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## Physico-Chemical Characterization of Avocado (*Persea americana* Mill.) Oil from Three Indonesian Avocado Cultivars

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### ABSTRACT

A study was carried out to determine the physico-chemical characteristics of avocado oil derived from three Indonesian avocado cultivars, namely Bantul (MAB), Purwokerto (MAP) and Garut (MAG). The extraction of avocado oil from avocado fruit was carried out using solvent extraction method. The avocado oil obtained from all samples had a green yellowish color. The iodine value of MAG is 88.7 g I<sub>2</sub>/100 g oil, slightly higher than MAB (87.0 g I<sub>2</sub>/100 g oil) and MAP (77.09 g I<sub>2</sub>/100 g oil) indicated that MAG contains more unsaturated fatty acid. The saponification values of avocado oil were 193.1 mg KOH/g oil for MAB, 198.4 mg KOH/g oil for MAP and 153.17 mg KOH/g oil for MAG, respectively. The peroxide values of MAB, MAP and MAP were 166.1, 124.7 and 14.9 meq kg<sup>-1</sup> oil, respectively. The Conjugated Dienes (CDs) and Conjugated Trienes (CTs) value of MAB, MAP and MAG were significantly different in the specific absorptivity range value from 2.6-3.7. The MAG had lowest CDs and CTs value. The anisidine value for avocado oil samples ranged from 10.59-11.36. There were no significant differences in the anisidine value among avocado oil samples. Avocado oil samples had high amounts of total unsaturated fatty acids, i.e., MAB (55.7%), MAP (62.8%) and MAG (68.9%), respectively. Thermal analysis by Differential Scanning Calorimetry (DSC) showed that avocado oil from three different cultivar had different melting and crystallization profile. Principal component analysis was used to classify each sample based on their DSC parameters. The results showed that by using the melting and crystallization profiles the discrimination of three avocado oils was very clear.

**Key words:** Fatty acid composition, avocado oil, differential scanning calorimetry, thermal profile, principal component analysis

### INTRODUCTION

The avocado (*Persea americana* Mill.) is a polymorphic tree species that originated in a broad geographical area from the Eastern and central highlands of Mexico through Guatemala to the Pacific (Storey *et al.*, 1986; Bost *et al.*, 2013; Athar and Nasir, 2005). Currently, avocado is a fruit that has been cultivated in many parts of the world, especially tropical countries. Avocado are existed in different shape, size, color depending on their variety. Avocado fruit can be consumed directly as a high energy food source because of content of lipids that significantly higher than in other fruit. Besides, avocado fruit is also a good source of oil (Quinones-Islas *et al.*, 2013).

Indonesia is one of the leading producing countries of avocado fruit. According to the Food and Agriculture Organization of the United Nations in 2012, Indonesia became the second leading producing countries (294,200 t) after Mexico (1,316,104 t) and followed by Dominican Republic

(290,011 t) and Chile (160,000 t) (FAOSTAT., 2013). Avocado fruit is a good oil source that has many health benefits, therefore, avocado oil can be considered as a functional oil (Gunstone *et al.*, 2007). Depending on the variety and the growth conditions, mesocarp of the avocado fruit contained 8-30% amount of oil (Quinones-Islas *et al.*, 2013). Nevertheless, studies about oil composition and thermal characteristics of Indonesian avocado cultivars is not available (Rohman *et al.*, 2015). Information of this would be important for the development of the use of avocado oil directly or product applications.

Avocado oil has been reported to lower cholesterol level (Moreno *et al.*, 2003), maintain skin elasticity (Athar and Nasir, 2005) and reduce the coronary heart risk (Berasategi *et al.*, 2012). Avocado oil is also widely used in the food industry, cosmetics and health products because of its unique characteristics and functions (Swisher, 1988), especially high content of unsaturated fatty acid. Unsaturated fatty acids include monounsaturated fatty acids (MUFAs) approximately 71%, which is dominated by oleic acid and polyunsaturated fatty acids (PUFAs) amounted to 13% of the total fat (Lu *et al.*, 2009).

There has been a considerable interest with regard to the oil potential of avocado mesocarp. Avocado oil is reported to have the beneficial health properties (Ding *et al.*, 2007; Swisher, 1988). Characterization studies of avocado oil has been reported using proximate analysis, physico-chemical properties (refractive index, specific gravity, iodine, saponification, acid and peroxide values) (Bora *et al.*, 2001; Moreno *et al.*, 2003), fatty acid profile (Berasategi *et al.*, 2012; Takenaga *et al.*, 2008), triacylglycerol profile (Yanty *et al.*, 2011a), volatile profile (Haiyan *et al.*, 2007) and thermal profile using Differential Scanning Calorimetry (DSC) (Yanty *et al.*, 2011a). Thermal analysis using DSC has been reported by Yanty *et al.* (2011a, b) to compare characteristics of three Malaysian avocado cultivars with Hass avocado variety and to compare stearin with olein fraction of avocado oil. From literature review, DSC has not been applied to characterize Indonesian avocado cultivars combined with multivariate statistical techniques (chemometrics).

DSC is one of thermal analysis method that has been widely used for determining various physico-chemical properties of oils in quality control. Differential Scanning Calorimetry (DSC) give the information about melting and crystallization phenomena of oils that is directly influenced by their physico-chemical properties such as fatty acid, TAG composition and chemical structure (Tan and Man, 2000). Melting and crystallization curve also correlated with quality, origin and storage history of the oil (Ferrari *et al.*, 2007). The DSC is suitable to discriminate between commercial EVOO and other samples of known geographical origin (Angiuli *et al.*, 2006).

Since the DSC method is rapid and does not require sample preparation or solvent utilization, it has more advantages than classical characterization methods that based upon the chromatographic (Chiavaro *et al.*, 2008). Nevertheless, only few reports available in the literature on the application of DSC to assess characterization of avocado oil. Trend studies about DSC lead to the combination of DSC with chemometrics. The multivariate classification (pattern recognition) strategies using Principal Component Analysis (PCA) coupled with DSC has been applied by Dahimi *et al.* (2014) to differentiate pig fat from chicken and beef fat. The results are quite satisfactory even at low doses <1%. The aim of this study was to evaluate the oil characteristics of Indonesian avocado varieties with physico-chemicals constant, conjugated dienes and trienes, p-anisidin value, fatty acid composition and volatile profile, the influence of major and minor chemicals component on DSC heating and cooling thermogram profiles and the use of DSC parameters-combined with PCA approach for differentiate native Indonesian avocado oil.

## MATERIALS AND METHODS

Three avocado cultivars of Bantul (MAB), Purwokerto (MAP) and Garut (MAG) were collected from three locations in Java, Indonesia. They were randomly selected from Sewon, Bantul, Yogyakarta with a round shape (MAB), Patikraja, Banyumas, Central Java with a bottle shape (MAP) and Samarang, Garut, West Java with round fruit and bottle shape (MAG). The MAB, MAP and MAG were harvested in February 2014, March 2014 and February 2015, respectively. The pieces of the mesocarp were dried manually using direct sunlight (MAB and MAP) and oven for 18 h at 70°C (MAG). Oil extraction from finely ground samples of dried avocado fruits was carried out by the cold percolation extraction method using n-hexane. Single and mixture of 37 standard fatty acids methyl ester were purchased from Sigma Aldrich (St Louis, MO). All the chemicals and solvents used in this study were of general and analytical grades.

**Determination physico-chemical parameters:** The characterization of physico-chemical of properties of oil samples were determined according to the standard analytical methods of the American Oil Chemists' Society (AOAS., 1996).

**Determination of Conjugated Dienes (CDs) and Conjugated Trienes (CTs):** The determination of CDs and CTs as the specific absorption coefficients in UV region was carried out according to Frankel *et al.* (1994). The oil samples (0.05 g) were accurately weighed into 10 mL volumetric flasks, dissolved in isoctane and made up to the mark with the same solvent. The mixtures were shaken thoroughly and their absorbances were measured for the determination of CDs and CTs using UV-Vis spectrophotometer U-2810 (Hitachi, Tokyo, Japan) at 234 and 270 nm, respectively, on a quartz cuvette (1 cm). These absorbances at specified wavelengths in UV region is related to the formation of CDs and CTs in avocado oil system due to oxidation processes.

**Determination of p-anisidin value (p-AV):** The p-AV was determined according to AOCS and Erkan *et al.* (2009). An aliquot of 0.5 g of oil samples was accurately weighed and dissolved with 25 mL of n-hexane. This solution was read using a UV-Vis spectrophotometer U-2810 (Hitachi, Tokyo, Japan) against n-hexane as a reference and recorded as  $A_1$ . In another separate run, a 5 mL portion of the sample solutions was mixed with 1 mL of p-anisidine solution (2.5 g L<sup>-1</sup> glacial acetic acid). Accordingly, a blank solution composed of 5 mL of n-hexane instead of sample solution was prepared. The sample and blank solutions were kept in the dark at ambient temperature for 10 min and the absorbance of the sample solution at 375 nm ( $A_2$ ) was measured against the blank solution. The p-AV was calculated as follows:

$$p-AV = 25 \frac{1.2A_2 - A_1}{m}$$

where,  $A_1$  and  $A_2$  are the measured absorbancies as described above, m is the mass of oil.

**Determination of fatty acid analysis:** Fatty acid composition of avocado oil was determined as Fatty Acid Methyl Esters (FAMES) according to the method described by Rohman and Che Man (2011) and Kumar *et al.* (2014). The oil samples (50 µL) were dissolved with 1 mL n-heptane and added with solution of 0.2 mL sodium methoxide 2 M in anhydrous methanol, place it in a test tube capped and then heated at a temperature 70°C for 10 min while occasionally shaken. The mixture

was added 1.5 mL of  $\text{BF}_3$  and then repeated the heating for 10 min. The mixture was added saturated solution of NaCl and mixed for 1 min using a vortex mixer. After sedimentation of sodium glycerolate, 1  $\mu\text{L}$  of the clear supernatant was injected into an Agilent HP-5 capillary column (0.25 mm internal diameter, 30 m length and 0.25 mm film) and analyzed using a gas chromatograph Agilent GC7890B (Agilent Technologist, USA) equipped with flame ionization detector. The temperature programme for GC was modified to achieve a good resolution of the peaks with a starting temperature of 160°C (hold time 2 min) and temperature flow rate 10°C/min to achieve a final temperature of 270°C in 11 min. The run was hold at 270°C for 7 min; hence, the total run time was approximately 20 min. A split-ratio was adjusted to 15:1 to prevent column-overloading.

**Determination of volatile compound:** An Agilent GC 6890N 5975B gas chromatograph with a capillary column HP-5 Agilent (0.25 mm internal diameter, 30 m length and 0.25 mm film) was used to determination of volatile compounds. The column temperature was programmed from an initial temperature of 80°C for 2 min, increased at 10°C/min to 300°C with a final isothermal period of 16 min. The flow rate of nitrogen carrier gas was 2 mL min<sup>-1</sup>. For identification, the volatile compounds were analyzed by Mass Spectrometry (MS) detector. Mass spectra were scanned from 30.00-700.00 m/z.

**Thermal analysis by DSC:** Thermal analysis was carried out on a Mettler Toledo differential scanning calorimeter DSC-60 Plus (Shimadzu, Jepang) equipped with a thermal analysis data station (TA60WS). Nitrogen (99.99% purity) was used as the purge gas at a rate of 20 mL min<sup>-1</sup>. The DSC instrument was calibrated with indium (m.p. 157.99°C, Hf = 28.62 J/g). Approximately of 9-12.5 mg (15  $\mu\text{L}$ ) of molten avocado oil samples consisting of MAB, MAP and MAG was placed in a standard DSC aluminum pan and then hermetically sealed. An empty, hermetically sealed DSC aluminum pan was used as the reference. The oil samples were subjected to the following temperature program: The sample was held at 80°C isotherm for 3 min to eliminate the thermal history of the samples then cooled at 5°C/min to -80°C and held for 3 min. The sample was then heated from -80 to 80°C at the same rate (Tan and Man, 2000). The DSC parameters of melting and crystallization curve were determined to characterize each sample. The DSC parameters consisted of the onset temperature (Ton, °C), the offset temperature (Tof, °C) (points where the extrapolated leading edge of the endotherm/exotherm intersects with the baseline), the range (range temperature between Ton and Tof), enthalpy (H, J/g) and the various temperature transitions (peak temperatures between To and Tf) were determined.

**Statistical analysis:** All analyses for physico-chemicals parameter were carried out in duplicate and the results were expressed as the mean value±standard deviation or Relative Percentage Difference (RPD). Data were statistically analyzed by one-way analysis of variance (ANOVA) followed by Tukey's test using MINITAB (version 16). The software MINITAB (version 16) also was used to construct thermal data analysis for pattern recognition with Principal Component Analysis (PCA).

## RESULTS AND DISCUSSION

**Physico-chemical parameters:** Avocado oil from all samples posses a dark green color due to the presence of pigments chlorophyll as dominant. The highest total yield was owned by avocado oil

Table 1: Some parameter constant of three cultivars avocado oil

Constants	Avocado oils		
	Bantul (MAB)	Pwt (MAP)	Garut (MAG)
Iodine value (g I <sub>2</sub> /100 g)	87.01±0.17 <sup>a</sup>	77.09±0.74 <sup>b</sup>	88.68±0.71 <sup>a</sup>
Saponification value (mg/KOH/g AO)	193.12±2.07 <sup>a</sup>	198.36±1.48 <sup>a</sup>	153.17±0.88 <sup>b</sup>
Acid value (mg/KOH/g AO)	31.87±0.14 <sup>a</sup>	3.05±0.27 <sup>b</sup>	2.17±0.40 <sup>b</sup>
Peroxide value (mequiv O <sub>2</sub> / kg AO)	166.10±0.38 <sup>a</sup>	124.65±0.51 <sup>ab</sup>	14.85±2.18 <sup>c</sup>
Conjugated dienes (g/100 mL cm <sup>-1</sup> )	3.707±0.005 <sup>a</sup>	3.743±0.003 <sup>b</sup>	3.595±0.006 <sup>c</sup>
Conjugated trienes (g/100 mL cm <sup>-1</sup> )	3.210±0.001 <sup>a</sup>	3.026±0.005 <sup>b</sup>	2.578±0.002 <sup>c</sup>
Anisidin value	11.36±0.65 <sup>a</sup>	10.87±0.12 <sup>a</sup>	10.59±0.23 <sup>a</sup>

<sup>a,b</sup>Each value in the table represents the Mean±SD of triplicate except \* represent duplicate analyses±RPD. Mean within each column with different superscripts are significantly (p<0.01) different, AO: Avocado oil

MAG (49.73%) followed by MAP (31.36%) and then MAB (24.46%). The MAG easily being lipids in the semisolid form at the room temperature. On the other hand, MAB and MAP being liquid oil. The differences form and yield of the oils from three local avocado cultivars could be attributed to the differences in their drying methods and their fatty acid composition. Avocado with oven drying method ables to maintain the oil content compared to that dried with direct sun-drying. Table 1 exhibited some parameter constants of three cultivars of avocado oil obtained from hexane cold percolation. The Iodine Value (IV) indicates the unsaturation degree of fats and oils (Lusas *et al.*, 2012). The slightly higher iodine value of MAG gives the indication that MAG contains more unsaturated fatty acid than MAB and MAP. The Iodine Value (IV) of all samples were closer to the values of other varieties reported by Bora *et al.* (2001), Moreno *et al.* (2003), Yanty *et al.* (2011a) and Quinones-Islas *et al.* (2013). Generally, these results are consistent with some reports stating that the number of IV for avocado oil was in the range of 65-95 (AOCS., 1998).

Different from IV as presented in Table 1, Saponification Value (SV) of MAG, MAB and MAP generally was slightly lower than the SV of avocado oil from Mexico and Brazil that reported by Bora *et al.* (2001) and Moreno *et al.* (2003). The SV is inversely proportional to the mean molecular weight of the fatty acid in oil. The MAG has the lowest saponification value. But, only MAP and MAB that has SV which corresponds to the range of SV of avocado oil in AOCS. (1998) i.e., 170-198 mg/KOH/g oil. The data indicated that MAG has unsaponifiable matter more than MAB and MAP. Acid value indicated the free fatty acids present in fats and oils. High degree acid value can be related with degree of oxidation during preparation or storage. The good quality of oil generally has low acid value. Since MAP and MAG has lower acid value than MAB, it indicated that the quality of MAP and MAG was better than MAB. The highest acid value of MAB may be indicated by high degree of oxidation because of MAB was stored longer than MAP and MAG. Acid value of avocado oil has been reported to be 0.65 (Moreno *et al.*, 2003) and 1.23 mg/KOH/g oil.

The extent of oxidative activity in oil may be estimated by Peroxide Value (PV). The number of peroxides present in vegetable oils reflect its oxidative level and thus tend to become rancid. The oxidation products generate hidroperoxide as primary oxidation product that was quantified with PV (Shahidi and Wanasundara, 2008). The peroxide values of MAB and MAP are definitely high, indicating that the samples are unstable against oxidation. A product with peroxide value above 10 meq kg<sup>-1</sup> is classified at high oxidation state (Moigradean *et al.*, 2012). Garut has the lowest PV, but PV also relatively high. This result maybe due to the presence of chlorophylls as photo-sensitizers which have not been removed in crude avocado oil samples. Besides, avocado oil should exhibit a high rate of oxidation due to its high content of unsaturated fatty acids. Unsaturated fatty acids easily react with oxygen to form peroxides. On other hand, the iodometric method might fails to adequately measure low PV because of difficulties encountered in

determination of the titration end point (Shahidi and Wanasundara, 2008). Peroxide values of avocado oil have been reported in the range of 5.1-12.3 meq kg<sup>-1</sup> (Quinones-Islas *et al.*, 2013).

During the early stages of oxidation, the increase in UV absorption due to the formation of CDs and CTs is proportional to the uptake of oxygen and to the production of peroxides. Therefore, the content of CDs and CTs also can serve as a relative measurement of oxidation. The contents of CDs and CTs were expressed with the specific absorptivity values of MAB, MAP and MAG. There was a significant difference ( $p < 0.01$ ) of all CDs values for MAB, MAP and MAG. The lowest CDs and CTs value was possessed by MAG that indicated lower stage of oxidation. While, the presence of malonaldehyde as secondary oxidation product may be measured by p-Anisidine Value (p-AV). In the second phase of oxidation, the primary product of oxidation, peroxides decompose and develop substances such as aldehydes, which are responsible for the rancid smell and taste. The P-AV determines the amount of aldehyde (principally 2-alkenals and 2,4-alkadienals) in vegetable oils (Shahidi and Wanasundara, 2008). The P-AV for avocado oil samples ranged from 10.59-11.36. There were no significant differences in the anisidine value among the avocado oil samples. These results were not in line with PV, CDs and CTs.

**Fatty acid analysis:** Table 2 showed fatty acid composition of avocado oil samples. All oils of three local cultivars are found to have oleic acid as the most dominant fatty acid. The main fatty acids composed of samples were oleic (C18:1), palmitic (C16:0), linoleic (C18:2) and palmitoleic acids (C16:1). These main fatty acid composition agreed with previous studies (Haiyan *et al.*, 2007; Moreno *et al.*, 2003; Yanty *et al.*, 2011a). Table 2 showed that the fatty acid composition of avocado oil samples especially MAG is very similar to that reported by Yanty *et al.* (2011a). All oils had high

Table 2: Composition of fatty acid in avocado oil from three cultivars

Fatty acid	Relative fatty acid of avocado oil (%)			
	MAB	MAP	MAG	Yanty <i>et al.</i> (2011a)
C6:0	0.14	0.05	ND	-
C8:0	0.06	0.03	ND	-
C10:0	0.02	ND	ND	-
C12:0	0.09	0.01	0.01	-
C14:0	0.15	0.11	0.11	-
C15:0	0.03	0.02	0.05	-
C16:1	10.06	7.68	7.42	7.44±0.03
C16:0	30.91	28.73	25.28	26.41±0.1
C17:1	0.10	0.08	0.10	-
C17:0	0.09	0.06	0.04	-
C18:2	8.67 <sup>b</sup>	10.65 <sup>b</sup>	11.95 <sup>b</sup>	12.75±0.5
C18:3n3	0.82 <sup>b</sup>	1.00 <sup>b</sup>	1.13 <sup>b</sup>	1.20±0.18
C18:3n6	-	-	-	-
C18:1	34.79 <sup>b</sup>	42.77 <sup>b</sup>	47.99 <sup>b</sup>	51.18±0.8
C18:1n9t	-	-	-	-
C18:0	1.17	1.04	1.09	1.03±0.05
C20:4	0.16	0.07	ND	-
C20:1	1.14	0.58	0.35	-
C20:0	0.25	0.24	0.18	-
C22:0	0.12	0.06	0.09	-
C23:0	0.04	0.02	0.04	-
C24:0	0.15	0.12	0.12	-
Total USFA	55.73	62.84	68.94	
Total SFA	33.21	30.5	27.01	

<sup>b</sup>Relative percentage of fatty acid for C18:1, C18:2 and C18:3 were compared with relative percentage of fatty acid reported by Yanty *et al.* (2011a) due to co-elution a number of peaks at retention time 8.17 min, MAG: Avocado oil from Garut, MAB: Avocado oil from Bantul and MAP: Avocado oil from purwokerto



amounts of total unsaturated fatty acids, MAB (55.73%), MAP (62.84%) and MAG (68.94%). Meanwhile, the highest level of saturated fatty acids is owned by MAB (33.21%) followed by MAP (30.50%) and MAG (27.01%). Fatty acid composition of avocado oil depends upon the cultivar, stage of ripening and the geographical growth location and different sample processing (Ahmed and Barmore, 1980; Bora *et al.*, 2001; Moreno *et al.*, 2003).

**Composition of volatile compound of avocado oil:** Analysis of volatile compounds using GC showed 63 peaks (MAB), 43 peaks (MAP) and 69 peaks (MAG). The volatile compounds are identified by mass spectra dataset that supplied by the instrument of GC-MS. Only peak with similarity index >80% that was identified. There were significantly fewer identified volatile compounds in MAG sample (8.01%) than MAB (83.01%) dan MAP (95.04%).

The analysis of volatile compounds showed that some identified components were product of secondary oxidation and degradation of fatty acids, such us aldehyde, alkane and alcohols. The samples contained terpenoids ( $\beta$ -caryophyllene,  $\alpha$ -copaene), aldehydes (hexanal, octanal and nonanal), tridecane and undecane. Hexanal has been reported to be a volatile compound present in avocado oil, together with octanal and nonanal in the avocado oils that were extracted with hexane (Haiyan *et al.*, 2007; Sinyinda and Gramshaw, 1998). This result is in concordance with the data reported by Moreno *et al.* (2003) which avocado oil contained 2,4-decadienal. The compound of 2,4-decadienal was found only in MAB and MAP. This compound indicates that lipid oxidation took place.

**Thermal characteristics by DSC:** Thermal characteristics of three cultivars of avocado oils is compared with palm oil (MKS) as shown in Fig. 1. In Fig. 1 (X), DSC cooling curves of MAB, MAP and MAG are represented by the curves a, b, c and d, respectively. From this, it is clear that the cooling profiles of each local avocado cultivars are different. For instance, the initial point of crystallization transition of MAB, MAP and MAG and MKS (palm oil) are A1 (5.22°C), B1 (7.72°C), C1 (21.67°C), dan D1 (0.89°C), respectively. While all three local cultivars displayed a high melting exothermic thermal transition above 0°C. As the final melting transition of MAB, MAP and MAG and MKS are A7 (13.76°C), B7 (8.94°C), C7 (39.00°C), D3 (9.21°C), respectively. The thermal behavior differences among avocado local cultivars and palm oil could be mainly due to the differences in their fatty acid and TAG molecular (Tan and Man, 2000). Cooling and melting thermogram of palm oil 100% samples were similar to those previously reported by Man *et al.* (1999) as refined bleached deodorized palm superolein. While, DSC cooling and melting thermograms of avocado oil were different with previous study reported by Yanty *et al.* (2011a). This might be due to different temperature scanning and type of calorimeter. All DSC cooling and heating data are summarised in Table 3.

Oils and fats do not have specific melting and crystallization temperatures, but they show melting/crystallization profiles (Tan and Man, 2012). The DSC crystallization curve, which is influenced only by the chemical composition of the sample and not by the initial crystalline state is more reproducible and simpler than the melting curve (Tan and Man, 1999). The complex features in the DSC melting curves of oils were not easily interpretable due to the consequence of the polymorphism phenomenon of oils that is strongly dependent on the thermal history of the sample (Tan and Man, 2000). The polymorphism transformations in avocado oil samples have not been reported in this study because of the polymorphism study of TAG can only be achieved by X-ray analysis.



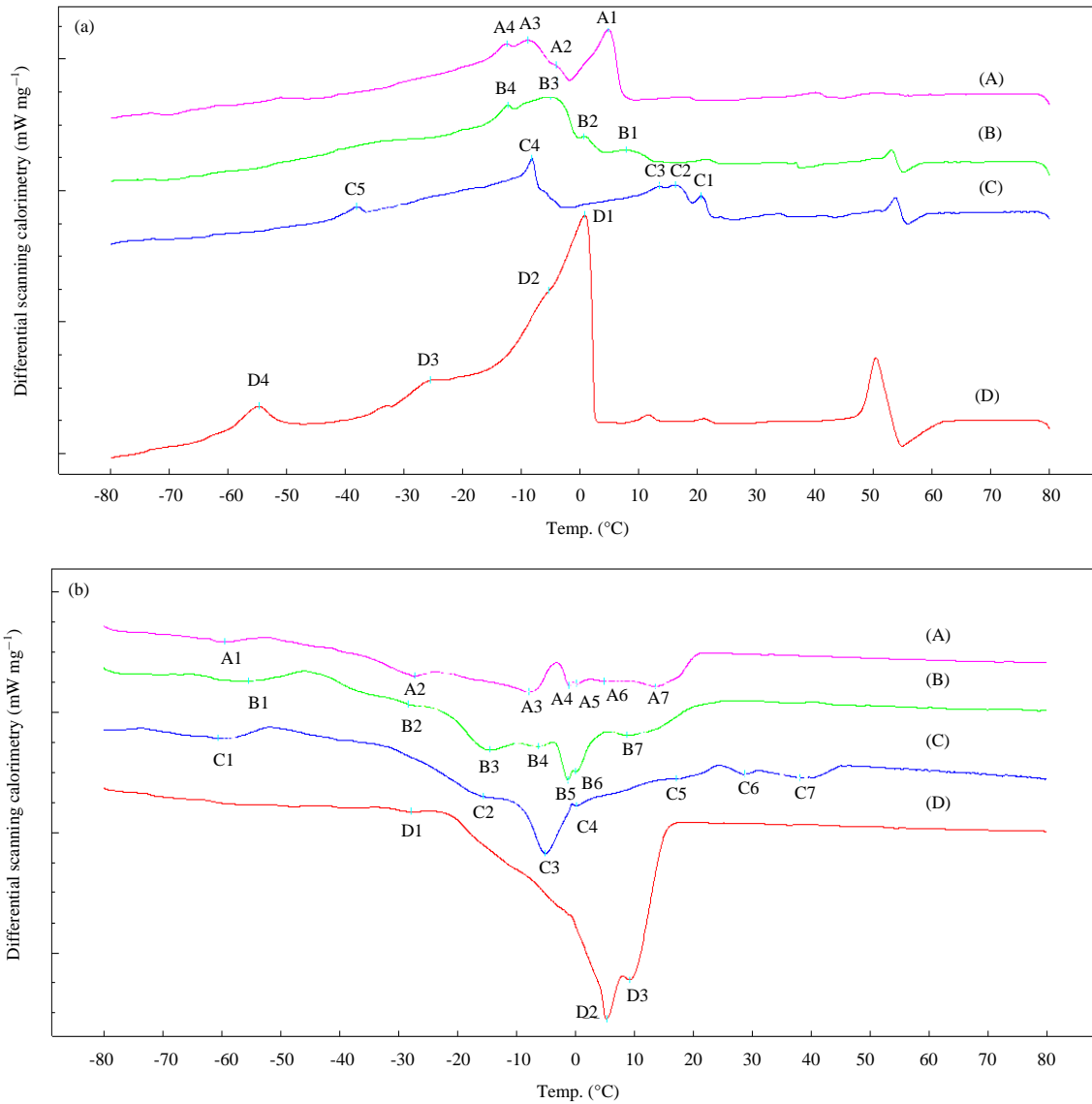


Fig. 1(a-b): (a) Crystallization profile (X) and (b) Melting profile (Y) of oil samples of A: MAB, B: MAP, C: MAG and D: Palm oil (MKS) 100%

Exothermic events were observed on DSC cooling thermograms of avocado oil samples, a major and a minor peaking associated with the crystallisation of TAG. Generally, the highly saturated TAG melted at higher temperatures than the highly unsaturated TAG. Man *et al.* (1999) identified that peak at -11.22 until -1.6°C was peak of disaturated TAG and then another peaks under these temperature were peak of TAG which more unsaturated. Compared with the crystallisation curve of MKS, generally indicated that avocado oil samples have more unsaturated TAG fractions than MKS. Meanwhile, MAG has significantly different cooling profile with MAB and MAP. The mayor peak in the range 13-22°C indicated that MAG has more saturated TAG than other samples. MAG might contain more stearin fraction as reported by Yanty *et al.* (2011b).

Table 3: Comparison of differential scanning calorimetry-measured transition temperatures for four different samples

Samples	Transition temperature (°C) <sup>a</sup>						
	1	2	3	4	5	6	7
<b>Cooling</b>							
MAB	5.22	-4.05	-9.22	-11.69			
MAP	7.72	0.65	-4.35	-10.60			
MAG	21.67	17.05	*	*	*	*	*
MKS	0.89	-5.94	-25.52	-54.48			
<b>Heating</b>							
MAB	-59.17	-27.48	-8.17	-1.12	0.21	5.12	13.76
MAP	-55.67	-28.36	-14.42	-6.21	-1.16	0.03	8.94
MAG	-60.46	-14.75	-5.33	0.12	15.11	25.47	39.00
MKS	-27.93	-1.13	5.32	9.21			

<sup>a</sup>Each value in the table represent the mean for two determinations. Relative percent difference of the reported results are in the range of 0-1.53°C, 0.19-3.35°C, 1.36-1.9°C, 0-0.70°C for cooling MAB, MAP, MAG and MKS (palm oil), respectively and in the range of 0.08-0.74°C, 0-0.52°C, 0.15-6.57°C, 0-0.31°C for heating MAB, MAP, MAG and MKS, respectively, <sup>b</sup>\*Value could not be averaged because of unreproducible, MAB: Bantul, MAP: Purwokerto, MAG: Garut

Table 4: Differential scanning calorimeter data obtained from the cooling and heating thermograms of avocado oil samples

Sample	DSC analysis parameters <sup>a</sup>			
	Onset (°C)	Enthalpy (J/g)	Offset (°C)	Range (°C)
<b>Cooling</b>				
MAB	7.82±12.28	32.39±0.77	-64.15±0.55	71.97±0.85
MAP	12.21±0.16	37.28±1.26	-64.39±0.19	76.60±0.18
MAG	23.40±9.57	37.97±17.9	-64.40±1.15	87.80±3.39
MKS	2.63±5.71	54.26±1.73	-64.23±0.16	66.86±1.41
<b>Heating</b>				
MAB	-72.79±0.14	-37.78±1.14	20.32±0.25	93.11±0.05
MAP	-63.44±0.22	-49.32±2.15	21.45±0.19	84.89±0.21
MAG	-73.25±0.53	-58.20±4.62	45.29±1.19	118.54±0.10
MKS	-62.13±0.53	-61.82±0.16	15.33±0.13	77.46±0.45

<sup>a</sup>Each value in the table represent the mean for two determinations±RPD, MAB: Bantul, MAP: Purwokerto, MAG: Garut

**Chemometric analysis:** In order to differentiate and classify three avocado oil samples from different cultivars, DSC parameters including onset temperature (Ton), enthalpy, end set temperature (Tof) and range as shown in Table 4 was subjected to Principal Component Analysis (PCA). Scores and loadings matrices were generated using 4 Principal Components (PCs) in exothermic and endothermic event. An eigenvalue of exothermic event about 76% was achieved using 1 PCs. This is a much greater proportion than any of the original variables. While PC2 described 22% of the variation but with eigenvalue <1. An eigen value of endothermic event about 99.9% was achieved using two PCs where PC1 accounted for 73.1% of the variation, while PC2 described 26.8% of the variation; therefore, the remaining two (<1% total) did not explain significant variability in the data.

The scores plot the samples were displayed in three groups as shown in Fig. 2 for heating data. The first group MAB, second group MAP and the last group MAG. The distribution of these groups helped to observe difference of avocado oil samples from different cultivars. Only MAG that has different location among two determinations of cooling profile. This could be due to MAG has not reproducible crystallization data as shown in Table 3. While, PCA result of heating data better than cooling data. Conversely, PCA score plot of heating data showed very clear discrimination. The DSC analysis may be a useful tool for differentiate avocado oils from three different cultivars. The DSC combined with chemometrics represent a rapid and attractive option for avocado oil quality screening without sample pretreatments.

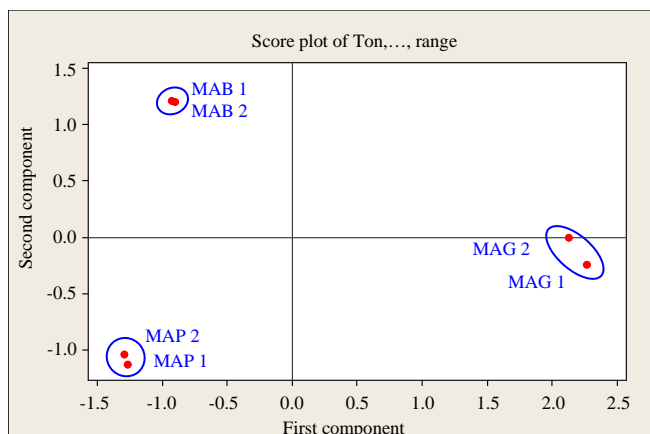


Fig. 2: Principal component analysis score plot of PC1 versus PC2 from heating thermogram data

## CONCLUSION

Among avocado oils evaluated, MAB is being the most unstable oxidative avocado oil with the highest peroxide value, conjugated dienes, conjugated trienes and Anisidin value. The MAG is found to have the highest proportion of unsaturated fatty acids when compared to the other studied oils of MAB and MAP. The compound of 2,4-decadienal was found only in MAB and MAP in which both oils were processed with direct sun-drying. Based on thermal profile, both cooling and heating DSC thermograms could characterize each sample of avocado oils. The cooling thermogram of MAG showed that MAG has more saturated TAG than MAB and MAP. Finally, this study has shown the ability of DSC coupled with PCA in discriminating avocado oil from different cultivars.

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