Harvest Influence in Volatile Composition of Chocolates Produced with Hybrid Varieties of Bahia's Cocoa was Investigated using GC×GC-QMS and Chemometrics

Soraia Cristina Gonzaga Neves Braga¹✉ Luciana Fontes Oliveira², Adriana Reis de Andrade Silva³, Priscilla Efraim³, Ronei de Jesus Poppi², Fabio Augusto²

¹Instituto Federal do Espírito Santo, Campus Viana, Rodovia BR-262, Universal, 29134-400 - Viana, ES - Brazil

²Instituto de Química, Universidade Estadual de Campinas (Unicamp), Cidade Universitária Zeferino Vaz s/n, Barão Geraldo, 13084971 - Campinas, SP, Brazil

³Faculdade de Engenharia de Alimentos, Universidade Estadual de Campinas (Unicamp), Rua Monteiro Lobato, 80, Cidade Universitária Zeferino Vaz, Barão Geraldo, 13083-862 - Campinas, SP, Brazil

Graphical Abstract

Cocoa from eleven different varieties harvested from two different harvests was used to produce chocolate that had its volatile composition analyzed by GC×GC and interpreted by multivariate statistical procedure.

Cocoa varieties are important in Brazil due to their resistance to “witch’s broom” disease. In this work, the chromatographic profiles of chocolates produced from eleven cocoa varieties harvested from two different harvests were analyzed. A total of 22 samples were analyzed by comprehensive two-dimensional gas chromatography coupled to mass spectrometry (GC×GC-MS), using headspace solid phase microextraction (HS-SPME). Multiway principal component analysis (MPCA) were conducted to study the differences between the two harvests. Two principal components were responsible for the separation of the profiles between the harvests and their respective loadings were evaluated. Hydrocarbons were the principal group responsible for this separation, as some pyrazines and aldehydes.

Keywords: comprehensive two-dimensional gas chromatography, volatile organic compounds, cocoa varieties, multiway principal component analysis.

INTRODUCTION

Until the 1980s, Brazil had a large cocoa production, but a disease known as witch’s broom, caused by the fungus *Moniliophthora perniciosa* [1], reached plantations in Bahia, a state located in the Brazilian

Northeast Region, dramatically reducing the production of cocoa [1]. Two ways were adopted to combat this disease in Brazil: the selection of clones and the selection of hybrid varieties [2]. Currently, new works have been conducted using biological control to reduce witch's broom infestation [3].

The first cocoa clones were selected in the 1930s and a series called ICS were originated from these clones [4]. Some genotypes of this ICS series were selected and crossed with resistant clones, generating the series TSH [4]. A cross of Scavinas with Amazonian clones generated the TSA series [4]. In Ecuador, CCN 51 clones have high productivity and are resistant to witch's broom.

Researches developed in Brazil by CEPLAC — The Executive Commission for Cocoa Cultivation Planning, an agency of the Brazilian Ministry of Agriculture, Livestock and Food Supply — obtained hybrid cocoas of the types Forastero x Forastero and Forastero x Trinitario. These cocoas' hybrid have been used by producers in the Bahia region since 1995 [4]. In 2009, Efraim [4] characterized hybrid cocoas of a CEPLAC experimental farm, studding the varieties: CEPEC 42 (hybrid of TSA 644 x SIC 19), EET 397 (coming from SCA), TSA 654 (hybrid of SCA6 x IMC 67), TSA 656 (hybrid of SCA6 x IMC 67), TSAN 792 (hybrid of TSA 641 and unknown genotype), TSH 516 (hybrid of SCA6 x ICS1), TSH 565 (hybrid of SCA6 x ICS1), TSH 774 (unknown origin) e TSH 1188 (unknown origin) [4]. Ramos *et al.* [5] evaluated, in 2014, the fermentation of four cocoa varieties through the volatile composition in the fermentation. In the new hybrids developed, it is necessary to characterize the volatile profile in chocolates obtained with these cocoas.

Cocoa and chocolate are complex samples, and analysis of volatile compounds in such samples usually requires complex analytical methods. Analysis of volatile compounds is usually conducted by gas chromatography; another technique that has been used is the comprehensive two-dimensional gas chromatography (GC×GC), which is widely used in the characterization of complex biological [6-8] and food [9-11] samples. It is also used to obtain chromatographic profiles with less sample preparation. GC×GC has a higher analyte resolution power, besides facilitating the identification of analyzed components by the chromatographic structuring that can occur with some sets of chromatographic columns.

In this work, eleven varieties of cocoa developed by CEPLAC were studied. The differences in the volatile composition of the chocolates obtained from two harvests of cocoa clones were investigated using HS-SPME and GC×GC-MS, analyzed with MPCA as chemometric tool. With this work it was possible to characterize the chocolate obtained by the new cocoa varieties, showing that the cocoa harvest influences the volatile composition in the final product, not only depending on the cocoa variety.

MATERIALS AND METHODS

Chocolate Samples

Cocoa was obtained from an experimental farm in Bahia, in a CEPLAC study. The produced nibs belonged to different cocoa varieties, and the samples were collected from two different crops, at harvests with a difference of six months between them. The varieties studied were: TSH 1188, TSH 565, LP 06, EET 397, OS 13.19, CCN 10, CA 1.4, CCN 51, PH 129, FM 31 and COMUM.

For the chocolate production, the nibs were processed under the same conditions used at the School of Food Engineering, University of Campinas (FEA-Unicamp). Dark chocolate with 65% cocoa and 35% sugar was produced according to Reis [12]. After production, the chocolate was stored in a cold chamber at -18 °C until analysis.

SPME Extraction and Chromatographic Conditions

Collection of the volatile fraction of the samples was performed by HS-SPME using a previously optimized procedure [13]. Aliquots of 1.000 ± 0.005 g of the chocolate samples were weighed into 15 mL vials. After a 5 min period for sample/headspace equilibration at 60 °C, a 50/30 μ m divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) fiber was exposed to the sample headspace for 50 min; the fiber was then immediately inserted into the GC×GC system injector and held there for 5 min. Before

the extractions, the fiber was conditioned for 2 h at 250 °C according to the manufacturer's instructions. The method optimization, including the fiber selection, was the same used by Oliveira *et al.* [14].

GC×GC-QMS

GC×GC-QMS analyses were carried out on a prototype based on a Shimadzu QP2010 + fast GC-QMS gas chromatograph fitted with a lab-made 4-jet cryogenic modulator, already described in the literature [7]. The column set consisted of a 30 m × 0.25 mm × 0.25 µm HP-5 column (Agilent Technologies, Wilmington, DE) connected to a 0.80 m × 0.1 mm × 0.1 µm Solgel Wax column (SGE Analytical Science, Ringwood - Victoria, Australia). The split-splitless injector was operated in splitless mode at 260 °C. The column oven temperature was programmed as follows: 60 °C (3 min) to 70 °C (7 min) at 3 °C min⁻¹; 70 °C to 110 °C at 3 °C min⁻¹; and 110 °C to 240 °C at 10 °C min⁻¹. The carrier gas used was high purity (99.9999%) hydrogen at 0.6 mL min⁻¹, and the modulation period was 6 s. The interface temperature was 260 °C and the photomultiplier power was programmed to 0.8 kV up to 10 min of the chromatographic run, and then increased to 0.9 kV until the end of the analysis. The scanned mass range was set from $m/z = 40$ D to 340 D, resulting in a data collection frequency of 25 spectra s⁻¹.

Data Processing

Peaks in chromatograms were identified by NIST 2010 mass spectral library and 1st dimension linear temperature programming retention indexes (LTPRI) performed using GC Image software (Zoex Corp., Houston – TX, USA), and samples spiked with n-alkanes mixture (C8-C20).

Chemometric analyzes were performed using the softwares: MATLAB environment (Mathworks, EUA) version R2011b, and PLS_toolbox (Mathworks, EUA) version 654. Differences between chromatographic profiles of chocolates were evaluated by MPCA. Before the use of MPCA, the unfolded chromatograms were aligned using the COW (Correlation Optimizing Warping) algorithm [15,16].

RESULTS AND DISCUSSION

Chocolate samples were produced with cocoa of 11 varieties and from two harvests, named harvest 1 and harvest 2, totaling 22 samples. After obtaining the chromatographic profiles by GC×GC-QMS, variations in these samples related to harvests were investigated. Chromatograms of harvest 1 and harvest 2 presented differences in a specific region of the chromatogram near to the retention time of 1.0 second in the 2^D dimension. MPCA was developed with the entire chromatograms.

Chromatographic profiles obtained were exported as TIC (total ion current) in .txt format and were investigated with MATLAB. Alignment was conducted with the unfolded chromatogram and in small segments independently, using the COW algorithm [15,16].

After the alignment, a MPCA model was developed using the PLS_toolbox. Three principal components were required and they explained 83,47% of data variance (PC1 69,70%, PC2 10,36% and PC3 3,41%). Hotelling (T²) versus Q residual (Q) showed no outliers.

Two principal components were responsible for separation of chromatographic profiles in harvest 1 and harvest 2: PC1 and PC2. A score graphic between these principal components is shown in Figure 1.

Figure 1. Graphic of scores of principal components 1 and 2, showing the separation between harvest 1 (triangles) and harvest 2 (asterisks).

To identify the compounds responsible for the class separation, the loadings graphics of PC1 and PC2 were evaluated and the compounds found were identified by matching their mass spectra with the NIST 10 library and confirmed by comparing the LTPRI with the standard. Table I and Table II present the chromatographic peaks identified in loadings of PC1 and PC2.

Table I. Compounds corresponding to loadings of PC1 in chocolate samples of two different harvests

t_R^{1D} (min)	t_R^{2D} (s)	Retention index calculated	Identified compounds
10.5	1.84	961	Benzaldehyde
12.9	1.52	1000	Decane
13.9	1.48	1015	NI
14.6	1.48	1025	NI
15.7	1.44	1042	3-methyl-decane
16.7	1.52	1056	2-methyl-decane
17.3	1.52	1065	(Z)-5-methyl-dec-2-ene
18.2	1.52	1078	2,5-dimethyl-decane
19.7	1.44	1105	NI
20.4	1.44	1112	2,3,5-trimethyl decane
21.9	1.44	1138	4-methyl-undec-1-ene
22.8	1.44	1154	3-methyl-undecane
24.3	1.44	1180	3,8-dimethyl-undecane
13.2	4.20	1006	Trimethylpyrazine
18.9	3.36	1088	Tetramethylpyrazine
20.0	2.44	1105	Nonanal
28.5	1.36	1285	2,3-dimethyldodecane

NI: Not identified

Table II. Compounds corresponding to loadings of PC2 in chocolate samples of two different harvests

t_R ¹ D (min)	t_R ² D (s)	Retention index calculated	Identified compounds
5.1	1.44	843	NI
5.8	1.40	863	4-methyl-octane
8.2	1.68	903	Dihydro-2(3H)-furanone
10.1	3.32	952	(E)-hept-2-enal
12.1	2.60	975	Octan-3-one
12.5	3.48	994	(Z)-hex-2-enyl acetate
17.4	4.04	1066	3-methoxybutanoic acid
18.1	5.04	1089	Octan-1-ol
19.0	4.24	1090	NI
19.7	4.04	1099	Linalool
19.9	3.56	1101	Nonan-2-ol
21.7	3.28	1129	Dihydrolinalol
27.1	1.84	1245	NI

NI: Not identified

Analyzing the identified compounds by loadings of PC1, it was possible to verify that hydrocarbons were responsible for the separation between the chocolate samples produced firstly (harvest 1), and those harvest later (harvest 2). In addition to this group of compounds, other compounds such as benzaldehyde, trimethylpyrazine, tetramethylpyrazine and nonanal were identified as the major compounds (after the hydrocarbons) in the chromatograms of chocolate samples, also being known as important contributors to chocolate aroma [2]. In PC2 there is a predominance of alcohols (4 compounds) and 1 compound of each class: furanone, aldehyde, ester, ketone, acid and alkane. Figure 2 shows the characteristic chromatograms of the COMUM cocoa variety from harvest 1 (A) and harvest 2 (B). The red overlapping rectangle in chromatogram (A) highlight some analytes not solved.

All the components in chromatograms were identified and separated in chemical classes. In Figure 3, the graphics show this classification for harvest 1 (A) and harvest 2 (B). Table III shows all the compounds identified in harvest 1 and harvest 2.

The developed MPCA showed that hydrocarbons are the compounds with the greatest difference between the two harvests, being this showed by the chemical class studies and presented in Figure 3. There was no change in the number of compounds in the classes of acids (1), aldehydes (8), ketones (11) and compounds containing nitrogen (6) between the two harvests. A decrease of 3 alcohols and an increase of 2 esters from harvest 1 to 2 were also observed.

Figure 2. Chromatograms obtained for the COMUM cacao variety from harvest 1 (A) and harvest 2 (B). The region in (A) surrounded by red corresponds to some analytes not resolved (splitless mode).

Figure 3. Classes of compounds found in chocolates from harvest 1 (A) and harvest 2 (B) and the number of compounds in each class.

Regarding the distribution of compounds in Table III, in the alcohol class, hept-4-en-1-ol, 3-methylheptan-2-ol and triethylene glycol were observed in harvest 1 and not observed in harvest 2. Among the esters, in harvest 2 there were 2-pentyl acetate, prop-2-ene-1,1-diol diacetate, isoamyl pyruvate and 3,4-dihydroxy-3-methyl-butyl acetate, while harvest 1 presented ethyl octanoate and ethyl benzene acetate. Regarding the aldehydes and N-compounds there were no variation in number and distribution of them. For the other classes, there was no variation in the number of compounds, but their distribution was different. In the ketone class, harvest 1 had undecan-2-one, pentan-2-one and 2-(formyloxy)-1-phenyl-ethanone, while harvest 2 had heptan-2-one, decan-2-one and 4-hidroxy-3-methyl-butanone.

The compound octan-1-ol was present only in FM 31 and LP 06, while dihydrolinalool and undecan-2-one were present in EET 397 and PS 13.19, both in harvest 1; o-cimene was present in the varieties EET397 and PH 129 - in the latter, triethylene glycol was identified. Furthermore, in harvest 1 variety PS13.19, only the ethyl octanoate compound was found. In harvest 2, the varieties FM31, CCN551, PH129, TSH and PS13.19 contained single compounds, with the last two varieties presenting N,N,O-triacetylhydroxylamine, the first containing prop-2-en-1,1-diol acetate, the second having 1-methyl-4-(1-methylethyl)-benzene and the third containing tetrahydrolinalol.

Table III. Compounds identified in all the chromatograms obtained in the two harvests

t_R^D (min)	t_R^{2D} (s)	Compound	FM 31	TSH1188	TSH565	LP06	COMUM	EET 397	PS 13.19	CCN10	CA 1.4	CCN551	PH129
3.8	1.84	Hexanal	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)
3.8	5.76	Acetic acid	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)
4.2	3.40	2,3-Butanediol	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)
4.7	2.96	Methyl-pyrazine	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)
5.4	1.76	2-pentyl acetate	X(2)	X(2)	X(2)	X(2)	X(2)	X(2)	X(2)	X(2)	X(2)	X(2)	X(2)
5.8	1.40	4-methyl-octane	*	X(2)	X(2)	X(2)	X(2)	*	X(2)	X(2)	*	X(2)	*
5.8	4.00	Hept-4-en-1-ol	X(1)	X(1)	X(1)	X(1)	X(1)	*	X(1)	X(1)	*	X(1)	X(1)
5.9	1.88	Ethyl benzene	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)
6.1	2.32	2-hydroxy-3-methyl-2-cyclopenten-1-one	X(1,2)	X(1,2)	X(1,2)	X(1)	X(1,2)	X(1)	X(1,2)	X(1,2)	X(1)	X(1,2)	X(1)
6.8	5.84	1,2-Diacetoxethane	X(1,2)	X(1,2)	X(1,2)	X(1)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(2)	X(1,2)	X(1,2)
7.0	2.60	Etheryl benzene	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)
7.0	2.12	Heptan-2-one	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)
7.2	1.40	Nonane	X(1)	X(1)	X(1)	X(1)	X(1)	X(1)	X(1)	X(1)	X(1)	X(1)	X(1)
7.3	2.12	Heptan-2-ol	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)
7.3	3.28	3-methyl-heptan-2-ol	X(1,2))	X(2)	X(1,2)	X(2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(2)	X(2)	X(1,2)
7.6	4.60	Butyl propanoate	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1)	X(1,2)	X(1,2)
7.9	3.24	2,6-dimethyl-pyrazine	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)
7.9	1.40	2,4,6-trimethyl-octane	X(1)	X(1)	X(1)	*	*	X(1)	*	X(1)	X(1)	X(1)	*
8.2	1.64	Dihydro-5-methyl-2 (3H) -furanone	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)
8.3	3.52	2,3-dimethyl-pyrazine	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)
8.7	1.48	Non-2-ene	X(1)	X(1)	X(1)	X(1)	X(1)	X(1)	X(1)	X(1)	X(1)	X(1)	X(1)
8.8	5.92	4-Hydroxy-butan-2-one acetate	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)
9.3	0.88	5-hydroxy-2-methyl-hexan-3-one	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)
10.0	2.24	Benzeneacetaldehyde	X(1,2)	X(1,2)	X(1,2)	X(1)	X(1,2)	X(1)	X(1,2)	X(1)	X(1,2)	X(1)	X(1)
10.1	3.28	(E)-hept-2-enal	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(2)	X(2)	X(2)	X(1)
10.4	2.32	1-ethyl-3-methyl-benzene	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)
10.5	1.84	Benzaldehyde	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)
11.3	3.12	Prop-2-ene-1,1-diol diacetate	X(2)	*	*	*	*	*	*	*	*	*	*
12.0	2.56	Octan-3-one	X(1,2)	X(2)	X(1,2)	X(1,2)	X(2)	X(2)	X(1,2)	X(2)	X(2)	X(1,2)	X(2)
12.1	3.48	5-hepten-2-one	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)

Table III. Compounds identified in all the chromatograms obtained in the two harvests (Cont.)

t _R ^{1D} (min)	t _R ^{2D} (s)	Compound	FM 31	TSH1188	TSH565	LP06	COMUM	EET 397	PS 13.19	CCN10	CA 1.4	CCN551	PH129
12.4	2.36	2-pentyl-furan	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)
12.5	2.76	Octan-2-one	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)
12.5	3.44	(Z)-2-hex-2-enyl acetate	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1)	X(2)	X(1,2)	X(2)	X(2)	X(2)	X(2)
12.7	5.20	4-hydroxy-3-methyl-butan-2-one	*	X(2)	X(2)	X(2)	X(2)	*	X(2)	*	*	*	*
12.9	1.52	Decane	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)
13.2	2.72	Octanal	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)
13.2	4.20	Trimethylpyrazine	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)
13.7	1.48	3-ethyl-2,7-dimethyloctane	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)
14.6	2.84	1,2,4-trimethyl-benzene	X(1)	X(1)	X(1)	X(1)	X(1)	X(1)	X(1)	X(1)	X(1)	X(1)	X(1)
14.7	2.32	o-Cimene	*	*	*	*	*	X(1)	*	*	*	*	X(1)
14.8	2.38	1-methyl-4-(1-methylethyl)-benzene	*	*	*	*	*	*	*	*	*	X(2)	*
15.0	1.92	Limonene	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)
15.2	5.12	2-ethyl-hexan-1-ol	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)
15.8	3.32	oct-3-en-2-one	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(2)	*
16.0	2.16	Isoamyl Pyruvate	*	X(2)	X(2)	X(2)	*	*	X(2)	*	*	*	X(2)
16.1	1.44	5-methyl-decane	X(1)	X(1)	X(1)	X(1)	X(1)	X(1)	X(1)	X(1)	X(1)	X(1)	X(1)
16.2	2.68	Phenylacetaldehyde	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1)	X(1,2)	X(1,2)	X(1)
16.7	1.48	2-methyl-decane	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)
17.3	1.48	5-methyl-dec-2-ene	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)
17.4	4.00	3-Methoxybutanoic acid	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(2)	X(1,2)	X(2)	X(1,2)
18.0	5.04	Octan-1-ol	X(1,2)	X(2)	X(2)	X(1,2)	X(2)	X(2)	X(2)	X(2)	X(2)	X(2)	X(2)
18.3	4.24	1,3-Butanediol diacetate	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(2)	X(1,2)	X(2)	X(1,2)	X(2)	X(1,2)
18.9	3.36	Tetramethyl-pyrazine	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)
19.3	2.48	Nonan-2-one	X(1,2)	X(2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(2)	X(1,2)
19.6	1.48	Undecane	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)
19.7	4.04	Linalool	X(1)	*	X(1,2)	X(1,2)	X(1)	X(1,2)	X(1,2)	X(2)	*	*	X(1,2)
19.8	2.76	Tetrahydro-linalool	*	*	*	*	*	*	*	*	*	*	X(2)
19.9	3.56	Nonan-2-ol	X(1)	*	X(1,2)	*	X(1)	X(1,2)	X(1,2)	X(2)	*	*	X(1,2)
20.0	2.44	Nonanal	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)

Table III. Compounds identified in all the chromatograms obtained in the two harvests (Cont.)

¹ D _R (min)	² D _R (s)	Compound	FM 31	TSH1188	TSH565	LP06	COMUM	EET 397	PS 13.19	CCN10	CA 1.4	CCN551	PH129
20.3	5.84	N, N, O-Triacetylhydroxylamine	X(1)	X(1)	X(1,2)	X(1)	X(1)	X(1)	X(1,2)	X(1)	X(1)	*	X(1)
20.4	1.44	2,3,5-trimethyl-decane	X(1)	X(1)	X(1)	X(1)	X(1)	X(1)	X(1)	X(1)	X(1)	X(1)	X(1)
20.7	1.48	2,5,6-trimethyl-decane	X(1)	X(1)	X(1)	X(1)	X(1)	X(1)	X(1)	X(1)	X(1)	X(1)	X(1)
20.9	4.72	Benzeneethanol	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)
20.9	1.48	2,6,7-trimethyl- decane	X(1)	X(1)	X(1)	X(1)	X(1)	X(1)	X(1)	X(1)	X(1)	X(1)	X(1)
21.3	1.44	Tetradecane	X(2)	X(2)	X(2)	X(2)	X(2)	*	X(2)	X(2)	*	X(2)	X(2)
21.7	3.32	Dihydrofuralol	*	*	X(2)	*	*	X(1,2)	X(1,2)	*	*	X(2)	X(2)*
21.9	1.44	4-methyl-undec-1-ene	X(1)	X(1)	X(1)	X(1)	X(1)	X(1)	X(1)	X(1)	X(1)	X(1)	X(1)
22.8	1.48	3-methyl-undecane	X(1)	X(1)	X(1)	X(1)	X(1)	X(1)	X(1)	X(1)	X(1)	X(1)	X(1)
23.0	1.52	5-methyl-undecane	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)
23.5	1.44	5-methyl-undec-2-ene	X(1)	X(1)	X(1)	X(1)	X(1)	X(1)	X(1)	X(1)	X(1)	X(1)	X(1)
23.7	1.48	2,2,6-trimethyl-decane	X(1)	X(1)	X(1)	X(1)	X(1)	X(1)	X(1)	X(1)	X(1)	X(1)	X(1)
24.3	1.44	3,8-dimethyl-undecane	X(1)	X(1)	X(1)	X(1)	X(1)	X(1)	X(1)	X(1)	X(1)	X(1)	X(1)
25.1	2.36	Decan-2-one	*	X(2)	X(2)	X(2)	X(2)	X(2)	X(2)	X(2)	X(2)	*	*
25.3	2.04	Ethyl octanoate	*	*	*	*	*	*	X(1)	*	*	*	*
25.4	1.48	Dodecane	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)
25.7	2.24	Decanal	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)
26.5	3.24	Benzene Ethyl acetate	X(1)	X(1)	X(1)	X(1)	X(1)	X(1)	X(1)	X(1)	X(1)	X(1)	X(1)
26.5	1.40	5,5-dimethyl-undecane	X(1)	X(1)	X(1)	X(1)	X(1)	X(1)	X(1)	X(1)	X(1)	*	X(1)
26.5	3.36	3,4-Dihydroxy-3-methyl-butyl acetate	X(2)	X(2)	X(2)	X(2)	X(2)	X(2)	X(2)	X(2)	X(2)	X(2)	X(2)
27.4	1.24	Triethylene glycol	*	X(1)	*	*	*	*	*	*	*	*	X(1)
27.9	3.16	2-Phenylethyl acetate	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)	X(1,2)
28.5	1.38	2,3-dimethyl-dodecane	X(1)	X(1)	X(1)	X(1)	X(1)	X(1)	X(1)	X(1)	X(1)	X(1)	X(1)
28.9	1.84	Undecan-2-one	*	*	*	*	*	X(1)	X(1)	*	*	*	*
29.1	1.36	Tridecane	X(1)	X(1)	X(1)	X(1)	X(1)	X(1,2)	X(1,2)	X(1)	X(1,2)	X(1)	X(1,2)
31.2	2.00	2- (formyloxy) -1-phenyl-ethanone	X(1)	X(1)	X(1)	X(1)	X(1)	X(1)	X(1)	X(1)	X(1)	X(1)	X(1)
31.3	1.40	Tetradecane	X(1)	X(1)	X(1)	X(1)	X(1)	X(1)	X(1)	X(1)	X(1)	X(1)	X(1)

X: compound present in the sample; *Compound missing in the sample; (1): Harvest 1; (2): Harvest 2; (1,2): Harvest 1 and 2.

Regarding the quantity of volatile compounds in each variety, in harvest 1 the order was as follows: FM31 = PS13.19 > TSH565 = EET397 > LP06 = PH129 > COMUM > TSH 1188 > CA 1.4 > CCN10 > CCN 51. In harvest 2, the order was PS13.19 > TSH565 > TSH1188 > EET397 > LP06 = CCN10 > FM31 = PH129 = CCN551 > COMUM > CA1.4.

It is possible to verify that in the two harvests the richest sample in volatiles compounds was PS13.19, followed by TSH565, being these two samples the ones that had smallest variation between the harvests. There was no pattern among the other varieties, so the distribution was random.

CONCLUSIONS

Applying the MPCA to the chocolate chromatographic profiles obtained from cocoa beans of 11 varieties and two harvests, it was possible to verify the differences in these profiles according to the harvest — the largest difference observed was in the hydrocarbon class found in higher quantity in harvest 1. PS13.19 was found to be the richest variety in volatile compounds, regardless of the original harvest. Furthermore, it was found that even within the same variety, the volatile composition of chocolate may change according to the cocoa harvesting period.

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