

OIL OF PANDAN KELAPA HUTAN (*Pandanus julianettii* Martelli): PHYSICOCHEMICAL PROPERTIES, TOTAL PHENOLS, TOTAL CAROTENE, VITAMIN E AND ANTIOXIDANT ACTIVITY

MINYAK PANDAN KELAPA HUTAN (*Pandanus julianettii* Martelli): SIFAT FISIKOKIMIA, TOTAL FENOL, TOTAL KAROTEN, VITAMIN E DAN AKTIVITAS ANTIOKSIDAN

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Diterima 7 Mei 2017. Disetujui 13 Oktober 2017

INTISARI

Tumbuhan pandan merupakan salah satu tanaman penting di Papua dan Papua New Guinea. Secara tradisional tumbuhan pandan digunakan oleh masyarakat untuk berbagai keperluan sehari-hari, mulai dari penyedap makanan, obat hingga keperluan upacara keagamaan. Buah pandan kelapa hutan (*Pandanus julianettii* Martelli) adalah salah satu jenis kelompok *Pandanus* yang telah dimanfaatkan secara turun temurun oleh masyarakat yang tinggal di dataran tinggi Papua dan Papua New Guinea sebagai bahan makanan. Penelitian ini bertujuan menentukan sifat fisikokimia, total fenol, total karoten, vitamin E dan aktivitas antioksidan minyak pandan kelapa (*P. julianettii* Martelli) yang diekstrak menggunakan wajan aluminium. Sifat fisikokimia yang dianalisis adalah bilangan iod, bilangan penyabunan, kadar asam lemak bebas, bilangan asam dan bilangan peroksida. Total fenol, total karoten dan vitamin E masing-masing diukur menggunakan metode Folin-Ciocalteu, metode spektrofotometer dan metode kolorimetri. Aktifitas antioksidan diukur menggunakan metode DPPH. Hasil penelitian menunjukkan minyak pandan kelapa mempunyai berat jenis 1,00059, bilangan iod 65,87 g/100 gr, bilangan penyabunan 201,28 mg/g, kadar asam lemak bebas 0,91%, bilangan asam 4,26%, bilangan peroksida 5,64 meq/kg dan total karoten 2,75 µg/g. Kandungan total fenol, total karoten masing-masing adalah 48,55 ppm, 2,75 µg/g and 5.0303 mg/100g. Hasil pengukuran antioksidan IC₅₀ menunjukkan nilai 45,83 mg/mL.

Kata kunci: Pandanus Julianettii, karakter fisikokimia minyak, total fenol, DPPH method.

ABSTRACT

Pandanus plant is one of the important plant in Papua and Papua New Guinea. Traditionally *pandanus* plant is used by local people for lots of daily activities and daily needs, start from food flavoring, medicine, to religious ceremony needs. *Pandan kelapa hutan* fruits (*P. julianettii* Martelli.) is one of a kind of *pandanus* that been used from generation to generation the highlands of Papua and Papua New Guinea for the food ingredients. This research aims to determine the physicochemical properties, total phenol, total carotene, vitamin E and antioxidant activities of the forest coconut pandan oil (*P. julianettii* Martelli.) extracted using an aluminum pan. Physicochemical character that been analyzed is specific gravity, iod value, saponification value, free fatty acid content, acid value and peroxide value. Total phenol, total carotene and vitamin E measured respectively using Folin-Ciocalteu method, spectrophotometry method and colorimetric methods. Antioxidant activities is measured using a method that called DPPH. The result shows the pandan coconut oil has 1,00059 of specific gravity, 65.87 g/100gr of iod, 201,28 mg/g of saponification, 4.26% of acid and 5.64 Meq/kg of peroxide. The content of total phenols, total carotene and vitamin E respectively are 48.55 ppm, 2.75 µg/g and 5.0303 mg/100g. The antioxidant IC₅₀ measuring result shows the value is 45.83 mg/mL.

Keywords: Pandanus julianettii, antioxidant, total phenols.

BACKGROUND

Pandanus plant has been used for a long time by the people who live in the highlands of Papua and Papua New Guinea, one of them is pandan kelapa hutan that consist from a variety of cultivars. Those people who lives on mountain areas of Jayawijaya know and consume pandan kelapa hutan as daily food and also as alternative if the sweet potatoes are failed to harvest. Sweet potatoes are the main food for the people who live at Jayawijaya Mountains (Kogoya, 2012).

Pandan kelapa hutan (*P. julianettii* Martelli.) is one of a variety of pandan which is the fruit was been used as a food. Not just at Papua Mountains, but also at Papua New Guinea mountains (Stone, 1982; Rose, 1982). Tribe Dani and tribe Lani respectively know pandan kelapa hutan with local name *tuke* and *woromo*. According to those who live in Jayawijaya, the taste of *P. julianettii* Martelli is savory and the scent is like a coconut. The kernel of this fruit can be eaten raw or cooked. The way to cook is burning it in hot ashes or steaming in burn stone (Milliwen, 1992).

The utilization of fat and oil as food material or in food processing need to be considered the physicochemical character that desirable. That character is being the basic of selection of fat or oil

source at the time will be used as food materials or in food processing (Kusnandar, 2010).

Chemical composition of pandan kelapa hutan fruit (*P. julianettii* Martelli.) is dominated by 47% fat, it means potentially become a source of oil fat (Kogoya et al, 2014). But, there is no information about physicochemical character antioxidant capacity of pandan kelapa hutan oil. This research aims to know how to obtained physicochemical character such as iod value, saponification value, acid value, free fatty acid content, peroxide value, carotene, and antioxidant capacity from pandan kelapa hutan oil.

MATERIAL AND METHODS

Plant materials

Samples were from a fruit of pandan kelapa hutan (*P. julianettii* Martelli.) that mature and the hood is still wrapped (Fig. 1a). Samples obtained from Lanny Jaya district and transported to Biology Laboratory, Cenderawasih University. The fruit of pandan kelapa hutan has separated from the hood, then the protective layer of the fruit got cracked to get the endosperm inside. The colour of endosperm is white. The endosperm obtained is then extracted to get the pandan kelapa hutan oil (Fig.1b).

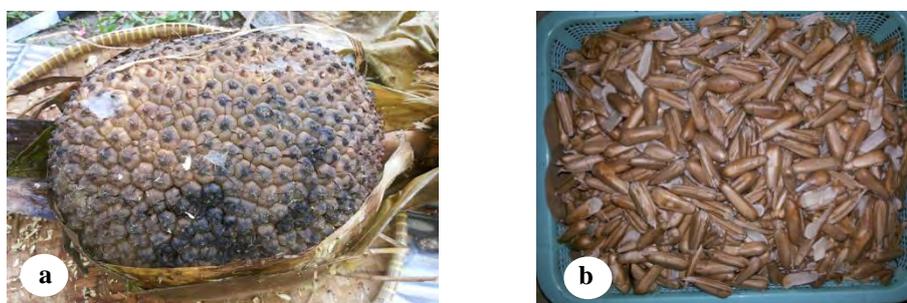


Figure 1. a and b. *Pandanus julianettii* Martelli. a. The fruits mature; b. Endosperms (white colour).

The ingredients that used iodine value, saponification value, acid, free fatty acid content, peroxide value, carotene and antioxidant activity is iodium, KOH, n-hexane, ethanol, eter, phenolphthalein indicator, HCl, KI, Na₂S₂O₃, starch indicator, NaOH, Br₂, acetic acid glacial, diethyl ether, DPPH, methanol and aquadest.

Oil Extraction of forest coconut fruits

Oil of pandan kelapa hutan fruits extracted using the method of wet rendering with aluminum pan. Endosperm of pandan kelapa hutan fruit (*P. julianettii* Martelli.) that has been separated from the protective layer of the fruit washed with water

flowing. Endosperm then in blender with the addition of water 1:1. Coconut milk can be obtained then heated at a temperature between 60°C-70°C until we can obtain the oil. Then the oil is separated from dregs of pandan kelapa hutan milk using centrifuge. The oil that obtained is stored in a dark color bottle before used to determine the nature of physicochemical and antioxidant capacity.

Determination of iodine value, saponification value, acid value, peroxide value, and levels of free fatty acids.

Iodine value of pandan kelapa hutan oil determined by Hanus method (AOAC 920.158, 2011).

Briefly, 0.1-0.5 g of oil was placed in a erlenmeyer and dissolved in 30 mL CHCl_3 . Added 25 mL Hanus I_2 solution with pipet and let stand 30 min in the dark with shaking occasionally. Added with 10 mL 15% KI solution and 100 mL aquadest thus shaken thoroughly. The solution titrated with 0,1M $\text{Na}_2\text{S}_2\text{O}_3$ with constant shaking until yellow solution turns almost colourless. Added few drops starch indicator and continue titration until blue entirely disappears. A blank was made the same way without the sample. The amount of titration of $\text{Na}_2\text{S}_2\text{O}_3$ recorded. Number of mL 0,1M $\text{Na}_2\text{S}_2\text{O}_3$ required by blank minus mL used in determination sample gives $\text{Na}_2\text{S}_2\text{O}_3$ equivalent of I_2 (Hanus solution) absorbed by the oil. Calculated % by weight of I_2 absorbed.

Saponification value of pandan kelapa hutan oil determined by titrimetric methods (AOAC 920.160, 2011). Acurately weighed 5 g filtered oil into erlenmeyer and added 50 mL alcoholic KOH solution. Connected flask with air condensor and boiled until fat was completely saponified (ca 30 min). Cooled and titrated with 0,5M HCl using indicator phenolphthalein. Conducted blank determination along with that on test portion. Calculated saponification number (mg KOH required to saponify 1 g fat) = $28.05 (B-S)/g$ oil. B = mL 0.5M HCl required by blank and S = mL 0.5 HCl required test portion.

Acid value of pandan kelapa hutan oil determined by titration method (AOAC 969.17, 2011). Weighed 5-10 g oil into 250-300 mL erlenmeyer. Added 50-100 mL alcohol-ether mixture and phenolphthalein solution. The solution titrated with 0,1N alcoholic KOH until permanent faint pink appears and persists **for ≥ 10 s. Acid value = mL alcohol solution X normality alcoholic KOH solution X 56.1/g sample.**

Free fatty acids content determined by titration methods (AOAC 940.28, 2011). Weighted 7.05 g oil into 250 mL flask. Add 50 mL of alcohol previously neutralized by adding 2 mL phenolphthalein solution and enough 0.1M NaOH to produce faint permanent pink. The oilition titrated with 0.25M NaOH, then vigorous shaking until permanent faint pink appears

and persists ≥ 1 min. Report as percent free fatty acids expressed as oleic acid; mL 0.25M NaOH used in titration corresponds to this percent.

Peroxide value of pandan kelapa hutan oil determined with titration method (AOAC 965.33, 2011). Weighed 5 ± 0.05 g oil into 250 mL erlenmeyer. Added 30 mL $\text{CH}_3\text{COOH-CHCl}_3$ and swirl to dissolve. Added 0.5 mL saturated KI solution, let stand with occasional shaking 1 min and added 30 mL aquadest. Slowly titrated with 0.1M $\text{Na}_2\text{S}_2\text{O}_3$ with vigorous shaking until yellow was almost gone. Added ca 0.5 mL 1% starch solution and continue titration, shaking vigorously to release all I_2 from CHCl_3 layer, until blue just disappears. Determination of blank conducted daily. Peroxide value (milliequivalent peroxide/kg oil) = $S \times M \times 1000/g$ test portion, where S = mL $\text{Na}_2\text{S}_2\text{O}_3$ (blank corrected) and M = molarity $\text{Na}_2\text{S}_2\text{O}_3$ solution.

Determination of total carotenoid

Total carotene determined with spectrophotometry method (AOAC, 2011). Approximately 15 g of the samples, plus 3 g of celite were weighed in a mortar on a digital balance. For the carotenoid extraction, successive additions of 25 mL of acetone were made to obtain a paste, which was transferred into a sintered funnel (5 μm) coupled to a 250 mL Buchner flask and filtered under vacuum. This procedure was repeated three times or until the sample became colorless. The extract obtained was transferred to a 500 mL separatory funnel containing 40 mL of petroleum ether. The acetone was removed through the slow addition of ultrapure water to prevent emulsion formation. The aqueous phase was discarded. This procedure was repeated four times until no residual solvent remained. Then, the extract was transferred through a funnel to a 50 mL volumetric flask containing 15 g of anhydrous sodium sulfate. The volume was made up by petroleum ether, and the samples were read at 450 nm. The total carotenoid content was calculated using the following formula:

$$\text{Carotenoids content } (\mu\text{g/g}) = \frac{A \times V (\text{mL}) \times 104}{A_{1\text{cm } 1\%} \times P (\text{g})}$$

where A = Absorbance; V = Total extract volume; P = sample weight; $A_{1\text{cm } 1\%} = 2592$ (β -carotene Extinction Coefficient in petroleum ether).

Determination of total phenol

The total phenol in the pandan kelapa hutan oil was determined by using Folin-Ciocalteu reagent. The phenol component was extracted by dissolving 0,1 g oil of forest coconut pandan in 1 mL of 80% methanol, centrifuging at 1100 rpm for 10 min then collecting the filtrate. The extraction process was repeated four times (Seneviratne *et al.*, 2009) and the filtrate

collected was added with 80% methanol to 5 mL. Measurement of total phenols was done by adding 0.5 mL Folin-Ciocalteu reagent to the 1 mL oil of forest coconut pandan fruit and the content mixed thoroughly. After 5 min, 1 mL of 5% Na_2CO_3 and 5 mL deionized water was added then the mixture was allowed to stand for 1 h at normal temperature. Absorbance was measured by spectrophotometry at a

wavelength of 745 nm. A blank was made with the same procedure without sample. Gallic acid was used as the standart for the calibration curve.

Determination of vitamin E

Vitamin E of pandan kelapa hutan oil determined by colorimetric method (AOAC 971.30, 2011). Briefly, 1 g oil of forest coconut pandan, 10 mL absolute alcohol and 20 mL, 1M alcoholic sulphuric acid was reflux for 45 min in a condenser and cooled for 15 min. Unsaponifiable matter in the mixture was extracted with dimethyl ether. The extracts evaporated at a low temperature and the residues obtained were dissolved

in 10 mL absolute alcohol. Absolute alcohol (5 mL) and 1 mL HNO₃ was added to aliquots of the sample and standart (0.3-3.0 mg vitamin E). The mixture was evaporated in a water bath at 90°C for 3 min from the time the alcohol starting boiling. A series of standard solutions of known concentration were determined with reference to their absorbance from which average was recorded. Its absorbance was measured at 290 nm using a spectrophotometer against a blank containing 5 mL absolute alcohol and 1 mL HNO₃ and treated in similar manner. The vitamin E content was calculated using the following formula:

$$\text{Vitamin E } (\mu\text{g}/100 \text{ g}) = \frac{\text{absorbance of sample} \times \text{dilution factor} \times \text{gradient}}{\text{weight sample}}$$

Measurement of 2,2-diphenil 1-picrylhydrazyl (DPPH)-radical scavenging activity

Radical scavenging activity of pandan kelapa hutan oil determined by DPPH method. 2,2-diphenyl 1-picrylhydrazyl (DPPH) used to source of free radical. DPPH radical scavenging activity was assessed according to Kikuzaki *et al.* (2002). In this test 50µ of pandan kelapa hutan oil with different concentration, added with 1 mL solution 0,4 mM DPPH, methanol

and 5 mL of methanol. Mixture solution and forest coconut pandan oil then mixed using vortex and left it for 30 minutes at room temperature in the dark room. Measurement scavenging effect on the DPPH radical done using spectrophotometer on the wavelength of 517 nm. The radical scavenging activity was expressed as the radical scavenging percentage using the following equation:

$$\text{Percentage } (\%) \text{ cleaning radical DPPH} = \left(1 - \frac{As}{Ac}\right) \times 100$$

Where, AS = Absorbance samples, AC = absorbance control, Control = DPPH solution without sample (forest coconut pandan oil).

Value IC₅₀ is the number of samples (antioxidant) required for 50% scavenging of DPPH radical and calculated from the graph describe the relationship scavenging activity the radical to the number of samples. The DPPH solution without sample solution of pandan kelapa hutan oil used as a control.

fatty acids of pandan kelapa hutan oil can be seen in Table 1. The acid value of forest coconut pandan oil represent the free fatty acids present in oil or fat. Saponification value can be used to estimate the molecular weights of fatty acids that compose forest coconut pandan oil. Iodine value indicate unsaturation degree oil or fat (Arumsari *et al.*, 2013). Peroxide value, levels of free fatty acids and acid value is often used as parameter damage of oil or fat (Kusnandar, 2010).

RESULTS

Physicochemical character

The determination of iodine value, saponification value, acids value, peroxide value and levels of free

Table 1. Physicochemical characteristic of pandan kelapa hutan oil

Parameter	Value	Unit
Iodine value	65.87	g/100 g
Saponification value	201.28	mg KOH/g
Acid value	4.26	%
Peroxide value	5.64	meq O ₂ /kg
Free fatty acid content	0.91	%

Total phenol, total carotene, vitamin E and antioxidant activity of forest coconut pandan oil

The total phenol, total carotene, vitamin E and antioxidant activity content of pandan kelapa hutan

oil are shown in Table 2. Total phenol of pandan kelapa hutan oil are 48,55 ppm. The way of determination of the level of total phenolic is based on their chemical reducing capacity relative to gallic acid.

Table 2. Total phenol, total carotene, vitamin E and antioxidant activity of forest coconut pandan oil

Pandan kelapa hutan	Total phenol (ppm)	Total carotene (µg/g)	Vitamin E (mg/100 g)	Antioxidant activity IC ₅₀ (mg/g)
Oil ^a	48.55	2.75	5.03	45.83
Fruit ^b	-	8.42	0.46	-

^a Primery data

^b Kogoya *et al.* (2014)

Levels of the total carotene contains inside pandan kelapa hutan oil is 2,75 µg/g. Carotenoids is pigment fat-soluble. In additions, it works the pigment that make up the colour such is yellow and orange (Fig. 2). Carotenoids is also a compound provitamin A. Carotenoid has many kind of variety in plant, there are **β-carotene, α-carotene, β-kriptoxantin, lutein, and lycopene** (Bendich and olson, 1989).

Content of vitamin E of pandan kelapa hutan oil measured as **α-tokoferol**. Oil of pandan kelapa hutan contained **5.0302 mg/100 g α-tocopherol**. Vegetable

oil are important compounds of source of energy, fatty acids and fat-soluble vitamins such as vitamin A and vitamin E. Tocopherols are natural antioxidants that **also present vitamin E activity, especially α-tocopherol** (De Greyt and Kellens, 2005).

Measuring the antioxidant activity IC₅₀ (inhibition concentration)₅₀ of pandan kelapa hutan oil with DPPH shows the value of 45.83 mg/g. That is to clear 50% of free radicals DPPH required a pandan kelapa hutan oil with a concentration of 45.83 mg/g.



Figure 2. The oil of pandan kelapa hutan

DISCUSSIONS

Oil yield with wet rendering extraction of pandan kelapa hutan oil is yellow (Figure 2). The colors correspond to the Indonesian National Standard (SNI) 2006 for palm oil colors yellow, orange to red.

The results of the measurement of iodine value of oil palm fruit in the pandan kelapa hutan oil (Table 1) indicates the degree of unsaturated fatty acids that compose the oil. Kogoya *et al.* (2014) stated that pandan kelapa hutan fruit dominated by palmitic acid (28,66%), trans oleic acid (9,29%) and stearic acid (3,77%) as well as cis oleic acid (1,84%). The fatty acid composition of pandan kelapa hutan fruit in accordance with the iodine value of the oil is not too high, however unsaturated fatty acid more dominant.

Saponification value indicates the molecular weight of the oil or fat. The lower the molecular weight, the

greater the saponification; the larger molecular weight, the smaller the saponification. Result of determination saponification oil palm fruit in the forest (Table 1) indicates the average triglyceride oil constituent pandan kelapa hutan has a low molecular weight.

Acid value, peroxide value and free fatty acid content is quality parameters for oil or fat. Fatty acid content of pandan kelapa hutan oil is relatively high (Table 1) compared to the standard CPO (maximum fatty acid level of 0.5%; SNI (2006) and palm oil standard (maximum fatty acid content of 0.3%; SNI (2012). Differences in levels of fatty acid free, indicates the start of the hydrolysis of the pandan kelapa hutan oil that severing of ties between the fatty acid ester and glycerol produce free fatty acids. Hydrolysis can be triggered by the presence of water and lipase activity of fruit (Ngando *et al.*, 2006), which

may occur during harvesting or during the extraction process. The level of ripeness at the time harvesting indirectly affect the occurrence of hydrolysis.

Salvador *et al.* (2001) reported that the olive oil produced from olives which is really measured has a high acidity because of the activity of lipase internally, the sensitivity of the pathogen and because of mechanical damage. For it is necessary to overcome postharvest handling and extraction process which can reduce and even suppress the occurrence the hydrolysis. Possibility the start of the hydrolysis on a pandan kelapa hutan oil also indicate woods by value and acid number peroxide number (table 1). Number acid indicates the presence of free fatty acids in the oil of pandan kelapa hutan, while the peroxide value showed the oil oxidation (Kusnandar, 2010).

Oil yield extraction of pandan kelapa hutan fruit contains carotene indicated by the total pigment levels of carotenes (table 1). Carotene is associated with yellow, orange and red in plants and vegetable and is soluble fat (Muchtadi, 2012). Carotene content in the oil of pandan kelapa hutan fruit associated with the color yellow oil produced from the extraction fruit.

The one of important parameters associated with functionality and quality of oil is content of phenolic. Phenolic compounds may contribute to oil flavor and protect the fatty acids from oxidation (Servili and Montedoro, 2002). The total phenol content of pandan kelapa hutan oil (table 2) was lower than that red fruits oil (90-705 ppm) according to Sarungallo *et al.* (2015).

The content of vitamin E of pandan kelapa hutan oil (*P. julianettii*) is higher than content of vitamin E of pandan kelapa hutan fruits (table 2). The high variability in the amount of vitamin E in vegetable oil has been widely reported and depends on several factors, such as genetic, agronomic, enviromental, extraction procedural and others (Cimato, 1990; Mousa *et al.*, 1996).

Measurement IC₅₀ pandan kelapa hutan oil (table 1) shows that low antioxidant activity due to scavenging activity 50% of free radical DPPH required pandan kelapa hutan oil as much forest 45.83 mg/mL. IC₅₀ is concentration of antioxidants that is needed to clear the DPPH radical timed. Low IC₅₀ value indicates high activity of antioxidant, in the other hands high IC₅₀ value indicates low activity of antioxidant (Maisuthisakul *et al.*, 2007). Low antioxidant activity is associated with the total phenol content of forest coconut pandan fruits oil (Table 1).

CONCLUSION

Oil extraction from pandan kelapa hutan is yellow, and the composition of saturated fatty acids is dominant than unsaturated fatty acid. It also means

triglyceride constituent has a low molecular weight. Allegedly began to form free fatty acid in the oil of pandan kelapa hutan by acid value, peroxide value and fatty acid levels free. Pandan kelapa hutan oil contains 48.55 ppm of total phenol, 5.03 mg/100g of vitamin E, 2.75 µg/g of total carotene and 45.83 mg/mL of antioxidant activity IC₅₀.

ACKNOWLEDGEMENT

Thank you to Ministry of Research, Technology and Higher Education, that funded this research through Competitive Grant Funds in 2015 to 2016.

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