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Influence of cabruca and full sun cultivation systems on the chemical quality of cocoa kernel (*Theobroma cacao* L.) from Southern Bahia: an integrated analysis of the metabolic profile and element transfer

Hélia de Barros Kobi
Doctor Scientiae

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Thesis submitted to the Food Science and Technology Graduate Program of the Universidade Federal de Viçosa in partial fulfillment of the requirements for the degree of *Doctor Scientiae*.

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Dedico este trabalho a mim, como reconhecimento da jornada percorrida; aos meus familiares e amigos, pelo apoio incondicional. Estendo esta homenagem a todos e todas cacauicultores da Bahia, pesquisadores, extensionistas e, por fim, a todos os amantes do cacau e chocolate, inspiração constante desta pesquisa.

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Não basta saber ler que "Eva viu a uva". É preciso compreender qual a posição que Eva ocupa no seu contexto social, quem trabalha para produzir a uva e quem lucra com esse trabalho.

A leitura do mundo precede a leitura da palavra.

Paulo Freire

ABSTRACT

KOBI, Hélia de Barros, D.Sc., Universidade Federal de Viçosa, December, 2024. **Influence of cabruca and full sun cultivation systems on the chemical quality of cocoa kernel (*Theobroma cacao* L.) from Southern Bahia: an integrated analysis of the metabolic profile and element transfer**. Adviser: Paulo Cesar Stringheta. Co-advisers: Raphael Braganca Alves Fernandes and Marcia Cristina Teixeira Ribeiro Vidigal.

The search for chemically high-quality cacao is driven by the global demand for chocolate but is threatened by the accumulation of heavy metals. The objective of this thesis was to investigate the metabolic profile (fatty acids, phenolic compounds, and methylxanthines) and the transfer of chemical elements in beans, leaves, and soils of the cultivars CCN 51 and PS13.19, produced in cabruca and full sun systems in southern Bahia. In the first chapter, it was observed that the cultivation system influenced the fatty acid composition, with higher values of linoleic acid (3.21±0.05%) associated with the full sun system, although higher levels of total lipids (54.92±0.45%) were obtained in the cabruca system. The cultivation system also influenced PUFA composition, with higher values in the full sun system (3.21±0.05%). On the other hand, CCN 51 beans showed higher SFA content (61.74±0.50%) and a higher S/U ratio (1.62±0.03%). The PS13.19 cultivar exhibited higher levels of MUFA (36.71±0.34%) and UFA (39.62±0.82%). Lower levels of total phenolic compounds (193.33±50.18 mg GAE·100g^{1}), total anthocyanins (52.35±21.67 mg AntT·100g^{1}), and antioxidant properties (ABTS: 200.74±110.68 mol TE/g; DPPH: 249.39±82.12 mol TE/g) were found in the full sun system, especially in the PS13.19 cultivar. Higher levels of epicatechin (155,626.67±15,802.19 EIC) and catechin (23,625.57±15,802.19 EIC) were found in CCN 51 beans. In summary, genetic and management factors significantly influenced cacao chemical composition, indicating that the transition from cabruca to full sun may reduce total lipid and phenolic compound content. In the second chapter, the physicochemical characterization of the soil revealed the prevalence of acidic soils (pH 4.5 to 6.1), with deficiencies in fertility components (P, K, Ca^{2+}, and Mg^{2+}). Heavy metal levels in the soils were predominantly low; however, some areas showed Zn and Cr levels above the reference values established by COPAM (2011). Regarding the beans, no sample presented quantifiable levels of As, Pb, or Cd, and Cu levels (15.2 mg·kg^{1}) were below the maximum tolerated limit (40 mg·kg^{1}) established by Anvisa (2022). Additionally, the beans exhibited high levels of K (9.75±0.59 g·kg^{1}) and P (5.23±0.37 g·kg^{1}), and notable amounts of Fe (25.88±10.42 mg·kg^{1}), Zn (50.92±7.23 mg·kg^{1}), Cu (15.20±5.32

mg·kg^{1}), and Mn (17.25±12.12 mg·kg^{1}). A positive and consistent correlation was observed between Mn content in soil, beans, and leaves. The integrated PCA showed that the cabruca system is characterized by factors related to soil fertility, such as Ca^{2+}, Mg^{2+}, and SB. Therefore, the results provide evidence that cacao beans from southern Bahia do not present contamination by Cd, Pb, or As and possess high mineral richness; however, the insufficiency of essential nutrients and the potential environmental risk of soil Cr represent challenges for the sustainability of the crop.

Keywords: cacao trees; CCN 51; PS 13.19; bioactive compounds; cocoa butter; antioxidant properties; primary metabolites; secondary metabolites; flavonoids; alkaloids; nutrients; minerals; heavy metals

RESUMO

KOBI, Hélia de Barros, D.Sc., Universidade Federal de Viçosa, dezembro de 2024. **Influência dos sistemas de cultivo cabruca e pleno sol na qualidade química de amêndoas de cacau (*Theobroma cacao* L.) do Sul da Bahia: uma análise integrada do perfil metabólico e da transferência de elementos.**. Orientador: Paulo Cesar Stringheta. Coorientadores: Raphael Braganca Alves Fernandes e Marcia Cristina Teixeira Ribeiro Vidigal.

A busca por cacau de qualidade química é impulsionada pela demanda global de chocolate, mas é ameaçada pelo acúmulo de metais pesados. O objetivo desta tese foi investigar o perfil metabólico (ácidos graxos, compostos fenólicos e metilxantinas) e a transferência de elementos químicos em amêndoas, folhas e solos, de cultivares CCN 51 e PS13.19, produzidas em sistemas cabruca e a pleno sol no Sul da Bahia. No capítulo um observou-se que o sistema de cultivo influenciou a composição de ácidos graxos, com maiores valores de ác. linoleico (3,21±0,05%) associados ao sistema pleno sol, embora maiores teores de lipídios totais (54,92±0,45%) tenham sido obtidos no sistema cabruca. O sistema de cultivo influenciou também a composição de PUFA, com valores mais elevados no sistema a pleno sol (3,21±0,05%). Por outro lado, amêndoas CCN 51 apresentaram maior teor de SFA (61,74±0,50%) e maior relação S/U (1,62±0,03%). A cultivar PS13.19 exibiu níveis mais elevados de MUFA (36,71±0,34%) e UFA (39,62±0,82%). Menores níveis de compostos fenólicos totais (193,33±50,18 mg GAE.100g^{1}), antocianinas totais (52,35±21,67 mg AntT.100g^{1}) e propriedades antioxidantes (ABTS: 200,74±110,68 mol TE/g e DPPH: 249,39±82,12 mol TE/g) foram encontrados no sistema pleno sol, especialmente na cultivar PS 13.19. Maiores níveis de epicatequina (155626,67±15802,19 EIC) e catequina (23625,57±15802,19 EIC) foram encontrados em amêndoas CCN 51. Em suma, os fatores genéticos e de manejo influenciaram significativamente a composição química do cacau, indicando que a transição de cabruca para o pleno sol pode reduzir o teor de lipídios totais e compostos fenólicos. No segundo capítulo a caracterização físico-química do solo revelou a prevalência de solos ácidos (pH de 4,5 a 6,1), com deficiências em componentes de fertilidade (P, K, Ca^{2+} e Mg^{2+}). Os níveis de metais pesados nos solos foram predominantemente baixos; no entanto, algumas áreas apresentaram níveis de Zn e Cr acima dos valores de referência da COPAM (2011). Em relação às amêndoas, nenhuma amostra apresentou níveis quantificáveis de As, Pb e Cd, e os níveis de Cu (15,2 mg.kg^{1}) estavam abaixo do limite máximo tolerado (40 mg.kg^{1}) pela Anvisa (2022). Adicionalmente, as amêndoas apresentaram altos níveis de K

(9,75±0,59 g.kg^{1}) e P (5,23±0,37 g.kg^{1}) e teores notáveis de Fe (25,88±10,42 mg.kg^{1}), Zn (50,92±7,23 mg.kg^{1}), Cu (15,20±5,32 mg.kg^{1}) e Mn (17,25±12,12 mg.kg^{1}). Observou-se correlação positiva e consistente entre o teor de Mn no solo, nas amêndoas e nas folhas. A PCA integrada demonstrou que o sistema cabruca é caracterizado por fatores relacionados à fertilidade do solo, como Ca^{2+}, Mg^{2+} e SB. Portanto, os resultados fornecem evidências de que as amêndoas de cacau da região sul da Bahia não apresentam contaminação por Cd, Pb e As e possuem alta riqueza mineral; no entanto, a insuficiência de nutrientes essenciais e o potencial risco ambiental do Cr no solo representam desafios para a sustentabilidade da cultura.

Palavras-chave: cacaueiros; CCN 51; PS 13.19; compostos bioativos; manteiga de cacau; propriedades antioxidantes; metabólitos primários; metabólitos secundários; flavonoides; alcaloides; nutrientes; minerais; metais pesados

SUMMARY

General introduction

The cacao tree (*Theobroma cacao* L.) is a perennial plant native to the tropical forests of the Upper Amazon River region in South America, and its production has spread to several countries located in the equatorial zone around the world (Figueroa-Hernández et al., 2019; Bertoldi et al., 2016).

In Brazil, the state of Bahia stands out as one of the largest producers, with cacao farming in the region being a historical activity, especially in the south of the state, where cultivation developed in the cabruca system, characterized by the cultivation of cacao intercropped with native vegetation (Oliveira & Assis, 2023). However, with the growing global demand for cacao beans and the search for greater productivity, many cacao farmers have opted for monoculture cacao cultivation systems in full sun, which, although they increase productivity, require intensive use of inputs (Niether et al., 2020).

The chemical composition of cocoa beans has attracted increasing interest due to its potential health benefits, as they are rich in bioactive compounds, such as polyphenols, which have antioxidant and anti-inflammatory properties (Sorrenti et al., 2020). In addition, cocoa is an important source of lipids, especially cocoa butter, which directly influences the quality and texture of derived products, such as chocolate (Figueira & Luccas, 2022). The abundance of these substances, however, can be affected by environmental conditions and cultivation systems (Fajardo et al., 2022), which, to date, have been little explored in the scientific literature, especially with regard to the comparison between cabruca and full sun systems.

Furthermore, the contamination of cocoa beans by heavy metals, such as arsenic, cadmium, and lead, has become a growing concern, since these elements can accumulate in the soil and plants, compromising the quality of final cocoa-based products and posing risks to human health (Maddela et al., 2020).

In the context of cocoa farming, agricultural practices and environmental conditions can influence this accumulation of metals in the beans, and despite international regulations that limit the presence of these contaminants, there is still a scarcity of information on heavy metal contamination in the southern region of Bahia (Mohamed et al., 2020).

Given this, the objective of this study was to investigate in an integrated way the metabolic profile (fatty acids, phenolic compounds and methylxanthines) and the transfer of elements (elemental composition and potential for heavy metal contamination) in cocoa cocoa kernels, leaves and soils (cultivars CCN 51 and PS 13.19), under cabruca and full sun cultivation systems. In order to understand the impacts of different management systems and cultivar types on the quality and chemical composition of cocoa kernels produced in Southern Bahia.

This thesis was organized into two research chapters that are presented in the format of a scientific article. It is worth highlighting that the first scientific article presented in this document was accepted and published in the journal Food Research International.

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Chapter 1. Metabolic profile of fatty acids, phenolic compounds, and methylxanthines of cocoa kernels (*Theobroma cacao* L.) from different cultivars produced in cabruca and full sun farming systems

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Abstract

The demand for high-quality cocoa beans has increased in line with the growing global demand for chocolate. The chemical composition of cocoa beans can vary according to their origin and growing conditions. In this context, this study evaluated the influence of the cultivar type (CCN51 and PS1319) and the cocoa management system (cabruca and full sun) on the chemical composition of unfermented cocoa kernels. The cultivation system influenced the fatty acid composition of cocoa kernels, with higher values of linoleic acid associated with the full sun system, although higher total lipid contents were obtained in the cabruca system. The cultivar influenced the content of saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), total unsaturated fatty acids (UFA) and the saturated/unsaturated ratio (S/U). Lower levels of total phenolic compounds, total anthocyanins and antioxidant properties were found in the full sun system, especially in the PS1319 cultivar. Higher levels of epicatechin and catechin were found in the cocoa kernel of the CCN51 cultivar. Theobromine and caffeine were not influenced by the treatments. Neither the PCA of total lipids and fatty acids, nor the PCA of antioxidante properties, phenolic compounds and methylxanthines indicated an isolated clustering between cultivar and cultivation system. The results showed that the factors under study influenced the chemical composition of the unfermented cocoa kernel. Furthermore, they indicated that the migration from traditional systems, such as cabruca, to full sun systems can reduce the total lipids and phenolic compounds content of the cocoa beans. When planning new plantations, the choice of genetic material should also be carefully considered to produce higher-quality cocoa butter and beans with a higher phenolic compound content.

Keywords: Bioactive compounds; Cocoa butter; Antioxidant properties; Primary metabolites; Secondary metabolites; Flavonoids; Alkaloids.

1. Introduction

The cacao tree (Theobroma cacao L.) is a plant native to the tropical forests of the Upper Amazon River basin in South America (Motamayor et al., 2008). Its cultivation has spread throughout Central and Eastern America (Afoakwa, 2016) and is currently produced in various countries within the equatorial zone of the planet (Figueroa-Hernández et al., 2019). Brazil is the sixth largest cocoa producer in the world (ICCO, 2022), and the state of Bahia is one of the country's largest producers (IBGE, 2022). In southern Bahia, in the mid-19th century, cocoa cultivation was initially established under the shade of trees in the original vegetation of the Brazilian Atlantic Forest, locally known as the cabruca system (Sambuichi et al., 2012). In this system, forest strata are suppressed for the cultivation of the economically important crop, and cocoa trees are planted in the understory surrounded by natural vegetation, with reduced density of the upper tree canopy (de Almeida et al., 2007).

The cocoa tree thrives in shade, both during establishment and productive phases, and proper shading can result in relatively high photosynthetic rates, growth, and seed production (de Almeida et al., 2007). However, recently, due to increased global demand and the pursuit of higher productivity, many cocoa producers have implemented cocoa plantations in full sun monoculture systems. These systems can cause adverse socioecological impacts and require intensive use of agricultural inputs and irrigation (Niether et al., 2020).

Traditional cocoa cultivation systems, such as cabruca, are associated with shading, low input consumption, and non-irrigation, resulting in reduced wind speed, evapotranspiration, and reduced extreme variations in soil temperature (Moser et al., 2010). However, these systems have been questioned due to potential increased competition for water, higher incidence of pests and diseases, and reduced cocoa yield compared to full sun cultivation (da Silva Branco et al., 2017). In this sense, the development of different cocoa clones has so far focused on productivity and tolerance to pests and diseases, with limited focus on the influence of cultivation system on the chemical composition of the beans.

Cacao is a food raw material rich in bioactive compounds associated with various health benefits (Martin & Ramos, 2021). Phenolic compounds, for example, have been widely studied for their effects against oxidative stress, as well as possessing anti-inflammatory properties, and cytotoxic effects (Cinar et al., 2021). Cocoa beans contain about 12 to 18 % of their dry weight in phenolic compounds (Kim & Keeney, 1984), with the main classes being tannins and flavonoids. Flavonoids include flavanols, flavonols, anthocyanins, flavones, and flavanones. (+)-Catechin and (-)-epicatechin are the most abundant flavanols, representing approximately 35 % of the total phenolic content (Efraim, Alves, & Jardim, 2011). Additionally, phenolic acids, flavonol glycosides, and proanthocyanidins (PAs) (B1-B5, C1, and larger oligomers and polymers) are also found (Febrianto & Zhu, 2019). Cocoa beans are also rich in alkaloids from the methylxanthine family, approximately 1 % to 2 % (w/w) theobromine and 0.1 % to 0.8 % caffeine (Becerra et al., 2024), which are associated with reducing total serum cholesterol, improving levels of lipoproteins and insulin sensitivity, cognitive protection Against Alzheimer’s and Parkinson’s effects (Fanning et al., 2023), reducing cellular oxidative stress, and regulating gene expression (Debnath et al., 2018).

The most abundant component in cocoa beans is lipids, representing approximately 50 % of its chemical constituents (Sirbu et al., 2018). This lipid fraction is commonly known as cocoa butter and is composed of fatty acids in the form of triacylglycerols (TAG), mainly palmitic and stearic acids (Afoakwa, 2016). Cocoa butter is an expensive and important ingredient in the composition of chocolate. It can influence several technological parameters during the processing and storage of chocolate, such as viscosity, in addition to influencing the texture, melting behavior and flavor of the final product (Melo et al., 2020). The composition of cocoa butter in cocoa beans varies according to the genetic material of the plant and its geographical origin (Sirbu et al., 2018).

Both lipids and phenolic compounds are molecules produced by the primary and secondary metabolism of cocoa and are stored in cocoa beans (Wang et al., 2016). However, environmental conditions can cause changes in the functional characteristics associated with the physiological and ecosystem processes of the plant (Saavedra et al., 2020).

In this context, it is important to understand the cocoa plant’s responses to environmental variables and how these variables interact and influence the production of primary and secondary metabolites stored in the seeds. At a time when climate change is being discussed, this is an important future demand. However, most studies conducted so far have evaluated the chemical composition of cocoa beans from diferente geographical origins without considering cultivation systems (Fajardo et al., 2022; Ostrowska-Ligęza et al., 2021; Samaniego et al., 2021; Sirbu et al., 2018; Torres-Morenoet al., 2015), thus excluding the significant distinction resulting from the characteristics of each type of farming systems.

Data on the characterization and comparison of cocoa beans produced under shade and in full sun are scarce. In addition to the lack of data, there are no references describing the influence of climate and local geomorphic conditions on bean composition. Therefore, knowledge about the chemical composition of cocoa beans produced in cabruca and full sun systems is relevant, especially for cocoa beans and chocolate producers in the state of Bahia, who aim to obtain higherquality products within a sustainability context, particularly in the face of impending climate changes. Given the above, the aim of this study was to evaluate the influence of cocoa cultivar and farming systems on the chemical composition of unfermented cocoa kernel.

2. Materials and methods

2.1. Cocoa kernel samples

In Brazil, during the 1990 s, several resistant clonal cultivars were introduced after the arrival of cocoa witches’ broom. In this scenario, cocoa producers in Brazil have widely cultivated the CCN51 cultivar from Ecuador and the hybrid PS1319 from farm Porto Seguro, Uruçuca, Brazil (Ramos et al., 2014). We selected both cultivars because they are most cultivated and preferred by Brazilian farmers in cocoa crops. The characteristics of the fruits and cocoa beans of the CCN51 and PS1319 cultivars are described by Bojacá et al. (2019) and Ramos et al. (2014), respectively.

Cocoa fruits were collected from 12 cocoa-producing farms located in eight different municipalities in the state of Bahia, Brazil (Teolândia, Gandu, Ibirapitanga, Ilhéus, Itajuípe, Uruçuca, Belmonte, and Eunápolis).

Among the studied farms, six have cocoa production in the cabruca system, and six farms cultivate in full sun system. The geographical coordinates of the sample collection point on each farm are described in Table S1 (supplementary materials). Samples of fruits from two cocoa cultivars (CCN51 and PS1319) were collected from all farms. The collection was carried out in July and August 2022. At each sampling location, four cocoa trees of approximately the same size and age were randomly selected, around which 3 to 4 ripe fruits were collected to compose a sample of cocoa kernel. Cocoa kernel samples were obtained by opening the fruit husks and removing the pulp and cocoa shell.The whole cocoa kernel were then dried in an oven at 70 °C for 8 h, until they reached approximately 5 % moisture content. After drying, they were packed in plastic bags and frozen in a freezer (approximately -18 ◦C) until analysis.

2.2. Materials

All chemicals used in this study were of analytical grade. The following reagents and standards were purchased from Sigma Aldrich Co. (St. Louis, MO, EUA): Folin-Ciocalteu reagent, 1,1-Diphenyl-2-picrylhydrazyl (DPPH), 2,2′-Azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) (ABTS), 6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), gallic acid, cyanidin-3-glucoside, sodium carbonate, acetonitrile, anhydrous sodium sulfate > 99 %, caffeine, (+)-catechin, (-)-epicatechin, caffeic acid, theobromine, 4-hydroxybenzoic acid, 4-hydroxyflavone, 4-hydroxy-3-methoxy-cinnamaldehyde, 7-hydroxyflavone, chalcone, chlorogenic acid, coniferyl alcohol, coumarin, curcumin, daidzen, ferulic acid, genistein, isoferulic, naringenin, neochlorogenic acid1, N-propil galato, p-coumaric acid, protocatechuic acid, quercetin, synaptic acid, sinapyl, alcohol, syringic acid, trans-cinnamic acid, vanillin,. petroleum ether (30–70) PA-ACS 100 %, hydrochloric acid (Synth, São Paulo, Brazil), potassium persulphate, potassium hydroxide 85 % and hydrochloric acid 37 % (Vetec Química Fina Ltd, Rio de Janeiro, Brazil), gallic acid (Analyticals Carlo Erba, France), methanol 99,8% (AlphaTec Systems, USA), dichloromethane 99.5 % and hexane 99 % (Química moderna Indústria e Comércio, São Paulo), absolute ethyl alcohol and acetone P.A. ACS (Êxodo científica, São Paulo, Brasil), acetic acid P.A. (ISOFAR ltda., Rio de Janeiro, Brasil), PVDF

Whatman qualitative filter paper, Grade 1 (Merck, Darmstadt, Germany). Aqueous solutions were made with Milli-Q water (18.2 mΩ) (Millipore, Bedford, MA, USA).

2.3. Total lipids

According to method 963.15 AOAC (1973), cocoa kernel samples were pre-digested by refluxing with 4.5 mol/L HCl for 30 min. The resulting residue was vacuum-filtered and washed with water until the pH was between 6 and 7, then dried in an oven at 100 °C for 6 h. Subsequently, the residue was defatted by continuous extraction in a Soxhlet apparatus, using petroleum ether as the solvent for a period of 6 h. The calculation of the total lipid content in the samples was based on the weight loss of the material subjected to reflux.

2.4. Extraction of phenolic compounds and methylxanthines

Before the process of obtaining extracts, the unfermented cocoa kernel was defatted. For this purpose, 5 g of crushed cocoa kernel were subjected to continuous extraction in a Soxhlet apparatus using 250 mL of petroleum ether as a solvent for 8 h.

The extraction of phenolic compounds and methylxanthines from defatted cocoa kernel was carried out by adapting the methodology described by (Counet & Collin, 2003). In this method, 2 g of crushed beans were added to 25 mL of an extracting solution containing acetone, water, and acetic acid (70:28:2 v/v), and this mixture was subjected to ultrasound treatment at 25 kHz and 25 ◦C for 1 h using an ultrasonic bath (Elmasonic TI-H-10, Elma, Germany). Subsequently, the mixture was vacuum-filtered through Whatman No.1 filter paper in a Büchner funnel. The extract was then concentrated in a rotary evaporator (RV 10 digital, IKA, Staufen, BW, Germany) at 40 °C until approximately 28 % of the original volume was reached. Next, distilled water was added to the concentrated extract to obtain a final volume of 10 mL, and the mixture was centrifuged (RA 280 rotor, Model NF 1200 R, Nüve, Akyurt/Ankara, Turkey) at 3899 g force for 20 min and 18 °C. The supernatant (extract) was stored in amber vials and frozen in a freezer (approximately -18 °C) until analysis. For HPLC analysis, the extract was filtered through a 0.22 µm polyvinylidene difluoride (PVDF) membrane.

2.5. Preparation of fatty acid methyl esters (FAMEs)

To analyze the fatty acids (FAs) derived from triacylglycerides (TAGs) present in the cocoa kernel, cocoa butter was initially extracted from the crushed bean. Following the fat removal protocol of the Official Methods of Analysis (AOAC 1990), the cocoa butter removal process was carried out in a semi-automatic soxhlet (JP Selecta F-6, Barcelona, Spain) containing petroleum ether as solvent for 8 h. Subsequently, the defatted cocoa mass was discarded, and the butter solvent was removed using a rotary evaporator (RV 10 digital, IKA, Staufen, BW, Germany) at 60 °C. The solvent-free butter was then quantified and stored in amber bottles at -20 ◦C until further analysis. Next, their respective fatty acid methyl esters (FAMEs) were obtained according to the method described by Jham, Teles, & Campos (1982). Initially, basic hydrolysis of the triacylglycerides was performed. For this, 100 mg of the obtained cocoa butterwas transferred to a 50 mL flask and dissolved in 10.0 mL of a 1.0 mol L^{-1} KOH solution (10 % methanol). This mixture was heated to 100 ºC with stirring for 1 h. Then, esterification of the obtained fatty acids was carried out. For this, 3.20 mL of hydrochloric acid (37 %) and 800 μL of methanol were added to the flask. This mixture was maintained at 100 ºC with stirring for another 1 h. At the end of this period, 20.0 mL of distilled water was added to the mixture, and the methyl esters formed were extracted by liquid–liquid extraction with dichloromethane (2 x 30.0 mL). After removing the residual water from the organic phase using anhydrous sodium sulfate, the dichloromethane was evaporated under reduced pressure using a rotary evaporator, yielding FAMEs. From this FAMEs, 5 mg were separated and transferred to a vial. After dissolving this material in 1.0 mL of hexane, it was analyzed by gas chromatography-mass spectrometry (GC–MS).

2.6. Bioactive compounds and antioxidant properties

The quantification of total phenolic content (TPC) was performed using the Folin-Ciocalteu colorimetric assay (Singleton & Rossi, 1965) with a UV–Vis spectrophotometer (UV-M51, Bel Photonics, Monza, Italy) at 760 nm. A calibration curve with increasing concentrations of gallic acid was used to calculate the TPC values. The results were expressed in mg of gallic acid equivalents (GAE) per 100 g of cocoa kernel.

The total anthocyanin content (TAC) was measured according to the method described by Fuleki & Francis (1968), and absorbance was measured at 535 nm (UV-M51, Bel Photonics, Monza, Italy). The results were expressed in mg of total anthocyanins per 100 g of cocoa kernel (mg AntT. 100 g^{-1}).

The antioxidant properties of the extracts were measured by DPPH (2,2-diphenyl-1-picrylhydrazyl radical) and ABTS (2,2-azino-bis-(3-tylbenzothiazoline-6-sulfonic acid) assays. The DPPH method was performed according to Kim et al. (2002), and the absorbance was determined at 517 nm. The ABTS assay was performed according to Re et al. (1999), with modifications, and the absorbance was measured at 734 nm. In both methods, the analysis was conducted using a spectrophotometer (UV-M51, Bel Photonics, Monza, Italy), and the results were obtained from a calibration curve using Trolox and expressed in micromoles of Trolox equivalent per gram of sample (μmol TE g^{-1}). All samples were analyzed in triplicate.

2.7. Analysis of fatty acid methyl esters by GC–MS

The quantification of fatty acid methyl esters was performed using a gas chromatograph coupled to a mass spectrometry detector (Shimadzu GCMS-QP2010C Ultra).The samples were analyzed using a method with the following specifications: (SBP-5 column, 30 m, ID 0.25 mm); helium carrier gas; injection volume: 1 μL; injector temperature: 290 ºC; oven temperature: 40 ºC (2 min), with a ramp of 30 ºC min^{-1} to 280 ºC (maintained for 5 min); interface detector and ion source temperature: 280 ºC; detector using electron impact ionization (70 eV), operating with a scan range of 50–700 m/z (scan time of 0.5).

The identification of components was based on comparing their mass spectra with the mass spectra of the libraries from the US National Institute of Standards and Technology (NIST), considering base peak, molecular masses, and characteristic mass fragmentations. The determination of fatty acid methyl esters was performed considering the relative areas of the peaks, expressed as the percentage relative area of each individual peak in relation to the total area of the compound peaks in the chromatogram.

2.8. Analysis of profile of phenolic compounds and methylxanthines by targeted LC/MS

Approximately 400 uL of the extracts were placed in vials and 5.0 uL were injected into the LC/MS system from NuBioMol (Center for Biomolecules Analysis-UFV, Brazil) using a chromatography column (Agilent Eclipse Plus, RRHD, 1.8 um, 2.1x150 mm) with a flow of 0.25 mL min^{-1}, coupled online to a mass spectrometer QQQ triple quadrupole (Agilent). The mobile phase consisted of buffers A (water 100 % and acetic acid 0.02 % pH 4.5 in water at 0.02 %) and B (acetonitrile 100 % and acetic acid in acetonitrile at 0.02 %acetic acid 0.02 % pH 4.5) and the following gradient program was used: a linear ramp starting at 2 % of (A), increasing to 19 % of (B) for 5 min, 60 % of (B) for 13 min and 98 % of (B) at 14 min, 98 % of (B) was maintained during 3 min (17 min) and a descent linear gradient starting at 98 % of B, decreasing to 2 % of (B) by 1 min (18 min), followed by an equilibrium condition at 2 % of (B) for 2 min.

The mass spectrometer was operated at negative/positive alternating mode and the sample scanned by Multiple Reaction Monitoring (MRM) using the mass transitions for phenolic compounds as describe in the Table S2 (supplementary material). The mass spectra generated were processed using Skyline software to obtain the extracted ion chromatograms

EIC of each transition and the chromatogram area values from EIC, as indicative of the abundance of each phenolic compound.

2.9. Statistical analysis

The effect of cultivation system and cultivars on the Chemical composition of non-fermented cocoa kernel was evaluated using a twoway analysis of variance (ANOVA), considering a 2x2 factorial design. The first factor corresponded to the cultivation system (cabruca and full sun) and the second to the cultivars (CCN51 and PS319).

Results were expressed as mean ± standard deviation, considering six replications (farms). When appropriate, Pearson correlation studies (r) were conducted, with coefficients considered significant for $r > 0.6$ (Asuero, Sayago & González, 2006).

For all analyses, R software version 4.3.3 (R Core Team, 2024) w used, considering a significance level of $p < 0.10$.

Data were also subjected to principal component analysis (PCA) to identify possible clusters based on the chemical composition data of cocoa kernel samples. The objective was to evaluate the effect of different cocoa cultivars (CCN51 and PS1319) and different farming systems (cabruca and full sun) on cluster formation. Only variables with explanatory power exceeding 85 % of the total variance of the sample set were selected. This procedure was repeated until only variables meeting this criterion remained. Two PCAs were conducted, one initially based on total lipid content and fatty acids data, and a second one based on antioxidant properties, phenolic compounds, and methylxanthines data of cocoa kernel.

3. Results and discussion

3.1. Total lipids and fatty acid methyl esters CG-MS

The total lipid content in cocoa kernel varied between 36.20 ± 7.73 and 55.24 ± 7.04 g/100 g (Table 1) and was influenced by the cultivation system, with higher values in the cabruca system, where there is greater shading. The cocoa tree cultivar factor did not significantly influence lipid levels ($p > 0.1$). According to Beckett, Fowler, & Ziegler (2017), the chemical composition of cocoa beans can be affected by geographic origin, genotype, management, or climatic conditions. In plants, lipid biosynthesis begins in the chloroplasts and continues in the cytosol, endoplasmic reticulum (ER), and oil bodies. Lipids are used in building cell membranes and stored as triacylglycerols (TAGs) (Fan et al., 2019). Therefore, fatty acid production, which constitutes TAGs, is a light-dependent reaction that utilizes reducing power and adenosine triphosphate (ATP) generated during the photochemical phase of photosynthesis (Garrido et al., 2023).

From the above, the results observed in this study do not support the idea that higher solar radiation guarantees higher lipid content in cocoa beans. This may be because even low light levels can provide suficiente reducing ATP to sustain fatty acid biosynthesis for accumulation in seeds (Chapman & Ohlrogge, 2012). However, it is not fully understood how plants control these mechanisms (Ye et al., 2020). It is known that cocoa trees have low irradiance saturation (lighting rate), low photosynthetic rate, and high sensitivity to photoinhibitory stress (Pinto et al., 2022). Perhaps this is why cocoa trees do not feel the reduction in brightness in the cabruca system, where solar radiation is approximately 50 to 60 % of the intensity of available light (Piasentin & Saito, 2014). Additionally, other factors must be considered, such as temperature, which in the cabruca system can be up to 6 ºC lower than the maximum temperature observed in cocoa plantations without shade (Heming et al., 2022).

The lipid content of cocoa kernel was similar to that obtained by other authors. Samaniego et al. (2021) obtained average levels ranging from 45.61 ± 1.27 to 52.13 ± 0.58 g/100 g for Nacional cocoa beans from different regions of Ecuador. Although the authors did not describe the cultivation conditions of each sampling region, higher lipid levels were associated with cocoa beans collected in the Amazon region and from locations with slightly lower average temperatures.

The results of fatty acids present in the lipid extracts (Table 1) indicated that the most abundant in cocoa kernel were oleic (34 to 36 %), stearic (29 to 33 %), and palmitic (28 to 29 %) acids. The fatty acid content are consistent with those found in other studies (Firmanto et al., 2019; Lipp et al., 2001; Ramos-Escudero et al., 2021; Řezanka & Řezanková, 1999; Torres-Moreno et al., 2015). Ribeiro et al. (2012) obtained values similar to those observed in this study for cocoa butters from the Brazilian states of Bahia, Amazonas, and Espírito Santo: oleic acids (32 to 35 %), stearic acids (34 to 36 %), and palmitic acids (24 to 27 %).

The cultivation system affected the content of some of the evaluated fatty acids (Table 1), with higher values associated with the full sun cultivation system for dodecanoic (C12:0), tridecanoic (C13:0), and linoleic (C18:2) acids. Furthermore, higher levels of dodecanoic (C12:0), palmitic (C16:0), and oleic (C18:1) acids were found in the cocoa kernel of the PS1319 cultivar, while higher levels of tridecanoic (C13:0) and stearic (C18:0) acids were obtained in the CCN51 cultivar. The levels of palmitic (C16:0), elaidic (trans-C18:1), and eicosanoic (C20:0) acids were not influenced by the cultivation systems and cultivar types ($p > 0.1$).

Table 1. Total lipid and fatty acid content of cocoa kernel from cultivars CCN51 and PS1319 grown in cabruca or full sun systems.

Constituent		**Cultivation system**	**Cultivar**		
			CCN51	**PS1319**	**Mean**
Lipids	g/100 g	Cabruca	54.60 ± 10.15	55.25 ± 7.04	54.92 ± 0.45^{A}
		Full sun	45.79 ± 11.11	36.20 ± 7.73	41.00 ± 6.78^{B}
		Mean	50.20 ± 6.23^{a}	45.72 ± 13.46^{a}	
(E)-dodecenoic acid (C12:0)	%(*)	Cabruca	0.03 ± 0.02	0.09 ± 0.04	0.06 ± 0.04^{B}
		Full sun	0.05 ± 0.02	0.11 ± 0.06	0.08 ± 0.04^{A}
		Mean	0.04 ± 0.02^{b}	0.10 ± 0.01^{a}	
Palmitic acid (C16:0)	%(*)	Cabruca	28.26 ± 0.76	29.73 ± 0.68	29.00 ± 1.04^{A}
		Full sun	28.30 ± 0.79	29.71 ± 0.68	29.01 ± 0.99^{A}
		Mean	28.28 ± 0.03^{b}	29.72 ± 0.02^{a}	
Tridecanoic acid (C13:0)	%(*)	Cabruca	0.10 ± 0.02	0.08 ± 0.02	0.09 ± 0.01^{B}
		Full sun	0.13 ± 0.02	0.10 ± 0.02	0.12 ± 0.03^{A}
		Mean	0.12 ± 0.03^{a}	0.09 ± 0.01^{b}	
Linoleic acid (C18:2)	%(*)	Cabruca	3.03 ± 0.13	2.57 ± 0.22	2.80 ± 0.33^{B}
		Full sun	3.18 ± 0.83	3.25 ± 0.63	3.21 ± 0.05^{A}
		Mean	3.10 ± 0.10^{a}	2.91 ± 0.48^{a}	
Elaidic acid (trans-C18:1)	%(*)	Cabruca	0.55 ± 0.12	0.64 ± 0.21	0.60 ± 0.06^{A}
		Full sun	0.59 ± 0.15	0.64 ± 0.10	0.62 ± 0.04^{A}
		Mean	0.57 ± 0.03^{a}	0.64 ± 0.00^{a}	
Oleic acid (C18:1)	%(*)	Cabruca	34.30 ± 1.10	35.83 ± 0.65	35.07 ± 1.08^{A}
		Full sun	34.85 ± 1.29	36.31 ± 1.89	35.58 ± 1.03^{A}
		Mean	34.58 ± 0.39^{b}	36.07 ± 0.34^{a}	
Stearic acid (C18:0)	%(*)	Cabruca	33.03 ± 1.05	30.37 ± 0.52	31.70 ± 1.89^{A}
		Full sun	32.17 ± 1.60	29.18 ± 2.68	30.67 ± 2.12^{A}
		Mean	32.60 ± 0.61^{a}	29.77 ± 0.84^{b}	
Eicosanoic acid (C20:0)	%(*)	Cabruca	0.67 ± 0.08	0.70 ± 0.08	0.68 ± 0.02^{A}
		Full sun	0.73 ± 0.10	0.71 ± 0.08	0.72 ± 0.01^{A}
		Mean	0.70 ± 0.04^{a}	0.70 ± 0.01^{a}	

Means followed by the same lowercase letters in the line do not differ from each other using the F-test ($p>0.1$). (*) Results expressed as relative percentage (%) of fatty acids in relation to the area of all the peaks in the chromatogram. There was no significant effect ($p>0.1$) of the interaction between the cultivation system and cultivars. Therefore, the effects of these factors were analyzed separately.

In a study with cocoa butters from different regions of Indonesia, Firmanto et al. (2019) reported that regional climatic conditions, including precipitation, temperature, and humidity, influenced palmitic acid (C16:0) levels more than oleic acid (C18:1), and that temperature, in particular, showed a good positive correlation with palmitic acid content. In the case of these two fatty acids (palmitic and oleic), our study did not identify significant differences between the cultivation systems, where at least the temperature was expected to be different due to reduced insolation in the cabruca.

The fatty acids were analyzed in groups, considering saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), total unsaturated fatty acids (UFA), and the saturated/unsaturated fatty acid ratio (S/U) (Figure 1). The cultivation system influenced only the composition of PUFA, with higher values associated with the full sun system (3.21 ± 0.05%) compared to the cabruca system (2.80 ± 0.33%). The effect of cocoa tree cultivar influenced the other variables (SFA, MUFA, UFA, and S/U). The cocoa kernel of the CCN51 cultivar showed a higher content of SFA (61.74 ± 0.50%) compared to PS1319 (60.38 ± 0.82%) and a higher S/U ratio (1.62 ± 0.03% versus 1.53 ± 0.05%). Meanwhile, cocoa kernel of the PS1319 cultivar exhibited higher levels of MUFA (36.71 ± 0.34%) and UFA (39.62 ± 0.82%) than those of the CCN51 cultivar (35.15 ± 0.41% and 38.25 ± 0.51%, respectively).

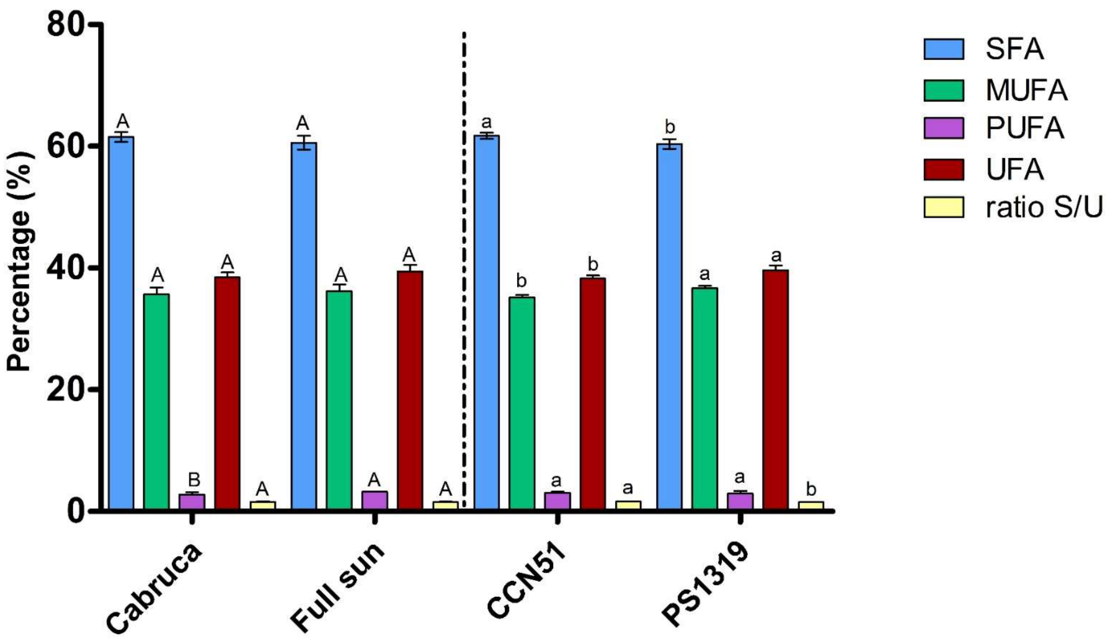

Figure 1. Relative percentage of saturated and unsaturated fatty acids in CCN51 and PS1319 cocoa kernel produced in cabruca and full sun systems. Means followed by the same uppercase letters (cabruca and full sun) and means followed by the same lowercase letters (CCN51 and

PS1319) do not differ from each other, as determined by the F test ($p > 0.1$). SFA: saturated fatty acids, MUFA: monounsaturated fatty acids, PUFA: polyunsaturated fatty acids, UFA: total unsaturated, S/U: saturated/unsaturated fatty acid ratio.

Cocoa butter is typically composed of more than 70% symmetrical triglycerides, which have an unsaturated fatty acid esterified to glycerol in the sn-2 position, being oleic acid and, to a lesser extent, linoleic acid. Saturated fatty acids, mainly palmitic (P, C16:0) and stearic, generally occupy the sn-1 and sn-3 positions in the glycerol structure. This type of triacylglycerol is often classified as SUS (Saturated-unsaturated-saturated) (Colella et al., 2023).

In general, cocoa butter contains oleic (O), stearic (St) and palmitic (P) acids in proportions that vary approximately between 32.5–36.5%, 33.0–37.5% and 24.0–28.0%, respectively. The main triacylglycerols found include POP (1-palmitoyl-2-oleoyl-palmitin), StOSt (1-stearyl-2-oleoyl-stearin) and POSt (1-palmitoyl-2-oleoyl-stearin), with oleic acid being preferentially esterified at the sn-2 position of the glycerol molecule (Buscato, Grimaldi & Kieckbusch et al., 2017).

Therefore, this symmetric configuration of triacylglycerols (TAG) and the high content of saturated fatty acids (SFAs) are the main factors responsible for the functionality of cocoa butter (Afoakwa, 2016; Vieira et al., 2015; Żyżelewicz et al., 2014). Small variations in the percentages of fatty acids caused by the geographic origin of cocoa, genotype, management techniques, climatic conditions, and post-harvest can lead to moderate variations in the physical properties of cocoa butter (Colella et al., 2023). It is known that the physical properties of cocoa butter directly affect the technological behavior of chocolate during the manufacturing process and the characteristics of the final product, such as texture, viscosity, melting properties and flavor (Afoakwa, 2016). High-quality chocolates must have a shiny surface, a crunchy and pleasant texture in the mouth, as well as good resistance to heat and fat bloom (Bresson et al., 2021).

Vieira et al. (2015) observed that the geographic origin and harvesting period of cocoa affected the composition of SFAs and TAGs of butter in Brazil; however, they did not find effects of the adopted cultivation, whether organic or conventional. Hence, knowing the composition of cocoa beans is crucial for the optimal use in the cocoa industry. In this study, the results obtained indicated that the cabruca system presented cocoa kernel with a lower percentage of linoleic acid, which belongs to the group of polyunsaturated fatty acids (PUFA).

The degree of saturation of fatty acids in cocoa beans can be estimated by the S/U ratio (Torres-moreno et al. 2015; Samaniego et al., 2021). In this sense, the higher S/U ratio found for CCN51 cocoa kernel (1.62 ± 0.03) compared to PS1319 cocoa kernel (1.53 ± 0.05) suggests that the cocoa kernel of this cultivar are more suitable for chocolate production. The effect of the origin on the S/U ratio was observed by Torres-Moreno et al. (2015), who noted a higher proportion (1.72) for Ghana cocoa beans compared to cocoa beans from Ecuador (1.65). According to these authors, given the distance between the two countries, environmental factors may be involved in the result, such as precipitation and solar radiation.

However, any cause and effect relationship between environmental conditions and the fatty acid composition of cocoa beans is complex. No evaluations of different light intensities in cocoa cultivation systems have been found in the literature. Furthermore, the variability of different cocoa genotypes in relation to temperature has yet to be observed, which can impact on decision-making regarding the planting of suitable plants in different local environments (Daymond & Hadley, 2008).

Thus, this study indicated that the cultivation system with higher solar radiation incidence resulted in cocoa kernel with lower total lipid content and higher unsaturated fatty acid content. Additionally, the type of cultivar influenced the content of saturated and monounsaturated fatty acids, suggesting that in planning new plantations, consideration should also be given to the genetic material to be acquired when aiming for the production of higher-quality cocoa butter.

3.2. Antioxidant properties and profile of phenolic compounds and methylxanthines by targeted LC/MS

The contents of total phenolic compounds (TPC), anthocyanins (TAC) and antioxidant properties (DPPH, ABTS) of cocoa kernel are shown in Table 2. Lower levels of phenolic compounds and antioxidant properties were obtained in the full sun system, especially in cultivar PS1319. The results suggest that the CCN51 cultivar has a higher production of total phenolic compounds in its secondary metabolism when compared to PS1319. Our results confirmed the ones obtained by Polanía-Hincapié et al. (2023), who found higher levels of phenolic compounds (64.56 ± 11.74 mg g^{-1}), flavonoids (3.30 ± 0.57 mg g^{-1}), catechin (1.95 ± 0.06 mg g^{-1}), epicatechin (0.49 ± 0.01 mg g^{-1}) in CCN51 beans when analyzing eight different cocoa clones from southern Colombia.The consumption of cocoa beans is often linked to various benefits for human health, especially due to their high concentrations of phenolic compounds with antioxidant properties, including the monomeric flavan-3-ols epicatechin and catechin (Fernández-Romero et al., 2020). In addition, nitrogen compounds such as methylxanthines may also be responsible for the antioxidant properties of cocoa beans (Cortez et al., 2023). However, the concentrations of these compounds can vary according to the origin and genotype of the cocoa, soil factors, climatic conditions and post-harvest handling (Borja Fajardo et al., 2017).

When analyzing fermented cocoa beans from Peru, Ramos-Escudero et al. (2021) obtained TPC contents between 19.85 and 33.39 mg GAE/g and antioxidant properties (DPPH) between 103.38 and 372.11 mmol TE/g. Farjado et al. (2022) found TPC values ranging from 44.51 ± 0.90 to 106.77 ± 5.21 mg GAE/g, with a significant difference between the four regional unfermented cocoa clones from Colombia evaluated, while Fernández-Romero et al. (2020) found TPC contents of 110.98 ± 1.43 mg GAE/g in fermented Criollo cocoa beans from Peru. Batista et al. (2016) evaluated three different cocoa varieties (PH15, PS1319 and CEPEC 2004) and observed TPC contents between 6.30 and 26.05 mg GAE/g, and antioxidant properties from 0.24 to 1.17 mM TE/g (DPPH) and from 1.29 to 4.83 mM TE/g (ABTS). However, no studies were found that evaluated the content of phenolic compounds, total anthocyanins and antioxidant properties comparing different types of cocoa beans cultivation.

Table 2. Content of total polyphenols, anthocyanins and antioxidant properties of cocoa kernel from cultivars CCN51 and PS1319 grown in cabruca or full sun systems.

Response variables	**Cultivation system**	**Cultivar**		
		CCN51	**PS1319**	**Mean**
TPC	Cabruca	316.66 ± 42.29	257.46 ± 46.50	287.06 ± 41.86^{A}
	Full sun	223.32 ± 60.03	193.33 ± 50.18	208.32 ± 21.21^{B}
	Mean	269.99 ± 66.01^{a}	225.39 ± 45.35^{b}	
TAC	Cabruca	147.53 ± 53.85	90.41 ± 21.47	118.97 ± 40.39^{A}
	Full sun	67.06 ± 56.16	52.35 ± 21.67	59.70 ± 10.40^{B}
	Mean	107.29 ± 56.90^{a}	71.38 ± 26.91^{b}	
ABTS	Cabruca	411.65 ± 87.46	320.62 ± 121.73	366.14 ± 64.37^{A}
	Full sun	238.18 ± 59.51	200.74 ± 110.68	219.46 ± 45.24^{B}
	Mean	324.92 ± 122.67^{a}	260.68 ± 84.77^{a}	
DPPH	Cabruca	516.42 ± 158.24	404.14 ± 90.62	460.28 ± 79.39^{A}
	Full sun	313.37 ± 132.09	249.39 ± 82.12	281.38 ± 45.24^{B}
	Mean	414.90 ± 143.57^{a}	326.77 ± 109.42^{b}	

Averages followed by the same lowercase letters in the row and uppercase letters in the column do not differ by the F-test ($p>0.1$). TPC: mg GAE. 100g^{-1}; TAC mg AntT. 100g^{-1}; ABTS: µmol TE/g; DPPH: µmol TE/g. There was no significant effect ($p>0.1$) of the interaction between the cultivation system and cultivars. Therefore, the effects of these factors were analyzed separately.

The profile of phenolic compounds and methylxanthines in the cocoa kernel of the CCN51 and PS1319 cultivars grown in cabruca and full sun systems is shown in Table 3. The most abundant compounds found were coniferyl alcohol, epicatechin, theobromine, caffeine and catechin. Higher levels of epicatechin, catechin, protocatechuic acid, quercetin and synaptic acid were found in CCN51 cocoa kernel, and higher levels of p-coumaric acid in PS1319 cocoa kernel. The cocoa kernel produced in the full sun system had higher levels of 7-hydroxyflavone, naringenin, p-coumaric acid and quercetin.

The other compounds analysed were not affected by the treatments, so there was no influence of the cultivar or the cultivation system on the levels of these compounds. The high standard deviation values may have contributed to the absence of effects (largest pure error that is obtained by the amount of difference between replicate runs). As the cocoa samples were collected in different locations, many environmental variables could not be controlled.

Table 3. Phenolic compounds and methylxanthines of cocoa kernel from cultivars CCN51 and PS1319 grown in cabruca or full sun systems.

Compound	Cultivation system	Cultivar		
		CCN51	PS1319	Mean
Caffeine	Cabruca	63726.33 ± 20272.95	74004.33 ± 18546.90	68885.33 ± 7267.64[A]
	Full sun	79818.83 ± 27027.37	93789.33 ± 43221.28	86804.08 ± 9878.64[A]
	Mean	71772.58 ± 11379.12[a]	83896.83 ± 13990.11[a]	
Catechin	Cabruca	24786.83 ± 12791.64	16045.17 ± 4464.00	20416.00 ± 6181.29[A]
	Full sun	22464.50 ± 10074.03	14776.00 ± 3270.29	18620.25 ± 5436.59[A]
	Mean	23625.57 ± 15802.19[a]	15410.58 ± 897.44[b]	
Epicatechin	Cabruca	166800.50 ± 50448.09	116266.33 ± 18633.12	141533.42 ± 35733.05[A]
	Full sun	144452.83 ± 60393.42	109623.67 ± 25028.70	127038.25 ± 24627.94[A]
	Mean	155626.67 ± 15802.19[a]	112945.00 ± 15802.19[b]	
Caffeic acid	Cabruca	547.17 ± 200.05	524.67 ± 119.36	535.92 ± 15.91[A]
	Full sun	604.17 ± 374.69	706.00 ± 437.22	655.08 ± 72.01[A]
	Mean	575.67 ± 40.31[a]	615.33 ± 128.22[a]	
Theobromine	Cabruca	151581.83 ± 29065.34	135659.67 ± 12481.85	143620.75 ± 11258.67[A]
	Full sun	172485.83 ± 60362.54	167811.00 ± 58758.89	170148.42 ± 3305.61[A]

	Mean	162033 ± 14781.36[a]	151735.33 ± 14781.36[a]	
4 – hydroxybenzoic acid	Cabruca	123.17 ± 21.12	127.33 ± 17.60	125.25 ± 2.95[A]
	Full sun	147.00 ± 55.08	152.67 ± 69.10	149.83 ± 4.01[A]
	Mean	135.08 ± 16.85[a]	140.00 ± 17.91[a]	
4 - hydroxyflavone	Cabruca	25.33 ± 13.13	31.17 ± 24.49	28.25 ± 4.12[A]
	Full sun	33.33 ± 15.12	39.50 ± 13.03	36.42 ± 4.36[A]
	Mean	29.33 ± 5.66[a]	35.33 ± 5.89[a]	
4-hydroxy-3-methoxy-cinnamaldehyde	Cabruca	157.83 ± 61.14	166.33 ± 63.60	162.08 ± 6.01[A]
	Full sun	194.83 ± 62.13	191.50 ± 50.29	193.17 ± 2.36[A]
	Mean	176.33 ± 26.16[a]	178.92 ± 17.80[a]	
7 - hydroxyflavone	Cabruca	29.50 ± 20.22	33.00 ± 17.49	31.25 ± 2.47[B]
	Full sun	49.67 ± 11.59	41.00 ± 10.14	45.33 ± 6.13[A]
	Mean	39.58 ± 14.26[a]	37.00 ± 5.66[a]	
Chalcone	Cabruca	26.83 ± 6.11	32.17 ± 13.32	29.50 ± 3.77[A]
	Full sun	35.17 ± 11.14	33.50 ± 10.60	34.33 ± 1.18[A]
	Mean	31.00 ± 5.89[a]	32.83 ± 0.94[a]	
Chlorogenic acid	Cabruca	147.17 ± 64.18	136.67 ± 56.46	141.92 ± 7.42[A]

	Full sun	164.33 ± 57.93	142.50 ± 61.11	153.42 ± 15.44^{A}
		155.75 ± 12.14^{a}	139.58 ± 4.12^{a}	
Coniferyl alcohol	Cabruca	247157.67 ± 52644.38	218843.83 ± 20660.71	233000.75 ± 20020.90^{A}
	Full sun	281791.00 ± 105022.59	273046.83 ± 100461.18	277418.92 ± 6183.06^{A}
	Mean	264474.33 ± 24489.46^{a}	245945.33 ± 38327.31^{a}	
Coumarin	Cabruca	8.00 ± 4.82	12.67 ± 9.54	10.33 ± 3.30^{A}
	Full sun	10.67 ± 8.96	11.33 ± 6.95	11.00 ± 0.47^{A}
	Mean	9.33 ± 1.89^{a}	12.00 ± 0.94^{a}	
Curcumin	Cabruca	382.50 ± 101.88	504.83 ± 368.39	443.67 ± 86.50^{A}
	Full sun	401.67 ± 211.76	339.00 ± 194.53	370.33 ± 44.31^{A}
	Mean	392.08 ± 13.55^{a}	421.92 ± 117.26^{a}	
Daidzen	Cabruca	18.67 ± 9.20	19.33 ± 9.50	19.00 ± 0.47^{A}
	Full sun	20.00 ± 13.58	16.83 ± 8.66	18.42 ± 2.24^{A}
	Mean	19.33 ± 0.94^{a}	18.08 ± 1.77^{a}	
Ferulic acid	Cabruca	15.17 ± 9.15	10.83 ± 7.49	13.00 ± 3.06^{A}
	Full sun	17.33 ± 6.77	16.50 ± 5.54	16.92 ± 0.59^{A}
	Mean	16.25 ± 1.53^{a}	13.67 ± 4.01^{a}	

Genistein	Cabruca	8.83 ± 8.42	16.67 ± 12.18	12.75 ± 5.54^{A}
	Full sun	8.33 ± 6.89	10.67 ± 9.42	9.50 ± 1.65^{A}
	Mean	8.58 ± 0.35^{a}	13.67 ± 4.24^{a}	
Isoferulic	Cabruca	2.33 ± 1.37	2.67 ± 1.63	2.50 ± 0.24^{A}
	Full sun	2.50 ± 2.35	1.50 ± 1.52	2.00 ± 0.71^{A}
	Mean	2.42 ± 0.12^{a}	2.08 ± 0.82^{a}	
Naringenin	Cabruca	1150.33 ± 410.16	1085.67 ± 411.55	1118.00 ± 45.73^{B}
	Full sun	1517.17 ± 474.96	1611.83 ± 713.76	1564.50 ± 66.94^{A}
	Mean	1333.75 ± 259.39^{a}	1348.75 ± 372.06^{a}	
Neochorogenic acid	Cabruca	8.00 ± 7.72	3.67 ± 2.73	5.83 ± 3.06^{A}
	Full sun	9.00 ± 8.20	8.67 ± 7.03	8.83 ± 0.24^{A}
	Mean	8.50 ± 0.71^{a}	6.17 ± 3.54^{a}	
N-propil galato	Cabruca	56.33 ± 42.28	44.83 ± 68.90	50.58 ± 8.13^{A}
	Full sun	81.17 ± 88.14	70.50 ± 86.00	75.83 ± 7.54^{A}
	Mean	68.75 ± 17.56^{a}	57.67 ± 18.15^{a}	
p-coumaric acid	Cabruca	304.33 ± 79.41	338.00 ± 78.26	321.17 ± 23.81^{B}
	Full sun	339.33 ± 95.25	436.83 ± 96.08	388.08 ± 68.94^{A}

	Mean	321.83 ± 24.74^{b}	387.42 ± 69.89^{a}	
Protocatechuic acid	Cabruca	1950.83 ± 467.00	1130.17 ± 330.20	1540.50 ± 580.30^{A}
	Full sun	2354.17 ± 1565.46	1744.33 ± 752.53	2049.25 ± 431.22^{A}
	Mean	2252.50 ± 285.20^{a}	1437.25 ± 434.28^{b}	
Quercetin	Cabruca	1397.17 ± 333.02	959.33 ± 324.98	1178.25 ± 309.59^{B}
	Full sun	1534.17 ± 265.48	1396.50 ± 456.58	1465.33 ± 97.35^{A}
	Mean	1465.67 ± 96.87^{a}	1177.92 ± 309.12^{b}	
Synaptic acid	Cabruca	6.17± 4.36	4.50 ± 2.35	5.33 ± 1.18^{A}
	Full sun	6.33 ± 2.58	3.33 ± 1.86	4.83 ± 2.12^{A}
	Mean	6.25 ± 0.12^{a}	3.92 ± 0.82^{b}	
Sinapyl alcohol	Cabruca	5.83 ± 4.83	3.50 ± 3.39	4.67 ± 1.65^{A}
	Full sun	6.17 ± 2.04	6.17 ± 4.54	6.17 ± 0.00^{A}
	Mean	6.00 ± 0.24^{a}	4.83 ± 1.89^{a}	
Syringic acid	Cabruca	4.33 ± 4.13	1.67 ± 0.82	3.00 ± 1.89^{A}
	Full sun	2.50 ± 3.21	3.17 ± 4.36	2.83 ± 0.47^{A}
	Mean	3.42 ± 1.30^{a}	2.42 ± 1.06^{a}	
Trans-cinnamic acid	Cabruca	2.83 ± 2.56	2.50 ± 2.59	2.67 ± 0.24^{A}

	Full sun	1.00 ± 0.89	3.00 ± 8.33	2.00 ± 1.41^{A}
	Mean	1.92 ± 1.30^{a}	2.75 ± 0.35^{a}	
Vanillin	Cabruca	6.67 ± 7.97	8.00 ± 4.47	7.33 ± 0.94^{A}
	Full sun	4.17 ± 2.56	8.33 ± 6.47	6.25 ± 2.95^{A}
	Mean	5.42 ± 1.77^{a}	8.17 ± 0.24^{a}	

Averages followed by the same lowercase letters in the row do not differ according to F-test (p>0.1). Results expressed as area of the extracted chromatogram (EIC). There was no significant effect (p>0.1) of the interaction between the cultivation system and cultivars. Therefore, the effects of these factors were analyzed separately.

Phenolic compounds are secondary metabolites stored in the pigment cells of seed cotyledons and accumulated during fruit ripening (Oracz & Nebesny, 2016). These compounds have great physiological and morphological relevance for plants, playing an important role in defense against abiotic stresses, such as high light, temperature variations and protection against pathogens and predators (Naikoo et al., 2019). However, the concentration of phenolics in plants changes mainly depending on the genetics of the cacao tree, as well as other factors such as geographical origin, environmental growing conditions, maturity level and fruit harvest time (Batista et al., 2016; Cortez et al., 2023; Hernández-Hernández et al., 2022; Oracz, Żyżelewicz, & Nebesny, 2015). In this context, the data obtained in our study corroborate this information, since the genetic material factor affected the abundance of six phenolic compounds, including the two main compounds in cocoa kernel, epicatechin and catechin.

Exposure to ambient UV-B solar radiation (280-320 nm) in open fields is known to be an abiotic stress factor and, as a result, many plants synthesize phenolic compounds that act to protect and adjust antioxidant systems at the cellular and organismal level (Naikoo et al., 2019). In addition, environmental temperature variations can affect the processes of plant secondary metabolism and impact biomass and biosynthesis of compounds (Isah, 2019). As in the full sun cultivation system, plants are exposed to a higher incidence of solar radiation and greater temperature variation throughout the day, one possible assumption would be that these factors may influence the abundances of the compounds 7-hydroxyflavone, naringenin, p-coumaric acid and quercetin.

Cocoa beans are mainly rich in catechins or flavan-3-ols, anthocyanins and proanthocyanidins (Batista et al., 2016). The main flavan-3-ols are (-)-epicatechin (Hernández-Hernández et al., 2022), (-)-epigallocatechin, quercetin, chlorogenic acid, clovamide, naringenin, luteolin, biapigenin, ferulic and gallic acids (Rodríguez-Carrasco et al., 2018). Cocoa anthocyanins, another group of phenolic compounds, mainly involve cyanidin-3-O-galactoside and cyanidin-3-O-arabinoside. Flavanones such as naringenin have also recently been identified in cocoa beans, as have the non-flavonoid polyphenols hydroxybenzoic and hydroxycinnamic acid (Oracz & Nebesny, 2016). The data obtained in the present study confirm the great diversity of bioactive compounds present in cocoa beans, with twenty-nine different compounds being identified.

Theobromine (3,7-dimethylxanthine) is the main methylxanthine in cocoa beans, followed by caffeine (1,3,7-trimethylxanthine) which is generally found in smaller quantities (Cortez et al., 2023; Hernández-Hernández et al., 2022). In this study, theobromine (TB) and caffeine (CF) are in the group of substances that were not influenced by the type of cultivar and cultivation system. However, a greater abundance of theobromine (135660 ± 12482 to 172486 ± 60.362) was observed in all samples compared tocaffeine (63726 ± 20273 to 93789 ± 43221). These results are consistent with those usually found in the literature.

It has been previously reported that the variation in the composition of methylxanthines in cocoa beans depends on the plant genotype, and that the proportion of methylxanthines is related to the quality and sensory characteristics of the derived products (Febrianto & Zhu, 2019).

Brunetto et al. (2007) proposed the existence of a relationship between the theobromine/caffeine ratio (TB/CF) of different cocoa beans and the genotype of the cocoa tree, which were classified as Forastero, Trinitario, and Criollo. Overall, the authors observed that Criollo cocoa had a lower theobromine concentration and a higher caffeine content, in contrast to Forastero, which had a higher theobromine content and a lower caffeine concentration. Trinitario cocoa showed an intermediate level. In their study, TB/CF values were observed between 10 and 12 for Forastero cocoa, 6 and 8 for Trinitario cocoa, and 2 and 4 for Criollo cocoa. However, it is worth noting that the researchers analyzed only one Forastero cocoa cultivar and one Trinitario cultivar, compared to five Criollo cocoa cultivars.

Carrillo, Londoño-Londoño & Gil (2014), when analyzing cocoa samples from 18 farms located in different regions of Colombia, proposed classifying cocoa beans with a TB/CF above 9 as Forastero, those with a TB/CF between 3 and 9 as Trinitario, and those below 3 as Criollo.

Davrieux et al. (2007) observed that most Trinitario cocoa samples had TB/CF ratios between 2 and 6, while samples with ratios higher than 6 were predominantly Forastero cocoa. Therefore, it is considered that cocoa beans with a fine flavor generally have a low theobromine to caffeine ratio (Febrianto & Zhu, 2019).

In the present study, no significant differences were observed in the TB/CF ratio between treatments (Figure 2), with an average value of 2.15. The TB/CF ratios obtained in cocoa kernels were 1.94 (PS1319), 2.06 (cabruca), 2.24 (full sun), and 2.36 (CCN51), all below the values frequently reported in the literature, such as 6.0 (Batista et al., 2016), 5.0 to 7.0 (Cortez et al., 2023) and 4.0 to 37.0 (Febrianto & Zhu, 2019).

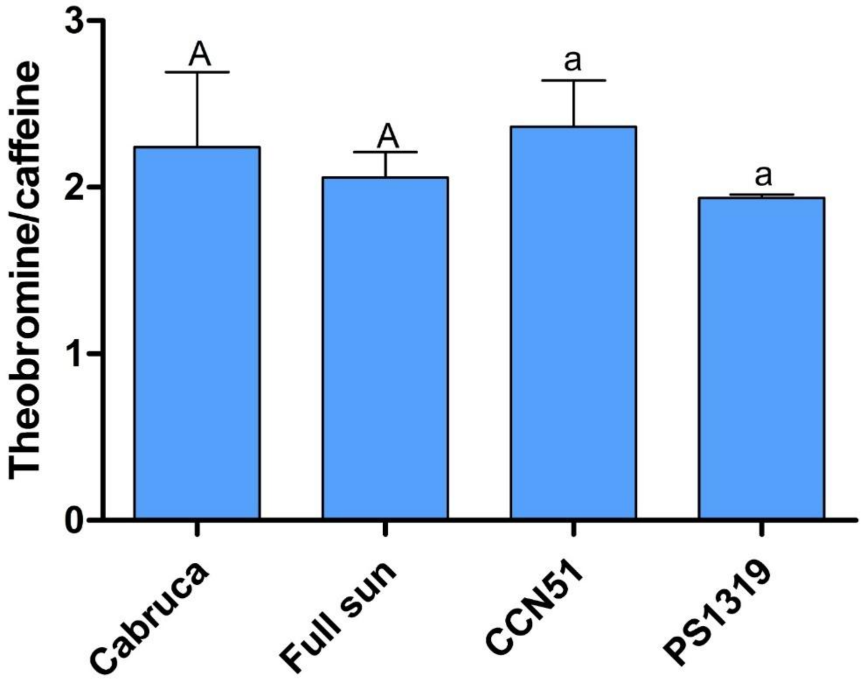

Figure 2. Theobronine/Caffeine ratio of cocoa kernel from cultivars CCN51 and PS1319 produced in cabruca and full sun systems. Bars followed by diferente lowercase letters (CCN51 and PS1319) and uppercase letters (cabruca and full sun) differ according to the F-test ($p < 0.1$).

As previously mentioned, values lower than 3 for the TB/CF ratio are generally associated with the Criollo genotype or fine-flavor cocoa. However, the CCN51 variety, which does not exhibit fine-flavor characteristics, showed a TB/CF ratio compatible with fine cocoa. This finding suggests that in addition to the genotype, the origin and characteristics of the cocoa cultivation site may influence the proportion of methylxanthines in cocoa beans. Therefore, it is recommended that additional studies be carry out on the classification of the type of cocoa based exclusively on the theobromine/caffeine ratio.

3.3. Correlation analysis

For the correlation studies (Supplementary Material), two groups were considered for analysis. The first group compared total lipid content and fatty acids, and the second group looked at correlations between the antioxidant properties, phenolic compounds and methylxanthines in cocoa kernel.

In the analysis of the lipid profile of cocoa kernel, a negative correlation was observed between total lipids and linoleic acid (C18:2) (-0.66***), between stearic acid (C18:0) and oleic acid (C18:1) (-0.91***), dodecanoic acid (C12:0) (-0.72***) and palmitic acid (C16:0) (-0.62**). Positive correlations were observed between tridecanoic acid (C13:0) and eicosanoic acid (C20:0) (-0.62***) and between oleic acid and dodecanoic acid (-0.61**) (Figure 3). This result suggests that under the conditions of the study, the higher the lipid content of the cocoa kernel, the lower the content of polyunsaturated fatty acids. It should be noted that the most abundant fatty acids in cocoa kernel (stearic and oleic) showed a strong negative correlation, indicating the inverse relationship between these two fatty acids, which are saturated and unsaturated, respectively.

The correlation matrix between antioxidant properties and the phenolic and methylxanthine profile (Table S3, supplementary material) indicated a predominance of positive correlations, except for the one found between naringenin and TPC. This finding is consistent with the direct relationship between antioxidant properties and the content of phenolic compounds in cocoa beans (Hernández-Hernández et al., 2022; Ramos-Escudero et al., 2021).

Among the antioxidant property variables, the highest correlations ($p<0.10$) were obtained between the DPPH variable with epicatechin ($r = 0.77$), TPC ($r = 0.85$), TAC ($r = 0.65$) and catechin ($r = 0.75$); between TAC and TPC ($r = 0.88$); between epicatechin with TPC ($r = 0.63$), and protocatechuic acid ($r = 0.65$) and catechin ($r = 0.94$). These results corroborate the study of Oracz & Nebesny (2016), who observed a positive correlation between the antioxidant properties (DPPH) and total phenolic content ($r = 0.96$; $p < 0.001$) of cocoa beans. Brito et al., (2017) observed a positive correlation between total phenolic compounds and total anthocyanins ($r = 0.91$), and both bioactive compounds showed a positive correlation with antioxidant properties using the ABTS assay ($r = 0.89$ and $r = 0.75$, respectively). Batista et al. (2016) found positive correlations between the content of total phenolic compounds and antioxidant properties (DPPH and ABTS) and TPC ($r \sim 0.99$, $p < 0.05$).

Among the alkaloid compounds, the theobromine content was correlated with coniferol alcohol ($r \sim 0.99$), 4-hydroxybenzoic ($r = 0.80$), caffeine ($r = 0.69$) and with protocatechuic (0.88), chlorogenic ($r = 0.61$), 1-neochlorogenic ($r = 0.74$) and caffeic ($r = 0.77$) acids. In turn, caffeine was correlated with 4-hydroxybenzoic ($r = 0.76$) and coniferol alcohol ($r = 0.69$). Among the phenolic compounds, conniferol alcohol correlated with 4-hydroxybenzoic ($r = 0.78$) and protocatechuic ($r = 0.88$) and 1-neochlorogenic ($r = 0.73$) acids. Protocatechuic acid also correlated with 4-

hydroxybenzoic (r = 0.71) and chlorogenic (r = 0.61) and 1-neochlorogenic (r = 0.78) acids.

In summary, it is suggested that the positive correlations between TPC, TAC and DPPH with epicatechin and catechin may explain the higher levels of these variables found in CCN51 cocoa kernel grown in the cabruca system (Table 2). Furthermore, the correlation results suggest that cocoa kernel with higher levels of theobromine have higher levels of phenolic acids.

3.4. Principal component analysis (PCA)

The Principal Component Analysis (PCA) study initially considered the total lipid and fatty acid content of the cocoa kernel (Figure 3), as well as data related to antioxidant properties, phenolic compounds and methylxanthines of the cocoa kernel (Figure 4).

In the PCA of the total lipid and fatty acid content of the cocoa kernel (Figure 3), the first two principal components explained 73.46% of the data variability. Correlations were observed between the total lipid content (-0.73), dodecanoic acid (0.84), oleic acid (0.76) and stearic acid (-0.91) with PC1, while tridecanoic acid (0.89), linoleic acid (0.59), eicosanoic acid (0.641) and palmitic acid (-0.71) correlated with PC2 (Figure 3A).

The scatter plot for the type of cultivation system did not indicate a clear separation between the groups of samples from the cabruca and full sun systems (Figure 3B). When analyzing the effect of the cultivar type, most of the CCN51 samples were related to the stearic acid content, while the PS1319 samples showed a greater relationship with palmitic and oleic fatty acids (Figure 3C).

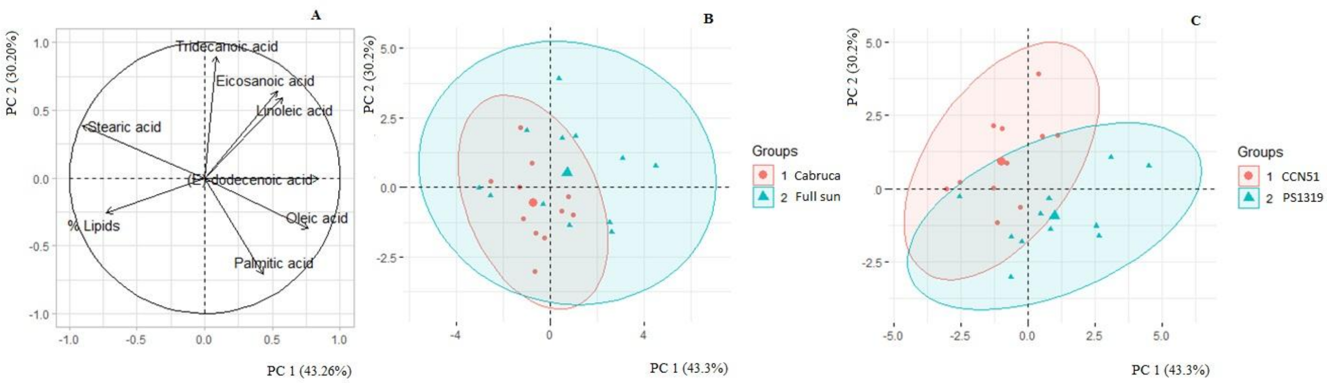

Figure 3. PCA of the total lipid and fatty acid content of unfermented cocoa kernel of cultivars CCN51 and PS1319 produced in cabruca and full sun systems. (A) Scatter diagram of the variables under study; (B) Scatter diagram of the samples separated by type of cultivation system (cabruca and full sun); (C) Scatter diagram of the samples

separated by type of cultivar (CCN51 and PS1319). *Larger symbols represent the mean values of the samples and smaller symbols represent the value of each sample under study.

In the PCA of the antioxidant properties, phenolic compounds and methylxanthines, PC1 and PC2 explained 67.2% of the variability present in the data (Figure 4). From the scatter plot, it was observed that the variables correlated with PC1 were protocatechuic acid (0.92), caffeic acid (0.80), neochlorogenic acid (0.81), chlorogenic acid (0.73), coniferyl alcohol (0.95), theobromine (0.96) and 4-hydroxybenzoic acid (0.82). The variables with the greatest contribution to PC2 were TPC (0.91), TAC (0.77), ABTS (0.79), DPPH (0.92), catechin (0.68) and epicatechin (0.70) (Figure 4A).

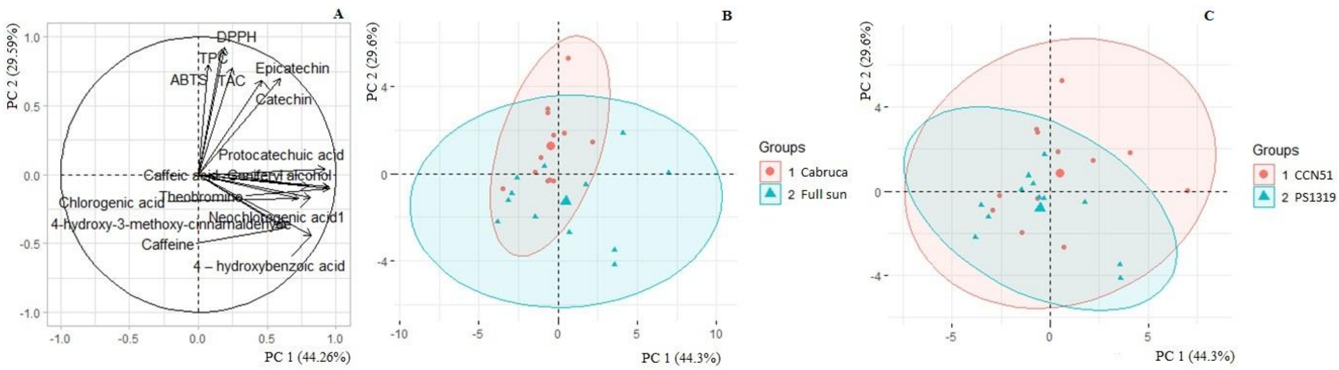

Fig. 4. PCA of variables related to antioxidant properties, phenolic compounds, and methylxanthines of unfermented cocoa kernel of cultivars CCN51 and PS1319 produced in cabruca and full sun systems. (A) Scatter diagram of the variables under study; (B) Scatter diagram of the samples separated by cultivation system (cabruca and full sun); (C) Scatter diagram of the samples separated by cultivar (CCN51 and PS1319). *TPC: Total phenolic compounds; TAC: Total anthocyanins; DPPH and ABTS: Antioxidant properties. **Larger symbols represent the mean values of the samples and smaller symbols represent the value of each sample under study.

Although no strong separation was observed between the different types of cropping systems, most of the samples from the cabruca system were positively related to PC2, while most of the full sun samples were negatively related to PC2 (Figure 4B). No separation was observed between the CCN51 and PS1319 sample groups (Figure 4C).

4. Conclusions

The cultivation system and the genetic material of the cocoa tree influenced the chemical composition of unfermented cocoa kernel. The cabruca cultivation system was associated with the production of cocoa kernel with higher levels of lipids, total phenolic compounds, total anthocyanins and antioxidant properties, while the full sun system was related to cocoa kernel with lower total lipid content and higher content of the unsaturated fatty acid linoleic.

Furthermore, the type of cultivar influenced the content of saturated and monounsaturated fatty acids, suggesting that when planning new plantations, the genetic material to be acquired should also be considered when aiming for the production of higher-quality cocoa butter. In addition, the cultivar CCN51 associated with differences in the levels of catechin, epicatechin, quercetin, protocatechuic acid and synaptic acid in cocoa kernels.

The results obtained indicated a concern that the cocoa production chain in Brazil should consider with special attention. The migration from traditional cabruca systems to more intensive full sun systems can reduce the levels of lipids and total phenolic compounds, affecting the quality of cocoa kernel produced in the country.

On the other hand, further research is needed to investigate the chemical composition and sensory acceptance of cocoa beans from different cultivars and different cultivation systems after processing.

In summary, the results indicated that the quality attributes related to the chemical composition of cocoa beans are more valued in agroforestry systems, such as the cabruca system. These discoveries can be used by Brazilian farmers to increase the value of their products, combining the best quality of cocoa beans with more sustainable production in the cabruca system, which preserves the biodiversity of flora, fauna and other diverse natural resources.

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CRediT authorship contribution statement

Hélia de Barros Kobi: Writing – original draft, Validation, Supervision, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Raphael Bragança Alves Fernandes:** Methodology, Validation, Writing - Review & Editing. **Davi Salgado de Senna:** Investigation. **Larissa Lorrane Rodrigues Borges:** Investigation, Formal analysis, Writing – review & editing. **Márcia Cristina Teixeira**

Ribeiro Vidigal: Methodology, Validation, Writing - Review & Editing. **Paulo Cesar Lima Marrocos:** Methodology, Resources, Investigation. **Valdeir Viana Freitas:** Investigation, Writing – review & editing. **Mariane Sampaio da Silveira de Souza:** Investigation. **Gabriel Abranches Dias Castro:** Resources, Investigation. **Sergio Antonio Fernandes:** Resources, Investigation. **Karin da Costa Ribeiro Ferraz:** Formal analysis. **Paulo Cesar Stringheta:** Validation, Supervision, Methodology, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Supplementary data

Supplementary data to this article can be found online at Appendix A.

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Appendix A. Supplementary material

Table S1. Geographical coordinates of the cocoa kernel sample collection sites.

Farm	Municipality (Bahia, Brazil)	Cultivation system	Geographic coordinates (UTM)	
			Latitude (S)	Longitude (E)
1	Uruçuca	Cabruca	8382898.00	473749.00
2	Ilhéus	Cabruca	8386195.00	473368.00
3	Ilhéus	Cabruca	8392298.00	484574.00
4	Teolândia	Full sun	8497128.00	448369.00
5	Gandú	Full sun	8480165.00	449832.00
6	Gandú	Full sun	8469773.00	446122.00
7	Ibirapitanga	Cabruca	8446928.00	458542.00
8	Itajuípe	Cabruca	8382818.00	456150.00
9	Ilhéus	Cabruca	8367821.00	475122.00
10	Belmonte	Full sun	8223743.81	479803.56
11	Eunápolis	Full sun	8224101.41	483224.03
12	Eunápolis	Ful sun	8189253.24	442664.07

Table S2. Transition list used as input in the Skyline to the analysis of the phenolic compounds.

Molecule list name	**Precursor Charge**	**Product m/z**	**Product charge**	**Precursor RT (min)**	**Precursor m/z**
4 - hydroxybenzoic acid	-1	93	-1	6.9	137.12
4 - hydroxybenzoic acid	-1	59	-1	6.9	137.12
4 - hydroxybenzoic acid	1	121	1	6.9	139.12
4 - hydroxyflavone	-1	117	-1	12.9	237.07
4 - hydroxyflavone	1	137	1	12.9	239.07
4-hydroxy-3-methoxy-cinnamaldehyde	1	161	1	10.2	179.1
4-hydroxy-3-methoxy-cinnamaldehyde	1	147.04	1	10.2	179.1
7 - hydroxyflavone	1	137	1	12.9	239.07
7 - hydroxyflavone	1	129	1	12.9	239.07

Cafeine	1	138.1	1	7.3	195.1
Cafeine	1	110.1	1	7.3	195.1
Caffeic acid	-1	135	-1	7.4	179
Caffeic acid	-1	89	-1	7.4	179
Catechin	1	123	1	6.8	291
Catechin	1	139	1	6.8	291
Chalcone	1	103	1	15.7	209.1
Chalcone	1	77.1	1	15.7	209.1
Chlorogenic acid	-1	191.2	-1	5	353
Chlorogenic acid	-1	176.2	-1	5	353
Chlorogenic acid	1	163	1	5	355.1
Chlorogenic acid	1	145	1	5	355.1
Coniferyl alcohol	1	138.2	1	5.3	181.1
Coniferyl alcohol	1	67.2	1	5.3	181.1
Coumarin	1	103	1	11.2	147.06
Coumarin	1	91	1	11.2	147.06
Coumenstrol	1	241	1	9	269
Coumenstrol	1	197	1	9	269
Curcumin	-1	173.06	-1	14.9	367.11
Curcumin	1	177	1	14.9	369.11
Curcumin	1	145	1	14.9	369.11
Daidzein	1	137	1	10.8	255
Epicatechin	1	123	1	7.5	291
Epicatechin	1	139	1	7.5	291
Daidzein	1	91	1	10.8	255
Ferulic acid	-1	149	-1	9.1	193.05
Ferulic acid	-1	134.1	-1	9.1	193.05
Genistein	1	215	1	12.3	271
Genistein	1	153	1	12.3	271
Isoferulic	-1	134	-1	9.3	193
Isoferulic	-1	134	-1	9.3	193
Naringenin	1	153	1	12.1	273
Naringenin	1	119	1	12.1	273

Neochlorogenic acid1	-1	191.2	-1	5.15	353.2
Neochlorogenic acid1	-1	176.2	-1	5.15	353.2
Neochlorogenic acid1	1	135.3	1	5.15	355.1
N-propil galato	1	153.3	1	10	213
N-propil galato	1	127.1	1	10	213
P-coumaric acid	-1	119	-1	8.8	163.04
P-coumaric acid	-1	93	-1	8.8	163.04
Protocatechuic acid	-1	109.1	-1	5.5	153
Protocatechuic acid	-1	91	-1	5.5	153
Quercetin	1	165.2	1	11.2	303
Quercetin	1	153.3	1	11.2	303
Sinapic acid	-1	208	-1	9.1	223
Sinapic acid	-1	164.1	-1	9.1	223
Sinapyl alcohol	1	91	1	8.5	211.1
Sinapyl alcohol	1	72.2	1	8.5	211.1
Syringic acid	-1	153	-1	7.6	197
Syringic acid	-1	121.2	-1	7.6	197
Theotrombine	1	138.4	1	5.3	181.3
Theotrombine	1	110.1	1	5.3	181.3
Trans-cinnamic acid	-1	117	-1	11.6	147
Trans-cinnamic acid	-1	77	-1	11.6	147
Trans-cinnamic acid	-1	74	-1	11.4	147
Vanilic acid	-1	152.1	-1	5.2	167.1
Vanilic acid	-1	108.1	-1	5.2	167.1
Vanilic acid	-1	152.1	-1	5.2	167.1
Vanillin	-1	136	-1	8.9	151
Vanillin	-1	92	-1	8.9	151
Vanillin	1	93	1	8.9	153

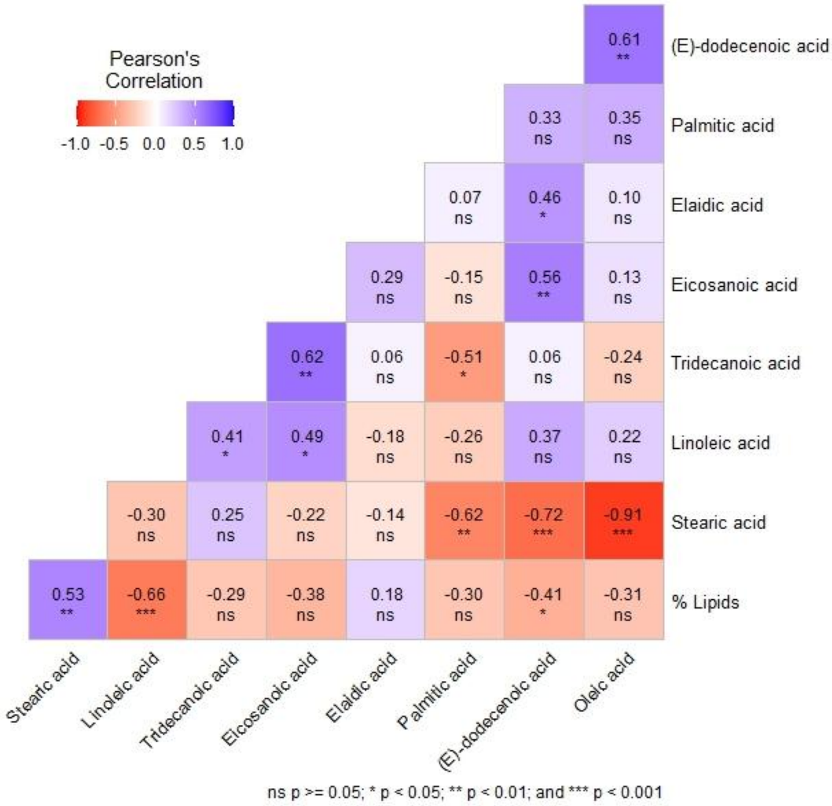

Figure S1. Perason's correlation matrix between total lipids (%) and fatty acids in CCN51 and PS1319 cocoa kernel produced in cabruca and full sun systems.

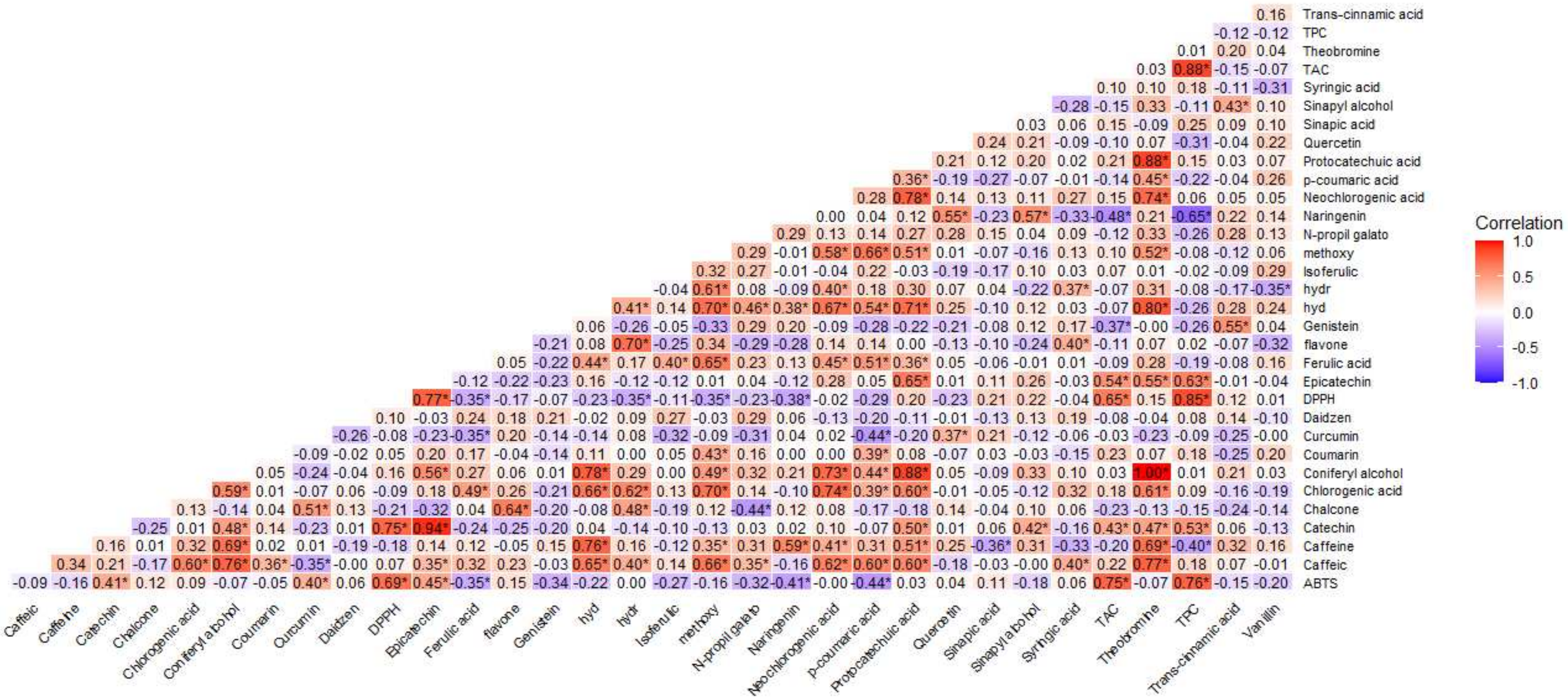

Figure S2. Pearson correlation matrix (r > 0.6) between the variables related to the antioxidant properties of cocoa kernel and the phenolic compounds analyzed by LM-MS. TPC: total phenolic compounds; TAC: total anthocyanin content; ABTS: antioxidant properties; DPPH: antioxidant properties; Hyd: 4-hydroxybenzoic; Hydr: 7-hudroxyflavone; Methoxy: 4-hydroxy-3-methoxy-cinnamaldehyde. (*): (p< 0,01).

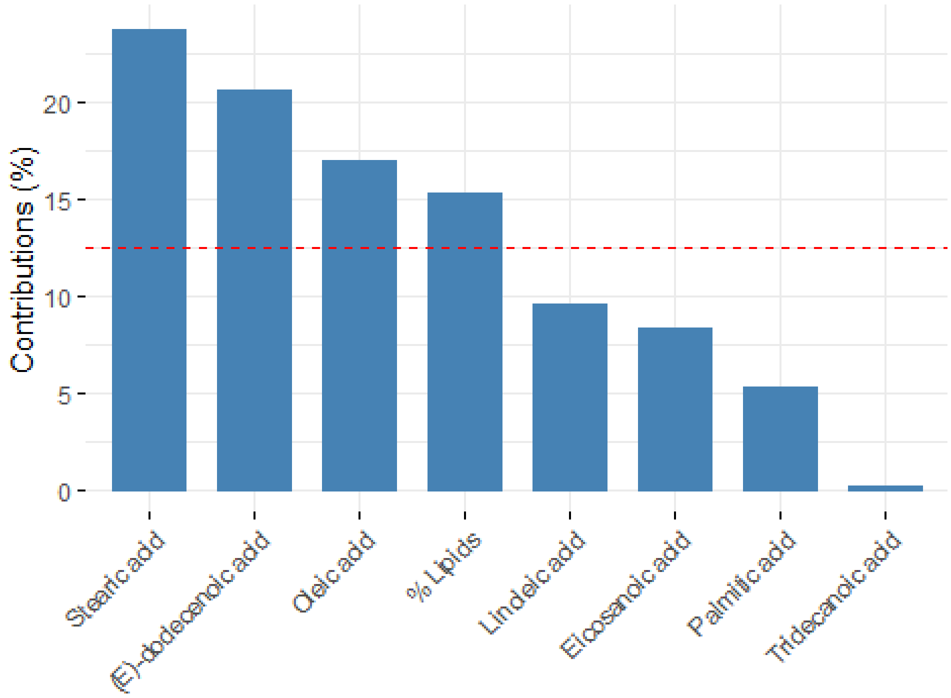

Figure S3. Variables with explanatory power greater than 85% of the total variance of the sample set related to the PC1 of the PCA of total lipids (%) and fatty acids in cocoa kernel.

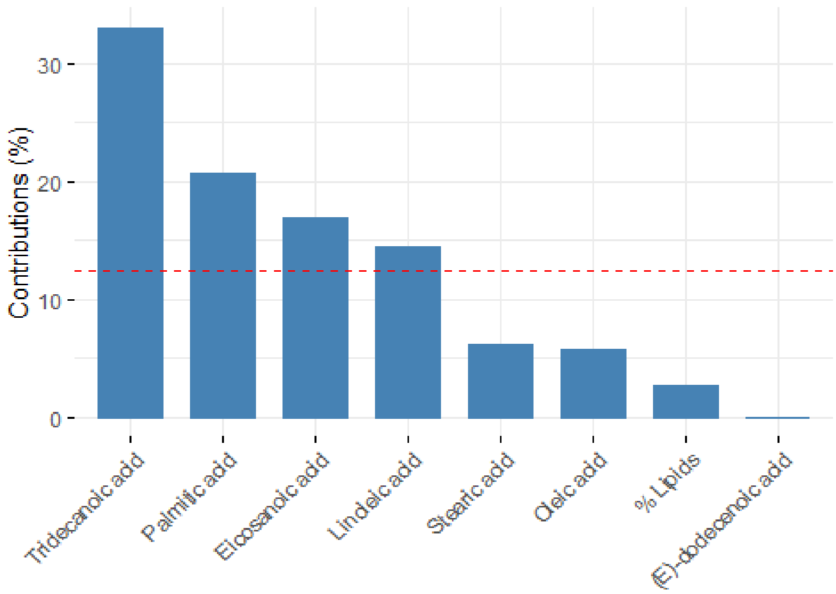

Figure S4. Variables with explanatory power greater than 85% of the total variance of the sample set related to PC2 of the PCA of total lipids (%) and fatty acids in cocoa kernel.

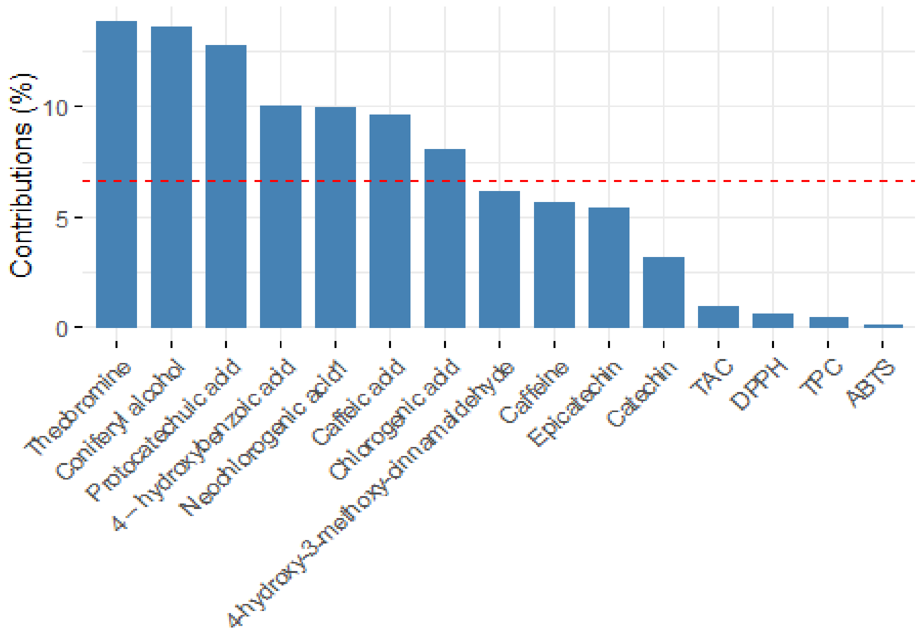

Figure S5. Variables with explanatory power greater than 85% of the total variance of the sample set related to PC1 of the PCA of phenolic compounds and antioxidant properties of cocoa kernel.

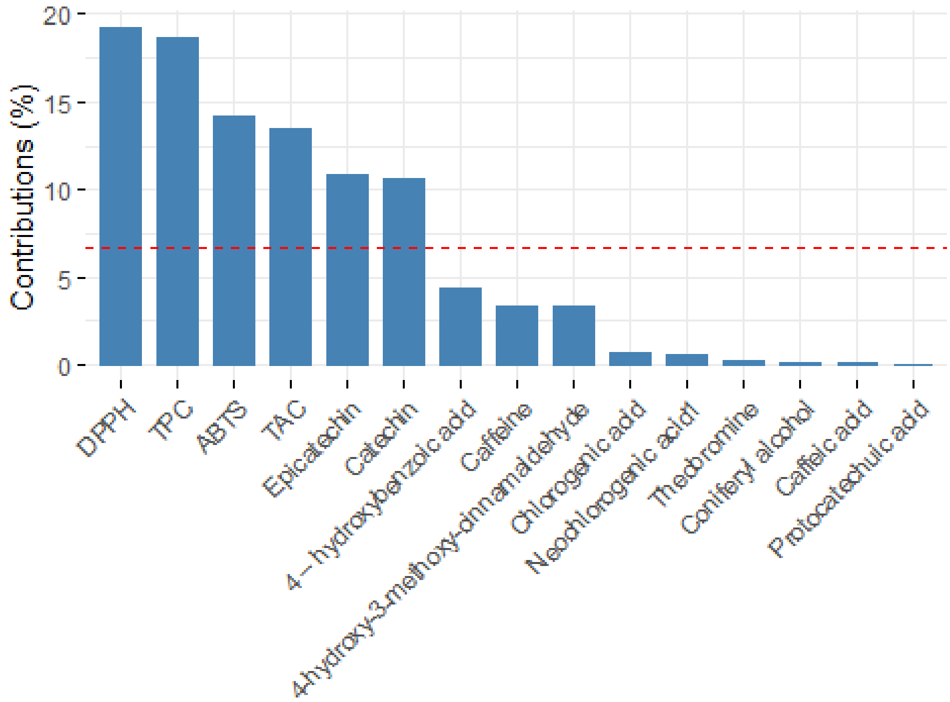

Figure S6. Variables with explanatory power greater than 85% of the total variance of the sample set related to PC2 of the PCA of phenolic compounds and antioxidant properties of cocoa kernel.

Chapter 2. Soil-plant relationships in the chemical quality of cocoa kernels: Evaluation of the transfer of essential elements and contaminants under different cultivation systems in Southern Bahia

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Abstract

Global cocoa production faces challenges associated with the accumulation of heavy metals in cocoa beans and derived products. This study evaluated the physicochemical characteristics of soils, leaves, and kernels of the cocoa cultivars CCN 51 and PS 13.19 under cabruca and full-sun systems. The physicochemical characterization of the soils revealed variations between farms and cultivation systems. The pH ranged from 4.5 to 6.1, indicating the prevalence of acidic soils with deficiencies in fertility components (P, K, Ca^{2+}, and Mg^{2+}), a more pronounced condition observed in the full-sun system. Soil texture varied between medium and clayey, with a significant presence of sand in the samples. Heavy metal levels in the soils were predominantly low; however, some areas presented Zn and Cr levels above the COPAM (2011) reference values of 46.5 $mg.kg^{-1}$ and 75 $mg.kg^{-1}$, respectively. Regarding cocoa kernels, none of the samples showed quantifiable levels of As, Pb, and Cd, and the Cu levels were below the maximum tolerated limit. Additionally, the cocoa kernels presented high levels of K (9.51±0.57 to 9.75±0.59 $g.kg^{-1}$) and P (5.14±0.38 to 5.23±0.37 $g.kg^{-1}$) and notable concentrations of Fe (18.86±3.56 to 25.88±10.42 $mg.kg^{-1}$), Zn (42.62±7.40 to 50.92±7.23 $mg.kg^{-1}$), Cu (11.64±3.51 to 15.20±5.32 $mg.kg^{-1}$) and Mn (8.96±4.41 to 17.25±12.12 $mg.kg^{-1}$), reinforcing the nutritional potential of the product. Statistical analysis indicated isolated effects of the management systems and/or cultivars evaluated, with the interaction effect verified only for the K content in the cacao leaves. A positive and consistent correlation was observed between the Mn content in the soil, in the kernels, and in the cacao leaves. However, the correlations between the nutrient contents in the leaves and kernels did not show significant direct relationships. Although the PCA of soil and leaf variables did not show groupings between the management types or cultivars, the integrated PCA (with soil, leaf, and kernel data) demonstrated that the cabruca system is characterized by factors related to soil fertility such as Ca^{2+}, Mg^{2+}, and SB, in addition to the presence of Ni in the leaves. Full sun management, in turn, did not show the same influence of these variables. Therefore, the results obtained provide evidence that cocoa kernels from the southern region of Bahia do not present contamination by the heavy metals Cd, Pb, and As and have high mineral richness; however, the insufficient state of essential nutrients (K, P, Fe, Zn, and Cu) and the potential environmental risk of Cr in the soil present themselves as challenges for the sustainability of the crop.

Keywords: Cacao trees, CCN 51, PS 13.19, Nutrients, Minerals, Heavy metals, Cabruca, Full sun.

1. Introduction

The cacao tree (*Theobroma cacao* L.) is a perennial species native to the tropical region of South America and is currently cultivated in several countries in the equatorial zone of the planet (Figueroa-Hernández et al., 2019; Bertoldi et al., 2016). In the Americas, cacao is produced by countries such as Ecuador, Brazil, Peru, Colombia, Dominican Republic, Mexico, Bolivia, Nicaragua, Costa Rica, Panama, Trinidad and Tobago, Cuba, Honduras, Guatemala, Belize, and El Salvador (FAO 2024; ICCO 2024; Ramos-Escudero et al., 2021; WCF, 2023).

Brazil ranks sixth among the world's largest cocoa producers (ICCO, 2022), with the state of Bahia being one of the main producers in the country (IBGE, 2022). Cocoa farming in southern Bahia is carried out in various cultivation systems, ranging from recent plantations to older ones, with or without intercropping, and with different management intensities. Among these practices, the system known as cabruca cocoa (hereinafter referred to as cabruca) stands out both for its historical tradition and for its widespread occurrence in the southern region of Bahia (Oliveira & Assis, 2023).

Cabruca is a traditional agroforestry system for cocoa cultivation, characterized by planting cacao trees under the canopy of native Atlantic Forest trees. Its name originated from the popular expression "cá brocar," which means "to open holes in the forest," describing the process of planting cocoa without deforesting the area. Considered a model of productive conservation, the cabruca system allows for the sustainable agricultural production of cocoa while protecting the forest. By maintaining the original ecosystem, it preserves the remnants of the Atlantic Forest, its biodiversity, and essential ecosystem services (Lobão et al., 2007; Lobão & Setenta, 2002). However, in recent years, a notable contrast has emerged in cocoa production. While the traditional cabruca system is still practiced, there has been growthin cacao cultivation in full-sun systems, especially in the south and far south of Bahia and other regions of Brazil. This method, which eliminates the need for permanent shading, requires more resources, such as irrigation and the intensive use of fertilizers (Pinto et al., 2022). Despite this change, agricultural scientific research focused on cacao production has concentrated efforts only on developing clones that offer higher productivity and resistance to pests and diseases, without considering the influence of the cultivation system, such as cabruca or full sun, on the chemical composition and quality of cocoa beans (Kobi et al., 2024).

Moderate consumption of cocoa beans, rich in polyphenols, has been associated with several health benefits (Sorrenti et al., 2020). However, there is serious global concern about the safety of the product, as the accumulation of heavy metals, such as arsenic, cadmium, and lead, in cocoa beans and their derivatives can represent a significant risk to human health (Maddela et al., 2020). Heavy metals are naturally found in soils due to rock weathering, atmospheric deposition, biogenic material, and volcanic origin, but high concentrations can also originate from anthropogenic actions, such as mining, the use of fertilizers, pesticides, etc. (Mohamed, Zainudin and Yaakob, 2020, Nnuro et al., 2020).

The accumulation of heavy metals in plants is influenced by factors such as soil pH, organic matter, plant genotype, and the concentration of metals in the growth medium (Alloway, 2013). Furthermore, this accumulation varies among metals, species, and interactions between elements, as in the case of zinc (Zn) and cadmium (Cd), which can have synergistic or antagonistic effects, and phosphorus (P) with arsenic (As), exhibits antagonistic behavior (Nan et al., 2002; Kabata-Pendias, 2011; Alloway, 2013).

Contaminants such as metals can persist after the post-harvest process, being found in semi-finished products and in final products, such as cocoa powder and chocolate (Mohamed, Zainudin and Yaakob, 2020). Although the consumption of cocoa derivatives is generally in small quantities, they can represent a source of heavy metals (Vanderschueren et al., 2020). According to the European Food Safety Authority (EFSA), dietary exposure to cadmium (Cd) in the adult population of Europe had an estimated contribution of 4.3% from the consumption of cocoa products. It is crucial to note that this contribution was more pronounced in vulnerable subgroups, such as the child population, where exposure levels potentially reached above 9% (EFSA, 2012). In response to this concern, international organizations have established acceptable maximum levels of heavy metals in chocolates and cocoa-derived products.

In this regard, the European Commission defined, effective from 2019, the maximum limits for cadmium (Cd) and lead (Pb) in chocolate and cocoa powder. The Codex Alimentarius Commission also adopted similar restrictions for cocoa-based products (Codex Alimentarius Commission, 2017). In Oceania, the 2016 Food Standards Code of Australia and New Zealand established a specific maximum level for Cd in chocolates. China set maximum limits for Pb and As applicable to cocoa products and confectionery (Malaysian Cocoa Board, 2018). In the United States, the Food and Drug Administration (FDA) issued recommendations for a maximum limit of Pb in sweet products, such as chocolate, in order to protect children's consumption (US Food and Drug Administration, 2006). In Brazil, the National Health Surveillance Agency (ANVISA) established Maximum Tolerable Limits (LMT) for As, Cd, Pb, and Cu in cocoa products through Normative Instruction (IN) No. 160, of July 1, 2022. However, despite the recently defined national and international standards, there is still little information on the contamination of cocoa produced in the country, especially in southern Bahia. Given the above, the objective of this study was to evaluate the potential for heavy metal contamination and the elemental composition in soils, leaves, and cocoa kernels (CCN 51 and PS 13.19), under two cultivation systems: cabruca and full sun.

2. Materials and methods

2.1. Study area

Cocoa cultivation was introduced to Bahia in 1746 and expanded mainly in the southeastern region of the state. The activity began in privileged areas, on the banks of large rivers, due to their edaphic and hydrological suitability. It then quickly expanded to forest ecosystems, favored by the high fertility of the soil and abundant rainfall, marking the commercial establishment (Piasentin & Saito, 2014).

This primary area, the Traditional Cocoa Zone, which stood out for its rich soils, concentrated the initial production and adopted the cabruca system. Subsequently, with the total occupation of the best lands, in the 1970s, there was an expansion to the Mixed Zone, a transitional frontier with less favorable edaphoclimatic conditions (Piasentin & Saito, 2014).

The climate of the Southern region of Bahia is tropical rainforest, without a dry season, with average monthly rainfall exceeding 60 mm and annual rainfall exceeding 1,500 mm. The average temperature of the coldest month is above 18ºC and the summers are long and hot, with the average temperature of the hottest month exceeding 22ºC (SEI, 1998).

For this study, 12 cocoa-producing farms located in eight different municipalities in the Southern and extreme regions of the state of Bahia, Brazil, were selected, covering areas of the aforementioned zones: Teolândia, Gandu, Ibirapitanga, Ilhéus, Itajuípe, Uruçuca, Belmonte, and Eunápolis. Of these farms, six produce cocoa in a cabruca system and six in a full sun system (Figure 1).

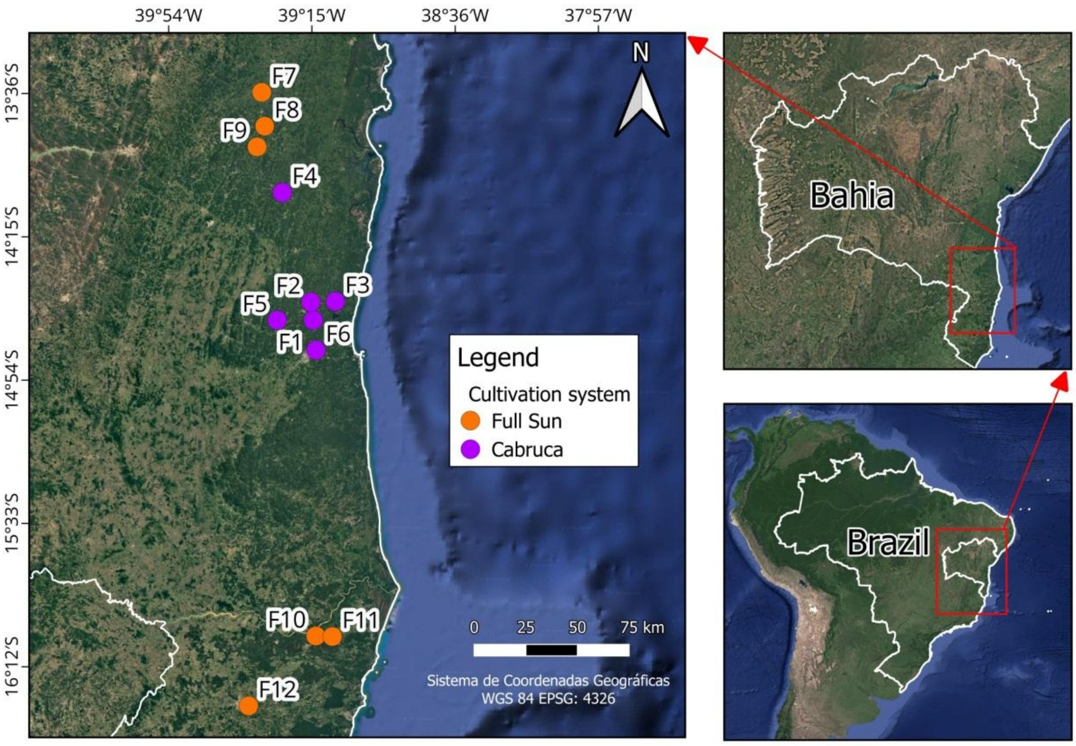


Figure 1. Location map of farms with soil, cocoa kernel, and leaf samples collected from CCN 51 and PS 13.19 cocoa trees in cabruca (F1 to F6: purple dots) and full sun (F7 to F12: orange dots) systems in Southern Bahia, Brazil.

On farms that adopt the cabruca system, the management practices observed included: pruning and thinning (for renewal or maintenance); manual or mechanized mowing (carried out three times a year); foliar fertilization and soil fertilization (twice a year); application of glyphosate herbicide (*Roundup*) (two to three times a year); and, on some properties, the use of fungicides as needed for phytosanitary purposes. The agroforestry system with cacao trees (*Theobroma cacao* L.) included several tree species, among which the following stood out: Jequitibá (*Cariniana* sp.), Sapucaia (*Lecythis pisonis*), Erithrina (*Erythrina* spp.), Cajá (*Spondias mombin*), Louro (*Cordia* spp.), Pau-brasil (*Paubrasilia echinata*), Jatobá (*Hymenaea courbaril*), Vinhático (*Plathymenia reticulata*), Cedro (*Cedrela odorata*), Araçá-d'água (*Psidium acutangulum*), Castanha-do-brasil (*Bertholletia excelsa*), Juerana (*Myrciaria cauliflora*), Seringueira (*Hevea brasiliensis*) and Bananeira (*Musa* spp.).

On properties that employ the full-sun cultivation system, intensive management practices predominate, such as: thinning and pruning (for renewal or maintenance); mechanized mowing complemented by the application of the herbicide glyphosate (*Roundup*); application of insecticides and fungicides (twice a year); modular fertilization (carried out four to five times a year); and controlled irrigation (by drip or micro-sprinkler).

Two cocoa cultivars were evaluated: CCN 51 and PS 13.19. The selection of these cultivars was due to their widespread use in the region, combined with the characteristics of high productivity and resistance to witches' broom disease (*Moniliophthora perniciosa*).

2.2. Soil and plant sampling

Soil, leaf, and fruit samples from cocoa trees of the two cocoa cultivars (CCN 51 and PS 13.19) were collected from selected farms in July and August 2022.

At each sampling site, four cocoa trees of approximately the same size and age were randomly selected, from which three to four ripe fruits from each plant were collected to compose a composite sample of cocoa kernels. The kernels were obtained after opening the fruit shells, removing the pulp and shell from the cocoa seed, and drying in an oven at 70°C for eight hours, until they reached approximately 5% moisture. After drying, the kernels were packaged in plastic bags and frozen in a freezer (approximately -18°C) until analysis. Therefore, two composite samples of cocoa kernels were obtained from each farm, one from the CCN 51 cultivar and the other from the PS 13.19 cultivar.

Samples of cacao leaves were collected from the same cacao plants where the fruits were collected. Four leaves were collected from each plant, considering the third leaf from the apex of a newly matured shoot, at mid-height of the plant's canopy. Leaves attacked by pests, diseases, or with mechanical injury were not collected. The leaves from the four plants of the same cultivar were mixed to obtain a composite sample. Thus, two composite samples of cacao leaves were obtained from each farm, one from each type of cultivar studied.

The leaves were then placed in paper bags and sent to the laboratory on the same day. When the time between collection and arrival at the laboratory exceeded two hours, the samples were kept in a thermal box with ice. Upon arrival at the laboratory, the leaves were washed with deionized water and dried in an oven at 70ºC until constant weight (Chepote et al., 2013; Marrocos et al., 1999). Next to each cocoa plant, within the canopy projection area, three mini trenches were dug to collect soil subsamples from 0 to 20 cm deep. After collection, the soil material was homogenized to obtain a composite sample. Two composite soil samples were collected from each farm, one for each cultivar evaluated. The samples were then air-dried and placed in plastic bags for laboratory analysis.

2.3. Sample analyses

For chemical characterization of the soils, the samples were analyzed for pH (in water); available P and K content (Mehlich^{-1}), Ca^{2+}, Mg^{+2} and Al^{3+} (1 mol.L^{-1} KCl) and total organic carbon (wet combustion). With the data from these analyses, the total cation exchange capacity at pH 7.0 was calculated. The physical characterization of the soils was carried out from the granulometric analysis, with separation of the fractions according to the method in Embrapa (2017).

Soil samples were dried in an oven (70 ºC for > 72 h), crushed, passed through a 2 mm sieve, weighed and subjected to digestion by the EPA 3051 method (USEPA, 2007), using nitric and hydrochloric acid solutions (3:1 v/v), in a microwave oven (Anton Paar Multiwave ECO, Software Version: 1.47). The digestion extract was used to determine the total concentrations of nutrients and heavy metals in the soil samples.

Leaf samples, after drying, were ground, passed through a 20 mesh sieve, and weighed. Cocoa kernel samples were thawed, crushed, and weighed. Both materials were digested in a nitro-perchloric acid solution (4:1 v/v) on a heating plate with an initial temperature of 80°C, gradually increasing the temperature to 180°C (Sarruge & Haag, 1974).

The chemical elements of cocoa kernels, cocoa leaves, and soils were quantified using an inductively coupled plasma optical emission spectrometer (ICP-OES) (Perkin Elmer Model Optima 8300 DV).

Reference plant and soil samples were included in the analyses for quality control. The recovery rates in the analysis of the plant sample (NIST - SRM 1575a, Pine Needles) were: P: 109 ± 0.18%; K: 97 ± 0.84%; Ca: 102 ± 0.53%; Al: 99 ± 0.66%; Cd: 99 ± 1.68%; Cu: 108 ± 0.34%; Fe: 100 ± 0.43%; Zn: 107 ± 0.44%.

2.4. Assessment of possible soil and cocoa kernel contamination

The levels of chemical elements found in the soil samples were compared with the Soil Quality Reference Values (VRQ), established by Normative Deliberation 166/2011 of the State Council for Environmental Policy of Minas Gerais (COPAM, 2011). The choice of Minas Gerais legislation is justified because the state of Bahia does not have a specific standard for this topic.

The levels of heavy metals in the cocoa kernels samples were compared with the maximum limits established by Brazilian legislation (ANVISA, Normative Instruction N°. 160/2022) and by the European Union (Commission Regulation), in addition to the regulatory values defined by other international jurisdictions.

2.5. Soil characterization

The physical and chemical characterization of the soil samples, segregated by farm, cultivation system, and cultivar, is presented in Table 1. Variabilities in soil attributes were observed, both between farms that share the same cultivation system (cabruca or full sun) and between farms with different systems.

It is essential to highlight the sampling methodology adopted: in each farm, two distinct composite samples were analyzed. Each composite sample represented a specific area, both under the same cultivation system (cabruca or full sun), but associated with different cocoa cultivars. Thus, this approach allowed the evaluation of two representative areas per farm.

The results demonstrated a prevalence of acidity in the soils that compromises the development of cocoa cultivation on the farms studied. Soil pH values ranging between 4.5 and 6.1 were observed. Although the ideal pH range for cocoa cultivation is stipulated between 5.7 and 6.2 (Souza Jr., Sodré and Neves, 2018), it was found that only 25% of the samples (four areas in the cabruca system and two in full sun) presented values above 5.7. These results indicate a problem of generalized acidity that demands correction.

As a consequence of the low base saturation, exchangeable Al^{3+}, recognized as the main limiting chemical factor in acidic soils due to its phytotoxicity, presented levels above the recommended value ($Al^{3+} < 0.3$ cmolc.dm^{-3}) (Souza Jr., Sodré and Neves, 2018) in six of the areas analyzed. It was observed that the problem is more pronounced in the full sun system (four areas) compared to the cabruca system (two areas).

Soil performance, with regard to fertility, also fell short of the values considered ideal for cocoa cultivation (P > 16 (mg.dm^{-3}), K > 90 (mg.dm^{-3}), $Ca^{2+} > 3$ (cmolc.dm^{-3}), $Mg^{2+} > 1$ (cmolc.dm^{-3}), CTC > 9 (cmolc.dm^{-3}), MO > 3.5 (dag.kg^{-1}) (Souza Jr., Sodré and Neves, 2018). Most of the essential components for fertility showed levels outside the recommended range, which indicates the need for attention in the management of cocoa trees (Table 1).

The results showed that in the cabruca system, only one of the 12 areas presented a level above the recommended value. In contrast, the full system proved to be more favorable to the availability of P in the soils, with values above the ideal in five areas.

Regarding K, satisfactory levels were observed in the soils of only four areas in each cropping system, highlighting a common risk to both management practices. The availability of Ca^{2+} in the soils was also low, with only two areas in the cabruca system and one in full sun showing values above the ideal. On the other hand, Mg^{2+} was the nutrient with the best performance in the cabruca system, where 83% of the samples recorded values above the recommended level. However, in the full sun system, only 16% (two areas) showed satisfactory levels.

The Cation Exchange Capacity (CTC) of the soils, an index of nutrient retention capacity, was mostly inadequate, although the cabruca system showed superior retention (four areas with adequate values) compared to full sun (only one area).

Similarly, the Organic Matter (MO) content was above the ideal in only one area for each cropping system. In summary, the predominance of acidic soils and the deficiency of essential nutrients demonstrate that, although the cabruca system presents better retention of Mg^{2+} and cations in general, both systems require urgent corrective interventions, especially in relation to acidity and the supply of P, K and Ca.

Regarding physical characterization, the soil texture varied between medium (n=14) and clayey (n=10), with a significant amount of sand at the different collection points.

Table 1. Chemical and physical characterization of the soils studied.

Farm	Cultivar	pH H_2O	P	K	Ca^{2+}	Mg^{2+}	Al^{3+}	CTC	MO dag.kg^{-1}	Clay	Silt	Sand
			-- mg.dm^{-3} --		--------- cmolc.dm^{-3} ---------					--------- % ---------		
						Cabruca system						
F1	CCN 51	5.0	14.5	145	2.6	1.9	0.4	12.1	4.4	34	18	48
	PS 13.19	5.3	3.4	24	3.9	2.1	0.2	10.4	2.0	21	17	62
F2	CCN 51	6.0	3.8	38	2.9	1.4	0.0	7.0	0.9	30	7	63
	PS 13.19	5.1	4.7	106	1.9	1.1	0.2	7.4	1.6	44	21	35
F3	CCN 51	6.1	29.0	60	5.7	2.0	0.0	12.2	3.3	34	15	51
	PS 13.19	6.1	2.5	20	2.4	1.3	0.0	7.0	2.4	40	15	45
F4	CCN 51	5.9	2.0	108	2.4	0.7	0.0	5.7	1.5	16	9	75
	PS 13.19	5.6	1.1	10	2.5	1.2	0.0	7.4	3.1	37	9	54
F5	CCN 51	4.9	1.5	14	1.8	0.9	0.1	7.5	2.7	42	5	53
	PS 13.19	5.4	1.2	36	2.6	2.0	0.1	8.9	2.8	35	7	58
F6	CCN 51	5.2	0.7	18	1.8	1.1	0.1	6.9	2.7	39	11	50
	PS 13.19	4.7	6.8	225	1.9	1.2	2.6	11.8	1.5	34	26	40
						Full sun system						
F7	CCN 51	5.3	0.2	14	1.2	0.6	0.1	5.5	2.1	61	4	35
	PS 13.19	5.1	0.4	14	0.9	0.4	0.4	6.1	2.7	61	5	34
F8	CCN 51	5.4	1.1	16	1.5	0.8	0.1	7.5	4.1	52	4	44
	PS 13.19	5.8	19.7	50	2.8	1.7	0.0	7.3	2.4	49	4	47
F9	CCN 51	4.9	4.7	62	1.4	0.6	0.3	6.7	2.3	30	3	67

	PS 13.19	5.6	2.7	80	2.3	0.8	0.0	5.9	1.9	22	5	73
F10	CCN 51	5.2	12.0	64	4.3	1.8	0.7	12.1	1.7	22	18	60
	PS 13.19	5.8	18.0	70	1.4	0.4	0.1	5.0	1.5	15	3	82
F11	CCN 51	5.6	35.0	66	2.0	0.2	0.1	5.4	1.3	14	1	85
	PS 13.19	5.1	127.7	94	1.1	0.1	0.1	5.5	1.5	8	2	90
F12	CCN 51	4.5	47.4	132	0.7	0.2	0.9	7.8	2.0	23	2	75
	PS 13.19	5.6	9.0	134	1.4	0.6	0.0	5.2	1.5	18	3	79
Reference (*)		5.7-6.2	>16	>90	>3	>1	<0.3	>9	>3.5			

Soil samples collected at a depth of 0 to 20 cm.

(*) Values considered good for cocoa cultivation (Souza Jr. et al., 2018).

2.6. Statistical analysis

The data were analyzed in a 2x2 factorial design, with the first factor being the management of cocoa systems (cabruca and full sun) and the second factor being the plant cultivar CCN 51 or PS 13.19. All statistical analyses were conducted using R software version 4.3.2 (Core Team, 2023).

The analysis of variance (ANOVA) considered $p<0.10$. When the data did not meet the normality of the residuals, the data were transformed. If they still did not meet the ANOVA assumptions, the data were compared using the non-parametric Kruskal-Wallis test ($p<0.10$). After the ANOVA, the means were compared ($p<0.10$) using Tukey's test using the ExpDes.pt package and the fat2.dic function.

For correlation analysis between variables, Spearman correlation was performed. Only correlation coefficients < - 0.70 or > 0.70 with $p<0.10$ were considered. The correlation matrix was created using the psych package and the corTest function. In the correlation studies, four comparison groups were considered. The first group analyzed the relationship between the total levels of chemical elements present in the soils and cocoa kernels; the second group analyzed the correlations between the total levels in the soils and leaves; the third group analyzed the relationship between the total level in the leaves and kernels, and in the fourth group, all the variables studied were analyzed.

Principal Component Analysis (PCA) was performed to relate the nutrient and metal levels of cocoa kernels, leaves, and soil with the chemical variables of the soil in each management and cocoa cultivar evaluated. For the PCA, the most important variables were used, considered those with eigenvectors correlated with the eigenvalues between - 0.70 and 0.70. In the PCA analysis, the FactoMineR, factoextra, and dendextend packages were used, and ggplot2 was used for graphical representation.

3. Results and discussion

3.1. Assessment of potential soil contamination

The assessment of potential soil contamination and the risks associated with the environment and human health is commonly carried out using reference values established in legal standards (Santos et al., 2013). For this study, the Reference Quality Values (RQV) for soils, defined by Normative Deliberation 166/2011 of the State Council for Environmental Policy of Minas Gerais (COPAM, 2011), were adopted, since the state of Bahia does not have specific legislation on the subject.

The results for the average content of nutrients and metals in soils, leaves and cocoa kernels produced in cabruca (Cab) and full sun systems (FS) are presented in Table 2. Considering the normative limits of COPAM (2011), 46.5 $mg.kg^{-1}$ for Zn and 75 $mg.kg^{-1}$ for Cr, the Zn and Cr contents in soil samples from some of the investigated farms exceeded their respective RQVs. Although the overall average of Zn contents in the soil samples did not exceed the RQV (Table 2), individual results from three of the 24 evaluated areas were higher than the limit. Specifically, one farm in the cabruca system (Farm 2 PS 13.19) recorded 172.37 ± 2.0 $mg.kg^{-1}$, while two in the full sun system (Farm 10 CCN 51 and Farm 11 CCN 51) presented 56.4 ± 3.0 $mg.kg^{-1}$ and 155.47 ± 1.0 $mg.kg^{-1}$, respectively. Although some Zn levels in the soil exceeded the RQV, this fact presents less immediate environmental concern, as the maximum values found did not exceed the Prevention Value (PV) of 300 $mg.kg^{-1}$ (COPAM, 2011).

Similarly, the average Cr content in the analyzed areas remained below the reference RQV (Table 2). However, this compliance was not maintained in eight of the farms studied, which recorded high levels. Among these, four operate in the cabruca system (Farm 3 CCN 51: 153.54 ± 3.0 $mg.kg^{-1}$; Farm 3 PS 13.19: 232.73 ± 10.0 $mg.kg^{-1}$; Farm 4 CCN 51: 112.03 ± 4.0 $mg.kg^{-1}$; and Farm 5 CCN 51: 88.71 $mg.kg^{-1}$). The four remaining areas utilize the full sun system (Farm 7 CCN 51: 77.80 ± 2.0 $mg.kg^{-1}$; Farm 7 PS 13.19: 143.97 ± 1.0 $mg.kg^{-1}$; Farm 8 CCN 51: 102.00 ± 3.0 $mg.kg^{-1}$; and Farm 8 PS 13.19: 123.15 ± 12.0 $mg.kg^{-1}$). The Cr situation requires greater attention, since the PV is also 75 $mg.kg^{-1}$. It is crucial to note that, for Cr in agricultural soils, the VRQ and VP are equivalent, which intensifies the need for vigilance. Cr levels above 100 mg.kg-1 in the soil already signal the need for specific procedures with the competent environmental agency for monitoring and managing the area, and may indicate a potential risk of contamination.

Zn is an essential micronutrient for the growth and development of various crops, including the cacao tree, which has a high demand for this element (Marrocos et al., 2020). In plant physiology, Zn acts as an enzymatic cofactor in important metabolic processes, playing a crucial role in protein synthesis and cell growth (Marschner, 1995; Alloway, 2013, Neto et al., 2015). Although fundamental, the nutritional balance of Zn in the soil is delicate, as high concentrations can generate toxicity for plants and microorganisms, negatively impacting fertility and agricultural productivity (Rutkowska et al., 2015).

Zn deficiency has been frequently reported in cacao plantations in southern Bahia (Chepote et al., 2013). To correct this deficiency, zinc sulfate ($ZnSO_4$), due to its high solubility in water, is the most commonly used fertilizer (Malavolta, 1987; Chepote et al., 2013), especially in fertigation systems. However, managing Zn fertilization in cocoa trees, particularly in full-sun cultivation systems, represents a significant challenge. The application of Zn requires precise management, based on soil and foliar analyses. Inadequate dosage, especially when the ideal concentration for the plant is exceeded, can lead to excessive accumulation of Zn in the soil, a phenomenon observed on some farms in this study. This accumulation represents a risk of toxicity to the cocoa tree and the microbial community, compromising soil health and the long-term sustainability of production. The need for Zn and the potential for excess in the soil therefore represent a fine line that requires attention and careful management.

Similarly, high concentrations of Cr in soils can accumulate in plant tissues, promoting toxic effects that result in molecular, biochemical, and ultrastructural alterations (Nascimento et al., 2018). Cr toxicity in plants depends on its speciation and manifests itself at various levels, including reduced productivity, negative effects on growth (leaves and roots), enzyme inhibition, and mutagenesis (Shanker et al., 2005). According to Bastos et al. (2024), a notable point in the geochemical profile of Bahia is the variation in elements typically associated with mafic rocks. Although the area has rocks rich in minerals that are primary sources of Cr and Ni, some assessments of geochemical background levels in the soils of the Atlantic Forest have indicated that the concentrations of Cr and Ni may be lower compared to other potentially toxic elements found in the same soil. This observation highlights the complexity of the regional distribution, which is highly influenced by the specific type of parent rock and the degree of leaching, requiring the determination of local VRQs (Steffens et al., 2025). The use of fertilizers, soil amendments, and especially some pesticides in the extensive cocoa-growing area (and other crops) can introduce Cr into the soil, since many phosphate and limestone fertilizers may contain Cr as an impurity (Zayed and Terry, 2003).

In view of the above, it is suggested that the geogenic source is the main responsible for the presence of Cr in the soils of Southern Bahia, resulting from the weathering of the rocks of the São Francisco Craton. However, for the purposes of monitoring and protecting human health, it is essential that soil studies continue to isolate and quantify potential anthropogenic sources, such as agricultural inputs and organic waste, in order to ensure food security and soil quality in the Atlantic Forest biome (Bastos et al., 2024).

3.2. Assessment of potential contamination of cocoa kernels

There is currently serious global concern regarding the presence of heavy metals in cocoa beans and their derivatives (Maddela et al., 2020). In response to this situation, several international bodies have established maximum acceptable levels of heavy metals in chocolates and cocoa-based products. In this study, all cocoa kernel samples analyzed did not present quantifiable levels of As, Pb, and Cd (Table 4). In the case of Cu, the levels obtained were from 11.6 to 15.2 $mg.kg^{-1}$, below the maximum limit tolerated by Anvisa. Regarding Ni, the total levels found in the cocoa kernels were between 1.7 and 3.4 $mg.kg^{-1}$, all also below the limit recommended by Regulation (EU) 2024/1987. These results indicated that the kernels grown in the Southern region of Bahia do not present contamination by Cd and Pb in their composition. The fact that levels of As, Cd, and Pb were quantified in the soils studied, but not in the kernels, suggests a low translocation or bioavailability of these metals to the fruit.

In Brazil, Normative Instruction No. 160, of July 1, 2022, from Anvisa, establishes the maximum tolerated limits (MTL) of contaminants in food. In this case, for chocolates and cocoa-based products with more than 40% cocoa in their total composition, the maximum limits are 0.30 $mg.kg^{-1}$ of Cd and 0.40 $mg.kg^{-1}$ of Pb or As. For chocolates and products with less than 40% cocoa, the maximum limits are 0.20 $mg.kg^{-1}$ of Cd, Pb, or As. While cocoa paste has higher limits of 0.50 $mg.kg^{-1}$ of Cd, Pb, or As. In addition, cocoa beans may contain 40 $mg.kg^{-1}$ of Cu (ANVISA, 2022).

The European Commission, through Regulation (EU) N°. 488/2014, defined maximum levels for Cd and Pb that came into force in 2019. In milk chocolates with less than 30% cocoa solids, the permitted limit for Cd is 0.10 $mg.kg^{-1}$ of product; in chocolates with more than 30% and less than 50% cocoa, the limit is 0.30 $mg.kg^{-1}$; in dark chocolates with more than 50% cocoa, the limit is 0.8 $mg.kg^{-1}$; and for cocoa powder sold to the final consumer, the limit for

Cd is 0.60 mg.kg^{-1} of product. The maximum limit for Pb in chocolate and cocoa products is 0.10 mg.kg^{-1} (COMMISSION REGULATION EU, 2014).

Australia and New Zealand have established a maximum level of 0.5 mg.kg^{-1} of Cd for chocolates and cocoa products (Food Standards Code, 2016). China has introduced maximum levels of 0.5 mg.kg^{-1} for Pb and As in cocoa products, chocolates, and candies (Malaysian Cocoa Board, 2018). The US Food and Drug Administration (FDA) has issued guidelines for a maximum limit of 0.1 mg.kg^{-1} of Pb in candies, including chocolates (US Food and Drug Administration, 2006). The Codex Alimentarius has adopted a limit of 2.0 mg.kg^{-1} of Cd in cocoa beans (Codex Alimentarius Commission, 2022).

Recently, the European Union (EU) adopted Regulation (EU) 2024/1987, which amends Regulation (EU) 2023/915, establishing new maximum Ni levels for various food categories, including cocoa and its derivatives. These regulatory limits came into effect on July 1, 2025. The maximum Ni limits in cocoa products were differentiated according to the concentration of cocoa dry solids, as detailed below: cocoa powder (sold to the end consumer, or as an ingredient in sweetened cocoa powder, or as drinking chocolate): 15 mg.kg^{-1} of Ni; milk chocolate with a total cocoa dry solids content of less than 30%: 2.5 mg.kg^{-1} of Ni; chocolate with a total cocoa dry solids content equal to or greater than 30% and less than 50% and milk chocolate with a total cocoa dry solids content equal to or greater than 30%: 7 mg.kg^{-1} of Ni.

It is imperative to highlight that in this study, all cocoa kernel samples analyzed did not present quantifiable levels of As, Pb, and Cd (Table 3). In the case of Cu, the levels obtained were from 11.6 to 15.2 mg.kg^{-1}, below the maximum limit tolerated by Anvisa. Regarding Ni, the total levels found in the cocoa kernels were between 1.7 and 3.4 mg.kg^{-1} of Ni, all also below the limit recommended by Regulation (EU) 2024/1987.

These results indicate that the kernels grown in the Southern region of Bahia do not present contamination by Cd and Pb in their composition. The fact that levels of As, Cd, and Pb were quantified in the soils studied, but not in the kernels, suggests a low translocation or bioavailability of these metals to the fruit.

In addition to these elements, other heavy metals were identified in the analyzed cocoa kernels, with levels ranging from 4.3 to 7.7 mg.kg^{-1} of Al and from 0.51 to 0.54 mg.kg^{-1} of Cr. Currently, the European Commission does not establish mandatory maximum limits for Al and Cr in cocoa and its derivatives. However, the European Food Safety Authority (EFSA) has been monitoring the occurrence of these metals, observing that cocoa and chocolate have shown high levels of Al compared to most other foods.

Although Al monitoring is an area of attention and some national authorities, such as those in Belgium, have already established action levels, there is still no harmonized regulation in the EU (EFSA, 2008). It is important to emphasize that Al^{3+} is absorbed by plants in soils with very acidic pH, suggesting that proper liming management could reduce or eliminate the Al content in cocoa kernels, since the ideal pH for cocoa cultivation is between 5.7 and 6.2 (Souza Júnior, Sodré & Neves, 2018).

Studies indicate that, in addition to the total Cd content in the soil, pH, clay content, the presence of competing metals (such as Zn), and organic matter content are crucial factors that influence the availability of Cd to plants (Chavez et al., 2015). Additionally, Cd absorption can vary between different cocoa cultivars (Clemens & Ma., 2016) and different types of cultivation (Gramlich et al., 2017). In this specific study, a significant influence of the type of cultivation system was observed only on the total Ni and As contents of the soils and on the total Ni content in cocoa kernels.

The interaction between metals in the absorption process is complex, with conflicting reports of synergism and antagonism between Zn and Cd, while interactions between P and As appear to be antagonistic (Kabata-Pendias, 2011; Alloway, 2013). Blommaert et al. (2022), studying Cd translocation in a *Theobroma cacao* L. cultivar with high cadmium accumulation, observed a positive correlation between Cd, Zn, Mn, Ca, and Mg, suggesting that these tend to be absorbed and/or distributed together. On the other hand, P, Cu, S, and K showed a negative correlation with Cd. In terms of distribution and specific retention, P exhibited markedly higher retention relative to Cd (high P/Cd) in the cocoa bean than in other tissues, and K showed remarkably high retention relative to Cd (high K/Cd) in the pod shell.

In cocoa cultivation, K is required in large quantities, with 824.2 kg ha^{-1} needed to produce 1,000 kg of dry seeds per year (Thong & Ng, 1980). K is the nutrient with the highest accumulation in plant tissue, with 8% of the total accumulated being used in fruit production, and 80% of this nutrient retained in the husk. Results obtained by Humphries (1950) suggest that fruit abortion is caused by competition for nutrient utilization, mainly K, which influences fruit size. Given these findings, it is suggestive that adequate K content in soils can decrease cadmium absorption by the plant or, at least, reduce the distribution/retention of cadmium in the bean and husk of the fruits.

3.3. Effects of treatments

Overall, the statistical analysis indicated only isolated effects of the evaluated factors, i.e., the cultivation systems and/or the evaluated cultivars. The interaction effect between cultivation system and cultivar was verified only for the K content in the cocoa leaves.

3.3.1. Effect of the cultivation system

The total analysis of the chemical elements present in the soils collected in the cabruca system and in full sun indicated few differences between the systems (Table 2). While the cabruca system was associated with higher levels of Mg, Fe, Mn, and Ni, in full sun the superiority was only verified for As.

Table 2. Average content of nutrients and metals in soils, leaves and cocoa kernels produced in cabruca (Cab) and full sun systems (FS).

		Soil		Leaves		Cocoa kernels	
		Cab	FS	Cab	FS	Cab	FS
P	$g.kg^{-1}$	0.78±0.55^{A}	0.83±1.19^{A}	1.38±0.17^{A}	1.44±0.18^{A}	5.14±0.38^{A}	5.23 ±0.37^{A}
K	$g.kg^{-1}$	0.93±1.36^{A}	0.46±0.28^{A}	mi*	mi*	9.51±0.57^{A}	9.75±0.59^{A}
Ca	$g.kg^{-1}$	2.31±1.66^{A}	1.90±0.96^{A}	13.75±3.20^{A}	12.72±2.99^{A}	0.69±0.13^{A}	0.55±0.07^{B}
Mg	$g.kg^{-1}$	1.76±1.47^{A}	0.58±0.33^{B}	6.05±0.63^{A}	5.30±1.17^{B}	3.35±0.17^{A}	3.42±0.08^{A}
S	$g.kg^{-1}$	-	-	1.82±0.19^{A}	1.85±0.16^{A}	1.34±0.11^{A}	1.38±0.08^{A}
Cu	$mg.kg^{-1}$	-	-	4.77±1.22^{A}	4.90±1.95^{A}	15.20±5.32^{A}	11.64±3.51^{B}
Fe	$mg.kg^{-1}$	4.26±1.14^{A}	3.00±2.31^{B}	19.77±0.14^{A}	19.72±0.13^{A}	25.88±10.42^{A}	18.86±3.56^{B}
Zn	$mg.kg^{-1}$	37.57±44.26^{A}	38.80±38.97^{A}	37.38±23.10^{A}	39.05±17.20^{A}	42.62±7.40^{B}	50.92±7.23^{A}
Mn	$mg.kg^{-1}$	537.80±571.63^{A}	81.09±57.24^{B}	197.14±168.88^{A}	121.21±149.43^{B}	17.25±12.12^{A}	8.96±4.41^{B}
Na	$mg.kg^{-1}$	288.86±98.31^{A}	248.61±39.98^{A}	198.04±41.51^{B}	250.94±76.03^{A}	33.61±4.14^{A}	39.77±14.41^{A}
Cr	$mg.kg^{-1}$	67.23±69.43^{A}	48.16±49.73^{A}	nq	nq	0.54±0.15^{A}	0.51±0.09^{A}
Ni	$mg.kg^{-1}$	5.80±2.92^{A}	4.18±3.23^{B}	2.00±2.03^{A}	0.92±1.38^{A}	3.37±1.98^{A}	1.76±1.93^{B}
Al	$g.kg^{-1}$	40.5±14.01^{A}	38.6±14.25^{A}	-	-	7.71±8.83^{A}	4.33±3.03^{A}
Cd	$mg.kg^{-1}$	0.11±0.12^{A}	0.09±0.12^{A}	nq	nq	nq	nq
Pb	$mg.kg^{-1}$	5.99±4.33^{A}	6.90±4.78^{A}	nq	nq	nq	nq
B	$mg.kg^{-1}$	-	-	33.45±4.16^{B}	40.00±5.27^{A}	nq	nq
As	$mg.kg^{-1}$	1.99±1.92^{B}	3.75±1.89^{A}	-	-	nq	nq
Co	$mg.kg^{-1}$	-	-	3.65±3.73^{A}	1.21±1.81^{B}	-	-
Se	$mg.kg^{-1}$	-	-	0.11±0.28^{A}	0.13±0.46^{A}	-	-

Different capital letters in the lines indicate differences ($p<0.10$) between cultivation systems. (*mi) = significant interaction. nq: not quantified. (-) element not analyzed in the sample.

The nutrient content in cacao leaves can be influenced by several factors, such as nutrient availability in the soil, soil characteristics, management practices, genotype, climate, plant age, among others. Plant tissue analysis is an important tool for diagnosing the nutritional status of the plant, with the leaf being the most commonly used tissue for this assessment (Souza Jr., Marrocos and Neves, 2018).

Similarly to soil samples, the analysis of the chemical composition of cacao leaves in different management systems did not vary much. Leaf samples from the cabruca system showed higher levels of Mg, Mn, and Co, while in full sun, higher levels of Na and B were observed (Table 2). The average leaf contents obtained in this study indicated adequate nutrition for the nutrients Ca (8-12 $g.kg^{-1}$), Mg (4-7 $g.kg^{-1}$), S (1-2 $g.kg^{-1}$), Mn (100-400 $mg.kg^{-1}$) and B (30-70 $mg.kg^{-1}$), regardless of the cultivation system, according to the recommendation of Souza Jr., Marrocos and Neves (2018) presented in parentheses. It is noted, however, that on average the leaf contents of P (1.7-2.3 $g.kg^{-1}$), K (18-23 $g.kg^{-1}$), Cu (10-20 $mg.kg^{-1}$), Fe (50-150 $mg.kg^{-1}$) and Zn (60-140 $mg.kg^{-1}$) do not reach the nutrient sufficiency range for the cocoa tree.

Evaluating the treatments adopted in this study, as mentioned, an interaction effect between cultivation system and cultivar was observed on the K content obtained in the leaves of cocoa trees (Table 3). A higher content of this element was obtained in the leaves of cocoa trees grown in full sun than in the cabruca system, an effect that was observed in the CCN 51 cultivar, while no differences were observed between the cultivation systems for PS 13.19. The differences in K content of the different treatments are not so significant from a practical point of view and, as already reported, all are below the range of nutritional sufficiency for the cocoa tree. This is concerning, as K is a nutrient known to promote the growth of plant roots, favoring water absorption under drought conditions (Kaba et al., 2022). Additionally, K is the second most effectively exported nutrient by the cacao tree, with a large part concentrated in the fruit's husk, and a smaller part in the seed, although some returns as residue to the crop (Silva, 2009; Souza Jr., Marrocos and Neves, 2018).

In any case, the differences in K content of the different treatments are not so significant from a practical point of view and, as already reported, all are below the range of nutritional sufficiency for the cocoa tree. This is worrying, as K is a nutrient known to promote the growth of plant roots, favoring water absorption under drought conditions (Kaba et al., 2022). Additionally, K is the second most effectively exported nutrient by the cacao tree, with a large part concentrated in the fruit's husk, and a smaller part in the seed, although some returns as residue to the crop (Silva, 2009; Souza Jr., Marrocos and Neves, 2018).

Table 3. Potassium content in leaves of CCN 51 and PS 13.19 cocoa trees produced in cabruca and full sun systems.

		Cabruca	**Full sun**
K (leaves)	**CCN 51**	4.83^{Ab}	6.6^{Aa}
($g.kg^{-1}$)	**PS 13.19**	5.49^{Aa}	4.46^{Ba}

Different uppercase letters in the columns indicate differences between cultivar types, and different lowercase letters in the rows indicate differences between cultivation systems ($p<0.10$).

Cocoa beans are a highly rich source of various minerals essential for human health. However, as with other plant-based foods, the mineral content of cocoa reflects the characteristics of the soil in which it is grown and the management practices adopted, in addition to other possible sources of variability (Cinquanta et al., 2016). In this study, differences in mineral content in cocoa kernels were limited to a few chemical elements (Table 2). While higher levels of Ca, Cu, Mn, Fe, and Ni were observed in cocoa kernels in the cabruca system, superior levels of Zn were observed in the full sun system. In descending order, the levels of the most abundant mineral nutrients obtained in cocoa kernels were: K (9.5-9.7 $g.kg^{-1}$), P (5.1-5.2 $g.kg^{-1}$), Mg (3.3-3.4 $g.kg^{-1}$), S (1.34-1.38 $g.kg^{-1}$), Ca (0.55-0.69 $g.kg^{-1}$), Zn (42.6-50.9 $mg.kg^{-1}$), Na (33.6-39.7 $mg.kg^{-1}$), Fe (18.7-24.9 $mg.kg^{-1}$) and Cu (11.2-15.2 $mg.kg^{-1}$).

In descending order, the levels of the most abundant mineral nutrients obtained in cocoa kernels were: K (9.5-9.7 $g.kg^{-1}$), P (5.1-5.2 $g.kg^{-1}$), Mg (3.3-3.4 $g.kg^{-1}$), S (1.34-1.38 $g.kg^{-1}$), Ca (0.55-0.69 $g.kg^{-1}$), Zn (42.6-50.9 $mg.kg^{-1}$), Na (33.6-39.7 $mg.kg^{-1}$), Fe (18.7-24.9 $mg.kg^{-1}$) and Cu (11.2-15.2 $mg.kg^{-1}$).

K was the element with the highest concentration and notably the most exported to the cocoa kernels, highlighting its importance in the composition of the fruits and the nutritional status of the crop. It is relevant to note that, although the leaf levels of K, P, Fe, Zn, and Cu were observed to be below the sufficiency range for the cocoa tree, the cocoa kernels presented significant amounts of these elements. This observation suggests that, under ideal conditions of nutritional sufficiency for the plant, the potential for enriching the cocoa beans with minerals essential for human health would be even greater.

The mineral levels found in this study are comparable to or higher than those reported in other studies in the literature. When analyzing cocoa fruits of the Criollo and hybrid types, originating from Peru, Ecuador, and Ghana, Grassia et al. (2019) verified an average mineral abundance, with emphasis on K (4.1-5.2 $g.kg^{-1}$), P (2.5-4.2 $g.kg^{-1}$), Mg (1.9-2.6 $g.kg^{-1}$), Ca (0.40-0.56 $g.kg^{-1}$), and Na (35-64 $mg.kg^{-1}$). In contrast, Cinquanta et al. (2016) reported mineral contents in Ghanaian cocoa beans closer to our results for some elements, such as K (7.2 $g.kg^{-}$

$^{-1}$), P (5.2 g.kg^{-1}) and Mg (2.9 g.kg^{-1}), while the contents of Fe (32 mg.kg^{-1}) and Cu (31.2 mg.kg^{-1}) found by them were higher than those in the present study.

3.3.2. Effect of cultivar type

As observed in the results (Table 4), the impact of the genotype on the levels of chemical elements was predominantly restricted to a few elements, indicating that mineral variability may have been more effectively controlled by edaphic or management factors, acting as limiting or dominant factors. However, the differences found for Ca, Zn, B, and S indicate the importance of genetic control in the absorption and translocation of nutrients in cocoa trees.

The superiority of the CCN 51 cultivar in the accumulation of Zn in the cocoa kernels and of Ca in the soil and leaves is particularly relevant. Zn and Ca are vital elements with structural and enzymatic functions in the plant (Lewis et al., 2018). The higher concentration of Zn in CCN 51 suggests that this cultivar has greater efficiency in the translocation of this micronutrient from vegetative structures to seeds compared to PS 13.19.

In turn, the PS 13.19 cultivar showed higher levels of B in the leaves and S in the kernels. PS 13.19 is recognized for its stress tolerance characteristics and exhibits distinct physiological strategies compared to CCN 51 (Santos et al., 2014; Araújo et al., 2024). This difference may be linked to its genetic strategy of absorption and preferential allocation of nutrients in specific tissues.

The results found in this study suggest the methodological relevance of evaluating different cultivars under similar edaphoclimatic conditions. Observing the variations in the absorption and partitioning of chemical elements between the CCN 51 and PS 13.19 genotypes on the same farm allowed us to observe the influence of the genotype. This evidence underlines the need for future research aimed at determining specific nutritional demands and formulating genotype-adjusted fertilization programs for high-yielding cultivars.

Table 4. Average content of nutrients and metals in soils, leaves and cocoa kernels of CCN 51 and PS 13.19.

		Soil		**Leaves**		**Cocoa kernels**	
		CCN 51	**PS 13.19**	**CCN 51**	**PS 13.19**	**CCN 51**	**PS 13.19**
P	g.kg^{-1}	0.91±1.07^{a}	0.70±0.56^{a}	1.46±0.17^{a}	1.35±0.16^{a}	5.23±0.26^{a}	5.14±0.46^{a}
K	g.kg^{-1}	0.63±0.79^{a}	0.76±1.19^{a}	mi*	mi*	9.60±0.44^{a}	9.67±0.71^{a}
Ca	g.kg^{-1}	2.57±1.57^{a}	1.64±0.91^{b}	14.73±2.80^{a}	11.75±2.66^{b}	0.62±0.09^{a}	0.62 ±0.15^{a}
Mg	g.kg^{-1}	1.28±1.01^{a}	1.07±1.41^{a}	5.84±0.93^{a}	5.52±1.07^{a}	3.39±0.12^{a}	3.37±0.14^{a}
S	g.kg^{-1}	-	-	1.87±0.17^{a}	1.80±0.17^{a}	1.31±0.05^{b}	1.42±0.10^{a}
Cu	mg.kg^{-1}	-	-	5.16±1.78^{a}	4.51±1.38^{a}	14.08±4.57^{a}	12.76±5.07^{a}

Fe	mg.kg-1	3.69±1.86a	3.57±2.01a	19.77±0.17a	19.72±0.10a	23.82±9.94a	20.92±6.68a
Zn	mg.kg-1	37.86±39.69a	38.51±43.62a	32.63±10.71a	43.81±25.46a	49.83±7.64a	43.71±8.12b
Mn	mg.kg-1	282.37±466.02a	336.52±474.38a	165.41±167.60a	152.94±160.68a	12.19±7.20a	14.02±12.25a
Na	mg.kg-1	261.15±66.27a	276.32±87.35a	213.12±41.80a	235.86±83.67a	34.98±6.77a	38.41±13.90a
Cr	mg.kg-1	57.23±49.65a	58.16±71.59a	nq	nq	0.51±0.12a	0.54±0.13a
Ni	mg.kg-1	5.13±3.11a	4.85±3.26a	1.51±2.04a	1.41±1.58a	2.51±1.86a	2.63±2.36a
Al	g.kg-1	42.74±15a	36.35±12.41a	-	-	6.87±8.65a	5.17±4.14a
Cd	mg.kg-1	0.09±0.11a	0.11±0.13a	nq	nq	Nq	nq
Pb	mg.kg-1	6.91±4+97a	5.98±4.11a	nq	nq	Nq	nq
B	mg.kg-1	-	-	34.10±4.66b	39.35±5.62a	Nq	nq
As	mg.kg-1	2.75±2.23a	2.99±1.99a	-	-	Nq	nq
Co	mg.kg-1	-	-	2.92±3.97a	1.94±2.03a	-	-
Se	mg.kg-1	-	-	0.21±0.51a	0.038±0.13a	-	-

Different lowercase letters in the lines indicate differences (p<0.10) between cropping systems. (*mi) = significant interaction. nq: not quantified. (-) element not analyzed in the sample.

3.4. Correlations and multivariate analyses

3.4.1 Correlations

In this study, correlations ($r > 0.70$ or $r < -0.70$ and $p < 0.10$) between the total levels of chemical elements found in soil, leaf, and cocoa kernel samples were analyzed. The results are presented in Table 5. Mn was the only element to show a significant positive correlation between total levels in the soil and its concentration in plant tissues. This association was observed both between the soil and the kernels and between the soil and the leaves, indicating a direct absorption and translocation of this element as a function of its edaphic availability.

In addition, cross-correlations were obtained between different elements. The positive association between Cr and Fe levels in the soil and Mg levels in the leaves stands out, as well as the positive correlation between available Mg in the soil and the total Ni content in the kernels.

Analysis of the relationships between plant tissues revealed a significant direct correlation between Ni content in leaves and Ni content in cocoa kernels, suggesting efficient translocation of this element. A similar positive correlation was observed for Mn (leaves vs. cocoa kernels). Additionally, in cocoa kernels, a positive correlation was found between total Mn and Ca contents.

Finally, significant positive correlations between total contents of different chemical elements in the soil were found between: K with P; Fe with Cd; Zn with P and K; and Cr with Cd and K. The only significant negative correlation detected in the soil occurred between total Cr and K contents.

Table 5. Spearman correlation coefficients between nutrient and heavy metal content of soils, cocoa leaves and cocoa cocoa kernels CCN 51 and PS 13.19 produced in cabruca and full sun systems.

		Correlation coefficient	**p-value***
Mn_soil_total	Mn_kernels	0.759	0.0392
Mn_soil_total	Mn_leaf	0.710	0.0001
Cr_soil_total	Mg_leaf	0.709	0.0001
Fe_soil_total	Mg_leaf	0.743	0.0746
Mg_disp	Ni_kernels	0.704	0.0001
Ni_leaf	Ni_kernels	0.858	0.0085
Mn_leaf	Mn_kernels	0.835	0.0393
Mn_kernels	Ca_kernels	0.809	0.0040
K_soil_total	P_soil_total	0.777	0.0189
Fe_soil_total	Cd_soil_total	0.830	0.0531
Zn_soil_total	P_soil_total	0.740	0.0822
Zn_soil_total	K_soil_total	0.768	0.0274
Cr_soil_total	Cd_soil_total	0.749	0.0688
Cr_soil_total	K_soil_total	-0.825	0.0016

(*): ($p < 0.1$).

Nutrient absorption and element accumulation in different parts of the plant are modulated by a complex network of edaphic, climatic, and genetic factors (Rana and Maiti, 2018; Macedo et al., 2020). However, a result of particular agronomic and environmental interest is the only significant negative correlation detected, which occurred between the total Cr and K contents in the soil. This relationship suggests that an increase in Cr concentration in the soil may be associated with a reduction in the availability or pool of K in the same compartment, or vice versa.

This interaction can be explained by ionic competition or by the impact of Cr on K dynamics in the soil. Although Cr is a heavy metal and K is an essential macronutrient (monovalent cation), the presence of Cr in its ionic forms (Cr (III) or Cr (VI)) can affect the retention of cations (K^+) in the exchange complex, especially in soils with low Cation Exchange Capacity (CEC) or in competition for adsorption sites (Shanker et al., 2005). The negative correlation between Cr and K is especially concerning in the context of cocoa. As discussed, potassium is the most exported nutrient by the fruit and is often limiting in the crop (Araujo et al., 2017). If high levels of Cr in the soil are inhibiting or indirectly competing with available K, this could worsen the nutritional status of the cocoa tree with respect to K and compromise the productivity and quality of the beans.

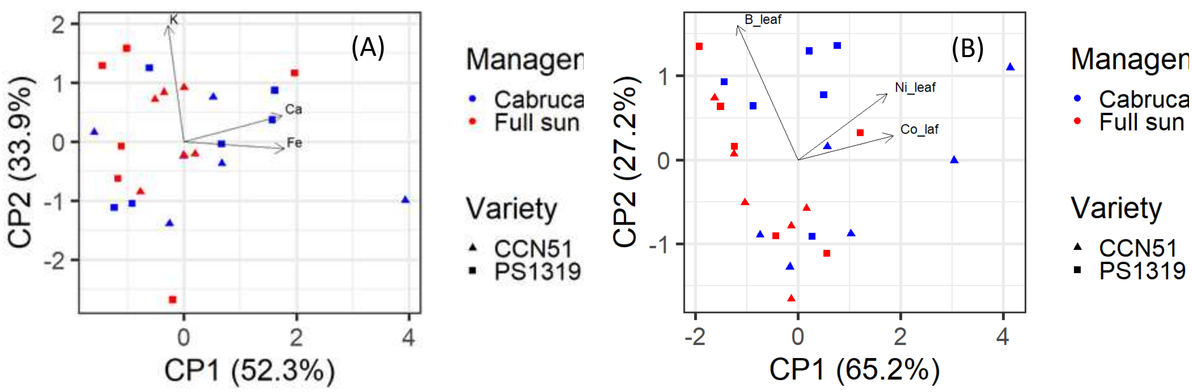
In this sense, it seems reasonable to suggest that maintaining an adequate level of K in the soil is crucial for plant health. However, Cr tends to be strongly adsorbed and complexed by organic matter (Ertani et al., 2017). An integrated management strategy that includes correcting soil acidity and increasing CEC and organic matter (for example, by incorporating the fruit peel, observing phytosanitary precautions) may be the best way to increase available K and, simultaneously, reduce the mobility and potential toxicity of Cr to the plant.

The direct and significant correlation between Ni content in leaves and cocoa kernel content suggests an efficient and non-limiting translocation of this micronutrient in the cocoa tree. Ni is recognized as an essential micronutrient for nitrogen metabolism, acting as an activator of the urease enzyme and being crucial for biological nitrogen fixation in some crops (Fabiano et al., 2015). The strong leaf-cocoa kernel correlation indicates that the Ni absorbed and accumulated in the leaves is being remobilized and partitioned to the cocoa beans, which are the plant's storage structures. This efficient Ni translocation pattern is biologically plausible, given that the element is mobile in the phloem and necessary in seeds (Chen et al., 2009).

From the point of view of product quality, the concentration of Ni in the cocoa kernels (and, consequently, in the chocolate) is relevant, as it is a trace element that has regulatory limits. Although this study shows that translocation is efficient (strong correlation), the average Ni levels found in cocoa kernels from Southern Bahia remained within safe ranges (1.76±1.93 to 3.37±1.98 mg.kg-1). However, the strong Ni leaf-cocoa kernel correlation underlines the need to monitor the foliar Ni content, since any increase in absorption would be directly reflected in the cocoa kernels, influencing the nutritional value and compliance with potential regulatory limits for trace elements.

3.4.2 Principal component analysis

The PCA of the elemental composition of the cocoa kernels (Figure 2A) explained 86.2% of the total variance (CP1=52.3%; CP2=33.9%). The elements that contributed most to the separation of points were Ca and Fe (correlated to CP1) and K (to CP2), with their eigenvalues ranging from 0.87 to 0.92. Regarding the leaves of the cocoa plants, the PCA showed a high percentage of explanation (92.4%; with CP1=65.2% and CP2=27.2%) (Figure 2B). The eigenvectors Ni, Co, and B were the most important for the variability. It is noteworthy that there was no well-defined grouping between the management types (cabruca and full sun) or cultivars (CCN 51 and PS 13.19) in both the PCA of the cocoa kernels and the PCA of the leaves. This result suggests that the management practices adopted and clonal variability were not the primary determining factors in the variability of the final elemental composition of the fruit and leaf tissue. The total levels of soil nutrients and metals showed 91.5% of the variance explained in the PCA (CP1=54.4%; CP2=37.1%) (Figure 2C). The main eigenvectors were K, Na, Cr, and Fe. In general, the points from the cabruca system showed a greater influence of Fe and Cr levels in the soil, which were positively correlated with CP2. On the other hand, in general, the samples from the full sun system were less influenced by these metals. Regarding the chemical properties of the soil (Figure 2D), the PCA showed 90.3% of the total variance, with CP1 being positively correlated with Ca^{2+}, Mg^{2+}, SB, and V. CP2 was negatively correlated with pH but positively correlated with Al^{3+}, H+Al, and m. Thus, the cabruca system showed higher values for the nutritional properties offered by the soil to the plants. The PCA of the total set of variables (soil, leaves and cocoa kernels) explained 82.2% of the variance (CP1=46.8%; CP2=35.4%) (Figure 2E). This integrated analysis showed a clearer separation of the influence of management. Cabruca management was mainly influenced by eigenvectors linked to soil fertility (Ca^{2+}, Mg^{2+}, SB) and by Ni in the leaves. Full sun management tended to be little influenced by these variables. Additionally, it also showed little relationship with total soil K and total soil, leaf and cocoa kernel Mn, two points of high influence of these variables. The eigenvectors correlated with CP2 were K and Mn in all compartments (Mn in cocoa kernel (0.95), leaf (0.89) and soil (0.84)), reinforcing the result of the simple correlation that Mn was one of the most sensitive elements in the soil-plant-cocoa kernels continuum.



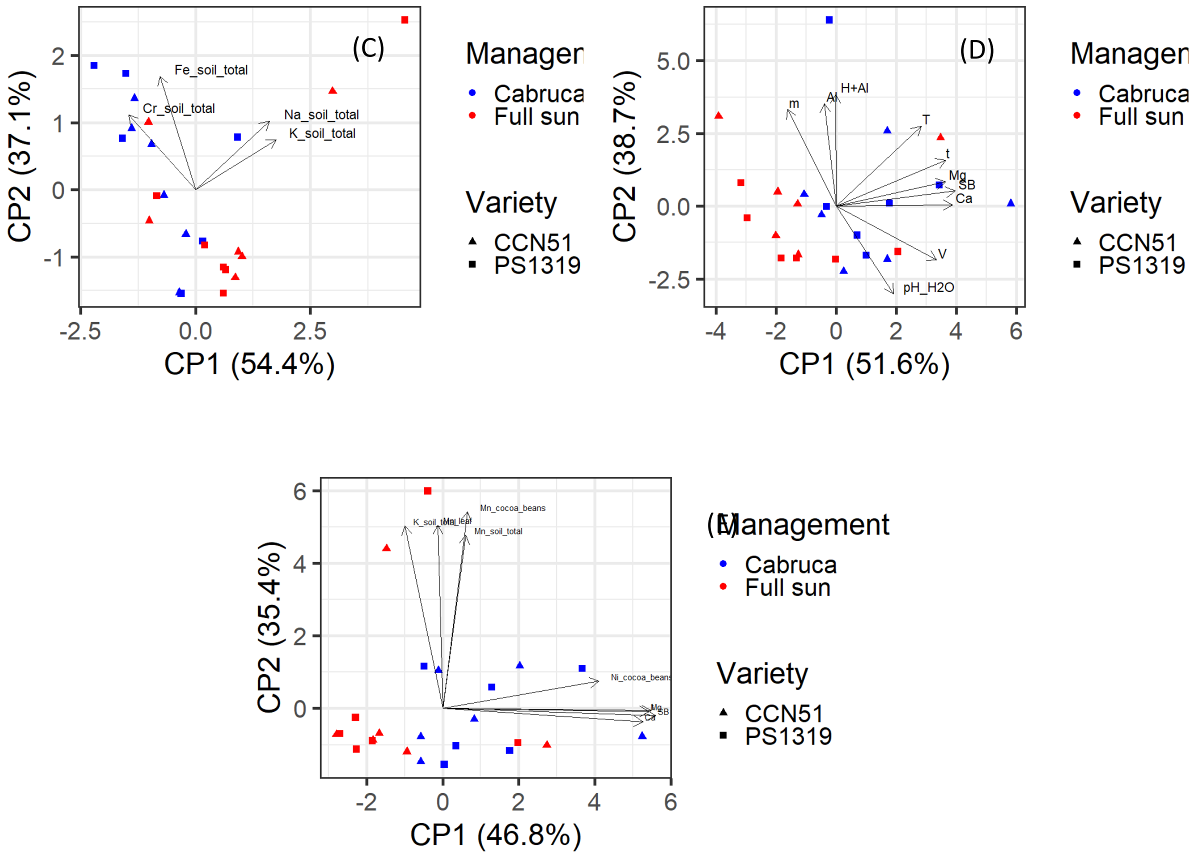


Figure 2. Scatter plot of total nutrient and heavy metal content in cocoa kernels (A); cocoa plant leaves (B); crop soils (C); Scatter plot of available nutrients and chemical properties of soils (D); and of the total set of variables (soil, leaves and kernels) (E) of cocoa trees of the cultivars CCN 51 and PS 13.19 produced in cabruca and full sun systems, Bahia, Brazil.

4. Conclusion

The results obtained provide crucial findings on the food safety of cocoa kernels and on the dynamics of chemical elements in the soil-leaf-kernel system of the evaluated areas Regarding Food Safety, all cocoa kernel samples presented non-quantifiable levels of Cd, Pb, and As. The levels of Cu and Ni found are below the maximum limits tolerated by ANVISA and the European Union, indicating that the kernels cultivated in the Southern region of Bahia, in the evaluated areas, do not present contamination by these heavy metals in the final product. Furthermore, the cocoa kernels from the region present high levels of minerals important for human nutrition, consistent with other studies. Regarding soil contamination, concentrations of Cr and Zn in some samples exceeded the Quality Reference Values (VRQ) of COPAM. For Cr, whose VRQ and Prevention Value (VP) are equivalent, the presence of high levels (> 100 mg.kg^{-1}) signals a potential environmental risk and requires monitoring. The analysis of the influence of the cultivation system demonstrated significant effects on the total levels of elements in the soil, leaves, and kernels, which was corroborated by the integrated multivariate analysis. The cabruca system was associated with higher levels of Mg, Fe, Mn, and Ni in the soil and Mg, Mn, and Co in the leaves. In the kernels, the cabruca system showed higher levels of Ca, Cu, Mn, Fe, and Ni. On the other hand, the full sun system showed higher levels of As in the soil and Zn in the kernels, in addition to being less influenced by soil fertility eigenvectors in the integrated PCA. In the soil-plant dynamics, Mn was the only element to show a strong and consistent positive correlation between the soil, leaves, and kernels. In contrast, the negative correlation between Cr and K in the soil is a worrying finding, as it suggests a potential ionic competition that could exacerbate the suboptimal nutritional status of the cocoa tree with respect to K, a nutrient that is highly exported by the fruit. In addition, the traditional nutritional sufficiency ranges proved insufficient for K, P, Fe, Zn, and Cu, indicating the need for adjustments in soil fertilization management in both systems. Furthermore, the unsatisfactory nutritional status of cocoa trees should be a point of attention, as this may limit the mineral enrichment of the kernels. In conclusion, cocoa from the Southern Bahia region presents high quality in terms of the absence of contamination by Cd, Pb, and As, as well as adequate levels of chemical elements important for human nutrition. However, the management of K and the potential risk of Cr in the soil represent the greatest challenges for the sustainability and productivity of cocoa farming in the region.

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CRediT authorship contribution statement

Hélia de Barros Kobi: Writing – original draft, Validation, Supervision, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Raphael Bragança Alves Fernandes:** Methodology, Validation, Writing - Review & Editing. **Davi Salgado de Senna:** Investigation. **Larissa Lorrane Rodrigues Borges:** Investigation, Formal analysis, Writing – review & editing. **Márcia Cristina Teixeira Ribeiro Vidigal:** Methodology, Validation, Writing - Review & Editing. **Paulo Cesar Lima Marrocos:** Methodology, Resources, Investigation. **Valdeir Viana Freitas:** Investigation, Writing – review & editing. **Mariane**

Sampaio da Silveira de Souza: Investigation. **Hugo Felipe Silva:** Formal analyses, Data curation. **Victor Martins Maia**: Formal analyses, Data curation. **Elpídio Inácio Fernandes Filho**: Formal analyses, Data curation. **Paulo Cesar Stringheta:** Validation, Supervision, Methodology, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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General conclusion

The results of this thesis establish an intrinsic relationship between the cultivation system and the metabolic quality of *Theobroma cacao* L. cocoa kernels, while also providing a preliminary overview of food safety in relation to heavy metals in the South of Bahia region.

The traditional agroforestry system known as cabruca proved to be significantly more efficient in promoting quality attributes compared to the full-sun cultivation system. Cocoa kernels grown in the cabruca system consistently showed higher levels of lipids, total phenolic compounds, and methylxanthines (theobromine and caffeine), substances that are recognized as essential for the development of flavor and functional properties valued by the fine cocoa market.

On the other hand, the transition to more intensive production models, such as full-sun cultivation, is associated with a reduction in the levels of lipids and total phenolic compounds, which entails a potential decrease in the chemical quality and added value of the cocoa kernels produced.

The assessment of the presence of heavy metals demonstrated that elements such as Cd, Pb, and As were not detected in cocoa kernels and leaves. Although these contaminants were present in the soils of the region, the occurrence was in low levels, not indicating an immediate risk of transfer to the crop.

The factors analyzed, cultivation systems (cabruca and full sun) and cultivars (CCN 51 and PS 13.19), did not establish themselves as determining variables for the presence or accumulation of these contaminants in the plant matrix.

For a complete assessment of food safety, it is suggested that the studies be expanded to quantify the total levels of Zn and Cr in the soils, and of Ni and Al in cocoa kernels from the region.

In short, the results confirm that the cabruca system not only ensures the preservation of local biodiversity, but is also intrinsically linked to the production of kernels with superior chemical quality attributes. These findings provide essential technical support for the Brazilian production chain to utilize the superior quality of agroforestry cocoa as a tool for differentiation and value addition in the global scenario.

It is recommended that future research establish the correlation between the initial metabolic profile of the kernels and their sensory acceptance after processing (fermentation and drying), a crucial step for validating the quality perceived by the end consumer.