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Thermal properties and volatile compounds profile of commercial dark-chocolates from different genotypes of cocoa beans (*Theobroma cacao* L.) from Latin America

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ABSTRACT

There is a growing interest in the identification of chemometric markers that allow the distinction and authentication of dark-chocolates according to their cocoa geographical origin and/or genotype. However, samples derived from Latin American cocoa, including specimens from North and South America, have not been studied in this context. An exploration of the melting behavior, fat composition, bioactive content, and volatile profile of commercial darkchocolates was conducted to identify possible patterns related to the genotype and/or origin of cocoa from Latin America. The melting properties were evaluated by DSC and related to fat content and fatty acids profile. Total polyphenol, anthocyanin, methylxanthine, and catechin content were analyzed. Finally, the volatile compounds were extracted and identified by HS-SPME/GC-MS and were analyzed through Principal Component Analysis (PCA) and the Hierarchical Cluster Analysis Heatmap (HCA Heatmap). The fatty acids profile showed a relationship with the melting properties of dark chocolate. The samples exhibited two glass-transition temperatures (T_g) at ≈19 °C and ≈25.5 °C, possibly related to traces of unstable polymorphic forms of monounsaturated triacylglycerides. The analysis of bioactive compounds demonstrated great variability among samples independent of the cocoa origin, genotype, and content. The PCA and HCA Heatmaps allowed discriminating against the chocolates in relation to the cocoa origin and genotype. Compounds like tetramethylpyrazine, trimethylpyrazine, benzaldehyde, and furfural could be considered as dark-chocolate aroma markers derived from Latin American cocoas (North American region). The 2-phenylethyl alcohol, 2-methylpropanoic acid, 2,3-butanediol, 2-nonanone, and limonene for derived from South America. And the 2-phenylethyl acetate, 3-methyl-butanal, and cinnamaldehyde could allow to distinguishing between regional genotypes.

1. Introduction

Chocolate is one of the most consumed and appreciated foodstuffs of the world due to the pleasant sensory effect associated with its texture, flavor, and aroma. However, recently, its nutritional and functional properties as a source of healthy compounds have been reconsidered, rendering the consumption of dark chocolates with high cocoa content as trending in the current market (Agibert & Lannes, 2018; Di Mattia et al., 2017; Torres-Moreno et al., 2012). Cocoa intake is associated with biological effects, such as anti-inflammatory, antiatherosclerotic, and antiplatelet aggregation activities, improved insulin sensitivity, as well as modulation of blood pressure and immune-function attributed to its rich polyphenol content (Oracz & Nebesny, 2016). Dark chocolate is studied as a base for fortification with microcapsules (Agibert & Lannes, 2018) and enrichment with probiotics (Eor et al., 2019), offering complimentary functional properties. Dark chocolate is a product obtained from fermented, roasted, and milled cocoa beans, and is basically formulated with cocoa liquor (at different percentages) without ingredients other than sugars, cocoa butter, emulsifiers, and flavoring agents, which are mixed, refined, and subsequently subjected to conching and tempering process for the development of the final texture, appearance, and flavor, key attributes in consumer choice and acceptability (Bordiga et al., 2015; Di Mattia et al., 2017; Torres-Moreno et al., 2012). These attributes are related to the melting properties, the

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polyphenol and methylxanthine content, and the volatile compounds profile (Tran et al., 2016).

During chocolate-making, a thermal process known as tempering is used to obtain a desirable crystalline state of the cocoa butter with a melting temperature around of 32–34 °C, conferring a desired glossy appearance, good snap, contraction, and enhanced shelf life (Ostrowska-ligęza et al., 2019). However, the melting behavior of dark chocolate can be influenced by fat content and the fat composition (the saturated and unsaturated fatty acids content), together with particle size, the content of emulsifiers, and storage time, which could exert an impact on the texture of the chocolate, thus on its quality (Afoakwa et al., 2008, 2009; Ostrowska-ligęza et al., 2019). Recent work revealed great difference between the composition of cocoa butter from Brazilian regions, which was associated with extrinsic factors (climate conditions, harvest time, geographical location, and agricultural method) that affected its melting behavior (Vieira et al., 2015). Torres-Moreno et al. (2015) evaluated the effect of different processing conditions (roasting and conching time) on the fatty acids profile of dark chocolate produced with cocoa from Ghana and from Ecuador, observing differences in the fatty acids profile associated with its geographical origin, but not with the processing effect. Further researches are needed to evaluate differences in the fatty acids profile of dark chocolate produced from Latin American cocoas and its relation with melting behavior.

Polyphenols and methylxanthines are the non-volatile compounds that are mainly responsible for the astringency and bitterness of dark chocolates, important attributes of cocoa flavor (Elwers et al., 2009; Peláez et al., 2017). The conditions of the chocolate-making process (roasting and conching) and the cocoa genotype of the cocoa influence the amounts of polyphenols and methylxanthine in the final product (Cambrai et al., 2017; Oracz & Nebesny, 2019). The bioactive compounds of dark chocolates have been evaluated (Belščak et al., 2009), including samples from origins such as Ecuador, Ghana, Ivory Coast, Cameroon, and Nigeria (Bordiga et al., 2015). In spite of this, there is little information and work is focused on being able to identify bioactive markers related to characterizing dark chocolates produced with cocoa genotypes from Latin America.

The aroma of chocolate is another important quality marker, a very complex matrix that can include more than 500 volatile compounds of several chemical classes, such as the acids, aldehydes, esters, ketones, and pyrazines that resulted from a sophisticated technological process applied to the cocoa bean (Braga et al., 2018; Frauendorfer & Schieberle, 2008). The volatile compounds of the chocolate depend on the genotype and origin of the cocoa beans, the agro-climatic growing conditions, and the post-harvest fermentation, together with manufacturing steps such as roasting and conching, which exert an influence on their sensory attributes, quality, and acceptance (Acierno et al., 2016; Braga et al., 2018; Engeseth & Ac Pangan, 2018). However, recent studies reveal that the fruity and floral notes of some esters and alcohols are stable along the dark chocolate processing chain; therefore, the final product maintains the fine flavor aroma characteristic of the cocoa bean (Ascrizzi et al., 2017). Chemometrics is applied when there is a large and complex dataset, in terms of sample numbers, types, and responses, in search of optimization of the experiment design and the extraction of useful information from complex matrixes employing mathematical and statistical tools, such as Principal Component Analysis (PCA) and Hierarchical Cluster Analysis (HCA) (Granato et al., 2018). In food science, the chemometrics analysis, such as PCA, has been utilized to evaluate the effects of the processing of foods, food adulteration, and food authentication through chemical markers (Cavalcanti et al., 2019). Recently, Cambrai et al. (2017) employed chemometric analysis by means of the PCA of a phenolic compounds profile in commercial dark-chocolate samples, the latter allowing discrimination of the origin (Madagascar, Caribbean, and different countries from South America and Africa) and cocoa genotype (Criollo, Trinitario, Nacional, and Forastero). Braga et al. (2018) used PCA to differentiate the compounds at each step of the chocolate manufacturing (nibs, liquor, and chocolate) through volatile compounds. In addition, Acierno et al. (2016) evaluated, through PCA, the volatile compounds of dark chocolates with different cocoa genotypes (Criollo, Forastero, and Trinitario), origins (Africa, Asia, Oceania, South America, and mixed origin), and brand, identifying volatile compounds related to the botanical and geographical origin of cocoa, despite the brand formulation and processing conditions. However, there is a lack of research focused on evaluating the relationship of the dark-chocolates volatile profile as a final product with native cocoa genotypes from Latin America, including exemplary genotypes from North America, which have not been studied.

Therefore, the aim of this study was to explore melting behavior and its relation with fat content and the fatty acids profile, the polyphenol and methylxanthine content, and the volatile profile of commercial dark chocolates of different genotypes (Criollo, Trinitario, and Nacional) and origin (North and South America). This is to identify possible patterns or markers that could be related to the cocoa genotype and/or Latin American origin, regardless of its intrinsic manufacturing process and formulation. The volatile compounds profile, due to its complexity, was analyzed through Principal Component Analysis (PCA) and the Hierarchical Cluster Analysis Heatmap (HCA Heatmap).

2. Materials and methods

2.1. Chemicals

The Folin–Ciocalteu phenol reagent (F9252), sodium carbonate (S7795), gallic acid (91215), epicatechin standard (E1753), catechin standard (43412), caffeine standard (C1778), theobromine standard (42993), boron trifluoride-methanol solution (B1252), and the analytical grade solvents (heptane, acetonitrile, and methanol) were obtained from Sigma (Sigma-Aldrich GmbH, Sternheim, Germany).

2.2. Samples

Five commercial samples of dark chocolates, with known cocoa-bean genotype and geographical origin, were studied. Table 1 presents the coding of the different samples and their general composition presented on their labels. The 100% cocoa sample (CA100) is a bar of chocolate that only contains cocoa liquor with the minimal content of emulsifier (lecithin) that has been processed like any chocolate according to the information provided by its manufacturer.

Table 1
Chocolate samples coding and composition (according to the manufacturer's declaration).

Sample coding	Cocoa genotype	Cocoa Origin	Cocoa liquor (%)	Sugar content (%)	Protein content (%)	Soy Lecitin	Other emulsifier
CA100	Criollo	Amazon	100	–	n/s	yes	–
CV74	Criollo	Venezuela	74	25.6	4.8	yes	–
CM70	Criollo	Mexico	70	28.7	10	yes	Sorbitan tristearate
TM66	Trinitario	Mexico	66	34	6.5	yes	–
NE60	Nacional	Ecuador	60	40	7.1	yes	–

n/s = not specified; - does not contain.

2.3. Total fat and fatty acids profile

Fat content in the sample was performed according to Jacquot et al. (2016). For the fatty-acids profile analysis by Gas Chromatography with Flame Ionization Detector (GC-FID), the Fatty Acids Methyl Esters (FAME) of samples were prepared and analyzed according to Torres-Moreno et al. (2015). Analysis of FAME was performed on GC (Model 7820; Agilent Technologies, Inc., Santa Clara, CA, USA) equipment with a DB23 column (6 m × 0.25 i.d. × 0.25 μm of stationary phase). Quantification of fatty acids was performed using external standards.

2.4. Determination of melting properties and glass-transition temperature (T_g)

The melting properties and glass-transition temperature (T_g) of the dark-chocolate samples were evaluated by a Differential Scanning Calorimeter (DSC Q100; TA Instruments, Wilmington, New Castle, DE, USA). Samples (~3 mg) were loaded into hermetic aluminum pans. DSC curves were obtained in the subsequent heating process from −60 to 70 °C using a heating rate of 10 °C/min in an N_2 stream. An empty aluminum pan was used as a reference. A rapid heating rate was employed to prevent annealing (transition of polymorphs of lower stability to more stable forms during heating) (Nightingale et al., 2011). Melting properties including onset Temperature (T_{onset}), peak Temperature (T_{peak}), end Temperature (T_{end}), and enthalpy-of-melting (ΔH_{melt}) were calculated automatically by the software, and Melting index (T_{index}) was computed as ($T_{end} - T_{onset}$) (Afoakwa et al., 2008).

2.5. Analysis of bioactive compounds

2.5.1. Extraction of catechins and methylxanthines

To eliminate lipids from the chocolate samples, 0.1 g of each sample was extracted twice with 1 mL of petroleum ether. The defatted solids of samples were air-dried during 24 h to remove the residual organic solvent. The catechins and methylxanthines were extracted three times with 1 mL of aqueous methanol (70%) acidified with 0.1% TriFluoroacetic Acid (TFA). After each extraction, the mixture was centrifuged for 10 min at 3,500 rpm and the supernatant was decanted. The chocolate extractions were filtered through a 0.45-μm filter (Polyvinylidene difluoride membranes; Agilent Technologies, USA) before total phenolics and HPLC analyses.

2.5.2. Total polyphenol and anthocyanin content analysis

Samples were analyzed for total polyphenol content according to the Folin-Ciocalteau method, measuring absorbance at 750 nm using a spectrophotometer (Singleton & Rossi, 1965). A calibration curve was performed using gallic acid (R^2 = 0.992) and epicatechin as standard (R^2 = 0.997). The results were expressed as mg of Gallic Acid Equivalents (GAE) and as mg of EpiCatechin Equivalents (ECE) per g of chocolate. Total anthocyanin content was measured by the pH differential method described by Peláez et al. (2016). The result was expressed in mg of Cyanidin-3-glucoside Equivalents (CyE) per $g_{d.d.m}$.

2.5.3. HPLC analysis of catechins and methylxanthines

HPLC analysis of catechins and methylxanthines was evaluated according to a modification of the method described by Li et al. (2012). Forty μL of each sample was injected into HPLC equipment (Varian Pro Star Solvent Delivery System 240 (Varian, Walnut Creek, CA, USA) and a Varian Pro Star 325 UltraViolet (UV) detector (Varian, Walnut Creek, CA, USA) using an RP-18 LiChrosorb column (Restek, USA) (250 × 4.6 mm, 7 μm i.d.). The solvents consisted of water acidified with 0.1% TFA (solvent A) and acetonitrile acidified with 0.1% TFA (solvent B) at a flow rate of 0.45 mL/min. Elution was performed with a gradient starting at 0% B to reach15% B at 2 min, maintaining 15% B at 10 min and 25% B at 24 min, becoming isocratic for 5 min, and finally decreasing to 0% B at 30 min. Chromatograms were recorded at 280 nm. Catechins and methylxanthines were identified comparing the retention times of the corresponding standards.

2.6. Analysis of volatile compounds by HS-SPME/GC–MS

The extraction of volatile compounds was performed by Head Space-Solid Phase Micro-Extraction (HS-SPME) using a 50/30 μm DiVinylBenzene/CARboxene/PolyDiMethylSiloxane (DVB/CAR/PDMS) fiber from Supelco. Briefly, the sample (2 g) was transferred into a head-space vial, which was sealed with a PolyTetraFluoroEthylene (PTFE) cap. The sample was equilibrated at 60 °C for 15 min. Then, the fiber was exposed into the vial and heated to 60 °C for 30 min under constant agitation; after this exposure time, the fiber was retracted and inserted immediately into the inlet of the GC. The volatile compounds were analyzed by Gas Chromatography-Mass Spectrometry (GC–MS) (Hewlett Packard Model 5890 Series II; Palo Alto, CA, USA) equipped with an Innowax capillary column (60 m × 0.25 mm id × 0.25-μm film thickness), performing the method described by Rodríguez-Campos et al. (2012). Volatile compounds were identified comparing the mass spectra of the compounds in the samples with the database of the National Institute of Standards and Technology (NIST Library, Gaithersburg, MD, USA) with a match of at least 80%. Aroma descriptors for the compounds identified were obtained by bibliographic review.

2.7. Data processing and chemometrics analysis

All analyses were performed in triplicate (n = 3). The experimental data are presented as the mean and Standard Deviation (SD). Data analysis was performed using the XLSTAT 2019.2.3 (Addinsoft, Boston, MA, USA). We performed an ANalysis Of VAriance (ANOVA) and Kruskal-Wallis multiple range tests were conducted to determine significant differences among the means. Mean differences were considered significant at $p < 0.05$.

To visualize the possible logical relations among the dark-chocolate samples, a Venn diagram was performed based on the total volatile compounds identified by GC-MS. Initially, the samples were classified, according to geographical region into two groups: 1) samples with cocoa from North America (CM70 and TM66), and 2) samples with cocoa from South America (CA100, CV74, and NE60). For each class, an independent Venn diagram was constructed to identify relations in volatile compounds by geographical region. Subsequently, a second diagram was constructed from volatile compounds that shared each geographic area to identify compounds that marked differences and similarities between the two regions.

The variables used in the chemometrics analysis represent the normalized area of the chromatographic peaks obtained by GC-MS. Each variable corresponds to the average of experimental points (analysis) carried out in triplicate. The data was processed with XLSTAT 2019.2.3 (Addinsoft, Boston, MA, USA). The variables were compared with ANOVA and Tukey test with a significance level of $p < 0.05$ to evaluate the most significant compounds among the chocolate samples. A Principal Component Analysis (PCA) bi-plot, and Hierarchical Cluster Heatmap Analysis (HCA-Heatmap) (Double Dendrograms) were constructed to demonstrate similarities and differences among the genotypes (Trinitario, Nacional, and Criollo) and geographical origin (North and South America) in terms of the volatile profile of five dark-chocolate samples. The dataset consisted of five observations (chocolate samples) and 63 variables (volatile compounds) No pre-treatment of the data was applied for the analysis. Filtration of the aligned data based on a Standard Deviation (SD) threshold of 0.5% was applied for the HCA-Heatmap.

Table 2
Fat content and fatty acids profile of different dark chocolate samples.

Parameter	Sample				
	CA100	CV74	CM70	TM66	NE60
Total fat content	57.90 ± 0.61^{a}	42.23 ± 0.28^{b}	39.26 ± 1.25^{c}	39.95 ± 0.38^{c}	32.71 ± 2.81^{d}
Fatty acids profile					
Myristic acid (C14:0)	n/d	0.05 ± 0.00^{c}	0.06 ± 0.00^{b}	0.07 ± 0.00^{a}	0.05 ± 0.00^{c}
Palmitic acid (C16:0)	27.85 ± 0.01^{b}	27.13 ± 0.05^{c}	25.24 ± 0.02^{e}	27.0 ± 0.00^{d}	28.41 ± 0.05^{a}
Palmitoleic acid (C16:1)	0.22 ± 0.01^{a}	0.15 ± 0.01^{c}	0.17 ± 0.01^{bc}	0.18 ± 0.00^{b}	0.23 ± 0.00^{a}
Margaric acid (C17:0)	0.26 ± 0.00^{a}	0.20 ± 0.01^{c}	0.23 ± 0.00^{b}	0.25 ± 0.00^{a}	0.23 ± 0.00^{b}
Estearic acid (C18:0)	34.82 ± 0.08^{c}	34.70 ± 0.06 ^{cd}	35.67 ± 0.05^{a}	35.19 ± 0.05^{b}	34.62 ± 0.06^{d}
Oleic acid (C18:1)	32.85 ± 0.11^{c}	33.13 ± 0.08^{b}	34.43 ± 0.08^{a}	33.14 ± 0.13^{b}	32.18 ± 0.11^{d}
Linoleic acid (C18:2)	2.66 ± 0.04^{c}	2.75 ± 0.02^{b}	2.78 ± 0.02^{b}	2.56 ± 0.01^{d}	2.83 ± 0.02^{a}
Linolenic acid (C18:3)	0.17 ± 0.00^{b}	0.20 ± 0.00^{a}	0.15 ± 0.00^{c}	0.17 ± 0.00^{b}	0.15 ± 0.00^{c}
Arachidonic acid (C20:4)	1.01 ± 0.00^{e}	1.45 ± 0.00^{a}	1.08 ± 0.00^{d}	1.24 ± 0.01^{b}	1.12 ± 0.01^{c}
Behenic acid (C22:0)	0.15 ± 0.00^{d}	0.25 ± 0.00^{a}	0.19 ± 0.00^{b}	0.19 ± 0.00^{b}	0.17 ± 0.00^{c}
total	100.00	100.00	100.00	100.00	100.00
Saturated fatty acids	63.08 ± 0.28^{a}	62.28 ± 0.10^{b}	61.33 ± 0.08^{c}	62.44 ± 0.10^{b}	63.44 ± 0.12^{a}
Monounsaturated fatty acids	33.07 ± 0.13^{b}	33.27 ± 0.11^{b}	34.60 ± 0.20^{a}	33.21 ± 0.12^{b}	32.41 ± 0.11^{c}
Polyunsaturated fatty acids	3.84 ± 0.04^{d}	4.39 ± 0.02^{a}	4.01 ± 0.02^{c}	3.96 ± 0.03^{c}	4.10 ± 0.03^{b}
total	100.00	100.00	100.00	100.00	100.00

Total fat value is expressed in sample percentage, and the fatty acids profile is expressed in percentage of total fat. Data are expressed as means ± SD. Different letters within the same row mean a significant difference ($p < 0.05$, $n = 3$). n/d = no detected.

3. Results and discussion

3.1. Fat content, melting properties, and glass-transition of dark chocolates

Fat content and composition were previously evaluated to find a possible relationship with its melting behavior due to that the fat content of dark chocolate exerts an influence on the degree of crystallinity and crystal-size distribution (Tan & Kerr, 2018). The results obtained in the determination of total fat content and fatty acids profile are depicted in Table 2. The percentage of total fat in the samples ranged between 32.71 and 57.90%. The main fatty acids identified in all samples were stearic acid (34.62–35.67%), oleic acid (32.18–34.43%), and palmitic acid (25.24–28.41%), which are reported as the majority of cocoa butter at similar proportions (Winkelmeyer et al., 2016). Significant differences were observed in the fatty acids profile of different chocolates ($p < 0.05$). Differences the composition of the in fatty acids profile among the chocolate samples can be mainly explained by the effect of the geographical origins of the cocoa, but not by the processing conditions (as roasting and conching time) employed in chocolate-making (Torres-Moreno et al., 2015). The dark-chocolate sample with Nacional cocoa beans from Ecuador (NE60) and the sample with Criollo cocoa beans from the Amazon (CA100) exhibited as slightly higher amount of saturated fatty acids (63.44 and 63.08%, respectively), attributed mainly to the content of palmitic acid as compared to the other samples. The dark-chocolate sample with Criollo cocoa from Mexico (CM70) demonstrated a higher amount of monounsaturated fatty acids (34.60%), mainly oleic acid. The sample with Criollo cocoa from Venezuela (CV74) contained a higher amount of polyunsaturated fatty acids (4.39%), such as arachidonic acid and linolenic acid, compared with the other samples.

The majority of cocoa butter fatty acids presented as TriAcylGlycerols (TAG), mainly comprising three key symmetrical TAG (saturated–unsaturated-saturated) account for over 90% of all cocoa butter, including 1-Palmitate-2-Oleate-3-Stearate triacylglycerol (POS), 1,3-diStearate-2-Oleate triacylglycerol (SOS), and 1,3-diPalmitate-2-Oleate triacylglycerol (POP), with incidence depending on the cocoa's geographic origin (de Silva Souza & Block, 2018; Vieira et al., 2015). TAG of cocoa are packed at the molecular level in a characteristic manner, crystallized in different polymorphic forms (I-VI) that are differentiated at their melting point (Afoakwa et al., 2008). Good-quality chocolate requires certain a polymorphic fat crystal that confers desirable mechanical and thermal properties (Biswas et al., 2017; Delbaere et al., 2016). The melting properties provide information related to crystal type, polymorphic stability, and chocolate quality. Depending on composition and crystalline-state distribution, these provide information on likely oral-melting performance, flavor release, and the texture of the chocolate (Afoakwa et al., 2008). Melting properties and degree of crystallinity were evaluated by Differential Scanning Calorimeter (DSC). DSC permits the identification of phase transitions of polymorphic forms in fat systems by their melting points (ranging between 10 and 36 °C) (Le Révérend, Fryer, & Bakalis, 2009). The melting properties of the different chocolate samples are presented in Table 3. For analysis by DSC, the chocolate samples were initially cooled at a maximal temperature in a search for the rearrangement of the components of the chocolates that could be in their amorphous state starting from room temperature. This is the case of the triolein, a minor triacylglyceride of cocoa butter with a melting point of −31 °C (Campos, Ollivon, & Marangoni, 2010), considering that this could generate a rearrangement of the structure of each chocolate. All DSC chocolate melting curves were characterized by the presence of a prominent endothermic transition peak (Fig. 1) with an onset temperature (T_{onset}) of 29.45–30.29 °C, and a maximal (T_{peak}) at 33.16–34.31 °C.

Table 3
Melting properties of the different dark chocolate samples measured in the heating curves of the thermogram analyzed by DSC.

Sample	T_{onset}	T_{peak}	T_{end}	T_{index}	ΔH_{melt}	C_p
CA100	30.21 ± 0.25^{a}	33.95 ± 0.09^{b}	35.89 ± 0.18^{b}	5.68 ± 0.21^{b}	77.57 ± 1.34^{a}	3.453 ± 0.11^{a}
CV74	30.12 ± 0.30^{a}	33.77 ± 0.11^{b}	35.84 ± 0.21^{b}	5.72 ± 0.18^{b}	53.37 ± 1.07^{b}	2.465 ± 0.09^{b}
CM70	30.29 ± 0.19^{a}	34.31 ± 0.16^{a}	36.41 ± 0.24^{a}	6.12 ± 0.19^{a}	46.54 ± 2.30^{c}	1.895 ± 0.13^{c}
TM66	30.32 ± 0.22^{a}	33.71 ± 0.15^{b}	35.63 ± 0.09^{b}	5.31 ± 0.15^{bc}	50.88 ± 1.75^{b}	2.649 ± 0.11^{b}
NE60	29.45 ± 0.17^{b}	33.16 ± 0.20^{c}	34.76 ± 0.12^{c}	5.31 ± 0.20^{c}	46.03 ± 2.05^{c}	2.533 ± 0.09^{b}

T = temperature expressed in °C; ΔH_{melt} data are expressed in J/g; C_p data are expressed in W/g. Different letters within the same column mean a significant difference ($p < 0.05$, $n = 3$).

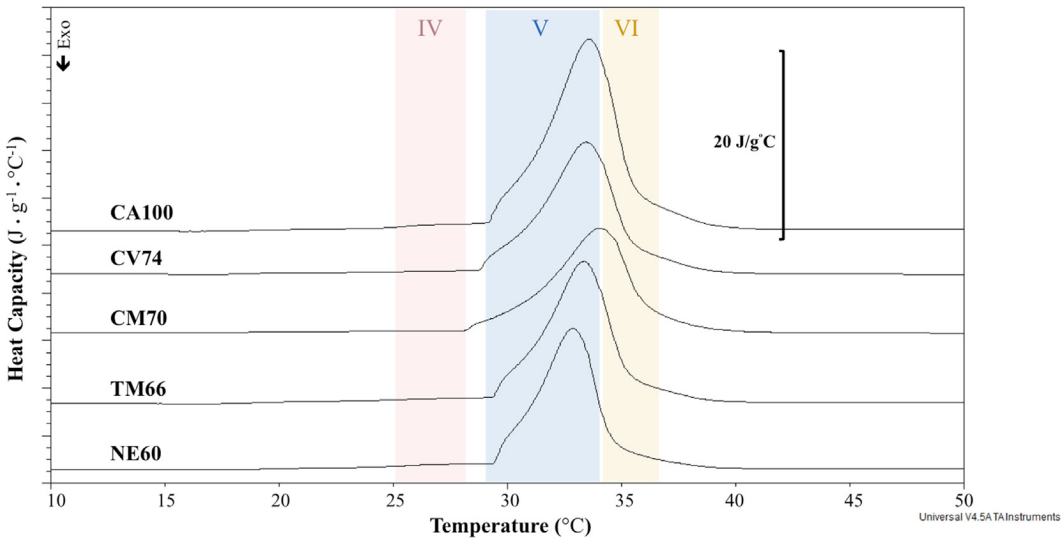

Fig. 1. Differential scanning calorimetric measurements of the different dark-chocolate samples. Melting temperature ranges for different cocoa-butter polymorphs (forms IV, V, and VI) are indicated in Fig. 1 with rectangles of a different color.

The T_{onset} parameter revealed the temperature at which a specific crystal form begins to melt in the chocolate samples, while T_{peak} indicates the temperature at which the greatest melting rate occurs (Afoakwa et al., 2008). This is followed by the end temperature of the melting (T_{end}), at which complete liquefaction occurs, observed at 34.76–36.41 °C. Significant differences ($p < .005$) were observed in the T_{onset}, T_{peak}, and T_{end} values of the samples. The results showed a similar melting process of the chocolate with a high content of cocoa liquor (Ostrowska-Ligęza et al., 2019). Tan & Kerr (2018) reported a T_{peak} value of 33.57–34.28 °C. The melting temperature of all samples corresponds to TAG in the polymorphic form V or β_2 (Fig. 1), which melts at between 29 and 34 °C according to Smith et al. (2016), indicating preponderance over the forms IV or β_1' (which melt at 26–28 °C), and the forms VI or β_1 (with a melting range between 34 and 36 °C). The polymorphic form V is expected for well-tempered chocolate, because it confers a good snap, glossy appearance, and it melts in the mouth, providing a pleasant sensation (Agibert & Lannes, 2018; Biswas et al., 2017). The sample with Criollo cocoa from Mexico (CM70) exhibited a higher T_{peak} value (34.31 °C), while the Nacional cocoa from Ecuador (NE60) sample demonstrated lowest T_{onset} and T_{peak} values compared with those of the other samples. The differences among between maximal melting temperatures of the samples could be due to the variability of their formulation. However, previous studies showed that neither particle size, nor fat content, nor the addition of lecithin exerts an influence on T_{onset} or T_{peak} values in chocolates (Afoakwa et al., 2008). Previous studies reported that the time and storage conditions of chocolate modify its crystalline structure through a polymorphic transition from form V to form VI (more stable), increasing the T_{peak} value of the phase transition (Afoakwa et al., 2009). If this was the case for the CM70 sample, an increase in the amount of energy would be required to melt (greater ΔH_{melt} and C_p) that is not evident in the results. The latter indicates a high T_{peak} value must be related to the ΔH_{melt} and the C_p must be associated with a phase transition of the polymorphic form V. An alternative interpretation of the data lies the fat composition, which is related to the origin of the cocoa bean (Talbot, 2012; Vieira et al., 2015) The CM70 sample is characterized by a higher amount of Stearic acid (S) and Oleic acid (O) and a lower amount of Palmitic acid (P), which is inversely to the NE60 sample (Table 2), considering that the principal TAG on the samples are POP, POS, and SOS. The CM70 sample data might have a greater more proportion of TAG POS and/or SOS, while the NE60 sample could present a higher proportion of POP TAG. POS and/or SOS TAG present a polymorphic form V or β_2 that is more stable, permitting the displacement of the maximal melting point of the fat at a higher temperature compared to the polymorphic form V of the POP TAG (Arishima, Sagi, Mori, & Sato, 1991; Dimick & Manning, 1987). On the other hand, in addition to the three main TAG (POP, POS, and SOS), monounsaturated TAG such as 1,2-dioleoyl-3-palmitoylglycerol (POO) and 1,2-dioleoyl-3-stearoylglycerol (SOO) have been reported as minorities in cocoa butter, and their content entertains a negative correlation with the T_{peak} (the more the monounsaturated TAG, the lower the T_{peak} value) (Vieira et al., 2015). This could indicate that sample NE60 contains a higher proportion of monosaturated TAG, compared with other samples, while CM70 could have a lower content. However, a study of the real composition of the TAG profile would be required to validate either of these hypotheses.

The melting profile of dark chocolate should possess a narrow melting peak leading, to a quick melt, producing a cool sensation and a smooth mouth-feel (Biswas et al., 2017). The T_{index} value, which if reflective of melting-peak width, resulted within the range of 5.31–6.12 °C. Significant differences ($p < 0.05$) were observed among the T_{index} value of the samples. The sample with Nacional cocoa (NE60) showed the lowest T_{index} value, but no difference with the sample with Trinitario cocoa (TM66). These results can be attributed to the lower fat content that requires a shorter heating time to be completely melted (Tan and Kerr, 2018). The dark-chocolate sample with Mexican Criollo cocoa (CM70) revealed a higher T_{index} value compared that of to all samples. The CM70 sample was the only sample that indicated the addition of a different emulsifier than lecithin in its label: the sorbitan tristearate. The incorporation of sorbitan tristearate in the chocolate formulation as an emulsifier has been studied, demonstrating the widening the melting point of the polymorphic form V of cocoa butter (Smith et al., 2011).

In enthalpy-of-melting (ΔH_{melt}), the energy required for changing the structure state of the chocolate in order to complete the liquefaction fell within the range of 46.03 and 77.57 J/g. Significant differences were observed in the ΔH_{melt} values ($p < 0.05$), reflecting differences in heat transfer rates between samples. The CA100 chocolate presented the highest ΔH_{melt} value compared with that of the other samples, while the CM70 and NE60 samples demonstrated a lower melting enthalpy. There is a direct relationship between fat content and ΔH_{melt} (Ostrowska-Ligęza et al., 2019). A higher amount of crystalline cocoa butter increases the energy necessary for melting due to the greater fat-fat interactions and the lower amount of non-fat solid particles suspended in the fat network (Zhao et al., 2018). Similar results were observed in the specific heat capacity (C_p) of the chocolate samples,

observing that the CA100 sample presented the highest C_p value (3.453 W/g), while the CM70 sample showed the lowest C_p (1.895 W/g). These results can be attributed to the higher saturated fatty acids content in the CA100 sample and the higher amount of monounsaturated fatty acids in the CM70 sample. Himawan et al. (2006) explained that TAG with saturated fatty acids form a more compacted structure, which is more difficult to melt, while TAG with more unsaturated fatty acids with the same chain length hamper the formation of crystals, facilitating its fusion. In particular, the NE60 sample, despite presenting a lower fat content compared with that of the other samples, presented a higher C_p (2.553 W/g) compared with the CM70 sample. This result could be attributed to the composition of a cocoa fat rich in saturated fatty acids (Table 2), reflecting that the melting enthalpy and the specific heat capacity of the chocolate samples could provide more information related to the composition and origin of the fat, regardless of its fat content. In fact, the microstructure of the non-fat solid particulate network has no direct effect on the melting behavior of the fat (Zhao, Li, & James, 2018).

The glass-transition temperature (T_g) of the chocolates was measured in the heating curve of the thermogram. The T_g values of the chocolate samples are presented in Table 4. The majority of samples presented two T_g values: the first T_g at 18.51–19.47 °C, and a second at 25.06–25.96 °C. This was with the exception of the CA100 sample, which only presented the second transition, and the CM70 sample, which presented only the first transition. Differences in the fatty acids profile affect the cocoa-butter polymorphism, exhibiting different solid-state transitions mediated by the melt (Biswas et al., 2017; Winkelmeyer et al., 2016). The first T_g could be related to the phase transition corresponding to traces of the unstable polymorphic form α or II of TAG, such as POS with a melting point near 19 °C, formed during the chocolate tempering process. The second T_g could be associated with traces of the unstable polymorphic form γ or I of the POP TAG with a melting temperature near 25 °C (Arishima et al., 1991). Assuming the aforementioned hypothesis, if the CA100 sample possesses a greater content of Palmitic acid (P), and a lesser content of Stearic acid (S) and Oleic acid (O) (Table 2), it could have a lower proportion of POS TAG, therefore the transition of the unstable form II of POS is hardly present. Otherwise, the CM70 sample could contain a lower proportion of POS TAG and could hardly identify its corresponding phase transitions. The melting point of the monounsaturated SOO TAG in their most stable form is 19.5 °C (Ghazani et al., 2018); this is the temperature at which the first T_g characteristic of the NE60 sample is observed, which could support the presence of SOO TAG and explain its lower T_{peak} value. It is noteworthy that these are only some hypotheses, in that, to our knowledge, this is the first time that T_g values have been reported in dark-chocolate samples, and that they could possibly be observed due to the initial cooling of the samples at −70 °C.

3.2. Total polyphenol, anthocyanin, and methylxanthine content

Initially, the content of the bioactive compounds of each chocolate bar was analyzed (in terms of bioactive mass/chocolate mass) in order to have an overview of the bioactive potential of dark chocolate with cocoa from Latin America. The results obtained are presented in Fig. 2A. Significant differences ($p < 0.05$) were observed in the polyphenol, catechin, anthocyanin, and methylxanthine content of the samples. The total polyphenol content of the samples ranged from 8.94 −21.17 mg GAE/g, and 2.15–5.63 mg ECE/g. Similar results were reported in dark chocolates with cocoa from Cameroon, Nigeria, and Ghana (4.9–12.5 mg ECE/g) Cambrai et al. (2017), Ecuador (26.18 ± 1.29 mg GAE/g), Madagascar (29.17 ± 1.21 mg GAE/g), Tanzania (28.72 ± 1.18 mg GAE/g), and Trinidad (25.83 ± 1.09 mg GAE/g) (Vertuani et al., 2014). Fig. 2A reveals that the lowest polyphenol content was found in the CV74 sample (8.94 ± 0.75 mg GAE/g), despite the higher percentage of cocoa contained (74% cocoa) compared with samples TM66 (66% cocoa) (13.97 ± 1.42 mg GAE/g), and NE60 (60% cocoa) (14.55 ± 0.94 mg GAE/g), observing that, regardless of the cocoa content in dark chocolates, cocoa quality and origin affect the polyphenol content (Vertuani et al., 2014). In the analysis of the content of flavan-3-ols, the result demonstrated that epicatechin was found at a greater proportion (0.45–1.03 mg/g) compared to catechin (0.06–0.25 mg/g). The results were lower than those reported by Martini, Conte, & Tagliazucchi, (2018) in commercial dark chocolate with 70% cocoa content (2.03 mg of epicatechin/g, and 0.66 mg of catechin/g). The differences of the results could be associated with the effect of the intrinsic cocoa process (roasting and conching) or could be associated with the cocoa genotype of the chocolate sample (Cambrai et al., 2017; Oracz & Nebesny, 2019). The content of flavan-3-ol expressed in the defatted dry mass ($_{ddm}$) of dark chocolate was also evaluated by Belščak et al. (2009) (0.75–1.68 mg of epicatechin/g_{ddm} and a catechin content of 0.02–0.59 mg of catechin/g_{ddm}), and by Tuenter et al. (2020) (0.541.90 mg of epicatechin/g_{ddm} and a catechin content of 0.16–0.66 mg of catechin/g_{ddm}), similar values to the equivalent results of this study (0.78–2.28 mg of epicatechin/g_{ddm}, and 0.100.42 mg of catechin/g_{ddm}). The anthocyanin content of the chocolates was 0.04–0.08 mg CyE/g. Previous studies reported an anthocyanin content of 0.002–0.005 mg of cyanidin-3-galactoside equivalents/g of dark chocolates with cocoa from different geographical origins determined by HLPC-DAD (Bordiga et al., 2015). Therefore, a comparison cannot be made with the results obtained in this study.

The predominant methylxanthine in all chocolate samples was theobromine. The range of the theobromine concentration was 9.25–12.19 mg/g, while the caffeine content was 1.49–2.34 mg/g. Values for theobromine and caffeine, of 6.14–8.26 mg/g, and 0.164–0.347 mg/g, respectively, in dark chocolates with cocoa from different geographical origins were reported (Bordiga et al., 2015). The methylxanthine content expressed in the defatted dry mass ($_{ddm}$) of dark chocolate was also evaluated by Belščak et al. (2009) (theobromine content of 7–13 mg/g_{ddm} and a caffeine content of 0.5–2.5 mg/g_{ddm}), and by Tuenter et al. (2020) (7–13 mg of theobromine/g_{ddm} and a caffeine content of 0.5–2.5 mg/g_{ddm}). The equivalent results were similar to those employed to evaluate the theobromine (13.82–29.03 mg/g_{ddm}), and caffeine (2.04–5.58 mg/g_{ddm}) in the chocolate samples (Fig. 2B).

In a second phase, the content of bioactive compounds in the cocoa liquor of the chocolates (bioactive mass/cocoa liquor mass) was analyzed, assuming that all chocolate samples constituted 100% cocoa liquor and to identify a relationship between bioactive compounds with the origin and/or genotype of cocoa. The results are shown in Fig. 2C.

Table 4
Glass-transition temperature (T_g) value of the different dark chocolate samples.

	Sample				
	CA100	**CV74**	**CM70**	**TM66**	**NE60**
T_{g1}	–	18.94 ± 0.13^{b}	18.51 ± 0.15^{c}	18.58 ± 0.17^{c}	19.47 ± 0.15^{a}
T_{g2}	25.06 ± 0.34^{b}	25.57 ± 0.23^{ab}	–	25.96 ± 0.30^{a}	25.78 ± 0.16^{a}

T_g = glass-transition temperature (°C) at the heating curve of DSC analysis. Different letters within the same row mean a significant difference ($p < 0.05$, $n = 3$).

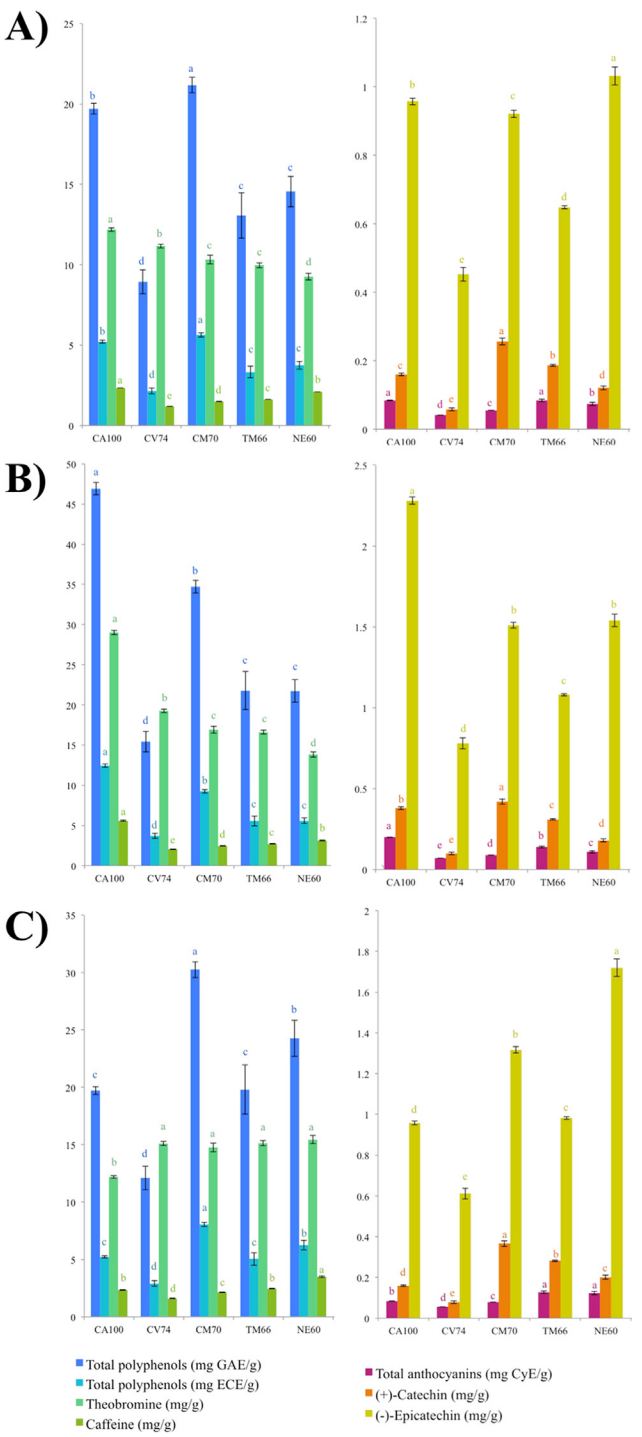

Fig. 2. The polyphenol, anthocyanin, catechin, and methylxanthine content of the dark-chocolate samples is expressed in A) Bioactive content per chocolate mass; B) Bioactive content per defatted dry chocolate mass, and C) Bioactive content per cocoa liquor mass (assuming that all chocolate samples are 100% cocoa liquor). Columns with different letters differ by Kruskal-Wallis multiple-range tests ($p < 0.05$; $n = 3$).

Significant differences ($p < 0.05$) were observed in the bioactive content of the samples. In relation to the genotype of the samples, the anthocyanin content of the Trinitario sample (TM66) and the National cocoa sample (NE60) was higher than that of the chocolates produced with Criollo cocoa (CA100, CV74, and CM70), while the Nacional cocoa sample (NE60) exhibited a higher amount of epicatechin and caffeine compared with that of all samples. These results could be associated with a pattern typical of the cocoa genotype. The Nacional cocoa genotype has been characterized by a higher epicatechin content compared to Criollo and Trinitario genotypes, and Criollo cocoa contains lower or no anthocyanin content compared to Trinitario and Nacional genotypes (Oracz et al., 2015).

The bioactive-compound content in relation to the cocoa's geographic origin demonstrated that the samples from North America (CM70, and TM66) revealed a higher catechin content (Fig. 2C), and the Venezuelan sample (CV74) showed a lower content of all of the bioactives analyzed, except for theobromine. Dependencies between the polyphenol content and the origin were reported by Redovniković et al. (2009), observing a higher content of polyphenols in cocoa liquor from Mexico and Ecuador compared to samples from Venezuela. Similar results were observed by Oracz & Nebesny (2016), in which a Venezuelan Trinitario cocoa exhibited a lower polyphenol content compared to the same cocoa genotype from other origins. The latter results may be associated to a greater degree with an effect of the processing of cocoa employed in the production of cocoa liquor, therefore of chocolate, in which polyphenols are strongly affected, and in which the Criollo cocoa polyphenols are those most susceptible compared to other genotypes (Elwers et al., 2009; Tuenter et al., 2020).

The Mexican (TM66) and Ecuatorian (NE60) samples showed a higher anthocyanin content. Previous studies reported a higher anthocyanin content in Ecuadorian Nacional cocoa samples, compared to samples from Ghana, Nigeria, and Cameroon (Bordiga et al., 2015; Oracz et al., 2015). The Ecuatorian sample (NE60) had a greater amount of epicatechin and caffeine. Previous studies reported a higher caffeine and epicatechin content in samples of pre-roasted cocoa nibs, cocoa liquor, and dark chocolate from Ecuador compared to dark chocolates from Ghana, Nigeria, Ivory Coast, and Cameroon (Bordiga et al., 2015). For theobromine, the chocolate sample from the Amazon (CA100) revealed the lowest content, while no significant differences were observed among the other samples. Previous studies did not observe significant differences among the theobromine content in cocoa liquor from Venezuela, Mexico, and Ecuador, but they did show a lower content compared to those of origins such as Ghana, Sao Tome, and Madagascar (Redovniković et al., 2009), which could indicate that the theobromine content could be a marker to differentiate Amazonian dark chocolate. The results appear to indicate that the anthocyanin, caffeine, theobromine, and epicatechin content could play a role in identifying the origin of cocoa from North America and South America in dark-chocolate samples, even after processing. Further studies are necessary to evaluate differences in the profile of the bioactive compounds associated with origin and genotypes from Latin America, suggesting a more complete analysis of the profile of polyphenols, including caffeic-acid derivatives gallocatechin, and epigallocatechin, which entertain marked differences among samples of cocoa liquor from Mexico, Ecuador, and Venezuela (Redovniković et al., 2009).

3.3. Volatile compounds profile of dark chocolates

The volatile profile of dark-chocolate samples was evaluated in order to identify possible key-aroma markers that could be associated with differentiating between the geographical origin and/or the cocoa genotype of the Latin American samples. The 85 volatile compounds identified among the different samples are presented in Table 5. The samples differed in the number of volatile compounds detected in their composition. In general, the sample of chocolate with Amazonian Criollo cocoa (CA100) presented the highest number of volatiles (62 compounds) compared with that of the other samples. Among the chemical classes of the detected compounds, the volatile acids, alcohols, aldehydes, esters, ketones, pyrazines, furans, among others, can be mentioned. Several of these groups of compounds have been identified in the chocolate aroma as a result of the fermentation and drying of cocoa beans, or during the roasting process through Maillard reactions and the Strecker degradation (Braga et al., 2018; Tran et al., 2016).

Table 5
Volatile compounds identified in the dark chocolate samples by HS-SPME/GC-MS.

Rt	Compound name	Odor descriptor*	Percentage (%)[a]				
			CA100	CV74	CM70	TM66	NE60
Acids							
17,86	Phenylmalonic acid	–	–	0,20	–	–	–
18,63	**Acetic acid**	*Sour, vinegary*[abd]	22,40	27,24	24,47	22,24	22,80
20,21	**2-Methylpropanoic acid**	*Rancid, sweaty*[abc]	–	2,66	–	–	3,27
20,97	**Butanoic acid**	*Sweaty, rancid*[bd]	–	0,60	–	0,46	–
21,48	**3-Methylbutanoic acid**	*Rancid, cheesy*[abc]	2,74	–	–	1,77	–
22,35	Pentanoic acid	*sweaty*[d]	–	–	–	–	0,43
25,49	Heptanoic acid	*Sour, rancid*[hi]	0,29	0,50	0,39	0,36	0,35
27,57	Octanoic acid	*Sweaty, fatty*[di]	0,79	–	0,56	0,67	–
29,98	Sorbic acid	–	–	–	–	0,34	0,37
30,24	Nonanoic acid	*Sweaty, waxy, green*[di]	0,16	0,33	–	0,13	–
42,46	Benzoic acid	–	0,45	0,80	0,44	0,61	0,65
	total percentage		**26,81**	**32,33**	**25,86**	**26,57**	**27,88**
Alcohols							
13,13	3-Methyl-2-butanol	–	0,08	–	–	–	–
16,61	2-Pentadecanol	–	–	–	–	–	0,27
16,61	**2-Heptanol**	*Citrusy, fresh, lemon grass-like*[bdc]	0,42	–	–	–	–
19,46	2-Nonanol	*No smell* [h]	0,94	1,80	–	–	1,40
19,83	2,3-Butanediol	*Sweet, floral, cocoa butter-like*[efh]	9,74	7,70	3,00	6,98	6,41
255,00	3-Methyl-2-heptanol	–	0,55	–	–	–	–
22,02	ρ-Menth-1-en-8-ol	–	0,98	–	–	–	–
22,45	1,3-Propanediol diacetate	–	0,41	–	–	–	0,25
23,62	1-Phenylethyl alcohol	*Honey-like, floral*[i]	0,48	–	0,21	0,26	0,28
24,49	Guaiacol	*Spicy*[d]	0,27	0,60	0,28	0,32	0,34
24,64	Benzyl alcohol	*Sweet, fruity, flowey*[di]	1,20	1,10	0,50	0,75	1,19
25,27	**2-Phenylethyl alcohol**	*Flowery, spicy, honey-like*[bcd]	12,37	9,34	6,93	6,71	9,51
31,04	Eugenol	–	0,46	0,24	–	–	0,28
36,04	4-Methyl-5-thiazoleethanol	–	0,12	–	0,20	0,19	0,28
	total percentage		**28,03**	**20,77**	**11,13**	**15,21**	**20,20**

Rt	Compound name	Odor descriptor*	Percentage (%)[a]				
			CA100	CV74	CM70	TM66	NE60
Aldehydes							
8,93	**3-Methyl-butanal**	*Chocolate-like, malty* [abc]	0,16	0,31	–	0,23	0,15
18,00	Nonanal	*Fatty, waxy, pungent, soapy*[de]	1,22	1,08	0,46	0,70	1,73
19,10	**Furfural**	*Sweet, bread-, potato-like*[dg]	2,17	0,96	7,76	1,32	2,03
19,25	Vanillin	*vanilla-like*[a]	–	–	–	–	0,18
20,11	Benzaldehyde	*Bitter, almond, burnt sugar, bean-like*[cde]	6,61	7,40	8,01	7,26	5,86
21,60	**Phenylacetaldehyde**	*Honey-like*[abd]	2,76	2,54	1,63	4,29	2,46
23,54	2-Methyl-3-phenylpropanal	–	0,48	0,18	–	–	2,04
25,84	α-Ethylidene-benzeneacetaldehyde	*Caramel-like, smooky, nutty* [h]	0,68	0,99	0,92	0,87	1,01
27,65	1H-pyrrole-2-carboxaldehyde	*Caramel, cocoa*[g]	–	–	–	0,86	–
28,18	3-Phenyl-2-propenal	–	0,11	–	–	–	–
28,19	Cinnamaldehyde	–	0,11	0,13	–	–	0,21
28,63	5-Methyl-2-phenyl-2-hexenal	*Sweet, roasted, cocoa-like*[c]	0,42	0,30	0,37	0,53	0,50
29,40	1-Methyl-1H-pyrrole-2-carboxaldehyde	–	0,19	0,23	0,25	0,42	0,37
	total percentage		**14,92**	**14,12**	**19,41**	**16,48**	**16,54**
Ketones							
14,54	2-Heptanone	*Fruity, sweet, cheese-like*[cd]	0,18	–	–	–	–
16,44	**3-Hydroxy-2-butanone**	*Buttery*[d]	1,64	–	–	–	–
17,91	2-Nonanone	*Milk, green, fruity*[e]	0,34	1,06	0,35	0,14	2,03
19,73	1-(2-Furanyl)-ethanone	–	–	–	0,25	0,25	0,41
21,75	Acetophenone	*Sweet, almond-like, flowery*[cef]	1,05	–	0,72	0,80	0,73
26,41	1-(1H-pyrrol-2-yl)-ethanone	*Sweet, caramel-, honey-like, nutty*[h]	3,01	2,99	5,71	5,31	4,69
28,01	2-Pyrrolidinone	–	0,20	0,71	0,42	0,91	0,45
34,70	2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one	–	1,22	1,72	2,70	3,07	3,32
35,19	3,5-Dihydroxy-2-methyl-4H-pyran-4-one	–	0,21	0,28	–	0,51	0,90
46,37	Benzophenone	–	0,12	–	–	–	–
46,66	3,4-Dihydro-8-hydroxy-3-methyl-(R)-1H-2-benzopyran-1-one	–	–	–	–	0,15	0,24
	total percentage		**7,98**	**6,75**	**10,15**	**11,14**	**12,76**

Rt	Compound name	Odor descriptor*	Percentage (%)[a]				
			CA100	CV74	CM70	TM66	NE60
Esters							
18,47	Ethyl octanoate	*Fruity, floral, pineapple*[c]	0,77	0,33	0,79	0,45	0,81
21,11	Ethyl decanoate	*Brandy, pear-, grape-like*[c]	0,66	–	0,41	0,38	0,83
21,85	Ethyl benzoate	*Fatty*[h]	–	–	–	0,21	–
22,58	Methyl phenylacetate	*Sweet, honey, jasmine*[ae]	0,30	0,29	0,36	0,25	0,30

(*continued on next page*)

Table 5 (*continued*)

Rt	Compound name	Odor descriptor*	Percentage (%)[a]				
			CA100	CV74	CM70	TM66	NE60
23,33	Ethyl phenylacetate	*Fruity, sweet, honey-like*[cei]	0,72	–	0,63	0,42	0,69
23,80	**2-Phenylethyl acetate**	*Flowery (rose), honey-like*[abc]	5,53	–	7,30	–	–
24,16	Butyl benzoate	*Flowery* [f]	0,88	–	–	–	–
33,02	Ethyl palmitate	*Waxy, green*[i]	0,18	0,19	–	0,08	0,14
	total percentage		**9,04**	**0,82**	**9,50**	**1,80**	**2,77**
Pyrazines							
16,11	**Methylpyrazine**	*Earthy, cocoa, hazelnut, green*[df]	0,24	0,64	0,37	0,72	1,14
17,00	**2,5-Dimethylpyrazine**	*Cocoa, rhum-like, roasted-nuts*[c]	0,56	0,72	0,84	1,03	1,79
17,08	**2,6-Dimethylpyrazine**	*Earthy, fried potato-like*[dg]	0,36	0,47	0,61	0,51	–
17,21	**Ethylpyrazine**	*Earthy, popcorn-like*[dg]	–	0,23	0,16	0,26	–
17,39	**2,3-Dimethylpyrazine**	*Caramel, cocoa, earthy*[cd]	0,83	1,12	0,70	0,62	0,87
17,94	**2-Ethyl-6-methylpyrazine**	*Roasted, cocoa-like, earthy*[cd]	–	–	–	0,20	–
18,06	**2-Ethyl-5-methylpyrazine**	*Earthy*[d]	0,12	–	0,28	0,48	0,36
18,22	**Trimethylpyrazine**	*Cocoa, roasted-nuts, peanuts*[c]	2,19	1,82	4,98	2,48	1,89
18,78	3-Ethyl-2,5-dimethylpyrazine	*Roasted, nutty*[g]	0,52	–	0,66	0,72	1,77
19,03	2,6-Diethylpyrazine	–	–	0,43	–	0,30	–
19,03	2,3-Dimethyl-5-ethylpyrazine	–	–	0,21	0,57	0,48	0,23
19,17	**Tetramethylpyrazine**	*Cocoa, coffe, roasted, earthy*[cd]	4,62	10,43	11,78	11,76	6,07
19,47	2-Ethenyl-6-methylpyrazine	–	–	–	0,75	0,25	–
19,67	Trimethyl-6-ethylpyrazine	*Chocolate-, cocoa-, hazelnut-like*[f]	0,46	0,88	1,31	0,85	0,45
22,23	2-Acetyl-3-methylpyrazine	–	–	–	–	0,28	–
25,70	Piperazine	–	0,12	–	0,22	0,31	–
	total percentage		**10,02**	**16,96**	**23,22**	**21,27**	**14,57**
Lactones							
26,74	δ-Octenolactone	*Coconut-like*[ab]	–	–	–	–	0,26
27,73	Pantoic lactone	–	–	–	–	0,17	–
	total percentage		**0,00**	**0,00**	**0,00**	**0,17**	**0,26**

Rt	Compound name	Odor descriptor	Percentage (%)				
			CA100	CV74	CM70	TM66	NE60
Furans							
15,40	2-Pentyl furan	*Buttery, green bean-like, vegetable*[de]	–	–	–	0,15	–
20,63	5-Methyl-2-furancarboxaldehyde	–	0,58	3,51	0,37	0,60	0,72
21,45	**2-Furanmethanol**	*Burned, faint burning-like*[de]	–	0,76	–	–	1,41
22,22	5-Methyl-furanmethanol	–	–	–	–	0,18	–
27,34	4-Hydroxy-2,5-dimethyl-3(2H)-furanone	*Caramel-like*[ad]	0,22	–	–	0,33	–
	total percentage		**0,80**	**4,27**	**0,37**	**1,27**	**2,13**
Terpenes							
14,14	β-Myrcene	*Balsamic, must, spicy, sweet*[e]	0,06	0,17	–	0,07	–
14,90	Limonene	*Citrus-, mint-like*[def]	1,49	2,76	0,35	2,33	2,89
23,12	α-Curcumene	–	0,34	–	–	–	–
	total percentage		**1,89**	**2,92**	**0,35**	**2,39**	**2,89**
Others							
22,74	Acetamide	–	–	0,74	–	–	–
24,03	Anisole	–	0,50	0,32	–	3,70	–
	total percentage		**0,50**	**1,06**	**0,00**	**3,70**	**0,00**

Rt = Retention time in minutes. The percentage of a compound is based on the area normalization. - Indicates compound not detected. Cocoa technological and key-aroma markers according Magagna et al. (2017) are highlighted with bold letters. Odor descriptors show according to:
[b]Frauendorfer and Schieberle (2008).
[a] Frauendorfer and Schieberle (2006).
[c] Ascrizzi et al. (2017).
[d] Magagna et al. (2017).
[e] Hinneh et al. (2019)9.
[f] Tran et al. (2016).
[g] Liu et al. (2017).
[h] Misnawi and Ariza (2011).
[i] Rodríguez-Campos et al. (2012).

Within the volatile acids group, 11 compounds were found in the samples. Short-chain carboxylic acids, such as acetic acid, 2-methylpropanoic acid, butanoic acid, 3-methylbutanoic acid, and heptanoic acid were those most identified. Among these, acetic, 2-methylpropanoic, butanoic, and 3-methylbutanoic acid have been recognized as key aroma markers of cocoa (Magagna et al., 2017). Acetic acid is the highest odor-active compound in cocoa, and is characterized by a vinegar-like odor descriptor (Aprotosoaie et al., 2016). Among the rest, volatile fatty acids are also important to the chocolate aroma, imparting sour and buttery notes (Nightingale, Cadwallader, & Engeseth, 2012). The chocolate sample with Venezuelan Criollo cocoa (CV74) was characterized by the highest percentage of acetic acid (27.24%) compared with all of the other samples, which could indicate that the vinegar-like odor will impact more intensely on the aroma. However, the presence of minority acids, such as 3-methylbutanoic acid, detected in the CA100 and TM66 samples cannot be ignored that, due to its very-low-odor threshold and its having greater stability during processing compared to that of acetic acid, may exert an additive impact on the general aroma of the product and may denote in its characteristic aroma (Frauendorfer & Schieberle, 2008).

Short-chain primary alcohols (such as 2-heptanol, 2-nonanol, and 2,3-butanediol) and phenyl propanoid derivatives (such as 1-phenylethyl alcohol, 2-phenylethyl alcohol, benzyl alcohol, and guaiacol) were identified in the samples. The chocolate sample with Amazonian Criollo cocoa (CA100) was characterized by the highest alcohol percentage (28.03%) compared with that of the other samples, with mainly a higher content of 2-phenylethyl alcohol, and 2,3-butanediol, while the chocolate samples with Mexican cocoa (CM70 and TM66) were characterized by a lower alcohol content (11.13% and 15.21%, respectively). A high alcohol content is desirable in cocoa products because they impart flowery and candy notes (Rodríguez-Campos et al., 2012). For example, 2-phenylethyl alcohol, present in all dark-chocolate samples, is recognized as a cocoa key-aroma marker characterized by a honey-like odor descriptor (Magagna et al., 2017) and as an odor-active volatile responsible for imparting the typical floral note to the chocolate (Hinneh et al., 2019). 2-Heptanol, identified only in the CA100 sample, is a key-aroma marker that confers a citric (lemongrass-like) and fresh aroma (Ascrizzi et al., 2017). 2,3-Butanediol, another desirable volatile for high-quality cocoa products related to sweet and floral notes, is present in substantial amounts to impact the aroma of chocolate due to its good stability during the manufacture of chocolate (Ascrizzi et al., 2017; Tran et al., 2016). Among the minority alcohols identified, the presence is noteworthy of guaiacol in all of the samples analyzed. Guaiacol is a compound that has been reported as characteristic of the Bahía cocoa beans, which are known for their typical smell of smoked ham (Counet et al., 2004).

From the group of aldehydes and ketones, compounds such as 3-methyl-butanal, nonanal, furfural, benzaldehyde, phenylacetaldehyde, α-ethylidene-benzeneacetaldehyde, 5-methyl-2-phenyl-2-hexenal, 3-Hydroxy-2-butanone, 2-nonanone, 1-(1H-pyrrol-2-yl)-ethanone, and 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one were mainly identified in the samples. In general, the chocolate sample with Mexican Criollo cocoa (CM70) showed the highest percentage of aldehydes (19.41%), mainly furfural and benzaldehyde, while the samples with Amazonian Criollo (CA100) and Venezuelan Criollo cocoa (CV74) presented the lowest concentration, with 14.92% and 14.12%, respectively. In the case of ketones, the chocolate with Ecuatorian Nacional cocoa (NE60) demonstrated the highest content (12.76%), 2-nonanone mainly, while the samples that presented the lowest percentage were CA100 (7.98%), and CV74 (6.75%). Aldehydes and ketones are formed during cocoa-bean roasting by Strecker degradation of free amino acids (Aprotosoaie et al., 2016). Among the main aldehydes and ketones detected in the dark-chocolate samples, the 3-methyl-butanal and phenylacetaldehydes, are recognized as key cocoa-aroma markers related to malty and honey-like odor descriptors, respectively. Furfural has a flavor-active low-odor threshold in the air that imparts a bread-like odor, and 3-hydroxy-2-butanone, which is associated with a buttery perception, have been classified as a technological marker for being considered analytes sensitive to cocoa processing (Magagna et al., 2017; Tran et al., 2016). Aldehydes are also important reactants that generate, via aldol condensation, compounds such as the 5-methyl-2-phenyl-2-hexenal, a minority compound detected in all chocolate samples, but which is associated with a desirable roasted-cocoa odor and an intense bitter taste regardless of bean origin (Aprotosoaie et al., 2016; Ascrizzi et al., 2017). However, this compound was not detected in Brazilian varieties of cocoa, while it was detected in Ecuadorian cocoa varieties (Menezes et al., 2016). Also noteworthy is the presence of minor ketones, such as 2-heptanone, detected only in sample CA100 that, due to its low-odor threshold in the air, could exert a substantial impact in conferring a banana-like aroma to the sample, a compound identified in dark chocolate from Vietnamese cocoa (Tran et al., 2016).

Esters are the second most important group of volatile compounds in roasted cocoa after pyrazines, and these confer ta fruity aroma on o the final cocoa product (Ascrizzi et al., 2017; Hinneh et al., 2019). Eight esters were identified in the dark-chocolate samples, including ethyl octanoate, ethyl decanoate, methyl phenylacetate, ethyl phenylacetate, and 2-phenylethyl acetate, mainly. Of these, only the 2-phenylethyl acetate is recognized as key-aroma marker with a honey-like odor descriptor (Magagna et al., 2017) and it has been identified as odor-active in Ecuatorian and Vietnamese chocolates (Hinneh et al., 2019). However, ethyl octanoate (described as a pineapple-like odor), ethyl decanoate (grape-like), and ethyl phenylacetate (honey-like) are also odor-active compounds that remain stable during the chocolate manufacturing process, and the last compound is defined as one of the most important in cocoa aroma (Ascrizzi et al., 2017). It should be noted that samples CA100 and CM70 revealed a higher percentage of esters, 9.04% and 9.50%, respectively, associated with a greater amount of 2-phenylethyl acetate in their composition compared to the other samples, in which the percentage of esters was less than 3%. Santandér-Muñoz et al. (2019) reported that the group of esters is present in a greater quantity and with greater peculiar diversity in Criollo genotypes.

Sixteen pyrazines were found in the dark-chocolate samples, among we can highlight methylpyrazine, ethylpyrazine, trimethylpyrazine, tetramethylpyrazine, 2,3-dimethylpyrazine, 2,5-dimethylpyrazine, 2,6-dimethylpyrazine, 2-ethyl-6-methylpyrazine, and 2-ethyl-5-methylpyrazine. All of these are considered as technological markers of cocoa processing, are associated with coffee- and cocoa-like attributes, and are considered as the most important volatile compounds in roasted cocoa (Magagna et al., 2017; Nightingale et al., 2012). The chocolate samples with Mexican Criollo cocoa (CM70), and Mexican Trinitario cocoa (TM66) showed the highest pyrazine content with respect to the remainder of the samples, with 23.22% and 21.27%, respectively.

Furans are volatile compounds generated during the drying and roasting process by the degradation of monosaccharides, favoring their formation at moderate temperatures and relatively high humidities (Aprotosoaie et al., 2016). Five furans were identified in the dark-chocolate samples. The 5-methyl-2-furancarboxaldehyde was the only common furan compound for all samples and was present at a higher proportion in the sample with Venezuelan Criollo cocoa bean (CV74). Other furan compounds-of-interest, such as 2-furanmethanol associated with burned-odor descriptor and considered a technological key marker (Magagna et al., 2017), was only identified in two samples: CV74, and NE60, while 4-hydroxy-2,5-dimethyl-3(2H)-furanone (furaneol), an important furan that imparts pleasant caramel notes (Aprotosoaie et al., 2016), was identified in the CA100 and TM66 samples. Due to the fact that 4-hydroxy-2,5-dimethyl-3(2H)-furanone has a very-low-odor threshold in the air, it could generate a contribution to the aroma of these chocolate samples (Frauendorfer & Schieberle, 2008).

Lactones such as δ-octenolactone, related to a coconut-like odor, were found in the NE60 sample. However, its high-odor threshold in the air would indicate that its contribution to the aroma of the sample would be insignificant (Frauendorfer & Schieberle, 2008). Terpenes such as limonene were identified in all samples. Although this compound is associated with citrus and mint odors, its impact on the overall aroma of the chocolate could probably not be evaluated as an active flavor in the chocolate samples due to its relatively high-odor threshold in the air (Tran et al., 2016). β-Myrcene was another terpene identified in the majority of samples, and α-curcumene was only identified in the CA100 chocolate. However, its contribution to the aroma of cocoa-derived products has not yet been reported, as well as that of acetamide and anisole, also identified. Although the volatile compounds profile in dark-chocolate samples is shown, as well as compounds considered as odor-active in certain products throughout the cocoa process (Frauendorfer & Schieberle, 2006; Tuenter et al., 2020), an evaluation of Odor Activity Values (OAV) is necessary to estimate whether odorants are present in concentrations above their odor thresholds in dark chocolates and whether they play a key role in the authenticity of cocoa produced in Latin America.

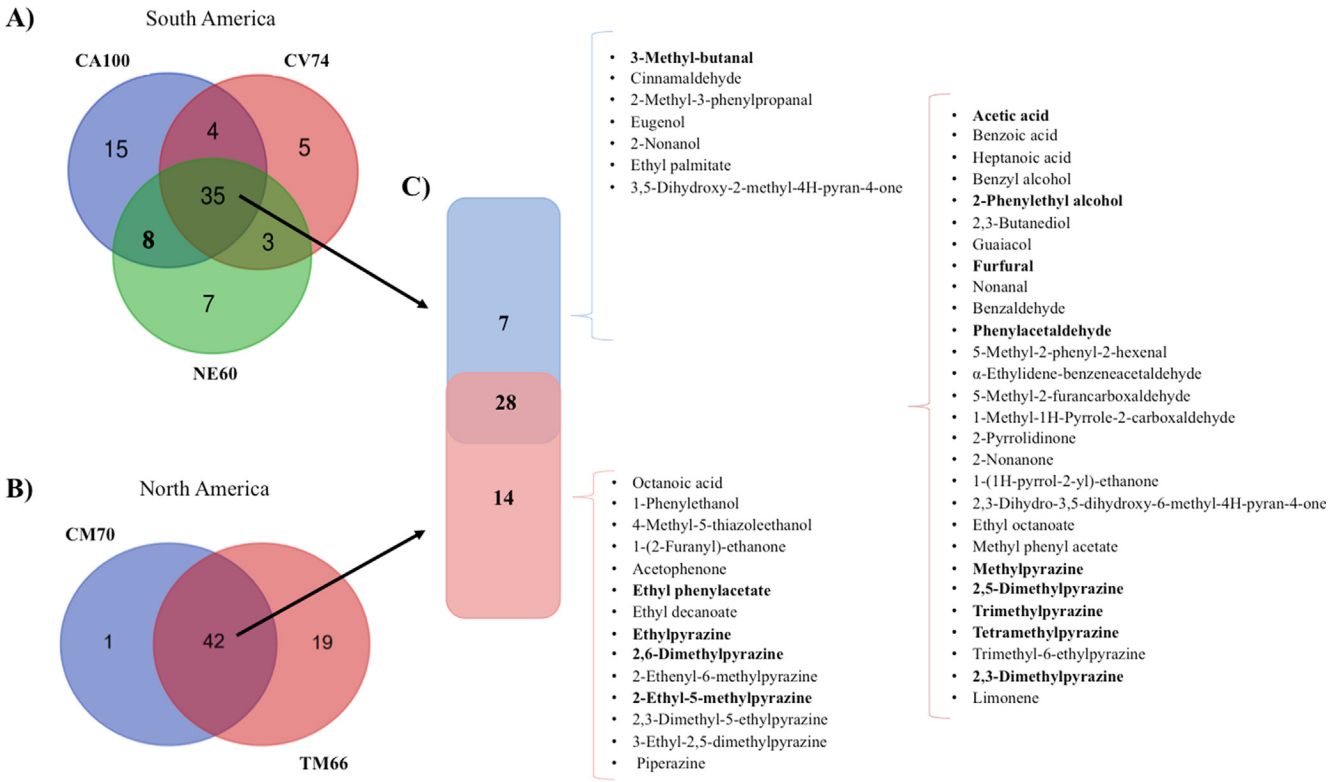

Fig. 3. Venn diagram showing the similarities and differences of volatile compounds between South- and North-American dark chocolates. A) The number of unique and common compounds in dark chocolates with cocoa from South America, B) The number of unique and common compounds in dark chocolates with cocoa from North America, and C) the number of unique and common compounds between South and North America with the corresponding list of compounds.

3.3.1. Differences and similarities between dark chocolates from different geographical origins

To illustrate the compositional differences and similarities among chocolates, we constructed Venn diagrams with all of the identified volatile compounds (Fig. 3). Of the 90 volatile compounds identified, 77 compounds were found in South-American samples (CA100, CV74, and NE60) with 35 compounds in common (Fig. 3A), while in North-American samples (CM70 and TM66) 62 compounds were found with 42 volatile compounds in common (Fig. 3B). On comparing the compounds in common between South-American and North-American dark chocolates, only 28 volatiles overlapped (Fig. 3C). Among the find the following: cocoa key-aroma compounds, such as acetic acid, 2-phenylethyl alcohol, furfural, phenylacetaldehyde, methylpyrazine, 2,5-dimethylpyrazine, trimethylpyrazine, tetramethylpyrazine, and 2,3-dimethylpyrazine, and among other compounds, such as 2,3-butanediol, guaiacol, benzyl alcohol, nonanal, benzaldehyde, 2-nonanone, 1-(1H-pyrrol-2-yl)-ethanone, ethyl octanoate, methylphenyl acetate, trimethyl-6-ethylpyrazine, and limonene, suggesting an important role in the composition of the dark-chocolates aroma profile from Latin American cocoas. The unique compounds in South-American chocolate samples were 3-methyl-butanal, cinnamaldehyde, 2-methyl-3-phenylpropanal, eugenol, 2-nonanol, ethyl palmitate, and 3,5-dihydroxy-2-methyl-4H-pyran-4-one, while, octanoic acid, 1-phenylethanol, acetophenone, ethylphenyl acetate, ethyldecanoate, and pyrazines, like ethylpyrazine, 2,6-dimethylpyrazine, 2-ethyl-5-methylpyrazine, and 3-ethyl-2,5-dimethylpyrazine, were exclusively identified in North-American chocolate samples (Fig. 3C).

3.4. Principal component analysis (PCA) and hierarchical cluster analysis heatmap (HCA heatmap)

PCA was utilized to map the natural groupings of dark-chocolate samples and to localize informative chemicals responsible for variations. PCA was performed using the area unit of the volatile compounds identified in the samples as analytical variables. In general, charges in the relative abundance and distribution of the different volatile compounds will reflect differences observed among the chocolate samples. Fig. 4 presents the PCA bi-plot of the variation in the volatile profile between each sample in triplicate. Overall variance is 78.29%, of which 40.86% relates to PC1 and 37.43% to PC2. Origin dominates group conformation, in particular, PCA bi-plot clustered dark-chocolate samples in two main subgroups. The first component (PC1) with positive loading separated the first subgroup (highlighted with a wine-colored circle) of samples from North America (CM70 and TM66) with respect to the second group (highlighted with a yellow circle) that includes the Ecuatorian (NE60), Venezuelan (CV74), and Amazonian (CA100) samples with a negative loading on PC1 (Fig. 4). PCA discriminates differences in the samples by cocoa genotype. In the first subgroup (PC1 with positive scoring), PC2 plotted the Criollo cocoa (CM70) sample in the upper quadrant (with positive loading), while the Trinitario (TM66) sample was located in the lower quadrant with negative loading. The South-American samples were also separated, placing the Amazonian Criollo (CA100) sample in the upper quadrant of PC2 (positive loading), while the Venezuelan Criollo cocoa (CV74) sample and the Ecuadorian National cocoa (NE60) sample, regardless of their genotype, were plotted in the negative quadrant of PC1 and PC2. The North-American samples (CM70 and TM66) were highly related to tetramethylpyrazine, but CM70 demonstrated a greater relationship to furfural and 2-phenylethyl acetate compared to TM60. These three compounds are cocoa key-aromas and processing markers. These volatile compounds exhibited significant differences ($p < 0.05$) in a greater peak area. Tetramethylpyrazine has been related to South American cocoa samples compared to dark-chocolate samples from Africa and Asia through PCA chemometric analysis (Acierno et al., 2016). Therefore, this compound could play a key role in distinguishing samples derived from Latin American cocoa, and mainly with cocoa

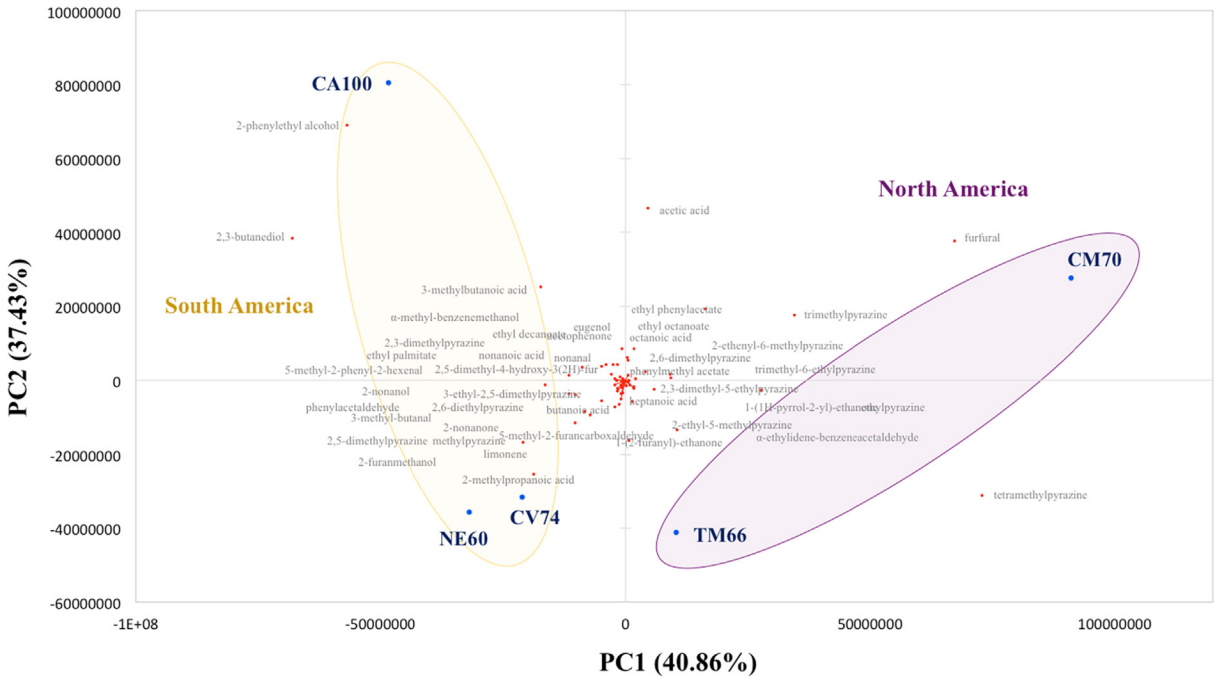

Fig. 4. Principal Component Analysis (PCA) and its associated bi-plot of the abundances of volatile compounds detected by HS-SPME/GC-MS. PCA showing the distribution of different dark chocolates with cocoa of different geographical origins from North America (highlighted with a wine-colored circle) and from South America (highlighted with a yellow circle).

genotypes from North America. The CA100 sample was highly influenced by 2,3-butanediol and 2-phenylethyl alcohol, both important cocoa aroma markers, while NE60 and CV74 were highly influenced by 2-nonanol, 2-nonanone, cinnamaldehyde, 3-ethyl-2,5-dimethylpyrazine, 1-methyl-1H-Pyrrole-2-carboxaldehyde, limonene, 2-methylpropanoic acid, and 3-methyl-butanal. The latter compound is recognized as an important key-aroma marker of cocoa. Among the volatile compounds that were related to South American cocoa chocolates, 2-phenylethyl alcohol and 2-methylpropanoic acid, were previously related to samples of dark-chocolate from South American cocoa, with respect to samples from from different origins (Acierno et al. 2016), sustaining the key role of both compounds in distinguishing the origin of American dark-chocolate samples, properly derived from South America.

Although PCA showed a natural separation of dark-chocolate samples by geographical origin, it did not demonstrate a clear separation between samples based on their genotype, except the case of cocoa samples from North America, in which the Trinitario and the Criollo genotype was clearly separated. This can be explained because the total volatile matrix was included in the PCA, and many of these compounds, mainly from the group of acids, aldehydes, ketones, and even some pyrazines, are drastically affected (increasing or decreasing their concentration) along the chocolate processing chain. In addition, depending on the roasting and conching conditions employed, used, they generate variability in the original aroma profile (Ascrizzi et al., 2017; Santandér-Muñoz et al., 2019). However, previous studies identified a distinctive signature of alkyl pyrazines, like tetramethylpyrazine in Mexican cocoa, that make it different from other genotypes (such as the Nacional genotype from Ecuador), and the tetramethylpirazine content, in particular, is maintained throughout the chocolate processing chain (Aprotosoaie et al., 2016; Ascrizzi et al., 2017; Magagna et al., 2017), indicating its strong diagnostic role in cocoa origin and genotype. The 2,3-butanediol and 2-phenylethyl alcohol that characterized the CA100 sample have also shown to remain stable during the chocolate manufacturing process (Ascrizzi et al., 2017), indicating a strong relationship of these compounds with the geographical origin of cocoa, regardless of the intrinsic chocolate processing. This suggests that the identification of the origin and genotype of the cocoa bean in dark-chocolate requires a detailed selection of volatiles, based on their lower variability, throughout the processing, such as roasting and conching, among the entire profile matrix.

Hierarchical Cluster Analysis Heatmap (HCA Heatmap) was constructed to illustrate the (dis)similarities among the different dark chocolates in terms of their volatiles profile. Two-way HCA and its associated Heatmap diagram of the five dark-chocolate samples are shown in Fig. 5. Results by HCA were somewhat different from PCA. Three main clusters can be identified by HCA. The dark chocolates CA100, CM70, and TM66 were identified in cluster one. These chocolates were characterized by a higher content of benzaldehyde, octanoic acid, and trimethylpyrazine. The North-American cocoa samples (TM66 and CM70) were identified in cluster two, distinguished by higher concentrations of pyrazines, mainly such as 2-ethenyl-6-methylpyrazine, trimethyl-6-ethylpyrazine, trimethylpyrazine, and tetramethylpyrazine from the Amazonian Criollo cocoa sample. Additionally, among these, CM70 differs from TM66 in terms of a higher content of acetic acid, and others such as benzaldehyde, 2-phenyl acetate, furfural, 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one, and limonene. It has been observed that in chocolates produced from Criollo cocoa, the content of acetic acid is greater compared to chocolates obtained with Trinitario cocoa (Acierno et al., 2016). The dark-chocolates with South-American cocoa (CV70 and NE60) were grouped in cluster three, confirming their similarities, and these were distinguished by higher concentrations of 2-furanmethanol, 2-nonanone, and 2-methylpropanoic acid. And the sample of Nacional cocoa (NE60) can be differentiated from Venezuelan Criollo (CV74) with a higher concentration of 2,5-dimethylpyrazine, 3-ethyl-2,5-dimethylpyrazine, ethyl decanoate, ethylphenyl acetate, and 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one.

Therefore, considering only those volatile compounds that were common among all the chocolate samples, tetramethylpyrazine, furfural, trimethylpyrazine, and benzaldehyde could be mentioned as potential aroma markers for dark-chocolate derived from Latin American cocoa (North American region), and the 2-phenylethyl alcohol, 2-methylpropanoic acid, 2,3-butanediol, 2-nonanone, and limonene as potential aroma markers for chocolates derived from South

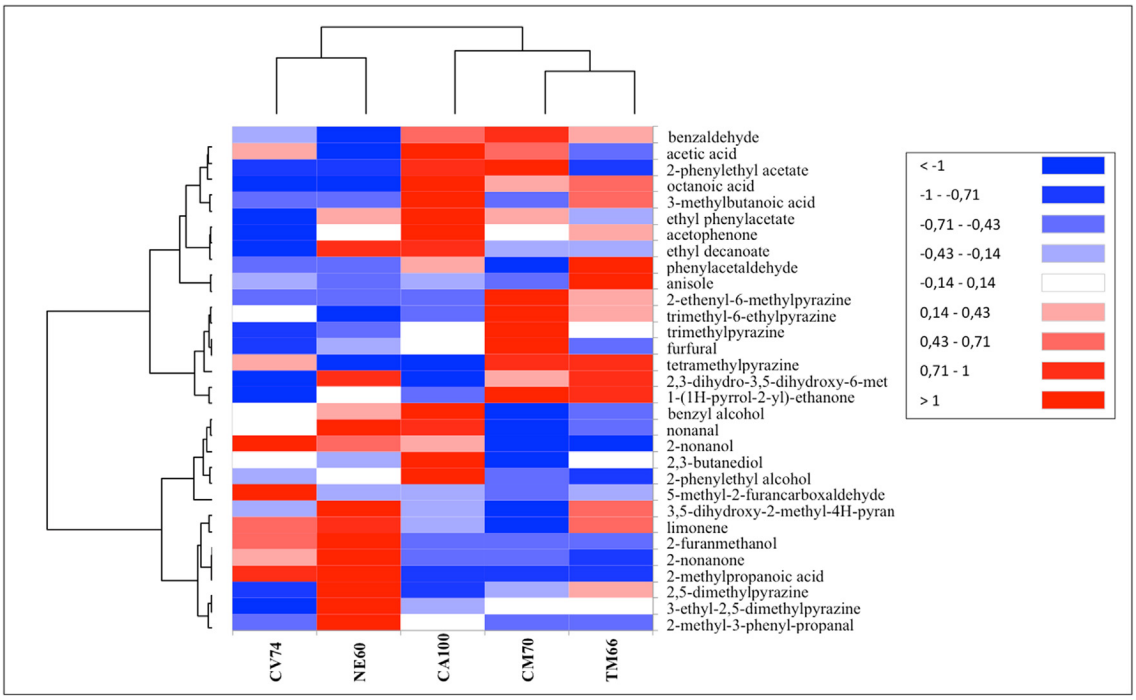

Fig. 5. Hierarchical Cluster Analysis Heatmap (HCA Heatmap) (Double Dendrogram) constructed from the normalized area of the chromatographic peaks obtained by GC-MS of the main volatile compounds found in dark-chocolate samples. The color of each tile of the Heatmap indicates the type/strength of the correlation for a given origin-compound combination. The red color indicates major abundance, while the blue color indicates minor abundance.

American. The 2-phenylethyl acetate could have a role in the particular distinction between samples derived from North American cocoas, while cinnamaldehyde and 3-methyl-butanal between samples derived from South American cocoas.

4. Conclusion

The general melting behavior of dark-chocolates was not affected by the formulation (fat content, sugar, protein, and lecithin) and its intrinsic manufacturing process, suggesting that the melting properties (specifically the T_{peak}, ΔH_{melt}, and C*p* values), the identification of glass-transition temperatures (T_g), and fatty acids profile could identify information related to the origin or genotype of dark-chocolate cocoa.

The analysis of bioactive compounds demonstrated great variability among samples independent of the origin and genotype of cocoa, which is possibly associated with the strong effect of the intrinsic processing of each chocolate. However, the profile of remaining bioactives, such as the anthocyanin, epicatechin, theobromine, and caffeine content in the final product, could provide information relating to cocoa genotype and origin.

The PCA of volatile compounds allowed a grouping of chocolates in relation to the origin of cocoa (from North America to South America) and discriminated against the Trinitario genotype of the Criollo in North-American chocolates, while HAC permitted the discrimination between geographical origins and genotypes. Although compounds like 2-methylpropanoic acid, 2-phenylethyl alcohol, and tetramethylpyrazine have been related to chocolates derived from South America cocoas in previous research, the present study included samples from North America, allowing to identify aroma markers typical of cocoas from Latin America, as well as of potential regional distinction, such as furfural, trimethylpyrazine, benzaldehyde, 2,3-butanediol, 2-nonanone, 2-phenylethyl acetate, cinnamaldehyde, 3-methyl-butanal, and limonene. However, a robust study that included a larger number of samples of commercial dark chocolates made with cocoa of native genotypes from these American regions is necessary to identify that volatiles that play a key role in the unique profile of its dark chocolates and that these determine consumer preference.

Declaration of Competing Interest

There are no conflicts of interest to declare.

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