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Comparative Study of Flavor Precursors, Volatile Compounds and Sensory between Malaysian and Ghanaian Cocoa Beans

(Kajian Perbandingan Kandungan Bahan Pelopor Perisa, Sebatian Meruap dan Sensori antara Biji Koko Malaysia dan Ghana)

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ABSTRACT

The aim of this work was to compare quantitatively the flavor precursor constituents between unroasted Ghanaian and Malaysian cocoa beans. Furthermore, the effect of roasting on the flavor development was also characterized based on the volatile compounds and sensory analysis. Sensory analysis results showed a significant difference ($p<0.05$*) for the cocoa attribute, sour and overall acceptability between samples. Ghanaian cocoa beans showed higher scores for cocoa and overall acceptability attributes, while Malaysian samples showed a higher acidic attribute. Meanwhile, other attributes such as fruity, bitterness, astringent, raw and viscosity do not show a significant difference (*$p>0.05$*) between samples. Effects of flavor pre-cursors such as organic acids (citric acid, lactic acid, acetic acid), sugars (glucose, sucrose, fructose), free amino acids (asidic, bases, hydrophobic, polyphenols), and volatile compounds (alcohol, ketones, aldehydes, esters, pyrazine, acetic acid) are presented. Ghanaian cocoa beans were found to be much more acceptable in term of taste and flavors. Importantly, the amount of organic acids found in the beans could be a potential marker, contributed to the overall acceptability level for cocoa beans.*

Keywords: Cocoa flavor; cocoa nibs; fermentation; flavor precursors; free amino acids

ABSTRAK

Tujuan kajian ini adalah untuk membandingkan secara kuantitif kandungan bahan pelopor perisa antara biji koko kering Ghana dan Malaysia yang tidak dipanggang. Seterusnya, pengaruh pemanggangan terhadap pembentukan ciri-ciri perisa turut diperinci berdasarkan analisis kandungan sebatian meruap dan analisis sensori. Keputusan analisis sensori menunjukkan perbezaan yang bererti ($p<0.05$*) bagi atribut koko, rasa masam dan penerimaan keseluruhan antara kedua-dua sampel. Biji koko Ghana menunjukkan skor yang lebih tinggi untuk atribut perisa koko dan penerimaan keseluruhan, manakala sampel Malaysia menunjukkan atribut masam yang sedikit tinggi. Manakala, bagi atribut lain seperti rasa buah, pahit, kelat, rasa mentah dan kelikatan tidak menunjukkan perbezaan bererti (*$p>0.05$*) antara kedua-dua sampel. Pengaruh kandungan bahan pelopor perisa seperti asid-asid organik (asid sitrik, asid laktik, asid asetik), kandungan gula (glukosa, sukrosa, fruktosa), kandungan asid amino bebas (asidik, bes & hidroforbik) kandungan polifenol, serta kandungan sebatian meruap (alkohol, keton, aldehida, ester, pirazina & asid asetik) dikuantifikasi dan dibentangkan. Didapati bahawa biji koko Ghana memberikan ciri rasa dan perisa koko yang lebih baik berbanding biji koko Malaysia. Jelasnya, kehadiran serta kuantiti asid organik boleh dijadikan penanda aras dalam penentuan tahap penerimaan sesuatu biji koko daripada aspek sensori.*

Kata kunci: Asid amino bebas; bahan pelopor perisa; fermentasi; nib koko; perisa koko

Introduction

Cocoa originates from beans of the cocoa tree (*Theobroma cacao* L.) and is the main ingredient in chocolate manufacturing. The popularity, value and quality of cocoa products are much related to unique and complex flavors contributed by various volatile and non-volatile compounds. These volatile compounds comprised of nitrogen and oxygen heterocyclic compounds, aldehydes and ketones, esters, alcohols, hydrocarbons, nitriles and sulphides, pyrazines, ethers, furans, thiazoles, pyrones, acids, phenols, imines, amines, oxazoles, and pyrroles (Afoakwa et al. 2008), and together with polyphenols as non-volatile compounds, play critical roles in cocoa flavor quality. These specific cocoa aromas strongly depends on various processing stages, started during post-harvest stage; specifically the fermentation and drying stages. The influences of both processes in the development of chocolate flavor were well reported and documented. Fermentation caused the death of the bean and initiation of flavor precursor formation namely amino acids, oligopeptides and, reducing sugars as well as organic acids and some flavor compounds (Thompson et al. 2001). These flavor precursors were further participated in Maillard reactions or non-enzymatic browning reactions during roasting which were responsible to the development of typical and unique chocolate flavors.

European cocoa and chocolate manufacturers have traditionally depended upon West African countries for their supplies of cocoa beans. However, there was increased concern amongst European manufacturers about the adequacy of West African supplies. Currently, the most obvious alternative sources of cocoa beans are from Asia and South America regions. Unfortunately, cocoa beans from this region have an excessively acidic flavor, weak chocolate flavor and certain other off-flavors.

Malaysian cocoa beans are perceived as lower quality compared to the Ghanaian cocoa due to the higher shell content and relatively higher acidity (Idris et al. 2011), low chocolate flavor and the presence of undesirable flavors. Acetic acid has been shown to contribute significantly to the high residual acidity in Malaysian cocoa beans compared to Ghanaian which affected the quality of cocoa products as well. However, this argument is mainly based on sensory analysis point of view and the relationships between all chemical components that are likely to play a role in development of cocoa flavor, their sensory properties, and the sources and mechanisms of flavor formation are not well explained. This paper provides the analytical information of flavor precursors and flavor compounds between Malaysian and Ghanaian cocoa beans and addressed the above concerns.

Materials and Methods

SAMPLE PREPARATION

Two kg of dried fermented Ghanaian cocoa beans from 2 different batches was purchased from Guan Chong Cocoa Manufacturer Sdn. Bhd, Johor Baharu. Meanwhile, Malaysian cocoa beans were obtained from Malaysian Cocoa Board (MCB) Research and Development Centre, Jengka, Pahang, which was fermented and dried according to the normal practices. Depending on the nature of the analysis, samples were divided into two categories which were unroasted and roasted samples. The unroasted cocoa samples were mixed homogeneously and divided into 3 laboratory sample prior to analysis. Meanwhile, roasted samples were prepared by roasting approximately 300 g dried cocoa samples in a convection drying oven UFE500ULM (Memmert, Germany) controlled at 140°C for 40 min as dictated by Ramli et al. (2006). Roasted samples were deshelled and ground using waring blender prior to analysis. Sample for precursors analysis was treated according to method described by Camu et al. (2007) and Tomlins et al. (1990) with slight modifications. About 20 g ground cocoa nib sample (less than 1 mm particle sizes) was mixed with 100 mL ultrapure water and homogenized using tissue homogenizer IKA Ultra Turrax T25 for 5 min. Homogenized solution were transferred into centrifuge tube 50 mL and centrifuged at 12,000 rpm, 4°C for 15 min. The upper layer was collected and filtered thru membrane filter 0.20 µm and aliquot obtained was kept in a refrigerator prior to further analysis.

CHEMICALS AND STANDARD SOLUTIONS

All solvents were HPLC grade and supplied by Merck Chemicals (Darmstard, Germany). Certified Reference Material (CRM) of sucrose, glucose, fructose, lactic acid, citric acid and acetic acid were purchased from Dr. Ehrenstorfer GmbH, Germany. Standard mixture solutions of amino acids containing 17 compounds (Trace CERT) were obtained from Merck, Germany.

DETERMINATION OF SUGAR

The sugars content was determined according to method described by Salman et al. (2014) using liquid chromatography-tandem mass spectrometry (LC-MS/MS) ABSciex 3200 QTrap (Applied Biosystem, Toronto, Canada). Chromatographic separation was achieved using Luna 3µ NH_2, 2 mm × 150 mm (Phenomenex, USA) column and controlled at 35°C. A 10 µL of sample extract was introduced via autosampler into mobile phase of 5mM ammonium formate in water (A) and acetonitrile (B) each containing 0.1% formic acid at flow rate 1.0 mL/min. The linear gradient programme was set as follows; 10% B to 90% B from 0 - 3 min, followed by 1 min elution time before re-equlibration to 10% B for 2 min. The measurements for sucrose, glucose and fructose were performed under negative electrospray ionization (ESI) and multiple reaction mode (MRM) using following parameters; ion spray voltage (IS)-4500 V; auxiliary gas temperature (TEM) 500°C; curtain gas (CUR), nebulizer gas (GS1) and auxiliary gas (GS2) 10, 40 and 40 arbitrary units, respectively, other compound dependant parameters were optimized using individual sucrose, glucose and fructose standard solution of 5.0 ppm. Data were acquired using data processor Analyst 1.5.2. Quantitative values were obtained by relating chromatographic peak areas to those derived from calibration curve of standards solution ranging from 2.5 - 50 ppm. Calibration curves were plotted using a weighted regression (1/x) and the concentration of each sugar was calculated using the following equation:

$$\text{Sugar (ppm)} = R \times DF,$$

where R is reading from the standard curve; and DF is dilution factor.

DETERMINATION OF ORGANIC ACIDS

Analysis of citric, malic and acetic acid was carried out according to the method described by Tomlins et al. (1990) using HPLC system (Shimadzu, Japan) with UV-Vis detector at 210 nm, and Aminex HPX-87H cation-exchange column (7.8 mm × 300 mm, Bio-rad Laboratories, UK) controlled at 45°C. A 20 µL of sample aqueous extracts was introduced via autosampler into mobile phase of 0.004 M sulfuric acid H_2SO_4 set at isocratic flow rate 0.6 mL/min with acquisition time was about 25 min. Quantitative values were obtained by relating chromatographic peak areas to those derived from externally run calibration standards ranging from

250-5000 ppm and the concentration of each organic acid was calculated using the following equation:

$$\text{Organic acid (ppm)} = R \times DF,$$

where R is reading from the standard curve; and DF is dilution factor.

DETERMINATION OF FREE AMINO ACIDS

The analysis was carried out based on Le et al. (2014) using liquid chromatography-tandem mass spectrometry (LC-MS/MS) ABSciex 3200 QTrap (Applied Biosystem, Toronto, Canada) equipped with electrospray ionization (ESI) source, solvent delivery system, autosampler, and nitrogen gaseous. Chromatographic separation was achieved using column C-18 Esclipse 5 µm, 4.6 mm ID × 50 mm (Agilent, USA) column. Sample extract of 10 µL was introduced via autosampler into mobile phase of 5 mM ammonium formate in water (A) and acetonitrile (B) each containing 0.1% formic acid at flow rate 1.0 mL/min. The linear gradient programme was set as follows; 5% B to 20% B from 0-3 min, followed by 1 min elution time before re-equlibration to 10% B for 2 min. ESI-positive ionization and MRM mode were used for detection. The precursor and product ions for each amino acids was predetermined using multiple reaction monitoring (MRM) with dwell time 50 ms. Data were acquired using data processor Analyst 1.5.2. Quantitative values were obtained by relating chromatographic peak areas to those derived from calibration curves of standards solution ranging from 0.001-0.01 mM. Calibration curves were plotted using a weighted regression (1/×) and the concentration of each amino acid was calculated using the following equation:

$$\text{Amino acid (ppm)} = R \times DF,$$

where R is reading from the standard curve; and DF is dilution factor.

DETERMINATION OF POLYPHENOLS

Total polyphenol or total phenolic content was determined using modified calorimetric technique Folin-Ciocalteu adapted by Singleton and Rossi (1965). About 0.1 g of defatted sample (previously prepared from milled cocoa nib and defatted with petroleum ether for 16-18 h) was extracted with 10 mL acetone (70%, v/v) in 50 mL centrifuge tube. The tubes were sonicated in ice water to prevent any browning. The extract was centrifuged for 15 min at 5000 rpm. For the total polyphenol assay, 100 µL of extract was added with 7.9 mL of distilled water. A 500 µL Folin-Ciocalteau reagent was added and mixed. The mixture was left for 8 min before 1.5 mL of 20% sodium carbonate (Na_2CO_3) solution was added and left for 2 h at room temperature. Absorbance was read at 765 nm using a UV-Vis spectrophotometer (Agilent, Cary 60). A calibration curve was prepared using gallic acid in the range of 100-1400 mg/L. Total polyphenol content was calculated using the following equation, expressed as mg gallic acid equivalent (GAE) per gram defatted sample.

$$\text{Total polyphenol (mg GAE/g)} = (CF \times C_{aliquot}) / W_1,$$

where CF is a unit conversion factor equal to 0.01; $C_{aliquot}$ is concentration of aliquot from calibration curve; and W_1 is sample weight.

Meanwhile, catechin and epicatechin were analyzed according to method described by Kim and Keeney (1983). About 0.5 g defatted cocoa powder (previously prepared from milled cocoa nib and defatted with petroleum ether for 16-18 h) was transferred into 125 mL conical flask mixed with 80 mL 80% acetone (v/v). This was then sonicated for 30 min in ice water to prevent browning. The extract was then filtered through a Whatman no. 4 filter paper. The residue and glassware were washed with 80% aqueous acetone and the filtrate was made up to 100 mL in a volumetric flask. Then, 10 mL of the extract was dried in a rotary evaporator (Buchi, Germany) at 45°C. The resulting residue was resuspended with 5 mL distilled water by swirling the flask in a 45°C water bath. The resuspended extract was then loaded into the SPE C18 cartridge (Alltech, Illinois, USA) was initially preconditioned with 1 mL methanol followed by 5 mL distilled water. The catechin and epicatechin retained in SPE cartridge were eluted with 10 mL 40% aqueous methanol into a 10 mL volumetric flask. 10 µL of this final solution was injected into high performance liquid chromatography, HPLC (Agilent 1100 Series, Agilent, USA). A reversed phase Genesis C18 column (150 mm × 0.46 mm, Jones Chromatography, USA) was used with mobile phase mixture of water, methanol and acetic acid (87:8:5) at flow rate 1.5 mL/min. Detection was carried out using Diode Array detectors (DAD) by monitoring absorbance at 280 nm. Quantitative values were obtained by relating chromatographic peak areas to those derived from externally run calibration standards ranging from 1-5 ppm and 5-10 ppm for catechin and epicatechin, respectively. The concentration of catechin/epicatechin was calculated using the following equation:

$$\text{Catechin/epicatechin (mg/g)} = R \times DF,$$

where R is reading from the standard curve; and DF is dilution factor.

DETERMINATION OF VOLATILES COMPOUNDS

Ghanaian and Malaysian dried cocoa nibs with particle sizes less than 1 mm were obtained using grinder Retsch ZM200. About 0.02 g powdered cocoa sample was weighted into stainless steel sample cup and was spiked with 1 µL of 8000 ppm of N,N-dimethyl aniline as an internal standard. Sample cup containing sample was introduced into Tandem Reactor RTX3050TR (Frontier Laboratories Ltd, Japan) which was heated at 140°C and hold for 15 min. Volatile compounds released from the heating process will be trap in cooling zone with cryogenic liquid nitrogen inside the

GC oven. Once the heating cycle completed, the GC oven heating temperature programme will start automatically and the trapped volatile components will start to flow into the gas chromatography system Agilent Technologies 7820A GC System equipped with a mass selective detector GC-5977E MSD in an electron ionization (EI) mode at 70 eV. A Ultra Alloy Capillary Column UA-5MS 100% dimethylpolysiloxane column (P/N UA1-30M-1.OF, Frontier Laboratories Ltd, Japan) with an inner diameter of 250 µm, 0.25 µm film thickness and a length of 30 m was used. The GC temperature program was: 50°C for 0.1 min, and increased to 200°C at heating rate 10°C/min, followed by second heating at 25°C/min to 300°C and hold for 4 min. The volatiles components (represented as a peaks) were identified by comparison of their mass spectra to the reference mass spectral database of the National Institute of Standards and Technology library (NIST 14.lib) and tentatively assigned as the closest match when the similarity of the mass spectrum of the peak and the library was at least 50%. The concentration was quantified as a relative concentration by comparing the response/area of individual peak against the response of the internal standard used and reported as relative concentration using the following equation:

$$\text{Volatile compound (ppm)} = (R_{cpd} / R_{is}) \times C_{is},$$

where R_{cpd} is response of the compound; R_{is} is response of the internal standard; and C_{is} the concentration of internal standard.

SENSORY ANALYSIS

Roasted and deshelled cocoa nibs sample were ground using laboratory blender to obtain particle sizes less than 5 mm prior to cocoa liquor preparation. Cocoa liquor for sensory evaluation was prepared using Labscale Mortar Pestle Concher. The sensory evaluation of cocoa liquor samples were conducted by trained panelists of Sensory Laboratory of Malaysian Cocoa Board (MCB), using a Quantitative Descriptive Analysis (QDA) method (Aminah. 2000). Cocoa liquor samples were given a score on a 0-10 scale for attributes of cocoa taste, astringent, bitter, sourness/acidity, raw/green, fruity/floral, mouldy/earthy, smoky, viscosity and overall acceptability. Panelist were assessed the intensity of each attributes based on reference cocoa liquor.

STATISTICAL ANALYSIS

Data were statistically analyzed using Minitab software version 16.1.1 (2010). Analysis of variance (ANOVA) was carried out on the results obtained to determine statistical differences between the studied parameters. The significance was established using Tukey's Pairwise Comparisons at a confidence level of 95% ($p \leq 0.05$).

RESULTS AND DISCUSSION

SUGARS CONTENT

The amount of sugar (sucrose, glucose and fructose) and organic acids present in Malaysian and Ghanaian cocoa beans is presented in Table 1. According to Aprotosoaie et al. (2016), raw cocoa beans contain about 2% to 4% (dry weight) free sugars consisting of fructose, glucose, sucrose, galactose, sorbose, xylose, arabinose, mannitol, and inositol. During fermentation, sucrose was converted into reducing sugars (fructose and glucose) due to invertase activity (Afoakwa et al. 2008). Reducing sugars are critically involved in the development of typical chocolate flavor through Maillard reactions with amino acids during roasting (Aprotosoaie et al. 2016). Table 1 shows there was no significant difference in sugar content except for Malaysian cocoa nib which was found to be 13.5% higher sucrose content compared to Ghanaian sample. Higher content of sucrose in Malaysian sample might be associated to a larger amount of pulp (Hashim et al. 1999).

Total reducing sugar (glucose and fructose) found in this study was in good agreement with data reported by Hashim et al. (1999). Interestingly, sugars content reduced significantly in roasted sample due to Maillard reaction or non-enzymatic (Misnawi et al. 2004; Ramli et al. 2006;

TABLE 1. Sugars content in unroasted and roasted cocoa nib from different origin

	Sugars content (× 10^3 mg/kg)		
	Sucrose	Glucose	Fructose
Unroasted nib;			
- Malaysian	$2.44^a \pm 0.11$	$1.45^a \pm 0.10$	$1.38^a \pm 0.10$
- Ghanaian	$2.15^b \pm 0.11$	$1.42^a \pm 0.10$	$1.37^a \pm 0.10$
Roasted nib;			
- Malaysian	$2.11^c \pm 0.64$ (-13.5%)	$0.50^c \pm 0.05$ (-65.5%)	$0.49^c \pm 0.12$ (-64.5%)
- Ghanaian	$1.33^d \pm 0.10$ (-38.1%)	$0.38^d \pm 0.02$ (-73.2%)	$0.56^c \pm 0.02$ (-59.1%)

The results are the means ± s.d. Mean values followed by same alphabet in the same column are not significantly different at $p>0.05$

$n = 5$

Ziegleder 1991). The concentration of sucrose and glucose content in roasted Ghanaian sample were significantly lower compared to Malaysian sample. The concentration level of sucrose, glucose and fructose in Malaysian sample reduced 13.5%, 65.5%, 64.5%, respectively. Similar trend was also observed in roasted Ghanaian sample, reduced at 38.1%, 73.2%, 59.1% for sucrose, glucose and fructose, respectively.

ORGANIC ACIDS CONTENT

The organic acids content in unroasted samples were significantly different for citric acid and acetic acid, however, the concentration of acetic acid in Malaysian nibs was found to be 24.1% higher compared to Ghanaian nibs. This finding corresponded well with literatures which reported that cocoa beans from South East Asia region has more acidic characteristics compared to South African beans (Voigt et al. 1994) and contributed to the weaker cocoa flavor.

Lactic acid was found to be the determining factor for the acid taste compared to acetic acid (Biehl & Ziegleder 2003). Even though lactic acid in both sample is not significantly different, however, the amount present in Malaysian sample is slightly higher compared to Ghanaian sample. High acidic content in cocoa beans is associated with excessive acidification and proteolysis during fermentation. The acids produced were further infused into cotyledons and resulted in high acid content. The results clearly indicated that Malaysian cocoa nibs have gone through strong acidification process due to the amount of acids quantified. In summary, Malaysian sample contained slightly higher organic acids which contributed to the acidic taste.

FREE AMINO ACIDS

In this study, four groups of free amino acids (acidic, basic, hydrophobic and others) present in Malaysian and Ghanaian cocoa samples were quantified against peak areas and retention time derived from calibration standards. In total, 14 amino acids (aspartic acid, glutamic acid, lysine, arginine, histidine, alanine, isoleucine, phenylalanine, tyrosine, valine, proline, methionine, serine & threonine) were successfully quantified, while 3 others (cysteine, histidine, glycine) were detected at very low level. Free amino acids detected were mainly due to the degradation of protein and peptide inside the cotyledons during fermentation process (Kirchoff et al. 1989). The cotyledons of ripe cocoa beans contain between 10% and 16% (dry weight) proteins: An albumin fraction (52%) and a globulin fraction (43%) represented by a (7S)-class globulin (Kirchoff et al. 1989), however, only vicilin (7S)-class globulin is involved in proteolysis during fermentation. The enzymatic proteolysis of globulin, under the combined action of cocoa aspartic endoprotease and serine carboxyexopeptidase, yields cocoa-specific flavor precursors like oligopeptides and free amino acids which will play important role in flavor formation during roasting. Table 3 shows that the amounts of amino acid in all groups in this study were not significantly different in term of concentration between Malaysian and Ghanaian unroasted samples. Acidic free amino acid in both Malaysian and Ghanaian cocoa are approximately at 1 mg/kg, basic free amino acid at less than 1 mg/kg, hydrophobic amino acids at less than 10 mg/kg, while others free amino acids are at approximately 3 mg/kg.

Hydrophobic amino acids which constitutes the larger number of individual amino acids, contributed to the higher content present in both samples. However, the higher concentration in Ghanaian sample (9.50±0.72 mg/kg) compared to Malaysian sample (9.03±0.75 mg/kg) was observed. Aprotosoaie et al. (2016) explained that these free amino groups of amino acids involved in cocoa flavor development through several steps namely Schiff bases reactions, Amadori rearrangement and Strecker degradation which lead to formation of volatile ketones, aldehydes, pyrazine and other heterocyclic compounds. Besides that, hydrophobic amino acids e.g. proline can also interact with polyphenols which caused astringency and reduce lubricating action of the saliva and causes a less viscous sensation on tongue (Burdock 2010). Meanwhile, after roasting most of the amino acids except

TABLE 2. Organic acids content in unroasted and roasted cocoa nib from different origin

	Organic acids content (× 10^3 mg/kg)		
	Citric	Lactic	Acetic
Unroasted nib;			
- Malaysian	21.57^a±0.46	13.30^a±1.05	4.23^a±0.34
- Ghanaian	24.97^b±1.40	12.50^a±1.10	3.41^b±0.36
Roasted nib;			
- Malaysian	18.13^c±1.60	11.81^c±0.34	4.11^c±0.10
- Ghanaian	12.76^d±1.50	10.97^d±0.50	3.43^d±0.30

The results are the means ± s.d. Mean values followed by same alphabet in the same column are not significantly different at p>0.05

n = 5

acidic group increased in both samples as shown in Table 3 and the concentration level are significantly different. The concentration of basic, hydrophobic and others amino acids in Malaysian cocoa increased 10.9%, 26.6% and 66%, respectively. Similar trend was also observed in roasted Ghanaian sample, whereby the concentration increased tremendously with 218%, 166.6%, 211% for basic, hydrophobic and others amino acids, respectively.

POLYPHENOLS AND FLAVANOL

The results from spectrophotometric determination of total phenolic and flavonoids (catechin and epicatechin classified as flavan-3-ol monomer units) are summarized in Table 4 as mg of gallic acid equivalent per gram defatted sample. Changes are represented as the mean taking into account the standard deviation. Total polyphenol and epicatechin content are significantly different in both unroasted samples and vary from 63.15 - 70.84 mg GAE/g, and from 0.93 - 1.59 mg/g, respectively. Meanwhile, the catechin content is not significantly different between samples and varies between 0.12 and 0.15 mg/g. The unroasted Malaysian sample contains 12.2% higher in total polyphenol content, but 41.5% lower in epicatechin content compared to unroasted Ghanaian sample. Most likely, these variables occurred due to several factors such as difference in varieties, geographical origin, and post harvest techniques as reported in the literature (Adam et al. 1931; Miller et al. 2009; Ortega et al. 2008). Azizah et al. (2007), also claimed that Malaysian cocoa beans contain highest phenolic content compared to those beans from Sulawesi, Ghanaian and Cote d'Ivoire.

After roasting, the quantified total polyphenol content decreased, ranging from 45.3 - 47.8 mg GAE/g. There is no significant difference in both samples were observed, while the total polyphenol decreased 36.1% and 24.3% in Malaysian and Ghanian samples, respectively. This decreasing trend in the total polyphenol content during roasting has been described quite well in the literature (de Brito et al. 2000; Kealey et al. 1998) and could probably due to the high redox-activity of polyphenols under such high oxygen and temperature condition. The loss of polyphenols content will reduce the level of bitterness and astringency of cocoa beans as claimed by Misnawi et al. (2005) and Robinson et al. (1961).

In contrary, the epicatechin content in roasted samples are significantly different and increased by 42.0% and 82.4% in Malaysian and Ghanaian samples. Data also showed that catechins was not affected by the roasting process in both samples. Similar result was also reported by Zyzelewicz et al. (2016) in their study on the effects of nib roasting in catechin content.

VOLATILES COMPONENTS

In the present study, the volatiles compounds in cocoa nibs of Malaysian and Ghanaian was extracted, isolated and identified using GC-MS. Altogether 30 compounds were successfully identified as shown in Table 5 which composed of different classes such as alcohols, aldehydes, ketones, pyrazines, esters, terpenes and acid. Those compounds are commonly identified as a key odorant compounds that contribute to the quality of cocoa or cocoa specific flavor (Bonvehi 2005; Burdock 2010; Rodriguez et al. 2012). Generally, alcohols, ketones and esters exhibit some kinds of fruity, green and floral aroma. Alcohols level in the cocoa nibs conferred cocoa products with flowery and candy like sweetness flavors (Rodriguez et al. 2012). Ketones and some aldehydes are favorable for cocoa quality with floral, fruity, sweet and cocoa-like flavor note (Rodriguez et al. 2012). Meanwhile, as well as alcohol and ketones, esters also exhibit fruity, floral and sweet aroma or taste (Rodriguez et al. 2012).

The major alcohols were 2,3-butanediol and iso-propyl alcohol. Both of its concentration was significantly higher ($p<0.05$) in Malaysian sample. Both compounds contribute to sweet chocolate characteristics taste (Rodriguez et al. 2012). Meanwhile, 1-(1H-pyrrol-2-yl)-ethanone is the only ketone was detected and their concentration is not significant difference between samples. There are 8 esters compounds detected in both samples and their concentration were not significantly different except for 2-phenylethyl ester acetic acid, pent-2-yl ester benzoic acid and hexadecanoic acid methyl ester which contributes

TABLE 3. Free amino acids of unroasted and roasted cocoa nib from different origin

	Free amino acids content (mg/kg)			
	Acidic	Basic	Hydrophobic	Others
Unroasted nib;				
- Malaysian	$1.13^{a} \pm 0.11$	$0.55^{a} \pm 0.08$	$9.03^{a} \pm 0.75$	$2.49^{a} \pm 0.22$
- Ghanaian	$1.23^{a} \pm 0.17$	$0.56^{a} \pm 0.05$	$9.50^{a} \pm 0.72$	$2.37^{a} \pm 0.20$
Roasted nib;				
- Malaysian	$0.76^{c} \pm 0.13$	$0.61^{c} \pm 0.07$	$11.43^{c} \pm 1.53$	$4.14^{c} \pm 0.98$
- Ghanaian	$1.15^{d} \pm 0.17$	$1.78^{d} \pm 0.25$	$25.30^{d} \pm 3.26$	$7.37^{d} \pm 1.25$

The results are the means ± s.d. Mean values followed by same alphabet in the same column are not significantly different at $p>0.05$

$n = 4$

TABLE 4. Polyphenols content in unroasted and roasted cocoa nib from different origin

	Total polyphenol (mg GAE/g)	Flavanol monomers (mg/g)	
		Catechin	Epicatechin
Unroasted nib;			
- Malaysian	70.84 ^{a} ± 0.83	0.15 ^{a} ± 0.02	0.93^{a} ± 0.06
- Ghana	63.15 ^{b} ± 3.33	0.12 ^{a} ± 0.01	1.59 ^{b} ± 0.16
Roasted nib;			
- Malaysian	45.26 ^{c} ± 0.53	0.15^{c} ± 0.02	1.32^{c} ± 0.22
- Ghanaian	47.82 ^{c} ± 3.70	0.19^{c} ± 0.07	2.90^{d} ± 0.37

The results are the means ± s.d. Mean values followed by same alphabet in the same column are not significantly different at $p>0.05$

$n = 4$

to fruity and floral taste. The concentrations of those 3 compounds were found slightly higher in Ghanaian sample. Pyrazines and aldehydes are generally known as a key flavor compounds that contribute to the good quality cocoa aroma and chocolate taste. In the present study, there are 10 aldehydes and 5 pyrazines compounds identified and each of them confer specific flavor characteristic as shown in Table 5. However, only 4 aldehydes that were reported to be related and responsible for the chocolate flavor which are benzeneacetaldehyde, 5-Methyl-2-phenyl-2-hexenal, 2-methyl-propanoic acid and 2-methyl propanal. Interestingly, 5-Methyl-2-phenyl-2-hexenal is a major aldehyde in both sample, however, its concentration in Malaysian sample is lesser compared to Ghanaian sample. 5-Methyl-2-phenyl-2-hexenal exhibits sweet chocolate taste (Bonvehi 2005) and deep bitter cocoa note (Burdock 2010). Besides that, 2-methyl propanal and 2-methyl-propanoic acid were not present in Malaysian beans.

Meanwhile, pyrazines conferred nutty, earthy, roasty or green aroma flavors, which determined the overall cocoa flavor (Afoakwa et al. 2008). Most of the pyrazines developed from α-aminoketones by Strecker degradation and Maillard reactions during roasting (Burdock 2010). In the present study, 6 pyrazines were identified in the Malaysian and Ghanaian sample as shown in Table 5 and most of them confer nutty, roasted and high cocoa aroma characteristics (Bonvehi 2005).

Besides those compounds, caffeine and acetic acid were also present significantly ($p<0.05$) in both samples. Caffeine is terpene that contributes to the bitter taste of cocoa and also involved in the palatability of food products containing them. Acetic acid is considered as a residue from the fermentation process, but it is not totally removed during roasting. It is responsible to bring sour or vinegar aroma with strong and pungent taste to cocoa beans or cocoa products containing it. Table 5 shows that the content of caffeine in Malaysian cocoa bean sample is 27% higher compared to Ghanaian sample as well as acetic acid which is 21% higher compared to Ghanaian sample. The higher content of acetic acid in Malaysian sample is in good agreement with the data of organic acids presented in Table 2.

SENSORY EVALUATION

The QDA results obtained from trained panelists were evaluated by ANOVA and the results of mean scores for each attributes were presented as in Table 6. The result showed that both samples are differed significantly ($p<0.05$), which Ghanaian sample showed higher score in cocoa taste, less acidity/sourness and better overall acceptability compared to Malaysian sample. Other attributes such as fruity, bitterness, astringent, raw and viscous were not significant different ($p>0.05$) between samples. Meanwhile, the mouldy and smoky notes which were known as off-flavor characteristics were considerably absent in both samples.

Generally, it was found and confirmed that Ghanaian cocoa samples was significantly much better in term of overall quality and acceptability. The results showed that the roasting gave the largest impact on the flavor precursors compounds and positive correlation with regards to flavor characteristics were observed. After roasting, most of the sugars were decreased in both samples as shown in Table 1. The reduction of reducing sugar glucose and fructose were mainly due to their involvement in Maillard reaction with oligopeptides and free amino acids. The abundance of free amino acids especially hydrophobic during roasting (Table 3) in Ghanaian cocoa also indicate that the proteins and oligopeptides in Ghanaian sample undergo excessive thermal degradation. Hydrophobic amino acids are believed an important contributor to the flavor formation as claimed by Voigt et al. (1994, 1993) and thus consequently contribute to the specific flavor quality of Ghanaian cocoa. Higher level of free amino acid content especially hydrophobic type amino acids gave specific flavor precursors which chemically affects the formation of taste specific flavors in the form of higher content of aldehydes, 5-methyl-2-phenyl-2-hexenal (sweet chocolate taste), 2-methyl hexanal (green/herbal), 3-methyl-butanoic acid (fruity), 2-methyl-propanoic acid (intense sweet/ vanilla-like milk chocolate) whose concentrations were significantly higher in Ghanaian sample. Uniquely, there are 2 types of aldehydes, 2-methyl-propanal and 2-methyl-propanoic acid which both contributes to sweet and vanilla like chocolate taste that were only found in Ghanaian beans. Roasted Ghanaian beans also contain significantly

TABLE 5. Volatile compounds in roasted cocoa nibs from different origin

Volatile compounds	Relative concentration, (ppm)			Flavor/taste characteristics Afoakwa et al. 2008; Bonvehi 2005; Burdock 2010; Rodriguez et al. 2012
	Ghanaian	Malaysian		
Alcohol				
4-methyl-1-hexanol	[a] 7.2±1.3	[b] not detected [b]	-	fruity, green / fruity, herbal
Isopropyl alcohol	[a] 13.2±5.2	33.0±10.7	-	sweet candy / sweet chocolate
Phenylethyl alcohol	[a] 25.7±2.1	[a] 29.0±11.6	-	honey, floral / floral
2,3-Butanediol	[a] 209.9±28.5	[b] 446.2±73.2	-	sweet chocolate
Ketones				
1-(1H-pyrrol-2-yl)-ethanone	[a] 14.1 ± 1.3	[a] 12.7 ± 6.4	-	musty, nutty / sweet, musty, nutty and tea-like
Aldehydes				
2-methyl-propanal	[a] 6.7 ± 0.5	[b] not detected	-	chocolate/sweet chocolate
benzaldehyde	[a] 6.8 ± 1.1	[a] 5.7 ± 1.3	-	taste bitter pungent
benzeneacetaldehyde,	[a] 7.7 ± 2.24	[a] 7.7 ± 1.4	-	honey, floral / flora
α-ethylidene-benzeneacetaldehyde	[a] 10.3 ± 3.7	[a] 16.3 ± 2.5	-	cocoa/sweet chocolate
5-methyl-2-phenyl-2-hexenal	[a]59.7 ± 6.5	[b]26.5 ± 8.7	-	chocolate/sweet chocolate
2-methyl hexanal	[a] 34.1 ± 7.8	[b] 31.2 ± 2.6	-	green / herbal
3-methyl-butanal	[a] 28.57 ± 6.7	[a] 35.6 ± 2.3	-	intense fruity, ether-like odor
2-methyl-propanoic acid-	[a] 2.9 ± 0.7	[b] not detected	-	intense sweet, creamy, vanilla-like milk chocolate
2-methyl-butanoic acid	[a] 17.1 ± 1.2	[a] 13.2 ± 4.1	-	green, fruity, sweet, juicy
3-methyl-butanoic acid	[a]45.3 ± 9.3	[b]24.9 ± 0.84	-	ether-like odor and a sweet, apple-like, fruity
Pyrazines				
3,5-diethyl-2-methyl-pyrazine	[a] 1.1 ± 0.4	[b] 1.8 ± 0.6	-	nutty, meaty/sweet, fruity
methyl-pyrazine	[a] 1.2 ± 0.2	[a] 3.1 ± 1.2	-	nutty, sweet chocolate, roasted
2,5-dimethyl-pyrazine	[a] 4.7 ± 1.8	[a] 6.3 ± 0.8	-	nutty, sweet chocolate, roasted
trimethyl-pyrazine	[a] 10.6 ± 1.5	[a] 12.9 ± 2.5	-	nutty, sweet chocolate, roasted
tetramethyl-pyrazine	[a] 74.1 ± 6.2	[b] 51.7 ± 11.9	-	chocolate, cocoa, coffee/sweet chocolate
Esters				
ethyl acetate	[a]12.6 ± 3.2	[a]8.55 ± 2.2	-	pineapple/fruity
propanoic acid, 2-methyl propyl ester	[a]4.5 ± 0.55	[a]5.46 ± 1.7	-	fruity/sweet, rummy, pungent, bubblegum
benzeneacetic acid	[a]16.1 ± 2.12	[a]13.75 ± 4.1	-	fruity, honey and rose-like odor
acetic acid, 2-phenyl ethyl ester	[a]17.1 ± 0.96	[b]8.53 ± 0.5	-	pleasant, strong, sweet/honey, bittersweet taste
benzoic acid, pent-2-yl ester	[a]8.6 ± 0.50	[b]2.54 ± 0.7	-	sweet, rose, honey-like/floral taste
dodecanoic acid, ethyl ester	[a]9.0 ± 1.2	[b]6.07 ± 1.4	-	floral, fruity odor
hexadecanoic acid, methyl ester	[a]77.8 ± 9.9	[a]90.49 ± 7.1	-	having slight characteristic odor/taste
hexadecanoic acid, ethyl ester	[a]124.6±15.5	[a]185.4±16.2	-	has a mild, waxy sweet odor/tasteless
Terpenes: Caffeine	[a]574.8±54.6	[b]694.8±44.9	-	cocoa, powdery, bitter, beany, brown and roasted
Acids: Acetic acid	[a]1441.9±89.5	[b]1943.9±52.8	-	vinegar with a strong, pungent, characteristic

The results are the means ± s.d. Mean values followed by same alphabet in the same row are not significantly different at p>0.05

n = 3

higher in tetramethyl pyrazine (chocolate/cocoa/sweet chocolate) and esters namely acetic acid, 2-phenylethyl ester (pleasant/bittersweet taste) and benzoic acid, pent-2-yl ester (sweet/rose/floral taste) as compared to Malaysian roasted sample. Besides low in acidic taste in Ghanaian sample, sensory results also indicated that there are no significant different in term of astringent and bitterness attributes between Ghanaian and Malaysian sample, thus was in good correlation with the data of phenolic compounds presented in Table 4.

Meanwhile, sensory result found that Malaysian sample tastes quite acidic and contribute to the less acceptability among sensory panelist. This is due to the amount of organic acids presence in roasted Malaysian cocoa sample as presented in Table 2 and was also confirmed via GC-MS data shown in Table 5. Approximately 34.05 × 10^3 mg/kg of total organic acid content was detected in Malaysia sample while Ghanaian beans showed lesser amount at approximately 27.2 × 10^3 mg/kg. The higher organic acids in Malaysian cocoa sample is in good agreement with the result reported by Tomlins et al. (1990) and adversely affects the quality of cocoa taste and flavors (Dimick & Hoskin 1999; Reed 2010). Even though Afoakwa et al. (2008) argues that sourness can be reduced

TABLE 6. Sensory attributes and overall acceptability of cocoa liquor samples evaluated by trained panelists

Sensory attributes	Malaysian	Ghanaian
Acidity/sourness	$2.9^{a} \pm 0.8$	$2.3^{b} \pm 0.6$
Cocoa	$4.4^{a} \pm 0.9$	$6.0^{b} \pm 1.1$
Fruity/floral	$0.1^{a} \pm 0.3$	$0.5^{a} \pm 0.6$
Mouldy/earthy	0.1	0.0
Smoky	0.0	0.0
Bitter	$3.9^{a} \pm 0.6$	$3.5^{a} \pm 0.7$
Astringent	$4.3^{a} \pm 0.8$	$3.9^{a} \pm 0.7$
Raw/green	$0.4^{a} \pm 1.0$	$0.2^{a} \pm 0.5$
Viscosity	$6.5^{a} \pm 1.0$	$6.0^{a} \pm 0.9$
overall acceptability	$3.9^{a} \pm 1.6$	$5.2^{b} \pm 1.1$

The results are the means ± s.d. Mean values followed by same alphabet in the same row are not significantly different at $p>0.05$

$n = 14$

by evaporation of volatile acids during roasting, however, our findings showed that the organic acids are not fully removed during roasting.

CONCLUSION

This study successfully quantified the important flavor precursors and volatile compounds in the dried cocoa beans from Malaysian and Ghanaian. It was found that there are some differences in term of concentration level in specific flavor precursors under this study, and these has contributed and add further information to the already existing knowledge on the factors determining the beans quality between of Ghanaian and Malaysian beans. In particular, the Malaysian cocoa beans contained higher amount of citric, lactic and acetic acid which contributed to acidic taste as reported in literatures. Lactic acid and acetic acid could potentially be significant markers to determine the overall sensory acceptability of cocoa liquor in term of taste and flavors. Additionally, the Ghanaian beans was found to contain higher amount of hydrophobic free amino acids (phenylalanine) and phenolic constituents (total polyphenol and epicatechin) compared to Malaysian beans which might be the determinant factors that contributed to the higher cocoa taste during roasting, especially on the specific cocoa taste of aldehydes and pyrazines. Finally, our results suggested that the flavor quality of Malaysian cocoa beans is closer to Ghanaian cocoa and can be improved through secondary processing technique especially on the roasting process.

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