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## Research Article

# Authentication of Avocado Oil (*Persea americana* Mill.) Using Differential Scanning Calorimetry and Multivariate Regression

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## Abstract

The potential application of Differential Scanning Calorimetry (DSC) combined with multivariate calibration was used to verify adulteration of avocado oil from Indonesian avocado cultivars with Refined Bleached Deodorized Palm Superolein (RBDPSO). Avocado oil and adulterant were characterized by significantly different cooling and heating DSC thermal profiles. The addition of RBDPSO makes the shift of overall transitions temperature toward lower temperature, enhancing crystallization, melting enthalpy and developing both process over a narrower temperature range. The change of characteristic exothermic and endothermic event in avocado oil with increasing adulterant was possibly associated with the increase of oleic and stearic acids along the decrease of palmitoleic acid. The multivariate calibration approach was applied to DSC data in order to build the quantitative calibration model for adulterant concentration in a range of 0-50%, (v/v). Partial Least Square Regression (PLSR) and Stepwise Multiple Linear Regression (SMLR) were tested to these mixtures. Generally, both calibration models showed good correlation coefficient (R) with low errors in both calibration and validation sets. But, SMLR model showed better criteria values than PLSR, not only on DSC crystallization profile data but also on heating profiles data. The crystallization models of SMLR and PLSR showed the higher R value (above 0.99) than melting calibration models. The results presented in this study suggest that DSC analysis may be a useful tool for detecting adulteration of avocado oil with RBDPSO. The DSC represent a rapid, environmentally friendly and alternative option for avocado oil quality screening without sample pretreatments.

**Key words:** Adulteration, crystallization profile, partial least square, stepwise multiple linear regression, RBDPSO

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**Competing Interest:** The authors have declared that no competing interest exists.

**Data Availability:** All relevant data are within the paper and its supporting information files.

## INTRODUCTION

Differential Scanning Calorimetry (DSC) is one of thermal analysis methods that is the most widely used for analysis of oils and fats, especially for authentication studies of oil as a quality control. Besides, thermal analysis has long been used in material science and testing, particularly in the field of polymer (Warne, 1992). The DSC give the information about melting and crystallization phenomena of oils that is directly influenced by their physicochemical properties such as fatty acid, triglyceride (TAG) composition and chemical structure (Tan and Man, 2000).

The application DSC in the field of oils and fats have a great interest in authentication and detection of adulteration. Each edible oil has fingerprint profile in their thermal behaviors including melting and crystallization profile that is closely related to the chemical composition of the oil (Tan and Man, 2000, 2002). Several studied have evaluated DSC application to detection of adulteration of edible oils and fats, such as detection of animal fat in canola oil (Marikkar *et al.*, 2002), soybean, sunflower and canola oils in olive oil (Jafari *et al.*, 2009), refined hazelnut oil in extra virgin olive oil/EVOO (Chiavaro *et al.*, 2008) and sunflower oil in EVOO (Angiuli *et al.*, 2009; Van Wetten *et al.*, 2015) but as far as the author knowledge, DSC has not been applied to detection adulteration of avocado oil (Chiavaro, 2014).

Indonesia is one of the leading producing countries of avocado fruit (*Persea americana* Mill.). According to the Food and Agriculture Organization of the United Nations (FAO., 2015), Indonesia became the third leading producing countries in 2013 (276,311 t) after Mexico (1,467,837 t) and Dominican Republic (387,546 t) and then followed by Chile (164,750 t). Avocado fruit is a good source of nutritious oil, which posses many health benefits. Mesocarp of avocado fruit contains 8-30% oil, depending on the variety and growth conditions (Quinones-Islas *et al.*, 2013). Avocado oil is widely used in the food industry, cosmetics and health products because of its unique characteristics and functions (Swisher, 1988), especially due to high content of monounsaturated fatty acid (oleic acid). Due to various benefits of avocado oil, it makes avocado oil has relatively high prices in the oil market (Quinones-Islas *et al.*, 2013). As a consequence, there is the potential adulteration of avocado oil with cheaper and lower quality oil. Most of analytical techniques to detect adulteration are based upon the chromatographic methods. These methods usually laborious, require chemical treatments and have high environmental impact. Since the DSC method is rapid and does not require sample preparation or solvent

utilization, it has more advantages than classical methods that based upon the chromatographic methods (Chiavaro *et al.*, 2008).

Recent studies about DSC lead to the combination of DSC with chemometrics of multivariate statistical techniques. The use of chemometrics to evaluate the quality of edible oils is extensively reported in literature. Mathematical model based on a regression procedure was developed to correlate thermal parameters to major and minor components or concentrations of adulterant. Cerretani *et al.* (2011) reported the use of combination of DSC-Partial Least Square (PLS) to construct a predictive model for fatty acid composition in 63 samples of oil (olive oil, hazelnuts, sunflower and canola). The results are quite satisfactory with high coefficient determination ( $R^2$ ) and low Root Mean Square Error of Calibration (RMSEC) and Root Mean Square Error of Prediction (RMSEP). Using literature review, DSC applications for detection adulteration of avocado oil in combination with multivariate analysis has not been reported before. Adulteration of avocado oil with different edible oils (sunflower, canola and soybean) has been reported previously using Fourier Transform Infrared Spectroscopy (FTIR) combination with PLS (Quinones-Islas *et al.*, 2013). The aim of this study was to use of DSC to discrimination between avocado oil and adulterated samples (avocado oil-refined bleached deodorized palm superolein in seven level concentration) and to develop and validate an analytical method based on DSC data, in combination with multivariate calibration of Partial Least Square (PLS) and Stepwise Multiple Linear Regression (SMLR) for the prediction of adulterant concentration.

## MATERIALS AND METHODS

Two of avocado cultivars (AO1 and AO2) were collected from two locations in Java, Indonesia and harvested in 2014. They were randomly selected from Sewon, Bantul, Yogyakarta with a round shape (AO1) and Patikraja, Banyumas, Central Java with a bottle shape (AO2). The pieces of the mesocarp were dried manually using direct sunlight. Oil extraction from finely ground samples of dried avocado fruits was carried out by the cold percolation extraction method using n-hexane. The Refined Bleached and Deodorized Palm Superolein (RBDPSO) were purchased from local supermarket. Avocado oil samples from two cultivars (AO1 and AO2) were mixed (AO). Admixtures of AO:RBDPSO were prepared at different ratios (90:10, 80:20, 70:30, 60:40, 50:50, v/v) to build calibration models and validation models (90:10, 80:20, 70:30, 50:50, v/v). Samples were stored in dark place at room temperature

before analysis. All the chemicals and solvents used were of analytical grades (Merck, Germany). Fatty acids methyl ester standards (single and mixture 37 std. FAME) were purchased from Sigma Aldrich (St Louis, MO).

**Analysis of fatty acid composition:** Fatty acid compositions of avocado oil were determined as Fatty Acid Methyl Esters (FAMES) according to the method described by Rohman and Man (2011) and Kumar *et al.* (2014). Oil samples (50  $\mu$ L) was 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 BF<sub>3</sub> and then repeated the heating for 10 min. The mixture was added saturated NaCl and mixed for 1 min using a vortex mixer. After sedimentation of sodium glycerolate, 1  $\mu$ L of the clear supernatant was injected into an Agilent HP-5 capillary column (30 m  $\times$  0.25 mm id; 0.25  $\mu$ m film thickness) and analyzed using a gas chromatograph Agilent GC7890B (Agilent Technologist, USA) equipped with flame ionization detector. The column temperature programme was 160°C held for 2 min and increased at rate 10°C min<sup>-1</sup> to achieve a final temperature of 270°C in 11 min. The run was held 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.

**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,  $\Delta H_f = 28.62$  J g<sup>-1</sup>). Approximately 9.0-12.5 mg (15  $\mu$ L) of oil samples (AO and RBDPSO) 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<sup>-1</sup> 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 consisting 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 ( $\Delta H$ , J g<sup>-1</sup>) and the various temperature transition (peak temperatures between To and Tf) were determined.

**Statistical analysis:** All thermal analyses were carried out in duplicate and the results were expressed as the Mean value  $\pm$  RPD (Relative Percent Difference). All statistical analyses were performed using Minitab software (version 16, Minitab, USA). Data were statistically analyzed by one-way analysis of variance and Tukey's multiple comparison test with family error rate of 5%. Multivariate regression of DSC thermal data were evaluated with PLS and SMLR. Quantification models that offering the highest values of coefficient of determination ( $R^2$ ) and the lowest values of Root Mean Square Error of Calibration (RMSEC) were selected for developing PLS and SMLR calibration models. The calibration models were further used to predict the concentration level of oil adulterants in samples. The values of  $R^2$  and Root Mean Square Error of Prediction (RMSEP) were used for prediction criteria.

## RESULTS AND DISCUSSION

**Fatty acid analysis:** Table 1 showed fatty acid composition of Avocado Oil (AO), RBD Palm Superolein (RBDPSO) and their admixtures. The mixture of avocado oils from two local cultivars are found to have oleic acid as the most dominant fatty acid. The main fatty acids composed of AO were oleic (C18:1), palmitic (C16:0), palmitoleic (C16:1) and linoleic acids (C18:2). These main fatty acid composition agreed with previous studies (Haiyan *et al.*, 2007; Moreno *et al.*, 2003; Yanty *et al.*, 2011a). The relative percentage of fatty acid of the avocado oils samples are similar with that reported by Yanty *et al.* (2011a) for Malaysian avocado cultivars. According to previous report, the fatty acid composition of avocado oil depends on the geographical growth condition, variety (Quinones-Islas *et al.*, 2013), cultivars and stage of ripening (Ahmed and Barmore, 1980; Bora *et al.*, 2001). While, the RBD Palm Superolein (RBDPSO) has highest concentration of total saturated fatty acid. The main fatty acids in RBDPSO are oleic (C18:1), palmitic (C16:0), linoleic (C18:2) and stearic acids (C18:0). This result agreed well with Man *et al.* (1999) and Tan and Man (2000). The admixture 50% RBDPSO showed that three principles fatty acids that were clearly affected by the addition of RBD palm superolein. The progressively decreasing of the content of palmitoleic acid (C16:1) and the slightly increasing of oleic (C18:1) and stearic acid (C18:0) existed in the addition of RBD palm superolein.

Table 1: Distribution of fatty acid in avocado oil, RBD palm superolein and their 50% admixture

Fatty acids	Relative fatty acids (%)		
	AO	RBDPSO 50%	RBDPSO
C6:0	0.22	ND	ND
C8:0	0.08	0.02	0.01
C10:0	0.01	0.01	0.02
C12:0	0.07	0.13	0.20
C14:0	0.14	0.60	0.98
C15:0 <sup>a</sup>	0.04	0.03	0.04
C16:1 <sup>a</sup>	8.84	3.94	0.22
C16:0	32.50	33.75	36.99
C17:1 <sup>a</sup>	0.10	0.06	0.03
C17:0 <sup>a</sup>	0.04	0.07	0.09
C18:2 <sup>a</sup>	8.52 <sup>b</sup>	10.45 <sup>b</sup>	10.99 <sup>b</sup>
C18:3n3 <sup>a</sup>	0.80 <sup>b</sup>	0.98 <sup>b</sup>	1.03 <sup>b</sup>
C18:3n6 <sup>a</sup>	-	-	-
C18:1 <sup>a</sup>	34.21 <sup>b</sup>	41.95 <sup>b</sup>	44.12 <sup>b</sup>
C18:1n9t <sup>a</sup>	-	-	-
C18:0	1.12	2.78	4.01
C20:4 <sup>a</sup>	0.17	ND	ND
C20:1 <sup>a</sup>	0.97	0.37	0.21
C20:0 <sup>a</sup>	0.19	0.30	0.37
C22:0	0.08	0.13	0.07
C23:0 <sup>a</sup>	0.04	0.02	0.02
C24:0	0.14	0.10	0.08
<b>Total USFA</b>	<b>53.61</b>	<b>57.75</b>	<b>56.62</b>
<b>Total SFA</b>	<b>34.68</b>	<b>37.95</b>	<b>42.88</b>

AO: Mixture of avocado oil from two cultivars, RBDPSO: Refined bleached and deodorized palm superolein, USFA: Unsaturated fatty acid, SFA: Saturated fatty acid, <sup>a</sup>Predicted fatty acid compared with single standards (bold), <sup>b</sup>Relative fatty acid (%) for C18:1, C18:2 and C18:3 were compared due to co-elution a number of peaks at retention time 8.17 min

Table 2: DSC parameters obtained from crystallization thermograms of AO, RBDPSO and their admixtures

Samples (%)	DSC parameters			
	Onset (°C)	Enthalpy (J g <sup>-1</sup> )	Offset (°C)	Range (°C)
AO 100	9.90 ± 1.31 <sup>a</sup>	35.07 ± 1.25 <sup>d</sup>	-64.73 ± 2.10	74.63 ± 1.65 <sup>a</sup>
RBDPSO 10	9.03 ± 0.33 <sup>a</sup>	37.08 ± 3.21 <sup>d</sup>	-64.54 ± 0.85	73.56 ± 0.71 <sup>ab</sup>
RBDPSO 20	7.91 ± 4.68 <sup>b</sup>	38.33 ± 3.03 <sup>cd</sup>	-65.11 ± 0.40	73.02 ± 0.15 <sup>ab</sup>
RBDPSO 30	7.76 ± 5.54 <sup>b</sup>	42.17 ± 1.40 <sup>b</sup>	-63.84 ± 1.71	71.94 ± 2.13 <sup>bc</sup>
RBDPSO 40	6.37 ± 7.85 <sup>c</sup>	41.44 ± 4.63 <sup>bc</sup>	-64.25 ± 0.02	70.62 ± 0.72 <sup>c</sup>
RBDPSO 50	6.40 ± 5.94 <sup>c</sup>	44.65 ± 2.89 <sup>b</sup>	-63.69 ± 0.27	70.09 ± 0.78 <sup>c</sup>
RBDPSO 100	2.63 ± 5.71 <sup>d</sup>	54.26 ± 1.73 <sup>a</sup>	-64.23 ± 0.16	66.86 ± 0.37 <sup>d</sup>

Each value in the table represents the mean for two determinations ± RPD. Means within each column with different superscripts are significantly different (p < 0.05), DSC: Differential scanning calorimetry, AO: Mixture of avocado oil from two cultivars, RBDPSO: Refined bleached and deodorized palm superolein

**DSC analysis of crystallization profile:** The DSC crystallization profile is obtained for AO, RBDPSO and their admixtures (10-50% RBDPSO in AO). The measured parameters are the beginning of crystal formations (onset, Ton), the end of crystallization (offset, Tof), the amount of energy that lost from samples during crystallization (enthalpy) and the range temperature between Ton and Tof (range). Table 2 summarizes the DSC parameters that characterize the crystallization profile. The crystallisation thermograms of AO (mix AO1 and AO2), RBDPSO and their admixtures are shown in Fig. 1a. The DSC crystallization profile of RBDPSO samples were similar to those previously reported by Man *et al.* (1999) and Tan and Man (2000). While, crystallisation profile of

avocado oil was different with previously reported by Yanty *et al.* (2011a, b). This may be due to the different nature of samples, method of preparations and treatment of avocado oils that influenced avocado oil composition. Generally, DSC crystallisation thermogram was easier to interpret than melting thermogram because it was influenced only by chemicals composition of samples (Tan and Man, 2000).

Thermogram profile of AO 100% exhibited two principal peaks (peak 1 and peak 2) while, RBDPSO exhibited three principal peaks. The differences of thermal behavior could be mainly due to the differences in their fatty acid and triacylglycerol (TAG) composition (Tan and Man, 2000). The AO crystallization thermograms showed two well defined

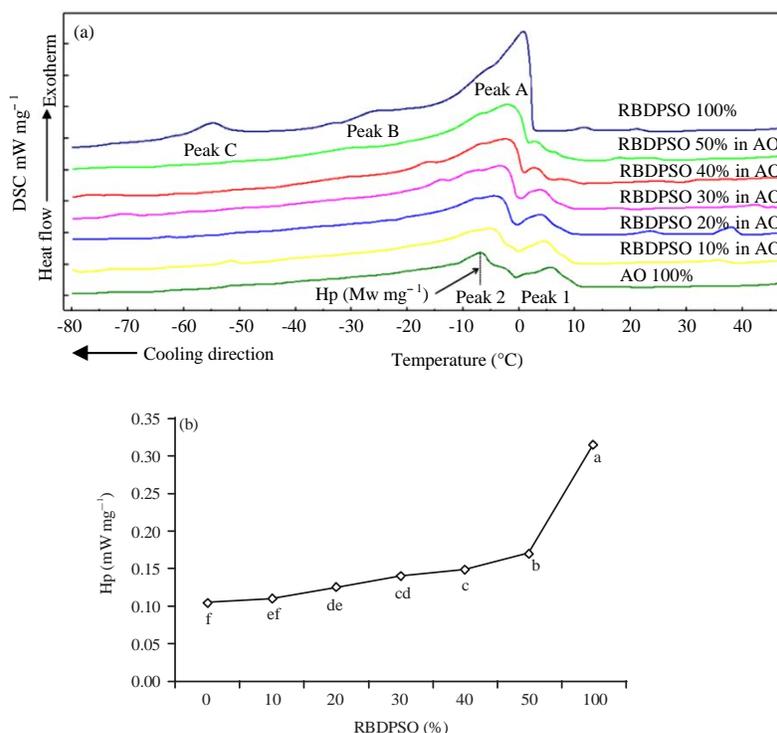


Fig. 1(a-b): (a) Representative DSC crystallization thermograms of AO, RBDPSO and their admixtures, (b) Peak height ( $\text{Mw mg}^{-1}$ ) of peak 2 of the crystallization thermograms at different RBDPSO percentages added. Scatters with the same letters are not significantly different ( $p < 0.05$ ), DSC: Differential scanning calorimetry, AO: Mixture of avocado oil from two cultivars, RBDPSO: Refined bleached and deodorized palm superolein

exothermic events, two major peaks at  $5.49^\circ\text{C}$  (peak 1) and  $-6.78^\circ\text{C}$  (peak 2), respectively. Furthermore, three well defined exothermic events of RBDPSO were observed, one major and two minor peaks at  $0.89^\circ\text{C}$  (peak A),  $-25.52^\circ\text{C}$  (peak B) and  $-54.48^\circ\text{C}$  (peak C), respectively. According to Man *et al.* (1999) and Tan and Man (2002), the major peak (peak 1) of RBDPSO was associated with the crystallisation of disaturated TAG while the minor peak (peak B and C) was attributed to the crystallisation of more unsaturated TAG fractions of RBDPSO (monosaturated and triunsaturated TAG). Generally, the higher degree of saturated TAG melted at higher temperature than the higher unsaturated TAG. Compared with RBDPSO, the thermograms of AO 100% gives the indication that avocado oil mainly contains disaturated TAG (peak 1 and 2). Peak 1 of AO may be associated with crystallisation of disaturated TAG that contains palmitoleic acid.

The DSC parameters of RBDPSO 100% were shown in Table 2. They were similar to those previously reported by Tan and Man (2000) with slightly higher value, where onset temperature started at  $2.63 \pm 5.71^\circ\text{C}$  and developed over a  $66.73\text{--}66.98^\circ\text{C}$ . This may be due to the different isothermal time programme and/or the type of DSC instrumentation. Thermal curve depends on the scanning rate, so it difficult to

compare thermal curves with other experiment results with different scanning programme and different calorimeter (Tan and Man, 2000). The addition RBDPSO cause the shift of overall transitions toward lower temperature, enhancing crystallisation enthalpy and developing the crystallisation process over a narrower temperature range. The shift of crystallization transitions and narrowed transition range may be due to the addition of more unsaturated fractions of RBDPSO in AO as reported by Tan and Man (2000) and Chiavaro *et al.* (2008) for oils with higher degree of unsaturation. Formation of crystal structure compactly may affect the increase of enthalpy crystallisation (Chiavaro *et al.*, 2008).

The addition RBDPSO also affect the shape of cooling thermograms. Peak 1 of avocado oil samples at  $5.49^\circ\text{C}$  was slightly increased in terms of peak heights as illustrated in Fig. 1b. Peak height increased significantly at RBDPSO concentration more than 40% v/v. An opposite effect was observed for peak 2 at  $-6.78^\circ\text{C}$ , where progressively disappeared with increasing addition of RBDPSO. This may be associated with the increase of oleic acid (C18:1) and stearic acid (C18:0) and the decrease of palmitoleic acid (C16:1), respectively.

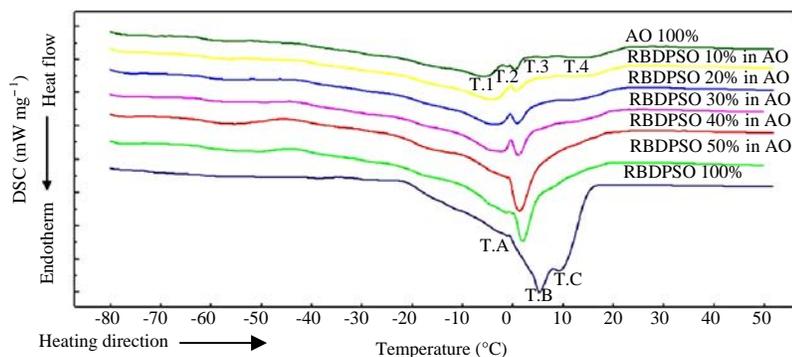


Fig. 2: Representative DSC melting thermograms of AO, RBDPSO and their admixtures, DSC: Differential scanning calorimetry, AO: Mixture of avocado oil from two cultivars, RBDPSO: Refined bleached and deodorized palm superolein

**DSC analysis of melting profile:** Melting profile of oils and fats were not easily interpretable like crystallization profile due to the phenomenon of polymorphism of TAG as a major content of oils which depends on thermal history of samples (Tan and Man, 2000). Melting thermogram of RBDPSO 100% samples exhibited one major endotherm as shown in Fig. 2 that were similar to those previously reported by Man *et al.* (1999). The major endothermic event of RBDPSO contained three overlapping peaks (endothermic transitions) at  $-1.13^{\circ}\text{C}$  (T.A),  $5.32^{\circ}\text{C}$  (T.B) and  $9.21^{\circ}\text{C}$  (T.C).

While, melting profiles of AO were further complicated by multiple endothermic transitions as shown in Table 3. Among the transitions, three mainly endothermic transition were detected, namely at  $-5.84^{\circ}\text{C}$  (T.1),  $-1.06^{\circ}\text{C}$  (T.2),  $0.42^{\circ}\text{C}$  (T.3) and  $12.42^{\circ}\text{C}$  (T.4).

According to Man and Swe (1995) low-temperature peaks region represent polymorphs  $\beta'_2$  and  $\alpha$ . Three main polymorphs ( $\alpha$ ,  $\beta'$  and  $\beta$ ) are correlated with the subcell structure, hexagonal, orthorhombic-perpendicular and triclinic-parallel, respectively (Lawler and Dimick, 2008). Compared with that previously study, the endothermic transition of RBDPSO at  $5.32^{\circ}\text{C}$  (T.B) corresponds to the melting of the  $\beta'_2$  form and  $9.21^{\circ}\text{C}$  (T.C) corresponds to the melting of the  $\alpha$  form. But the further observation was required to evaluate polymorphism in samples using X-ray diffraction technique. While, peak height of T.3 of AO samples progressively increased especially when the RBDPSO ratio exceeded 40% v/v. The peak of T.3 is gradually developed to become T.B peak of RBDPSO and shifted towards higher temperatures, indicating the formation of melting of  $\beta'_2$  form as reported by Man *et al.* (1999). The decreasing T.4 peak and the appearance T.C peak were also clearly observed by the addition of RBDPSO above 40% v/v.

The increase of the higher unsaturated and lower melting fraction (oleic acid) and the decrease of lower unsaturated and

higher melting fractions (palmitoleic acid in TAG disaturated) may be induced by RBDPSO addition to AO samples. Generally, melting point of fatty acids decrease with increasing unsaturation and increase with increasing chain length (Tan and Man, 2012). The variation of TAG composition makes oils and fats do not have specific melting point but have a range of melting profiles (Tan and Man, 2000). Besides the endothermic events, the exothermic event was observed at 40 and 50% v/v approximately at  $\pm 58^{\circ}\text{C}$ . This event may be related to the rearrangement and recrystallization of TAG into more stable structure that melt at higher temperature (Tan and Man, 2002). The  $\alpha$  form that has the lowest stability can easily transforms into  $\beta'$  and  $\beta$  form, depending on composition and thermal treatment (Sato and Ueno, 2005).

The summary of the DSC parameters that characterise the melting profile of avocado oil and RBDPSO pure oil and their admixture is shown in Table 3. Different from DSC crystallization parameters that used onset (Ton) to differentiate edible oils in melting profile, offset (Tof) parameter was used to characterise edible oils, since onset of the crystallization curve and offset of the melting curve were previously reported for differentiation of 17 edible oil samples. Offset of AO sample significantly shifted toward lower temperature, starting from 10% of RBDPSO added and slightly increased starting from 40% of RBDPSO added. Enthalpy of overall heating transition significantly increased every 20% added of RBDPSO. The addition of RBDPSO also narrowed the range of transition making the endothermic event more similar to the RBDPSO.

**Authentication of avocado oil from RBDPSO:** In order to build the quantitative calibration model for adulteration study, the mixtures of avocado oil and RBDPSO as adulterants were prepared in a range of 0-50% v/v. All DSC parameters for both the crystallization and melting profiles including onset, offset,

Table 3: DSC parameters obtained from melting thermograms of AO, RBDPSO and their admixtures

Samples (%)	DSC parameters			
	Onset (°C)	Enthalpy (J g <sup>-1</sup> )	Offset (°C)	Range (°C)
AO 100	-63.43±0.60	-38.48± 2.03 <sup>a</sup>	23.13±0.78 <sup>a</sup>	86.56±0.23 <sup>a</sup>
RBDPSO 10	-63.09±1.57	-39.10±6.19 <sup>a</sup>	22.31±0.18 <sup>b</sup>	85.40±1.11 <sup>ab</sup>
RBDPSO 20	-63.41±1.01	-42.87±2.24 <sup>b</sup>	20.89±0.62 <sup>c</sup>	84.30±0.91 <sup>bc</sup>
RBDPSO 30	-63.26±0.19	-44.36±0.95 <sup>b</sup>	20.21±0.54 <sup>d</sup>	83.47±0.28 <sup>c</sup>
RBDPSO 40	-63.71±0.47	-50.58±3.80 <sup>c</sup>	19.65±1.93 <sup>de</sup>	83.36±0.10 <sup>c</sup>
RBDPSO 50	-62.67±0.86	-49.62±1.47 <sup>c</sup>	19.07±2.10 <sup>e</sup>	81.74±0.17 <sup>d</sup>
RBDPSO 100	-62.13±0.53	-61.82±0.16 <sup>d</sup>	15.33±0.13 <sup>f</sup>	77.46±0.45 <sup>e</sup>

Each value in the table represent the mean for two determinations±RPD. Means within each column with different superscripts are significantly (p<0.05) different

Table 4: Multivariate statistical summary from DSC-PLSR and DSC-SMLR calibration for and melting thermograms of AO, RBDPSO and their admixtures

Calibration models		Factors		Validity criteria					
				Internal validation			External validation		
		R (adj.)	PRESS	R Pred.	RMSEC	RMSECV	PRESS	R Pred.	RMSEP
<b>Crystallization</b>									
SMLR	2	0.9974	28.38	0.9957	2.66	2.18	42.38	0.9940	3.76
PLS	3	0.9989	42.44	0.9935	3.76	2.66	46.39	0.9910	3.93
<b>Melting</b>									
SMLR	2	0.9983	23.03	99.65	2.40	1.96	23.78	0.9760	2.82
PLS	3	0.9994	21.87	99.67	2.70	1.91	109.83	0.9410	6.05

PRESS: Predicted residual error sum of square, RMSEC: Root mean square error of calibration, RMSECV: Root mean square error of cross-validation, RMSEP: Root mean square error of prediction, Pred: Predicted, Rh

enthalpy and range of 7 samples (i.e., the pure of AO, RBDPSO and their mixtures) were subjected to Partial Least Square (PLS) regression and Stepwise Multiple Linear Regression (SMLR). The PLS and SMLR were included in multiple linear regression that different on the way to choose linear combinations of the predictor variables. In PLS, variables that show a high correlations with the response variables are chosen and given extra weight for predictions (Miller and Miller, 2005). As with PLS, SMLR also choose the variables that highly correlated with response variables but SMLR has ability to enter and remove the variable predictors in a stepwise manner, until there is no justifiable reason to enter or remove more.

Table 4 compiled the performance of multivariate calibration of PLS and SMLR for the determination of RBDPSO concentration as avocado oil adulterant. In general, SMLR offers the highest value of correlation coefficient (R) and the lowest value of RMSEC and RMSEP compared with PLSR for both crystallization and melting profiles. The coefficient of determination (R<sup>2</sup>) and Root Mean Square Error of Cross Validation (RMSECV) of prediction were used for the internal validity criteria. These value obtained from cross validation technique using leave-one-out methods. The result of internal validation showed that both PLSR and SMLR have R<sup>2</sup> value above 0.99 that describes the goodness of fit of the predicted concentrations and actual values.

The difference between actual and predicted value is calculated. Then, the predictive value of the model is measured by the PRESS (Predicted Residual Error Sum of Square) value. The better performance of prediction models, the lower PRESS statistic value (Miller and Miller, 2005). The SMLR give better PRESS value than PLS, only using two factors. Meanwhile, PLSR model gave the higher PRESS with three factors (enthalpy, Ton and range). The regression equation of SMLR for DSC crystallization data follow the Eq. 1 while, the DSC melting data follow the Eq. 2. Figure 3 and 4 showed a good agreement between actual and predicted values of RBDPSO on validation data using PLSR and SMLR as analyzed using crystallization profile and melting profile:

$$\text{RBDPSO (\%)} = -22.52 - 7.08 \text{ Ton} + 2.60 \text{ Enthalpy} \quad (1)$$

$$\text{RBDPSO (\%)} = 569.6 - 7.24 \text{ Range} - 1.46 \text{ Enthalpy} \quad (2)$$

The capability of SMLR calibration model was also evaluated using external validation methods. For this purpose, four samples were prepared (10, 20, 30, 50%, v/v of RBDPSO in AO). The values of R<sup>2</sup> and RMSEP were used for the validity external criteria. Calibration models of SMLR and PLSR for DSC crystallization data showed the higher R<sup>2</sup> value (above 0.99) than calibration model of DSC melting data. As shown in

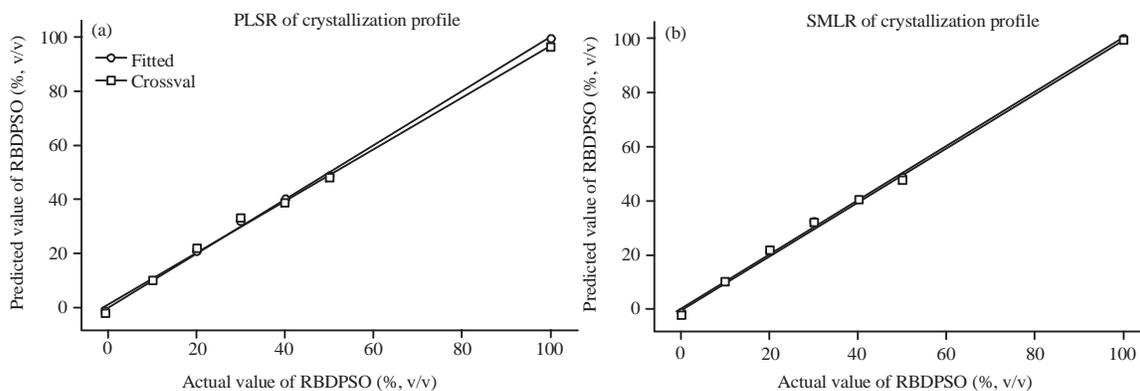


Fig.3(a-b): Scatterplot of actual vs. predicted value of RBD palm superolein as adulterant in avocado oil in the internal validation (cross validation) using Partial Least Square Regression (PLSR) and Stepwise Multiple Linear Regression (SMLR), (a) Calibration model, (b) Validation as analyzed using crystallization profile

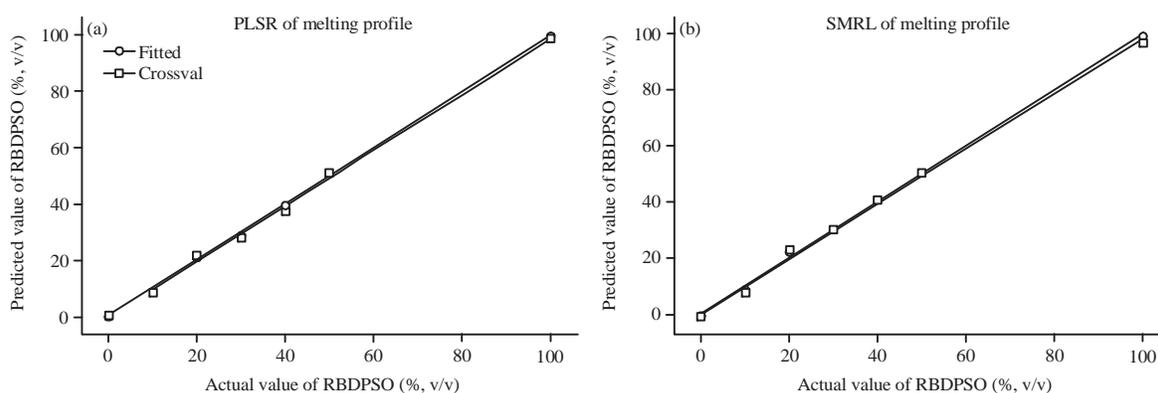


Fig.4(a-b): Scatterplot of actual vs. predicted value of RBD palm superolein as adulterant in avocado oil in the internal validation (cross validation) using Partial Least Square Regression (PLSR) and Stepwise Multiple Linear Regression (SMLR), (a) Calibration model, (b) Validation as analyzed using melting profile

Table 4, it can be stated that SMLR was more appropriate to predict adulterant (RBDPSO) than PLSR with acceptable  $R^2$  and RMSEP values.

### CONCLUSION

The results presented in this study suggest that DSC analysis can be a useful tools for detecting adulteration of avocado oil with RBDPSO. The DSC combined with multivariate calibration represent a rapid, environmentally friendly and attractive option for avocado oil authentication without sample pretreatments and the use of hazardous solvent. The results are satisfied for determination concentrations of adulterant with a good correlation coefficient, low RMSEC and RMSEP. The SMLR models showed better criteria values in both crystallization and melting calibration and validation sets than PLS regression.

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