdin ,H., Afrizal, Ekaprasada, M. T., Suciati, D., *Journal of Chemical and Pharmaceutical Research*, 2016, **8**(6), 156-159.
- Atomssa ,T., and Gholap,A. V., *Journal of Engineering and Technology Research*, 2015, **7**(1), 22-31.
- Anggraini, T, Tai, A., Yoshino, T., and Itai, T., *African Journal of Biochemistry Research*, 2011, **5** (1): 33-38.
- Kosanović, M. M., Seruga, M., Jakobek. L., and Novak, I.,*Croat. Chem. Acta* , 2010, **83**(2), 197–207.
- Hirasawa, M., and Takada, K., *Journal of Antimicrobial Chemotherapy*, 2004, **53**: 225–229.