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Gas chromatography/pulsed flame photometric detection monitoring of volatile sulfur compounds produced by metabolism of sulfur-containing amino acids in model Cheddar cheeses during ripening

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Introduction

In cheeses, volatile sulfur compounds (VSCs) such as **methanethiol**, **hydrogen sulfide**, methional and **methylsulfides** (**dimethyl sulfide**, -**disulfide** and -**trisulfide**) are regarded to be key flavor compounds. They mainly derive from the decomposition of the sulfur-containing amino acids cysteine and methionine.[1,2] In Cheddar for example, methanethiol, dimethyl disulfide and dimethyl trisulfide are reported to have a strong flavor impact, depending on the method of extraction used.[1-5] However, depending on the type of cheese and the ripening stage, their quantities are often low and may vary strongly due to high volatility and reactivity. Monitoring VSCs during cheese ripening hence remains a challenge. The use of an extraction technique such as headspace solid-phase microextraction (HS-SPME) with an appropriate fiber coating for highly volatile compounds combined with gas chromatography with sulfur specific detection (pulsed flame photometric detector, GC/PFPD) is recommended.[3-5]

Objectives

- Monitoring VSCs during Cheddar ripening over three months (sampling after 24h, 30 days, 60 days and 90 days of ripening)
- Extraction by HS-SPME and quantification by GC/PFPD using internal standards

Experimental: sample preparation and calibration curve

Five model Cheddar cheeses were manufactured using *L. casei* strains as adjunct cultures that possess the *cysK2-ctl1-cysE2* operon. VSCs were analyzed by GC/PFPD using HS-SPME. [6]
Two internal standards were used to correct for any variations of the detector and change of the fiber during the experiment. An external calibration curve was established to determine the correction factor between the target molecules and the internal standards.

Sample preparation

Ground Cheddar samples (2 g in a 20 mL-HS-vial) were spiked with 10 % (*w/w*) of a solution of internal standards (ISTD1: ethyl methyl sulfide, 100 µg/kg and ISTD2: methyl propyl sulfide, 50 µg/kg in Miglyol® 812, a caprylic/capric triglyceride). All vials were flushed with Argon for 30s after addition of the sample with closed caps using needles.

External calibration

Ground Cheddar model cheese containing a very low concentration of VSCs (ripening stopped after a maximum of 24 hours) was mixed with 10 % (*w/w*) of a calibration solution containing a mixture of target VSCs (dimethyl sulfide, -disulfide and -trisulfide) in Miglyol® 812 at five different concentrations.

HS-SPME sample extraction conditions

- Sample conditioning: 10 min at 60°C, $t_{adsorption}$= 30 min
- Fibre: **CAR/PDMS 85 µm 1 cm**

Monitoring of target VSCs during Cheddar cheese ripening

At very low concentrations, VSCs are not detected in MS-TIC mode. PFPD is a more suitable detector for the analysis of sulfur compounds.

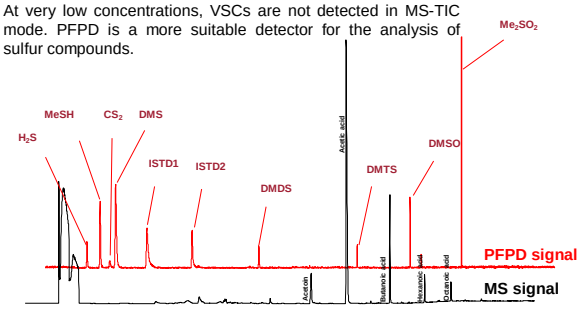

Fig. 1 Comparison of the MS-TIC (total ion count) signal *vs.* PFPD signal of Cheddar headspace (90 days of ripening) extracted with a **CAR/PDMS 85 µm** SPME fibre**.** H_2S: Hydrogen sulfide, MeSH: Methanethiol, CS_2 : Carbone disulfide, DMS: Dimethyl sulfide, ISTD1: Ethyl methyl sulfide (at 10 µg kg^{-1}), ISTD2: Methyl propyl sulfide (at 5 µg kg^{-1}), DMDS: Dimethyl disulfide, DMTS: Dimethyl trisulfide, DMSO: Dimethyl sulfoxide, Me_2SO_2: Dimethyl sulfone. ISTD1 was used for the quantification of H_2S, MeSH, DMS. ISTD2 was used for the quantification of DMDS and DMTS.

Sum of GC/PFPD signals as total target sulfur compounds amount in Cheddar

The concentration of sulfur compounds continuously increased during ripening. Only the sample taken after 24h of ripening and stored at –40°C remained unchanged. The very low storage temperature inhibits bacterial metabolism and as a result, the evolution of sulfur compounds.

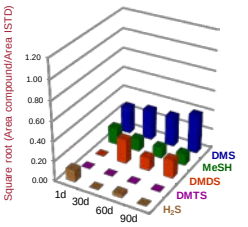

Fig 2: Cheese containing culture N° FAM18110 sampled after 24h of ripening and stored at –40°C over three months.

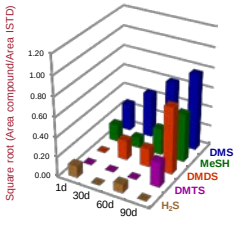

Fig 3: Cheese containing culture N° FAM18110. Normal ripening (The temperature is around 14°C with a humidity of 70–75%)

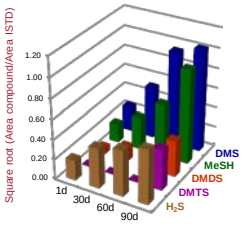

Fig 4: Cheese containing culture N°FAM18149 which showed the best performance for generation of VSCs.

Total amount of target VSCs in model Cheddar cheese

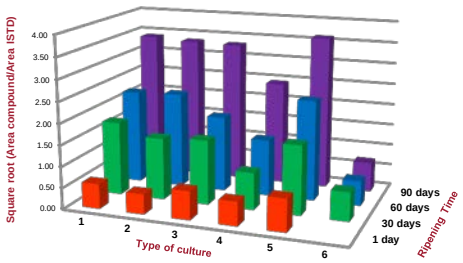

Fig. 5 Evolution of target VSCs during ripening (24 hours, 30, 60 and 90 days).
1: FAM4067, 2: FAM18101, 3: FAM18108, 4: FAM18110 (reference), 5: FAM18149, 6: Reference sampled after 24 hours of cheese ripening and stored at –40°C for 90d.

Quantification of target VSCs after 90 days of ripening in selected cheeses

Tab. 1 Quantification of target VSCs in selected cheeses using two internal standards and an external calibration curve. (n.d. : not determined)

		Sulfur compounds concentration [µg kg^{-1}] ppb				
Specific culture	**Relevant characteristics**	**H_2S**	**MeSH**	**DMS**	**DMDS**	**DMTS**
L. casei FAM4067	*ctl2*, Cheese isolate, Citrate$^{(+)}$	n.d.	n.d.	$38^{\pm 1.28}$	$1.68^{\pm 0.54}$	$2.43^{\pm 0.76}$
L. casei FAM18101	*cysK2-ctl1-cysE2*:ISLca2, Citrate$^{(-)}$	n.d.	n.d.	$40^{\pm 0.73}$	$1.52^{\pm 0.21}$	$2.64^{\pm 0.52}$
L. casei FAM18108	*cysK3-ctl2-cysE3*. Citrate$^{(+)}$	n.d.	n.d.	$36^{\pm 0.22}$	$1.37^{\pm 0.16}$	$2.28^{\pm 0.52}$
L. casei FAM18110 Ref	Overexpressed *cysK*, Citrate$^{(-)}$	n.d.	n.d.	$28^{\pm 0.83}$	$2.16^{\pm 0.00}$	$1.28^{\pm 0.02}$
L. casei FAM18149	*cysK2-ctl1-cysE2*, Milk isolate, Citrate$^{(+)}$	n.d.	n.d.	$41^{\pm 3.87}$	$1.12^{\pm 0.00}$	$3.28^{\pm 0.02}$

Conclusion

The developed headspace SPME-GC/PFPD method is very sensitive and accurate. It is suitable for the analysis and monitoring of the target VSCs hydrogen sulfide, methanethiol, dimethyl sulfide, dimethyl disulfide and dimethyl trisulfide in a semi-hard cheese matrix. Further VSCs such as carbon disulfide, dimethyl sulfoxide and dimethyl sulfone could also be monitoring. However, they were not considered as they are not regarded to be important aroma contributors.
At the end of the ripening period of 90 days, sensory evaluation with a trained panel (*n*= 8) and GC-Sniffing screenings revealed that despite a considerable and measurable increase in selected target VSCs shown by GC/PFPD, their concentrations were still below sensory detection threshold in the Cheddar matrix. A longer ripening period might eventually lead to perceptible sensory differences as the age of Cheddar cheeses found on the market starts at 90 days: three months for mild cheddar, five to six months for medium mature, nine months for mature, 15 months for extra mature, and 18 months for vintage cheddar.
The analytical method developed in this study will be applied to the quantification of VSCs in other semi-soft/hard cheeses.

Literature 1.P.M.G. Curioni and J.O. Bosset, *Int. Dairy J.*, **2002**, *12*, 959; 2. G. Smit *et al. FEMS Microbiol.Rev.*, **2005**, *29*, 591; 3. D.C. Frank *et al.*, *Lebensm.-Wiss- u.- Techn.*, **2002**, *37*, 139; 4. H.M. Burbank and M.C. Qian, *J. Chromatogr. A*, **2005**, *1066*, 149 ; *5.* H.M. Burbank and M.C. Qian, *Int. Dairy J.*, **2008**, *18*, 811; 6. B. Bogicevic *et al. Int. J. Food Microbiol.*, **2012**, *152*, 211.

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