Analysis of Gruyère type Cheeses by Purge&Trap GC-MS and Solvent Assisted Flavour Evaporation GC-O/MS

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Introduction

Microbial enzymes are essential for flavor development in cheese. Non starter lactic acid bacteria (NSLAB) originate from the factory environment or are already present in the raw milk. Selected NSLAB strains are also added as adjunct cultures during cheese production, since they have been reported to be crucial for flavor and aroma development in cheeses. NSLAB are able to convert amino acids produced from the starter cultures by milk protein degradation into aroma compounds [1]. Methional, 2- and 3-methylbutanal are examples for key aroma compounds in Gruyère cheese [2]. They originate from the amino acids methionine, leucine and isoleucine, respectively.
Facultatively heterofermentative lactic acid bacteria (FHL) are indigenous to raw milk. They inhibit growth of propionibacteria and are used in Switzerland to prevent late fermentation defects caused by the latter. FHL ferment hexoses mostly to lactic acid and grow in cheese by metabolising citrate into formic and acetic acids and CO_2 [3].
The aim of the present study was to compare the aroma compounds formed in Gruyère type hard cheeses manufactured with different FHL adjunct cultures (*Lactobacillus casei*) by different extraction methods.

Materials and methods

Samples: Gruyère type hard cheeses were produced from pasteurised milk using three different FHL non starter *Lactobacillus casei* strains (FAM 3228, FAM 6161, and FAM 8407) and ripened for 180 days. The control cheese was ripened without adjunct cultures.
Purge&Trap GC-MS: The cheeses (5g) were suspended in distilled water (20mL), and 10g of the suspension were extracted by dynamic headspace (purge&trap). The effluent was trapped on a tenax trap no.1 (Tekmar 3100). GC-MS was done using a SPB-1 sulfur capillary column with helium at 55kPa applying the following temperature program: 45°C (13min) → 240°C (5°C/min). Mass spectra were obtained in the EI mode at 70eV and a scan range from *m/z* 26-250.
SAFE: The cheeses (50 g) were extracted with diethyl ether/pentane (2:1), and the extracts were submitted to solvent assisted flavour evaporation (SAFE) according to [4].
GC-O/MS: GC-O/MS was performed with 1µL aroma extract using an Optima 1701 capillary column at a constant helium carrier gas flow of 55 mL/min. The column effluent was assessed sensorially by 4 panellists. Mass spectra were obtained in the EI mode at 70eV and a scan range from *m/z* 29-400.

Results

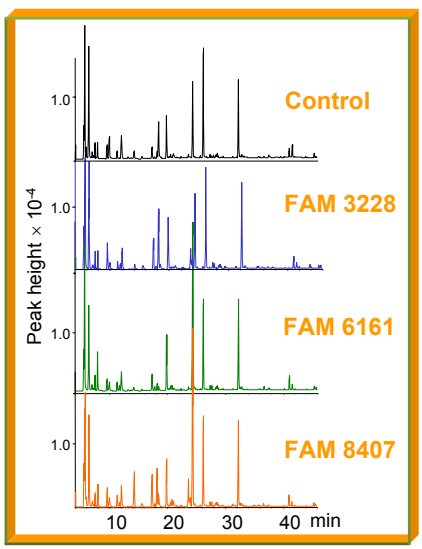

Figure 1. Purge&Trap GC profiles of different selected strains

2. The comparison of the peak heights obtained by Purge&Trap GC-MS shows that the facultatively heterofermentative strains FAM 6161 and FAM 8407 metabolise methionine into dimethyl disulfide and dimethyl trisulfide (*Figure 2*). FAM 3228, but also FAM 8407 produced the most intense signals for 2-methylpropanal and for 2- and 3-methylbutanal.

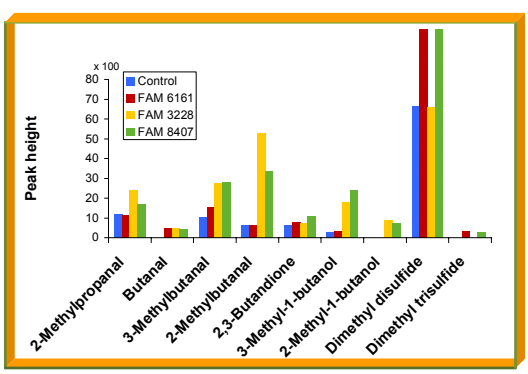

Figure 2. Selected volatiles produced by different FHL strains (Purge&trap GC-MS)

3. Solvent assisted flavour evaporation (SAFE) in combination with GC-MS and GC-O/MS was used in comparison. The extract reveals very intense signals for free fatty acids, such as acetic, butanoic, 2-methyl and 3-methyl butanoic acids (*Figure 3*). The less volatile, more polar caramel-like odorants 2,5-dimethyl-4-hydroxy-3(2*H*)-furanone and 2-ethyl-4-hydroxy-3(2*H*)-furanone were only detected by SAFE-GC-O/MS. Table 1 compares the peak heights for selected odorants. FAM 8407 produced intense fatty acid signals.

1. The Purge&Trap profile shown in *Figure 1* reveals volatile aldehydes and ketones which have been described as aroma compounds in Gruyère and other cheeses, such as 2- and 3-methylbutanal, 2,3-butandione, dimethyl disulfide and dimethyl trisulfide. Free fatty acids were not detected in the Purge&Trap chromatogram, probably because the sample consisted of an aqueous suspension of cheese.

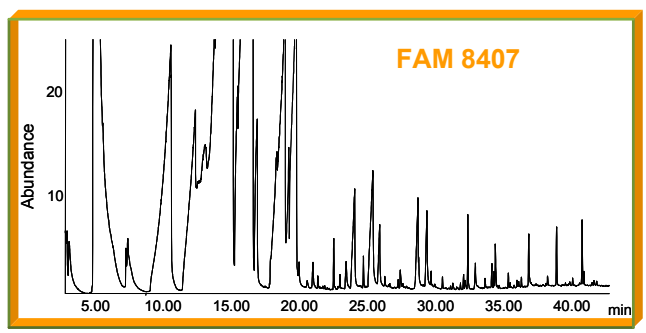

Figure 3. GC chromatogram of a cheese extract (SAFE) using FAM 8407 as adjunct culture

- Purge&Trap GC-MS retrieves highly volatile odorants whereas SAFE-GC-MS revealed more polar and less volatile compounds. Both can be used as complimentary techniques.
- Facultatively heterofermentative lactic acid bacteria strains are promising adjunct cultures for flavour production in Gruyère type cheese, since they produce important aroma compounds such as sulfides, aldehydes and furanones.

Table 1. Profiles of selected odorants analysed by SAFE-GC-O/MS

Compound	Odour quality	RI [a]	Peak height / 1000		
			8407	6161	3228
2,3-Butandione	Buttery, creamy	Co-elution with diethyl ether/pentane			
Acetic acid	Pungent	765	21873	20108	22083
Dimethyl disulfide	Onion, sulfury	802	180	165	153
Propanoic acid	Pungent, rancid	904	5817	6246	4899
2-Methyl propanoic acid	Goat-like	978	5285	4390	2653
Butanoic acid	Rancid, sweaty	1009	15338	13895	12367
Methional	Cooked potato	1033	No signal detected		
3-Methyl butanoic acid	Rancid, sweaty	1035	4473	3674	3888
1-Octene-3-one	Mushroom-like	Co-elution with 2-methyl butanoic acid			
2-Methyl butanoic acid	Sweaty, fatty	1054	8808	8000	6520
Hexanoic acid	Rancid, cheesy	1173	5862	6150	5854
2-Nonanone	Cheesy	1181	511	703	581
2,5-Dimethyl-4-hydroxy-3(2*H*)-furanone	Sweet, caramel-like	1237	183	143	266
2-Ethyl-4-hydroxy-5-methyl-3(2*H*)-furanone	Sweet, burnt sugar, caramel	1336	130	62	n.d.
δ-Decalactone	Coconut-like	1714	680	624	1017
δ-Dodecalactone	Fruity	1934	501	469	787

[a] linear retention index (OV 1701)

References

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