

# IDENTIFICATION, QUANTIFICATION AND ODOR-IMPACT OF SELECTED VOLATILE SULFUR COMPOUNDS (VSC) IN SWISS TILSIT CHEESES BY HEADSPACE SOLID PHASE MICROEXTRACTION-GAS CHROMATOGRAPHY/PULSED FLAME PHOTOMETRIC DETECTION (HS-SPME-GC/PFPD) AND HS-SPME-GC-OLFACTOMETRY (GC-O)

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## Abstract

Commercial Swiss Tilsit cheeses were analyzed by headspace solid-phase microextraction gas chromatography-mass spectrometry/pulsed flame photometric detection (HS-SPME-GC-MS/PFPD) and GC-Olfactometry (GC-O) to identify and quantify volatile sulfur compounds (VSC). In addition physiological information on odor quality and impact of target VSCs on the overall cheese flavor was investigated. Over a dozen VSCs were detected in ppb levels, but only a few influenced the cheese odor. Dimethyl trisulfide was the only VSC to show a strong odor impact.

## Introduction

Volatile sulfur compounds (VSC) such as methanethiol (MeSH), hydrogen sulfide (H<sub>2</sub>S), methyl thioacetate (MTA) and methylsulfides (dimethyl sulfide (DMS), -disulfide (DMDS) and -trisulfide (DMTS)) are key flavor compounds found in a variety of cheeses. In cheeses, they are mainly derived from the decomposition of the sulfur-containing amino acids cysteine and methionine. Due to their low odor thresholds, their sensory properties are very pronounced even at low concentrations. Hence a variation in VSC concentration can have a significant influence on cheese flavor. Since the VSCs are highly volatile and reactive, their analysis remains a challenge [1-6].

## Experimental

### *Samples and sample preparation*

Commercial Swiss Tilsit cheese was purchased at a local Swiss supermarket, cut into small 1 cm cubes and pulverized after frizzling with liquid nitrogen. Cheese powder (2 g ± 0.1) was weighed into 20 mL HS-vials. For GC-MS/PFPD, 5 ml phosphate buffer (0.1M; pH 6.0); for GC-O 5 ml of saturated sodium chloride solution (30%) were added. For GC-MS/PFPD, the mixture was spiked with 10 µl of internal standards in methanol (ISTD; ethyl methyl sulfide (EtMeS) 0.6 mg g<sup>-1</sup>; methyl propyl sulfide (MeProS) 0.6 mg g<sup>-1</sup>). The sample was homogenized in the vial by gentle agitation. The vials were closed with teflon-lined silicone caps and flushed with argon for 15 seconds.

### *Extraction of volatile compounds by headspace solid-phase microextraction*

Volatile compounds were extracted by HS-SPME with a fully automated MPS2 system (Gerstel, Sursee, Switzerland).

After preheating the samples for 10 min at 60°C (GC-MS/PFPD) or 10 min at 30°C (GC-O) at an agitation rate of 500 rpm, a 1 cm 85 µm CAR/PDMS StableFlex™ SPME-fiber (GC-MS/PFPD) or a 2 cm DVB/CAR/PDMS 50/30 µm StableFlex™ SPME-fiber (GC-O; both Supelco, Buchs, Switzerland) was exposed to the sample headspace for 30 min at 60 °C (GC-MS/PFPD) or 360 min at 30 °C (GC-O) at an agitation rate of 250 rpm. Prior to use, the fibers were conditioned according to the manufacturer's recommendations. Bound volatiles were thermally desorbed in the GC inlet for 1 min at 280 °C (GC-MS/PFPD) or 250 °C (GC-O; splitless mode for 30s; then opening of the split valve at a flow rate of 22 mL/min).

### *Separation and detection of VSCs by GC-MS/PFPD*

VSCs were determined on a Thermo Scientific Finnigan Trace Gas Chromatograph GC Ultra coupled to a Dual Stage Quadrupole DSQ II mass spectrometer (Thermo Fisher Scientific, Reinach, Switzerland) and equipped with a pulsed flame photometric detector PFPD 5380 (O.I. Analytical, Texas, USA). Compounds were separated on a TRB-FFAP fused silica capillary column (30 m × 0.32 mm × 1.0 µm film; Teknokroma, Switzerland), with helium as carrier gas at a constant flow of 2.2 mL min<sup>-1</sup>. Oven temperature program: 5 min at 35 °C, then heated to 150 °C at a rate of 10 °C min<sup>-1</sup>, held for 1 min, then heated to 220 °C at a rate of 20 °C min<sup>-1</sup> with a final hold time of 5 min.

A split of 1:2 between the MS and the PFPD was used. The settings of the PFPD were optimized for sulfur compounds.

PFPD signals were integrated using the Xcalibur 2.0.7 software (Thermo Fisher Scientific, Reinach, Switzerland) and the amount of each target sulfur compound was presented as a square root of the total peak area divided by the peak area of the corresponding internal standard. All samples were analyzed in triplicate.

### *Calibration curve and quantification*

Standard solutions (STD) containing MeSH, DMS, MTA, DMDS, DMTS were prepared separately in methanol to the final target concentration and further diluted to 1:2, 1:3, 1:4, 1:5, and 1:6 strengths.

Each sample containing 5 ml phosphate buffer (0.1M, pH 6.0) and 1 ml Mygliol® was spiked with 10 µl of STD and 10 µl of ISTD. Triplicate analysis at each concentration level and linear regression plots were used to construct calibration curves.

Quantification of target molecules was achieved using the calibration curve and the ratio with the internal standard to avoid fluctuations.

### *Separation and detection of odorant compounds by GC-MS/O*

Volatile compounds were determined on an Agilent 6890 Gas Chromatograph coupled to an Agilent 5973 mass spectrometer MS (Agilent, Basel, Switzerland) and equipped with a 2-way-GC-O-system where two panelists judge a sample simultaneously.

Compounds were separated on a TRB-FFAP fused silica capillary column (60 m × 0.32 mm × 0.5 μm film; Teknokroma, Switzerland), with helium as carrier gas at a constant flow of 2.3 mL min<sup>-1</sup>. Oven temperature program: 5 min at 35 °C, then heated to 150 °C at a rate of 10 °C min<sup>-1</sup>, held for 1 min, then heated to 220 °C at a rate of 20 °C min<sup>-1</sup> with a final hold time of 5 min at 220°C.

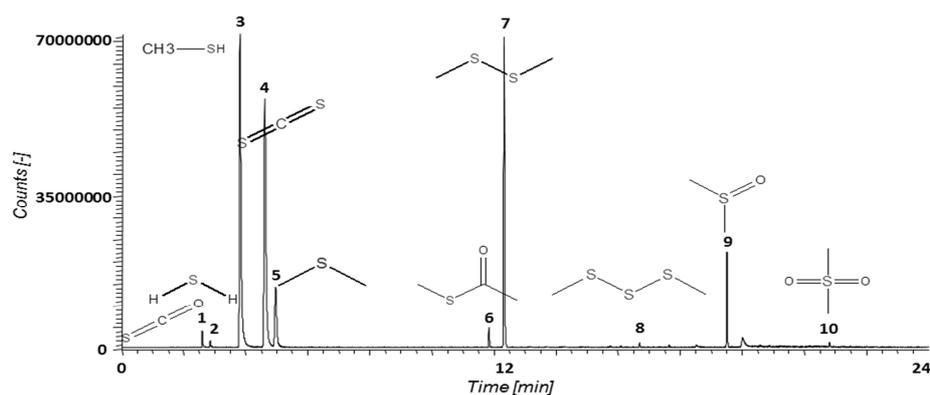
A split of 1:2 between the MS and the two sniffing ports was used.

Olfactometric signals were integrated using the Acquisniff<sup>®</sup> software (INRA, Clermont-Ferrand, France). Trained judges (total *n*= 8; two at a time) described the perceived odors and rated their intensity on a three-point-scale. Data were processed taking into account detection frequency and odor intensity [7,8].

## Results

### GC/PFPD analysis of Swiss Tilsit cheese

More than a dozen VSCs were detected in different concentrations in the cheeses (Figure 1). H<sub>2</sub>S, MeSH, DMS, MTA, DMDS and DMTS were found at ppb levels. In addition, carbonylsulphide (COS), carbon disulphide (CS<sub>2</sub>), dimethylsulfoxide (DMSO) and dimethylsulfone (methylsulfonylmethan, MSM) were identified (Figure 1, Table 1).



**Figure 1.** GC/PFPD chromatogram of Tilsit headspace Only VSCs are detected. The signal intensity is proportional to the amount of analyte and does not depend on the number of sulfur atoms in the molecule. Numbers in the graphic refer to Table 1

**Table 1.** Quantification of target VSCs in Tilsit using ISTDs and an external calibration curve

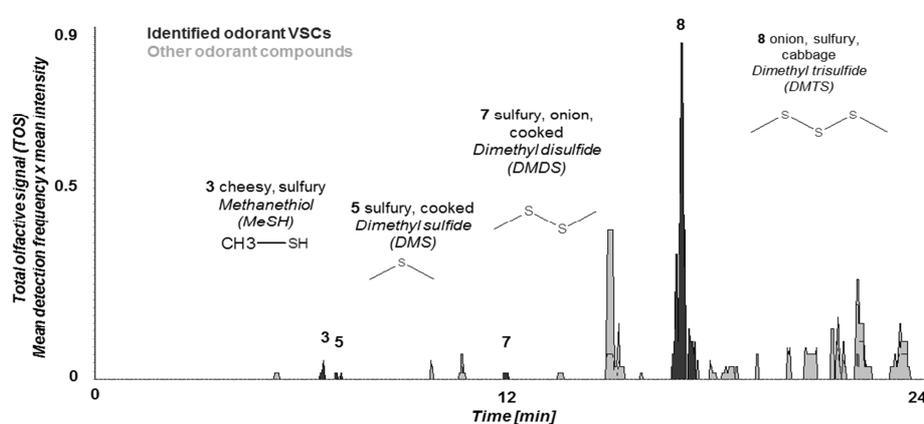
| N° Fig. 1&2 | Name             | Odor characteristics (literature) [9-11]          | Odor threshold in water [ppb] | Concentration [ $\mu\text{g kg}^{-1}$ = ppb] |
|-------------|------------------|---|-------------------------------|--|
| 1           | COS              | burnt rubber, carbamate                           | NA                            | <i>n.d.</i>                                  |
| 2           | H <sub>2</sub> S | rotten egg  | 5–10 <sup>11</sup>            | <i>n.d.</i>                                  |
| 3           | MeSH             | rotten cabbage, burnt rubber                      | 0.02–2 <sup>11</sup>          | <i>In progress</i>                           |
| 4           | CS <sub>2</sub>  | sweet, ethereal, slightly green                   | 0.05 - 0.5 <sup>11</sup>      | <i>n.d.</i>                                  |
| 5           | DMS              | canned corn, cooked cabbage, asparagus            | 0.3–1 <sup>9</sup>            | 4  |
| 6           | MTA              | sulfurous, egg, cheese, dairy, vegetable, cabbage | 50 <sup>11</sup>              | 0.1  |
| 7           | DMDS             | vegetal, cabbage, onion-like                      | 0.16–12 <sup>10</sup>         | 1.7  |
| 8           | DMTS             | sulfurous, alliaceous, cooked, savory, meaty      | 0.005–0.01 <sup>10</sup>      | 0.5  |

|    |      |                  |    |             |
|----|------|------------------|----|-------------|
| 9  | DMSO | garlic           | NA | <i>n.d.</i> |
| 10 | MSM  | sulfurous, burnt | NA | <i>n.d.</i> |

Only molecules with an impact on Tilsit odor were quantified.

### GC-Olfactometry of Swiss Tilsit cheese

According to GC-O, MeSH, DMS, DMDS and DMTS were the only VSCs found to have an impact on the olfactometry profile of the tested cheese samples (Figure 2). However, in the employed conditions, MeSH, DMS and DMDS only showed a minor, and solely DMTS a strong odor impact; the latter seeming particularly important due to its low sensory threshold. Other odorant VSCs seem to be below odor perception threshold in the employed conditions. Various other odorant compounds were also detected, but not further examined in the present study.



**Figure 2.** Aromagram of Tilsit cheese indicating identified odorant VSCs and odor descriptors used by the panel in this study. In light gray the other volatiles compounds with odorant impact

In conclusion, only DMTS was found to have an important odor impact in Tilsit cheese. Other VSCs are present in higher concentrations, but do not significantly affect the aromagram profile. However, they can still affect the perception and taste due to synergistic effects. and can therefore, not be ignored.

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