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Factors contributing to the variation in the volatile composition of chocolate: Botanical and geographical origins of the cocoa beans, and brand-related formulation and processing

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ABSTRACT

The intrinsic characteristics of chocolate and the complex technological process complicate the assessment of the typical features of this product and the verification of its authenticity. In this study, the influence of the botanical and geographical origin of the cocoa beans, as well as the impact of brand-related processing on the volatile organic compound (VOC) composition of the resulting chocolates was examined. A total of ninety dark chocolates available on the Dutch market were analysed using Proton-Transfer-Reaction-Mass Spectrometry (PTR-MS). The VOC profiles generated by PTR-MS (136 masses per sample) were used as fingerprints and investigated using chemometric tools to elucidate information on production factors of cocoa and subsequent processing, in the finished product. Principal component analysis (PCA) showed some clustering of the chocolates according to the botanical and geographical origins of beans as well as according to the brand. Partial least square discriminant analysis (PLS-DA) further discriminated the samples according to the three classes (botanical origin, geographical origin, brand) and the models with the best classification results were used to investigate the relevant masses for each class. PCA clustering and PLS-DA classification highlighted that chocolate profiles are strongly affected by the processing applied by the different brands. However, reflection of the botanical and geographical origins of the beans was also mirrored in the VOC composition of the chocolates. PTR-Time of Flight-MS (PTR-ToF-MS) was used to tentatively identify the VOCs of the chocolates. These measurements allowed the identification of 36 spectrometric peaks which relate to the main classes of chocolate odorant compounds, in particular, aldehydes and pyrazines, products of Maillard reactions. Several compounds already present in unroasted beans were tentatively identified in the chocolates as well, such as, acetic acid, methylpropanoic acid, 2- and 3-methylbutanoic acid, 2-phenylethanol, and tetramethylpyrazine. The results of this study emphasize the impact of the brand-related formulation and processing on VOC profiles of dark chocolates. However, using chemometrics, VOC reflection of the botanical origin and geographical origin of the beans in the chocolates was revealed, which may be useful for a future cocoa/chocolate traceability.

1. Introduction

Food authentication aims to protect consumers from being sold an inferior product, with a false description, and honest traders from unfair competition. Common authenticity issues in food are: (1) to ensure composition and constituents of foods; (2) to guarantee the country, regional, and production provenance of a product; and (3) to protect from counterfeits. Developing or improving analytical methods able to investigate these parameters are necessary steps in food analyses (Dennis, 1998).

The complex characteristics of chocolate related to the supply chain, the technology involved in the production, and the ingredients' composition complicate the assessment of the typical features of this product and the verification of its authenticity. Therefore, the discovery of markers related to e.g. the botanical and geographical origins of the beans would be a first step towards the development of a reliable method for authentication which in turn would underpin sustainable production and would help to preserve stakeholders' confidence (Saltini, Akkerman, & Frosch, 2013). Besides consumers, stakeholders include farmers, shipping organizations, processors, and distributers.

Mass spectrometry, spectroscopic and separation techniques have been applied to assess the food's geographical origin (Luykx & van

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Ruth, 2008) and species authentication (Bossier, 1999; Fajardo, González, Rojas, García, & Martín, 2010; Spaniolas, May, Bennett, & Tucker, 2006). Several studies in this area have been carried out on coffee (Downey, Briandet, Wilson, & Kemsley, 1997), wine (Liu, Cozzolino, Cynkar, Gishen, & Colby, 2006), fruit juice (Zielinski et al., 2014), and tea (Kovács et al., 2010). Chocolate authentication is especially focused on cocoa butter evaluation (Cheman, 2005; Dionisi et al., 2004; Ulberth & Buchgraber, 2003). Nevertheless, as stated before, other authentication issues are evenly relevant such as the assessment of the origin and verification of the brand.

Publications related to the geographical origin of chocolate and cocoa beans, and to the cocoa variety concern the differentiation of the products by their fatty acid profiles (Hernandez, Castellote, & Permanyer, 1991) and the examination of the volatile and non-volatile profiles (Afoakwa, Paterson, Fowler, & Ryan, 2008; Farah, Zaibunnisa, Misnawi, & Zainal, 2012; Hernandez & Rutledge, 1994; Jinap, Dimick, & Hollender, 1995). However, the majority of these studies aim to characterize and improve the aroma quality of cocoa and chocolate, and just few of them regard chocolate authenticity (Caligiani, Cirlini, Palla, Ravaglia, & Arlorio, 2007; Cambrai et al., 2010).

Considering the fact that chocolate is a complex matrix, it is difficult to pinpoint particular markers for its authenticity. Traditional techniques cannot fully satisfy the new needs of food authentication, as they just focus on specific markers or particular undesired compounds, which cannot characterize a product according to the origin or the production steps. Therefore, an analytical fingerprint approach may be more suitable. This methodology is a non-selective way of analysis and takes into account a complete spectrum or an image of the test material. Combining analytical techniques with statistical analyses, the fingerprint aims at having a more complete description of the product (Capuano & Van Ruth, 2012). This approach has been applied for the authentication of different foods such as organic eggs (Tres & van Ruth, 2011), olive oil (Araghipour et al., 2008), and cheese (Biasioli et al., 2006) among many others.

The volatile organic compound (VOC) composition of chocolate is influenced by the genotype and origin of the cocoa beans, the agro-climatic condition of growing, and post-harvest fermentation, drying, and storage (Afoakwa et al., 2008; Camu et al., 2008; Jinap et al., 1995; Rodriguez-Campos, Escalona-Buendía, Orozco-Avila, Lugo-Cervantes, & Jaramillo-Flores, 2011). Therefore, each bean variety will result in a characteristic flavour that will impact on the final product, together with the manufacturing steps as roasting and conching (Counet, Callemien, Ouwerx, & Collin, 2002; Frauendorfer & Schieberle, 2008). By analysing the volatile profile, we aim to reveal the reflection of origin factors and processing in the final chocolate product.

Several studies showed that volatile compounds are preserved during cocoa beans fermentation and drying. In the final product, even after the thermal treatments, it is possible to find compounds developed before and after cocoa fermentation (Aculey et al., 2010; Frauendorfer & Schieberle, 2008). This is an important consideration that underlines the possibility to detect the VOCs related to the raw material in a chocolate bar.

The aim of this study is to explore how the volatile profiles of dark chocolates are affected by the botanical and geographical origins of the cocoa beans used in the manufacturing and the different systems of processing applied by various brands, to extract information that may be useful for future authentication. The VOCs' investigation was carried out by High-sensitivity-Proton Transfer Reaction Mass Spectrometry (HS-PTR-MS). Headspace PTR-MS analysis requires no chemical pre-treatment of the sample and it is a sensitive (pptv, part per trillion by volume level) and fast technique (<1 min for a complete mass spectrum). PTR-MS is based on a "soft ionization" characterized by a proton transfer that generates protonated molecules with little or no fragmentation. Most commonly, H_3O^+ is used as protonating agent. One of the most important advantages in using H_3O^+ is the possibility to use the samples headspace air as buffer gas since H_3O^+ does not react with the main natural components of air (Lindinger, Hansel, & Jordan, 1998). To further characterize the chocolates, VOCs were tentatively identified using PTR-time of flight-MS (PTR-ToF-MS). Ninety dark chocolates available on the Dutch market were analysed to compare products available to consumers.

2. Materials and methods

2.1. Sampling

Ninety dark chocolate bars, available on the Dutch market, were collected from retail outlets in 2013 and were analysed. The chocolates were collected considering the botanical and geographical origins of the cocoa beans, and the brand presented on the labels. According to this information the set of samples was subdivided as shown in Table 1. Chocolate bars were stored at room temperature.

2.2. HS-PTR-MS analysis

Chocolate samples were powdered using an electrical grater for food and kept at 4 °C prior to analysis. For the measurements, 3.0 g of ground chocolate was weighed into clean and odourless flasks of 250 ml. The closed flasks were placed in a water bath at 40 °C for 30 min to equilibrate the samples with their headspace. Preliminary experiments showed that 30 min were sufficient for equilibration. The headspace of the powdered dark chocolate 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, 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} cm^2 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 prior to each sample. Five cycles per measurement were recorded and, specifically, the three last cycles for the blanks and the three 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. HS-PTR-MS analysis was run in triplicate.

Table 1
Sample subdivision according to the properties of interest.

Group	Total number of samples	Subgroup[a]	Number of samples
Botanical origin	16		
		Criollo	6
		Forastero	4
		Trinitario	6
Geographical origin	90		
		Africa	15
		Asia	11
		Oceania	2
		South America	30
		Mixed origin	11
		Unknown origin	21
Brand	46		
		A	4
		B	4
		C	5
		D	6
		E	5
		F	14
		G	4
		H	4

[a] For each group the total number of samples belonging and the subgroups with the related number of samples is specified.