Organoleptic properties of dark chocolates investigated by direct-injection mass spectrometry (PTR-ToF-MS) and GC-olfactometry

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Abstract

A preliminary sensory study conducted on a set of 187 dark chocolates varying in terms of cocoa origin and variety allowed their classification into four distinct sensory categories. Fingerprints in volatile organic compounds (VOCs) of these chocolates were obtained by a direct-injection mass spectrometry headspace method using Proton Transfer Reaction Mass Spectrometry (PTR-MS). This chemical analysis allowed discriminating the four sensory poles, so the sensory discrimination seemed to be mainly based on volatile compounds. Then, the key odorants responsible for chocolates differentiation were determined through identification of targeted aroma compounds by GC-MS after GC-O analyses of extracts representative of each subset of chocolates. Twelve dark chocolates were studied using the detection frequency method. The odour events generated by a panel of 12 assessors were grouped into 124 odorant areas (OAs). Correspondence analyses allowed distinguishing the samples while identifying 34 OAs that appear relevant to discriminate the chocolates sensory poles. Among these characteristic OAs, five were identified unambiguously with GC-MS and the remaining need to be resolved from numerous coeluted peaks.

Introduction

Dark chocolates develop several organoleptic characteristics depending on cocoa origin, cocoa variety and fabrication process. These parameters influence the chemical composition of the chocolates, and particularly their qualitative and quantitative content in volatile organic compounds (VOCs) responsible for their aroma [1]. A set of 187 dark chocolates varying in terms of cocoa origin and variety, obtained with exactly the same fabrication process, was submitted to sensory evaluation based on 36 descriptors (32 aromas and 4 tastes). Four distinct sensory poles (SPs) were subsequently clearly established. As their sensory differentiation was essentially based on aroma descriptors, we hypothesized that the sensory classification of the chocolates should be mainly based on their composition in VOCs. VOCs investigation can be carried out by headspace analysis using direct-injection mass spectrometry such as Proton-Transfer Reaction Mass Spectrometry (PTR-MS), an untargeted approach that leads to aroma profiles (fingerprints). Identification of targeted aroma compounds is possible using gas chromatography combined with olfactometry (GC-O) and GC-MS. GC-O has been commonly used to investigate key aroma compounds in several products, including cocoa and chocolate [2-4]. The aim of this study was to identify key aroma compounds of the four sensory poles. To achieve this goal, we first checked that the sensorial differentiation

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was mainly based on VOCs composition by studying the chemical fingerprints of the 187 chocolates. Then we identified the key odorants responsible for chocolates differentiation by GC-MS analyses of targeted aroma compounds selected after GC-O analyses of extracts representative of each subset of chocolates.

Experimental

Samples

Dark chocolates were provided by the Valrhona company. All the samples originating from different cocoa varieties and sources were produced using the same transformation process with the same mass of cocoa, sugar, soy lecithin and vanillin.

Headspace analysis using PTR-ToF-MS

Samples of chocolate (1 g) mixed with 1 mL of artificial saliva were transferred to 20 mL vials that were maintained under stirring at 36.2°C for 2 hours equilibration time. Headspace measurements of 187 samples were performed in triplicates using a Proton Transfer Reaction - Time of Flight - Mass Spectrometry (PTR-ToF-MS) instrument (PTR-ToF 8000, Ionicon Analytik GmbH, Innsbruck, Austria) with H_3O^+ as reagent ion. The instrument drift-tube was set to a pressure of 2.30 mbar, a temperature of 80°C and a voltage of 480 V, which resulted in E/N ratio (electric field strength to gas number density) of 111 Townsend (Td, 1 Td=10^{-17} V.cm^2). Total inlet flux was adjusted to 65 ml/min and the transfer line maintained at 110°C. To assure a constant flux into the PTR and avoid drift-tube depression, a flux of 100 ml/min of zero-air was used with a leak allowing the flux excess to escape. The designed experimental setup allowed analysing successively background air, the sample and the molecule used for mass calibration of the instrument (headspace of aqueous ethyl decanoate (Sigma-Aldrich)) just by twisting four three-way valves. A sample analysis lasted 5 minutes and was followed by cleaning the tubing by flushing the transfer line with zero-air until baseline recovery. This protocol allowed the analysis of successive samples every 10 min. The measurement order was randomized using a Latin square design to avoid possible systematic memory effects. The average areas under the curves obtained for the 2 min release of 314 significant ions present in the mass spectra were used to perform unsupervised (PCA) and supervised (PLS-DA) multivariate data analyses.

Extraction of the volatiles of 12 samples

30 g of chocolate were mixed with 100 mL ultra-pure water and 300 µl 2-methylheptan-3-one (93 ng/µl in water) as internal standard. This mixture was vacuum distilled under stirring for 1h45 using a SAFE apparatus [5] in a thermostated bath at 37°C. The aqueous distillate was extracted with dichloromethane (3 x 15ml). Finally, the extract was concentrated to 400µl with a Kuderna-Danish apparatus in a 70°C water bath.

Identification of odorous compounds with GC-O and GC-MS

Twelve assessors evaluated the extracts using detection frequency methodology. Samples were analysed using a 6890A gas chromatograph (Agilent Technologies, Massy, France) equipped with a flame ionization detector (FID) using a DB-FFAP column (30 m x 0.32 mm x 0.5 µm; J&W Scientific, Folsom, CA, USA). The effluent was split into two equal parts to the FID and the sniffing port via Y-type seal glass and two deactivated capillaries. The assessors generated sensorial attributes at the same time they detected an odour events. These were grouped into olfactive areas (OAs) on the basis of the closeness of their linear retention indices (LRIs). A detection filter of 30% was set to finally retain 124 significant OAs. A correspondence analysis (CA) was performed on the detection

frequencies of the discriminant OAs found in the 12 samples. Identification of the compounds responsible for OAs was done by gas chromatography-mass spectrometry (GC-MS) by injection on the same column as in the GC-O study. Reliability of compounds identification was assured by comparison of mass spectra to databases (NIST 08 and an in-house database, INRAMass) and by comparison of LRIs to LRIs on DB-FFAP cited in literature.

Results and discussion

A Principal Component Analysis (PCA) conducted on the PTR-MS data revealed partial separation of the four sensory poles (SPs) (data not shown). To go further a Partial Least Squares Discriminant Analysis (PLS-DA) was conducted on the 314 ions obtained in the PTR-MS study (X variables) to try to better distinguish the four sensory poles (Y variables) and identify the most explanatory ions used for the classification. PLS-DA revealed 7 significant latent variables with $R^2 = 0.847$.

Figure 1 displays the plane defined by the two first latent variables that carried out significant explained variance (28% for X and 26% for Y on the first factor and 9% for X and 20% for Y on the second). The robustness of the model was obtained using leave-one-out cross validation. The groups formed by samples of each SPs were differentiated, especially those from the SP 1 and 2, found in the positive side of the first factor while SP4 were find on the opposite side. The groups formed by samples affected to the SP3 and the SP4 are better distinguished on the plan defined by the factors 1 and 3. Explanatory ions could be inferred from the model and could be considered as molecular markers of SPs and could be used to predict to which SP an unknown sample belongs. This classification could be compared to the one obtained with the sensory data and globally revealed the same features (data not shown).

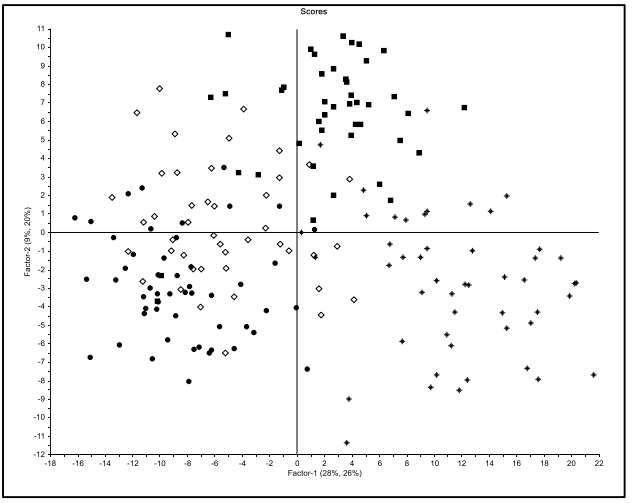

Figure 1: PLS-DA with chemical data (factors 1 and 2) 187 samples distributed in 4 sensory poles (Y variables) / 314 ions (X variables) (star: pole 1; box pole 2; dot: pole 3; open diamond: pole 4)

The GC-O experiment revealed 124 OAs after application of a 30 % threshold on the detection frequencies. Among them, 34 showed significant detection frequencies differences between samples and were included in a correspondence analysis in the aim to discriminate the samples and associate corresponding OAs. The samples were clearly

discriminated along factor 1 of the CA (Figure 2) and characteristic OAs were found for each SP. Factor 3 discriminated samples belonging to the poles 1 and 2 (data not shown). Furthermore 90 OAs exhibited no real changes in detection between samples and therefore may represent the background of the overall chocolate aroma. Only five OAs have been positively identified so far by comparing their experimental data to the literature data (retention indices, mass spectra and aroma descriptors).

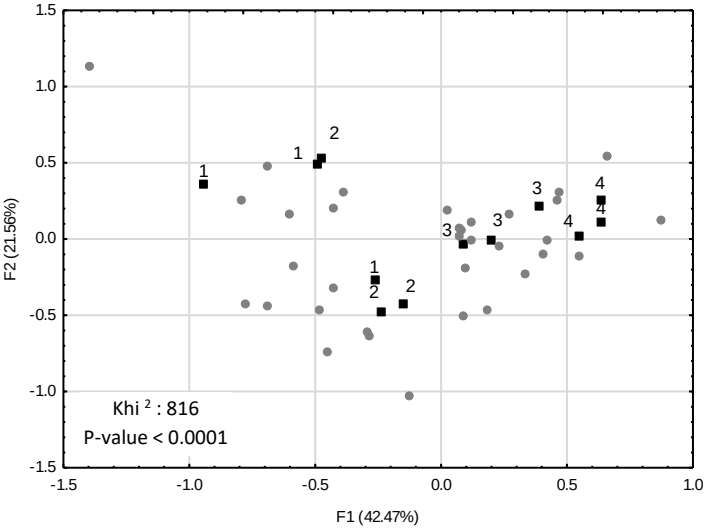

Figure 2: Correspondence analysis (factors 1 and 2): detection frequencies of 34 OAs (grey dots) within 12 samples (black diamonds). The different numbers (1, 2, 3 and 4) indicate the sensory poles.

To conclude, the "chemical map" obtained in the PTR-MS analyses of the chocolates headspace allowed retrieving the classification of the 187 samples into the four sensory categories previously determined. Thus, it could be deduced that the composition of chocolates in VOCs explained in a large part the sensory classification. Using GC-Olfactometry, discriminant OAs for each pole were identified thanks to a correspondence analysis. Some discriminant OAs have been positively identified using GC-MS. The remaining unidentified OAs required additional analyses for their identification (a different GC column, chemical ionization, 2DGC-MS-O…).

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