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IMPACT OF SOILS AND CROPPING SYSTEMS ON BIOCHEMICAL ATTRIBUTES OF DRY CACAO BEANS

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In the present technological context, the identification of substances that contribute to the quality of dry cacao (*Theobroma cacao* L.) beans is as important as the agricultural practices and processing of cacao beans for food safety and marketing. The objective of this study was investigating the influence of cropping sites, represented by different soils (Argisols, Cambisols and Latosols) and cropping systems (cacao cabruca agroforestry system, cacao tree shaded with erythrina tree, agroforestry system of cacao tree with rubber tree) on biochemical attributes of dry cacao beans of PH-16 clone, in the humid zone of cacao growing region of Bahia, Brazil. The average contents of dry cacao bean attributes total acidity, simple carbohydrates (sucrose, fructose and glucose), theobromine and epicatechin have varied according to the different cropping sites. For these attributes, the cropping site that correspond to typic Dystrophic Red-Yellow Latosol in the agroforestry system of cacao tree with rubber tree (400 trees ha^{-1}) showed the best quality characteristics in dry cacao beans. In contrast, the cropping site that correspond to the abrupt Dystrophic Red-Yellow Argisol in the cacao cabruca agroforestry system (60 shade trees ha^{-1}) showed the worst performance relative to these same cacao beans quality indicators.

Key words: *Theobroma cacao* L., cacao quality, total acidity, simple carbohydrates, theobromine, epicatechin.

Impacto de solos e sistemas de cultivo sobre atributos bioquímicos de amêndoas secas de cacau. No contexto tecnológico atual, a identificação de substâncias que contribuem para a qualidade das amêndoas secas de cacau (*Theobroma cacao* L.) é tão importante quanto as práticas agrícolas e o processamento do cacau para a segurança e comercialização dos alimentos. O objetivo deste estudo foi investigar a influência dos locais de cultivo representados por diferentes solos (Argissolos, Cambissolos e Latossolos) e sistemas de cultivo (sistema agroflorestal cacau cabruca, cacau sombreado com eritrina, sistema agroflorestal cacaueiro com seringueira) sobre os atributos bioquímicos das amêndoas secas do clone PH-16, na zona úmida da região de cultivo de cacau da Bahia, Brasil. Nas amêndoas secas de cacau, os teores médios dos atributos acidez total, carboidratos simples (sacarose, frutose e glicose), teobromina e epicatequina variaram de acordo com os diferentes locais de cultivo. Para estes atributos, o local de cultivo que corresponde ao Latossolo Vermelho-Amarelo Distrófico típico no sistema agroflorestal cacaueiro com seringueira (400 árvores ha^{-1}) mostrou as melhores características de qualidade nas amêndoas secas de cacau. Em contraste, o local de cultivo que corresponde ao Argissolo Vermelho-Amarelo Distrófico abrupto no sistema agroflorestal da cabruca (60 árvores de sombra ha^{-1}), apresentou o pior desempenho em relação a esses mesmos indicadores de qualidade de amêndoas de cacau.

Palavras-chave: *Theobroma cacao* L., qualidade do cacau, acidez total, carboidratos simples, teobromina, epicatequina.

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Introduction

Cacao (*Theobroma cacao* L.) is an important commodity cultivated over 10 million ha of land area in the tropical countries with world production over 4 million tones, of which about 6 million people worldwide depend directly on its cultivation to sustain their livelihood (FAO, 2003, 2017). The current prospects for the supply of chocolate makers industries and other industrial sectors benefited by seeds (beans), coming from various countries in different continents, suggest that the relationship between supply and demand tends to become increasingly fierce (FAO, 2003, 2017; ICCO, 2012; WCF, 2014). Besides increased production by raising productivity and price appreciation, the world's cacao is facing the challenge of quality of primary production and cacao by-products (EFSA, 2012a, 2012b, EU, 2006, 2014; ICCO, 2010; WCF, 2014).

In this context, in addition to all agricultural practices and the processing of cacao beans (ICCO, 2008), the need arises to identify internal substances that contribute to the quality of cacao. Many biochemical attributes are linked to the quality of cacao beans, such as pH and total acidity, acetic and lactic acids, sucrose, fructose, glucose, lipids, proteins, amino acids, theobromine and caffeine, catechin, epicatechin and the total phenols (Loureiro et al., 2016). Because of their importance, these attributes were included in the development methodology of cacao quality index (Araujo et al., 2014; Loureiro, 2014).

The pH during fermentation is crucial as it determines the rate of enzyme activities that are responsible for the production of flavour precursor as well as the development of the characteristic brown color of cacao beans (Afoakwa, 2014). Most of these enzymes are reported to have pH optimum between 4.5 and 5.5 (Biehl et al., 1989). In a study, cacao beans fermented with pH between 5.0 and 5.5 (Biehl et al., 1985) produced higher flavour potentials, whereas cacao beans fermented beans with pH 4.0 to 4.5 produced lower flavour potential (Biehl and Voigt, 1994).

The desired range of total acidity of fermented and dried bean by the chocolate industry is between 12-15 mEq NaOH 100 g^{-1} (Lopez and Passos, 1984). However, from the results obtained by Bonvehí and Coll (1997) could infer that optimally fermented cacao bean samples should have a maximum total fixed acidity of 39 mEq NaOH 100 g^{-1}, and a non-volatile fixed acidity of 35 mEq NaOH 100 g^{-1}. In different cropping systems, the reported range of average total acidity contents of fermented cacao beans was between 8.3 to 19.8 mEq NaOH 100 g^{-1} (Jinap and Dimick, 1990). Holm et al. (1993) also reported that the total acidity average contents ranged from 8 to 29 mEq NaOH 100 g^{-1} in cacao beans from different geographic regions.

The contents of most organic acids found in cacao beans are result of fermentation process (Holm et al., 1993; Jinap and Dimick, 1990). Aprotosoaie et al. (2016) explained that during fermentation the concentration of organic acids increases as a result of sugar metabolism. According this review, acetic acid with sour and vinegar-like aroma is considered the highest odor-active compound in fermented and unroasted beans. Besides acetic acid, other short-chain carboxylic acids (isobutyric, isovaleric, and propionic) predominate in fermented cacao beans. They produce off-odor notes (rancid, butter, and hammy) and they are eliminated during the roasting and conching stages (Aprotosoaie et al., 2016). Rodriguez-Campos et al. (2012) also explained that a prolonged fermentation (over 6 days) increases the level of organic acids and their off-flavour notes. Jinap and Dimick (1990) reported that the average acetic acid contents ranged from 4.19 to 8.09 mg g^{-1} in cacao beans from different geographic regions. Another study also reported that the acetic acid average contents ranged from 1.3 to 11.8 mg g^{-1} in cacao beans from different cropping regions (Holm et al., 1993). Despite their important role in the enzymatic processes (Schwan and Wheals, 2004), excess of acetic acid may impair important characteristics of chocolate flavour and aroma (Armijos Paredes, 2002; Schwan and Wheals, 2004).

Lactic acid has been claimed to be partly responsible for acidity of the cacao beans (Passos et al., 1984). Jinap and Dimick (1990) reported that the average lactic acid contents ranged from 2.1 to 5.0 mg g^{-1} in cacao beans from different geographic regions. Another study also reported that the average lactic acid contents ranged from 0.6 to 11.1 mg g^{-1} in cacao beans (Holm et al., 1993). The excess of lactic acid amounts in cacao beans also reduces the quality of products and by-products of cacao beans, being undesirable for the chocolate industry (Armijos

Paredes, 2002; Schwan and Wheals, 2004). Bonvehí and Coll (1997) showed that optimally fermented cacao samples should have a lactic acid content of 0.5 g 100 g^{-1} (5 mg g^{-1}).

The hydrolysis of the sucrose present in the mucilage of beans in the fermentation process, or enzymatic breakdown results in reducing carbohydrates such as fructose and glucose, which are found in the dry beans (Reineccius et al., 1972; Voigt and Biehl, 1995). Due to loss of selective permeability of cell membranes of fermented beans, carbohydrates migrate to the center of the beans (Reineccius et al., 1972; Voigt and Biehl, 1995). The simple carbohydrates sucrose, glucose and fructose are important to detect the quality and purity of the cacao nibs' by-products, such as cacao powder, and the deficiency of reducing carbohydrates (fructose and glucose) is a limiting factor for the development of the ideal flavour for chocolate during roasting (Pisaturo and Bisagno, 1981; Rohan and Stewart, 1966). The post-harvest fermentation process is a major factor affecting the sugars in cacao beans (Reineccius et al., 1972). Recent information on the simple carbohydrate contents in dry cacao beans are scarce. Reineccius et al. (1972) reported that the total free sugars in cacao beans from different cropping regions ranged between 8.58 to 18.56 mg g^{-1}. However, it was explained that the range of values found in this study was due to the different conditions of data collection, fermentation and drying process (Reineccius et al., 1972). The study by Brito et al. (2000) confirms the hypothesis of changes in the contents of simple carbohydrates from cacao beans during the fermentation, drying and roasting.

Sucrose disappears almost completely during cacao beans fermentation (Knapp, 1937). From the relative percentage of each single carbohydrate in total content of free sugars reported by Reineccius et al. (1972) it was possible to estimate a range of sucrose content in cacao beans from 0.54 mg g^{-1} (supposed ideal condition) to 9.94 mg g^{-1} (supposed not ideal condition). Brito et al. (2000) reported for Forastero cacao beans from Brazil a sucrose content of 1.84 mg g^{-1} in crude beans, 1.46 mg g^{-1} in fermented beans (72 hours), 0.82 mg g^{-1} in dry beans and 0.42 mg g^{-1} in roasted beans. The value of 1.12 mg g^{-1} of sucrose average content was reported in Common cacao beans from Bahia, Brazil (Loureiro, 2012).

For fructose contents in dry cacao beans it was possible to estimate a range from 3.6 mg g^{-1} (supposed ideal condition) to 4.9 mg g^{-1} (supposed not ideal condition) (Reineccius et al., 1972). Brito et al. (2000) reported for Forastero cacao beans from Brazil a fructose average content of 1.88 mg g^{-1} in crude beans, 2.59 mg g^{-1} in fermented beans (72 hours), 1.27 mg g^{-1} in dry beans and 0.50 mg g^{-1} in roasted beans. Average fructose content of 5.94 mg g^{-1} has been reported for cacao beans of Common cacao from Bahia, Brazil (Loureiro, 2012).

A range of glucose contents it was estimate from 0.43 mg g^{-1} (supposed ideal condition) to 3.4 mg g^{-1} (supposed not ideal condition) (Reineccius et al., 1972). Brito et al. (2000) reported for Forastero cacao beans from Brazil an average glucose content of 0.89 mg g^{-1} in crude beans, 0.90 mg g^{-1} in fermented beans (72 hours), 0.28 mg g^{-1} in dry beans and 0.27 mg g^{-1} in roasted beans. Average glucose content of 1.93 mg g^{-1} has been reported for cacao beans of Common cacao from Bahia, Brazil (Loureiro, 2012).

Despite the importance of the lipids content of dry cacao beans, the composition of the different fatty acids has been highlighted due to differences in texture and promotes the melting point of chocolate (Wood, 2001). In addition, it is important to note that different genetic material of cacao trees exhibit different values in the lipids content of their beans (Loureiro et al., 2016). It must be observed the significance of lipids for obtaining cacao butter and also superior quality chocolate (Timms and Stewart, 1999; Wood, 2001).

Proteins and amino acids are important for the quality of chocolate, especially because the proteolysis that occurs in processing steps, among other chemical reactions, are important to the flavour profile and chocolate aroma (Brito et al., 2000; Jinap et al., 2010; Possignolo, 2010; Rohsius et al., 2006; Yusep et al., 2002).

The total content of proteins in the cacao beans are reported in the range from 150 to 200 g kg^{-1} (Biehl et al., 1977). Brito et al. (2000) reported for Forastero cacao beans average total proteins content of 220 g kg^{-1} in crude beans, 157 g kg^{-1} in fermented beans (72 hours), 118 g kg^{-1} in dry beans and 138 g kg^{-1} in roasted beans.

Rohsius et al. (2006) reported that the total free amino acid contents of 110 cacao beans samples varied between 5.0 and 25.2 mg g^{-1} fat free dry matter (ffdm), with a mean value of 13.0 mg g^{-1} ffdm. Further they

also reported the total free amino acid content of 14.5 mg g^{-1} in cacao beans from Brazil (Rohsius et al., 2006). Brito et al. (2000) reported by Forastero cacao beans form Brazil of average total free amino acids content of 25.7 mg g^{-1} in crude beans, 32.6 mg g^{-1} in fermented beans (72 hours), 35.3 mg g^{-1} in dry beans and 24.1 mg g^{-1} in roasted beans. Jinap et al. (2010) reported that the total free amino acids in samples of under-fermented cacao beans treated with carboxypeptidase B were observed at a range from 46.55 to 61.19 mg g^{-1}, whereas those treated with carboxypeptidase Y contained 30.76 to 42.59 mg g^{-1}. Yusep et al. (2002) reported that the total free amino acids in samples of under-fermented cacao beans treated with carboxypeptidase B were observed at a range from 52.72 to 76.51 mg g^{-1}, whereas those treated with carboxypeptidase Y contained 34.82 to 86.04 mg g^{-1}. Without carboxypeptidase, the total free amino acids in cacao beans were ranged at 34.38 to 50.42 mg g^{-1} (Yusep et al., 2002). Free amino acids, oligopeptides, and reducing sugars are cacao aroma precursors formed during the fermentation process (Rohsius et al., 2006). Based on reported studies, the amount and composition of free amino acids changes during fermentation process (Jinap et al., 2010; Rohsius et al., 2006; Yusep et al., 2002). Unfermented cacao beans contain low amounts of total free amino acids with high percentages of acidic amino acids (Rohsius et al., 2006).

In the report for Schwan and Fleet (2015), the theobromine content in cacao beans ranged between 8 to 21 mg g^{-1}. In another study, the average theobromine contents in cacao beans ranged from 17.72 to 25.28 mg g^{-1} in cacao liquor, 16.23 to 26.64 mg g^{-1} in dry cacao beans and 16.63 to 29.38 mg g^{-1} in cacao powder (Ramli et al., 2001). The average theobromine content found in this study (Table 5) is very similar to the value found in Malaysian cacao bens (Ramli et al., 2001). Theobromine is a typical alkaloid of the cacao beans, and it is also important for the biochemical and sensory profile of chocolate (Araujo et al., 2014; Loureiro et al., 2016). However, because it is a substance stimulating the central nervous system, studies suggest limits for daily consumption (Araujo et al., 2014; Araujo et al., 2013; EFSA, 2008; Pimentel, 2007).

Ramli et al. (2001) reported that the caffeine average contents ranged from 3.13 to 4.12 mg g^{-1} in cacao liquor, 2.52 to 4.98 mg g^{-1} in dry cacao beans and 3.27 to 6.58 mg g^{-1} in cacao powder. These reported values for cacao beans (Ramli et al., 2001) are also lower than those found in this study (Table 5). Caffeine is also a purine alkaloid widely studied in the context of human health (Araujo et al., 2014; Araujo et al., 2013; Medeiros and Lannes, 2009), so it is an important attribute to the cacao quality (Loureiro et al., 2016).

In the literature, averages catechin content varied between 0.82 and 3.91 mg g^{-1} in dry cacao beans (Cruz et al., 2015; Loureiro et al., 2016; Ramli et al., 2001). The (-)-catechin average contents were reported ranged from 2.81 to 4.16 mg g^{-1} in cacao liquor, 2.25 to 3.91 mg g^{-1} in dry cacao beans and 1.74 to 7.53 mg g^{-1} in cacao powder (Ramli et al., 2001). A study related that found (-)-epicatechin and (+)-catechin in unfermented, dried, unroasted cacao beans (Kofink et al., 2007). In contrast, roasted cacao beans and cacao products additionally contained the atypical flavan-3-ol (-)-catechin, another stereoisomer (Kofink et al., 2007). These are generally formed during the manufacturing process by an epimerization which converts (-)-epicatechin to its epimer (-)-catechin (Kofink et al., 2007).

The monomer epicatechin is the main flavanol cacao, representing approximately 35% of the total content of phenolic compounds (Wollgast and Anklam, 2000). This value differs from the results of the current study (Table 5) which was approximately 9% of total phenols. The reports in literature shows that averages of epicatechin content range from 2.15 to 16.52 mg g^{-1} in dry cacao beans (Loureiro et al., 2016; Ramli et al., 2001). Ramli et al. (2001) reported that the (-)-epicatechin average contents ranged from 1.41 to 3.65 mg g^{-1} in cacao liquor, 3.49 to 5.27 mg g^{-1} in dry cacao beans and 0.48 to 7.78 mg g^{-1} in cacao powder.

It has been reported earlier that total phenolic content of 52.5 mg g was found in unfermented cacao beans of PH-16 clone (Cruz et al., 2013). According this report (Cruz et al., 2013), the PH-16 cultivar decreased total phenols content during fermentation to about 36.5%. The total phenols content of cacao beans corresponds to approximately 10% (100 mg g^{-1}) of dry matter, varying according to the geographical location (Oliveira, 2005). The literature shows that average of total phenols content varying between 30 and 215.5 mg g^{-1} in dry cacao beans (Loureiro et al., 2016). The polyphenols have bitter, astringent flavours and their antioxidant properties help protect the seeds

from damage and disease (Kim and Keeney, 1984; Kyi et al., 2005). Ramli et al. (2001) reported that the total polyphenols ranged from 45 to 52 mg g^{-1} in cacao liquor, 34.93 to 60.22 mg g^{-1} in dry cacao beans, and 20.59 to 61.58 mg g^{-1} in cacao powder. Brito et al. (2000) reported that in Forastero cacao beans average total phenols contents was 231 mg g^{-1} in crude beans, 213 mg g^{-1} in fermented beans (72 hours), 157 mg g^{-1} in dry beans and 131 mg g^{-1} in roasted beans. After the processing, the contents of total phenols in cacao beans decay to 10-15% - 5%, values equal to or higher than 10% that are considered signs of inadequate fermentation (Wollgast and Anklam, 2000).

Cacao genetic materials tolerant to witch's broom, disease caused by the fungus *Moniliophthora perniciosa* (Stahel) Aime & Phillips-Mora, has been propagated in Bahia, Brazil, as the main strategy to control this disease (Lopes et al., 2011; Monteiro and Ahnert, 2012; Pereira, 2001). Porto Hibrido 16 (PH-16) is a clonal variety selected from a population of crosses between cacao trees of the Forastero (Amazon) and Trinitario groups (whose parents are unknown), located at Porto Hibrido farm in São José da Vitória, Bahia, Brazil (Cruz et al., 2013). There is shortage of detailed available information on biochemical attributes of these new genetic materials grown in the last 20 years. Studies on the quality of cacao (Araujo et al., 2014; Loureiro, 2012, 2014) and its agri-environment nuances are important tools for the development of public policies aimed at recovery of cacao plantations in Bahia.

The objective of this study was to investigate the influence of cropping sites, represented by different soils and cropping systems on biochemical attributes of dry cacao beans of PH-16 clone.

Materials and Methods

Cropping sites and sampling

Twelve cropping sites were located in the humid zone of the cacao growing region of Bahia, Brazil were selected for this study (Table 1). The Thornthwaite climatic classification was B4r A', B3r A', B2r A', B2r B', B1r A', B1r' A', B1w A' (SEI, 1998). These

Table 1. Cropping sites cultivated with PH-16 cacao clone in the cacao growing region of Bahia, Brazil

Site	Geographic Coordinates	City	Acronym of the SiBCS[1]	Soil Classification	Soil Taxonomy	Cropping Systems	Average den -sity of shade trees/ha
1	13º 40' 30" S, 39º 14' 27" W	Nilo Peçanha	LAd cam	cambisolic Dystrophic Yellow Latosol	Hapludox	CxR[2]	150
2	13º 44' 38" S, 39º 30' 10" W	Gandú	PVAd	typic Dystrophic Red-Yellow Argisol	Hapludult	CxE[3]	60
3	13º 45' 21" S, 39º 20' 25" W	Piraí do Norte	PVAd	abrupt Dystrophic Red-Yellow Argisol	Hapludult	Cabruca[4]	60
4	13º 46' 07.0"S, 39º 17' 52.0"W	Ituberá	LAd	typic Dystrophic Yellow Latosol	Typic Hapludox	CxR[2]	350
5	13º 51' 08" S, 39º 17' 54" W	Ituberá	LVAd	typic Dystrophic Red-Yellow Latosol	Typic Hapludox	CxR[2]	400
6	14º 31' 14" S, 39º 15' 45" W	Uruçuca	PVAe cam	cambisolic Eutrophic Red-Yellow Argisol	Hapludalf	Cabruca[4]	50
7	14º 51' 36" S, 39º 14' 42" W	Itabuna	CXd	typic Dystrophic Haplic Cambisol	Dystropept	Cabruca[4]	35
8	14º 51' 47" S, 39º 06' 47" W	Ilhéus	LVAd arg	argisolic Dystrophic Red-Yellow Latosol	Hapludox	Cabruca[4]	70
9	15º 17' 04" S, 39º 28' 43" W	Arataca	PAd lat	latosolic Dystrophic Yellow Argisol	Hapludult	Cabruca[4]	35
10	15º 23' 08" S, 39º 26' 04" W	Camacan	PVAd	typic Dystrophic Red-Yellow Argisol	Hapludult	Cabruca[4]	35
11	15º 23' 15.1"S, 39º 25' 48.6" W	Camacan	PVA ali	typic Alitic Red-Yellow Argisol	Hapludult	Cabruca[4]	35
12	16º 29' 02" S, 39º 23' 56" W	Porto Seguro	PVAd coe	abrupt Cohesive Dystrophic Red-Yellow Argisol	Hapludult	CxR[2]	400

[1]SiBCS - Brazilian System of Soil Classification (Santos et al., 2013). [2]Intercropping with cacao (*Theobroma cacao* L.) and rubber tree (*Hevea brasiliensis* (Willd. Ex Adr de Juss.) Muell. Arg.). [3]Cacao with shade of erythrina (*Erythrina fusca* Lour). [4]Cabruca is an ecological agroforestry cropping system where cacao trees are grown under native trees of the Atlantic Forest of South of Bahia (Lobão et al., 2012).

cropping sites have been planted with PH-16 cacao clone under different cropping systems, with a range of shade tree densities and across three soil class: Argisols, Cambisols and Latosols. Soils were classified in the Brazilian System of Soil Classification (SiBCS) (Santos et al., 2013), and its corresponding classification in Soil Taxonomy (Soil Survey Staff, 2006).

Cacao pod sampling occurred on November of 2008. This month was chosen because it is representative of the second harvest period (August / 2008 to January / 2009). Each study site with approximately one hectare was divided into three collection areas, characterized by the same soil and same cropping system. Each single composite sample corresponds to 50 mature cacao pods from each of the three sampling areas of a given site for the post-harvest processing of fermentation and drying. The origin of each sample of pods and beans corresponds to a properly identified and classified soil in each study site.

Post-harvest Processing

In Styrofoam boxes (30 x 20 x 30 cm) with a capacity of approximately 8 kg were used and cacao beans were fermented with mucilage which corresponded to 50 cacao pods. The fermentation process of cacao bens occurred for 168 hours (7 days) with peak temperature of 51°C, occurred on the 3rd day. During the fermentation process, after 48 hours, for oxygenation beans were mixed on daily basis. After being fermented, cacao beans were continuously dried in forced ventilation oven with temperatures ranging between 35 and 45°C for 192 hours (8 days). After drying, the cacao beans contained approximately 7% of moisture. The dry cacao beans were manually peeled with tweezers for a complete separation between the seed coat and endosperm, and only the endosperm (cotyledons and embryo) were milled for chemical analysis. In this study the term dry cacao beans refer to the endosperm of dry cacao beans.

pH and Total Acidity

Dried and milled cacao endosperm were used to determine hydrogenic potential (pH) and total acidity by methods 970.21 and 942.15 of the Association of Official Analysts Chemists (AOAC, 2005), respectively.

Organic acids and simple carbohydrates

Organic acids (acetic acid and lactic acid) and simple carbohydrates (glucose, fructose, and sucrose) contents of dried and milled cacao endosperm were determined by High Performance Liquid Chromatography (HPLC) according to the method described by Schwan and Souza (1986) slightly modified by this study.

Lipids

Lipid content of dry cacao beans was determined according to the method 963.15 of AOAC (2005) slightly modified by this study.

Proteins

Samples of 0.2 g of dried and milled cacao endosperm was used for determination of total nitrogen and estimation of proteins. Protein content of dry cacao beans was estimated from the total nitrogen, according to the 31.1.08 method of micro-Kjeldahl (AOAC, 2005) slightly modified by Brazilian Agricultural Research Corporation (Embrapa) (Carmo et al., 2000), based on hydrolysis and subsequent distillation of the sample protein was calculated by factor 6.25 x % total N.

Amino Acids

Free amino acid contents of dried and milled cacao endosperm were analyzed according to the method described by Kirchhoff et al. (1989) and Rohsius et al. (2006).

Theobromine and Caffeine

Dried and milled cacao endosperm were used for quantifying simultaneously theobromine and caffeine by high performance liquid chromatography (HPLC) according to the method described by Brunetto et al. (2007).

Phenolic substances

The phenolic substances (-)-epicatechin and (+)-catechin contents of dried and milled cacao endosperm were determined with the procedure described by Elwers et al. (2009).

Total phenols

The total phenols content of dried and milled cacao endosperm was determined with the Folin-Ciocalteu procedure described by Singleton and Rossi (1965) and Elwers et al. (2009).

Statistical Analysis

A completely randomized experimental design was used, with 12 cropping sites characterized by different soil types and cropping systems, with three replicates at each site. The replicate measurements were taken during identical but different experimental runs, which were randomized. This multiple response measurement was taken at the same combination of factor settings (geographic coordinate, cropping system and shade tree densities), and mainly characterized by soil type. Statistical procedures used in this study were performed in the R Core Team program (2016). Package 'stats': Shapiro-Wilks normality test, Bartlett homoscedasticity test (R Development Core Team, 2016). Package 'nortest': Kolmogorov-Smirnov normality test (Lilliefors correction) (Gross and Ligges, 2015). Package 'MASS': Box-Cox transformation (Venables and Ripley, 2002). Package 'ExpDes': Analysis of Variance (ANOVA) and Scott-Knott test (Ferreira et al., 2015). Package 'Lattice': Graphics (Sarkar, 2016). Package 'bpca': Biplot applied to Principal Component Analysis (Faria et al., 2015).

Results and Discussion

The variables that correspond to the attributes of acidity and organic in dry cacao beans of PH-16 clone were subjected to verification of the normality assumptions and homoscedasticity of ANOVA. The attributes pH, lactic acid, total amino acids and caffeine did not meet the normal criteria and homoscedasticity of ANOVA, and therefore were transformed by normalization by the average and standard deviation (Jöreskog et al., 2000).

Acidity attributes

Statistically significant differences in averages of the total acidity content in dry cacao beans of PH-16 clone are associated with the cropping sites (Table 2). For pH, and lactic acid and acetic acid contents, no differences were observed (Table 2).

Table 2. Analysis of variance, Scott-Knott test and descriptive analysis of the acidity attributes in dry cacao beans of PH-16 clone

Source	DF	pH	Total Acidity	Acetic Acid	Lactic Acid
			mEq NaOH 100 g^{-1}		mg g^{-1}
		Mean Square			
Cropping Site[1]	11	0.04^{ns}	10.27**	0.56^{ns}	0.12^{ns}
Error	24	0.02	2.05	0.27	0.06
Total	35				
CV (%)	2.4	9.8	25	23.3	
Cropping Site[1]			**Average ± Standard Deviation (n = 3)**		
01 LAd cam		6.0 ± 0.2	**14.3 ± 1.5 b**	2.66 ± 0.96	1.36 ± 0.18
02 PVAd		5.9 ± < 0.1	**13.5 ± 1.8 b**	2.29 ± 0.61	0.72 ± 0.07
03 PVAd		5.9 ± 0.1	15.4 ± 0.5 a	1.73 ± 0.45	0.83 ± 0.31
04 LAd		6.0 ± < 0.1	**12.9 ± 0.6 b**	2.79 ± 0.22	0.63 ± 0.07
05 LVAd		6.2 ± 0.2	**12.4 ± 1.8 b**	2.58 ± 0.40	0.98 ± 0.14
06 PVAe cam		6.0 ± 0.1	17.0 ± 0.8 a	2.11 ± 0.26	0.97 ± 0.10
07 CXd		6.1 ± < 0.1	17.2 ± 2.4 a	2.14 ± 0.38	0.84 ± 0.12
08 LVAd arg		5.9 ± 0.1	17.7 ± 1.3 a	2.60 ± 0.41	0.81 ± 0.05
09 PAd lat		6.3 ± 0.3	**13.3 ± 1.6 b**	2.10 ± 0.53	0.83 ± 0.23
10 PVAd		5.9 ± 0.1	**13.5 ± 0.7 b**	1.32 ± 0.39	0.72 ± 0.24
11 PVA ali		6.0 ± 0.1	**13.0 ± 1.9 b**	1.98 ± 0.22	0.89 ± 0.19
12 PVAd coe		5.9 ± < 0.1	**14.2 ± 0.1 b**	2.59 ± 0.83	1.13 ± 0.62
			Overall Average (n = 36)		
Minimum		5.8	10.5	0.93	0.48
Average ± SD		6.0 ± 0.2	14.5 ± 2.1	2.24± 0.6	0.89 ± 0.28
Maximum		6.6	19.2	3.6	1.85

[1] Cropping site identified by soil type: 01 LAd cam - cambisolic Dystrophic Yellow Latosol; 02 PVAd - typic Dystrophic Red-Yellow Argisol; 03 PVAd - abrupt Dystrophic Red-Yellow Argisol; 04 LAd - typic Dystrophic Yellow Latosol; 05 LVAd - typic Dystrophic Red-Yellow Latosol; 06 PVAe cam - cambisolic Eutrophic Red-Yellow Argisol; 07 CXd - typic Dystrophic Haplic Cambisol; 08 LVAd arg - argisolic Dystrophic Red-Yellow Latosol; 09 PAd lat - latosolic Dystrophic Yellow Argisol; 10 PVAd - typic Dystrophic Red-Yellow Argisol; 11 PVA ali - typic Alitic Red-Yellow Argisol; 12 PVAd coe - abrupt Cohesive Dystrophic Red-Yellow Argisol. DF - Degrees of Freedom. CV - Coefficient of Variation. SD - Standard Deviation. Significance levels by test F: (**) = 1% of error, (^{ns}) = not significant. In bold are highlighted the average clusters positively correlated with cacao quality.

In the current study the observed pH ranged from 5.8 to 6.6 (Table 2) which is higher than the pH range of 5.0 to 5.5 required by the chocolate industry (Biehl et al., 1985; Biehl and Voigt, 1994). These pH values suggested by chocolate industry indicate that when the processing of beans at such pH ranges tend to produce good quality beans that tend to have a lower acidic character, which is an important feature for obtaining good quality chocolate (Amin et al., 2002; Amores and Jiménez, 2007; Voigt and Biehl, 1995).

This study highlights the group of lower total acidity averages generated by the Scott-Knott test (Table 2), because these values are within the total acidity contents standards required by the chocolate industry (Lopez and Passos, 1984). The differences between the total acidity contents demonstrate that this biochemical indicator is also influenced by the environment of the cropping site.

The overall average acetic acid content of cacao beans of PH-16 clone (Table 2) observed in the current study will not pose any problems in the fermentation process adapted.

The averages lactic acid content found in the current study (Table 2) are lower than this critical recommended value (Bonvehí and Coll, 1997).

Simple carbohydrates

The Table 3 show statistically significant differences between the averages of simple carbohydrates of dry cacao beans of PH-16 clone.

This current study considers that the groups of highest averages of simple carbohydrate sucrose, fructose and glucose contents of cacao beans (Table 3) are more closely related to the best cacao quality. Sucrose content varied from 0.99 to 2.23 mg g-1 with an average value of 1.59 mg g-1. Average fructose content of 6.11 mg g-1 in cacao beans of PH-16 (Table 3) was higher than the values reported in the previous literature. Average glucose content of 2.98 mg g-1 in cacao beans of PH-16 was observed in the current

Table 3. Analysis of Variance, Scott-Knott test and Descriptive Analysis of the simple carbohydrates in dry cacao beans of PH-16 clone

Source	DF	Sucrose	Fructose	Glucose
		mg g^{-1}		
		Mean Square		
Cropping Site[1]	11	0.48**	6.18**	1.76**
Error	24	0.08	0.86	0.51
Total	35			
CV (%)		17.4	15.2	24
Cropping Site[1]		**Average ± Standard Deviation (n = 3)**		
01 LAd cam		**1.88 ± 0.40 a**	**5.62 ± 1.27 a**	2.18 ± 0.56 b
02 PVAd		1.54 ± 0.07 b	4.66 ± 0.83 b	2.46 ± 0.48 b
03 PVAd		0.99 ± 0.37 c	2.85 ± 0.24 c	1.46 ± 1.00 b
04 LAd		1.54 ± 0.19 b	**6.07 ± 0.47 a**	**3.31 ± 0.13 a**
05 LVAd		**2.23 ± 0.44 a**	**6.42 ± 1.08 a**	**3.07 ± 0.22 a**
06 PVAe cam		1.48 ± 0.23 b	**7.19 ± 1.01 a**	**2.96 ± 0.17 a**
07 CXd		**2.13 ± 0.40 a**	**7.76 ± 1.12 a**	**3.56 ± 0.72 a**
08 LVAd arg		**1.86 ± 0.11 a**	**8.11 ± 1.61 a**	**3.42 ± 1.58 a**
09 PAd lat		**1.73 ± 0.30 a**	5.12 ± 0.75 b	2.13 ± 0.70 b
10 PVAd		1.03 ± 0.17 c	**6.37 ± 0.69 a**	**3.96 ± 0.71 a**
11 PVA ali		1.55 ± 0.09 b	**6.91 ± 0.57 a**	**3.82 ± 0.28 a**
12 PVAd coe		1.18 ± 0.19 c	**6.23 ± 0.63 a**	**3.41 ± 0.62 a**
		Overall Average (n = 36)		
Minimum		0.76	2.59	0.85
Average ± SD		1.59 ± 0.45	6.11 ± 1.59	2.98 ± 0.95
Maximum		2.73	9.82	5.21

[1] Cropping site identified by soil type: 01 LAd cam - cambisolic Dystrophic Yellow Latosol; 02 PVAd - typic Dystrophic Red-Yellow Argisol; 03 PVAd - abrupt Dystrophic Red-Yellow Argisol; 04 LAd - typic Dystrophic Yellow Latosol; 05 LVAd - typic Dystrophic Red-Yellow Latosol; 06 PVAe cam - cambisolic Eutrophic Red-Yellow Argisol; 07 CXd - typic Dystrophic Haplic Cambisol; 08 LVAd arg - argisolic Dystrophic Red-Yellow Latosol; 09 PAd lat - latosolic Dystrophic Yellow Argisol; 10 PVAd - typic Dystrophic Red-Yellow Argisol; 11 PVA ali - typic Alitic Red-Yellow Argisol; 12 PVAd coe - abrupt Cohesive Dystrophic Red-Yellow Argisol. DF - Degrees of Freedom. CV - Coefficient of Variation. SD - Standard Deviation. Significance levels by test F: (**) = 1% of error. In bold are highlighted the average clusters positively correlated with cacao quality.

study (Table 3). The glucose content was approximately 3 times lower than the sucrose content reported by Brito et al. (2000), and 1.5 times higher than the sucrose content reported by Loureiro (2012).

The ratio of sucrose: fructose: glucose was similar in both genetic materials, corresponding to 1:4:2 in PH-16 clone (Table 3), 1:5:2 in Common cacao (Forastero) from Bahia (Loureiro, 2012), and 3:4.5:1 in Forastero cacao from São Paulo, Brazil (Brito et al., 2000). The sugar content in the cacao hybrid variety PH-16 was higher compared to the common cacao Forastero group (Loureiro, 2012). Lower sucrose values in cacao beans (Table 3) indicate that the hydrolysis process was satisfactorily, contributing to the rise in fructose and glucose levels, which is an important aroma precursors and chocolate flavour (Voigt and Biehl, 1995).

Figure 1 shows a statistically significant positive correlation (r = 0.77) between glucose and fructose, which agrees with the information described in the literature on these important attributes associated with chocolate flavour (Pisaturo and Bisagno, 1981; Reineccius et al., 1972; Rohan and Stewart, 1966).

Structural substances

The attributes lipid, proteins and amino acids of dry cacao beans of PH-16 clone did not showed significant differences by F ANOVA test between averages related to different cropping sites (Table 4).

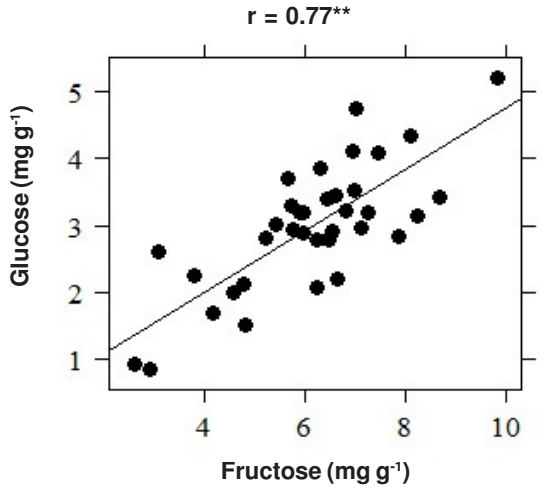

Figure 1. Correlation between glucose and fructose of dry cacao beans of PH-16 clone grown in the cacao region of Bahia (r = Pearson's linear correlation coefficient; **Significant at 1% level; n = 36).

Although a significant difference between the averages of lipids content in dry cacao beans of PH-16 clone for the different cropping sites was not detected, data in Table 4 shows the lipid average content of 366.5 g kg^{-1} is depicted, this value is lower than that the content of 500 g kg^{-1} related to the cacao quality (Ávila and Dias, 1993; Beckett, 2008, 2009; Biehl et al., 1977; Romanczyk et al., 1997). Another study with dry cacao beans of PH-16 clone showed a lipids content of 457.5 g kg^{-1} (Cruz, 2012), this value is higher than that found in the current study (Table 4).

Overall average of proteins content of 164.8 g kg-1 of dry cacao beans was observed in the current study (Table 4) is about 15% higher than the proteins content of 137.5 g kg^{-1} observed in another study with the same cacao clone (Cruz, 2012) and it is also higher than the proteins content reported by Brito et al. (2000).

The overall average content of amino acids contents in cacao beans of PH-16 clone corresponds to value of 14 mg g^{-1}, this value represents approximately 85% of proteins content (Table 4). The total amino acids content in Common cacao beans (Forastero) from Bahia, Brazil, corresponds to a value 168.6 mg g^{-1} (Lopes et al., 2003), this value is higher than that of the amino acids content of cacao beans samples of the PH-16 clone (Table 4). The overall average of free amino acids content observed in this study (Table 4) was similar to the average reported for cacao beans samples from Brazil (Rohsius et al., 2006), and higher than the overall average of samples from different countries (Rohsius et al., 2006). However, free amino acids content found in the current study was approximately 2 to 4 times lower than the contents found in other studies (Brito et al., 2000; Jinap et al., 2010; Yusep et al., 2002). Figure 2 shows a statistically significant positive correlation (r = 0.93) between the attributes proteins and amino acids content in dry cacao beans.

Purine Alkaloids and Phenolic substances

Statistically significant differences in averages of the theobromine and epicatechin contents in dry cacao beans of PH-16 clone are associated with the cropping sites (Table 5). No significant differences were observed in the caffeine, catechin and total phenols contents (Table 5).

Table 4. Summary of analysis of variance, Scott-Knott test and descriptive analysis of the structural substances in dry cacao beans of PH-16 clone

Source	DF	Lipids	Proteins	Total Free Amino Acids
		g kg^{-1}	g kg^{-1}	mg g^{-1}
		Mean Square		
Cropping Site[1]	11	707.01^{ns}	39.54^{ns}	4.87^{ns}
Error	24	878.0	36.71	5.57
Total	35			
CV (%)		8.09	11.6	15.5
Cropping Site[1]		Average ± Standard Deviation (n = 3)		
01 LAd cam		371.6 ± 12.7	169.2 ± 16.5	14.65 ± 1.89
02 PVAd		362.5 ± 9.6	157.1 ± 21.6	12.87 ± 3.42
03 PVAd		370.2 ± 31.1	165.0 ± 23.3	14.48 ± 3.41
04 LAd		359.5 ± 50.4	155.1 ± 15.0	13.75 ± 1.35
05 LVAd		354.7 ± 9.7	163.0 ± 5.2	12.75 ± 1.0
06 PVAe cam		355.1 ± 48.9	180.6 ± 39.0	15.79 ± 4.10
07 CXd		411.1 ± 30.4	183.4 ± 12.3	16.15 ± 3.2
08 LVAd arg		358.6 ± 30.5	182.1 ± 15.0	15.44 ± 1.38
09 PAd lat		366.3 ± 9.6	150.9 ± 5.1	12.99 ± 0.79
10 PVAd		368.0 ± 18.6	157.2 ± 21.2	12.37 ± 3.58
11 PVA ali		366.3 ± 18.2	159.1 ± 19.9	13.50 ± 2.02
12 PVAd coe		353.7 ± 40.7	155.1 ± 10.4	13.28 ± 0.91
		Overall Average (n = 36)		
Minimum		307.7	133.3	8.5
Average ± SD		366.5 ± 28.7	164.8 ± 19.4	14.0 ± 2.31
Maximum		436.8	206.4	18.94

[1] Cropping site identified by soil type: 01 LAd cam - cambisolic Dystrophic Yellow Latosol; 02 PVAd - typic Dystrophic Red-Yellow Argisol; 03 PVAd - abrupt Dystrophic Red-Yellow Argisol; 04 LAd - typic Dystrophic Yellow Latosol; 05 LVAd - typic Dystrophic Red-Yellow Latosol; 06 PVAe cam - cambisolic Eutrophic Red-Yellow Argisol; 07 CXd - typic Dystrophic Haplic Cambisol; 08 LVAd arg - argisolic Dystrophic Red-Yellow Latosol; 09 PAd lat - latosolic Dystrophic Yellow Argisol; 10 PVAd - typic Dystrophic Red-Yellow Argisol; 11 PVA ali - typic Alitic Red-Yellow Argisol; 12 PVAd coe - abrupt Cohesive Dystrophic Red-Yellow Argisol. DF - Degrees of Freedom. CV - Coefficient of Variation. SD - Standard Deviation. Significance levels by test F: (^{ns}) = not significant.

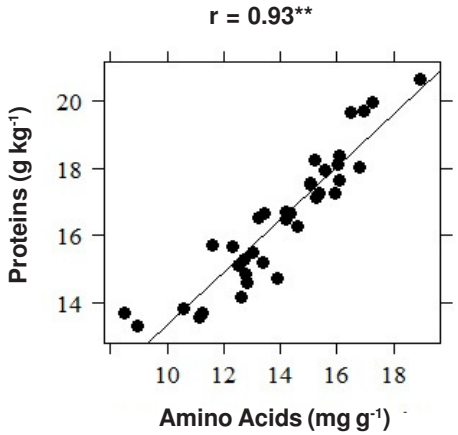

Figure 2. Correlation between proteins and amino acids of dry cacao beans of PH-16 clone grown in the cacao region of Bahia (r = Pearson's linear correlation coefficient; **Significant at 1% level; *n* = 36).

From previous reports theobromine content in cacao beans ranged between 8 to 21 mg g^{-1} (Schwan and Fleet, 2015), these values were lower than the value found in the current study (Table 5). Average theobromine content of 29.6 mg g^{-1} in dry cacao beans of PH-16 clone was recorded (Table 5) which is approximately 5 times lower than the content of 5.53 mg g^{-1} previously reported for the same cacao genotype (Cruz et al., 2015). This cacao quality study highlights the group of lower average contents of theobromine (Table 5).

Average caffeine content of 5.93 mg g^{-1} of dry cacao beans of PH-16 (Table 5) was approximately two times higher than the content of 3.02 mg g^{-1} reported in another study for the cacao beans of the same cacao genotype (Cruz et al., 2015). The content found in the current study (Table 5) is also higher than the caffeine content ranges from 0.8 to 2.3 mg g^{-1} reported in other studies (Schwan and Fleet, 2015).

Average catechin content of 1.94 mg g^{-1} of dry cacao beans in this study (Table 5) was approximately two times higher than 0.82 mg g^{-1} reported in another study with the same cacao clone (Cruz et al., 2015).

Table 5. Summary of analysis of variance, Scott-Knott test and descriptive analysis of the purine alkaloids and phenolic substances in dry cacao beans of PH-16 clone

Source	DF	Theobromine	Caffeine	Catechin	Epicatechin	Total Phenols
		mg g^{-1}				
		Mean Square				
CS[1]	11	10.71*	1.38[ns]	1.58[ns]	14.08*	159.6[ns]
Error	24	3.63	0.84	0.78	5.98	95.1
Total	35					
CV (%)		6.44	15.5	45.82	39.63	14.4
Cropping Site[1]		**Average ± Standard Deviation (n = 3)**				
01 LAd cam		**28.35 ± 1.00 b**	5.95 ± 0.43	1.58 ± 0.74	5.12 ± 2.22 c	62.20 ± 10.08
02 PVAd		31.43 ± 3.28 a	5.17 ± 0.30	2.12 ± 0.21	6.48 ± 2.00 c	70.94 ± 9.48
03 PVAd		29.57 ± 1.25 a	5.13 ± 0.31	2.16 ± 1.10	5.74 ± 1.20 c	67.29 ± 1.32
04 Lad		29.98 ± 1.96 a	5.97 ± 0.47	0.86 ± 0.21	4.30 ± 0.81c	62.45 ± 1.89
05 LVAd		**27.84 ± 2.79 b**	6.87 ± 0.21	1.46 ± 0.76	5.11 ± 2.41 c	65.43 ± 10.37
06 PVAe cam		29.73 ± 1.20 a	7.05 ± 2.32	3.69 ± 1.87	**11.58 ± 4.92 a**	78.06 ± 14.19
07 CXd		30.96 ± 1.59 a	5.90 ± 0.56	2.31 ± 1.13	7.71 ± 3.48 b	73.09 ± 11.84
08 LVAd arg		**26.45 ± 1.72 b**	6.20 ± 1.27	2.39 ± 1.22	7.41 ± 3.33 b	71.44 ± 12.20
09 PAd lat		**27.35 ± 1.63 b**	6.59 ± 1.16	1.85 ± 0.67	4.78 ± 1.86 c	66.71 ± 10.19
10 PVAd		29.38 ± 1.41 a	5.31 ± 0.75	1.73 ± 0.23	4.24 ± 0.56 c	52.04 ± 9.09
11 PVA ali		31.01 ± 2.05 a	6.01 ± 0.22	1.08 ± 0.23	3.98 ± 1.68 c	64.73 ± 10.76
12 PVAd coe		33.10 ± 1.68 a	5.02 ± 0.48	2.04 ± 0.50	7.59 ± 0.95 b	77.99 ± 6.39
		Overall Average (n = 36)				
Minimum		24.68	4.55	0.65	2.22	45.99
Average ± SD		29.60 ± 2.42	5.93 ± 1.01	1.94 ± 1.02	6.17 ± 2.92	67.70 ± 10.74
Maximum		35.21	9.61	5.72	16.58	92.37

[1]Cropping site identified by soil type: 01 LAd cam - cambisolic Dystrophic Yellow Latosol; 02 PVAd - typic Dystrophic Red-Yellow Argisol; 03 PVAd - abrupt Dystrophic Red-Yellow Argisol; 04 LAd - typic Dystrophic Yellow Latosol; 05 LVAd - typic Dystrophic Red-Yellow Latosol; 06 PVAe cam - cambisolic Eutrophic Red-Yellow Argisol; 07 CXd - typic Dystrophic Haplic Cambisol; 08 LVAd arg - argisolic Dystrophic Red-Yellow Latosol; 09 PAd lat - latosolic Dystrophic Yellow Argisol; 10 PVAd - typic Dystrophic Red-Yellow Argisol; 11 PVA ali - typic Alitic Red-Yellow Argisol; 12 PVAd coe - abrupt Cohesive Dystrophic Red-Yellow Argisol. DF - Degrees of Freedom. CV - Coefficient of Variation. SD - Standard Deviation. Significance levels by test F: (*) = 5% of error, ([ns]) = not significant. In bold are highlighted the average clusters positively correlated with cacao quality.

The cropping site 6 (cambisolic Eutrophic Red-Yellow Argisol) (Table 5) stands out for its high content epicatechin. The eutrophic character of the soil of this cropping site may have been an important factor for the increment of this phenolic substance. Average epicatechin content of 6.17 mg g^{-1} was observed in cacao beans in the current study (Table 5) which was approximately two times higher than the value of 2.98 mg g^{-1} observed in the same genotype (Cruz et al., 2015). Current cacao quality study highlights the group of higher average content of epicatechin (Table 5).

The overall average of total phenols content in dry cacao beans of the PH-16 clone was 67.70 mg g^{-1} (Table 5The total phenols contents observed in the current study with dry cacao beans of the PH-16 clone was less than 100 mg g^{-1}; that is, good indication that the adapted fermentation process was adequate.

Statistically significant positive correlations were observed between catechin and epicatechin (r = 0.91) (Figure 3a), between catechin and total phenols (r = 0.68) (Figure 3b), and between epicatechin and total phenol (r = 0.78) (Figure 3c).

Is worth emphasizing that the different genetic cacao materials can have higher variations in the contents of phenolic substances, therefore it is important to determine the existence of differences due to beans processing of different genotypes and regions from where they are grown (Cedeño, 2008; Cruz, 2012; Efraim et al., 2010; Kwik-Uribe, 2005; Loureiro, 2012).

Figures 4 and 5 shows the Biplot graphics of secondary metabolites of dry cacao beans. Table 6 list the summary of PCA secondary metabolites of cacao beans of PH-16 clone exploited by Biplot graphs (Figures 4 and 5).

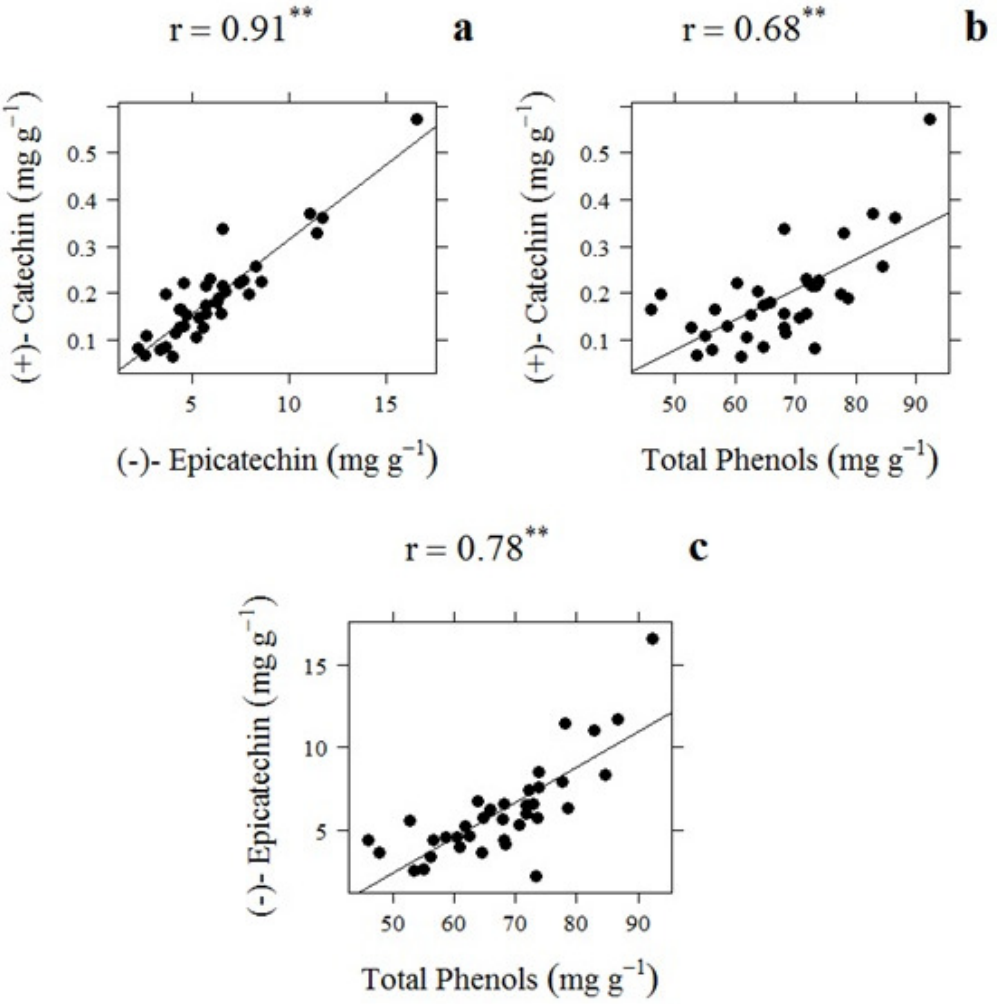

Figure 3. Correlation between phenolic substances of dry beans of PH-16 clone grown in 12 cropping sites in the cacao growing region of Bahia (r = Pearson's linear correlation coefficient. Catechin correlated with epicatechin (a). Catechin correlated with total phenols (b). Epicatechin correlated with phenols (c). **Significant at 1% level; *n* = 36).

Biplot graphs represent the secondary metabolites of dry cacao beans of PH-16 clone, which vary according to the cropping sites identified by soils (Figure 4a) and their classes (Figure 5a), the different cropping systems (Figures 4b and 5b), the average density of shade trees per hectare (Figure 4b) and different geographic coordinates (Figure 4a and b).

Graphs a and b of Figure 4 have the same structure between variables and objects because they are the same Principal Component Analysis (PCA). The objects were renamed for interpretation purposes (Figure 4a and b). In this same way the objects of graphs a and b of Figure 5 were also renamed.

The PCA represented in Biplot graphs a and b of Figure 4 have eigenvalue higher than 1, and retains 86% of the total variance of the data for interpretation based on the average cropping sites (Table 6). The PCA represented by the graphs a and b of Figure 5 also have eigenvalue higher than 1, and retains about 81% of the total variance of the data for interpretation based on the sample observations of the cropping sites (Table 6).

The variables catechin (CAT), epicatechin (EPI) and total phenols (PHE) were positively correlated (Figures 4 and 5). Graphically, the site 6 (cambisolic Eutrophic Red-Yellow Argisol) (Figure 4a) in cacao cabruca system (Cab) with an average density of 50 shade trees (Figure 4b) is positively correlated with these variables (CAT, EPI, PHE). According to Table 5, the higher epicatechin content also

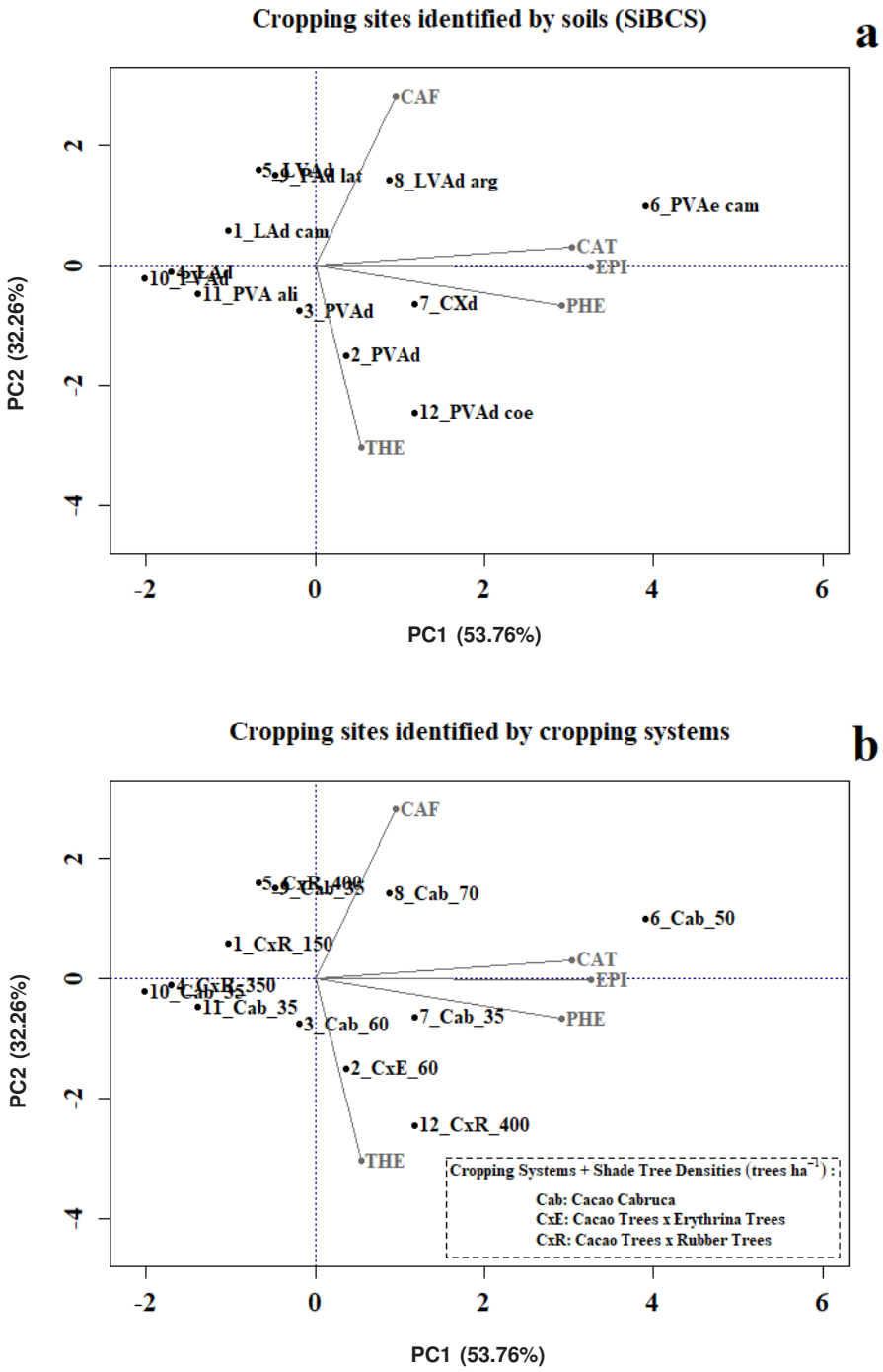

Figure 4. Biplots the Principal Component Analysis. Factors indicate the relative weight of the variables on the axes. Secondary metabolites of dry cacao beans of PH-16 clone: theobromine (THE), caffeine (CAF), catechin (CAT), epicatechin (EPI), total phenols (PHE). Cropping sites identified by soils (Brazilian System of Soil Classification - SiBCS) (a): cambisolic Dystrophic Yellow Latosol (1_LAd cam), typic Dystrophic Red-Yellow Argisol (2_PVAd), abrupt Dystrophic Red-Yellow Argisol (3_PVAd), typic Dystrophic Yellow Latosol (4_LAd), typic Dystrophic Red-Yellow Latosol (5_LVAd), cambisolic Eutrophic Red-Yellow Argisol (6_PVAe cam), typic Dystrophic Haplic Cambisol (7_CXd), argisolic Dystrophic Red-Yellow Latosol (8_LVAd arg), latosolic Dystrophic Yellow Argisol (9_PAd lat), typic Dystrophic Red-Yellow Argisol (10_PVAd), typic Alitic Red-Yellow Argisol (11_PVA ali), abrupt Cohesive Dystrophic Red-Yellow Argisol (12_PVAd coe). Sites identified by cropping systems (b). Numbered soils according to the longitudinal direction North-South.

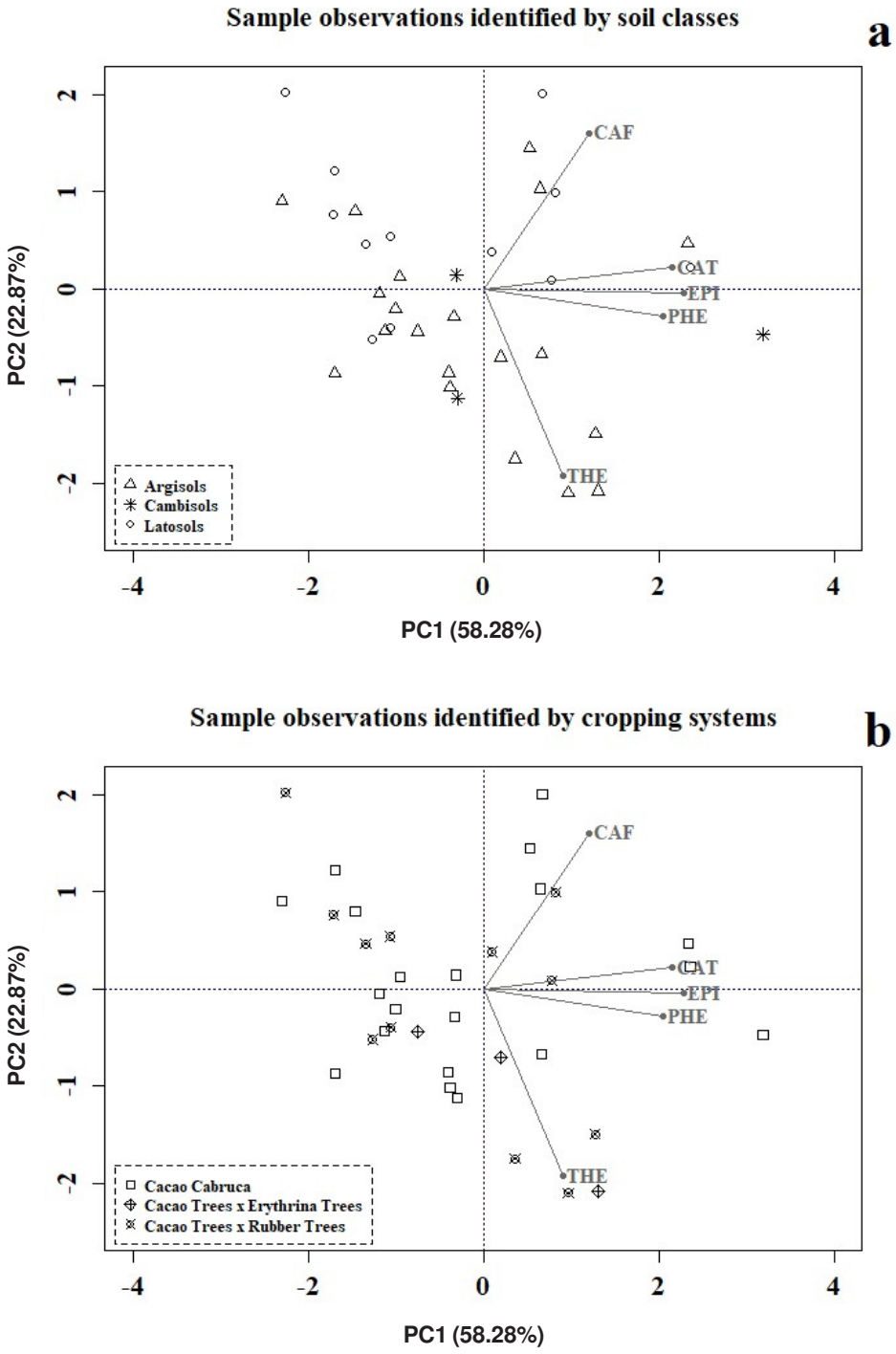

Figure 5. Biplots the Principal Component Analysis. Factors indicate the relative weight of the variables on the axes. Secondary metabolites of dry cacao beans of PH-16 clone: theobromine (THE), caffeine (CAF), catechin (CAT), epicatechin (EPI), total phenols (PHE). Sample observations identified by soil classes (Brazilian System of Soil Classification) (a). Sample observations identified by cropping systems (b).

Table 6. Summary of principal component analysis of secondary metabolites of dry cacao beans of PH-16 clone grown in 12 cropping sites in the cacao growing region of Bahia, Brazil

Summary	Averages of cropping sites (12 soils and cropping systems with average density of shade trees)		All sample observations (soil types and cropping systems)	
	PC1	PC2	PC1	PC2
THE - Theobromine	0.10	-0.72	0.22	-0.76
CAF - Caffeine	0.17	0.67	0.30	0.63
CAT - Catechin	0.56	0.07	0.53	0,09
EPI - Epicatechin	0.60	0.00	0.56	-0.02
PHE -Total Phenols	0.54	-0.16	0.51	-0.11
Eigenvalue	5.44	4.21	10.10	6.33
Retained Variance	0.54	0.32	0.58	0.23
Accumulated Variance	0.54	0.86	0.58	0.81

PC - Principal Component.

corresponded to site 6. The sites 4 (typic Dystrophic Yellow Latosol), 10 (typic Dystrophic Red-Yellow Argisol) and 11 (typic Alitic Red-Yellow Argisol) showed negative correlations with these same variables (CAT, EPI, PHE) (Figure 4a). The eutrophic character observed in the site 6 corresponding to the availability of nutrients for plants, both in the natural condition or the management of fertilization (Meurer, 2007; Oliveira, 2008). Good soil fertility conditions can increase plant production, provided that all other environmental and climatic factors are according to the ecophysiological requirements of plants (Meurer, 2007; Taiz and Zeiger, 2013). The production of secondary metabolites in plants are controlled by genetic and environmental factors (Taiz and Zeiger, 2013), and thus physiologically healthy plants have higher chances of survive if they produce substances that could act as defense against predators (Heitefuss and Williams, 1976). Phenolic substances are natural compounds that act on the protection of plants against diseases and pests (Strack, 1997; Taiz and Zeiger, 2013), so in seeds such as cacao beans, are found high levels of these substances (Calderón, 2002; Efraim et al., 2006; Kim and Keeney, 1984; Pereira-Caro et al., 2013). The correlation between phenolic substances with a eutrophic soil could be evidence of the influence of soil mineral nutrients in the production of these substances in cacao beans. However, the majority of the observations of these polyphenols contents proved uncorrelated or weak correlations, as well as with negative correlations with the observations corresponding to almost all samples of the cultivation sites represented by soil classes (Figure 5a) and cropping systems (Figure 5b).

Theobromine (THE) and caffeine (CAF) showed an inverse correlation (Figures 4 and 5). Graphically, no correlation was observed between phenolic compounds and purine alkaloids in cacao beans (Figures 4 and 5), and too weak correlations (not-significant) with the phenolic substances (CAT, EPI, PHE) (Figures 4 and 5). Site 12 (abrupt Cohesive Dystrophic Red-Yellow Argisol) showed positive correlation with theobromine (THE) (Figure 4a). The chemical and physical conditions that affect soil fertility influence on the different biochemical attributes in cacao beans (Loureiro, 2012, 2014), and another study indicate the differences between dry and rainy seasons influence the levels of purine alkaloids in cacao beans (Cedeño, 2008). From such information, it is possible that the different soil water levels (capacity of available water) and other soil characteristics (soil bulk density, clay content) of different cropping sites influence the contents of purine alkaloids in cacao beans. This same site was negatively correlated with the caffeine variable indicating the possibility of influence exerted by soil water conditions probably less favorable to the plants.

The different geographical locations (Table 1), implicit to the condition of identification of the cacao samples and the cropping sites, influenced on the content of purine alkaloids in cacao beans, as was be observed in the group of sites of 1 (cambisolic Dystrophic Yellow Latosol), 5 (typic Dystrophic Red-Yellow Latosol), 8 (argisolic Dystrophic Red-Yellow Latosol) and 9 (latosolic Dystrophic Yellow Argisol) from THE and CAF (Table 1 and 5; Figure 4a). The sites 10 and 11 (Table 1 and 5; Figure 4a) are close to each other and showed the influence of geographical locations on the phenolic substances CAT, EPI, PHE (Table 1 and 5; Figure 4a). The same influence of the cropping site was observed in the distancing of sites 6, 7 (typic Dystrophic Haplic Cambisol) and 12 from CAT, EPI, PHE (Table 1 and 5; Figure 4a) in relation to the other dry cacao beans samples.

Despite the low number of observations, a trend of positive correlation of theobromine with the samples corresponding to the class of argisols was observed, and inversely correlated with latosols (Figure 5a). This information needs to be confirmed in the future studies. However, differences in soil clay content, used for soil classification (Oliveira, 2008), directly affects the edaphic water conditions (Ferreira, 2010), and it seems to influence the theobromine content in the cacao beans (Table 5, Figures 4a and 5a).

Not observed clusters that enable associate these attributes with the cacao cropping systems (Figure 4b and 5b). However, although the level of detail of this study was not sufficient to explain these differences directly, or indirectly they indicate that the secondary metabolites of dry cacao beans could be also influenced by genotype x environment (Figures 4 and 5) interaction as evidenced by the univariate analysis (Table 5).

Conclusions

The average contents of total acidity, simple carbohydrates (sucrose, fructose and glucose), theobromine and epicatechin have varied according to the different cropping sites, indicating that due to discriminating power of these attributes they could be used for determination of the cacao beans quality.

With the exception of epicatechin, all the other attributes highlighted in the current study (total acidity, sucrose, fructose, glucose and theobromine) showed satisfactory contents for the cacao bean quality in the cropping site corresponding to typic Dystrophic Red-Yellow Latosol in the agroforestry system of cacao tree with rubber tree (400 trees ha^{-1}). In turn, the cropping site characterized by abrupt Dystrophic Red-Yellow Argisol in the cacao cabruca agroforestry system (60 trees ha^{-1}) did not showed any satisfactory content of these same cocoa beans quality indicators.

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