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Physico-Chemical Properties of the Oils and Fat from *Crotalaria cleomifolia* Seeds

(Sifat-sifat Fiziko-Kimia Minyak dan Lemak daripada Biji *Crotalaria cleomifolia)*

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ABSTRACT

The seeds of C. cleomifolia *(locally known as kacang hantu) collected along Simpang Pulai - Berinchang Road, Cameron Highlands, was defatted with hexane and the resulting oil was analysed for their physico-chemical properties. The percentage yield of the oil was calculated as 5.3%. The acid value (1.2%), iodine value (85), peroxide value (0.6), saponification value (192.0) and unsaponifiable matter (2.3%) were determined to assess the quality of the oil. The physico-chemical characterisation showed that* C. cleomifolia *seeds oil is unsaturated semi-drying oil, with high saponification and acidic values. The fatty acid composition of* C. cleomifolia *seed oil was determined by Gas Chromatography and Gas Chromatography-Mass Spectrometry (ToF). The seed oil of* C. cleomifolia *contained linoleic acid (57.59%) and palmitic acid (5.07%), the most abundant unsaturated and saturated fatty acids, respectively. The polyunsaturated triacylglycerol (TAG) in* C. cleomifolia *seed oil determined by reverse phase High performance Liquid Chromatography; contained as PLL (18.04%) followed by POL + SLL (11.92%), OOL (7.04%) and PLLn (6.31%). The melting and cooling point of the oil were 16.22°C and -33.54°C, respectively.*

Keywords: Crotalaria cleomifolia*; Leguminosae; minyak lemak*

ABSTRAK

Biji C. cleomifolia *(dikenali dengan nama tempatan sebagai kacang hantu) diambil di Jalan Simpang Pulai-Berinchang, Cameron Highlands, dinyah lemak dengan pelarut heksana dan hasil minyak lemak tersebut dianalisis sifat fiziko-kimia. Peratus minyak lemak yang diperoleh ialah 5.3%. Nilai asid (1.2%), nilai iodin (85), nilai peroksida (0.6), nilai penyabunan (192.0) dan nilai bahan tak tersabun (2.3%) telah ditentukan untuk menilai kualiti minyak biji* C. cleomifolia. *Sifat fiziko-kimia minyak ini menunjukkan minyak ini adalah jenis minyak taktepu 'semi-drying' dengan nilai asid dan nilai penyabunan yang tinggi. Komposisi asid lemak minyak biji* C. cleomifolia *ditentukan dengan menggunakan kromatografi gas dan kromatografi gas spektroskopi jisim (ToF). Minyak biji* C. cleomifolia *mengandungi asid linoleik (57.59%) dan asid palmitik (5.07%) yang merupakan asid major lemak taktepu dan tepu. Triasilgliserol (TAG) politaktepu minyak biji* C. cleomifolia *ditentukan oleh Kromatografi Cecair Prestasi Tinggi (HPLC) fasa balikan. Puncak major politaktepu TAG dalam minyak biji* C. Cleomifolia *ialah PLL (18.04%) diikuti oleh POL + SLL (11.92%), OOL (7.04%) dan PLLn (6.31%). Manakala takat peleburan dan penghabluran minyak adalah pada 16.22°C dan -33.54°C.*

Kata kunci: Asid lemak; Crotalaria cleomifolia*; Leguminosae*

INTRODUCTION

Seed oil consists of fatty acids which has high commercial value in food industries, pharmaceuticals, lubricants, cosmetics and others. *Crotalaria cleomifolia* seed oil has the potential to be explored to get an alternative source of polyunsaturated fatty acid such as linoleic acid and linolenic acid which are essential fatty acids since they cannot be synthesized by our body and has to be taken instead from dietary supplements (Lawson 1985). *C. cleomifolia* (Leguminosae) locally known as *kacang hantu* or *gegiring* is a shrub that can grow until 4 m high, with branches and yellow flowers. The plant was introduced in Malaysia in order to improve the quality of farm land (Burkill 1966). In Malaysia there are 20 *Crotalaria* species of which ten are endemic to Peninsular Malaysia and mostly found in Perak, Selangor, Pahang, Melaka, Negeri Sembilan and Johor (Turner 1995). In Asia, some of *Crotalaria* species are used as herbal tea and vegetables (Huxtable 1989). Previous studies have shown that the oil extracted from the seeds of *C. striata (syn. C. mucronata)* (Hosamani & Ramesh 2001) contained some interesting acids such as cylopropanoic fatty acids; ricinoleic, malvalic, sterculic, and normal fatty acids; myristic, palmitic, oleic, linoleic and linolenic. The present study was conducted to determine some of the physical and chemical properties including the fatty acids and TAGs composition of the *C. cleomifolia* seed oil since no report has been found on any chemical investigations of *C. cleomifolia*. Results from this

study will give us some information on nutritional values in *C. cleomifolia* seed oil which may have a high potential in food and the pharmaceutical applications.

Materials and Method

Seeds of *C. cleomifolia* (voucher specimen UKMB 29771 deposited in the Herbarium, UKM Bangi) were collected from Simpang Pulai-Berinchang Road, Cameron Highlands, Pahang. *Extraction*: *C. cleomifolia* seeds (1340 g) were extracted into using hexane at room temperature for 2 days (2 times). The extract was filtered and the solvent evaporated to give the crude oil which was stored in the freezer prior to analysis. The acid value, iodine value, peroxide value, saponification value, unsaponifiable matter, moisture content, slip melting point and cloud point were determined in accordance with the *Association of Official Analytical Chemists* (AOAC) and *American Oil Chemist's Society* (AOCS) standard procedures. Finally, the thermal characteristic of *C. cleomifolia* seed oil was measured by using a differential scanning calorimeter (DSC 822e Mettler Toledo). The fatty acid composition was determined by gas chromatography (GC) and GC × GC-MS (ToF). Before injecting sample into GC × GC-MS (ToF), the sample is first analysed by GC in order to get the whole profiles of peaks. GC: About 0.1 mL of the oil was converted to fatty acid methyl esters (FAME) by using NaOMe (1M) in 1mL hexane. 1 μL of FAME layer was injected into Gas Chromatography Shimadzu 17A-FID equipped with capillary column BPX-70 (30 m × 0.25 mm × 0.25 μm), using isothermal temperature at 120ºC in 0 min, 245ºC in 57 min, detector temperature at 260ºC and flow rate at 0.3 mL/min. Nitrogen gas was used as a carrier gas. Then, using the same sample about 0.5 μL of FAME layer was injected into GCxGC-MS (ToF). In this study, measurements and peak identifications were preliminarily made with 1D-GC, and followed by a LECO Pergasus GC × GC-MS (ToF) system. The GC × GC-MS (ToF) system consists of an Agilent 6890 gas chromatograph equipped with a LECO dual jet thermal modulator between the primary and secondary columns and a LECO Pegasus IV Time-of-Flight Mass Spectrometer (TOFMS) as a detector. The primary column is 30.0 m × 250 μm ID × 0.10μm df RTx-5MS (non-polar column), maximum temperature 360ºC and the secondary column is 0.790 m × 100 μm ID × 0.10 μm df RTx-17 (polar column), maximum temperature 300ºC and housed in the GC oven. The carrier gas was helium at constant rate of 1.0 mL/min. for the entire run. Transfer line temperature to MS (ToF) was 250ºC.

Triacylglycerol (TAG) composition was determined by High Performance Liquid Chromatography (HPLC). The separation method was based on equivalent carbon number (ECN) in TAG sample by using reverse phase liquid chromatography equipped with a refractive index detector. TAG composition was determined by comparing the retention time profile of standard TAG (Ooi & Salimon 2006) and reported as percentage of peak area (PORIM 1995). About 0.5 g of the sample was dissolved in acetone:acetonitrile (63.5:36.5), from which 20 μL of sample was injected into HPLC (Waters 1515) equipped with a capillary column (Spherisorb C18; 150 mm × 4.8 mm × 5 μm) and flow rate at 1 mL/min. Mobile phase used is acetone:acetonitrile (63.5:36.5) and time needed about 60 min.

Results and Discussion

Physico-chemical properties of oils

C. cleomifolia seeds yielded 5.3% of the oil (magenta colour) which was comparable to the amount produced by *C. striata* seeds (5.2%) in a previous study (Hosamani & Ramesh 2001). The percentage of moisture content of seeds was 5.1%, both of slip melting point of seeds oil was 27°C and cloud point 14°C. The oil had an iodine value of 85-95 indicating a degree of unsaturation that was comparable to the iodine value of *C. striata* seed oil (Hosamani & Ramesh 2001) and castor seed oil (Akpan et al. 2006). Since the IV value was less than 100, this seed oil is classified as non drying oil, which has a wide variety of industrial uses especially for preparing soaps and cleansers, cosmetics, lubricants, leather dressings, and candles. As a comparison *Elateriospermum tapos* seed oil (106) (Ooi & Salimon 2006), corn oil (103-128), cotton seed oil (99-119) and mustard seed oil (92-125) (Gunstone et al. 1994) are more suitable for use in the food industries. A low peroxide value (0.6) which is comparable to soybean oil (0.1), palm oil (4.0) and sunflower seed oil (4.2) (Gunstone 2004) shows that the oil is stable to relative oxidation. Instead, the oil which has peroxide value of more than 10.0 will go rancid such as castor seed oil (158.6) (Abitogun et al. 2009). All measured values are summarised in Table 1.

Saponification value of *C. cleomifolia* seed oil is 192.0, which was comparable to saponification value of *C. striata* seed oil (Hosamani & Ramesh 2001), grape seed oil, raspberry seed oil and safflower seed oil (Oomah et al. 2000). Besides, the unsaponifiable matter of *C. cleomifolia* seed oil is 3.5% which was comparable to *C. striata* seed oil, 2.1% (Hosamani & Ramesh 2001). This value indicates that the amount of undissolved matter in aqueous medium but will dissolve in hexane including sterol, tocopherol, hydrocarbon and long chain alcohols (Gunstone 2004).

TABLE 1. Analytical values of *C. cleomifolia* seed oil

Lipid content in seeds (%)	5.3
Moisture content in seeds (%)	5.1
Slip melting point (°C)	27
Cloud point (°C)	14
Acid value (AV) (%)	1.28
Iodine value (IV)	85.0
Peroxide value (PV)	0.6
Saponification value (SV)	192.0
Unsaponifiable matter (%)	2.3

Table 4 shows the comparison of some properties between *C. cleomifolia* and *C. striata* (Hosamani & Ramesh 2001) seed oil.

FATTY ACIDS COMPOSITION

The GC × GC-MS (ToF) software was used to define all the peaks in the raw GC × GC chromatograms. The typical chromatogram obtained for the *C. cleomifolia* seed oil and selective identification of the peaks done by the GC × GC-MS (ToF) Wiley data base library. Major fatty acids present in *C. cleomifolia* seed oil are linoleic acid, oleic acid, linolenic acid, palmitic acid and behenic acid. *C. cleomifolia* seed oil contains a high percentage of polyunsaturated fatty acid (PUFA) and monoenes. Linoleic acid ($C_{18:2}$), being the dominant PUFA (57.59%). This value was similar to sunflower, cottonseed, soybean, corn, poppy seed, sesame and *Capcicum* seed oil (Formo et al. 1979). Due to the high linoleic acid content, *C. cleomifolia* seed oil is suitable for industrial purposes, notably for preparing varnish (Gecgel et al. 2007). Other fatty acids present were oleic acid ($C_{18:1}$) 28.6% and linolenic acid ($C_{18:3}$) 5.53%. Minor polyunsaturated fatty acids are *cis*-9, *cis*-15-octadecadienoic acid ($C_{18:2}$) 0.03% and erucic acid ($C_{22:1}$) 0.02%. The major saturated fatty acids present in *C. cleomifolia* seed oil were palmitic acid ($C_{16:0}$) 5.07%, followed by behenic acid ($C_{22:0}$) 1.63% whilr minor amount of saturated fatty acids were detected; stearic acid ($C_{18:0}$) 0.45%, followed by 15-methyl palmitic acid ($C_{17:0}$) 0.40%, 2-hexyl-cyclopropaneoctanoic acid, ($C_{17:0}$) 0.32%, arachidic acid ($C_{20:0}$) 0.27% and heneicosanoic acid ($C_{21:0}$) 0.09%. The five fatty acids (linoleic, oleic, linolenic, palmitic and behenic acid) comprise more than 98% of the total fatty acid methyl esters (FAMEs) in *C. cleomifolia* seed oil. The proportion of unsaturated and saturated fatty acids of *C. cleomifolia* seeds oil and *C. striata* seed oil were calculated 92 : 8% and 60.2 : 39.8, respectively (see Table 2).

TAG COMPOSITION

TAG present in *C. cleomifolia* seed oil was analysed by reverse phase High Performance Liquid Chromatography (HPLC), whereby mechanism of the separation involves long chain and degree of unsaturated fatty acid (Gutierrez & Barron 1995). Due to the limitation on available standard TAG, triacylglycerols of *C. cleomifolia* seed oil was identified by comparing with the retention time profile of triacylglycerols standards (soybean oil) using the same parameters. Major peaks of TAG in *C. cleomifolia* seeds oil was identified as polyunsaturated TAG; PLL (18.04%) followed by POL + SLL (11.92%), OOL (7.04%) and PLLn (6.31%) as shown in Table 3.

TABLE 2. Fatty acids of *C. cleomifolia* seed oil (%)

Fatty acids	Composition (%)
Saturated	
Palmitic acid	5.07
Palmitic acid, 15-methyl	0.40
Cyclopropaneoctanoic acid, 2-hexyl	0.32
Stearic acid	0.45
Arachidic acid	0.27
Heneicosanoic acid	0.09
Behenic acid	1.63
Unsaturated	
Oleic acid	28.60
Linoleic acid	57.59
cis-9, cis-15-octadecadienoic acid	0.03
Linolenic acid	5.53
Erucic acid	0.02

THERMAL BEHAVIOUR

In differential scanning calorimetry (DSC) analysis, the ends of the melting and cooling ranges are determined by the position of the offset of the last peak. Each individual fatty acid has its own melting point, since fats and oils are essentially mixtures of various fatty acids such as TAGs, they do not have sharp melting points. Figures 1 and 2 show the DSC thermograms of melting and cooling of the same *C. cleomifolia* seed oil respectively after being heated until complete melting and maintained at 60°C.

As observed, profiles for phase change solid to liquid and vice versa are relatively simple and closely correspond to the similarly relatively simple fatty acid composition. It is shown that, during fusion, there is a low melting point fraction with fusion starting at a temperature of -54.39°C and a maximum of -17.37°C and stop melting at 16.22°C. The enthalpy change for this fusion process was -144.87 mJ. The cooling process for the melting *C. cleomifolia* seed oil starts the solidification step at 2.34°C and ends at -33.54°C, with a crystallization maximum at -16.46°C. A slight ‘‘shoulder’’ is observed at -5.5°C, that might be assumed to be the solidification of the low fusion point fraction, constituted by triacylglycerides with unsaturated

TABLE 3. TAG composition in *C. cleomifolia* seed oil

TAG	Retention Time (min)	Relative Composition (%)
PLLn	18.19	6.31
PLL	20.84	18.04
LnOO	22.15	2.35
OOL	24.75	7.04
POL+SLL	25.68	11.92

L: Linoleic acid, Ln: Linolenic acid, O: Oleic acid, P: Palmitic acid, S: Stearic acid

TABLE 4. Comparison of some properties between *C. cleomifolia* and *C. striata* seed oil

	C. cleomifolia	*C. striata* [a]
Lipid content in seeds (%)	5.3	5.0
Moisture content in seeds (%)	5.1	-
Slip melting point (°C)	27	-
Cloud point (°C)	14	-
Asid value (AV) (%)	1.28	-
Iodine value (IV) (%)	85.0	92.0
Peroxide value (PV)	0.6	-
Saponification value (SV)	192.0	203.0
Unsaponifiable matter (%)	2.3	2.1
Saturated fatty acids (%)	8.0	39.8
Unsaturated fatty acids (%)	92.0	60.2

[a] Hosamani & Ramesh (2001

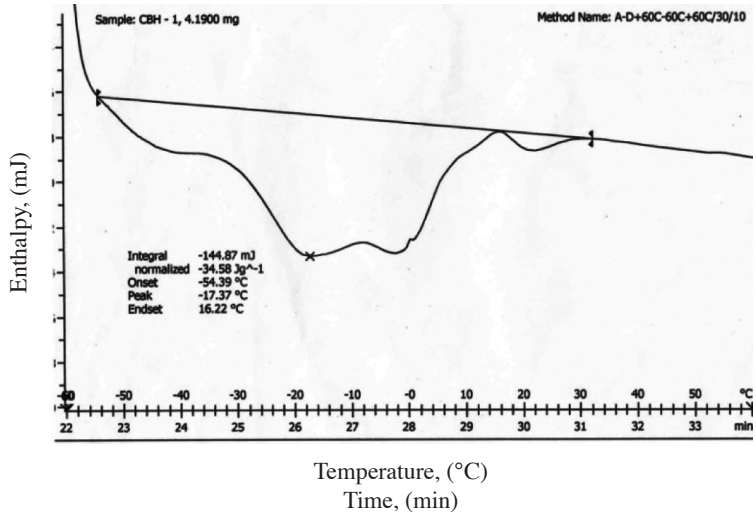

FIGURE 1. DSC melting thermogram of *C. cleomifolia* seeds oil

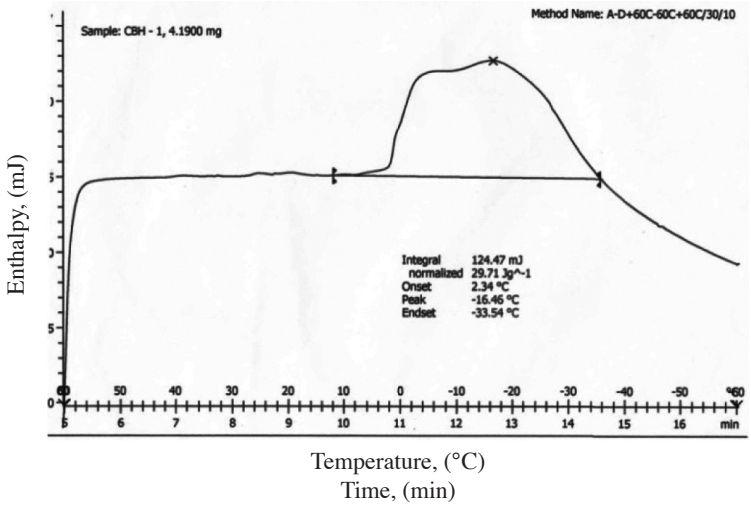

FIGURE 2. DSC cooling thermogram of *C. cleomifolia* seeds oil

fatty acids. The energy released for this solidification process was 124.47 mJ. This is consistent with the fact that the oil has a high degree of unsaturation which from linoleic and oleic acids.

CONCLUSION

The present investigation describes the composition of saturated and unsaturated fatty acids in *C. cleomifolia* seed oil. The high composition of unsaturated fatty acids; linoleic acid, (57.59%), oleic acid 28.6% and linolenic acid 5.53% and some physico-chemical properties of *C. cleomifolia* seed oil, shows this seed oil has potential to be exploited further for various applications or as a source for unsaturated fatty acids especially linoleic acid.

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