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Dynamics of volatile and non-volatile compounds in cocoa (*Theobroma cacao* L.) during fermentation and drying processes using principal components analysis

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ABSTRACT

Different volatile and non-volatile compounds produced during the fermentation-drying process are considered as indicatives of cocoa beans quality. We found thirty-nine different compounds identified by SPME-HS/GC–MS and related to the desirable notes and off-flavor that have been reported. Volatile and non-volatile compounds were associated with acidity and changes of pH, such as acetic and lactic acid. Using the principal component analysis (PCA), relations between compounds and fermentation and drying day were associated with dynamics of these compounds. The identification of principal compound produced during the fermentation and drying processes can be helpful in searching for off-flavor indicator and as a fermentation index, such as isobutyric, isovaleric and propionic acids. Oxidation of 3-methyl-1-butanol-to-3-methyl-1-butanol acetate can be of use in evaluating the degree of fermentation. At drying, the compounds with the highest levels were acetic and isobutyric acid, ethyl and 3-methyl-1-butanol acetate, pentanal and 2,3-pentanedione, and 1,3-butanediol and 2,3-butanediol. Therefore, acetic acid and isobutyric acid, due to their high levels and their low threshold value could play an important role in the aromatic quality of cacao drying.

1. Introduction

Since pre-hispanic times the cocoa bean (*Theobroma cacao* L.) has been used in Mexico to produce traditional beverages. Mexico is the 11th worldwide producer of cocoa beans with an annual production of 27,549 dry ton in 2008 (SIAP/SAGARPA, 2009).

Cocoa beans are classified by aromatic quality according to aromatic compounds presents (Frauendorfer & Schieberle, 2008). In Mexico, three varieties of cocoa are cultivated, the forastero, trinitario and criollo. Forastero cocoa is classified as low quality, the trinitario as intermediate quality and the criollo as higher quality (Ciferri & Ciferri, 1957). Genotype, agroclimatic conditions, fermentation, drying and industrialization process are factors that have important effects on the composition of volatile and non-volatile compounds and they define the quality of the final cocoa product (Brito et al., 2000).

During the fermentation process, the mucilaginous pulp surrounding the seed is removed, heat is generated and the pH is dropped to inhibit seed germination (Thompson, Miller, & Lopez, 2001). The cocoa mucilaginous pulp is rich in fermentable sugars such as glucose, fructose, sucrose and inorganic salts and has a pH from 3.0 to 3.5 due to the presence of citric acid. This composition is ideal to grow yeast and lactic bacteria (Ardhana & Fleet, 2003; Thompson et al., 2001).

The yeasts ferment carbohydrates of the cocoa mucilaginous pulp and produce mainly ethanol and carbon dioxide. In addition, this fermentation produces an increase in the temperature of the beans mass (Schwan & Wheals, 2004; Thompson et al., 2001). Schwan and Wheals (2004) found that *Kloeckera apiculata* and *Saccharomyces cerevisiae* var. *chevalieri* produced large amounts of isopropyl acetate, ethyl acetate, 1-propanol, isoamyl alcohol, 2,3-butanediol, diethyl succinate and phenylethanol. These are desirable compounds to high quality from cocoa products. Furthermore, they found that at the end of alcoholic fermentation lactic acid bacteria and acetic acid bacteria began to grow. The lactic acid bacteria consume glucose and citric acid from cocoa pulp to produce lactic acid (Schwan & Wheals, 2004; Thompson et al., 2001). Lagunes-Galvez, Loiseau, Paredes, Barel, and Guiraud (2007) reported that the highest content of lactic acid in the cocoa beans mass was found in fermentation at five day. The presence of lactic acid is not favorable for cocoa quality. The lactic acid content generated during fermentation remains in chocolate after processing, producing a chocolate with undesirable flavor (Thompson et al., 2001). The acetic acid bacteria oxidize the ethanol produced by alcoholic fermentation producing acetic acid and ethyl acetate. Acetic

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acid in high concentration may be detrimental to the quality of the cocoa products (Brito et al., 2000). During fermentation, acetic acid is diffused into the beans, which causes a decrease in the pH from 6.5 to 4.5 (Thompson et al., 2001). If cocoa beans have a high pH (5.5–5.8) after fermentation, they are considered poorly fermented with a low index of fermentation, while cocoa beans with lower pH (4.75–5.19) are considered well fermented (Afoakwa, Paterson, Fowler, & Ryan, 2008). Chocolates made from cocoa beans with a high pH (5.5–5.8) and lower pH (4.75–5.19) were evaluated sensorially, with lower notes of chocolate and higher notes of off-flavor descriptors. Chocolate manufactured with intermediate pH (5.20–5.49) was evaluated with higher notes of chocolate flavor (Jinap, Dimick, & Hollender, 1995).

Additionally, the drying process of fermented cocoa beans reduces the moisture content to less than 8% (Afoakwa et al., 2008; Hashim, Selamat, Muhammad, & Ali, 1998a). The drying of cocoa is carried out by sun or artificial methods. Drying reduces levels of acidity and the astringency in cocoa, decreasing volatile compounds content (Afoakwa, Paterson, Fowler, & Ryan, 2009). However, different authors reported that those volatile and non-volatile compounds were maintained after the fermentation, drying and roasting processes (Bailey, Mitchell, Bazinet, & Weurman, 1962; Frauendorfer & Schieberle, 2008; Ney, 1992).

The dynamics of volatile and non-volatiles compounds of cocoa beans during the fermentation and drying processes can be analyzed by principal component analysis (PCA), which can be used to discriminate the effect of both processes on the composition of compounds produced (Franca, Oliveira, Oliveira, Mancha-Agresti, & Augusti, 2009; Mancha-Agresti, Franca, Oliveira, & Augusti, 2008; Shin, Craft, Pegg, Phillips, & Eitenmiller, 2010).

The cocoa cultivated in Mexico has been classified as low quality; however, there are no studies that use the identification of volatile compound produced during fermentation-drying process to evaluate the cocoa beans quality.

The aim of this research was to identify the different volatile (alcohols, aldehydes, ketones, esters, carboxylic acids and pyrazines) and non-volatile (sugars and carboxylic acids) compounds during the cocoa beans fermentation and drying processes that are important in manufacturing chocolate with higher notes of chocolate flavor, using PCA.

2. Materials and methods

2.1. Chemicals and standards used

The HPLC-grade acetonitrile and methanol were obtained from Sigma-Aldrich, and analytical grade sulphuric acid, phosphoric and acetic acid from Fluka with purity higher than 98%. The standards for D-glucose, D-fructose and sucrose (>98% purity) were supplied by Sigma-Aldrich. Analytical grade oxalic, malic, lactic, citric and succinic acids were obtained from Sigma-Aldrich or Fluka.

2.2. Fermentation and drying processes

Cocoa beans of the Forastero variety were obtained from Cunduacan, Tabasco, México. The cocoa beans harvested by traditional methods were transported to the laboratory. Natural fermentation process was carried out with 1000 kg of raw cocoa (cocoa beans plus surrounding mucilaginous pulp), in batteries of wooden boxes of 1 m^3, at environmental temperature for 8 days. The cocoa beans were manually turning up moving the total mass from one box to other box once per day, to ensure aeration and uniform fermentation. Afterwards, fermented cocoa beans were placed on a concrete floor in layers from 5 to 10 cm thick and they were exposed to sun drying. The beans were mixed manually every day to obtain uniform drying for five days.

2.3. Sample collection

During the random sampling was done to analyze volatile and non-volatile compounds. Sub-samples of 2 kg from fermented cocoa beans fermentation and drying processes were taken every day, starting with the fermentation of raw cocoa beans at zero time (RC0) and at 1 (FC1), 2 (FC2), 3 (FC3), 4 (FC4), 5 (FC5), 6 (FC6), 7 (FC7) and 8 (FC8) days. Sampling was always at same time and depth in the mass (40 and 80 cm from upper surface). Afterwards, during the drying process sub-samples of 2 kg from dried cocoa beans were taken at 1 (DC1), 2 (DC2), 3 (DC3), 4 (DC4) and 5 (DC5) days. Sampling was done randomly and at the same time.

All sub-samples were frozen and transported to the laboratory; each sub-sample was subsequently fractioned into two parts; one of which was used for the analysis of non-volatile compounds. These fermented cocoa beans were manually separated from the testa to obtain the cocoa beans. Then, the cocoa beans were ground with a pestle and mortar until to obtain cocoa flour, and processed for freeze drying (Labconco corporation Kansas city, Missouri), and stored at -20 ± 2 °C until it was utilized for the extraction of sugars and carboxylic acid compounds. The other one sub-sample was immediately frozen at -40 ± 2 °C until it was analyzed for volatile compounds.

2.4. Determination of pH and titratable acidity

Five grams of cocoa beans sample were homogenized in 100 mL of hot distilled water, stirred manually for 30 s and filtered using Whatman No. 4® filter paper with vacuum assisted (Pump G. M., Model: 4C48GX28, O. 5 HP). The pH from filtrated liquid was measured using a pH meter (OAKTON, 2500 series) fitted with a glass electrode and an aliquot (25 mL) was used to determinate pH by titration with 0.1 N NaOH solution (Nazaruddin, Seng, Hassan, & Said, 2006).

2.5. Sugars and non-volatile organic acids extractions

Carbohydrates and non-volatile acids were isolated following the procedure established by Jinap and Dimick (1990). Five grams of ground fermented cocoa beans were mixed with 25 mL of deionized water using a vortex for 20 s and centrifuged (Sorvall RC 6 Plus, Thermo Scientific; Osterode, Germany) at $8000\times g$ for 45 min at 25 °C. The supernatant was extracted and 10 mL were adjusted from 8 to 9 pH with 5 N NH_4OH and 2 mL of the extract were applied to a column (5 mL syringe) containing 2 g of anion exchange resin from Cl^{-1} Bio Rex 5, 100–200 mesh (Bio-Rad Laboratories). The column was eluted with deionized water until to obtain a final volume of 10 mL. The collected eluate contained the natural compounds. Sulphuric acid solution (1 mL at 10% v/v) was added to the column and subsequently 25 mL of deionized water were passed through the column to obtain the acid fraction. Before high performance liquid chromatography (HPLC) analysis, each extract was filtered through a membrane of 0.45 μm (millipore®), and then analyzed by HPLC.

2.6. Separation and identification of sugar compounds

The carbohydrates extracted were analyzed by HPLC. A Varian ProStar (Model 230) chromatograph was used, equipped with autosampler Varian ProStar Model 430 and a Aminex HPX-87 C 300×7.8 mm column (Bio-Rad Laboratories). A Varian ProStar Model 355 differential refractometer performed detection of sugar compounds. Ultrapure water was the mobile phase for elution, with a flow rate of 0.6 ml/min and a temperature of 80 °C. Identification of compounds was performed using standards of D-glucose, D-fructose and sucrose. Furthermore, the sugars calibration curve for each standard was made for quantification.

2.7. Separation and identification of non-volatile organic acids

Non-volatile acids such as oxalic, malic, lactic, citric and succinic were separated using an HPLC (Varian ProStar Model 230) equipped with autosampler (Varian ProStar Model 420) and provided with a C-18 column Zorbax SB-Aq 4.5×150 mm, 5 μm (Agilent). The column operated at 30 °C (oven Varian ProStar Model 510). The mobile phases consisted of 99% (v/v) solvent (A); 0.02 M sodium phosphate monobasic adjusted to pH 2 with phosphoric acid; and 1% (v/v) solvent (B) acetonitrile at 100%. The flow rate was 0.3 ml/min and isocratic elusion. Absorbance of eluted sample was monitored at 210 nm. Identification of compounds was carried out with the retention times of standards for non-volatile acid compounds. The quantification was performed by calibration curves for each acid compound.

2.8. Extraction of volatile compounds

The volatile compounds from cocoa sample (2.0 g) were extracted using the technique of solid phase microextraction in the headspace (SPME-HS). The selected fiber was 50/30 μm divinylbenzene/carboxene/polydimethylsiloxane (DVB/CAR/PDMS), provided by Supelco. The extraction conditions were previously optimized combining the exposure time of the fiber at different temperatures. The final conditions selected were 15 min in order to reach equilibrium, with fiber exposition for 30 min to the cocoa aromas sample, in the HS at 60 °C.

2.9. Separation and identification of volatile compounds

The fiber with volatile compounds were analyzed by gas chromatography–mass spectrometry (GC–MS) (Hewlett Packard Model 5890 Series II, Palo Alto, Ca.), equipped with an Innowax capillary column (60 m*0.25 mm id*0.25 μm film thickness). The oven temperature was set at 40 °C for 5 min, increased until 200 °C at a rate of 10 °C min^{-1}, and finally maintained at 200 °C for 30 min. The carrier gas was helium with high purity at 0.7 mL min^{-1}. The splitless injection mode was at 240 °C (0.5 min). The selective mass detector was a quadrupole (Hewlett Packard, Model 5972), with an electronic impact ionization system at 70 eV and at 260 °C. Compounds identification was done with base to two criteria: (1) by comparing the mass spectra of each compound with the Wiley 275L library of mass spectra; and (2) by comparing the retention index with literature data.

2.10. Statistical analysis

Areas and concentrations of volatile and non-volatile compounds were subjected to principal component analysis (PCA). Compounds in each process were studied separately. Scores obtained from each PCA were analyzed to a one-way analysis of variance (ANOVA) to test for significant differences between samples. All statistical analysis were performed using Statgraphics XV Software (Statgraphics, 2007).

3. Results and discussion

3.1. Changes of pH during fermentation and drying processes

It is known that during fermentation, pH values of cocoa beans decrease in this traditional process. The pH decreased from 6.4 at zero time (RC0) to 4.5 value in eight day of fermentation. Nazaruddin et al. (2006) found a decrease of pH value from 6.54 to 4.35 in fermented cocoa beans at four days. It has been reported in several researches that this decrease is due to diffusion into the beans of acid produced by lactic and acetic bacteria during the fermentation process (Afoakwa et al., 2008; Thompson et al., 2001).

A very low pH can indicate lower quality cocoa beans. Jinap et al. (1995) reported that dry and roasted cocoa beans with lower pH values (4.75–5.19) were scored with lower notes for chocolate. In addition, Portillo, Graziani, and Betancourt (2007) found that pH values lower than 4.5 decreased cocoa beans aromatic potential. During the drying process, there is a loss of volatile acids and water when this process occurred slowly, with an increase of pH values (Nogales, Graziani, & Ortiz-Bertorelli, 2006). In contrast, we found that after two days of drying the pH decreases significantly from 4.7 to 4.6 ($P<0.05$). Garcìa-Alamilla et al. (2007) reported that when the drying temperature was near 60 °C, cocoa beans can have a higher concentration of acetic, propionic, isobutyric, and isovaleric acids. We had during the dying process average temperatures of 47±2 °C.

In this research the acidity percentage of cocoa beans had a significant increase from 0.0062% to 0.106% during eight days of fermentation ($P<0.05$). High values of the acidity parameter have relation with low values of pH. There is a high correlation between acetic and lactic acid and pH ($r=0.86$) titratable acidity ($r=0.91$), indicating that acid could be responsible for high values of acidity in cocoa beans (Jinap & Dimick, 1990).

3.2. Sugar and non-volatile acids

Cocoa beans have higher concentration of sucrose sugar and lower reducing sugar (glucose and fructose) concentrations at zero time of fermentation (RC0) (Fig. 1a), as was reported by Hashim, Selamat, Muhammad, and Ali (1998b). Furthermore, we found that the sucrose concentration decreased significantly ($P<0.05$) during the fermentation and drying process. In contrast, we found a significant rise in glucose and fructose concentration (Fig. 1a). Sucrose is converted to glucose and fructose during the fermentation process and their

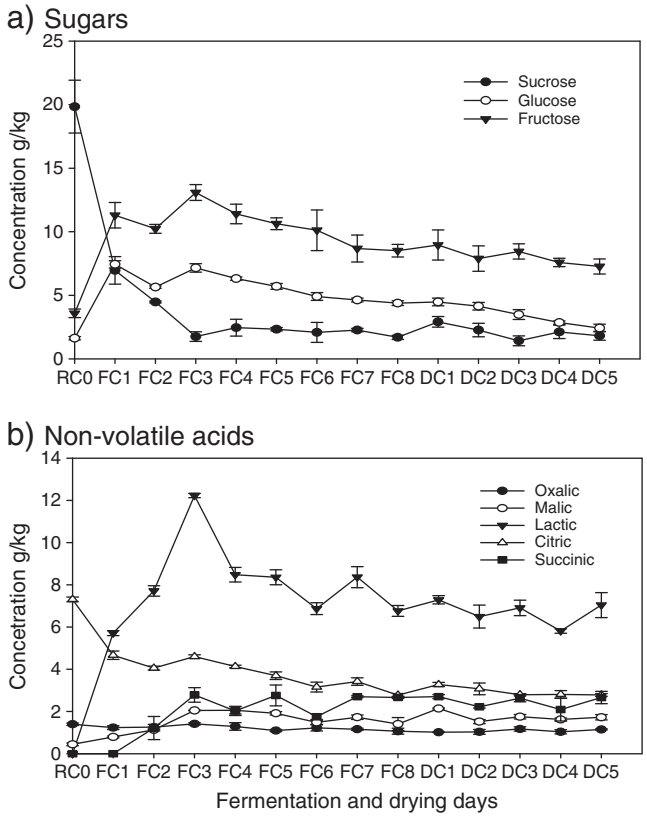

Fig. 1. Dynamics of a) sugar and b) non-volatile compounds during fermentation traditional process of the *Theobroma cacao* L. for raw cocoa at zero time (RC0); fermented cocoa beans from one to eight days (FC1, FC2, FC3, FC4, FC5, FC6, FC7 and FC8); and drying cocoa beans from one to five days (DC1, DC2, DC3, DC4 and DC5). Bars are ±standard deviation ($P<0.05$).

concentrations increased almost three fold, while sucrose is depleted (Hashim et al., 1998a; Thompson et al., 2001). We observed an increase of 2.7-fold and 2.4-fold in concentrations of glucose and fructose respectively, at the end of fermentation.

Additionally, organic acids were determined in raw cocoa beans at the beginning of fermentation (RC0), where citric acid concentrate was higher than malic, lactic, oxalic and succinic non-volatile acids, (Fig. 1b). Changes observed for oxalic and malic acids during fermentation and drying were not significant ($P<0.05$). However, malic and lactic acid concentrations have a significant increase during the total fermentation and drying process ($P<0.05$). Lactic acid concentration showed the highest value at 3 day of fermentation (Fig. 1b). Lagunes-Galvez et al. (2007) reported that the maximum development of lactic acid bacteria was found after 24 h with the highest population at two days. The presence of lactic acid is not favorable for cocoa quality, because high acid concentrations will produce excessive sourness that can to mask the chocolate flavor in manufactured products (Lagunes-Galvez et al., 2007; Thompson et al., 2001).

In addition, we observed that citric acid had a significant depleted during all the fermentation and drying processes (Fig. 1b). Citric acid can be metabolized to acetic acid, carbon dioxide and lactic acid (Thompson et al., 2001).

3.3. Volatile compounds produced during fermentation

Thirty-nine compounds were identified during the cocoa fermentation and drying processes. They are shown by functional chemical group in Table 1. Some of these compounds have been reported as

Table 1
Volatile compounds identified during traditional fermentation and drying cocoa beans process, using a gas chromatography couple with mass spectrometry (GC–MS).

Group	Retention time (min)	Compound	Odor quality[a]	Kovats Retention Index[b]	Process stage found[c]	Reference
Aldehydes and ketones	6.54	2-methyl-1-propanal	Malty, chocolate		RC0	Afoakwa et al. (2009)
	8.03	Pentanal	Almond, malt, pungent	935	RC0, FC6, FC7, DC1–DC5	Ducki, Miralles-García, Zumbe, Tornero & Storey, 2008 (2007)
	9.07	2,3-butanedione	Butter		FC3–FC8, DC1–DC5	Afoakwa et al. (2009)
	9.51	2-pentanone	Fruit	983	RC0, FC1	
	14.89	Acetoin	Butter, cream	1287	FC1–FC8, DC1–DC5	Afoakwa et al. (2009)
	20.25	Phenyl acetaldehyde	Honey, flowery, sweet		RC0, FC1–FC8, DC1–DC5	Afoakwa et al. (2009)
	20.51	Acetophenone	Must, flower, almond, sweet	1645	FC5–FC8, DC4	Serra-Bonvehí (2005)
	20.56	Phenylmethyl ketone			RC0, FC4	
Alcohols	10.14	1-propanol	Pungent, sweet candy	1037	FC1,FC2	Serra-Bonvehí (2005)
	11.41	2-methyl-1-propanol	Wine		FC1–FC7, DC1–DC5	Serra-Bonvehí (2005)
	11.75	3-methyl-2-butanol		1052	FC3–FC8, DC1	
	11.93	2-pentanol	Green, mild green	1118	RC0, FC1, FC2	Serra-Bonvehí (2005)
	18.31	3-methyl-1-butanol	Malty, bitter, chocolate		RC0, FC1–FC8, DC1–DC5	Frauendorfer & Schieberle (2008)
	18.43	2,3-Butanediol			FC1–FC8, DC1–DC5	Ducki et al. (2007)
	18.84	1,3-Butanediol		1692	FC1–FC8, DC1–DC5	
	22.38	Benzyl alcohol	Sweet, flower	1865	DC1–DC5	Afoakwa et al. (2009)
	22.95	Phenylethyl alcohol	Honey, spice, rose, lilac, flowery, caramel	1925	FC1–FC8, DC1–DC5	Frauendorfer and Schieberle (2006)
Esters	6.74	Methyl acetate			FC1–FC8, DC1–DC5	Ducki et al. (2007)
	7.43	Ethyl acetate	Pineapple	907	FC1–FC8, DC2–DC5	
	9.67	Isobutyl acetate	Fruit, apple, banana	1015	FC5–FC7, DC2–DC5	
	10.65	3-methyl-2-butanol acetate			FC2, DC2–DC5	
	10.82	2-phenylacetate			FC4	
	11.87	3-methyl-1-butanol acetate			FC2–FC8, DC2–DC5	
	15.63	Ethyl lactate	Fruit	1358	FC1–FC5	Jenning & Shibamoto (1980)
	21.82	Ethylphenyl acetate	Fruit, sweet, honey	1773	DC4, DC5	Serra-Bonvehí (2005)
	21.98	2-phenylethyl acetate	Rose, honey, tobacco, flowery	1829	FC4–FC8, DC1–DC5	Frauendorfer and Schieberle (2006)
	22.15	Ethyl laurate	Leaf, fruity, floral	1842	FC1–FC7, DC4	Serra-Bonvehí (2005)
	28.45	Ethyl palmitate	Waxy, mild green		FC3–FC4	Serra-Bonvehí (2005)
Acids	16.92	Acetic acid	Sour, astringent, vinegar	1450	FC1–FC8, DC1–DC5	Frauendorfer and Schieberle (2006)
	18.26	Propanoic acid	Pungent, rancid, soy	1523	FC5–FC8	Frauendorfer & Schieberle (2008)
	18.50	Isobutyric acid	Rancid, butter, cheese, hammy	1563	FC3–FC8, DC1–DC5	Serra-Bonvehí (2005)
	19.4	Butanoic acid	Rancid, cheese, sweat	1619	FC2	Frauendorfer and Schieberle (2006)
	19.75	Isovaleric acid	Sweat, acid, rancid	1665	FC2–FC8, DC1–DC5	Serra-Bonvehí (2005)
	21.75	Hexanoic acid	Sweat, pungent, sickening, rancid, sour	1829	FC2–FC8, DC1–DC5	Serra-Bonvehí (2005)
	24.40	Octanoic acid	Sweat, cheese, oily, fatty	2083	FC2–FC8, DC1–DC5	Serra-Bonvehí (2005)
	25.97	Nonanoic acid	Green, fat	2203	FC2–FC5, DC1–DC5	Jenning & Shibamoto (1980)
	34.43	Dodecanoic acid	Metal	2517	RC0, FC2–FC5	Jenning & Shibamoto (1980)
	37.87	Bencenacetic acid			DC3	–
Pyrazines	18.14	Tetramethylpyrazine	Milk-coffee, roasted, chocolate	1458	DC2–DC5	Afoakwa et al. (2009)

[a] Flavor notes reported.
[b] Obtained of literature.
[c] Sample of different stages: RC0 = Raw cocoa at zero time; FC1, FC2, FC3, FC4, FC5, FC6, FC7 and FC8 = Fermented cocoa from one to eight day; DC1, DC2, DC3, DC4 and DC5 = Dry cocoa from one to five days.

responsible for producing desirable note flavors and off flavors in cocoa beans during the fermentation, drying and roasting processes.

The amyl alcohols are common compounds in food flavor, which are found in the cacao fermentation process and some of these alcohols are already used to evaluate cocoa flavor and fermentation degree (Oberparleiter & Ziegleder, 1997). We identified six principal alcohols during cocoa fermentation, of which two were amyl alcohols; 3-methyl-1-butanol and 3-methyl-2-butanol; and the others were 2-methyl-1-propanol; 2, 3-butanediol; 1, 3-butanediol and phenylethyl alcohol (Fig. 2a). These alcohols are produced during fermentation of sugars present in cocoa beans. Also, alcohols such as 3-methyl-1-butanol, 2, 3-butanediol and phenylethyl alcohol have been reported in cacao fermentation with *K. apiculata* and *S. cerevisiae* var. *chevalieri* yeasts and these compounds are desirable for high quality cocoa products (Schwan and Wheals, 2004).

In addition, compounds such as 3-methyl-1-butanol and phenylacetaldehyde have been reported as derivates from amino acids catabolism realized during fermentation (Afoakwa et al., 2008).

Furthermore, we found that acetoin was the aldehyde with the highest area, and other compounds presented during cocoa fermentation were 2,3-butanedione, acetophenone and phenylacetaldehyde (Fig. 2b). Acetoin could be produced by alcohol fermentation from piruvate, and butanodiol (Pretorious, 2000). Phenylethyl alcohol is a precursor of phenylacetaldehyde, and is oxided to ester phenylethyl acetate (Smit, Smit, & Engels, 2005). We found seven esters during the fermentation, of which the ethyl acetate area was highest at three day of fermentation (Fig. 2c). The 3-methyl-1-butanol acetate could be produced from 3-methyl-1-butanol oxidation (Smit et al., 2005).

In this research, ethyl acetate was produced during cocoa fermentation and shows the highest area after three day of fermentation. We also found a high area from acetic acid during the time of all fermentation (Fig. 2d). Ethyl acetate is a product of esterification from acetic acid and ethanol (Pretorious, 2000).

Oberparleiter & Ziegleder, (1997) proposed ratios of aldehydes-amyl alcohols and acetates-amyl alcohols to evaluate fermentation degree. They suggested that a ratio of methyl-1-butyl acetate:methy-1-butanol higher than 1.5 indicates that the cocoa beans were over fermentation. It produces a hammy flavor defect, caused by isobutyric and isovaleric acids, which are formed enzymatically during the extended fermentation process (Oberparleiter & Ziegleder, 1997).

We found a 3-methyl-1-butyl acetate:methyl-1-butanol ratio of 0.99 at six days of fermentation and 1.68 at the end, and according to Oberparleiter & Ziegleder (1997) we had over fermentation with an increase of isobutyric and isovaleric acids after four day (Fig. 2d). Therefore, is recommended to stop fermentation at six days, when the 3-methyl-1-butyl acetate: methy-1-butanol ratio is lower than 1.5, and avoid esterification of amyl alcohols to amyl acetates. The presence of these compounds and a low concentration of methyl-1-butanol can be used as a flavor defects index. We also found propionic, hexanoic, octanoic, nonanoic and dodecanoic acids during the cocoa fermentation process (Fig. 2d). Propionic and butyric acid would have a negative effect on cocoa aromatic quality (Serra-Bonvehí, 2005).

3.4. Volatile compounds produced during dying process

Alcohols area for 2,3-butanediol and 1,3-butanediol increased during the drying process (Fig. 3a). Compounds such as 2-methyl-1-propanol, and 3-methyl-1-butanol did not present significant changes during drying by five days. Nevertheless, phenylethyl alcohol and benzyl alcohol showed a decrease in peak area. Latter, alcohol not was detected in the fermentation process. However, 3-methyl-2-butanol not was found during the drying process (Fig. 3a). These changes in

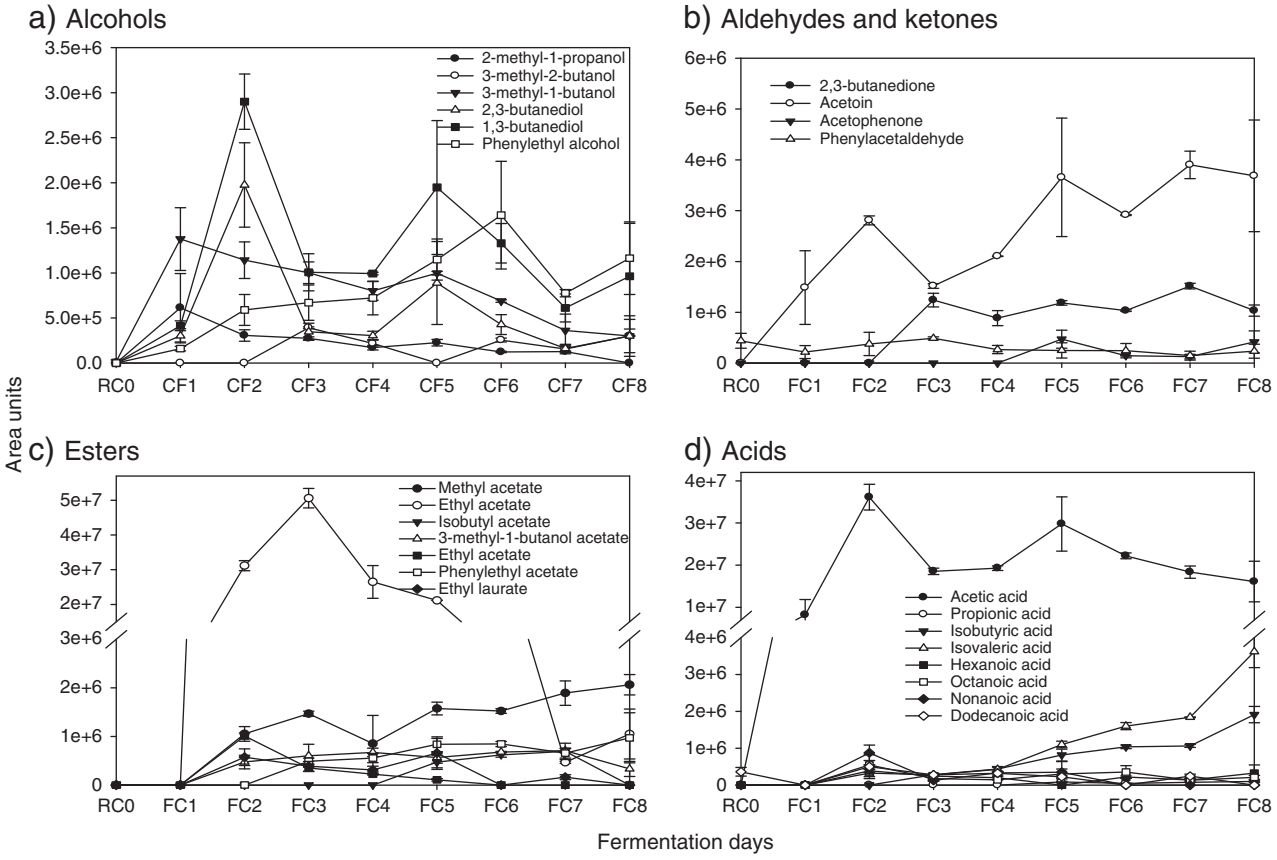

Fig. 2. Dynamics of a) alcohol, b) aldehydes and ketones, c) esters, and d) acids compounds during fermentation traditional process of the *Theobroma cacao* L. for raw cocoa at zero time (RC0); fermented cocoa beans from one to eight days (FC1, FC2, FC3, FC4, FC5, FC6, FC7 and FC8). Bars are ±standard deviation ($P<0.05$).

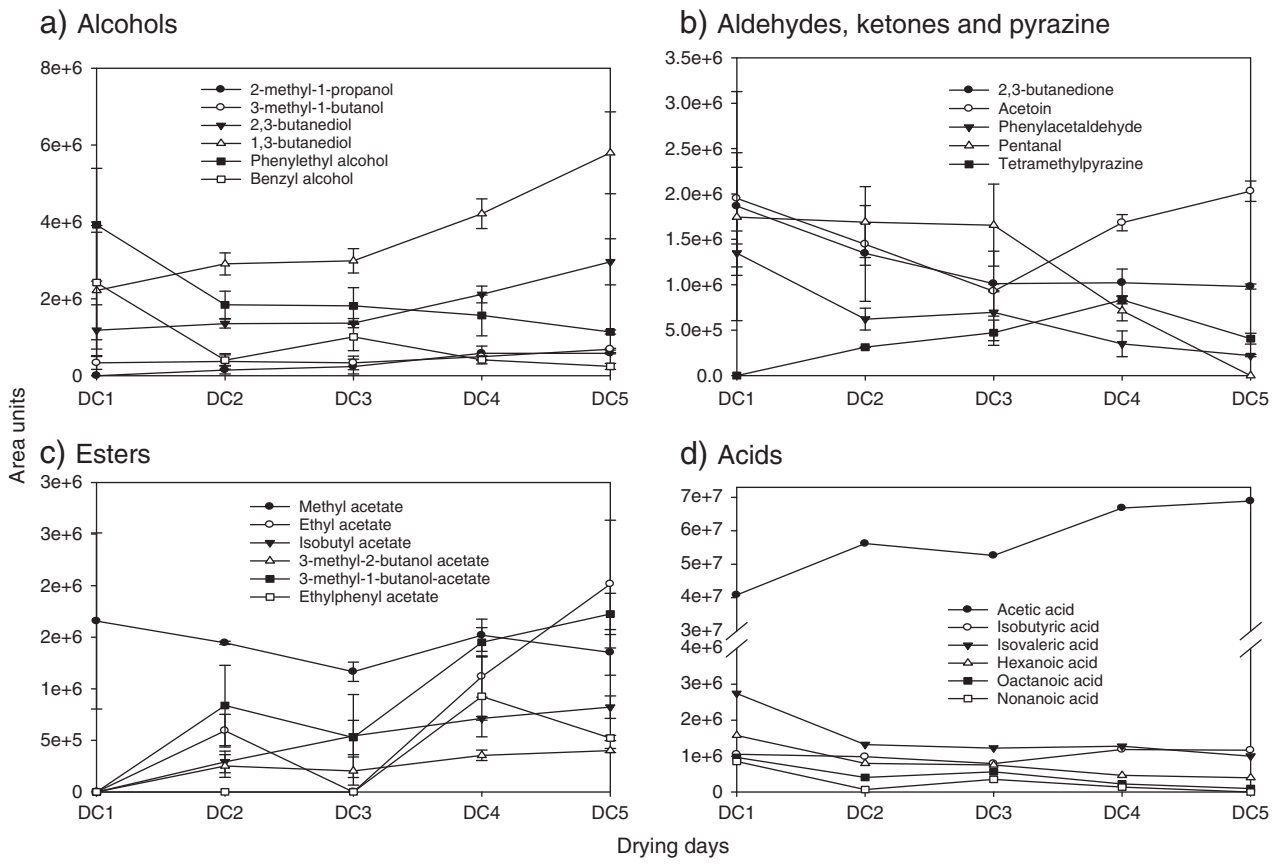

Fig. 3. Dynamics of a) alcohol, b) aldehydes and ketones, c) esters, and d) acids compounds during drying process of the *Theobroma cacao* L. from one to five days (DC1, DC2, DC3, DC4 and DC5). Bars are ± standard deviation ($P<0.05$).

compounds suggest that fermentation had not stopped and volatile alcohol was produced as a precursor to other compounds, i.e. 2,3-butanediol to produce 2,3-butanedione (Fig. 3b) (Smit et al., 2005). The peak area of 2,3-butanedione, acetoin, pentanal and phenylacetaldehyde decreased with drying (Fig. 3b).

In addition, we found tetramethylpyrazine with a significant increase in area during drying (Fig. 3b). The tetramethylpyrazine compound has been reported to produce notes characteristics of cocoa and coffee roasted on flavor quality and these are desirable for cocoa beans (Afoakwa et al., 2008; Serra-Bonvehí, 2005). Drying reduces volatile acids and total polyphenols and also converts the flavor precursors into two main classes of flavor-active components pyrazines and aldehydes, while flavor development continues during elimination of volatile acids and moisture (Ramli, Hassan, Said, Samsudin, & Idris, 2006).

Furthermore, we found that 3-methyl-1-butanol acetate and isobuthyl acetate areas increased during the drying process (Fig. 3c). As was mentioned before, 3-methyl-1-butanol acetate could be produced from 3-methyl-1-butanol oxidation (Smit et al., 2005). Also, isobuthyl acetate is a precursor of isobutyric acid, which is responsible for producing off-flavor notes such as rancid, butter, cheese and hammy in cocoa beans (Jenning & Shibamoto, 1980; Serra-Bonvehí, 2005). In addition, ethyl acetate is a product of esterification from acetic acid and ethanol (Pretorious, 2000). We observed that during the drying process, a variable behavior was found in the ethyl acetate area, which increased, decreased and finally increased again (Fig. 3c). This was similar to the behavior in acetic acid production with high areas during the drying process (Fig. 3d). These suggested that acids bacteria were present after fermentation and during the drying process, producing accumulative acetic acid (Ardhana & Fleet, 2003; Thompson et al., 2001).

We observed that the acetic acid area increased during the drying process, and was higher than isobutyric, isovaleric, hexanoic, octanoic and nonanoic acid, which diminished in the drying process (Fig. 3d). Propionic and dodecanoic acid disappeared after drying began, and they were not found after five days of drying (Fig. 3d). An increase in temperature and aeration favored compound volatilization of short chain volatile acids (Nogales et al., 2006).

3.5. Principal component analysis (PCA)

A principal component analysis (PCA) was performed to determine which the most important volatile were and non-volatile compounds on the cocoa fermentation and drying process. The PCA can compress the data based on their similarities and differences, by reducing the number of dimensions without much loss of information, and define the number of "principal components" (Shin et al., 2010). The two first principal components (PCs) are sufficient to explain the maximum variation in all original dates. In our case, the PCs explained 38.8 and 22.2% of date variance (PC1 and PC2 respectively) from the fermentation process. Loadings and scores plots are show in Fig. 4a and b. This analysis included pH, titratable acidity, sugars (sucrose, glucose and fructose), non-volatile acids (oxalic, malic, lactic, citric and succinic) and all volatile compounds. On the one hand, PC1 on the positive axis was highly influenced by some volatile compounds such as methyl acetate, acetoin, 2,3-butanedione, phenylethyl acetate and 3-methyl-1-butanol acetate; and also by succinic, lactic, malic acids and titratable acidity. These compounds presented a significant increase in concentration at middle fermentation (FC3, FC4 and FC5). Compounds with the highest concentrations were methyl acetate, acetoin, lactic acid, and 2,3-butanedione. On the other hand, PC1 negative axis grouped compounds with high concentration in

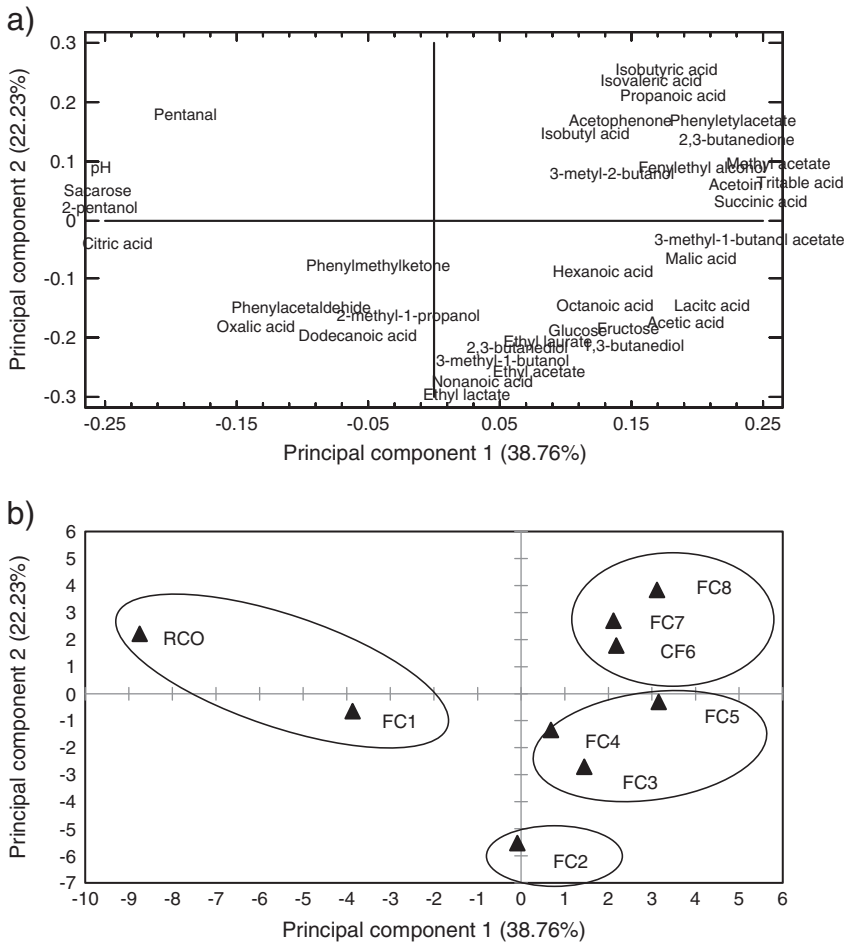

Fig. 4. Principal component analysis the first two principal components: a) Loading plot for pH, sugars, non-volatile acids and volatile compounds, b) Score plot from raw cocoa beans at zero time (RC0) and fermented cocoa beans from one to eight days (FC1, FC2, FC3, FC4, FC5, FC6, FC7 and FC8), producing by traditional fermentation of *Theobroma cacao* L.

cocoa beans at zero time (RC0) (Fig. 4b) such as sucrose, citric, oxalic and dodecanonic acids, 2-pentanol, phenylacetaldehyde and pH parameter. In addition, the concentration of these compounds decreased during the fermentation process. Regarding PC2, some volatile acids were located in the positive axis of the plane: isobutyric, isovaleric and propanoic acid. These acids increased their concentration in at last days of fermentation (FC6, FC7 and FC8) (Fig. 4b). However, we observed that were grouped the volatile compounds on the negative axis i.e. ethyl lactate, nonanoic acid, ethyl acetate, 3-methyl-1-butanol and 2,3-butanediol. These compounds were the most important at two days of fermentation (FC2) (Fig. 4b).

The scores distribution from the two first PCs (Fig. 4b) showed four separate groups, at eight days of fermentation. The fermentation middle (FC3, FC4 and FC5) were located on the positive side of PC1, while the first days of fermentation (RC0 and FC1) were found in the negative side. The last days of fermentation (FC6, FC7 and FC8) were clustered on the positive axis of PC2, but the second day of fermentation (FC2) was found in the lower-right quadrant.

Fig. 5a and b show plots of the loadings and scores obtained from PCs during the cocoa drying process, where PCs explained a 66.34% of the total date variance. In this analysis, we also included volatile and non-volatile compounds. The PC1 on the positive axis was strongly influenced by phenylacetaldehyde, glucose, octanoic acid, phenylethyl alcohol and hexanoic acid. The compounds in this group presented a decrease in concentration during the cocoa drying process. These compounds can be associated with cocoa composition at the beginning of drying (DC1) (Fig. 5b). In the negative axis plane volatile compounds were located i.e. 3-methyl-1-butanol acetate, 3-methyl-2-butanol acetate, 2-methyl-1-propanol, acetic acid and tetramethylpyrazine. This compounds group was clustered by increasing their concentration during the cocoa drying process and can be associated with the last days of drying (DC4 and DC5) (Fig. 5b). As regard at PC2 in the positive side, the titratable acidity, 2,3-butanedione, fructose and methyl acetate showed high loadings. In addition, these compounds presented a decrease in their concentrations during the middle drying process (DC2 end DC3) (Fig. 5b). Finally, in the negative side PC2 was markedly influenced by acetoin. The score plot (Fig. 5b) of PC1 and PC2 showed that the drying process was separated into three groups.

The beginning of drying (DC1) is located in the positive side of PC1, while the negative side are found the end days of drying (DC4 and DC5). The volatile and non-volatile compounds located at the extremes of PC1 (Fig. 5a) are associated with the volatile and non-volatile profiles found in cocoa beans at the beginning of drying and also in the lasts days of the process (Fig. 5b). On the other hand, DC2 and DC3 were positioned in the positive axis of PC2. These days can be related with a reduction of tritrable acidity, 2,3-butanedione and methyl acetate.

4. Conclusion

The PCA discriminated the stage of fermentation in four groups and into three groups for drying. The identification of principal compounds produced during the fermentation and drying processes can be of help in searching for indicators of off-flavor and as a fermentation index, such as isobutyric, isovaleric and propionic acids.

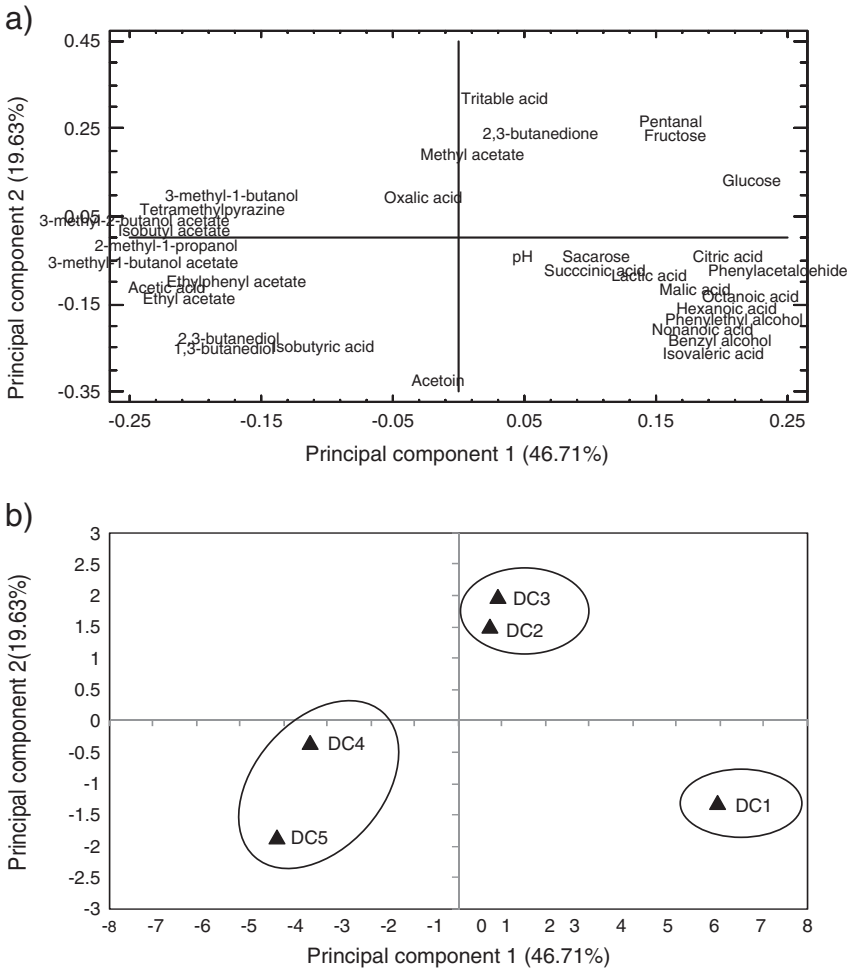

Fig. 5. Principal component analysis the first two principal components: a) Loading plot for pH, sugars, non-volatile acids and volatile compounds, b) Score plot from drying cocoa beans from one to five days (DC1, DC2, DC3, DC4 and DC5).

The oxidation of 3-methyl-1-butanol-to-3-methyl-1-butanol acetate can be used to evaluated the degree of fermentation. We found that the 3-methyl-1-butanol acetate: 3-methyl-1 butanol ratio at the end of fermentation was 1.68, indicating over fermentation. The critical point in traditional fermentation of cacao beans was at three days where formation of acetic and lactic acid increased. Identification of the main compounds produced during the fermentation and drying processes can be of help in deciding when to stop the fermentation process to avoid production of compounds with off-flavor.

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