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Identification of 2,5-dimethyl-3-furanthiol in coffee by two dimensional GC TOF MS

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Abstract

2,5-dimethyl-3-furanthiol is an important aroma constituent widely used in flavorings for food stuff. While its occurrence in food is mentioned several times, its unequivocal identification is not clear. In an SDE extract of ground coffee beans, using a pre-separation step and two dimensional GC TOF MS, its occurrence in ground coffee was confirmed. Targeted analysis with modern instrumentation proved to be a powerful technique to detect such a minor constituent in a complex matrix.

Keywords: *2,5-dimethyl-3-furanthiol, coffee, GC TOF MS*

1. Introduction

2,5-dimethyl-3-furanthiol (DMFT) is a strong meat like smelling aroma constituent with a threshold in air in the range of 0.0035 – 0.014 ng/L [1]. DMFT is widely used for imparting a

meaty flavor profile in foodstuff. Its use in food is approved by FEMA (FEMA No 3451) and a use level of 0.25 ppm in baked goods, meat sauces, meat soups, condiments and pickles is regarded as safe [2]. It is also listed in the EU Lists of Flavorings.

DMFT has been reported in chicken broth by Gasser et al. [1] based on GC O data and retention index on two different columns, but this finding was not confirmed by mass spectrometry. DMFT was also tentatively identified in beef [3], coffee [4] and extruded potato snacks [5]. Indeed, none of those publications supports an unequivocal identification of the substance in a food product as for example laid out by the International Organization of the Flavour Industry [6], which requires the use of an authentic reference sample and confirmation of identity by two independent methods, e.g. retention index and mass spectrum.

From a commercial perspective a clear identification of DMFT in food is desired, as this has implications from a regulatory perspective. Objective of this research project was to find unequivocal evidence for the occurrence of DMFT in a food.

Based on previous tentative identification of DMFT in coffee [4], and the fact that structurally related constituents of DMFT, i.e. 2,5-dimethyltetrahydrofuran-3-one and 2,5-dimethyl-3(2H)-furanone were reported in coffee [7], we selected ground coffee powder as a target. In this investigation we applied two-dimensional GC MS TOF to identify DMFT in roasted coffee beans.

2. Materials and Methods

2.1 Sample

Espresso coffee beans: Lavazza Torino, Italia, 1895, Espresso Classico 100% Arabica, light roasting, was obtained from a local store.

2.2 Chemicals

Analytical grade chemicals were commercially obtained: Pentane (Merck Darmstadt, Germany 1.00921.1000), diethyl ether (Merck Darmstadt, Germany 1.07177.1000), dichloromethane (Merck Darmstadt, Germany 1.06050.1000), Merck silica gel 60 (0.2-0.5 um) art 1.07733.1000. An authentic reference from DMFT was internally sourced from Givaudan. The structure was confirmed by NMR (Spectrum S1).

2.3 Instruments and settings

NMR Measurements

NMR-spectra were recorded on a JEOL ECZ/R 600 MHz spectrometer equipped with a 5 mm dual autotune probe cooled with liquid nitrogen. Sample was dissolved in CCl_4 and transferred to a 5 mm NMR tube. A small capillary filled with D_2O/TSP was inserted into the 5 mm tube in order to provide a deuterium lock signal and a chemical shift reference standard.

Gas Chromatography

GCMS analysis was performed on a two-dimensional GC MS QTOF system from Agilent. The precursor ion of DMFT was isolated followed by measuring the accurate MS^2 spectrum. Column 1: RTX35 (60 m, 0.32 mm, 0.5 µm), Column 2: DB1 (30 m, 0.25 mm, 0.25 µm).

GC settings

Column 1: Temperature program: 40 °C (1 min), 3 °C/min, 260 °C (23 min), Injector temp: 250 °C, Column flow: 0.75 mL/min, Injection: splitless, Injection volume: 3 µL, Detector: FID at 275 °C, Heart cut: 38.35-38.85 min; Column 2: Temperature program: 40 °C (40 min), 2 °C/min, 120° C, 15 °C/min, 300 °C (5 min), Column flow: 1.5 mL/min, Detector: TOF MS.

MS settings

Full scan EI: Quench Gas He: 5 mL/min, Collision Gas N2: 0 mL/min, Scan range: 30-450 amu, Acquisition rate: 3 spectra/s, Source temperature: 230 °C, Emission current: 5 µA, Electron Energy: 70 eV.

Targeted MS/MS: Quench Gas He: 4 mL/min, Collision Gas N2: 1 mL/min, Precursor ion: 128.0296 amu, MS/MS range: 35-150 amu, Acquisition time: 200 mS, Collision energy: 20 eV, Source temperature: 230 °C, Emission current: 5 µA.

2.4 Extraction method and clean-up

Espresso coffee was ground and extracted using Likens-Nickerson technique (simultaneous extraction distillation; SDE). Ground espresso coffee beans (1000 g) were mixed with water

(3 L) in a 5 L flask. Extraction solvent was dichloromethane (100 mL). SDE was run for 1 h. Sample was concentrated to a final volume of 1 mL using a Vigreux column.

Silica gel chromatography:

A cooled glass column (1.4 cm i.d.) was filled with silica gel (13.3 g). The concentrated sample was mixed with silica gel (0.5 g) until the solvent was evaporated. Then the mix was transferred on top of the silica gel column. Six fraction were obtained using the following solvent mixes (P = pentane, DE = diethyl ether). Fr. 1: P (40 mL), Fr. 2: P (24 mL) and DE (6 mL), Fr. 3: P (22.5 mL) and E (7.5 mL), Fr. 4: P (22 mL) and E (8 mL), Fr. 5: P (20 mL) and E (10 mL), Fr. 6: Dichloromethane (100 mL).

3. Results

The elution behavior of DMFT during silica gel chromatography was determined by spiking the reference into an extract. It turned out that DMFT was eluting in fraction 3. The spiked fraction was also used to determine the retention time on both columns and to optimize the GC MS method for DMFT detection. Retention time of DMFT on the first column was 38.6 min, retention time on the second column was 52.36 min.

Before analyzing the coffee sample, a blank sample was prepared using the entire isolation procedure (simultaneous extraction distillation, column chromatography) in order to check the system for contaminations of DMFT at threshold level. In the blank run no precursor ion of 128.029 at the known retention time of DMFT could be detected. The MS^2 spectra shows noise signals only, proofing the system was not contaminated with DMFT (Figure S1).

Then the isolated fraction 3 of the coffee bean SDE extract was injected and analyzed for the presence of DMFT. The previously determined heart cut, where DMFT would elute, was transferred to the 2nd column. The FID chromatogram of the 1st column and the TOF-MS full scan chromatogram of the 2nd column are displayed in Figure S2.

At the retention time of DMFT on the 2nd column the accurate mass of DMFT was detected (128.0292 au at 52.357 min), which is in line with the elemental composition of DMFT of C_6H_8OS. This precursor ion of DMFT was isolated followed by measuring the accurate MS^2 spectrum. The base peak of the MS^2 spectrum was 95.0491. The chromatogram of the base

peak of the MS^2 spectrum on the 2nd column is displayed in Figure S3 comparing coffee extract and authentic reference. Retention time matches the one of the authentic reference.

The full MS^2 spectra obtained from the espresso coffee extract and the authentic reference is displayed in Figure 1. The accurate mass spectrum obtained from coffee extract and DMFT reference match. The calculated mass differences of fragments and their calculated accurate mass are within the instruments accuracy. These data are given in Table 1. Based on the area of the authentic reference the concentration of DMFT was roughly estimated at 10 ng/kg coffee powder.

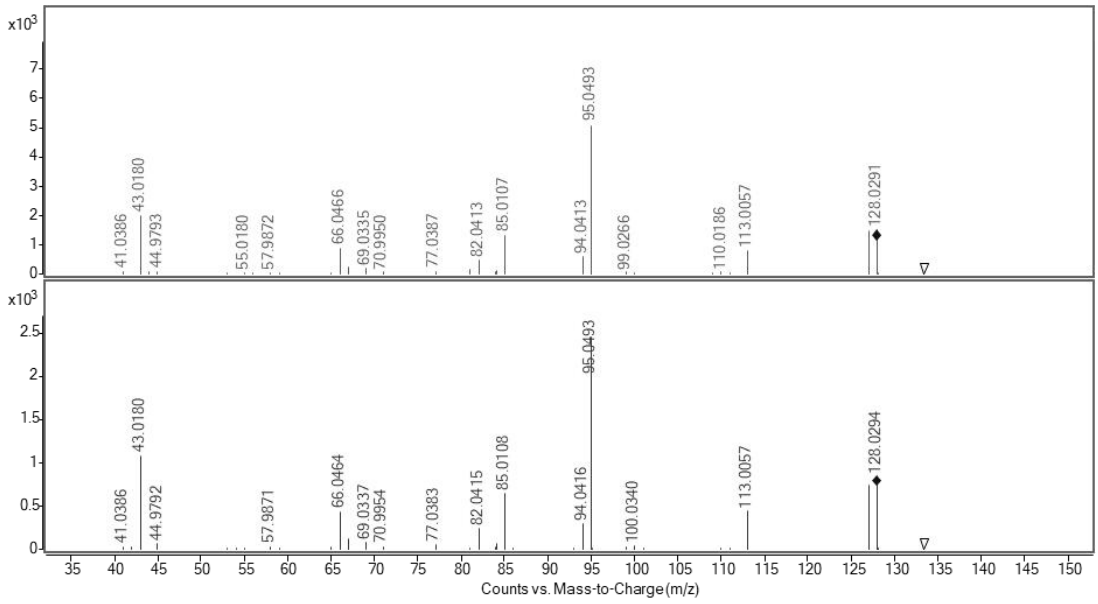


Figure 1. Espresso coffee extract MS^2 spectra (upper) versus reference of DMFT (lower)

Table 1. Mass differences of fragments to their calculated accurate values.

Sample name	**Lavazza coffee extract**	**Reference DMFT**
Retention time (min)	52.339	52.348
Precursor (m/z)	128.029	128.029
C_6H_7OS (delta ppm)	1.3	1.6
C_5H_5OS (delta ppm)	1.6	2.0
C_6H_7O (delta ppm)	1.5	1.5
C_4H_5S (delta ppm)	0.6	0.6
C_2H_3O (delta ppm)	3.9	3.9

4. Discussion

The investigation confirms the previous tentative identification of DMFT in coffee [4]. The identification is based on matching of the retention time on two different columns, accurate mass of the precursor ion and the full accurate MS^2 spectra with the authentic reference.

Formation of DMFT could proceed e.g. via 2,5-dimethyl-3(2H)-furanone or 2,5-dimethyl-4-hydroxy-3(2H)-furanone and hydrogen sulfide. These furanones have been identified in significant amounts in roasted rhamnose/cysteine reaction mixtures, in which also DMFT has been detected [8]. In other model systems, where thiamine was heated in propylene glycol, DMFT has been detected, too [9], however here the formation mechanism is not obvious. Latter results may indicate that formation of DMFT may more efficiently proceed from short chain carbonyl compounds, e.g. hydroxy acetone and 2-oxopropanal. Such carbonyls have been shown to be very efficient precursors of the homologue 2-methyl-3-mercaptofurane [10]. A better understanding of the formation pathways of DMFT could open avenues for optimized process flavor systems rich in DMFT.

Supplementary Materials: The following supporting information is available below: Spectrum S1: NMR spectrum of DMFT; Figure S1: Analysis of a blank by GC TOF MS; Figure S2: Espresso coffee extract, heart cutting and GC TOF MS chromatogram; Figure S3: Espresso coffee extract fraction 3: MS^2-spectra versus reference.

Author Contributions: Conceptualization, E.H.; methodology, E.H.; validation, E.H.; writing - original draft preparation, E.H.; writing - review and editing, K.G. All authors have read and agreed to the published version of the manuscript.

Data Availability Statement: The data presented in this study are available in this article.

Conflicts of Interest: The authors declare no conflict of interest.

References

[1] Gasser, U.; Grosch, W. Primary Odorants of Chicken Broth. Z. Lebensm. Unters. Forsch. 1990, 190, 3-8. https://doi.org/10.1007/BF01188254

[2] Oser, B.L.; Ford, R.A. Recent progress it the consideration of flavoring ingredients under the food additives amendment. 9. GRAS Substances. Food Technol. 1975, 29, 70-72.

[3] Giri, A.; Khummueng, W.; Mercier, F.; Kondjoyan, N.; Tournayre, P.; Meurillon, M.; Ratel, J.; Engel, E. Relevance of two-dimensional gas chromatography and high resolution olfactometry for the parallel determination of heat-induced toxicants and odorants in cooked food. J. Chromatogr. A 2015, 1388, 217–226. https://doi.org/10.1016/j.chroma.2015.01.045

[4] Quintanilla-Casas, B.; Dulsat-Serra, N.; Cortes-Francisco, N.; Caixach, J.; Vichi, S. Thiols in brewed coffee: Assessment by fast derivatization and liquid Chromatography - high resolution mass spectrometry. LWT - Food Sci. Technol. 2015, 64, 1085-1090. https://doi.org/10.1016/j.lwt.2015.07.010.

[5] Majcher, M.; Jeleń, H.H. Comparison of suitability of SPME, SAFE and SDE methods for isolation of flavor compounds from extruded potato snacks. J. Food Compos. Anal. 2009, 22, 606-612. https://doi.org/10.1016/j.jfca.2008.11.006

[6] International Organization of the Flavour Industry (I. O. F. I.). Statement on the identification in nature of flavouring substances, made by the Working Group on Methods of Analysis of the International Organization of the Flavour Industry (IOFI). Flavour Fragr. J. 2006, 21, 185. https://doi.org/10.1002/ffj.1721

[7] Ishikawa, M.; Ito, O.; Ishizaki, S.; Kurobayashi, Y.; Fujita, A. Solid-phase aroma concentrate extraction (SPACE™): a new headspace technique for more sensitive analysis of volatiles. Flavour Fragr. J. 2004, 19, 183–187. https://doi.org/10.1002/ffj.1322.

[8] Silwar, R. Gaschromatographisch-massenspektrometrische Untersuchung der im Modellsystem Cystein/Rhamnose unter Röstbedingungen gebildeten Aromastoffe. Z. Lebensm. Unters. Forsch. 1992, 195, 112-119. https://doi.org/10.1007/BF01201769

[9] Hartman, G.J.; Carlin, J.T.; Scheide, J.D.; Ho, C.T. Volatile products formed from the thermal degradation of thiamin at high and low moisture levels. J. Agric. Food Chem. 1984, 32, 1015-1018. https://doi.org/10.1021/jf00125a016

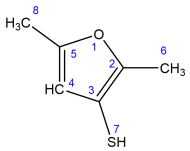
[10] Hofmann, T.; Schieberle, P. Quantitative model studies on the effectiveness of different precursor systems in the formation of the intense food odorants 2-furfurylthiol and 2-methyl-3-furanthiol. J. Agric. Food Chem. 1998, 46, 235−241.

https://doi.org/10.1021/jf9705983

Supplemental Materials

Spectrum S1. NMR spectrum of DMFT

The identity of the reference sample was confirmed by NMR analysis; numbers refer to structure below. 1H NMR (DEUTERIUM OXIDE, 600 MHz) δ = 5.89 - 5.97 (1H, s, H-4), 2.54 - 2.58 (1H, s, H-7), 2.39 - 2.42 (3H, s, H-8), 2.32 - 2.35 (3H, s, H-6); 13C NMR (DEUTERIUM OXIDE, 151 MHz) δ = 154.7, 152.1 (C-2, 3), 114.6, 113.5 (C-4), 105.4 (C-5), 99.1, 16.5, 16.4, 14.6 (C-8), 14.4 (C-6)



2,5-dimethyl-3-furanthiol

Figure S1. Analysis of a blank by GC TOF MS

Precursor Ion 128.029 (2nd column signal, upper signal) and MS^2-spectra at the retention time of DMFT (lower signal): No signal at the retention time of DMFT was observed in the blank

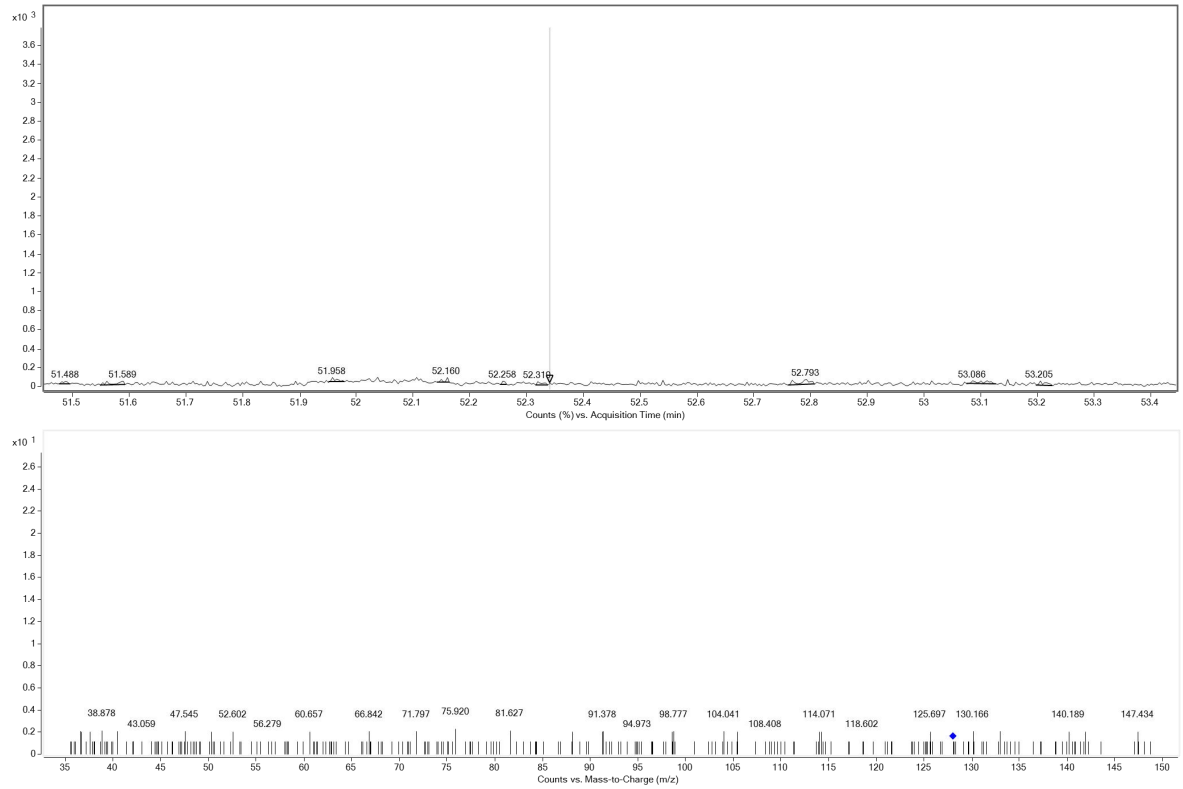
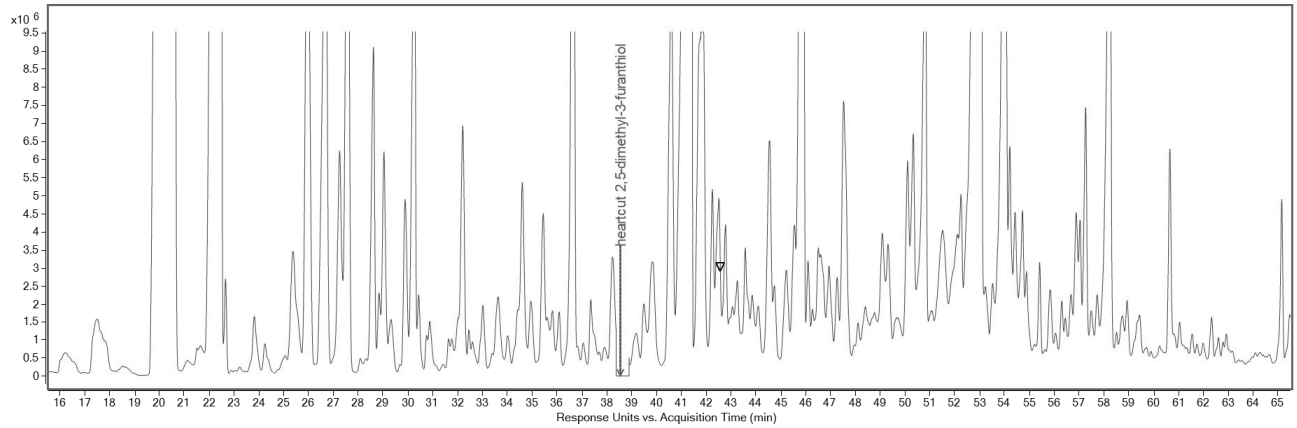


Figure S2. Espresso coffee extract, heart cutting and GC TOF MS chromatogram: The effluent from 38.35-38.85 min was transferred to the 2nd column.

1st Column: FID detection



2nd Column: TOF-MS full scan detection

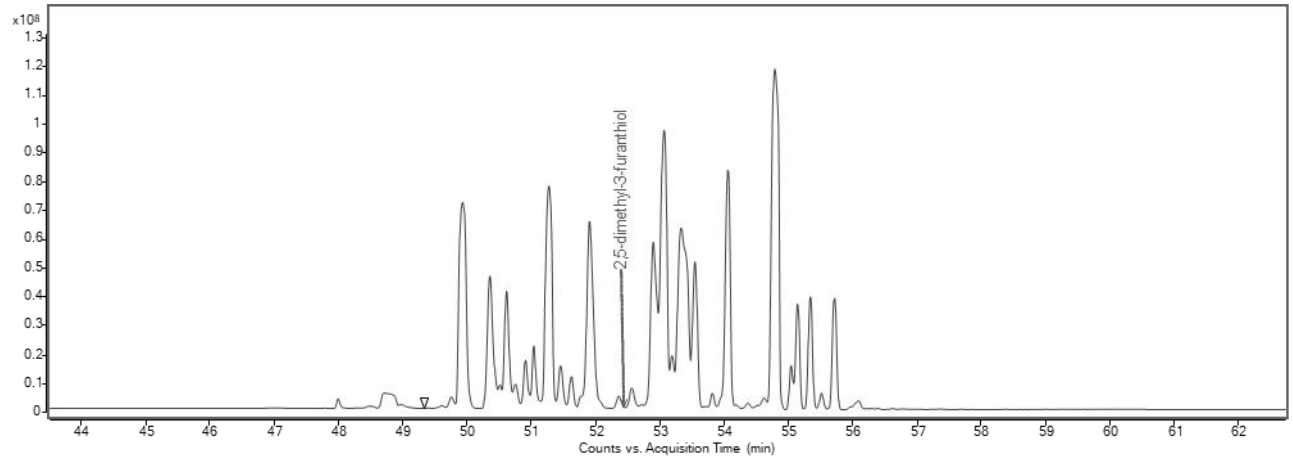


Figure S3. Espresso coffee extract fraction 3. 2nd column, MS^2-spectra, high resolution selected ion chromatogram of m/z 95.0491 versus reference of DMFT CAS 55764-23-3