Talanta 195 (2019) 676–682

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Which cocoa bean traits persist when eating chocolate? Real-time nosespace analysis by PTR-QiToF-MS

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ARTICLE INFO

Keywords:
Chocolate
Cocoa beans origin
Nosespace
PTR-QiToF-MS

ABSTRACT

More consumers have become aware of the existence of different cocoa genotypes and their origins, which resulted in a growing market of premium chocolates with single-origin beans. The question is whether traits of cocoa botanical and geographical origins still persist in the end product, especially when it is consumed. By analysing the concentrations of volatile organic compounds (VOCs) in the nose of subjects over time while they are eating, new insights about aroma release can be gained. In the current study, in vivo release of VOCs during consumption of dark chocolates with different botanical and geographical origins was examined. Proton Transfer Reaction-Quadrupole interface Time of Flight- Mass Spectrometry (PTR-Qi ToF- MS) was applied to analyse nosespace VOC profiles of 10 subjects while they were eating 10 different chocolates manufactured with beans of different botanical origins (Criollo-Forastero-Trinitario) and geographical origins (Africa-South America-Asia). The headspace of the chocolates were also analysed for comparison. Cocoa botanical information appeared to affect the nosespace profiles more than geographical information. The subjects varied considerable in their VOC release, and inter-individual differences were larger than cocoa beans differences. Nevertheless, the botanical origin was consistently reflected in the nosespace profile during eating. It was clearly possible to distinguish Criollo chocolates from the nosespace profiles despite inter-individual differences.

1. Introduction

To be called “chocolate”, a product must contain cocoa [1]. This statement is relevant both for the quality and sensory perception of chocolate. The complex flavour of cocoa beans depends on genotype/origin/processing and is generated by several processes, such as fermentation, drying, and roasting. After all processing steps, the beans are mixed with other ingredients to produce chocolate with its peculiar flavour. This raises the question about which of the flavour characteristics of the beans persist in the chocolate. Answering this question is important in order to assure chocolate quality and consumer satisfaction.

More consumers become aware of the existence of different cocoa genotypes and their origins. This is linked to the growing market of premium chocolates and the trend of many companies to launch new chocolates with single-origin bean, organic and fair-trade chocolate [2]. For this reason, the analytical verification of which raw material characteristics such as botanical and geographical origin persist in the final product is significant in ensuring high quality product.

Several studies focused on the volatile profiles of different cocoa genotypes [3] and different origins [4]. The volatile profiles of cocoa products such as cocoa liquor [5] and chocolate [6,7] were also evaluated according to the provenances of the raw materials. However, there are no studies focused on the persistence of cocoa beans botanical and/ or geographical origin flavour in the aroma released when eating chocolates; only a study optimised an organoleptic assessment protocol to describe and quantify different flavour attributes of cocoa liquors made from Ghana and Trinitario beans [8]. For this analysis a panel test was involved. An alternative approach is to use analytical techniques based on real time measurements, which can be applied for the determination of volatile organic compounds (VOCs) released during food consumption. By analysing concentration of VOCs over time, new insights about aroma release can be gained. Proton Transfer Reaction Mass Spectrometry (PTR-MS) has been used to quantify in-nose and in-mouth volatile flavour release from different foods. Several food products were tested such as custard [9], apple [10] and cereal bars [11]. Moreover, milk and aqueous sugar solution [12], flavoured vodka [13], lipid solutions [14], coffee [15,16], and aromatized air [17] were investigated. There is no literature about in-nose and/or in-mouth volatile flavour released from chocolate.

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https://doi.org/10.1016/j.talanta.2018.11.100
Received 30 July 2018; Received in revised form 25 November 2018; Accepted 28 November 2018
Available online 29 November 2018

The aim of this study was to assess the influence of the botanical and/or geographical origin of cocoa beans on the volatiles compounds released in-nose when eating dark chocolates. Ten identical chocolates manufactured with beans from different botanical and geographical origins were evaluated and the nosespace (NS) profiles - when eating those chocolates - were measured using PTR- Quadrupole interface Time of Flight (Qi ToF)-MS.

2. Materials and methods

2.1. Materials and subjects

2.1.1. Samples

Ten dark chocolates (75% cocoa) of a single manufacturer were analysed. The chocolate samples were manufactured with beans belonging to three different varieties (Criollo, Forastero, Trinitario) and from ten different single origins (Ghana, Madagascar, Sao Tome, Tanzania, Cuba, Brazil, Trinidad, Venezuela, Papua New Guinea and Java). Regarding the botanical origin the samples were grouped in Criollo (Madagascar, Java), Forastero (Brazil, Ghana, Sao Tome, Tanzania) and Trinitario chocolates (Cuba, Papua New Guinea, Trinidad, Venezuela). While for the geographical origin the chocolates were split in three clusters according to the continent of provenance Africa (Ghana, Madagascar, Sao Tome, Tanzania), South America (Cuba, Brazil, Trinidad, Venezuela) and Asia (Papua New Guinea, Java). The set of samples was used to carry out both headspace and nosespace measurements.

2.1.2. Subjects

The ten samples were presented to a panel of ten subjects (five females and five males, age 25–55 yrs.). Subjects were volunteers recruited from Wageningen University and Research and had no self-reported sensory issues. They were asked not to eat, drink or use persistent products at least 2 h before the session. Subjects attended six sessions on six different days. Each session lasted for about 25 min: 90 s of measurement followed by 5 min break before the next measurement. For each session a maximum of four chocolates were tested.

2.2. Methods

2.2.1. PTR-QiTOF-MS Instrumental conditions and raw data processing

All measurements were performed using a PTR-QiToF-MS (Ionicon Analytik G.m.b.H., Innsbruck, Austria) to investigate the VOCs. Ionisation was carried out under drift tube voltage 999 ± 1 V, drift pressure of 3.80 mbar, temperature 60 °C, and E/N value of 133 Td. Data acquisition was carried out at 1 spectrum per second. For each sample a mass range between 0.00 and 512.15 Th was measured using a dwell time of 0.1 s mass^{-1}. Sampling was performed at a flow rate of 60 ± 1 sccm for headspace and nosespace analysis.

2.2.1.1. Data processing. Mass scale calibration and peak extraction were performed using PTRwid [18].

2.2.2. Headspace analysis

Chocolate samples were powdered using an electrical grater and were kept at 4 °C prior to analysis. For the measurements, 3 g of ground chocolate were weighed into clean and odourless flasks of 250 ml. The closed flasks were placed in a water bath at 30 °C for 30 min to equilibrate samples with their headspace. For all samples, four repeated measurements were carried out. Data acquisition was performed over 60 spectra resulting in an analysis time of 60 s per sample. A blank (empty flask) was analysed prior to each sample. So eventually 10 samples x 4 replicates x 60 spectra = 2400 spectra were acquired. The first 30 scans per sample were averaged because the intensity of the signal was constant. The values obtained for the blank were subtracted from each sample.

2.2.2.1. Data treatment and analysis. A concentration threshold of 1 pbbv was set for the data set, and the mass peaks with lower concentrations were filtered out. Peaks attributed to ^{13}C isotopologues and water clusters, were considered to be redundant and therefore discarded.

The final data set was analysed using two-way ANOVA to understand whether there was an interaction between botanical and geographical origin of chocolate on the VOCs concentration. R 3.3.3 (the R Foundation for Statistical Computing, Vienna, Austria) was used to perform the analysis.

2.2.3. Nosespace analysis

The ten chocolates were presented in independent duplicates to the ten subjects. The pieces of chocolate tested had the same dimensions (2 cm × 2 cm) and weight (3 g). The order of chocolates and subjects was fully randomized. A maximum of four chocolates per day was given to the subjects. Sampling was carried out by applying an ergonomic nosepiece in glass to the nose of the subjects. The glass nosepiece was connected to the PTR-QiToF-MS by a PEEK tube (inner diameter = 0.5 mm); the nosepiece was changed for every test.

After positioning the nosepiece in the nostrils, subjects were asked to breath normally through the nose (mouth closed) for 10 s. Then subjects received the sample. As soon as subjects put the sample in the mouth, they were allowed to masticate and swallow freely. The mastication period and the first swallowing were recorded for each session. During all evaluations, subjects had to keep their mouth closed. In total, the NS measurement lasted 90 s. Sampling was carried out in duplicate. So, eventually 10 chocolates x 10 subjects x 2 replicate measurements x 90 spectra = 18,000 spectra were acquired.

2.2.4. Data treatment

For each compound 200 release curves, corresponding to the NS sessions, were acquired.

Within each release curve three time windows were selected: breathing session (1–10 s), mastication session (11 s to first swallowing point) and post-swallowing session (first swallowing point plus 20 s). For each window, the part of the curve selected were superposed and averaged to generate an average release curve. Differences between mastication and swallowing were expected as has been shown in a previous study [13] for this reason were analysed as a separate set.

According to Romano et al. [16], in order to select mastication and post swallowing relevant peaks, the breathing part was compared with the mastication and post-swallowing part by means of a Mann–Whitney test and p-values were corrected using the Rate of False Discovery (RDF) [16,19]. Mass peaks and corresponding release curves associated with a corrected p-value lower than 0.05 were retained for further analysis. After this selection step, peaks attributed to ^{13}C isotopologues and water clusters, were considered to be redundant and therefore discarded. From each selected peak, the baseline (obtained by averaging the first 10 cycles) was subtracted. A non-parametric two-way Friedman test comparing each compound released curve was used to investigate the interaction of botanical and geographical origin on the NS mastication and post-swallowing profiles.

For each selected peak five descriptors for mastication and post-swallowing were calculated: time-independent parameters, i.e. the maximum intensity (max), the area under the curve (area), and the average of the release curve (avg), as well as time-dependent parameters such as the time to reach the maximum (tmax), and the slope of the first descending section of the curve (slope) [20,21].

Non-parametric Kruskall Wallis tests were applied to the time-independent (max, area, avg) and time-dependent parameters (tmax, slope), in order to investigate statistical differences between the NS profile released during mastication and post-swallowing of the dark chocolates. p-Values were corrected using the Rate of False Discovery (RDF). For analysing the specific sample pairs for stochastic dominance in post hoc testing, pairwise Mann-Whitney tests was performed.

Non-parametric tests were preferred for the NS session analysis because when using human data there is a higher probability of not satisfying the ANOVA assumptions. Shapiro Wilk tests were conducted to examine normality within the groups. Data analysis and statistical analysis were performed using IBM SPSS Statistics 23.0 (IBM Corp., Armonk, NY, USA) for one-way non-parametric tests, R 3.3.3 (the R Foundation for Statistical Computing, Vienna, Austria) for mastication and post-swallowing descriptors extraction and non-parametric tests-Friedman Two-Way ANOVA, and Pirouette 4.0 Software (Infometrix, Seattle, WA, USA) for multivariate analysis (Principal Component analysis, PCA).

3. Results and discussion

3.1. Headspace analysis

A total of 695 mass peaks were measured in the range 19.081 – 505.086 *m/z*. After setting the 1 ppb threshold and having discarded 20 peaks related to ^{13}C isotopologues and water clusters, 205 peaks were retained.

The sum peaks concentration measured in the chocolate headspace was higher in Forastero samples (104.358 ± 32.695 ppbv) immediately followed by the Trinitario chocolates (102.391 ± 48.571 ppbv). Criollo showed the lowest headspace concentration (97.355 ± 67.651 ppbv). High concentrations for Forastero samples were found also in previous studies [5]. Regarding the geographical origin, the South American (112.068 ± 45.860 ppbv) and African (111.490 ± 45.860 ppbv) chocolates scored higher values compared to the Asian samples (63.737 ± 20.109 ppbv). General prime ions with *m/z* 33.033, 43.017, and 61.028 tentatively identified as methanol, fragment of diverse origin, and acetic acid, respectively, were observed in all samples (Table 1, supplementary material). These masses were considered predominant also in a previous study focused on the volatile compositions of chocolates using PTR-MS [22]. Mass 61.028 did not show significant differences related to botanical or geographical origin. However, its concentration was highest in Criollo samples, which underpins the acidic tones of Criollo beans [22–24].

From our previous studies [22], an interaction between geographical origin, botanical origin and brand is expected. To investigate the interaction between botanical and geographical origin, the dataset was submitted to two-way ANOVA tests ($p < 0.05$).

A subset of 56 significantly different peaks for geographical origin (Africa, Asia, South America) and 20 for botanical origin was obtained. Eleven masses were affected by an interaction of both parameters. Thus, a higher number of mass peaks were significant in differentiating the samples according to the geographical origin compared to the botanical one. A sum formula was applied to 48 of those masses, and 30 were tentatively identified based on literature knowledge of their occurrence in chocolate [4,22] (Table 1, supplementary material).

3.2. Nosespace analysis

3.2.1. PTR-ToF-MS spectra

The nosespace measurement generated 695 mass peaks over a total of 18,000 mass spectra, distributed over 200 sessions overall (90 spectra per session). Fig. 1 illustrates a typical NS release curve; the curve of *m/z* 87.080, methylbutanal, an important contributor to the chocolate aroma [3]. This plot supports the decision of analysing mastication and post-swallowing separately. Differences exist between intensities according to the mastication and post-swallowing phases. This trend can be supported by previous studies that demonstrated that the flavour release changes according to different phenomena occurring in mouth such as salivation, mastication, swallowing [25].

3.2.2. Impact of cocoa bean traits in general

The NS profiles resulted into 311 mass peaks for the mastication and 441 for the post-swallowing part. The data were not normally distributed (Shapiro-Wilk test < 0.05) and the two-way non parametric Friedman-tests were applied. The results showed that both in mastication and post-swallowing a higher number of masses showed significant differences for botanical origin compared to the geographical origin (Table 2 and 3, supplementary material). Taking into account this information, attention was focused on the botanical information of cocoa beans in the NS profile.

3.2.3. Influence of cocoa beans' botanical origin on NS profiles

3.2.3.1. Univariate characterization. The botanical origin of the beans in the chocolates affected the VOCs concentration of NS profiles during mastication and post-swallowing for the time-independent parameters (*area, avg, max*) (Kruskall Wallis test and Mann-Whitney post- hoc test). The data were not normally distributed (Shapiro-Wilk test < 0.05) and significance of differences between the samples was evaluated by non-parametric tests (Kruskall Wallis test and Mann-Whitney post- hoc test). Significant differences ($p < 0.05$) were found for 30 mass peaks for the mastication phase and 39 mass peaks for the post-swallowing phase. With help of data reported in literature [3,4,22,26–30] a tentative identification was proposed for all the significant mass peaks (46 tentative identifications). These masses can be linked to chemical classes known to be responsible for the typical chocolate aroma, including Maillard reaction products such as aldehydes and pyrazine and other compounds as pyrroles, furans, pyridines, oxazoles [4,16]. Table 4 (supplementary material) reports all mass peaks and parameters showing significant differences according to the botanical origins of the cocoa beans used for the manufacture of the chocolates.

Differences were found for parameters related to area under the curve (area, occurring 24 times for mastication and 34 for post-swallowing), maximum intensity (max, 5 times for mastication and 19 for post-swallowing), mean intensity (avg, 8 times for mastication and 12 for post-swallowing). The influence of the time-independent parameters has been shown also in a previous study about coffee NS measurements [15]. On the other hand, time-related parameters (i.e. slope, tmax) did not show significant differences; also in coffee NS they were scarcely represented [15].

As expected from Fig. 1, the aforementioned parameters were always higher in the post-swallowing session; the majority of the significant differences were found during the post-swallowing measurement as well (Table 4, supplementary material). An increase of the volatile released during the post-swallowing phase, when eating a fat matrix, was also found in another study measuring the flavour released from a lipid emulsion [14].

3.2.3.1.1. Mastication phase. During mastication the main NS profile differences are observed for the chocolates made from Criollo beans ('Criollo chocolates') versus the rest of the chocolates, and for chocolates produced from Forastero beans ('Forastero chocolates') versus those with Trinitario beans ('Trinitario chocolates'). Only *m/z* 93.067, tentatively identified as toluene/ terpene fragment was able to differentiate the three botanical origin at the same time (area). About two third of the compounds (Table 4, supplementary material) exhibit statistically different concentrations for 'Forastero chocolates' compared to 'Trinitario chocolates', ~20% show significant differences between 'Criollo chocolates' and the rest of the samples, and ca. 10% distinguish 'Trinitario chocolates' from the other samples.

Lower mass (lighter) compounds tend to characterise the differences between 'Criollo chocolates' from the others whereas those distinguishing 'Forastero and Trinitario chocolates' tend to be heavier. The group of compounds showing significant differences for the NS of 'Criollo chocolates' during mastication comprises mainly esters and acids. This was expected as according to the literature Criollo beans are characterized by acidic tones [23]. On the other hand, within the group of compounds relevant for the NS of 'Forastero chocolates' there are aldehydes, alcohols, furans, ketones, and pyrroles. The higher concentrations of these aroma compounds in the NS profiles of 'Forastero

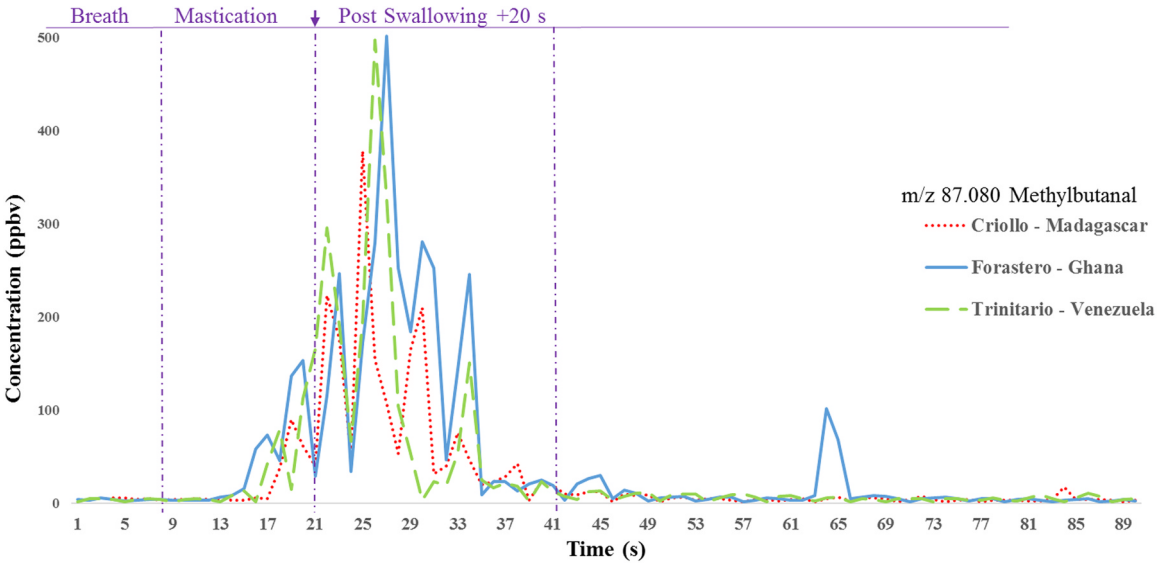

Fig. 1. Release curve of mass 87.080, related to a single person when eating 3 different chocolates (swallowing points were aligned at 24 s for curves visualization).

chocolates' can be attributed to different conditions of these beans fermentation compared to the Criollo beans. Usually, Forastero beans are more fermented; this maximizes the release of flavour precursors which subsequently increases the aroma compounds concentrations in the final product [4,5,24] and consequently in the NS profile.

The mean values for max, avg and area (Table 4,supplementary material) indicate that Forastero chocolate showed generally higher concentrations compared to the other samples. The high concentrations of VOCs of 'Forastero chocolates' in the NS is in alignment with the high VOC concentrations in the headspace of the same chocolates.

3.2.3.1.2. Post-swallowing phase. During the post-swallowing session, the situation changes. More compounds show significant botanical differences and the number of compounds exhibiting significant differences between 'Criollo chocolates' and the others increases. About 44% of the compounds show significant differences between 'Criollo chocolates' and the other chocolates. The number of mass peaks significantly differentiating between 'Forastero and Trinitario chocolates' is decreased during the post-swallowing phase to approximately 15%. Some of the peaks which distinguished 'Forastero chocolates' during the mastication phase now become in the post-swallowing phase significant in differentiating 'Trinitario chocolates' from the other samples (~25%) or 'Criollo chocolates' from the rest. During swallowing also the number of mass peaks differentiating 'Criollo chocolates' from 'Trinitario chocolate' increase (~15%).

This change in relevance of compounds can be related to the different way in which compounds are retained in the chocolates. For instance, a different structure of the chocolate matrix, such as cocoa butter hardness and lipids composition, depending on the cocoa beans provenance, is expected [31]; this could influence the release of the compounds.

According to Mann Whitney results, *m/z* 112.077 (Trimethyl-oxazole/2-acetyl-1-pyrroline), 123.092 (Trimethylpyrazine/2-ethyl-3-methylpyrazine) and 131.071 (Acetyloxy-butanone/ethanrdiol diacetate/oxopropoxy-propanone/ethyl-oxobutanoate) differentiated chocolate from the three different botanical origins simultaneously. Mass 112.077 and 123.092 are related to oxazoles and pyrazine. These compounds are generated during the cocoa beans roasting through Maillard browning [32] and they can be an indication of different roasting effects as a result of the cocoa beans botanical origins.

During post-swallowing the majority of the compounds show high concentrations for the 'Criollo chocolates'. Some of them do not so in the mastication phase (Table 4, supplementary material). They comprise ester/acids, oxazoles and pyrazines. The latter is known to be a more persistent group of compounds [16] and, most probably, for this reason is more relevant during post- swallowing phase.

Some of the other compounds showing significant differences due to origin during the mastication phase are indeed not showing these differences post-swallowing. They include for example alcohols, carbonyls, aldehydes, pyridines and acids. For instance, it is expected that aldehydes are released faster as they interact less with the mucosa [15]; this could affect their influence on the NS during swallowing.

These results emphasise that difference in the botanical origin of cocoa beans used to produce the chocolate influences the NS profile released by different subjects when eating chocolates. Especially chocolates manufactured from Criollo cocoa beans stand out in the post-swallowing phase.

3.2.3.2. Multivariate characterization. The *area* values of the peaks measured during the post-swallowing phase of the NS analyses were subjected to PCA; the results of the mean-centred and normalized data are displayed in Fig. 2. The PCA highlights a cluster of NS area data of 'Criollo chocolates'. Areas related to 'Forastero and Trinitario chocolates' are not well distinguished, supporting the univariate trend discussed in the previous paragraphs. PCAs based on *area*, *average* and *maximum* values, both for mastication and post swallowing phases, clearly highlighted differences in VOCs of NS profiles released when eating chocolate produced with Criollo (results not shown). A geographical trend in the distribution of chocolates is visible when analysing the cluster related to the botanical origin separately. Specifically for Criollo and Trinitario chocolate, it is possible to notice the NS of chocolate made with beans from the same single origin close together (Supplementary material Figs. 1 and 2).

3.2.4. Influence of subject on NS profiles

Evaluation of the plot of the PCA of the *area* values (Fig. 2) reveals subject clusters in the part of the plot corresponding to the mixed area of 'Forastero and Trinitario chocolates'. This indicates that for these samples consistent inter-individual patterns exist. The consistent patterns for subjects are probably related to other inter-individual parameters such as genetic factors, and physiological factors, or naso-oropharyngeal volumes, saliva composition, mucus composition, and breath flow [15,17].

To better understand the influence of each subject, Fig. 3 shows the release profiles of each participant, expressed as mean integrated release cumulated for all masses for the mastication and post-swallowing

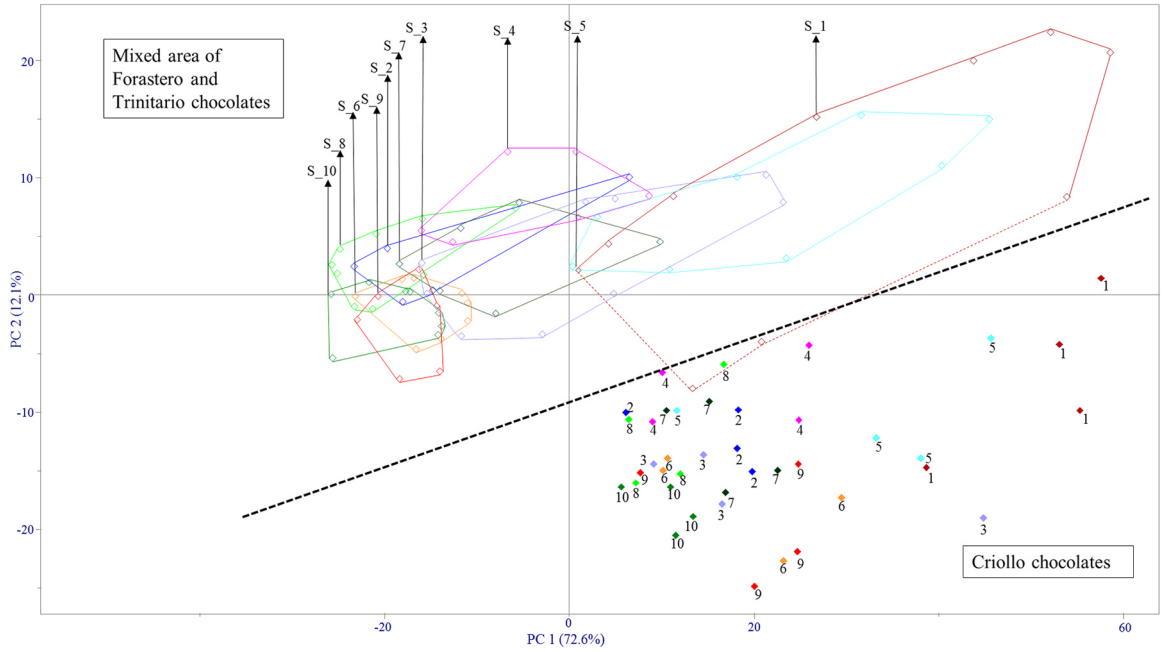

Fig. 2. PCA scores plot of the mean-centred and normalized NS (*area*) data of the post-swallowing phase collected from 10 subjects when eating 10 different chocolates (replicates are shown). Plot showing Criollo chocolate clusters in the lower part and clusters of subjects in the upper part. The lines connect the external points of the clusters related to each subject from S_1 to S_10. S: Subject. The chocolates highlighted are produced from Criollo beans while the numbers from 1 to 10 indicate the different subjects. Empty symbols are related to Forastero and Trinitario chocolates.

phases (area) for each cocoa botanical origin. Looking at the bar plot (Fig. 3), it is possible to notice subjects characterized by low (S_1-S_5) and high (S_6-S_10) NS post-swallowing release.

The PCA in Fig. 2 highlights that the distribution of the subjects is most probably concentration related. Subjects characterized by higher cumulative area (S_6, _8, _9,_10) overlapped in the left hand side of the plot. Subject 7, showing a high cumulative area, occupies a bigger area in the plot compare to the other high releasers. Controversial is the trend for the low releasers; Subject 2 overlaps with the high releaser occupying a relative small area compare to Subject 3 and 4. Subject 1, and_5 follow a different distribution compared to the rest. Apparently, the people characterized by a low cumulative area show more variation and a larger spreading of the chocolates compared to the subjects with a higher cumulative area (Fig. 2).

3.2.5. *Interactions between traits of cocoa beans and subjects*

Cumulative release shows a factor 3 difference between S1 and S10 (Fig. 3), but there appears also a sample effect. Larger differences are observed between 'Criollo chocolates' and others after swallowing; this trend is valid almost for each subject. The magnitude of difference between Criollo chocolate and the others is approximately a factor 2.

This confirms that the subject effect is probably larger than the bean influence on NS profile release when eating chocolate, emphasizing the distribution of subjects over the first dimension, and the separation of 'Criollo chocolates' and others in the second dimension in the PCA (Fig. 2). Moreover analysing PCA related to each subject, it is possible to notice that for certain subjects there is a good separation within the three chocolate samples. This is more evident for subject 1 and 5 (Supplementary material Figs. 3 and 4). Probably, for this reason they show a different distribution compared to the rest of the participants (Fig. 2). Despite the influence of the subjects, the chocolate-cluster in Fig. 2 and the plot in Fig. 3 can still be distinguished. In fact, it is important to highlight the consistent differences between NS profile when eating chocolate made with Criollo bean and the rest.

3.3. *Linking headspace and nosespace analysis*

In the headspace of the chocolates the geographical information was

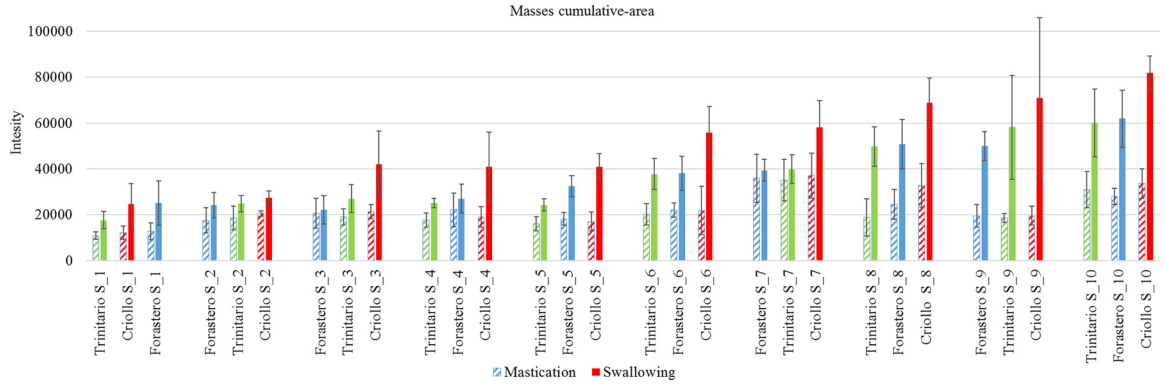

Fig. 3. Plot showing the cumulative release profiles of each subject (S), expressed as average of the cumulative areas of NS chocolates belonging to the same botanical cluster (Criollo, Forastero, Trinitario). The cumulative area includes all the masses. Mastication and post-swallowing comparison is shown. The chocolate areas are ordered according to the highest intensity per subject.

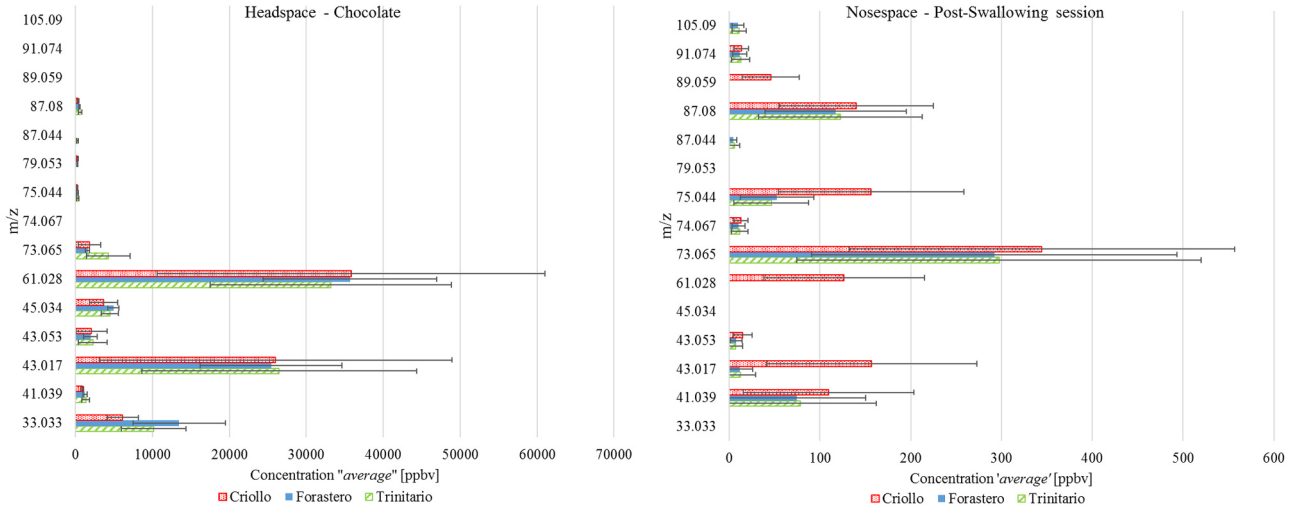

Fig. 4. Botanical origin comparison based on the ten highest compounds of the average concentration of all headspace and NS measurments. Left figure: Headspace 10 highest compounds for Criollo, Foratsero, Trinitario; Right figure: NS 10 highest compounds for Criollo, Forastero, Trinitario.

more dominant than the botanical traits (Section 3.1), whereas the order was reversed for the NS analyses (Section 3.2.2). The headspace concentrations of the VOCs showed highest total intensity for 'Forastero chocolates' (Section 3.1), even though some of the major compounds were present in higher concentrations in the headspace of the 'Criollo chocolates' (Fig. 4). The latter is reversed in the NS measurement (Fig. 4). This high release is in accordance with a previous study in which Criollo chocolate measured with PTR-MS showed higher concentration compared to the other botanical origins [22]. Dissimilarities in flavour delivery will depend on the affinity of the compounds for the food matrix. As mentioned before, taking into account the structure of the chocolate matrix according to the cocoa beans used, differences between the samples are expected [31]; this explain the variation in the NS concentration. In a multiphase system, such as chocolate, aroma compounds are partitioned in the fat phase that can influence the release of the volatile compounds according to their lipophilicity. For these reasons, differences in the cocoa butter hardness and lipids composition can cause differences in the release of VOCs, especially when the matrix is changing due to salivation and mastication. Looking at Fig. 4, comparing the 10 highest compounds in the headspace and NS of each botanical origin, it is possible to notice that for chocolate the VOCs release is almost homogeneous between the different botanical samples while in the NS the differences clearly increase.

Fig. 4 underlines the VOCs profile differences between headspace and NS. Whereas for the chocolates' headspace mass 61.028 is the one highest in concentration, in the NS mass 73.065 (methylpropanal) is predominant for all the chocolates together with mass 87.080 (methlbutanal), although overall concentrations are much lower due to dilution by the breath air flow. These compounds are typical contributors to the chocolate flavour. Masses 74.067, 91.074, 105.09 (methional) were not included in the chocolates' predominant compounds. Within them, mass 91.074 was found in a previous work of the authors of this study as important compound for the discrimination of Trinitario chocolate [22]. Moreover, in the aforementioned study mass 45.053 (acetaldehyde) and 105.09 (methional) were relevant in the discrimination of the chocolates belonging to the brand analysed in the NS measurements.

Evaluating the trend of the compounds, in the headspace is clear the prevalence of few compounds on the other hand in the NS measurements even though the compounds are more diluted, the predominance of certain compounds is less evident and heavier compounds are becoming more dominant during eating. They are probably more hydrophobic and are being pushed out from the matrix that is becoming more and more hydrophilic with continued salivation. When air/product distribution coefficients change as the 'product' phase becomes very hydrophilic with the saliva, they tend to accumulate and increase their concentrations in air. Mastication and swallowing cause significant changes to the food in terms of surface area, hydration and time in the mouth and these factors in turn effect the mass transfer phase in the mouth [33].

4. Conclusions

PTR-QiToF-MS was show to allow a rapid real-time screening of the cocoa beans characteristics that persist in the final product before and during consumption. Distinct profiles of the VOCs released during eating of chocolates of different origins (botanical and geographical) during mastication and post-swallowing were defined and show consistent differences due to inter-individual subject and beans' botanical origin factors as well as product/subject interactions. The inter-individual differences were larger than cocoa beans differences, and cocoa *botanical* traits were more pronounced in the profiles than *geographical* traits. On the other hand, in the chocolate headspace profiles the geographical information was more dominant than the botanical traits; this dissimilarity is mainly due to the affinity of the compounds for the food matrix and the air/product distribution changes caused during the mastication and swallowing.

This study underlines that the botanical origin is consistently reflected in the final chocolates and even in the breath of subjects during eating. Criollo chocolates were clearly distinguished from the other samples. Geographical differences were detectable within the three botanical origin groups. Further studies could focus on differences in VOC release due to cocoa bean origin and sensory properties to understand how sensory perception is eventually affected.

Acknowledgements

This study has been funded by Proton Ionization Molecular Mass Spectrometry (PIMMS) ITN which is supported by the European Commission Seventh Framework Programme under Grant Agreement Number 287382.

The authors wish to thank dr. S. Yener for sharing knowledge about PTR-ToF-MS and offering assistance with PTR-ToF-MS in-nose data analysis, and dr. T. Lloyd for comments that greatly improved the manuscript.

Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.talanta.2018.11.100.

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