



Sniffing out cocoa bean traits that persist in chocolates by PTR-MS, ICP-MS and IR-MS

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ARTICLE INFO

Keywords:

Cocoa beans

Chocolate

Fingerprint

High sensitivity-proton transfer reaction-mass spectrometry

Inductively coupled plasma-mass spectrometry

Isotope ratio-mass spectrometry

ABSTRACT

The cocoa botanical and geographical origin and the primary processing steps applied by cocoa farmers at the beginning of the supply chain influence the chemical compositional traits of the cocoa beans. These features are carried along the supply chain as intrinsic markers up to the final products. These intrinsic markers could be used for tracking and tracing purposes. In this study, we examined the retention and loss of compositional signatures from cocoa beans to chocolates. Volatile, elemental and stable isotope signatures of cocoa beans of 10 different origins and 11 corresponding chocolates were determined by high sensitivity-proton transfer reaction-mass spectrometry (HS-PTR-MS), inductively coupled plasma-MS (ICP-MS) and isotope ratio-MS (IR-MS), respectively. The volatile fingerprints provided mostly information on the origin and primary processing traits of the raw cocoa beans in the chocolates. Volatile compounds that are relevant markers include: acetic acid (m/z 61), benzene (m/z 79), pyridine (m/z 80), 2-phenylethanol (m/z 123), and maltol (m/z 127). On the other hand, the elemental and stable isotope characteristics are more indicative of the cocoa content and added ingredients. Possible elemental markers for cocoa origin include Fe, Cr, and Cd. VOCs appear to be the most robust markers carried from cocoa beans to chocolates of the groups examined. This provides the potential for track and trace of cocoa beans from farm to chocolates.

1. Introduction

The chain from cocoa beans to chocolate is long and complex. Production dispersion of the batches can already take place in the first steps. The cocoa beans produced by small-sized farmers are merged by the local collectors into larger batches causing loss of traceability. Saltini and Akkerman (2012), were able to trace the cocoa beans up to the local exporter. However, it was not possible to collect further information, indicating a poor data exchange between farmers and chocolate manufacturers. The cocoa botanical and geographical origin and the primary processing applied at the beginning of the supply chain characterize the quality of cocoa beans and influence the finished chocolate. This information could be used to establish a compositional link between the raw materials and finished chocolate and possibly traceback or verify the origin of the cocoa beans in the supply chain.

A few studies searched for relationships between cocoa beans and chocolate characteristics. These studies focused mainly on quality assurance in terms of flavour development (Ascrizzi, Flamini, Tessieri, & Pistelli, 2017) or quality prediction (Davies et al., 1991). Other studies

followed the evolution of volatile organic compounds (VOCs) (Braga et al., 2018), sugars, acids (Rodriguez-Campos, Escalona-Buendía, Contreras-Ramos, & Orozco-Avila, 2012) and amino acids (Pätzold & Brückner, 2006) along the chocolate production but they focused on optimizing production steps such as fermentation, drying, roasting and conching (Counet, Callemien, Ouwerx, & Collin, 2002). No studies establishing a compositional link between cocoa beans and chocolates are available to improve product traceability.

A direct link with the raw material could be represented by the botanical and geographical information. So far, botanical and geographical origin of cocoa beans has been largely analysed. Volatile organic compounds (Qin et al., 2017), triglyceride composition (Hernandez, Castellote, & Permanyer, 1991) and elements (Bertoldi, Barbero, Camin, Caligiani, & Larcher, 2016) have been used to differentiate cocoa beans of different origins. In our previous studies, through the analysis of the VOCs, we were able to detect the botanical and geographical origin of cocoa beans used to manufacture the chocolate into a finished chocolate bar (Acierno, Alewijn, Zomer, & van Ruth, 2018; Acierno, Yener, Alewijn, Biasoli, & Van Ruth, 2016) indicating

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<https://doi.org/10.1016/j.foodres.2020.109212>

Received 24 January 2020; Received in revised form 25 March 2020; Accepted 31 March 2020

Available online 02 April 2020

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the possibility to extract raw material information from a finished product. However, the magnitude of changing of potential origin markers along the chocolate production has never been tested. Other parameters that could be used to track and trace the production are the primary processing steps, such as fermentation and drying, as they are also characterising the final product. These procedures could give cocoa beans origin information as they differ between regions, countries and/or continents (Acierno, Fasciani, Kiani, Caligiani, & van Ruth, 2019; Caligiani, Marseglia, Prandi, Palla, & Sforza, 2016; Caligiani, Palla, Acquotti, Marseglia, & Palla, 2014).

Mass spectrometry (MS) is usually applied to detect origin information in food products (Luykx & van Ruth, 2008). Techniques such as inductively coupled plasma-MS (ICP-MS) for the detection of macro, micro- or trace-elements and isotope ratio-MS (IR-MS) for the measurements of stable isotope abundance could give information on factors both indicative of the origin and the production such as the use of fertilisers, and seasonal and geological variations (Isabel et al., 2009; Serra et al., 2005). In the specific case of cocoa, multi-element screening has been used to identify macro-, micro- or trace-elements indicative both of cocoa beans and chocolates provenance (Bertoldi et al., 2016; Levi et al., 2014), and soil and cropping system reflection on dry cocoa beans (De Araujo, Baligar, Loureiro, & Júnior, 2017). Only a few studies investigated stable isotopes for detection of the cocoa beans provenance. Diomande et al. (2015) used multi-compound isotope profiling to identify botanical and geographical information of fermented cocoa beans. Other analytical MS techniques have been studied to verify the origin of foods and the influence of the processing steps. These techniques include gas chromatography-MS and proton transfer reaction-MS (PTR-MS). GC-MS for detection of small and volatile molecules has been largely applied in the cocoa-chocolate sector both for VOCs identification (Frauendorfer & Schieberle, 2008; Rodriguez-Campos, Escalona-Buendía, Orozco-Avila, Lugo-Cervantes, & Jaramillo-Flores, 2011) and origin investigation (Caligiani, Cirlini, Palla, Ravaglia, & Arlorio, 2007; Cambrai et al., 2010).

PTR-MS allows fast analysis (< 1 min for a complete mass spectrum) of VOCs at low concentration (pptv, part per trillion by volume level); it has been used to distinguish the provenance of several food products. Specifically, in our previous studies it has been used for the origin detection of cocoa beans and chocolates (Acierno et al., 2016, 2019). Furthermore, this technique has been used to study the impact of processing such as coffee roasting (Yener et al., 2016) and it has been applied to assess the impact of different production systems in milk production (Liu, Koot, Hettinga, Jong, & Van Ruth, 2018). Therefore, PTR-MS seems a suitable technique for gathering information about the beginning of the production and for application at different stages of the supply chain. For this reason, this technique has been selected in this study, to evaluate the magnitude of VOCs changing from cocoa beans to chocolates and to further support the applicability of PTR-MS as a fast and reliable scan of the product along the supply chain. Specifically, in this study we examined the retention and loss of compositional signatures from cocoa beans to chocolates. The analysis of VOCs has been combined with the elements and isotope investigation to enlarge cocoa-chocolate characterization using robust and largely applied MS-techniques. Volatile, elemental and isotopic fingerprints of cocoa beans (fermented and dried) and chocolates produced thereof have been evaluated to investigate the magnitude of similarity and dissimilarity between cocoa bean raw materials and finished chocolate products. Ten samples of cocoa beans of different origin and eleven corresponding chocolates were analysed for their volatile, elemental and isotopic signatures using high sensitivity-PTR-MS (HS-PTR-MS), ICP-MS and IR-MS, respectively.

Table 1

Cocoa beans and corresponding chocolates.

Cocoa beans		Chocolates	
Origin	Code	Cocoa content (w/w (%))	Code
Congo	CG	60	CG(60)
Mexico	MEX	34	MEX(34)
Ecuador	EC	72	EC(72)
Venezuela	YV	45	YV(45)
Costa Rica 1	CR1	48	CR1(48)
Costa Rica 2	CR2	48	CR2(48)
Vanuatu 1	VU1	44	VU1(44)
Vanuatu 2	VU2	44	VU2(44)
Trinidad 1	TT1	81	TT1(81)
Trinidad 2	TT2	78	TT2(78)
		82	TT2(82)

2. Material and methods

2.1. Samples

Ten fermented and dried cocoa bean samples from different geographical origins and eleven chocolate samples industrially manufactured from those beans were sourced from a single manufacturer (Table 1) to decrease variability related to the ingredient added and the production steps highlighted in previous studies (Acierno et al., 2016, 2018). From each bean sample one type of chocolate was manufactured, except for one cocoa bean sample from Trinidad (TT2) which was used to produce two chocolates with different cocoa contents (TT2 (78% cocoa); TT2 (82% cocoa)). Chocolates generally varied in cocoa content. The samples were stored at room temperature (18–20 °C) until analysis. Cocoa beans and chocolates were ground a day prior to the start of the measurements. After grinding, they were stored in plastic bottles with screw caps at 4 °C.

2.2. HS-PTR-MS analysis

One gram of powdered cocoa bean and three grams of ground chocolate were weighed into 250 ml flasks. Cocoa beans and chocolates were placed in a water bath at respectively 30 °C and 40 °C for 30 min to equilibrate the samples with their headspace. Due to compounds saturation in the cocoa beans headspace, lower quantity and lower water bath temperature have been used for the cocoa beans compared to the chocolates. The headspace of the powdered cocoa beans and chocolates was measured by HS-PTR-MS (Ionicon Analytik G.m.b.H., Innsbruck, Austria). The PTR-MS conditions were as follows: drift pressure 2.20 mbar, inlet flow 60 ml/min, the reaction chamber and inlet temperature at 60 °C. The instrument was operated at an E/N (ratio of electric field strength across the reaction chamber, E, to buffer gas number density, N, within the chamber) of 119 Td (1 Td = 10^{-17} cm² V molecule⁻¹). For each sample, a mass range between 20 and 160 was measured using a dwell time of 0.2 s mass⁻¹. A blank (empty flask) was analysed before each sample. Five cycles per measurement were recorded and specifically the three last cycles for the blanks and the three cycles in the middle for the samples were used for data analysis. The values obtained for the blank were subtracted from each sample and all values were corrected for transmission. Three replicates of each sample were analysed. For each replicate a different sample of the same batch was taken.

2.3. ICP-MS

Cocoa beans and chocolate samples were pre-treated using acid digestion with a microwave oven (MARS express, CEM Corporation, Matthews, NC, USA). For the microwave digestion, 10 ml of concentrated nitric acid (69% Instra-analyzed nitric acid, J.T. Baker,

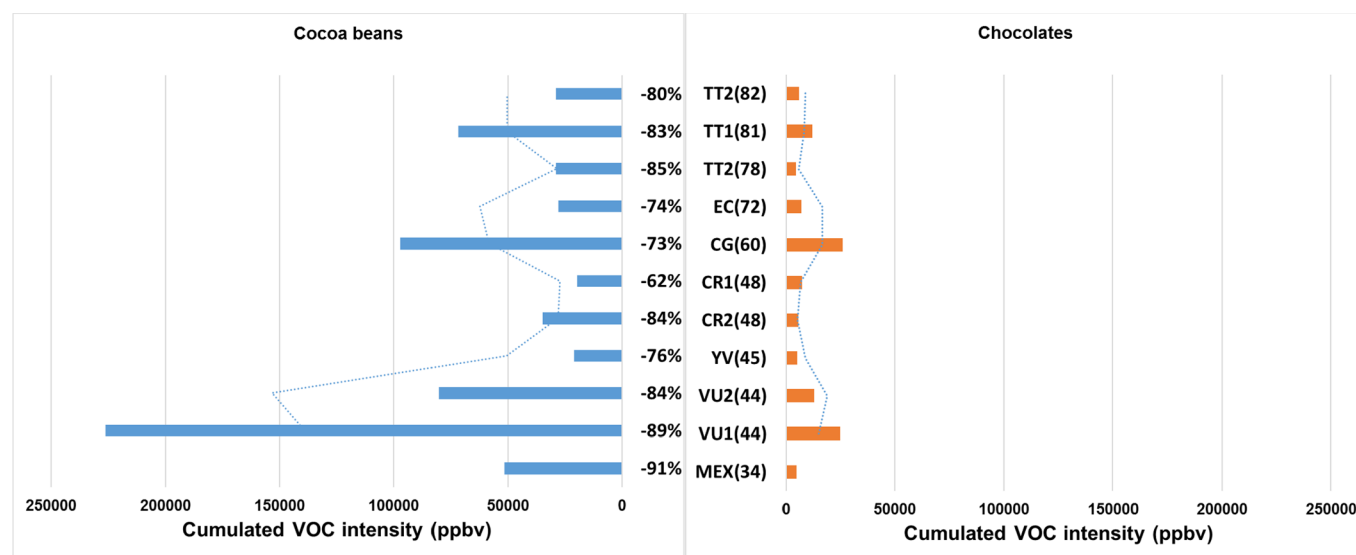


Fig. 1. Cumulated VOC intensities of cocoa beans and corresponding chocolates and the relative intensity differences between cocoa beans to chocolates. MEX: Mexico; VU1 Vanuatu1; VU2: Vanuatu 2; YV: Venezuela; CR1: Costa Rica1; CR2: Costa Rica2; CG: Congo; EC: Ecuador; TT2: Trinidad 2; TT1: Trinidad1. The percentage in brackets indicates the relative cocoa content of the chocolates.

Phillipsburg, NJ, USA) was added to 0.5 g of sample in Teflon digestion vessels. The samples were then placed in the microwave oven at a temperature of 210 °C. The digests were quantitatively transferred to 50 ml polypropylene (PP) centrifuge tubes (Greiner Bio-One, Frickenhausen, Germany) and diluted with de-ionized water to a final volume of 50 ml. The digests were diluted 5-fold and 200-fold before analysis with a final acid concentration of 2.8% v/v. Concentrations of 53 elements were determined using ICP-MS (NexION 300D, Perkin Elmer, Waltham, MA, USA). Samples were introduced into the ICP-MS using an SC-FAST autosampler (ESI, Omaha, NE, USA). The ICP-MS was equipped with a PFA-ST nebulizer, a quartz cyclonic spray chamber and a quartz torch with a sapphire injector. The ICP-MS was operated in collision mode (KED) with helium as collision gas for all elements, with a flow rate of 4.1 ml/min and an RPq of 0.25. Concentrations were determined using external calibration curves with rhodium as an internal standard.

2.4. IR-MS

Cocoa beans and chocolate samples were analysed for ^{13}C and ^{15}N enrichment by elemental analyses using an isotope ratio mass spectrometer (Flash 2000 in combination with Delta V advantage, Thermo Scientific, Waltham MA USA). All the samples were analysed in duplicate. For each replicate a different sample of the same batch was taken. The relative ^{13}C and ^{15}N enrichment are expressed as the $^{13}\text{C}:^{12}\text{C}$ and $^{15}\text{N}:^{14}\text{N}$ ratio in the samples relative (in ‰) to the $^{13}\text{C}:^{12}\text{C}$ and $^{15}\text{N}:^{14}\text{N}$ ratio of the Vienna Pee Dee Belemnite (VPDB) international standard.

For ^{18}O and ^2H determination, cocoa beans and chocolate samples were analysed on a HEKAtech high-temperature elemental analyser coupled with a GV Instruments IsoPrime mass spectrometer. Samples were pyrolyzed at 1450 °C, in silver capsules. Three replicates of each sample were analysed. For each replicate a different sample of the same batch was taken. All results are reported with respect to Vienna Standard Mean Ocean Water (VSMOW), normalized to international standards IAEA-SO-5 and IAEA-SO-6 with reported values of + 12.0‰ and - 11.3‰ for $\delta^{18}\text{O}$ values. The analytical precision for these measurements is 0.5‰.

2.4.1. Statistical analysis

2.4.1.1. Univariate analysis. For the entire data set normality within each group was checked by Shapiro-Wilk test and the assumption of

equal variances was assessed by Levine's test based on the median. To measure similarity and dissimilarity in volatile, elemental and isotopic signatures between cocoa beans and corresponding chocolates, data were subjected to non-parametric tests. Spearman's Rho was applied to correlate data of cocoa beans and chocolates, Mann-Whitney tests were used to investigate significant differences between groups. The Mann-Whitney tests were followed by a rate of false discovery (RFD) correction. A significance level of $p \leq 0.05$ was used throughout the study. All the statistical analyses of data were conducted using IBM SPSS (Statistic 23.0, IBM Corp., Armonk, NY, USA).

2.4.1.2. Multivariate analysis. Principal component analysis (PCA) was applied to visually explore the presence of any natural clustering in the data using Pirouette 4.0 Software (Infometrix, Seattle, WA, USA). A correlation between the score values of cocoa beans and corresponding chocolates was measured with Pearson's correlation coefficient using IBM SPSS (Statistic 23.0, IBM Corp., Armonk, NY, USA).

2.4.1.3. Correlations between volatile, elemental and isotopic data. Correlation tables between volatile and elemental and isotopic data, based on Spearman's Rho values, were built using R 3.3.3 (R Foundation for Statistical Computing, Vienna, Austria).

3. Results and discussion

3.1. Signatures of volatile compounds of cocoa beans and corresponding chocolates

The 10 cocoa beans and the 11 corresponding chocolate samples were subjected to HS-PTR-MS analysis. The mass spectra of both cocoa beans and chocolates obtained by the PTR-MS showed signals on most masses in the defined measurement range (20–160 amu). The spectra, used as signatures, comprised 133 masses after removal of O_2^+ (m/z 32) and water clusters (m/z 37). The data were not normally distributed (Shapiro-Wilk test, $p \leq 0.05$), hence both significant correlations and significant differences were evaluated by non-parametric tests, i.e. the Spearman's Rho and Mann-Whitney tests, respectively.

Cumulated intensities of the VOCs of each cocoa bean sample and the corresponding chocolate revealed a significant correlation between the two data groups (Spearman's Rho = 0.59; $p \leq 0.05$) (Fig. 1).

Cumulative VOC intensities of the chocolates were significantly

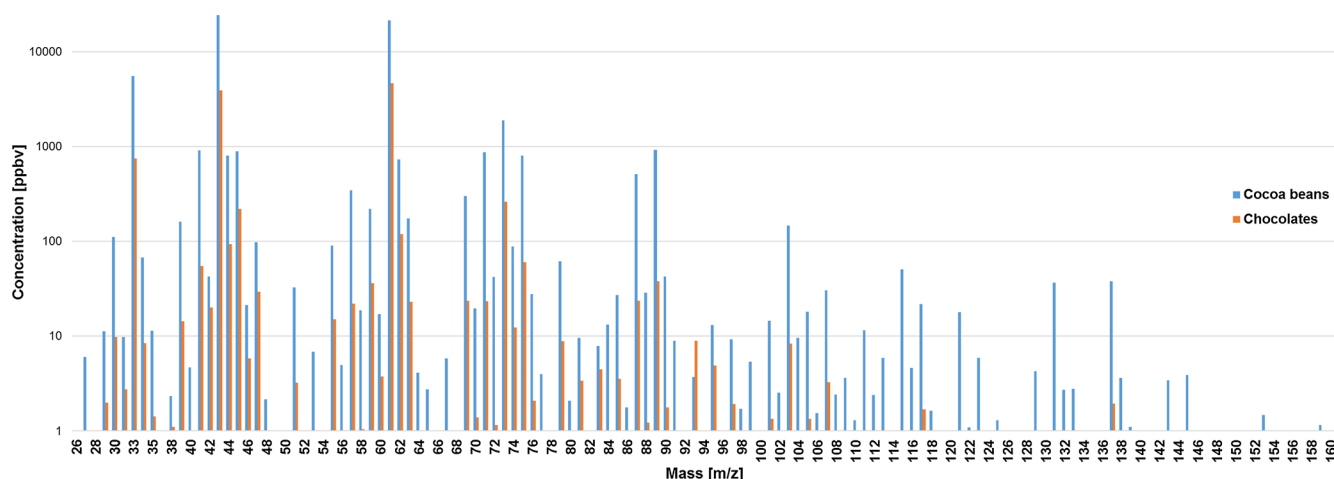


Fig. 2. HS-PTR-MS averaged profiles of cocoa beans and chocolates.

lower (Mann-Whitney test, $p \leq 0.05$) than those of the cocoa beans. This also holds for the individual compounds, for which significant differences were noted for ~93% of the 133 masses detected. Rather more quantitative than qualitative differences in profile intensities between cocoa beans and chocolates were observed (Fig. 2). For cocoa beans, highest intensities were observed for higher molecular weight compounds ($m/z > 100$). Within this group significantly higher intensities for cocoa beans were noted in comparison to the chocolates, e.g. for m/z 109 (4-methylphenol/benzyl alcohol), m/z 112 (trimethylxazole), m/z 121 (2-ethenyl-6-methylpyrazine), m/z 123 (2-phenylethanol), m/z 125 (guaicol), m/z 129 (furanol/heptanedione/octanal), m/z 131 (ethyl valerate/heptanoic acid), m/z 137 (various monoterpenes), m/z 138 (pyridine carboxylic acid methyl ester), m/z 153 (E,E-2,4-decadienal), and m/z 159 (nonanoic acid) (Mann-Whitney test, $p \leq 0.05$; Table 2). The tentative identifications of the masses were based on our previous studies focused on the analysis of cocoa beans and chocolates using PTR-Time of Flight-MS (PTR-ToF-MS) (Acierno et al., 2016, 2019).

The lower concentrations in the chocolate samples can be related to the influence of the chocolate processing and matrix effects which in turn alter the partitioning of VOCs from the product to the headspace (Ducki, Miralles-García, Zumbé, Tornero, & Storey, 2008). For instance, concentrations of organic acids such heptanoic acid (m/z 131) and nonanoic acid (m/z 159) typically decrease during conching; in general the acid concentration is expected to be low in chocolate so the flavour is mild and pleasant (Ascrizzi et al., 2017). Terpenes (m/z 137, various monoterpenes) usually decrease during roasting (Yener et al., 2016). 4-Methylphenol (m/z 109) and guaicol (2-methoxyphenol) (m/z 125) increase during fermentation and drying, however, phenolic compounds are undesirable in the cocoa product so the low level in the final product is considered to add to a better chocolate flavour (Rodríguez-Campos et al., 2012). Regarding the matrix effects, it is known that the headspace concentrations of volatiles are influenced by fat, sugar and additive contents (Romano, 2017). For instance, low concentrations of VOCs with caramel-like notes such as furaneol (m/z 129), 2-phenylethanol (m/z 123) and long aldehydes such as E/E-2,4-decadienal (m/z 153) depend on the fat content since a high lipid content reduces the volatility of lipophilic components (Afoakwa, Paterson, Fowler, & Ryan, 2009). Low concentration of 2-ethenyl-6-methylpyrazine (m/z 121) is mainly related to the fact that pyrazine, being a non-polar compound, tend to be trapped in the fat phase (Ziegleder, 1991).

Despite the difference in concentration, significant correlations (Spearman's Rho, $p \leq 0.05$) were established between mass intensities of the cocoa beans and chocolates (Fig. 1, Supplementary materials).

In Table 2 a tentative identification of the cocoa beans' masses that showed high correlation with chocolate masses (Spearman's Rho >

0.70; $p \leq 0.05$) is presented. The majority of the masses noted for cocoa beans are correlated with certain masses determined in the chocolates, i.e. m/z 43, m/z 61, m/z 79 (benzene), m/z 80 (pyridine), m/z 99 (hexanal), m/z 113 (methylfurfuryl alcohol), m/z 121, m/z 123, m/z 127 (maltol) and m/z 131. According to the tentative identifications in Table 2, these masses can be linked to compounds found in raw cocoa beans and/or related to an early stage of production, specifically during fermentation and drying. For instance, some of the highly correlated masses, tentatively identified as hexanal (m/z 99) and 2-phenylethanol (m/z 123), are already present in cocoa pulp and possibly influence the profile of fermented beans (Chetschik et al., 2018). Acetic acid (m/z 61) can also be found in raw beans (Frauendorfer & Schieberle, 2008), however, its concentration, together with m/z 43 can be influenced by different geographical conditions of fermentation and drying (Acierno et al., 2019; Caligiani et al., 2016). 2-Ethenyl-6-methylpyrazine (m/z 121) has been defined as a technological marker related to hot-air roasting (Magagna et al., 2017). As stated before, pyrazine is generated during roasting. However, we have previously detected this compound in fermented and dried cocoa beans (Acierno et al., 2019). Heptanoic acid (m/z 131) has been found at certain conditions of fermentation times and drying temperature as well, including sun drying, artificial drying and convection oven (Rodríguez-Campos et al., 2012). Because of the measured correlation (Fig. 1, Supplementary materials), knowing the different drying procedures applied at the growing location this mass could be further investigated as an origin marker (Beckett, 2008). Another interesting correlation was between maltol (m/z 127) in chocolates and most of the VOCs in cocoa beans. Maltol being a pyrone is released during drying and roasting via degradation of monosaccharides (Aprotosoaie, Luca, & Miron, 2016). It is usually produced during industrial drying and roasting and could be indicative of this kind of processes.

All the previous volatile compounds found in chocolate are correlated with the corresponding cocoa bean compounds, indicating the possibility to use VOCs for tracing back characteristics of cocoa in chocolate products along the supply chain. Specifically, compounds that are already present in non-fermented cocoa beans such as 2-phenylethanol can be used to directly link raw material and the finished chocolate. The compounds that are formed as a result of primary processes such as fermentation and drying could be used to track and trace the supply chain when the procedures applied at the location of cocoa beans origin are known.

3.2. Signatures of elements of cocoa beans and corresponding chocolates

The contents of 24 macro-, micro-, and trace elements were determined in all cocoa bean and chocolate samples (Table 3; Table 1 and

Table 2

Tentative identifications and concentrations of volatile compounds of cocoa beans and corresponding chocolates, median values and relative differences.

m/z	Tentative identification	Sum formula	Cocoa beans concentration (ppbv)	Chocolates concentration (ppbv)	Chocolates-Cocoa beans difference (%)
			Median	Median	Median
43	Fragment (diverse origin)	C ₃ H ₇ ⁺	13291.84	2176.65	-84
59	Acetone/propanal	C ₃ H ₇ O ⁺	165.84	34.66	-77
61	Acetic acid	C ₂ H ₅ O ₂ ⁺	13538.21	3069.98	-79
71	Terpene fragment/pentene	C ₅ H ⁺	346.85	14.17	-97
73	2-Methylpropanal/Butanone	C ₄ H ₈ O ⁺	1056.88	185.53	-84
75	Propanoic acid/methyl-acetate/acetol	C ₃ H ₇ O ₂ ⁺	287.63	53.77	-88
81	Methylpentene/terpene fragment	C ₆ H ₉ ⁺	5.49	3.19	-55
85	2(5H)-furanone/ methyl-butanal	C ₄ H ₅ O ₂ ⁺ / C ₅ H ₉ O ⁺	20.93	3.05	-82
89	Methylpropanoate/2-methylpropanoic acid/hydroxybutanone/acetoin	C ₄ H ₉ O ₂ ⁺	399.44	29.00	-95
91	2,3- Butanediol	C ₄ H ₁₁ O ₂ ⁺	4.17	0.76	-84
93	Terpene fragment/Toluene	C ₇ H ₉ ⁺	2.65	1.10	-15
95	Phenol/methylpyrazine	C ₆ H ₇ O ⁺	9.64	4.56	-80
96	Dimethyl-pyrrole/ethyl-pyrrole	C ₆ H ₁₀ N ⁺	0.66	0.32	-64
97	Furfural/dimethyl-furan	C ₅ H ₅ O ₂ ⁺	7.41	1.80	-77
98	Dimethyl-oxazole	C ₅ H ₈ ON ⁺	1.05	0.18	-85
99	Hexenal/methyl-pentenone ; Furfuryl alcohol/a-angelica lactone	C ₆ H ₁₁ O ⁺ / C ₅ H ₇ O ₂ ⁺	3.14	0.80	-78
101	Dihydro-2-methyl-3(2H)-furanone/2,3- pentanedione/pentanedione/methyl-tetrahydrofuranone	C ₅ H ₈ O ₂ ⁺	9.51	1.23	-88
103	2,3-methylbutanoic acid/2-hydroxy-3-pentanone/ 3-methylbutanoic acid	C ₅ H ₁₁ O ₂ ⁺	1.64	0.12	-95
104	Benzonitrile	C ₇ H ₆ N ⁺	118.57	7.50	-95
109	4-methylphenol/benzyl alcohol; 2-5 dimethylpyrazine	C ₇ H ₉ O ⁺ ; C ₆ H ₈ N ₂ ⁺	7.75	0.43	-69
110	Formil-methylpyrrole/acetylpyrrole/2-acetylpyrrole	C ₆ H ₈ ON ⁺	3.49	0.74	-46
112	Trimethyloxazole	C ₆ H ₁₀ ON ⁺	0.61	0.34	-95
113	Methylfurfuryl alcohol/ heptenone	C ₆ H ₉ O ₂ ⁺ / C ₇ H ₁₃ O ⁺	1.31	0.08	-91
121	2-ethenyl-6-methylpyrazine/ 6,7-dihydro-(5H)-cyclopentapyrazine	C ₇ H ₉ N ₂ ⁺	4.92	0.44	-90
123	2-phenylethanol / trimethylpyrazine	C ₈ H ₁₁ O ⁺ / C ₇ H ₁₁ N ₂ ⁺	5.62	0.61	-86
125	Guaiaicol/methyl-benzenediol/furyl acetone; Butylfuran/methylpropylfuran/octadienone/ trimethylcyclopentenone/alkylfurans/ E-2-octenal	C ₇ H ₉ O ₂ ⁺ ; C ₈ H ₁₃ O ⁺	2.96	0.41	-54
126	Acethyl-dimethylpyrrole/alkyloxazole	C ₇ H ₁₂ ON ⁺	0.79	0.39	-66
127	Maltol/methyl-furoate; 3-ethyl-1-2-cyclopentanedione	C ₆ H ₆ O ₃ H ⁺ ; C ₇ H ₁₀ O ₂ H ⁺	0.14	0.06	-59
129	Pentenylacetate/heptanedione; furaneol;octanal	C ₇ H ₁₃ O ₂ ⁺ ; C ₆ H ₆ O ₃ H ⁺ ; C ₈ H ₁₆ OH ⁺	0.62	0.26	-92
131	Ethyl valerate/heptanoic acid/C7 ester	C ₇ H ₁₅ O ₂ ⁺	2.10	0.28	-99
136	Butyl-pyridine/ethyl-propyl pyridine/ ethyl-dimethyl-pyrazine	C ₉ H ₁₄ N ⁺	14.36	0.20	-76
137	Various monoterpenes	C ₁₀ H ₁₇ ⁺	0.13	0.03	-86
138	Pyridinecarboxylic acid methyl ester	C ₇ H ₉ O ₂ N ⁺	9.98	1.75	-87
139	E,E-2,4-nonadienal/E-2-nonenal	C ₉ H ₁₅ O ⁺	1.04	0.20	-59
153	E/E-2,4-decadienal	C ₁₀ H ₁₇ O ⁺	0.89	0.30	-90
155	Linalool	C ₁₀ H ₁₈ O ⁺	1.40	0.12	-61
159	Nonanoic acid/C9 ester	C ₉ H ₁₉ O ₂ ⁺	0.24	0.08	-94

Significant relative differences between cocoa beans and chocolates are highlighted in blue (Mann-Whitney test, $p \leq 0.05$).**2, Supplementary materials).**

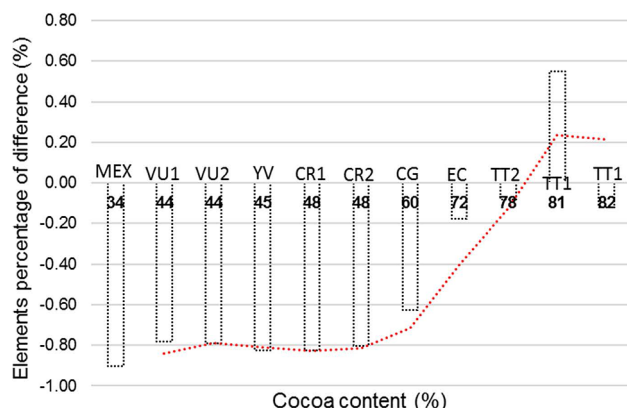
The chocolates showed a significantly lower content for ~72% of the 24 elements in comparison to the cocoa beans. These results are in agreement with previous studies which showed that finished and semi-finished products (chocolate and cocoa butter) had the lowest level of trace elements compared to the cocoa beans and cocoa shells and/or

cocoa nibs (Levi et al., 2014). Only the Na content was significantly higher in chocolates with low cocoa solid contents. A negative correlation between the chocolate cocoa content (Spearman's Rho = -0.81; $p = 0.002$) and Na content was measured. These results could be indicative of the influence of other ingredients on the Na content (the less cocoa content, the more extra ingredients added). Usually, most of the

Table 3

Macro-, micro-, and trace elements (mg/kg) determined in cocoa beans and corresponding chocolates: median and relative differences.

	Cocoa beans (mg/kg)	Chocolates (mg/kg)	Chocolates-Cocoa beans difference* (%)
	Median	Median	Median
Na	57.00	550.00	746
Cr	0.30	0.23	-6
La	0.025	0.000	-25
Ce	0.03	0.01	-33
Mo	0.27	0.16	-34
Cs	0.01	0.01	-35
Ga	0.01	0.00	-38
Ti	0.99	0.38	-43
Y	0.01	0.00	-50
Ba	5.40	1.60	-62
Rb	18.00	6.80	-67
Zn	56.00	17.00	-67
Sr	9.70	3.10	-73
Fe	92.00	11.00	-74
Mg	3200.00	780.00	-75
Al	38.00	2.40	-77
Co	0.48	0.11	-77
Cu	25.00	5.50	-79
Cd	0.56	0.11	-80
Mn	27.00	6.40	-80
Ni	4.70	0.99	-81
As	0.03	0.01	-82
Pb	0.06	0.00	-83
V	0.08	0.00	-100

*Significant relative differences between cocoa beans and chocolates are highlighted in blue (Mann-Whitney test, $p \leq 0.05$).**Fig. 3.** Relative differences in elemental composition between cocoa beans and corresponding chocolates. MEX: Mexico; VU1 Vanuatu1; VU2: Vanuatu 2; YV: Venezuela; CR1: Costa Rica1; CR2: Costa Rica2; CG: Congo; EC: Ecuador; TT2: Trinidad 2; TT1: Trinidad1.

essential trace metals are found in the cocoa bean core while the highest level of toxic trace metals is found in the cocoa bean shells (Pb, Bi and As) (Levi et al., 2014). In our study, fermented and dried beans

including the shell were analysed. Therefore, it is possible that some of the trace elements and metals are coming directly from the outer part of the beans. Nevertheless, the small amount of Pb and As found in the chocolate samples could result from small amounts of shells that are processed with the beans (Manton, 2010). The difference in element contents between cocoa bean and chocolate samples correlated well with the cocoa solids content (Spearman's $Rho = 0.81$; $p < 0.001$). This correlation was also found in previous studies (Bertoldi et al., 2016; Levi et al., 2014). Taking into account the median value of the percentage of difference from chocolates to cocoa beans, as presented in Fig. 3, it is evident that chocolates with a higher cocoa content differ less from their beans. Only Cd was not significantly correlated with the chocolate cocoa content (Spearman's $Rho = 0.41$; $p = 0.20$). This is in contrast with previous studies (Bertoldi et al., 2016), however, a significant correlation between Cd content in cocoa beans and corresponding chocolates has been measured (Spearman's Rho , $p \leq 0.05$) (Fig. 2, Supplementary materials). When correlating the elements contents of the cocoa beans and the chocolates, Fe and As contents in cocoa beans showed a significant correlation with the majority of the chocolate elements (Fig. 2, Supplementary materials) which makes them an interesting marker for linking beans and chocolates. According to literature, Fe and As are related to the geographical origin of cocoa beans (Bertoldi et al., 2016; De Araujo et al., 2017). Fe and As of cocoa

beans are significantly correlated with Fe and As of the corresponding chocolates. Other elements that showed this direct correlation are Cr, Mo, and, as stated before, Cd. Furthermore, the latter has been found to be related to the geographical origin of cocoa beans before (Bertoldi et al., 2016).

Elemental fingerprints showed to be mainly informative of the cocoa content. Fe, As, Cr, Mo, and Cd were potentially able to link cocoa beans to chocolates and could be used for tracing back characteristics of cocoa in chocolate products along the supply chain as they are informative of cocoa origin. However, it is important to take into account that the chemical elements such as Fe, As, Cr and Cd are potentially subject to environmental contamination and their concentration can change over time (Bertoldi et al., 2016).

3.3. Signatures of stable isotope ratios in cocoa beans and corresponding chocolates

The stable isotope ratio values of the various cocoa bean samples and chocolates varied considerably. They varied from -32.2 to -27.7 ‰ for $\delta^{13}\text{C}$, from 1.3 to 2.7 ‰ for $\delta^{15}\text{N}$, from -137.7 to -102.2 ‰ for $\delta^2\text{H}$, and from 22.2 to 25.5 ‰ for $\delta^{18}\text{O}$. For the chocolates, they varied from -30.1 to -27.2 ‰ for $\delta^{13}\text{C}$, from 2.1 to 3.5 ‰ for $\delta^{15}\text{N}$, from -117.9 to -105.8 ‰ for $\delta^2\text{H}$, and from 25.9 to 30.4 ‰ for $\delta^{18}\text{O}$ (Fig. 4; Table 3 and 4, Supplementary materials).

The mean value of each stable isotope ratio is higher in chocolates compared to the cocoa beans except for $\delta^2\text{H}$ as is shown in Fig. 4. The shift from beans to chocolates is most likely due to added ingredients. For instance, in the case of $\delta^{13}\text{C}$ the beans are included in the range of C3

plant within -33 and -22 ‰ (Spangenberg & Dionisi, 2001). However, the possible enrichment in chocolate can be related to the ingredient that is added, e.g. the addition of oxidizable compounds to cocoa butter as foreign fats. Evidence of this is that the chocolate $\delta^{13}\text{C}$ ratio is close to the one of cocoa butter equivalent made mainly from vegetable oils (-29.7 ‰ to -28.5 ‰) (Spangenberg & Dionisi, 2001). The $\delta^{13}\text{C}$ enrichment in chocolate samples can be also related to possible sugar added such as cane and/or corn sugar, derived from C4 plants (-23 ‰ to -6 ‰) (Bostic, Hagopian, & Jähren, 2018; Spangenberg & Dionisi, 2001). The influence of both fat and added sugars concentration on the carbon stable composition of other food products such as milk has been proven in previous studies (Bostic et al., 2018). The isotope ratios of cocoa beans and chocolates were significantly different for all the isotopes analysed except for $\delta^2\text{H}$ (Table 4).

The relative standard deviation (RSD) of $\delta^{13}\text{C}$ (RSD cocoa beans: -4 ‰; RSD chocolates: -4 ‰) and $\delta^{18}\text{O}$ (RSD cocoa beans: 5 ‰; RSD chocolates: 5 ‰) both for cocoa beans and chocolates is in the same order of magnitude, highlighting a stable dispersion of the values in relation to their means; on the other hand, this is not valid for $\delta^{15}\text{N}$ (RSD cocoa beans: 22 ‰; RSD chocolates: 13 ‰). In the latter case, the variation within the cocoa beans samples is mostly related to the N supplied from the soil/fertilizers that is assumed to differ according to the production area and annual temperature and precipitation. However, the large shift between cocoa beans and chocolates $\delta^{15}\text{N}$, supported also by the high relative differences (76 %) showed in Table 4. A trend within cocoa beans and corresponding chocolates can be noticed (Fig. 3, Supplementary materials). This is mostly valid for $\delta^{15}\text{N}$, $\delta^{13}\text{C}$, $\delta^{18}\text{O}$ and mainly for the chocolates with high cocoa content

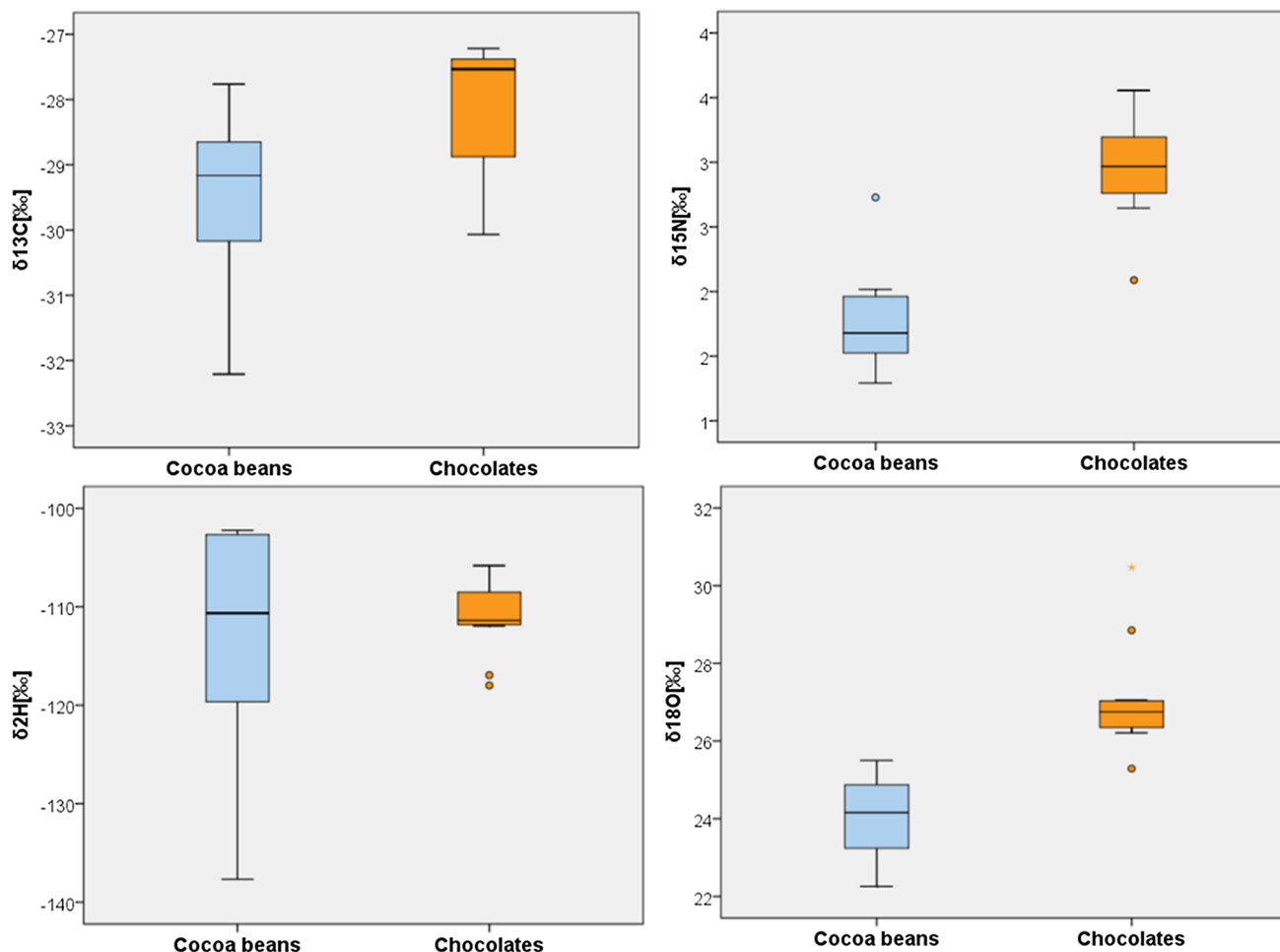


Fig. 4. Boxplots of $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, $\delta^2\text{H}$, $\delta^{18}\text{O}$ of cocoa beans and chocolates.

Table 4 $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, $\delta^2\text{H}$ and $\delta^{18}\text{O}$ stable isotope ratio values determined in cocoa beans and corresponding chocolates: median and relative differences.

	Cocoa Beans (‰)	Chocolates (‰)	Chocolates-Cocoa beans difference* (%)
	Median	Median	Median
$\delta^{13}\text{C}$ [‰]	-29.16	-27.53	-3
$\delta^{15}\text{N}$ [‰]	1.68	2.97	76
$\delta^2\text{H}$ [‰]	-110.64	-111.38	-3
$\delta^{18}\text{O}$ [‰]	24.16	26.75	12

*Significant relative differences between cocoa beans and chocolates are highlighted in blue (Mann-Whitney test, $p \leq 0.05$).

(60–80%). This set of samples does not show evident information related to the origin (Fig. 3, [Supplementary materials](#)). This could be related to the fact that in this study the whole beans (husk and nib) were analysed and previous studies showed that different part of the beans could have a different isotopic signature ([Diomande et al., 2015](#)). Furthermore, differences between cocoa beans from the same origin can be noticed (Fig. 3, [Supplementary materials](#)); this is particularly valid for the $\delta^2\text{H}$ of cocoa beans indicating a possible bean-specific differentiation related to the crop season and or weather condition. No significant correlations between stable isotope ratios of cocoa beans and chocolates were observed (Fig. 2, [Supplementary materials](#)). Therefore, stable isotope signatures appear less sufficient for tracing back characteristics of cocoa in chocolate products along the supply chain. To accurately estimate the isotopic signature of multi-ingredient food products the quantity of each ingredient should be available as shown by [Bostic, Palafox, Rottmueller, and Jähren \(2015\)](#).

3.4. Effect of cocoa contents on cocoa bean-chocolate relationships

The data of the various measurements were recalculated based on the cocoa contents of the chocolates ((measured value/cocoa content) * 100). Some minor changes in individual VOCs and elements

cocoa bean-chocolate correlations were observed (Figs. 4 and 5, [Supplementary materials](#)).

Before and after correction for the cocoa content, the relative difference in cumulative VOC intensities between cocoa beans and chocolate samples did not show a significant correlation with the cocoa content (Spearman's Rho = 0.42; $p = 0.19$). After considering the cocoa contents, most of the VOCs in the beans still present significant correlations with those in the chocolates, e.g. for masses 43, 61, 79, 80, 113, 123, and 127. However, some differences are evident compared to the non-corrected data thus indicating a possible dilution effect due to the differences in cocoa contents. Interestingly, other masses such as m/z 73 (methylpropanal), m/z 91 (2–3 butanediol) and m/z 109 (4-methylphenol/benzyl alcohol) show significant correlations with a higher number of cocoa bean masses compared to the non-corrected data (Fig. 1, [Supplementary materials](#)). After cocoa content correction, Fe and As show the same trend as previously. Cr, Fe, and Cd content of cocoa beans and corresponding chocolates are still significantly correlated (Spearman's Rho, $p \leq 0.05$).

Before and after correcting for the cocoa content, the stable isotope ratios did not show a significant correlation between cocoa beans and corresponding chocolates (Spearman's Rho, $p \leq 0.05$). Only $\delta^{13}\text{C}$, before the cocoa content correction, showed a significant negative

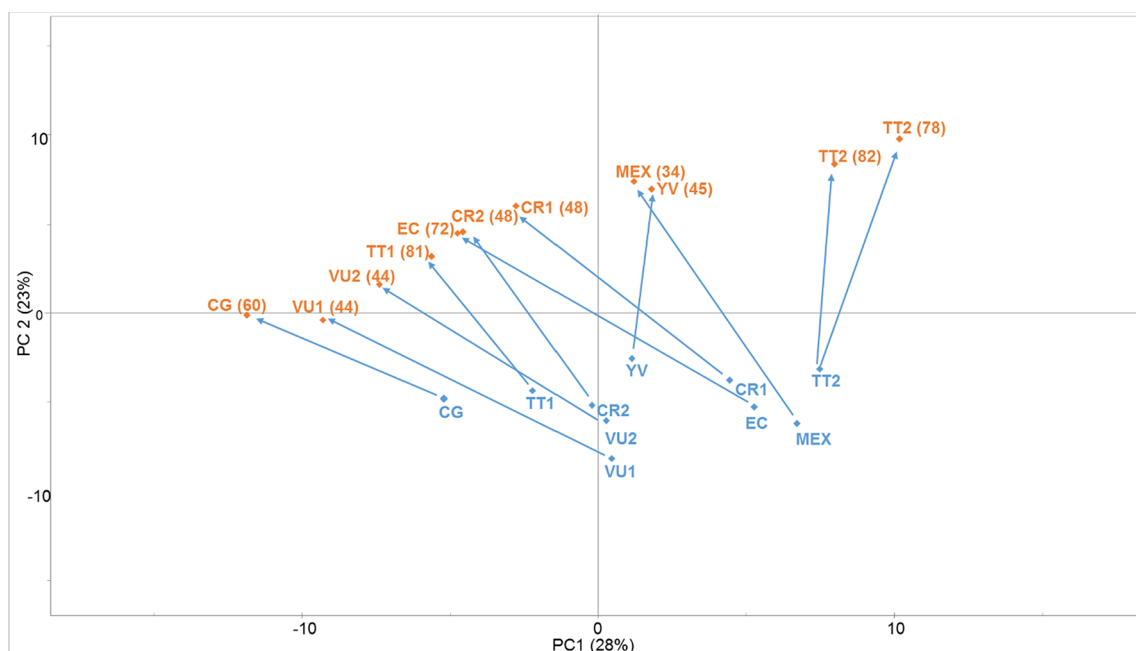


Fig. 5. First two dimensions of Principal Component Analysis plot of normalized and auto-scaled HS-PTR-MS data of cocoa beans from different geographical origins and their corresponding chocolates. The numbers in brackets indicate the cocoa content (% w/w). The arrows indicate the shift in VOCs from cocoa beans to chocolates. MEX: Mexico; VU1 Vanuatu1; VU2: Vanuatu 2; YV: Venezuela; CR1: Costa Rica1; CR2: Costa Rica2; CG: Congo; EC: Ecuador; TT2: Trinidad 2; TT1: Trinidad1. Blue colour indicates cocoa beans samples, orange colour indicates chocolate samples.

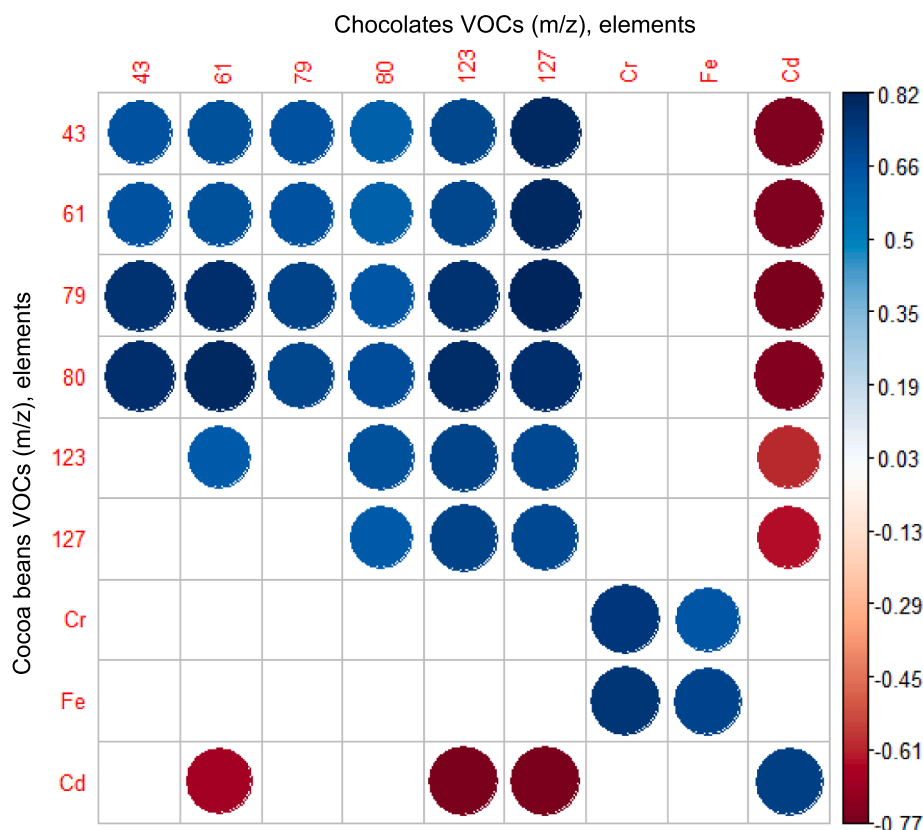


Fig. 6. VOCs and elements showing a significant correlation between cocoa beans and corresponding chocolates (Spearman's Rho, $p \leq 0.05$).

correlation with the cocoa content (Spearman's Rho = -0.75 ; $p < 0.001$). The negative correlation indicates that the trend in Fig. 6 might be partially due to the recipe (the less cocoa content, the more extra ingredients added).

3.5. Comparison of volatile, elemental and isotopic signatures

In order to link cocoa beans and chocolates, a marker should be able to significantly differentiate beans and chocolates but should also show a consistent correlation between them. Considering the masses showing significant differences in concentration between cocoa beans and chocolates, the VOCs showed a larger relative difference between cocoa beans and chocolates (125 masses, median value $\sim -85\%$) compared to the elemental fingerprints (median value $\sim -77\%$) and isotope ratios (median value $\sim 12\%$) (Tables 3 and 4). The VOC fingerprint showed more variance among the cocoa beans samples (RSD: median value $\sim 100\%$) compared to the chocolates (RSD median value $\sim 52\%$). Since cocoa beans are a natural product high variation is expected. The elemental composition highlights more variance among the chocolate samples (RSD: median value $\sim 72\%$) compared to the cocoa beans (RSD: median value $\sim 37\%$); considering the behaviour of Na (paragraph 5.3.2), this could be related to possible extra ingredients added during processing. On the other hand, the relative variance of the isotope ratio values was similar for cocoa bean samples (RSD: median value $\sim 5\%$) and chocolate samples (RSD: median value $\sim 5\%$). These results highlight that the magnitude of dissimilarity between cocoa beans and chocolate samples tends to be higher for the VOCs and elemental composition compared to the isotopic ratios. To link cocoa beans and chocolates, a consistent correlation between markers and beans should also exist. Table 5 highlights that VOCs measured by using HS-PTR-MS can distinguish bean and chocolate samples according to the origin and show a consistent shift from beans to chocolates. This can be better visualized in the PCA in Fig. 5 built with HS-PTR-MS results.

Table 5

Evaluation of differences between cocoa beans and chocolates for volatiles, elements, and stable isotope data.

	% Difference	Consistent shift	Cocoa content influence
VOCs	+ (~ 85)	+	-
Elements	\pm (~ 77)	\pm	+
Stable isotope	- (~ 12)	-	\pm

Prior to PCA, the data were auto-scaled and normalized to balance the differences in VOC concentration between cocoa beans and chocolates.

The variability within samples is mainly represented in PC1, while PC2 differentiates raw materials and the finished products. Along with PC1, connection points within the cocoa beans and the corresponding chocolates can be distinguished. The shift of the volatile signatures of the cocoa beans to corresponding chocolates is represented by the direction of the arrows. The latter underline a link between the cocoa beans and the corresponding chocolates which is supported by the significant correlations between the VOCs of cocoa beans and chocolates (Fig. 5; Fig. 1, Supplementary materials). Considering the possible connection points on PC1, a significant correlation (Pearson's correlation coefficient = 0.65) between cocoa beans and chocolates has been determined using the score values of each sample on PC1. This further highlights the consistent shift of VOCs fingerprints from cocoa beans to chocolates. The trend in the PCA in Fig. 5 is not visible for the elemental composition and the stable isotope ratio data nor exists a significant correlation between cocoa beans and chocolates (Pearson's correlation coefficient, $p \geq 0.05$).

When correcting for the cocoa content, the cocoa beans-chocolates scores correlation is still significant for VOCs (Pearson's correlation coefficient = 0.65). After the cocoa content correction, the correlations for the elemental data do not change. The correlations for the stable isotope ratios improve (Pearson's correlation coefficient = 0.37).

However, the correlation is weaker than the correlation for the VOCs.

Fig. 6 presents the selection of VOCs, elements that show significant correlations between beans and chocolates, both with and without cocoa content correction. These could be used as markers indicative of cocoa beans traits that significantly persist in the final product. Within the VOC group, it is possible to highlight compounds retained along with the production from both raw material and primary processing at the origin, while within the selected elements potential toxic elements appear informative of the geographical origin of the cocoa beans. m/z 79 and 123, together with Cr, Fe and Cd showed the highest direct correlation between cocoa beans and chocolates (Spearman's $Rho > 0.70$). Within the elements, Cd in particular, was able to statistically differentiate cocoa beans and chocolates (Mann-Whitney test, $p \leq 0.05$). At the same time, Cd shows a significant correlation between its contents in bean and corresponding chocolates (Spearman's Rho , $p \leq 0.05$) independently from the cocoa content. Apart from its toxicity, Cd could be informative of the soil at the growing location of the cocoa beans (De Araujo et al., 2017). The traits presenting strong correlations between beans and chocolates could be used to track and trace beans along the supply chain and support the consideration of applying the concept of 'terroir' to chocolate as an indication of processing location and a growing environment (Engeseth & Pangan, 2018; Sukha, Butler, Comissioning, & Umaharan, 2014).

4. Conclusions

When looking for intrinsic information carried from cocoa beans to chocolates, the volatile signatures generated by HS-PTR-MS revealed a constant shift between raw materials and the finished products as well as a high magnitude of dissimilarity between samples of different origins. The group of key VOC markers are represented by acetic acid (m/z 61), benzene (m/z 79), pyridine (m/z 80), 2-phenylethanol (m/z 123) and maltol (m/z 127). On the other hand, the elemental composition and isotopic data generally carried the information related to the cocoa content and ingredients added. Within the elemental group, potential geographical markers are Fe, Cr and Cd. However, we must keep in mind that their concentrations are potentially susceptible to environmental contamination. According to univariate and multivariate correlations, VOCs appear to be establishing the most robust link between cocoa beans and corresponding chocolates. This provides potential use of track and trace of cocoa beans from farm to chocolates. These results support the consideration of applying the concept of 'terroir' to chocolate and provides a scientific basis for cacao quality certification programmes.

CRedit authorship contribution statement

Valentina Acierno: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing - original draft. **Leon de Jonge:** Data curation, Formal analysis, Methodology, Writing - review & editing. **Saskia van Ruth:** Conceptualization, Funding acquisition, Investigation, Project administration, Resources, Supervision, Writing - review & editing.

Declaration of Competing Interest

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

Acknowledgements

This study has been funded by PIMMS (Proton Ionization Molecular Mass Spectrometry) ITN which is supported by the European Commission's 7th Framework Program under Grant Agreement Number

287382. The authors wish to thank John Verbunt for running ICP-MS measurements and GNS Stable Isotope Laboratory (GNS Science, Gracefield, New Zealand) for ^{18}O and 2H determination of cocoa beans and chocolate samples. The authors wish to thank prof. dr. Patricia Regal López for comments that greatly improved the manuscript.

Appendix A. Supplementary material

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.foodres.2020.109212>.

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