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Studies of Phenolic Compounds Coupled to Minerals in Cocoa Beans of the "*Mercedes*" and "*Forastero*" Varieties from the Divo and Abengourou Regions (Côte d'Ivoire)

Kouablan Jean Constant Karim[1,a,*], Suomion Justin Saki[1,b], Tehi Bernard Sea[1,c], Kouassi Aboutou Severin Kra[1,d], Guillaume Arthur Kouassi Loh Yoboue[1,e]

[1]*Laboratoire de Biotechnologie, Agriculture et Valorisation des Ressources Biologiques (LBAVRB), UFR Biosciences, Université Félix Houphouët-Boigny (UFHB), Cocody Abidjan, 22 BP 582 Abidjan 22, Côte d'Ivoire.*
[*]*Corresponding author*

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A B S T R A C T

The aim of this study is to contribute to the valorization of the cocoa bean varieties "*Mercedes*" and "*Forastero*" grown in Côte d'Ivoire. A comparative characterisation of phenolic compounds and minerals in the beans of the "*Mercedes*" and "*Forastero*" varieties from two major cocoa production areas of Côte d'Ivoire were determined. The phenolic compounds of cocoa beans were determined by high performance liquid chromatography (HPLC) and the minerals by atomic absorption spectrophotometer. The study showed that a significant difference at the 5% level was observed in the averages of minerals, cinnamic acid, protocatechic acid, quercetin, coumaric acid, rutin, ellagic acid, veratric acid, epicatechin, ferulic acid and naringenin. On the other hand, no significant difference at the 5% level was observed in the averages of arbutin, catechin, vanillic acid and caffeic acid in the bean kernels analysed in the Divo and Abengourou regions. The proportion of cinnamic acid is (6.18±0.63 mg/100g "*Mercedes*" Divo), that of ellagic acid is (18.48±0.44 mg/100g "*Mercedes*" Divo) and that of naringenin is (5.95±0.35 mg/100g "*Mercedes*" Divo). The amount of potassium is (7569.97±1.51 mg/kg "*Forastero*" of Abengourou) and that of iron is (179.9 ± 0.70 mg/Kg "*Forastero*" of Divo). The phenolic compounds and minerals in the cocoa bean samples analysed are highly correlated and rich in antioxidants. They would be beneficial for many biological functions. They can be recommended in pharmacology and cosmetics to fight against oxidative stress and cardiovascular diseases.

[a] *kouablan_jean@live.fr* *https://orcid.org/0000-0002-1457-8634*
[b] *justsakis@yahoo.fr* *https://orcid.org/0000-0002-3507-8575*
[c] *seatehi@yahoo.com* *https://orcid.org/0000-0002-9017-0892*
[d] *kra_severin@yahoo.fr* *https://orcid.org/0000-0002-9269-5465*
[e] *arthurloh59@gmail.com* *https://orcid.org/0000-0002-8621-1719*

Introduction

Cocoa is the mainstay of the economies of the various cocoa-producing countries. It generates large export revenues and is the main source of income for millions of smallholder farmers who grow 90% of the world's cocoa. West Africa is the main producer with more than 68% of world production (International Cocoa Organization: ICCO, 2012). With 40% of world production, Côte d'Ivoire remains the world's leading producer and exporter of cocoa with more than two million one hundred and eighty thousand tons (FAO, 2019). Cocoa's richness in nutrients (lipids, fiber, polyphenols, minerals) and economic elements make it and its by-products a highly prized food. According to Chartron (2005), chocolate is a source of energy that contains nutrients and flavonoids that are important for people's health. Its antioxidants and its richness in vitamin E help to fight against skin ageing. Cocoa is used in various foods (cakes, biscuits, chocolates, children's food, etc.) for its therapeutic and nutritional values, especially its high antioxidant content (Guehi et al., 2007).

Today, we note the resurgence of diseases linked to oxidative and cardiovascular stress such as high blood pressure and type 2 diabete, which are a public health problem. In Côte d'Ivoire, the National Metabolic Disease Control Program (PNLMM) estimated the prevalence of metabolic syndromes at 44.7% in 2015. As for its components, their prevalence was 4.2% for diabetes and 20.4% for hypertension (Ministry of Health and Public Hygiene, 2015; Kouakou et al., 2020).

Numerous studies have demonstrated the beneficial nutritional effects of a diet rich in cocoa and its derivatives, particularly chocolate. Consumed in large quantities, they are associated with a reduced risk of cardiovascular disease. These benefits are attributed to a high antioxidant content. An antioxidant is defined as a substance which, when present in low concentrations relative to oxidisable substrates, significantly retards or prevents the oxidation of that substrate (Gutteridge et al., 1994; Sevindik et al., 2017; Sevindik, 2018; Sevindik, 2019). Oxidisable substrates can be lipids, proteins, carbohydrates or nucleic acids. Phenolic compounds have in common the presence of one or more benzene rings bearing one or more hydroxyl functions. They can be divided into several classes, depending on the complexity of their basic skeleton, the degree of modification of this skeleton and the possible bonds of these compounds with other molecules. They are classified into simple phenols (C_6), hydroxybenzoic (C_6-C_1), hydroxycinnamic (C_6-C_3), naphthoquinones (C_6-C_4), stilbenes (C_6-C_2-C_6), flavonoids (C_6-C_3-C_6), lignans ($(C_6$-$C_3)_2$), lignins ($(C_6$-$C_3)_n$) and tannins ($(C_{15})_n$) (Macheix et al., 2005). The interaction of phenolic compounds with transition metal ions such as iron and copper are a phenomenon of great biological interest. It plays an important role in the antioxidant power of phenolic compounds.

Indeed, iron and copper ions are likely to enter redox cycles, which under aerobic conditions produce reactive oxygen species or ROS (superoxide, hydrogen peroxide, hydroxyl radical, Fe^{4+} species).

Phenolic compounds can inhibit this oxidative stress, not only by trapping ROS by reduction, but also by forming inert complexes with iron and copper ions (Nkhili, 2009; Sevindik, 2021).

This article aims to contribute to the valorization of the cocoa bean varieties "*Mercedes*" and "*Forastero*" grown in Côte d'Ivoire. To achieve this objective, a comparative characterisation of phenolic compounds and minerals in the beans of the "*Mercedes*" and "*Forastero*" varieties from the localities of Divo (Lôh-djiboua region) and Abengourou (Indenie-djuablin region) will be conducted.

To achieve this, we will need methods and materials.

Materials and Methods

Material

The plant material used in this study consisted of cocoa beans of the "*Mercedes*" variety from the National Center for Agronomic Research (CNRA) and the "*Forastero*" variety (Figure 1). The beans were sampled in the locality of Divo (5°50′ North latitude and 5°22′ West longitude) precisely in the villages of Behiri and Ble and in the locality of Abengourou (6°43′47″ North latitude and 3°29′47 West longitude) in the villages Kouacoudramankro and Tchegbekro.

Methods

High performance liquid chromatography (HPLC) was used to quantify phenolic compounds and their profile. The mineral content of cocoa bean kernels was determined by an atomic absorption spectrophotometer.

Chromatographic Profile and Phenolic Content of Cocoa Beans

The phenolic compound content of the foodstuffs was determined according to the method of Donovan et al. (1998) with some modifications. Twenty µL of methanolic extract diluted one third in distilled water and filtered through a millipore filter were analyzed on an HPLC system (Shimadzu Corporation, JAPAN) equipped with a binary pump (LC-6A) coupled to a UV-VIS detector (SPD-6A). The phenolic compounds twenty µL were separated on an ICSep ICE ORH-801 column (length 25 cm) at a temperature set at 30°C. The mobile phase consisted of 50 mM $NaH_4H_2PO_4$ pH 2.6 (eluent A), acetonitrile/ $NaH_4H_2PO_4$ solution (80:20, v/v) (eluent B) and o-phosphoric acid (200 mM) pH 1.5 (eluent C).

The running time was 70 min with a flow rate of one mL/min. All external standards were prepared under the same conditions from pure powders of the reference phenolic compounds (gallic acid, salicylic acid, benzoic acid, tannin-ol, cinnamic acid, ρ-coumaric acid, protocatechic acid, caffeic acid, parahydroxybenzoïcic acid, resveratrol, quercetin and catechin).

The concentrations of the individual phenolic compounds of the different samples were estimated using the average of the peak areas of each of the standards. Thus, the EC concentration of each phenolic compound of each sample expressed in mg/100g of dry matter is given by the following mathematical relationship

$$EC=\frac{\text{Area E} \times \text{TC}}{\text{Area T}}$$

With;

EC: Concentration of each phenolic compound in the sample

Area T: Average area of the standard phenolic compound peaks

Area E: peak area of the phenolic compound in the sample

TC: Concentration of standard phenolic compound

Mineral Content of Cocoa Beans

Mineralization

The AOAC method (1980) is used for the determination of minerals. A mass of 0.1 g of ash obtained after mineralization of the sample is dissolved in six mL of nitric acid (65%, v/v). The resulting mineralization is then transferred to a volumetric flask and the volume is made up to 50 mL with demineralized water.

Determination of the minerals

For the preparation of the mineral calibration solutions, dilutions of standard solutions of each mineral (100 mg/L) (Mn, Mg, Fe, Cu, Zn, K, P, Ca, Na, Cd, Pb) are carried out by making the various initial volumes (0.1; 0.25; 0.5; 1; 1.5 and 2 mL) up to 50 mL with demineralized water so as to obtain respective concentrations of 0.2; 0.5; 1; 2 and 4 mg/L. These calibration solutions are then used to calibrate the flame atomic absorption spectrophotometer.

To a five-mL solution of the previously obtained mineralisate, two mL of 3% lanthanum solution are added in a 50-mL volumetric flask and the whole is made up to the mark with 65% nitric acid. The determination of the individual minerals is carried out using an atomic absorption spectrophotometer (SPECTRAA-5, VARIAN) at specific wavelengths. Quantification of each mineral is performed using an external calibration based on the standard solution (100 mg/mL) of each mineral.

Statistical Analysis

The tests relating to the analysis of the minerals and phenolic compounds of the beans were carried out in triplicate and the numerical values obtained are expressed as the arithmetic mean affected by the corresponding statistical standard deviation. An analysis of variance (ANOVA) of the different characteristics studied was carried out using the R Studio software version 1.1.456. The separation of the means was done using Duncan's test at the 5% threshold. The Pearson correlation allowed us to see how the different parameters evolve between them. Principal component analysis (PCA) was used to compare the cocoa beans of the "*Mercedes*" and "*Forastero*" varieties from the Divo and Abengourou regions on the basis of the variables phenolic compounds and minerals measured. PCA allows the measured variables to be grouped into new variables called components. This grouping is based on the correlation of the variables. This analysis allowed us to determine the links between the parameters evaluated and the similarities and proximities between the varieties.

Results and Discussion

Results

Phenolic Compounds in Cocoa Beans of the "Mercedes" and "Forastero" Varieties

The phenolic compounds present in the beans of the "*Mercedes*" and "*Forastero*" varieties from the Lôh-Djiboua (Divo) and Indenie-Djuablin (Abengourou) regions were identified by High Performance Liquid Chromatography (HPLC).

The chromatographic profiles of the phenolic compounds of the beans from both regions are shown in Figure 2.

The phenolic contents of the "*Mercedes*" and "*Forastero*" beans from both regions are given in Table I.

The comparison of the standard chromatogram with those obtained with the samples clearly shows that there are fourteen (14) peaks. The retention times of these peaks correspond to the retention times of the standard. This shows the existence of fourteen compounds in the samples.

The different phenolic compounds listed according to their retention time are protocatechic acid ($C_7H_6O_4$), caffeic acid ($C_8H_{10}N_4O_2$), vanillic acid ($C_8H_8O_4$), veratric acid ($C_9H_{10}O_4$), coumaric acid ($C_9H_8O_3$), cinnamic acid ($C_9H_8O_2$), ferulic acid ($C_{10}H_{10}O_4$), ellagic acid ($C_{14}H_6O_8$), arbutin ($C_{12}H_{16}O_7$), quercetin ($C_{15}H_{10}O_7$), epicatechin ($C_{15}H_{14}O_6$), catechin ($C_{15}H_{14}O_6$), naringenin ($C_{15}H_{12}O_5$) and rutin ($C_{27}H_{30}O_{16}$).

In terms of hydroxycinnamic acids, cinnamic and ferulic acids are the most abundant phenolic compounds. The level of cinnamic acids varies from (1.08 ± 0.09 mg/100g) for the variety "*Mercedes*" of Abengourou to (6.18 ±0.63 mg/100g) for the variety "*Mercedes*" of Divo. A significant difference in cinnamic acid content at the 5% threshold was observed within the same region for both varieties.

Figure 1. Photograph of dried beans of the "Forastero" (A) and "Mercedes" (B) varieties. (shot: Karim Kouablan, 2017)

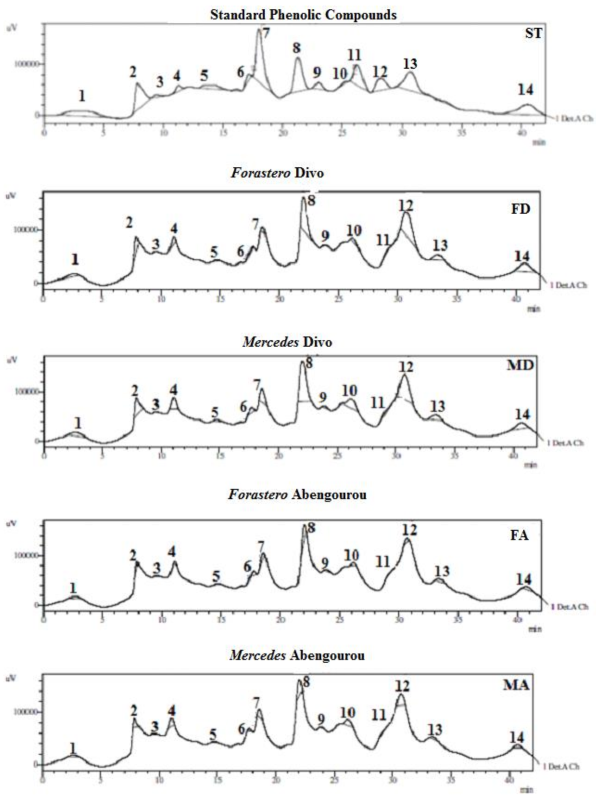

Figure 2. Chromatogram of phenolic compounds of beans of "Forastero" Divo (FD), "Mercedes" Divo (MD) and "Forastero" d'Abengourou (FA) varieties, "Mercedes" d'Abengourou (MA).

ST: standard phenolic compounds. Peaks detected: 1(Protocatechic acid); 2(Caffeic acid); 3(Vanillic acid); 4(Veratric acid); 5(Coumaric acid); 6(Cinnamic acid); 7(Ferulic acid); 8(Arbutin); 9(Ellagic acid); 10(Quercetin); 11(Epicatechin); 12(Catechin); 13(Naringenin); 14(Rutin).

The level of ferulic acids varied from (1.46 ±0.06 mg/100g) for the variety "*Mercedes*" from Abengourou to (3.09 ±0.02 mg/100g) for the variety "*Mercedes*" from Divo. A significant difference at the 5% threshold in ferulic acid content was observed in the Divo region for both varieties. On the other hand, in the Abengourou region, the level of ferulic acids was statistically identical for both varieties.

As regards hydroxybenzoic acids, ellagic and veratric acids are the most important. The level of ellagic acid is higher (18.48 ±0.44 mg/100g) for the variety "*Mercedes*" from Divo and lower (4.18 ±0.36 mg/100g) for the variety "*Mercedes*" from Abengourou. A significant difference at the 5% threshold in ellagic acid content was observed in the Divo region for both varieties. In contrast to the Abengourou region, the two varieties are statistically identical.

The veratric acid content varies from (0.63 ±0.01 mg/100g) for the Abengourou variety "*Forastero*" to (4.09 ±0.05 mg/100g) for the Divo variety "*Mercedes*". A significant difference at the 5% threshold in veratric acid content was observed in the Divo region for both varieties. Contrary to the Abengourou region, the two varieties are statistically identical.

Finally, in terms of flavonoids, the most important phenolic compounds are naringenin and epicatechin. The naringenin level varies from (0.95 ±0.06 mg/100g) for the Abengourou "*Forastero*" variety to (5.95 ±0.35 mg/100g) for the Divo "*Mercedes*" variety. In the same region, the naringenin level is statistically identical whatever the variety. A significant difference in naringenin content at the 5% threshold was observed from one region to another for the same variety.

The epicatechin content was higher (3.84 ±0.08 mg/100g) for the variety "*Mercedes*" from Divo and lower (0.95 ± 0.02 mg/100g) for the variety "*Forastero*" from Divo. A significant difference in epicatechin content at the 5% threshold was observed in the Divo region for both varieties. In contrast to the Abengourou region, the two varieties have statistically the same content. From one region to another the epicatechin content differs significantly for the variety "*Mercedes*" and is statistically identical for the variety "*Forastero*".

Minerals In Cocoa Beans of the "Mercedes" and "Forastero" Varieties

The mineral content of the beans of the "*Mercedes*" and "*Forastero*" varieties from the Lôh-Djiboua (Divo) and Indenie-Djuablin (Abengourou) regions is given in Table 2.

Bean minerals from both regions were grouped into macro and micro elements. The macro-elements are magnesium (Mg), sodium (Na), potassium (K) and calcium (Ca) and the microelements are iron (Fe), copper (Cu), manganese (Mn), zinc (Zn).

The magnesium content of the beans varies from (2410.34±0.98 mg/kg) for the "*Forastero*" variety of Divo to (4093.26 ± 0.41 mg/kg) for the "*Forastero*" variety of Abengourou. In the same region, a significant difference in magnesium content at the 5% threshold was observed for both varieties. The magnesium content of the "*Mercedes*" and "*Forastero*" varieties showed a significant difference at the 5% threshold from one region to another.

The sodium level of the beans was lower (352.57±3.06 mg/kg) for the "*Mercedes*" variety in Divo and higher (4056.73±1.22 mg/kg) for the "*Forastero*" variety in Abengourou. From one variety to another and from one region to another, sodium levels are statistically different at the 5% level.

The potassium level of the beans varies from (6426.60±1.09 mg/kg) for the variety "*Forastero*" of Divo to (7569.97±1.51 mg/kg) for the variety "*Forastero*" of Abengourou. The values are statistically different at the 5% threshold from one variety to another and from one region to another.

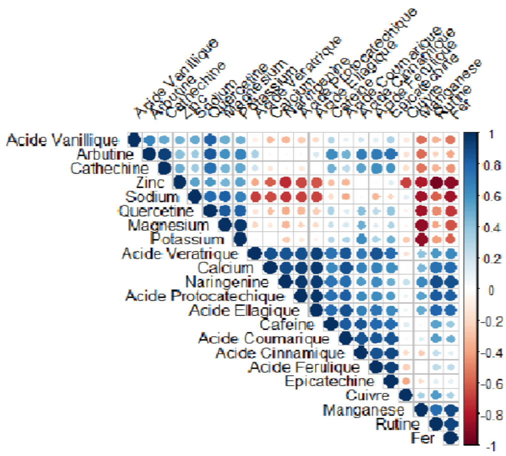

Figure 3. Correlation matrix of phenolic compounds and minerals in cocoa beans

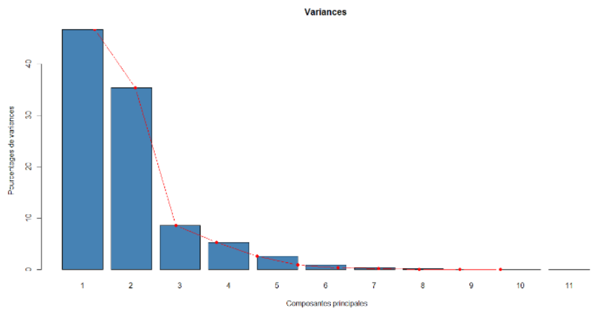

Figure 4. Scree plot

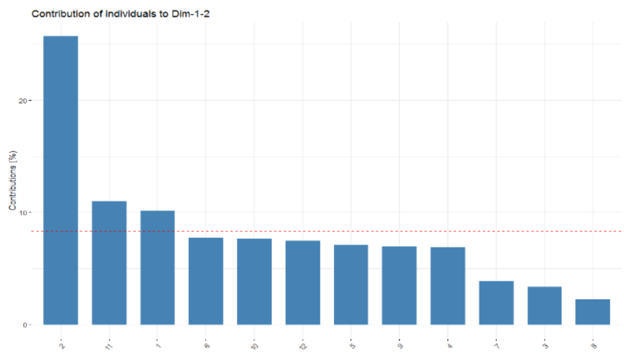

Figure 5. Contribution of bean samples to the formation of axes 1 and 2
Individuals 1, 2, 3 = "*Mercedes*" variety from Divo
Individuals 4, 5, 6 = "*Forastero*" variety from Divo
Individuals 7, 8, 9 = "*Mercedes*" variety from Abengourou
Individuals 10, 11, 12 = "*Forastero*" variety from Abengourou

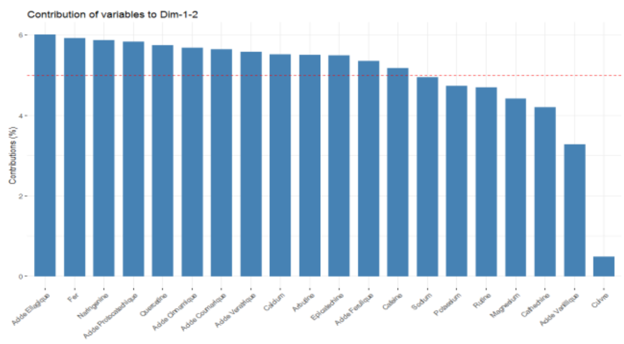

Figure 6. Contribution of phenolic and mineral compounds in cocoa beans to the formation of axes 1 and 2

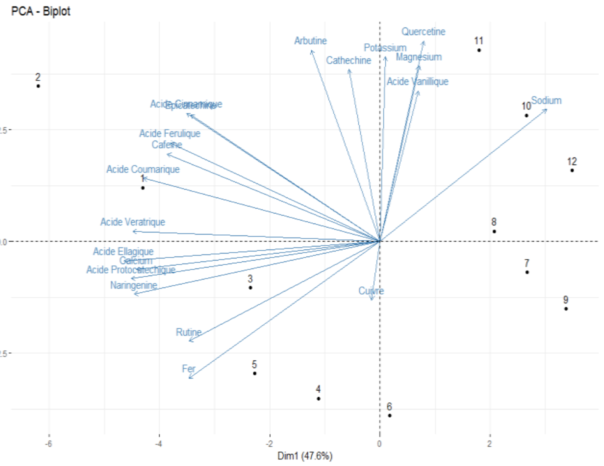

Figure 7. Distribution diagram of cocoa bean varieties in Divo and Abengourou according to phenolic and mineral compounds on axes 1 and 2 of the PCA at 82% inertia
Individuals 1, 2, 3 = "*Mercedes*" variety from Divo
Individuals 4, 5, 6 = "*Forastero*" variety from Divo
Individuals 7, 8, 9 = "*Mercedes*" variety from Abengourou
Individuals 10, 11, 12 = "*Forastero*" variety from Abengourou

The calcium content of the beans of the varieties varies from (396.58±0.70 mg/kg) for the variety "*Mercedes*" of Abengourou to (1045.77±1.61 mg/kg) for the variety "*Mercedes*" of Divo. Calcium levels are statistically different from one variety to another and from one region to another at the 5% level.

The iron content of the beans is lower (140.69±1.30 mg/kg) for the "*Forastero*" variety of Abengourou and higher (179.9±0.70 mg/kg) for the "*Forastero*" variety of Divo. The iron levels are statistically different from one variety to another and from one region to another at the 5% level.

Copper levels in beans ranged from (21.75±0.24 mg/kg) for the variety "*Mercedes*" from Abengourou to (26.34±0.15 mg/kg) for "*Forastero*" from Divo. The copper level is statistically identical for the variety "*Mercedes*" in both regions. On the other hand, a significant difference at the 5% level in copper content was observed for the variety "*Forastero*" in both regions.

The manganese level of the beans was lower (20.23±0.56 mg/kg) for the 'Forastero' variety of Abengourou and higher (52.85±1.36 mg/kg) for the "*Forastero*" variety of Divo. No significant difference at the 5% level was observed for the "*Mercedes*" variety in the two regions. On the other hand, a significant difference at the 5% level was observed for the "*Forastero*" variety in both regions.

Zinc levels in beans ranged from (12.28±0.48 mg/kg) for the "*Forastero*" variety in Divo to (21.57±1.09 mg/kg) for the "*Mercedes*" variety in Abengourou. From one variety to another and from one region to another, zinc levels are statistically different.

Interactions Between Phenolic Compounds and Minerals in Cocoa Beans

Pearson correlation of phenolic compounds and minerals in cocoa beans

The Pearson correlation matrix of all quantitative parameters is shown in figure 3. Only values greater than or equal to 0.50 for positive correlations and values less than or equal to -0.50 for negative correlations are shown.

This matrix indicated negative correlations between micro-minerals (iron, manganese and zinc) and flavonoids (quercetin, naringenin, catechin, rutin), hydroxybenzoic acids (veratric acid, arbutin, protocatechic acid, ellagic acid) and macro-minerals (magnesium, sodium, potassium, calcium). In addition, zinc is negatively correlated with the other micro-minerals (iron, copper, manganese). Negative correlations were observed between sodium and hydroxybenzoic acids (protocatechic acid, veratric acid, ellagic acid) and flavonoids (naringenin, rutin). Finally, a negative correlation between calcium and sodium.

All other parameters show positive correlations with each other. However, there is a group of variables that are strongly positively correlated with each other. These are flavonoids (epicatechin, catechin, naringenin, rutin, quercetin) which are positively correlated with hydroxycinnamic (caffeine, ferulic acid, coumaric acid, cinnamic acid) and hydroxybenzoic (protocatechic acid, veratric acid, ellagic acid, arbutin, vanillic acid). Then macro-minerals (magnesium, sodium, calcium, potassium) and micro-minerals (iron, manganese, and zinc) are strongly correlated to hydroxybenzoic and flavonoids. Finally, hydroxycinnamic acids are strongly correlated with each other. And hydroxybenzoic acids are strongly correlated with hydroxycinnamic acids.

PCA of Phenolic and Mineral Compounds in Cocoa Beans

Percentage of Inertia

A Principal Component Analysis (PCA) was carried out on the basis of the phenolic and mineral compounds measured in the bean samples from the regions studied. All variables were considered as active variables and all individuals were also considered as active. In our sample, the first two axes present a cumulative variance of 82% of the total variance with 47.6% of the variance explained by axis 1 and 34.4% of the variance carried by axis 2 (Figure 4).

Contribution of Individuals and Variables to the Construction of Axes 1 and 2

The "*Mercedes*" variety from the Divo region and the "*Forastero*" variety from the Abengourou region contribute most to the construction of axes 1 and 2 (figure 5). The variables that contribute most to the formation of axis 1 and 2 are the hydroxy-benzoic acids consisting of (ellagic acid, protocatechic acid, veratric acid and arbutin), the hydroxy-cinnamic acids consisting of (cinnamic acid, coumaric acid, ferulic acid and caffeic acid), flavonoids consisting of (quercetin, naringenin and epicatechin) and minerals such as iron and calcium (Figure 6).

Correlation Between the Variables and the Coordinates of Axes 1 and 2

Caffeic acid, ferulic acid, coumaric acid, cinnamic acid, protocatechic acid, veratric acid, ellagic acid, epicatechin, naringenin, rutin, calcium and iron are the variables that are negatively correlated with axis 1. Vanillic acid, arbutin, quercetin, catechin, magnesium and potassium are the variables positively correlated to axis 2 (Table 3).

Table 1. Phenolic composition of beans from Lôh-Djiboua (Divo) and Indenie-Djuablin (Abengourou) regions

Phenolic Compounds (mg/100g)	Retention times (min)	DM	AM	DF	AF
Caffeic acid	7.906	0.88 ±0.02[a]	0.24 ±0.06[b]	0.55 ±0.05[ab]	0.52 ±0.04[ab]
Coumaric acid	14.711	1.51 ±0.05[a]	0.35 ±0.05[c]	0.79 ±0.02[b]	0.65 ±0.08[bc]
Cinnamic acid	16.727	6.18 ±0.63[a]	1.08 ± 0.09[c]	1.75 ±0.20[bc]	3.39 ± 0.09[b]
Ferulic acid	17.734	3.09 ±0.02[a]	1.46 ±0.06[b]	1.48 ±0.02[b]	1.52 ±0.04[b]
Protocatechic acid	2.653	1.74 ±0.05[a]	0.41 ± 0.09[c]	1.32 ± 0.09[b]	0.43 ± 0.04[c]
Vanillic acid	9.573	1.30 ±0.02[a]	1.47 ±0.08[a]	0.70 ±0.25[a]	2.10 ±0.07[a]
Veratric acid	11.067	4.09 ±0.05[a]	1.35 ±0.02[bc]	2.18 ±0.03[b]	0.63 ±0.01[c]
Arbutin	18.575	0.31 ±0.05[a]	0.18 ±0.01[a]	0.07 ± 0.04[a]	0.33 ± 0.07[a]
Ellagic acid	22.028	18.48 ±0.44[a]	4.18 ±0.36[c]	13.08 ±0.11[b]	4.43 ±0.25[c]
Quercetin	23.846	0.49 ± 0.01[b]	0.41 ± 0.03[b]	0.19 ± 0.03[b]	0.85 ± 0.05[a]
Epicatechin	26.163	3.84 ±0.08[a]	1.44 ± 0.01[b]	0.95 ± 0.02[b]	1.68 ± 0.05[b]
Catechin	29.517	1.45 ±0.09[a]	1.35 ±0.08[a]	0.77 ±0.02[a]	1.62 ±0.04[a]
Naringenin	30.710	5.95 ±0.35[a]	1.11 ±0.05[b]	5.34 ±0.50[a]	0.95 ±0.06[b]
Rutin	40.697	1.41 ±0.05[b]	0.36 ± 0.01[c]	2.00 ± 0.02[a]	0.59 ±0.04[c]

For each component, on the rows, the means ± standard deviation assigned to different letters are significantly different from each other at the threshold of P≤0.05 according to the Duncan test. DM: Divo "Mercedes", AM: Abengourou "Mercedes", DF: Divo "Forastero", AF: Abengourou "Forastero"

Table 2. Mineral composition of "Mercedes" and "Forastero" beans from the Lôh-Djiboua (Divo) and Indenie-Djuablin (Abengourou) regions

	Minerals	DM	AM	DF	AF
Macroelements	Mg (mg/kg)	3277.86 ± 2.90[b]	2593.41 ± 3.90[c]	2410.34 ± 0.98[d]	4093.26 ± 0.41[a]
	Na (mg/kg)	352.57 ± 3.06[d]	1189.15 ± 0.27[b]	417.87 ± 1.44[c]	4056.73 ± 1.22[a]
	K (mg/kg)	7291.26 ± 2.76[b]	6718.10 ± 3.16[c]	6426.60 ± 1.09[d]	7569.97 ± 1.51[a]
	Ca (mg/kg)	1045.77 ± 1.61[a]	396.58 ± 0.70[d]	767.01 ± 1.99[b]	439.82 ± 1.85[c]
Microelements	Fe (mg/kg)	169.71 ± 1.03[b]	150.42 ± 1.40[c]	179.9 ± 0.70[a]	140.69 ± 1.30[d]
	Cu (mg/kg)	22.15 ± 0.89[c]	21.75 ± 0.24[c]	26.34 ± 0.15[a]	24.48 ± 1.54[b]
	Mn (mg/kg)	36.65 ± 0.64[b]	35.95 ± 0.54[b]	52.85 ± 1.36[a]	20.23 ± 0.56[c]
	Zn (mg/kg)	13.07 ± 0.59[c]	21.57 ± 1.09[a]	12.28 ± 0.48[d]	19.04 ± 0.43[b]

For each component, on the rows the assigned means ± standard deviations of different letters are significantly different from each other at the threshold of P≤0.05 according to the Duncan test. DM: Divo "Mercedes", AM: Abengourou "Mercedes", DF: Divo "Forastero", AF: Abengourou "Forastero"

Distribution Diagram of "Mercedes" and "Forastero" Varieties According to Phenolic Compounds and Minerals in Cocoa Beans in the Divo and Abengourou Regions

The distribution diagram of varieties in the two regions studied according to phenolic compounds and minerals in cocoa beans showed two groups of varieties according to geographical area: the Divo cocoa bean varieties and the Abengourou cocoa bean varieties. The "*Mercedes*" variety from Divo and the "*Forastero*" variety from Divo are very close in terms of the variables studied. The "*Mercedes*" variety of Abengourou and the "*Forastero*" variety of Abengourou are also very close in terms of the variables studied (figure 7).

The "*Mercedes*" variety of Divo and the "*Forastero*" variety of Abengourou are located on either side of axis 1. Moreover, the Divo "*Mercedes*" variety is richer in caffeine, ferulic acid, coumaric acid, cinnamic acid, protocatechic acid, veratric acid, ellagic acid, epicatechin, naringenin, rutin, calcium and iron than the Abengourou "*Forastero*" variety The "*Forastero*" varieties of the two regions are opposite with respect to axis 2. The Abengourou "*Forastero*" variety is richer in arbutin, quercetin, catechin, magnesium and potassium than the Divo variety.

Discussion

Phenolic compounds are secondary metabolites that represent an important source of molecules used by humans in pharmacology or food processing (Mohammed et al., 2019). The phenolic compounds found in the different bean samples were classified into hydroxycinnamic acids, hydroxybenzoic acids and flavonoids.

Concerning hydroxycinnamic acids, cinnamic and ferulic acids are the most important phenolic compounds. The level of cinnamic acids varies from (1.08 ± 0.09 mg/100g) to (6.18 ±0.63 mg/100g). The level of cinnamic acids in our beans is higher than that found by Ehala et al. (2005) which varies from 0.10 to 4.12 mg/100g in berries.

For ferulic acids, the level varies from (1.46 ±0.06 mg/100g) to (3.09 ± 0.02 mg/100g). The work of Counet et al. (2006) on dark chocolate found a high level of ferulic acid of 24 mg/100g.

The variable composition of hydroxycinnamic acids would be due to the composition of the soil or the use of pesticides or the substratum (Hoffmann, 2003).

Cinnamic acid is the precursor of a large number of phenolic compounds and has plant growth regulating properties such as elongation activities, inhibition of root growth in cereals, role in ripening of bananas or seed germination (Wong et al., 2005).

It is used in perfumery and pharmacology for its antiseptic and antifungal properties, as a flavour enhancer, and enters the biosynthetic pathway of shikimic acid and phenylpropanoids. It should also be noted that derivatives of cinnamic acid have better antioxidant activity (Cuvelier, 1992; Rice-Evans, 1996; Natella, 1999).

As for ferulic acid, it plays a preponderant role in the fight against oxidative stress and the reduction of blood cholesterol. It acts effectively against free radicals, lipid peroxidation and increases the level of trace elements during nicotine-induced toxicity (Sudheer et al., 2005). Ferulic acid is a super antioxidant. It has many physiological functions, including anti-inflammatory, antimicrobial, anticancer (lung, breast, colon and skin cancer), anti-arrhythmic, anti-thrombotic and anti-diabetic activity. Ferulic acid has a protective role for the main structures of the skin: keratinocytes, fibroblasts, collagen, elastin (Zduńska et al., 2018).

As far as hydroxybenzoic acids are concerned, ellagic and veratric acids are the most important. The ellagic acid content varies from (4.18 ±0.36 mg/100g) to (18.48 ±0.44 mg/100g). The veratric acid content varies from (0.63 ±0.01 mg/100g) to (4.09 ±0.05 mg/100g). This could be explained by the type of soil and its mineralogical composition. The work of Wang et al. (2002) and Wada et al. (2002) showed high levels of ellagic acid ranging from 1.24 to 43.67 mg/100g in berries. High levels of veratric acids up to 20 mg/Kg/day are safe for the body.

Ellagic acid is an antioxidant polyphenol with pharmacological properties; it combines with glucose to form tannins known as ellagitanins. It is hydrolysable tannin with the ability to scavenge free radicals and superoxide ion (Rios et al., 2018).

Veratric acid (3,4-dimethoxybenzoic acid), a simple benzoic acid derived from plants and fruits, has antioxidant, anti-inflammatory and hypotensive effects (Saravanakumar et al., 2011).

Veratric acid reduces the regulated expression of protein-expressing cyclooxygenase-2 (COX-2), prostanglandin-2 (PGE2) and interleukin (IL-6) levels after UVB (ultraviolet) irradiation. Veratric acid can reduce UV-induced skin damage (DNA damage, redox imbalance and inflammation) and is safe to use. Veratric acid may be an effective therapeutic agent offering protection against UVB-induced skin disorders (Seoung-Woo et al., 2013).

For flavonoids, the most important phenolic compounds are naringenin and epicatechin. The naringenin content varies from (0.95 ±0.06 mg/100g) to (5.95 ±0.35 mg/100g). The epicatechin content varied from (0.95 ± 0.02 mg/100g) to (3.84 ±0.08 mg/100g). These results are in agreement with those of Portillo (2006) who found epicatechin values between 0.96 and 3.27%. According to Hooper (2008), the acceptable daily intake of naringenin and epicatechin are 14.4 mg/day and 156.9 mg/day. The consumption of cocoa powders could meet the body's needs for naringenin and epicatechin.

Naringenin is a flavanone which is a subgroup of flavonoids. It is responsible for drug interactions through cytochrome inhibition. Several biological activities have been attributed to naringenin, including antioxidant, anti-tumour, antiviral, antibacterial, anti-inflammatory, anti-adipogenic and cardio-protective effects. The work of Salehi et al. (2019) showed the ability of naringenin to improve endothelial function. Naringenin is one of the flavonoids produced from phenylalanine and has many beneficial effects on the human body, including aiding metabolism, as well as preventing cardiovascular disease (Yao et al., 2004).

Epicatechin has various biological properties, such as anti-oxidant, anti-inflammatory antimicrobial, anti-tumour, anti-diabetic and cardio-protective activity (Prakash et al., 2019). Indeed, in vitro studies have suggested that the anti-oxidant activity of epicatechin is mainly due to its ability to scavenge free radicals through multiple attached phenolic groups. Similarly, epicatechins have also shown significant antimicrobial activity against various multi-drug resistant pathogens, which is a serious need in today's healthcare system. Preclinical studies have shown an excellent chemopreventive and anti-tumour effect of epicatechins present in green tea. In addition, epicatechin has also shown cardio-protective, anti-diabetic and neuro-protective activity (Bernatova, 2018).

Epicatechin is a flavanol monomer found in foods such as tea, apples, berries and cocoa. Work by Haskell-Ramsay et al. (2018) showed an association between consumption of these foods and cognitive function, as well as improved blood flow.

The cocoa bean kernels studied can be used in the treatment of cardiovascular disease and in the fight against oxidative stress. According to Scalbert and Williamson (2000), they are capable of fighting all types of cancer. Thus, numerous anti-carcinogenic, anti-inflammatory, anti-atherogenic, anti-thrombotic, analgesic, antibacterial, antiviral, anti-cancer (Babar et al., 2007), anti-allergenic, vasodilator (Falleh et al., 2008) and anti-oxidant (Gomez-aravaca et al., 2006; Azizah et al., 2007) activities are associated with them.

The cocoa bean kernels of the "*Mercedes*" and "*Forastero*" varieties are characterised by their richness in macro-elements, particularly potassium and magnesium, without neglecting sodium and calcium, although they have relatively lower contents (Karim et al., 2020).

The magnesium content of the beans varied from 2410.34±0.98 mg/kg to 4093.26 ± 0.41 mg/kg. The sodium level varied from 352.57±3.06 mg/kg to 4056.73±1.22 mg/kg. The potassium level of the beans varied from 6426.60±1.09 mg/kg to 7569.97±1.51 mg/kg. The calcium content of the beans varied from 396.58±0.70 mg/kg to 1045.77±1.61 mg/kg.

Table 3. Correlation between the variables and the coordinates of axes 1 and 2

Phenolic compounds / Minerals	Axis 1	Axis 2
Caffeic acid	-0.82	0.41
Ferulic acid	-0.81	0.47
Coumaric acid	-0.91	0.30
Cinnamic acid	-0.75	0.61
Protocatechic acid	-0.96	-0.17
Vanillic acid	0.15	0.72
Veratric acid	-0.95	0.04
Arbutin	-0.26	0.91
Ellagic acid	-0.98	-0.09
Quercetin	0.17	0.95
Epicatechin	-0.73	0.60
Catechin	-0.12	0.82
Naringenin	-0.95	-0.25
Rutin	-0.74	-0.48
Magnesium	-0.15	0.83
Sodium	0.64	0.62
Potassium	0.02	0.88
Calcium	-0.94	-0.13
Iron	-0.74	-0.65
Copper	-0.03	-0.28

These macroelements play very important roles in the body. Indeed, potassium is essential in the regulation of cell osmotic balance and the maintenance of membrane potentials, so it plays a very important role in cardiovascular health in toning up biochemical and vascular pathways (Murray et al., 2000).

Cocoa products are richer in magnesium than black tea, red wine and apples Steinberg et al. (2003). Magnesium is an important cofactor in many reactions of cellular metabolism, thus it catalyses many biological reactions, such as protein synthesis, transmission of nerve impulses, muscle movements, energy production and in the fixation of bones and teeth (Soetan et al., 2010 and Colombo et al., 2011).

Calcium is involved in muscle contraction, transmission of nerve impulses and in the formation of bones and teeth (Soetan et al., 2010).

Sodium and potassium form a pump that is involved in the restoration of membrane permeability. Cocoa bean kernels can therefore be recommended to ensure a good balance and proper functioning of the body (Askari, 2019).

Cocoa bean kernels of the "*Mercedes*" and "*Forastero*" varieties are characterised by their richness in mineral microelements, particularly iron and manganese (Karim et al., 2020).

The iron content of the beans varies from 140.69±1.30 mg/kg to 179.9±0.70 mg/kg. The copper content of the beans varied from 21.75±0.24 mg/kg to 26.34±0.15 mg/kg. The manganese content of the beans varied from 20.23±0.56 mg/kg to 52.85±1.36 mg/kg. The zinc content of the beans ranged from 12.28±0.48 mg/kg to 21.57±1.09 mg/kg.

Relatively high values of microelements have been reported by the USDA (2011) for dark chocolate containing 70-85% cocoa mass containing the proportions of 11.90 mg/100 g of iron (Fe), 1.948 mg/100 g of manganese (Mn), 1.766 mg/100 g of copper (Cu) and 3.31 mg/100 g of zinc (Zn).

These microelements are essential because they contribute to the proper functioning of the body through their involvement in physiological and metabolic functions.

In fact, the iron contained in the cocoa bean kernel samples studied can be recommended to pregnant and breastfeeding women because the quantity obtained (16 mg/100 g) is greater than that contained in beef or beef liver (3 mg/100 g).

However, the non-haem iron in cocoa limits intestinal absorption by 5 to 10%, compared with the higher intestinal absorption of haem iron contained in meat (20-30%). The low absorption of non-haem iron from cocoa can be noted in the presence of ascorbic acid (Colombo et al., 2011).

Manganese is an essential trace element involved in the constitution of many essential enzymes in the metabolism of amino acids, lipids, carbohydrates; participates in the production of insulin and in bone formation (Doctissimo, 2017).

Copper is involved in multiple enzymatic reactions including collagen and neurotransmitter synthesis (Arredondo et al., 2005)

A copper-deficient diet can lead to cardiovascular disease later in life. Divo "*Forastero*" cocoa is a good source of copper (Colombo et al., 2011).

Zinc is a cofactor in more than 300 biochemical reactions in our body. It is also involved in the growth of children, hair loss, liver detoxification and immunity. Zinc also plays an important role in fertility. In men it is important for sperm quality and in women it plays an important role in the implantation of the egg in the uterus (Anonymous 1, 2012).

Furthermore, according to the European Food Safety Authority EFSA (2013), the daily dietary allowances for trace elements are: Iron (adult: 9-15 mg), manganese (adult: 3 mg), copper (adult: 1.7 mg), zinc (adult: 8-13.5 mg). These nutrients can be provided by the "*Mercedes*" and "*Forastero*" varieties of cocoa beans.

These almonds can therefore be recommended to ensure a good balance and proper functioning of the body.

Conclusion

This study revealed that the cocoa bean samples analysed are rich in phenolic compounds, macro minerals (calcium, potassium, sodium, magnesium) and trace minerals (iron, copper, zinc and manganese). The profile of phenolic compounds showed the presence of flavonoids, hydroxy-benzoics and hydroxy-cinnamics. Phenolic compounds and minerals interact well to give cocoa bean kernels a strong antioxidant capacity to fight against oxidative stress and cardiovascular diseases. Cocoa bean kernels can be recommended in pharmacology and cosmetics.

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