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Fatty Acid Composition of Cocoa Beans from Yogyakarta Special Region for the Establishment of Geographical Origin Discriminations

Retno Utami Hatmi[1], Makhmudun Ainuri[2], Anggoro Cahyo Sukartiko[2*]

[1]Yogyakarta Assessment Institute for Agricultural Technology, Jl. Stadion Maguwoharjo No. 22 Wedomartani, Ngemplak, Sleman, Yogyakarta, Indonesia
[2]Department of Agro-Industrial Technology, Faculty of Agricultural Technology, Universitas Gadjah Mada, Jl. Flora No. 1, Bulaksumur, Yogyakarta 55281, Indonesia
*Corresponding author: Anggoro Cahyo Sukartiko, Email: cahyos@ugm.ac.id

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ABSTRACT

The composition of fatty acids in cocoa beans, which affect the characteristics of the processed products, is dependent on the geographic location. This research aimed to identify the fatty acid composition of cocoa beans from the Special Region of Yogyakarta (Patuk Gunungkidul and Kalibawang Kulon Progo), used as a geographical origin discrimination parameter. The data were analyzed using ANOVA and Partial Least Square – Discriminant Analysis (PLS-DA). The results showed that the average composition from Patuk Gunungkidul and Kalibawang Kulon Progo were dominated by stearic (35.23%), oleic (33.12%), and palmitate acids (27%). The three unsaturated fatty acids (palmitoleic, oleic, and linolenic acids) were significantly different in some origin regions of the cocoa bean sample, while the three types of saturated fatty acids (capric, caprylic, and lauric acids) were distinguishing factors due to their minimal availability in nature, as they were not detected in all regions. The developed method combined with PLS-DA was successfully employed the fatty acid composition to discriminate the geographic origin of cocoa beans in the Special Region of Yogyakarta.

Keywords: Cocoa bean; discriminant analysis; fatty acid; geographical origin; partial least square

INTRODUCTION

Fat is the main component of cocoa, which possesses the highest economic value compared to the other derivative products with an export value of 6.3 US $ per kilogram in 2015 (Directorate General of Estate Crops, 2016). The availability of fat in fermented cocoa beans for five days ranges between 43.18 and 58.40% of all its components (Jonfia-Essien and Tettey, 2016). Cocoa fat is an essential factor in the process of making chocolate, as it determines the rheology, texture, and chemical characteristics of chocolate products (Lipp and Anklam, 1998). Furthermore, the physical and thermal properties of cocoa fat are determined by the fatty acid type, as well as the position and distribution of the Triacylglyceride (TAG) molecule (Aronhime and Garti, 1988) in (Isyanti *et al*., 2015).

Fatty acids are a group of hydrocarbon compounds that contain long-chain, carboxylic groups at the ends. Based on their types of bonds, these acids are grouped into two, namely saturated and unsaturated fatty acids. The saturated have a single carbon atomic bond, including fatty acid caprylate (C8: 0), caprate (C10: 0), lauric (C12: 0), myristate (C14: 0), palmitate (C16: 0), stearic (C18: 0), and arachidic (C20: 0). In contrast, unsaturated fatty acids, such as palmitoleic (C16: 1), oleic (C18: 1), linoleic (C18: 2), and linolenic fatty acids (C18: 3), possess at least one double carbon bond.

The dominant types of fatty acids in cocoa beans are palmitic, stearic, and oleic acids (Ristanti *et al.,* 2016; Torres-Moreno *et al*., 2015; Vieira *et al.,* 2015) with levels> 25% (palmitate),> 33% (stearic), and> 34% (oleate) (Torres-Moreno *et al*., 2015). Additionally, Triacylglycerol (TAG) composition in cocoa butter is

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responsible for the physical properties of processed cocoa products, such as shiny appearance, hardness or ease of melting, and taste (Sirbu *et al*., 2018; Marty-Terrade and Marangoni, 2012). The dominant triglycerides found in cocoa fat include 1,3 dipalmitin-2-oleate glycerol (POP), 1-palmito, 2-olein, 3-stearin glycerol (POS), and 1,3 distearin-2-oleate glycerol (SOS) (Lipp and Anklam, 1998). The length of the short carbon chain, the level of saturation of fatty acids, and the free fatty acid content significantly influence the hardness/ melting point of cocoa fat (Guehi *et al*., 2008). High saturated fatty acids with short carbon chains result in a low melting point of the chocolate product (Tarigan *et al*., 2016).

The composition of cocoa bean varies according to their geographical origin (Torres-Moreno *et al*., 2015). Extrinsic factors aside geographical origin, which significantly influence the composition and physicochemistry of cocoa fatty acids in Brazil include climate conditions, harvest time, and farming methods (Vieira *et al*., 2015). According to Liyanda *et al.* (2012) and Alam *et al.* (2010) cocoa plants are highly susceptible to the elevation level of the place of growth, as the temperature at certain altitudes influence the optimization of the metabolic process of cocoa beans. The altitude of the growing area also affects several things, including production and fat content. Furthermore, Tresniawati *et al.* (2014) observed an interaction between the fat quality of cocoa beans and the altitude of growth from above sea level. Altitude is one of the characteristics of the land, which has a significant negative correlation with cocoa production and a positive correlation with fat content. A higher location will result in a decrease in production, alongside an increase in fat content (Liyanda *et al.,* 2012). Therefore, a higher location of growing cocoa plants causes an increased seed size and fat content. This agrees with Minifie (1989), which stated that higher fat content are obtained from more giant-sized cocoa beans.

Liendo *et al.* (1997) stated that the composition of cocoa beans varies according to geographical origin. The fatty acid profiles and the amount of cocoa fat in chocolate products depend on the conditions of growing cocoa plants. Furthermore, Lipp and Anklam (1998) mentioned that the geographical origin influences the composition of cocoa fatty acids. The palmitic, stearic, and mono-unsaturated acid triacylglycerol increases with increasing environmental temperature, while high rainfall affects the levels of stearic, oleic, and free fatty acids (Lehrian *et al.,* 1980); Mustiga *et al*. (2019). Fowler (2009) confirmed that the fat content and melting characteristics of cocoa beans are strongly influenced by the type of cocoa plants and environmental conditions, precisely the average daily temperature during the last few months. This environmental temperature affects the hardness of cocoa butter; the lower the environmental temperature, the softer the butter, alongside a lower melting point.

According to Foubert *et al.* (2004), differences in the composition of cocoa fatty acids cause differences in crystallization kinetics. Mono-unsaturated fatty acids, or oleic acids (C18:1) and polyunsaturated fatty acids (linoleic acid and linolenic acid) (C18:2 and C18:3) significantly affect the crystallization of cocoa butter (Marty-Terrade and Marangoni, 2012). In addition, cocoa fat is dominated by components of saturated fatty acids (palmitic and stearic) and unsaturated fatty acids (oleic, linoleate, and linoleate). Saturated fatty acids have a significantly higher melting point than the unsaturated (palmitic acid = 62 °C and stearic acid = 68 °C; while oleic acid = 160 °C) (Tymoczko *et al.,* 2015). This difference in melting point causes a hard or solid texture in chocolate products at room temperature and melts when placed in the mouth (McHenry *et al.,* 1987).

Apart from its intrinsic quality, the geographical origin of cocoa beans is one of the extrinsic factors which promote consumer confidence (Saltini *et al.,* 2013) and support sustainable production, especially with the discovery of markers to verify the authenticity (Acierno *et al*., 2016). Therefore, one of the cocoa-producing regions in Indonesia, the Special Region of Yogyakarta, can use these factors to increase consumer acceptance, providing added value to the processed products. This study aimed to identify the fatty acid composition of cocoa beans as a distinguishing parameter for their geographical origin, derived from three levels of elevation in the Patuk Gunungkidul and Kalibawang Kulon Progo, Special Region of Yogyakarta.

MATERIALS AND METHODS

Sample

The sampling technique refers to the modified study by (Bertoldi *et al*., 2016) adjusted to the research needs. Samples were obtained through stratified and simple random sampling. The stratified sampling was used to distinguish the elevation level of cocoa plant-suitable lands into three groups, i.e., 0-300 m asl, 301-450 m asl, and 450 m asl, respectively (Directorate General of Estate Crops, 2014), while simple random sampling was used for selecting the cocoa pod trees. As a dominant cocoa variety, bulk cocoa (*lindak*) was selected as a default sample in this study.

The primary samples of the study were cocoa pods taken from the Patuk Gunungkidul and Kalibawang

Table 1. Coordinate and elevation of the sample's origin location

Elevation (m asl)	Area codes	Sample's Origin	Coordinates Latitude (S), Longitude (E)	Elevation Range (m asl)	Number of pod samples
0 – 300	GK1	Patuk (Nglanggeran, Bunder, Putat)	7° 51′ – 7° 52′ S; 110° 30′ – 110° 32′ E	130 – 267	40
	KP1	Kalibawang (Banjaroya, Banjarharjo)	7° 39′ - 7° 41′ S; 110° 14′ – 110° 15′ E	173 – 261	40
301 – 450	GK2	Patuk (Ngoro-Oro, Putat, Nglanggeran)	7° 50′ - 7° 51′ S; 110° 30′ – 110° 33′ E	302 - 401	40
	KP2	Kalibawang (Banjaroya, Banjarharjo)	7° 39′ - 7° 41′ S; 110° 13′ – 110° 14′ E	302 – 449	40
>450	GK3	Patuk (Nglegi)	7° 50′ - 7° 51′ S; 110° 33′ – 110° 33′ E	469 – 658	40
	KP3	Kalibawang (Banjaroya, Banjarharjo)	7° 38′ - 7° 39′ S; 110° 12′ – 110° 13′ E	477 - 559	40

Kulon Progo, each with three levels of altitude (0-300 m asl, 301-450 m asl, and 450 m asl). Area codes were given for each combination of area and altitude, with GK and KP showing the Gunungkidul and Kulon Progo areas, respectively. Simultaneously, 1, 2, and 3 indicate an altitude range of 0-300 m asl, 301-450 m asl, and >450 m asl, respectively. For example, GK1 showed the Gunung Kidul region at an altitude of 0-300 m asl, while KP2 showed the Kulon Progo region at an altitude of 301-450 m asl. For each combination of area and altitude, twenty cocoa trees were randomly selected, and two cocoa pods were obtained from each tree. Therefore, a total of forty cocoa pods were obtained for each combination of area and altitude. The total number collected for the whole GK1, GK2, GK3, KP1, KP2, and KP3 regions, was 240 cocoa pods. Sampling locations were distributed in detail according to villages, sub-village, coordinates, and elevation levels in both regions (Patuk Gunungkidul and Kalibawang Kulon Progo). Sample sizes obtained from each region were in line with Fraenkel *et al.* (2012). Furthermore, the combination of regions and altitudes, area codes, the origin of samples, coordinates, elevation ranges, and the number of cocoa pods collected are shown in Table 1, while environmental characteristics of the location are shown in Table 2.

Sample Preparation

To represent one combination of area and altitude, forty randomly selected cocoa pods were pooled for fatty acid analysis. Therefore, there were six pooled triplicate samples, representing six combinations of areas and altitudes (GK1, GK2, GK3, KP1, KP2, and KP3), with a total of 18 samples for analysis. Sample preparation started by delaying the breakdown of cocoa pods for three days after picking for uniformity of maturity and minimizing pulp, the pod was broken down, and its seed was taken. Afterward, wet cocoa beans from each pod were dried separately in a solar drying container for 7-10 days (7 hours per day). Samples of dried cocoa beans were then used for the analysis of fatty acids. Each sample was stripped, with the nibs and skin weighed, and smoothed using a mortar to pass the 40-mesh sieve.

Sample Analysis

Each cocoa bean powder sample was weighed 50 g during fatty acid analysis, which was performed with Gas Chromatography (GC) according to (Park and Goins, 1994). The GC used was the Shimadzu brand, type GC 2010 Plus. Fatty acids were obtained from cocoa bean powder samples, placed in a test tube, and diluted with 100 µl of methyl chloride and 1000 µl of NaOH 0.5N in methanol. Furthermore, nitrogen gas (N_2) was inserted into the reaction tube and closed tightly to remove oxygen. The test tube was then heated in a water bath at 88-90 °C for 10 min and cooled, with an addition of 1000 µl BF_3 20% methanol. Nitrogen (N_2) gas was added to the test tube, closed tightly, and reheated with Waterbath at the same temperature and time. Afterward, the test tube was cooled at room temperature, with the addition of 1000 µl of distilled water and 1000 µl of hexane, further exported for 1 min to extract fatty acid methyl esters. The test tube was centrifuged for 10 min until the top layer formed was ready for analysis using GC.

The operational conditions of gas chromatography during fatty acid analysis were set as follows: using a

Flame Ionization Detector (FID) type at 240 °C, 240 °C injector temperature, RTX-Wax column dimension 30 meters long and 0.25 mm diameter, helium as a carrier gas, the column flow velocity of 0.80 mL/min, column temperature at 150 °C was raised to 200 °C with an average increase of 8 °C /min and maintenance of 220 °C temperature for 25 minutes. The identification of fatty acids was performed by comparing the standard fatty acid mix LC06457 Supelco C 8:0 - C 24:0.

This study used Garmin GPS map 62s series GPS for tracking pod sampling locations in the field. Several tools were used to analyze fatty acid components, including analytical balances AND FX-3000i Japan series, oven, water bath type WNB45 Memmert Germany, SHIBATA TTM-1 vortex mixer, K Centrifuge PLC series, and GC 2010 Plus brand Shimadzu.

Statistical Analysis

The differentiation of the geographical origin of cocoa beans was performed using ANOVA and Partial Least Square - Discriminant Analysis (PLS-DA). ANOVA was performed with SPSS Statistics Version 22 software (IBM Corporation, USA), while PLS-DA was applied with XLSTAT Basic + (Addinsoft).

RESULTS AND DISCUSSION

Gunungkidul and Kulon Progo are areas in Yogyakarta Special Region with the most significant potential for cocoa production. In Gunungkidul Regency, production mainly occurred in Patuk (51.37%), while Kalibawang in Kulon Progo District was the largest producer (32.77%) of cocoa in the nine sub-districts.

Characteristics of the Sample Origin Location

This study used a bulk cocoa (*lindak*) sample harvested from productive plants (10 to 25 years age range). Samples were obtained from smallholder plantations in the Patuk Gunungkidul and Kalibawang Kulon Progo. Cocoa plants in these two regions were not planted monoculture and did not use shade plants in their cultivation. Both regions, Gunungkidul and Kulon Progo, have different characteristics; therefore, there may be differences in the quality of cocoa beans produced. The determination of characteristics of the sample location of origin is essential for product traceability. Characteristics of sample locations, in general, can be identified according to the area, scope of the area, topography (height and slope), soil type, and climate (sun intensity, temperature, humidity, and rainfall). The general characteristics of the sample origin location are shown in Table 2.

Patuk has a larger area and village coverage compared to Kalibawang. The elevation and slope in both regions are dominated by the same class. Although there are similarities in the level of elevation and slope, a slight difference in climatic conditions in both regions exist due to the intensity of solar radiation and rainfall. Furthermore, differences in the geographical formation of these regions cause differences in the type of soil formed. Of the various kinds of soil that exist in both regions, the most suitable soil type for cocoa plant growth is latosol and regosol. In contrast, rendzina, lithosols, and grumosol are very minimal in nutrients and organic due to their formative process. They originate from weathering of large and hard rocks (lithosols), limestone (rendzina), and limestone formation. Additionally, the

Table 2. Characteristics of sample-origin location

Area Parameter	Unit	Patuk Gunungkidul	Kalibawang Kulon Progo
Area	ha	7204 [a]	5096.70 [b]
Number of villages	village	11 [a]	4 [b]
Altitude (dominance)	m asl	100 - 499 (93.02%) [a]	101 - 500 (96.16%) [b]
Slope (dominance)	degree (°)	Steep >250 (64%) [a]	Steep 16-400 (57.19%) [b]
Solar radiation	W/m^2/month	15.49 - 18.68 [c]	19 - 31.63 [c]
Temperature	°C	25.24 - 26.01 [c]	26.23 - 26.5 [c]
Humidity	%	82.60 - 98.31 [c]	81.84 - 85.97 [c]
Rainfall	mm/year	2348 – 3127 [c]	3709 – 3887 [c]
Soil type	-	Red Latosol - Lithosols; Lithosols; Lithosols - Rendzina [d]	Latosol, Grumosol, Regosol [e]

Note: (a) BPS Gunungkidul (2016); (b) BPS Kulon Progo (2016); (c) BMKG Special Region of Yogyakarta (2017); (d) Bakosurtanal (2011); (e) BP3K Kalibawang (2016) identification of the sampling location was also carried out in detail, covering the village area, coordinates, and elevation in this study for both the Patuk Gunungkidul region and Kalibawang Kulon Progo (Table 1).

The differences in land and climate characteristics in both regions caused corresponding differences in some quality parameters of cocoa beans, including seed sizes (physical) and their nutritional content (fatty acids). This result agrees with (Torres-Moreno *et al*., 2015), which stated that the amount of cocoa fat and fatty acid composition in chocolate products rely on the growth conditions of the cocoa plant which gives different physical properties, thereby influencing the processing and texture of the final product.

Fat Composition and Geographical Origin Discrimination

The determination of the composition of cocoa bean fatty acids originating from two regions with different land and climate conditions at three altitude levels is essential. It helps to ascertain the advantages of each cocoa bean product, to enhance precise use and processing techniques. Cocoa fat is an essential factor in the manufacture of chocolate as it determines the rheology, texture, and chemical characteristics of chocolate products (Lipp and Anklam, 1998). The physical and thermal properties of cocoa fat are determined by the fatty acid type and the distribution or position of the Triacylglyceride (TAG) molecule (Aronhime and Garti, 1988) in (Isyanti *et al*., 2015). Meanwhile, the dominance of components and proportions of fatty acids in different countries and regions vary. Cocoa beans from the Special Region of Yogyakarta (both from the Patuk Gunungkidul and Kalibawang Kulon Progo regions) were dominated by fatty acid components, with the largest portion being stearic acid, with an insignificant average value of 35.227%. This was followed by oleic at 33.121% and palmitic at 27%, as shown in Table 3. The composition and proportion of these main fatty acids of cocoa beans (stearic, oleic, and palmitic) from the Special Region of Yogyakarta possesses similarities with cacao beans originating from Ecuador, Ghana, Togo (West Africa), Brazil, and Venezuela with stearic acid levels (33.76%; 36.34%; 35.81%; 33.96%; 35.93%), oleic (34.73%; 34.31%; 33.74%; 33.84%; 36.55%), and palmitate (27.61%; 25.02%; 26.24%; 26.53%; 26.13%) respectively (Liendo *et al.,* 1997; Torres-Moreno *et al*., 2015; Vieira *et al*., 2015; Zyzelewicz *et al*., 2014). Meanwhile, Ristanti *et al.* (2016) analyzed cocoa beans from Sulawesi, and concluded that the

Table 3. Fatty acid composition

Fatty Acid Component (%)		Origin					
		GK1	GK2	GK3	KP1	KP2	KP3
Caprylic	C 8 : 0	0.096 ± 0.090	0.024 ± 0.022	0.024 ± 0.042	0.016 ± 0.028	0.000 ± 0.000	0.000 ± 0.000
Capric	C 10 : 0	0.044 ± 0.041	0.000 ± 0.000	0.018 ± 0.031	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Lauric	C 12 : 0	0.036 ± 0.031	0.000 ± 0.000	0.183 ± 0.317	0.037 ± 0.064	0.036 ± 0.062	0.035 ± 0.060
Myristic	C 14 : 0	0.154 ± 0.050	0.101 ± 0.025	0.212 ± 0.167	0.129 ± 0.043	0.168 ± 0.089	0.124 ± 0.038
Palmitic	C 16 : 0	27.922 ± 1.500	25.665 ± 0.277	26.202 ± 2.179	27.744 ± 0.808	27.547 ± 2.480	26.916 ± 2.609
Palmitoleic	C 16 : 1	0.300^{b} ± 0.045	0.230^{ab} ± 0.021	0.194^{a} ± 0.050	0.245^{ab} ± 0.023	0.279^{ab} ± 0.039	0.223^{ab} ± 0.029
Stearic	C 18 : 0	34.554 ± 1.806	36.004 ± 0.146	36.235 ± 2.537	34.763 ± 1.032	34.995 ± 2.757	34.809 ± 0.920
Oleic	C 18 : 1	32.368^{a} ± 0.401	33.886^{b} ± 0.428	32.838^{ab} ± 0.276	33.029^{ab} ± 0.576	32.960^{ab} ± 0.644	33.647^{ab} ± 0.504
Linoleic	C 18 : 2	3.411 ± 0.768	3.050 ± 0.171	3.070 ± 0.099	2.869 ± 0.301	2.855 ± 0.386	3.173 ± 0.153
Linolenic	C 18 : 3	0.222^{b} ± 0.016	0.191^{ab} ± 0.011	0.180^{ab} ± 0.004	0.197^{ab} ± 0.040	0.170^{ab} ± 0.013	0.162^{a} ± 0.008
Arachidic	C 20 : 0	0.894 ± 0.041	0.849 ± 0.008	0.844 ± 0.069	0.971 ± 0.076	0.990 ± 0.162	0.911 ± 0.104
SFA		63.700	62.643	63.718	63.661	63.736	62.794
MUFA		32.668^{a}	34.117^{b}	33.032^{ab}	33.274^{ab}	33.239^{ab}	33.869^{ab}
PUFA		3.633	3.241	3.250	3.066	3.025	3.335
UFA		36.301	37.358	36.282	36.340	36.264	37.204
S/U Ratio		1.755	1.677	1.756	1.752	1.758	1.688

Different letters on the same row denote significant differences (p<0.5). GK stands for Gunungkidul, while KP stands for Kulon Progo.

dominant component of fatty acids was the same. The lower percentage was still good for stearic (27.78%), oleic (24.77%), and palmitic acid (20.68 %).

The similarity in the composition of the primary fatty acids of cocoa beans from Special Region of Yogyakarta, Indonesia, with other countries such as Ecuador, Ghana, Togo, Brazil, and Venezuela may be due to the similarity in growing location latitude. This latitude ranges between 20 °N and 20 °S of the equator, such as South America, West Africa, and Southeast Asia (Fowler, 2009). Vieira *et al*. (2015) confirmed the statement of Fowler (2009) that cocoa-growing locations in Brazil possesses similar growing conditions with the ones in Indonesia, in terms of temperature conditions ranging between 23.4 - 27.0 °C, rainfall between 1193 - 2250 mm, and at elevations up to 284 m. Vieira *et al*. (2015) also stated that similar growing conditions produce cocoa fat characteristics identical to Asian and African ones. Furthermore, the temperature is an influential growing factor for the aspects of cocoa butter. The growth temperature of cocoa plants within a 20-30 °C range will produce hard cocoa butter, while below 20°C produces soft cocoa butter (Marty-Terrade and Marangoni, 2012). Kongor *et al*. (2016) stated that the chemical composition of the soil and plant age did not significantly influence the formation of flavor precursors and the final taste quality.

Statistical analysis showed that only three components of fatty acids (palmitoleic, oleic, and linolenic acids) were significantly different in the studied areas (Table 3). These three acids were components of unsaturated fatty acids. Furthermore, the minimum availability and significant difference in some regions can be used to distinguish the geographical origin of cocoa beans. For the Patuk Gunungkidul region at an elevation of 0-300 m above sea level, the highest percentage of palmitoleic (0.3%), linoleic (0.2%) and the lowest oleic acid (32.37%) were significantly different compared to other research areas.

There were several types of fatty acids with minor amounts that were only available in certain regions. Capric acid was a fatty acid type available in the Patuk Gunungkidul region at elevations of 0-300 m asl and above 450 m asl. Caprylic acid was also only available in all heights in the Patuk Gunungkidul and Kalibawang Kulon Progo regions at elevations of 0-300 m asl. The only area where no lauric fatty acids were detected was Patuk Gunungkidul at altitude 301-450 m asl. The availability of capric, caprylate, and laurate acids has been reported only in Patuk Gunungkidul and Kalibawang Kulon Progo areas, which enables this component to be a geographic origin identifier.

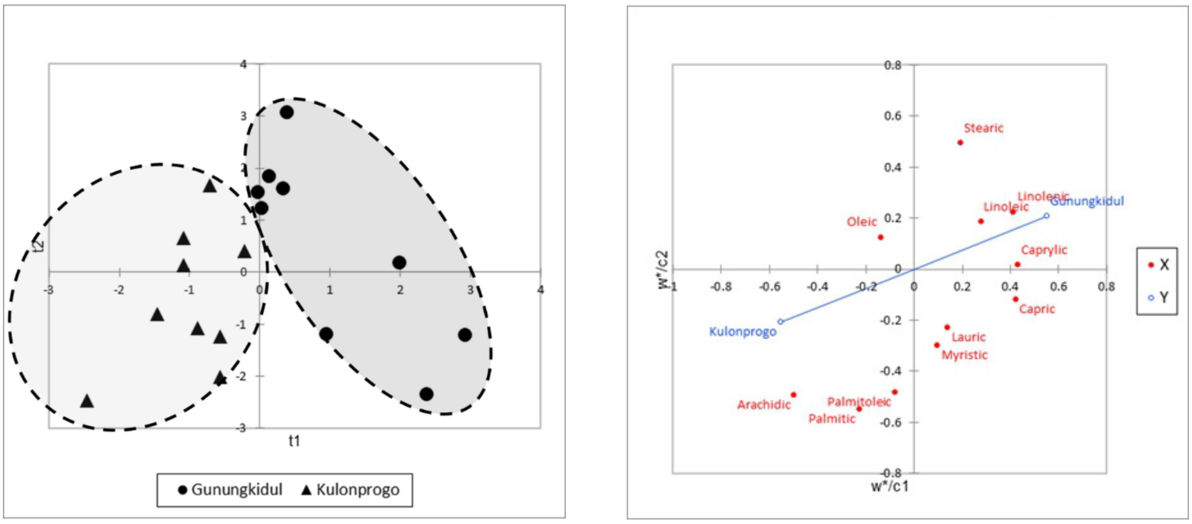
In this study, Ristanti *et al.* (2016) and Torres-Moreno *et al*. (2015) detected a number of minor fatty acid components in cocoa beans from Sulawesi, Ecuador, and Ghana that were not available in the Special Region of Yogyakarta, such as pentadecanoic, heptadecanoic, eicosanoic, eicosedien-oat, behenic, tricosanoic, and lignoceric acids. However, caprylic, capric, and lauric acids were detected in the Special Region of Yogyakarta, but not found in the Sulawesi region.

The statistical results also showed that the total saturated and unsaturated fatty acids of cocoa beans in the entire Special Region of Yogyakarta were not significantly different. The value of saturated fatty acids was slightly higher with an average value of 63.136%, compared to the unsaturated (36,623%) with an average S/U ratio of 1,731. The S/U ratio of the cocoa beans was classified as the highest when compared to Ghana, Ecuador, and Venezuela (1.72; 1.65; and 1.64). This ratio showed that the cocoa beans from the Special Region of Yogyakarta possess the highest composition of saturated fatty acids derived from palmitic and stearic acids.

Unsaturated fatty acids in this study were categorized into mono-unsaturated carbon bonds (MUFA) and polyunsaturated (PUFA) bonds. The MUFA were significantly different for the Patuk Gunungkidul region of 0-300 m asl and 301-450 m asl with other research areas. According to Torres-Moreno *et al*. (2015), PUFA has a maximum total calorie content of 10%, whereas MUFA should be the main component due to its excellent hypocholesterolemic effect for health. This agrees with the results of this study, which had an average PUFA value of 3.258% and MUFA of 33.336%.

The components of both saturated and unsaturated fatty acids detected in the study area include arachidic, linoleic, and linolenic acids. Arachidic acid (0.91%) had a lower value than the Brazilian (2.1%), while linoleic acid (3.07%) was almost on par with cocoa butter from Brazil (3.5%). This level was high as the average originating from Africa (Ghana and Ivory Coast) was only 2.8%. Linolenic acid (0.187%) was at the same level as a range of previous studies (0.1-0.2%) (Zyzelewicz *et al*., 2014).

The differentiation of the geographical origin of cocoa bean samples using ANOVA analysis (Table 3) cannot be performed without involving a combination of the various fatty acids. Principal Component Analysis (PCA), an unsupervised multivariate technique was used initially to reduce the number of variable and to detect sample grouping. The first two factors allow us to represent 55.61% of the initial variability of the data (figure not shown). As the result did not



(a) (b)

Figure 1. (a) Score plot of the PLS-DA model; (b) Loading plot of the PLS-DA model

showed a good clustering based on geographical origin, therefore, the use of multivariate analysis, such as PLS-DA, is performed. A supervised classification model was developed and evaluated by leave-one-out cross-validation to classify the cocoa samples based on origin (Jackknife LOO). Figure 1.a depicts the score plot of the PLS-DA model showing both sample origins. Each symbol, circle, and triangle represent the samples with varying origins. Two axes, score t1 and t2, represents two latent variables of the model, which were mathematically constructed and 'summed up' the fatty acid composition in this study. Complementing the previous figure, Figure 1.b depicts the loading plot of the PLS-DA model and shows how fatty acids composition (X) relate to each other as well as to sample origins (Y). Furthermore, the results of this study showed that cocoa bean samples from Gunungkidul could be distinguished from cocoa bean samples from Kulon Progo. Therefore, the ability of fatty acids to distinguish the geographical origin of cocoa beans shows that cocoa composition and fatty acid profile vary depending on geographic origin (Torres-Moreno *et al*., 2015).

CONCLUSION

The average composition of fatty acids from the Special Region of Yogyakarta (Patuk Gunungkidul and Kalibawang Kulon Progo) were dominated by stearic (35.23%), oleic (33.12%), and palmitic acids (27%), respectively. The types and composition of fatty acids that can be used to distinguish the geographical origin of cocoa beans in the Special Region of Yogyakarta include unsaturated (palmitoleic, oleic, and linolenic acids) and saturated (capric, caprylic, and lauric acids) fatty acids. The three unsaturated fatty acids were significantly different in some origin regions of the cocoa bean sample, while the three types of saturated fatty acids were distinguishing factors due to their minimal availability in nature, as they were not detected in all regions. When applied with PLS-DA, the fatty acid composition can be used to distinguish the cocoa bean geographic origin in the Special Region of Yogyakarta.

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CONFLICT OF INTERESTS

The authors declare no conflict of interest.

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