Rapid analysis of important taste active components in chocolate by ultra-high performance liquid chromatography coupled with high resolution mass spectrometry (UHPLC-QToF-MS)

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Abstract

This study illustrates a rapid approach to qualify and quantify taste active compounds in chocolate. Eleven commercially available chocolate samples were extracted with aqueous, acidified methanol and analysed by UHPLC-QToF-MS. This allowed the (semi)-quantification of a series of taste actives within a single injection, while the high resolution of the mass analyser allowed a high confidence in their identification. Dose-over-Threshold (DoT) values were calculated for key chocolate taste compounds allowing a deeper understanding of the orosensory properties of chocolate and creating a link with human-sensory investigations. The outcomes of this study illustrate the benefit of UHPLC in combination with high resolution mass spectrometry, while requiring minimal sample preparation for the extraction of key tastants from chocolate.

Introduction

Chocolate is appreciated by consumers around the globe due to its unique organoleptic properties. The combination of desirable textural features and a boost of flavours when melting in the mouth made chocolate become one of the most beloved treats in the human diet. Several decades of research have focussed on deciphering the flavour active components in cocoa and chocolate and deepening the understanding of their origin [1, 2, 3, 4]. In particular, the role of cocoa derived aroma active components and their fate during roasting has been studied in detail [2]. Fewer studies are available for the taste active components, but it is generally well understood that besides acidity and astringency, bitterness is a characteristic sensory attribute for cocoa taste [5].

Research applying sensory-guided techniques identified a range of chemically diverse molecules contributing to the bitter taste of roasted cocoa nibs (Figure 1) [5].

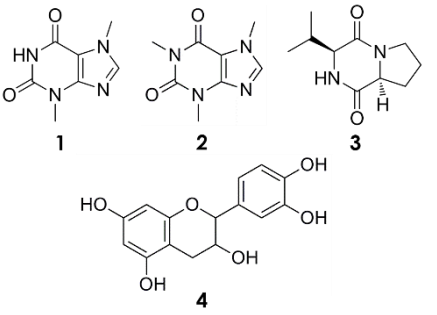

Figure 1: Structure of the bitter tastants theobromine (**1**), caffeine (**2**), cyclo(Pro-Val) (**3**) and epi-/catechin (**4a/b**)

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In particular, the alkaloids theobromine and caffeine, the diketopiperazine cyclo(Pro-Val) and the flavan-3-ol epicatechin were attributed with high Dose-over-Threshold (DoT) values [5]. Besides their intrinsic taste, selected diketopiperazines were found to modulate the taste of theobromine solutions resulting in increased bitterness intensity, as well as changing bitterness qualities [6, 7].

The aim of this work was to develop a fast method to identify and quantify key bitter compounds in chocolate and create a link with bitter sensory scores.

Experimental

Materials

Eleven chocolates from seven markets (with claimed cocoa content from 39 to 70%) were used. Methanol for LC-MS, acetonitrile for LC-MS, water for LC-MS and formic acid were from VWR (Lutterworth, United Kingdom). Caffeine, (+)-catechin, (-)-epicatechin, theobromine, and tyrosine methyl ester hydrochloride were from Sigma-Aldrich (Dorset, United Kingdom). cyclo(Pro-Val) was from Bachem (Bubendorf, Switzerland).

Extraction of key tastants and analysis by UHPLC-QToF-MS

Details of the experimental procedures on the isolation and quantification will be published elsewhere. Briefly, finely ground chocolate was spiked with the internal standard tyrosine methyl ester and extracted with a mixture of methanol/water/formic acid (80/20/0.1, v/v/v). The samples were filtered, diluted and analysed on an ekspert™ ultraLC 100 (AB Sciex, Warrington, United Kingdom) coupled to a Triple TOF 5600$^+$ Mass Spectrometer (AB Sciex, Warrington, United Kingdom). Chromatographic separation was achieved on an ACQUITY® BEH C18 column (2.1 x 150 mm, 1.7 µm) (Waters, Elstree, United Kingdom) equipped with corresponding pre-column using 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B) as mobile phases. Calibration curves were prepared by dilution of the commercially available reference substances. The peak areas of the target analytes were extracted using the *m/z* values calculated for $[M+H]^+$ with an extraction window of ± 5 ppm.

Quantification of theobromine by HPLC-UV

The quantitative determination of theobromine in chocolate was performed as described in literature [8].

Sensory evaluation

The bitter taste of the chocolates was scored by twelve trained sensory panellists on a scale ranging from 0 (not bitter) to 10 (very bitter), in duplicate repetition.

Results and discussion

Identification of the analytes

The developed UHPLC-QToF-MS method enabled analysis of a wide range of taste active components of different functional groups found in the chocolates. The tastants were identified based on their retention times and accurate masses in comparison to references. Figure 2 shows extracted ion chromatograms (EICs) for selected bitter compounds (Figure 1) in a dark chocolate.

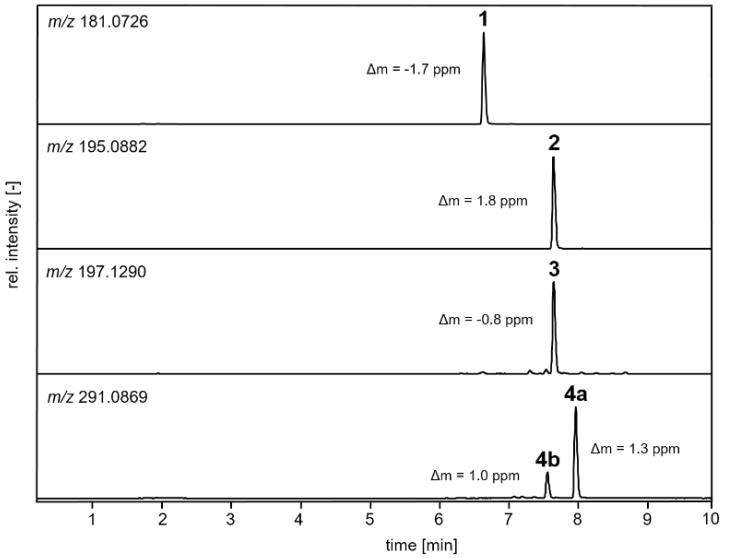

Figure 2: Extracted ion chromatograms (EICs) for the $[M+H]^+$ (±5 ppm) for theobromine (**1**), caffeine (**2**), cyclo(Pro-Val) (**3**), catechin (**4b**) and epicatechin (**4a**) from a dark chocolate sample

Quantification of the analytes

Following identification, the concentrations of the analytes were quantified using tyrosine methyl ester as internal standard. The values obtained for theobromine using the developed method were compared to that of a conventional HPLC-UV method (Figure 3). A good correlation of the results was observed (r^2=0.9171).

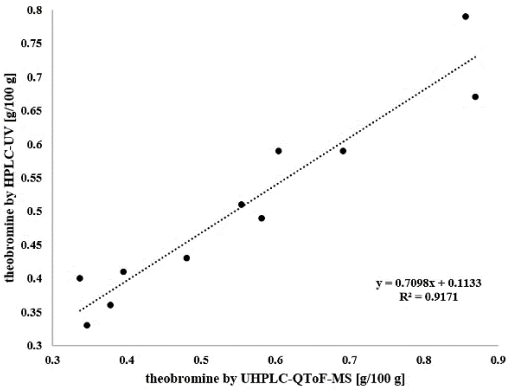

Figure 3: Correlation of theobromine values obtained by the developed UHPLC-QToF-MS method and the HPLC-UV reference method

Calculation of Dose over Threshold (DoT) values and correlation to sensory analysis

The quantification of key taste actives (theobromine, caffeine, epicatechin, catechin and cyclo(Pro-Val)) allowed the calculation of DoT values based on taste threshold values obtained in literature [5]. A link of the sum of the DoT values for compounds **1-4** (Figure 1) with values obtained by human-sensory assessment of bitter taste was then established (Figure 4). Figure 4 shows that in particular sample **I** is deviating from the

predicted values. This was attributed to the higher concentration of DKPs, shown for cyclo(Pro-Val) in Table 1. This agreed with literature on the taste modifying properties of DKPs [5, 6].

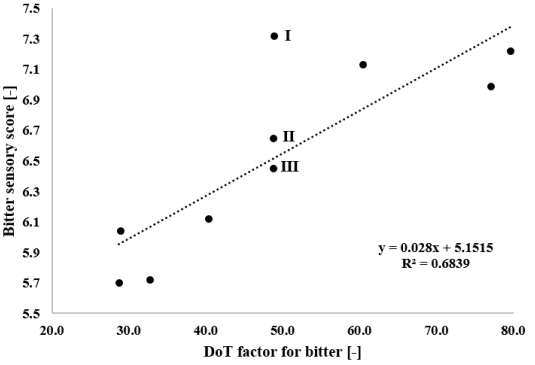

Figure 4: Correlation of the sum of the DoT values for bitter for the key tastants (**Figure 1**) and the observed sensory bitter scores

Overall a good correlation was observed between the sum of the DoT factors for the five taste compounds (Figure 1) and the bitter sensory scores. The results confirm in particular that **1** is the most important contributor to the bitterness of commercial dark chocolates (Table 1). Further investigation on the synergism of DKPs and theobromine (**1)** and the relevance of DKPs for the sensory perception of dark chocolates are on-going.

Table 1: DoT values for the key tastants and sensory scores for bitter in selected dark chocolate samples. Numbering of components refers to Figure 1.

Sample	*Dose over threshold factor (DoT) [-]*					*Sensory score bitter*
	1	*2*	*3*	*4a*	*4b*	
I	40.6	4.6	2.9	<0.5	2.9	7.3
II	38.7	4.9	0.8	0.9	0.8	6.7
III	42.2	4.4	1.9	<0.5	1.9	6.5

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