Analytical methods

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Nutritional composition and fatty acids profile in cocoa beans and chocolates with different geographical origin and processing conditions

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Abstract

Nutritional composition and fatty acids (FA) profile was determined in cocoa and chocolates with different geographical origin and processing conditions. In cocoa, fat was the major nutrient whereas in chocolates were the carbohydrates. Cocoa composition varied depending on geographical origin while in chocolates only carbohydrates and fat content varied significantly due to the effect of origin and no significant effect was observed for processing conditions. Both for cocoa and chocolates differences in FA profile were mainly explained as an effect of the geographical origin, and were not due to processing conditions in chocolate. For cocoa, differences in FA profile were found in C12:0, C14:0, C16:0, C16:1, C17:0, C17:1 and C18:0 while for chocolates only differences were found in C16:0, C18:0, C18:1 and C18:2. For all samples, C16:0, C18:0, C18:1 and C18:2 were quantitatively the most important FA. Ecuadorian chocolate showed a healthier FA profile having higher amounts of unsaturated FA and lower amounts of saturated FA than Ghanaian chocolate.

Keywords: cocoa beans, chocolate, total fat, fatty acids profile, GC-MS.

1. Introduction

Chocolate is a product obtained from cocoa beans, the fruit of the cocoa tree (*Theobroma cacao* L.) that grows in Central and South America and in West Africa (Rusconi and Conti, 2010).

In the formulation of chocolate the key ingredients are: cocoa solids, cocoa butter, sugar, and lecithin as an emulsifier. However, the wide diversity of products available on the market is achieved by incorporating other ingredients to the formulations, such as nuts, fruits or cereals. The main commercial chocolate categories are dark, milk and white chocolates, differing in their content of cocoa solids, milk fat and cocoa butter.

Chocolate and chocolate products are characterized as products with high energy and nutritional density. Energy content in chocolate and chocolate products reaches over 3000 kcal/kg of product, and the nutritional composition of chocolate corresponds to its high content in carbohydrates and fats. Carbohydrates are mainly represented as sugars, with a total content of up to 45%, and fat, with a total content of up to 30%. Chocolate also contains minerals, specially potassium, magnesium, copper and iron (USDA, 2010). The content of each nutrient in chocolate depends, among other factors, on the cocoa solids percentage of chocolate. When cocoa solids content of chocolate increases, the percentage of carbohydrates decreases with respect to an increase in total fat content. As a result, chocolate types with a higher content of cocoa solids are fattier and, in consequence, are high calorie products (USDA, 2010).

Regarding the chemical composition, chocolate and chocolate products can be considered as a dense suspension of solid particles (sugar, cocoa and milk mixture, depending on type) dispersed in a continuous fat phase, which is mostly composed of cocoa butter (Khampius, 2010; Nickless, 1996).

Cocoa butter is considered the most important cocoa by-product, due to its physical (rheology and texture) and chemical characteristics and also organoleptic qualities, (Lipp and Anklam, 1998) which produce widely demanded functional properties in the food industry. These special characteristics, which are not comparable with any other edible vegetable fat, are very useful in the manufacture of a great variety of products in the chocolate, cosmetic and pharmaceutical industries. Fatty acids (FA) identification and quantification in cocoa butter is of enormous interest for research and development laboratories, and also, in processing and quality control during manufacture.

The amount of cocoa butter and the fatty acid profiles in chocolate products depend on the growing conditions of cocoa beans. In cocoa butter, fatty acids are organized as triacylglycerol (TAG), the majority of these TAG's being 2-oleyl glycerides (O) of palmitic (P) and stearic (S) acids (POP, POS, SOS) (Simoneau, Hannaert and Anklam, 1999; Segall, Artz, Raslan, Ferraz and Takahashi, 2005). This TAG structure directly affects the way chocolate behaves in the manufacturing process and the characteristics of the final product (texture, viscosity, melting behaviour, flavour and taste) (Afoakwa, 2010).

Epidemiologic data have shown that fatty acids profile in food has a direct impact on human health (Hu, Stampfer, Manson, Ascherio, Colditz, Speizer, Hennekens and Willett, 1999; López-Huertas, 2009; Solfrizzi, D'Introno, Colaccio, Capurso, Palasciano, Capurso, Torres, Capurso and Panza, 2005). It has been generally accepted that unsaturated fats have a hypocholesterolemic effect, while saturated fats tend to raise total cholesterol and low-density lipoproteins (LDL) levels. The latest dietary reference intakes for fat for a healthy population are from 20 to 35% of total diet energy content (Food and Nutrition Board, 2005). Saturated fatty acid (SFA) content must be as low as possible while consuming a nutritionally adequate diet. Polyunsaturated fatty acids (PUFA) must represent a maximum of 10% of total calories and monounsaturated fatty acids (MUFA) should be the major ones.

In this sense, new perspectives for chocolate have recently been approached in the cardiovascular field; whereby dark chocolate consumption could provide a beneficial effect due to the characteristics of its chemical composition (Grassi, Lippi, Necozione, Desideri and Ferri, 2005; Grassi, Desideri and Ferri, 2010; Ding, Hutfless, Ding and Girotra, 2006).

The aim of the present study was to determine the total fat content and fatty acid profile of chocolate samples produced with cocoa beans from different geographical origin and with different processing conditions in order to assess their nutritional value.

2. Materials and methods

2.1. Samples

Theobroma cacao beans of different geographic origins (Ecuador and Ghana) were used. Six dark chocolate samples for each origin produced with different conditions (three roasting times: R1=30.5, R2=34.5 and R3=38.5 min; and two conching times: C1=24 and C2=42 h) were studied. Chocolates containing 51% (w/w) of cocoa were prepared in a chocolate factory (Chocolates Simón Coll, S.A. from Sant Sadurní d'Anoia, Barcelona, Spain) following a traditional chocolate manufacturing process (Beckett, 2008) as described in a previous study (Torres-Moreno, Tarrega, Costell and Blanch, 2012).

2.2. Proximate analysis

The unroasted cocoa beans and the chocolate samples were analyzed in duplicate for moisture, ash, protein, total fat and total dietary fibre content following the AOAC methods for cacao beans and its products (AOAC, 1995). Total carbohydrate content was estimated by difference.

Moisture contents of the samples was determined by the gravimetric method by drying 2 g of grinded sample at 103 ± 2ºC to constant weight in an air oven. Ash contents was determined by using a muffle furnace at 550-600ºC for 4 h. Fat was determined in a Soxhlet apparatus using petroleum ether as solvent of extraction. Total organic nitrogen was determined by using the macro Kjeldahl procedure. Protein content of samples was calculated using 6.25 as the conversion factor (Protein = Nitrogen * 6.25). Crude fibre content was determined by the AOAC method for cocoa products not containing dairy ingredients (AOAC, 1995).

Mineral composition of unroasted cocoa samples was analyzed in triplicates. First, a microwave digestion (Milestone Ethos plus, 220ºC; HNO_3: H_2O_2, 1:1 v/v) was used to eliminate organic matter before samples were analyzed. Calcium (Ca), Magnesium (Mg), Potassium (K), Phosphorus (P) and Iron (Fe) were all determined by using the ICP method (Thermo Jarrell-Ash model 61E Polyscan); Manganese (Mn), Copper (Cu), Zinc (Zn), Selenium (Se) and Sodium (Na) were analyzed using the ICP-MS method (Agilent model 7500 CE). Calibration was made with 5 acidic standard solutions in HNO_3 1% by volume, for each of the studied elements.

All reagents and solvents used were of analytical grade or chromatographic grade and were obtained from Panreac and Sigma-Fluka (Barcelona, Spain).

2.3. Preparation of fatty acid methyl esters (FAMEs)

FAMEs were prepared according to the AOAC official method 948.22 (AOAC, 1990b). For each sample 2 derivatizations were prepared.

The FAMEs were obtained from the lipid fractions after alkaline hydrolysis (NaOH/methanol 0.5 mol L^{-1}), followed by methylation with boron trifluoride 14% in methanol. Samples were extracted with heptane and washed with saturated sodium chloride solution. 1 mL of the organic phase was purified though a micro column fitted with anhydrous sodium sulphate and then eluted with 2 mL of heptane. Finally the eluate was evaporated to dryness over N_2 and reconstituted with 1 mL of dichloromethane for the chromatographic analysis.

2.4. Chromatographic conditions

Analysis of total FAMEs was performed on a GC 8000 Thermo Quest gas chromatograph coupled to a Voyager MD800 Finnigam Mass Spectrometer Detector.

The analytical column was a fused-silica TRB-WAX polyethylenglycol capillary column (60 m x 0.25 mm I.D., 0.2 µm film thickness; Teknokroma, Barcelona, Spain).

1 µL of sample was injected in a split/splitless injector set at 250ºC. The GC was setup with helium as a carrier gas at a constant flow of 1.2 mL/min. The oven temperature programme was: 40ºC (5 min); to 100ºC (10 min) at 5ºC/min; to 190ºC (20 min) at 3ºC/min; to 240ºC (5 min) at 5ºC/min.

Detection was carried out with a MD800 mass-selective single quadrupole, using electron-impact ionization (70eV), detector at 550V, source temperature at 200ºC, a scan range of 50-450 amu (scan time of 0.9 s and an inter scan delay of 0.1 s). Two replicates were injected for each sample.

Components identification was based on comparison of its mass spectra with those of the US National Institute of Standards and Technology (NIST) database 98 library of mass spectra, considering peak base, molecular masses and characteristic mass losses. Complementary, standard fatty acid methyl esters (FAMEs) of palmitic, stearic, oleic and linoleic acids were used for the confirmation of the GC-MS libraries results, by comparing the peaks in the analyzed chocolate samples with retention times and mass spectra of known standards (Supelco Bellefonte, USA). Quantification of the fatty acids methyl ester profiles was done considering the relative areas of peaks, expressed as the relative percentage of the individual area of each one versus the total area of compounds in the chromatogram.

2.5. Statistical analysis

A one-factor ANOVA was used to study the effect of cocoa origin on proximate and minerals content and on the fatty acid profile of unroasted cocoa bean samples.

A three-factor ANOVA with interactions was used to study the effect of cocoa origin, roasting time and conching time on proximates content and on the fatty acid profile of chocolate samples.

The significance of differences between means was established using Tukey's test ($\alpha \leq 0.05$).

All the analyses were carried out with XLSTAT Pro software version 2009 (Addinsoft, France).

3. Results and discussion

3.1. Chemical composition of unroasted cocoa beans and chocolate samples

The chemical composition of the unroasted cocoa beans from Ghana and Ecuador is shown in Table 1. For both origins fat was the major nutrient (> 40%), followed by carbohydrates (> 32%) and proteins (12-13%). Fibre content ranged between 11-19% and ash and moisture contents were less than 6%. Cocoa bean composition varied depending on geographical origin. Ghanaian and Ecuadorian bean samples have significantly different contents of moisture (F = 544.96, p = 0.002), carbohydrates (F = 499.50, p= 0.002), fibre (F = 1218.51, p = 0.001) and fat (F = 59.83, p = 0.016). Ecuadorian cocoa beans showed higher moisture (5.95%), fibre (19.47%) and total fat (43.45%) content than Ghanaian ones, and lower carbohydrates (33.78%). No significant differences between the two origins were observed in protein and ash contents (F = 0.78, p = 0.470 and F = 11.53, p = 0.077, respectively). The values found for total fat were similar to those obtained by Liendo (1997) when studying differences in cocoa fat composition between several cocoa origins.

Results related to mineral composition of the unroasted cocoa beans, as shown in Table 1, indicated that cocoa beans contain several minerals with a major role in different body functions, such as numerous enzymatic reactions, energy production, transmission of nerve impulses and multiple biologic reactions (Steinberg, Bearden and Keen, 2003). Significant differences due to the effect of origin were found for Phosphorus (F = 53.46, p = 0.002), Iron (F = 71.02, p = 0.001), Manganese (F = 642.32, p < 0.0001), Zinc (F = 33.16, p = 0.005), Sodium (F = 15.38, p = 0.017) and Selenium (F = 1960.03, p < 0.0001) content. Ecuadorian cocoa beans showed significantly higher values of Phosphorus, Iron, Zinc, Sodium and Selenium than Ghanaian ones, and significantly lower values of Manganese. No significant differences were observed in Calcium, Magnesium, Potassium and Copper content between the two origins. These values confirm, as in other plants, that the mineral content of cocoa reflects the mineral characteristics of the soil in which it has grown (Borchers, Keen, Hannum and Gershwin, 2000).

The chemical composition of chocolate samples is shown in Table 2. Unlike the results observed for cocoa beans, in chocolate samples carbohydrates (> 55%) were the main component and total fat (> 30%) ranked second, as could be expected, because in chocolate formulations the sugar added is significantly higher than the amount of additional cocoa butter incorporated. Furthermore, the addition of the other ingredients reduced protein (6-7%), moisture (< 2%), ash (< 2%) and fibre (< 2%) content in chocolates compared to values obtained in cocoa beans. Thus, chocolate consumption does not contribute significantly to protein and dietary fibre intake.

The values obtained in the chemical composition of chocolate samples analyzed were in line with the values reported in the USDA Database for dark chocolate with 45-59% of cocoa solids (USDA, 2010). Carbohydrates were the main nutrient and their content in chocolate was about 580-600 g kg^{-1}. Total fat and protein content was about 300-320 g kg^{-1} and less than 70 g kg^{-1}, respectively. Total dietary fibre, ash and water content were less than 20 g kg^{-1} for all samples.

Considering the composition of chocolate samples in this study (Table 2), only carbohydrates and fat content values varied significantly due to the effect of origin (F = 1179.73, p = 0.019; F = 996.74, p = 0.020 respectively) and no significant effect was observed for roasting and conching conditions. Ecuadorian samples have higher content in fat than Ghanaian ones and lower content in carbohydrates. No significant differences were observed between the samples in moisture, ash, protein and fibre content (F = 0.11, p = 0.986; F = 0.84, p = 0.70; F = 0.16, p = 0.966; F = 2.01, p = 0.504, respectively).

3.2. Fatty acids profile

Fatty acids from the different cocoa and chocolate samples under study were identified and quantified using GC-MS analyses, showing the separation of fifteen different fatty acid methyl esters, as illustrated in a typical chromatogram in Figure 1.

Unroasted cocoa beans

Fatty acids detected in unroasted cocoa beans were: C12:0 (dodecanoic acid), C14:0 (tetradecanoic acid), C15:0 (pentadecanoic acid), C15:1 (pentadecenoic acid), C16:0 (hexadecanoic acid), C16:1 (hexadecenoic acid), C17:0 (heptadecanoic acid), C17:1 (heptadecenoic acid), C18:0 (octadecanoic acid), C18:1 (octadecenoic acid), C18:2 (octadecadienoic acid), C18:3 (octadecatrienoic acid), C20:0 (eicosanoic acid), C20:1 (eicosenoic acid) and C22:0 (docosanoic acid), ranked in order of the retention time. For unsaturated fatty acids double bounds position and Z or E geometrical isomers were undifferentiated. Table 3 shows the average content of total fatty acids in unroasted cocoa beans from the two origins, Ghana and Ecuador.

Quantitatively C16:0 (>25%), C18:0 (>33%) and C18:1 (>34%) were the most important fatty acids for both origins in unroasted cocoa beans. These results were in agreement with the findings obtained by other authors such as Liendo (1997), Rezanka (1999) and Lipp (2001) who reported that C16:0, C18:0 and C18:1 were the most important fatty acids in cocoa butter.

The effect of geographical origin on the fatty acids profile of unroasted cocoa beans was studied with a one-factor ANOVA. Results obtained show that the origin only had a significant effect on the following fatty acids: C12:0 ($F = 45124.97$, $p = < 0.0001$), C14:0 ($F = 51.07$, $p = 0.019$), C16:0 ($F = 52.69$, $p = 0.002$), C17:0 ($F = 25.53$, $p = 0.037$), C18:0 ($F = 68.24$, $p = 0.014$), C16:1 ($F = 50.75$, $p = 0.019$) and C17:1 ($F = 182.99$, $p = 0.005$). These results confirm that the geographical origin had an influence on the fatty acid composition of cocoa butters as pointed out by Lipp and Anklam (1998). Ecuadorian cocoa beans had a higher content in: C16:0 (27.30%) and C16:1 (0.31%) than Ghanaian ones and a lower content in C14:0 (0.06%), C17:0 (0.26%), C18:0 (33.37%) and C17:1 (0.02%). C12:0 was found in trace amounts in Ecuadorian beans (0.015%), while not detected in Ghanaian samples.

Despite the abovementioned differences, the fatty acids profile was very similar in the two studied cocoa beans with a different geographical provenance. For both origins, palmitic acid (C16:0) and stearic acid (C18:0) were the maximum representatives of SFA. The predominant unsaturated fatty acid was oleic acid (C18:1) followed by linoleic acid (C18:2), which was present in a small amount if compared to stearic, palmitic or oleic acid contents.

When considering total percentage of saturated (SFA), monounsaturated (MUFA) and polyunsaturated fatty acids (PUFA) no differences were found between unroasted cocoa beans (Table 4). For both origins, SFA content was the highest, up to 60%, followed by MUFA > 32% and finally, a minor content of PUFA < 3% were found. The ratio of saturated/unsaturated FA showed the relationship between two major fatty acid groups in cocoa bean fat. Its ratio varied from 1.65 in Ecuadorian beans to 1.72 in Ghanaian beans, which clearly indicates a high proportion of SFA in the fatty acid profile for both origins. Nevertheless, no significant difference was observed when the ratio of saturated/unsaturated fatty acids for the two cocoa bean origins was considered.

Chocolate

Fatty acid profiles of the chocolate samples were similar to those obtained for the unroasted cocoa beans (Table 3), being quantitatively C16:0 (> 26%), C18:0 (> 35%) and C18:1 (> 31%) the most important fatty acids for both origins.

A three-factor ANOVA (origin, roasting and conching time) with interactions was performed in order to study if the fatty acid profile of chocolate samples was affected by these factors. Results of the ANOVA, as shown in Table 3, indicated that only the geographical origin had a significant effect in C16:0 (F = 24.17, $p < 0.0001$), C18:0 (F = 39.71, $p < 0.0001$), C18:1 (F = 5.878, $p < 0.0001$) and C18:2 (F = 35.14, $p < 0.05$) fatty acids. Ecuadorian samples had significantly higher content of C16:0 (27.98%), C18:1 (32.76%) and C18:2 (2.20%) than Ghanaian samples and lower content in C18:0 (35.25%).

Differences were also found between Ghanaian and Ecuadorian chocolate samples when considering total percentage of SFA (F = 33.51, $p < 0.0001$), MUFA (F = 20.35, $p = 0.001$) and PUFA (F = 23.99, $p < 0.001$) as shown in Table 4. For both origins, SFA content was the highest- up to 62%- in Ghanaian samples having significantly higher amounts (66.04%) than Ecuadorian samples (63.95%). MUFA were higher by 32%, and mean values were significantly higher for Ecuadorian chocolate (33.70%) than for Ghanaian chocolate (32.27%). Finally, a minor content of PUFA (< 3%) was found in Ecuadorian samples being significantly richer (2.36%) than Ghanaian ones (1.78%). C16:0 and C18:0 were quantitatively the maximum representatives of SFA, C18:1 the predominant MUFA and C18:2 the most important PUFA. When considering the ratio of saturated/unsaturated FA, significant differences were found between the two origins (F = 25.86, $p < 0.05$), 1.77 for Ecuadorian chocolate and 1.94 for Ghanaian chocolate. Therefore, these results indicate that Ecuadorian chocolate seems to have a healthier FA profile, as Ecuadorian chocolate contains more unsaturated and less saturated fatty acids than Ghanaian chocolate.

The prevalence of saturated fatty acids over unsaturated fatty acids is considered to be negative from the nutritional point of view. Saturated fatty acids whose chain length is: (C12:0-C16:0) have for many year been considered to have promoted atherosclerosis, and to have been associated with cardiovascular disease (CVD). Thus, because of its high SFA content, chocolate is often postulated to have a hypercholesterolemic effect. However, stearic acid (C18:0), a non-cholesterolemic and atherogenic type of dietary saturated fat, has been suggested to be neutral in recent clinical trials, which have shown that chocolate consumption has neutral effects on serum total cholesterol and LDL-cholesterol, as neither lowers HDL-cholesterol (Kris-Etherton and Mustad, 1994; Bonanone and Grundy, 1988; Thijssen and Mensink, 2005). Wan (2001) demonstrated in his study that after a daily consumption of 22 g of cocoa powder and 16 g of dark chocolate for 4 weeks the concentration of HDL cholesterol increased by 4%. Mursu (2004) in another study demonstrated that after a daily ingestion of 75 g of dark chocolate for 3 weeks the concentration of HDL cholesterol increased by 11-14%, and Kurlandsky (2006) demonstrated that in healthy women a dark chocolate intake of 41 g daily in addition to a self-selected diet followed for 6 weeks improved serum triacylglicerol levels.

Furthermore, evidence shows that the effects of stearic acid on lipids can even be considered similar to oleic and linoleic acids which have proved to have preventive effects on cardiovascular disease (Kris-Etherton, Pearson,Wan, Hargrove, Moriarty, Fishell and Etherton, 1999; Hu, Manson and Willett, 2001; Hopper, Thompson, Harrison, Summerbell, Moore, Worthington, Durrington, Ness, Capps, Davey Smith, Riemersma and Ebrahim, 2005).

Among the MUFA, oleic acid was the major fatty acid in chocolate samples. Oleic acid is considered to be responsible for lowering the LDL-cholesterol levels. Scientific evidence demonstrates that oleic acid present in foods has preventive effects on several chronic diseases (cardiovascular diseases, cancer or age-related cognitive decline) (Trichopoulou, Costacou, Barnia and Trichopoulos, 2003; Martínez-González, Fernández-Jarne, Serrano-Martínez, Martí, Martínez and Martín-Moreno, 2002; Alonso and Martínez-González, 2004; Serra-Majem, Ngo de la Cruz, Ribas and Salleras, 2003/2004; Fitó, de la Torre and Covas, 2007) and therefore, may increase human longevity (Solfrizzi, d'Introno, Colaccio, Capurso, Palasciano, Capurso, Torres, Capurso and Panza, 2005; Huang and Sumpio, 2008).

PUFA, C18:2 and C18:3, although in very small amounts compared to saturated or monounsaturated fatty acids, were found in the samples studied. Much scientific evidence has shown that daily consumption of these two fatty acids have protective effects on cardiovascular health, cancer, diabetes or immune functions (Fraser, Sabaté, Beeson and Strahan, 1992; Jiang, Manson, Stampfer, Willett and Hu, 2002; García-Lorda, Megías Rangil and Salas-Salvado, 2003).

Although evidence in the literature suggests that chocolate consumption may have beneficial effects on health, it must be noted that chocolate has a high total fat and sugar content; in consequence, daily consumption of large amounts of chocolate may increase weight in the long term (Mursu, Voutilainen, Nurmi, Rissanen, Virtanen, Kaikonen, Nyysso¨Nen and Salonen, 2004). That is why scientific evidence suggests that chocolate consumption should be considered in the context of a healthy diet and dark chocolate must be consumed in moderate amounts (20-25 g/daily) (Steinberg, Bearden and Keen, 2003; Mostofsky, Levitan, Wolk and Mittleman, 2010).

4. Conclusions

Cocoa beans and dark chocolate can be considered as products with an important nutritional density, because of their richness in carbohydrates and fats. Differences in fatty acids profile composition between Ecuadorian and Ghanaian samples were mainly explained by the effect of geographical origins, both for unroasted cocoa beans and chocolate, but not by the processing conditions (roasting and conching time). C16:0, C18:0, C18:1 and C18:2 were quantitatively the most important fatty acids in all of the samples studied. Ecuadorian chocolate showed a healthier fatty acid profile and saturated/unsaturated fatty acids ratio, having higher amounts of unsaturated fatty acids and lower amounts of saturated fatty acids. Although because of its fatty acid profile chocolate can be considered an important source of saturated fatty acids, especially stearic acid, it should nonetheless be noted that it has been suggested to have a neutral effect on human health. Due to its chemical composition one should take into account that dark chocolate consumption has to be moderate in the context of a healthy diet.

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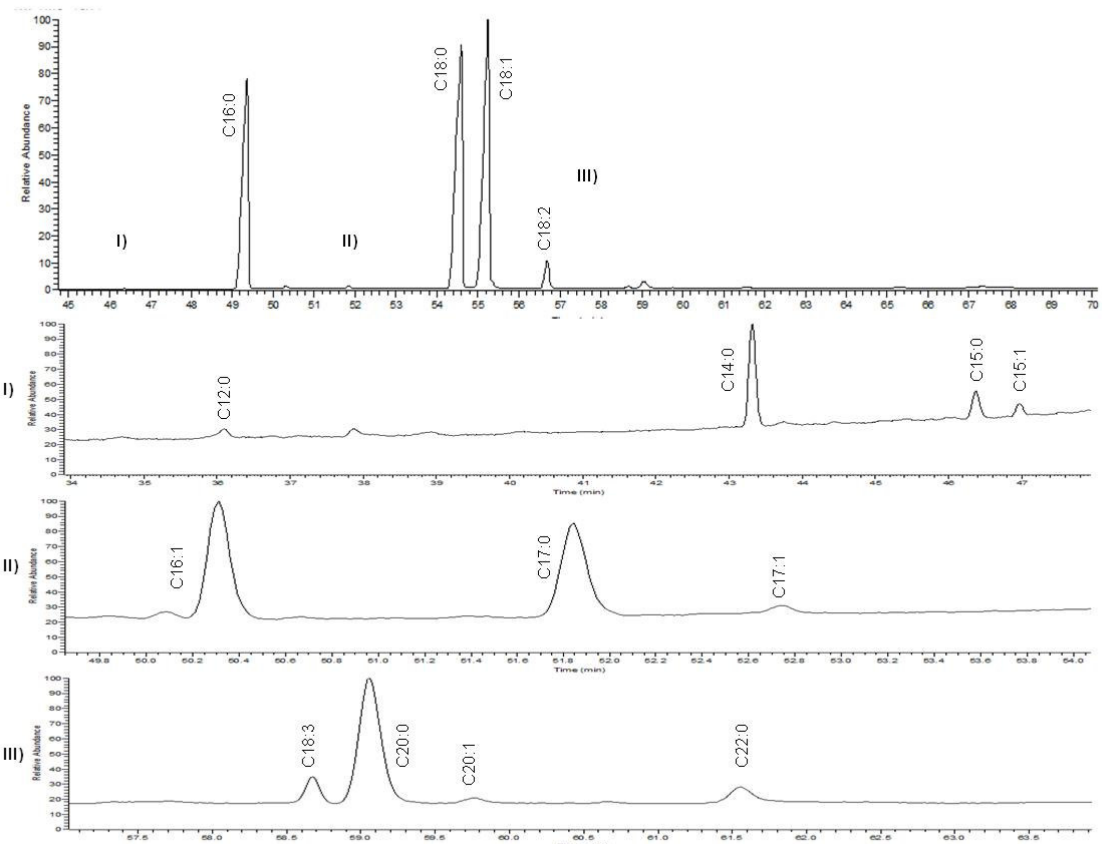
FIGURE CAPTIONS.

Figure 1. Characteristic GC-MS chromatogram of the fatty acids methyl esters from samples: (a) Ecuadorian and (b) Ghanaian chocolate.

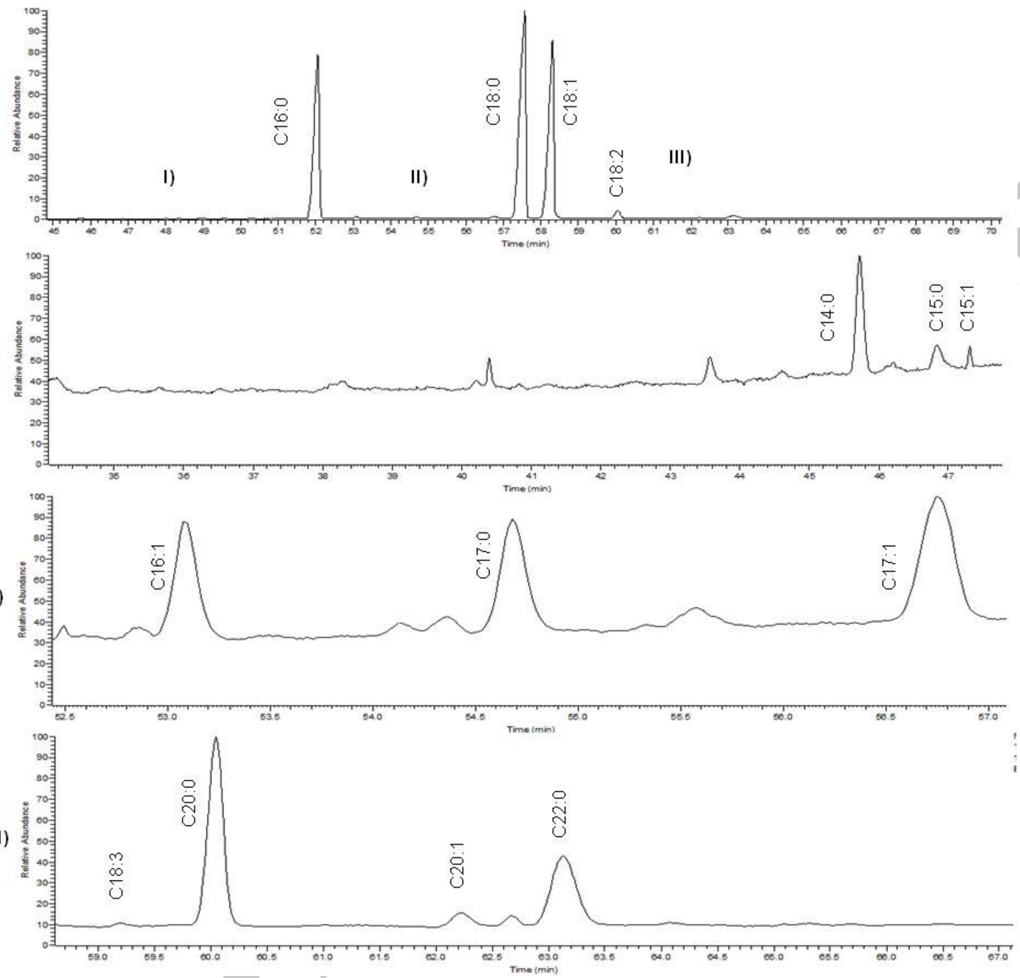
ACCEPTED MANUSCRIPT

Figure 1.

a)


Relative Abundance
C16:0
C18:0
C18:1
C18:2
I)
II)
III)
C12:0
C14:0
C15:0
C15:1
Time (min)
C16:1
C17:0
C17:1
C18:3
C20:0
C20:1
C22:0

b)



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TABLES

Table 1. Chemical composition of unroasted cocoa bean samples.

		Origin	
		ECUADOR	GHANA
Proximates *	Moisture (g)	59.51 ± 0.04 a	51.12 ± 0.03b
	Ash (g)	40.32 ± 0.03	35.65 ± 0.19
	Total protein (g)	127.91 ± 0.03	128.25 ± 0.05
	Carbohydrate by difference (g)	337.85 ± 0.24b	365.81 ± 0.15 a
	Fibre, total dietary (g)	194.74 ± 0.28 a	113.02 ± 0.17b
	Total fat (g)	434.56 ± 0.25 a	419.32 ± 0.13b
Minerals +	Calcium, Ca (mg)	1225.55 ± 18	1136.15 ± 11
	Magnesium, Mg (mg)	3075.24 ± 0.01	3195.74 ± 0.01
	Potassium, K (mg)	12486.21 ± 0.05	11996.62 ± 0.07
	Phosphorus, P (mg)	4231.43 ± 0.01 a	3694.83 ± 0.01b
	Iron, Fe (mg)	146.47 ± 12 a	67.80 ± 8 b
	Manganese, Mn (mg)	21.64 ± 0.50 b	39.32 ± 1.10 a
	Copper, Cu (mg)	26.1 ± 1.40	24.50 ± 0.91
	Zinc, Zn (mg)	44.65 ± 0.21 a	37.10 ± 1.20 b
	Sodium, Na (mg)	261.05 ± 0.34 a	237.02 ± 0.15 b
	Selenium, Se (mcg)	2.80 ± 0.03 a	< 1 b

Values expressed as means ± SD values per kg.

Composition analysis of *two, or + three samples.

Values with different letters within a column are significantly different (p<0.05) according to Tukey's test.

583 **Table 2.** Chemical composition of chocolate samples.

Samples description			Proximates (g Kg^{-1})					
Origin	**Roasting time (min)**	**Conching time (h)**	**Moisture**	**Ash**	**Total protein**	**Carbohydrates**	**Fibre**	**Total fat**
Ecuador	**30.5**	**24**	13.85 ± 0.02	15.55 ± 0.02	64.00 ± 0.01	600.60 ± 0.05 bcd	17.00 ± 0.04	306.00 ± 0.04 abcd
	30.5	**42**	14.35± 0.01	15.65 ± 0.02	64.05 ± 0.04	599.80 ± 0.03 cd	16.90 ± 0.04	306.15 ± 0.02 abcd
	34.5	**24**	14.70 ± 0.01	15.55 ± 0.01	64.20 ± 0.04	598.40 ± 0.03 d	16.75 ± 0.02	307.10 ± 0.01 abc
	34.5	**42**	14.25 ± 0.03	15.70 ± 0.05	64.10 ± 0.08	598.60 ± 0.01 d	16.45 ± 0.01	307.30 ± 0.06 ab
	38.5	**24**	13.95 ± 0.01	15.85 ± 0.01	64.05 ± 0.01	598.00 ± 0.01 d	16.15 ± 0.01	308.15 ± 0.05 a
	38.5	**42**	14.35 ± 0.11	15.05 ± 0.04	64.30 ± 0.03	598.20 ± 0.01d	16.21 ± 0.06	308.10 ± 0.04 a
Ghana	**30.5**	**24**	14.40 ± 0.06	14.50 ± 0.02	64.25 ± 0.05	605.35 ± 0.05 ab	16.82 ± 0.06	301.50 ± 0.02 d
	30.5	**42**	14.05 ± 0.12	15.95 ± 0.02	63.95 ± 0.02	605.55 ± 0.08 a	16.95 ± 0.04	301.50 ± 0.01 d
	34.5	**24**	13.90 ± 0.04	15.55 ± 0.01	63.75 ± 0.01	604.40 ± 0.02 abc	16.94 ± 0.05	302.40 ± 0.07 d
	34.5	**42**	14.35 ± 0.01	15.35 ± 0.05	64.05 ± 0.21	604.00 ± 0.05 abc	17.28 ± 0.02	302.25 ± 0.02 d
	38.5	**24**	14.55 ± 0.01	15.45 ± 0.01	64.40 ± 0.04	602.75 ± 0.01 abc	17.14 ± 0.04	302.90 ± 0.04 cd
	38.5	**42**	14.15 ± 0.01	15.45 ± 0.04	64.05 ± 0.01	603.10 ± 0.04 abc	17.05 ± 0.03	303.25 ± 0.01 bcd

584 Values expressed as means ± SD values per kg of two samples.

585 Values with different letters within a column are significantly different (p<0.05) according to Tukey's test.

Table 3. Mean content of fatty acids in unroasted cocoa beans and chocolate samples expressed as relative percentage of total fatty acid content.

Samples description			% Relative Fatty Acids														
			C12:0	C14:0	C15:0	C15:1	C16:0	C16:1	C17:0	C17:1	C18:0	C18:1	C18:2	C18:3	C20:0	C20:1	C22:0
Unroasted cocoa beans	Ecuador	Mean	0.015*	0.064*	0.027	0.011	27.612*	0.315*	0.268*	0.024*	33.757*	34.732	2.434	0.139	1.089	0.046	0.152
		SD	0.001	0.001	0.004	0.001	0.048	0.002	0.012	0.001	0.692	0.812	0.262	0.001	0.051	0.001	0.002
	Ghana	Mean	N.I.	0.091	0.033	0.011	25.018	0.200	0.321	0.032	36.398	34.309	2.020	0.128	1.240	0.038	0.144
		SD	-	0.001	0.001	0.001	0.161	0.010	0.001	0.001	0.420	0.561	0.052	0.023	0.051	0.001	0.001
Chocolate	Ecuador	Mean	0.017	0.089	0.026	0.014	27.983^{+}	0.246	0.247	0.038	35.249^{+}	32.761^{+}	2.199^{+}	0.102	0.826	0.067	0.101
		SD	0.001	0.002	0.001	0.001	0.792	0.042	0.031	0.001	0.761	1.025	0.056	0.004	0.125	0.008	0.003
	Ghana	Mean	0.018	0.097	0.035	0.014	27.003	0.198	0.250	0.072	37.590	31.979	1.622	0.155	0.883	0.014	0.165
		SD	0.001	0.002	0.001	0.001	1.032	0.002	0.003	0.001	1.641	1.685	0.005	0.002	0.011	0.002	0.001

Composition analysis of two samples.

* Significant difference (α=0.05) between cocoa bean samples. $^{+}$ Significant difference (α=0.05) between chocolate samples.

Table 4. Fatty acid content in unroasted cocoa beans and chocolate samples.

Samples description		Fatty acid content (relative percentage by groups)				
		% SFA	% MUFA	% PUFA	% UFA	Ratio S/U
Unroasted cocoa beans	Ecuador	62.28 ± 0.57	35.12 ± 0.82	2.57 ± 0.25	37.69 ± 0.57	1.65 ± 0.04
	Ghana	63.24 ± 0.63	34.59 ± 0.56	2.15 ± 0.07	36.74 ± 0.63	1.72 ± 0.05
Chocolate	Ecuador	63.95 ± 1.39 *	33.70 ± 0.94 *	2.36 ± 0.59 *	36.06 ± 0.05 *	1.77 ± 0.01 *
	Ghana	66.03 ± 2.09	32.27 ± 1.68	1.78 ± 0.48	34.05 ± 0.13	1.94 ± 0.06

Values expressed as means ± SD values.

Composition analysis of two samples.

SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; UFA: unsaturated fatty acids; ratio S/U: saturated/unsaturated fatty acids ratio.

* Significant difference (α=0.05) between chocolate samples.

HIGHLIGHTS

> Fatty acids profile in cocoa beans and chocolates with different geographical origin and processing conditions. > Differences in FAs profile were mainly due in all samples to the geographical origin effect. > Fifteen FA were identified in cocoa and chocolates samples studied. > For all samples, C16:0, C18:0, C18:1 and C18:2 were quantitatively the most important FAs. > Ecuadorian chocolates were those with healthier FAs profile. >