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Distribution of the volatile (flavour) compounds in Raclette cheese produced with different staphylococci in the smear

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Introduction

Smear coated cheese types such as Raclette, Gruyère AOC, L'Etivaz AOC, Tête de Moine AOC, Tilsiter, Appenzeller and Vacherin fribourgeois are submitted to a double ripening process: an inner ripening due to both, the lactic starters used for the manufacture as well as the native raw milk flora, and an outer ripening due to the microbial biofilm growing on the rind.

Previous studies have reported significant concentration gradients of volatile (flavour) compounds in Gruyère AOC cheese during ripening (1–4) but did not investigate the microbial activity of the smear in the different zones of the loaf. The enzymatic activities responsible for volatile aroma components of cheese are mainly derived from the three following major catabolic pathways: lactose, lipid and protein catabolism (5–7). The control of these metabolites is therefore necessary to guarantee consistent quality and satisfy consumer expectations. Therefore a better knowledge of the microbial growth on surface ripened cheese types (8, 9) is needed.

Stahnke (10) has shown the influence of different staphylococci on the flavour formation in sausages. *Staphylococcus* spp. are also dominant components of the biofilm of smear coated or smear ripened cheeses (11, 12). In order to better understand the complex development of flavour by staphylococci on cheese, the effect of *Staphylococcus succinus* subsp. *casei* and *Staphylococcus equorum* subsp. *linens* on the volatile products of Raclette cheese was investigated in this preliminary study.

Experimental

Sample material

Raclette cheeses were made from thermised milk and ripened over 5 months at 12 °C and 96 % relative humidity. Cheeses were smeared with a starter culture containing *Debaryomyces hansenii, Geotrichum candidum, Arthrobacter nicotianae* and *Corynebacterium casei*. One batch was smeared with additional *Staphylococcus succinus* subsp. *casei* DSM 15096^T (Raclette 1) and the other batch with *Staphylococcus equorum* subsp. *linens* DSM 15097^T (Raclette 2) (table 1). Each investigated cheese block was cut into five parts corresponding to inner, outer, and discarded zones, whereof only the inner and outer zones were used for further analysis (fig. 1). The inner and outer zones of a quarter cheese block were pooled for analysis.

Table 1
Composition of starter cultures

Raclette 1	*Raclette 2*
Staphylococcus succinus subsp. *casei* DSM 15096^T	*Staphylococcus equorum* subsp. *linens* DSM 15097^T
Brevibacterium linens SB108	*Brevibacterium linens* SB108
Corynebacterium casei SB67	*Corynebacterium casei* SB67
Arthrobacter nicotianae SB42	*Arthrobacter nicotianae* SB42
Geotrichum candidum GC15	*Geotrichum candidum* GC15
Debaryomyces hansenii GR3C9	*Debaryomyces hansenii* GR3C9

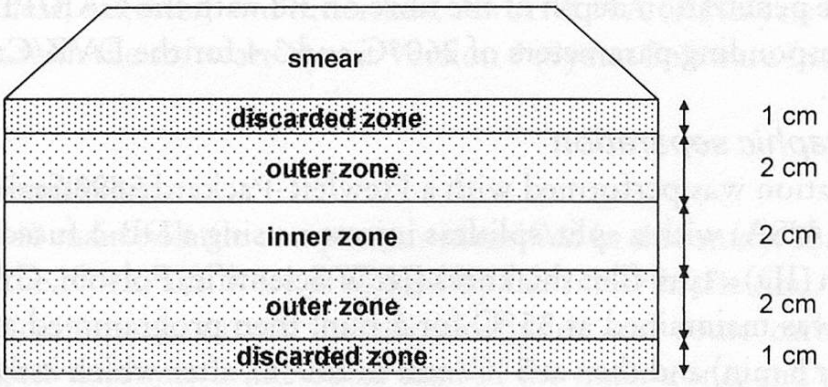

Figure 1 **Zone fractioning of Raclette cheese samples.**

Sample preparation for physicochemical analysis

The Raclette cheese samples were stored deep frozen at -20 °C packed in aluminium foil. Before their analysis, they were left for 10 h at approximately +6 °C and manually grated using a domestic rasp. The analyses were carried out twice using two different preparation techniques. For the samples A (grated), 10±0.01 g grated cheese were weighed. For the samples B (dispersed), 5±0.01 g grated cheese

were finely dispersed with 7.5 g boiled Milli-Q-water using a POLYTRON PT 30000 (Kinematica, Littau/Luzern, Switzerland) directly in the glass vials. The samples prepared on both ways were left for 12 h at room temperature and conditioned for 60 min (equilibration time) at 45 °C in 22 ml clear glass vials closed with screw top polypropylene cap with a hole and PTFE/silicon septa (Supelco, art no 27212, Bellefonte, PA, USA).

The high concentrations of volatile fatty acids in cheese is often responsible for peak overlapping. Therefore the different sample preparation techniques A and B mentioned above were employed. The former (A) is generally more efficient (higher extraction rate) but also more sensitive to fatty acid interferences than the latter (B) (i.e. peak overlapping preventing precise peak integration).

SPME technique

The Headspace Solid-Phase Microextraction (HS-SPME) was performed using two different types of fibres (Supelco, Bellefonte, PA, USA): StableFlex Fibre 85 µm Carboxen/Polydimethylsiloxane coating (CAR/ PDMS) and 50/30 µm Divinylbenzene/Carboxen on Polydimethylsiloxane (DVB/CAR/PDMS) on a 2 cm StableFlex fibre in order to analyse a greater number of volatile compounds. For both fibres a blank value was obtained.

The SPME fibres were introduced into the headspace of the vials containing the samples for 30 min at 45 °C (loading time). For loading the fibre, the penetration depth of the fibre was set on 2.0 (scale on the SPME halter) with the CAR/PDMS fibre, and on 1.6 with the DVB/CAR/PDMS. For the thermodesorption, the injector was switched for 5 min to splitless mode. The injector temperature was set at 280 °C and the penetration depth of the fibre on 3.8 with the CAR/PDMS fibre, and with the corresponding parameters of 260 °C and 3.4 for the DVB/CAR/PDMS.

Chromatographic separation

The separation was performed with a Hewlett-Packard 5890 Series II – (Agilent Technologies, USA) with a split/splitless injector, using a DB-1 fused silica column, 60m × 0.32mm (ID) × 1µm film thickness (J&W Scientific, Folsom, CA, USA). Oven temperature was maintained at 35 °C for 5 min, then programmed at 10 °C/min to 45 °C (held for 5 min) and then at 5 °C/min to 250 °C, after which it was held for further 10 min at 250 °C. The carrier gas was helium set at 110 kPa at 45 °C. Injector temperature depended on the type of SPME fibre used (280 °C or 260 °C, see *SPME technique*). FID temperature was set at 300 °C and MS-Interface at 280 °C.

Detection

Three detectors were used. Two were mounted simultaneously in parallel by splitting the flow at the end of the capillary column; one stream led to a flame ionisation detector (FID), the other to a mass-selective detector (MSD model HP 5971A) or a sniffing port for olfactometry. The MSD operated in the scan mode

(TIC) from 26 to 300 amu at 1.6 scan/s, ionisation was by EI at 70 eV by autotuning. The MSD was used for the identification of the volatile (flavour) compounds using the Wiley library (13). In addition the identity was confirmed by comparing the retention indices of authentic reference compounds. The FID signal was used for the semi-quantitative determination of the peak height. Only compounds with a FID peak height greater than the value of 50 arbitrary units (fixed threshold) have been considered for this study.

Microbiological analysis

The surface microflora of the cheeses was analysed after 2, 3, 4, 6, 8 and 12 weeks. For this an area of 10 cm^2 of the smear film was scraped of with a sterile swap and scalpel, serially diluted in 20 g/l NaCl solution and plated on mSK (12), mMA (14), PY Agar (Becton Dickinson), and VRBD (Merck). The mSK, mMA and PY plates were incubated under aerobic conditions at 25 °C for 6 days and the VRBD under aerobic conditions at 37 °C for 24 hours. The isolates were identified on the basis of biochemical characteristics with the ID 32 STAPH (bioMérieux) and MicroLog (Biolog) systems including activities of catalase and oxidase (15). All counts are given per cm^2 smear surface.

Sensory analysis

After five months of ripening, cheeses were subjected to sensory evaluation by a fifteen-member professional tasting panel familiar with Raclette cheese. The cheeses were tasted at room temperature under standard conditions. The panel graded on a scale from zero (weak) to four (strong) the smell when breaking as well as intensity of aroma, sourness, saltiness, and bitterness. An ANOVA (ANalysis Of VAriance) was carried out with the software Systat Version 9 (Systat Software Inc., Richmond, CA).

Results and discussion

Among the 40 main volatile compounds identified in this study, 23 depended on the location in the block, and were produced by the smear in the rind. Indeed, most volatile substances found, except for the aldehydes and some carboxylic acids, occurred in higher amounts in the outer than in the inner zone, and also in higher amounts in cheese made with *Staphylococcus succinus* subsp. *casei* than in those made with *Staphylococcus equorum* subsp. *linens*. Tables 2 to 7 indicate the relative concentrations (FID arbitrary unit) of these compounds listed according to their functional groups within each zone as well as their ratios (outer/inner). Table 2 lists the hydrocarbons, table 3 the aldehydes, table 4 the ketones, table 5 the alcohols, table 6 the carboxylic acids, and table 7 the miscellaneous substances. Figure 2 shows typical FID chromatograms of the outer and inner zones of cheeses made with *S. succinus* subsp. *casei*, using the SMPE fibre CAR/ PDMS.

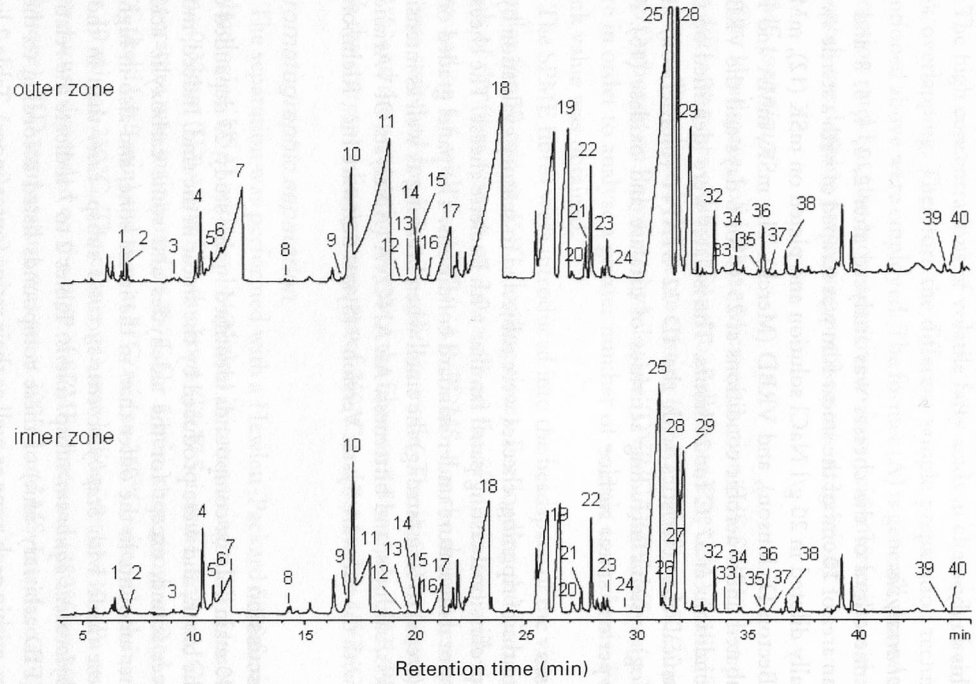

Figure 2 **Typical FID chromatograms of the outer and inner zone of grated (A) cheese using a DB-1 separation column with a CAR/PDMS fibre.** The cheese was made with *Staphylococcus succinus* subsp. *casei* (Raclette 1). The peak numbering in this figure corresponds to the peak numbering in table 2 to 7.

Due to the uncertainty of the chromatographic data (approx. +/-20% of the FID peak height), differences in the concentration of volatile components are considered as significant only when the peak ratio (outer/inner zone) was higher than 1.5 or lower than 0.6. The peaks generated by the SPME fibres themselves (artefacts) were omitted.

Unfortunately the addition of water in method B did not result in a decrease in overlapping of peaks as expected due to high concentrations of volatile fatty acids. This effect was reported by using the Purge and Trap method (16).

The following components 1-octanol, 1-hexanol, 2-undecanone, acetic acid, butanoic acid, 2-methyl butanoic acid, 3,7-dimethyl 1,6-octadiene, terpenes and trimethyl benzene were better extracted from dispersed samples with the addition of water (B), whereas all other compounds were extracted better from grated samples without any addition of water (A).

Hydrophobic compounds were isolated better with fibres coated with DVB/CAR/PDMS. Figure 3 shows typical FID chromatograms of the grated (A) outer zone of cheese made with *S. succinus* subsp. *casei*, using the SMPE fibre CAR/PDMS and DVB/CAR/PDMS, and dispersed samples (B) using the SMPE fibre DVB/CAR/PDMS.

The results had shown much more significant concentration gradients if the discarded zones had been introduced in this study. This part of the block was, however, discarded because it is not edible and may contain pathogens such as *Listeria monocytogenes* (17).

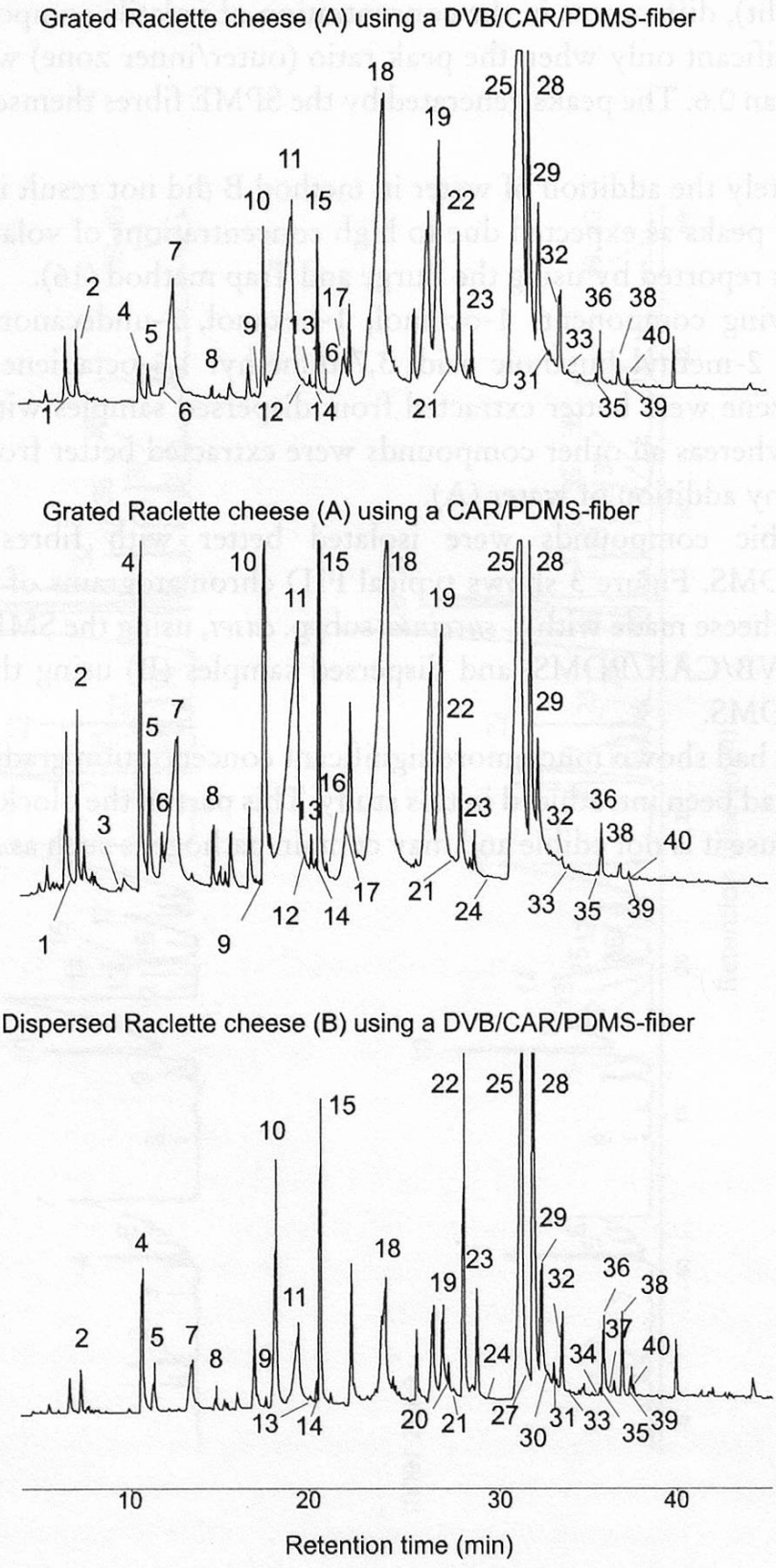

Figure 3 **FID chromatograms of different analytical methods used.** All samples derive from the outer zone of a cheese made with *Staphylococcus succinus* subsp. *casei* (Raclette 1). The peak numbering in this figure is the same as in figure 2 and corresponds to the peak numbering in table 2 to 7.

Hydrocarbons

The more pronounced occurrence of hydrocarbons (table 2) in the outer zone indicates their biosynthesis by micro-organisms of the cheese surface and is probably derived from lipids (18). Trimethyl benzene, 1,3- and 1,4-dimethyl benzene have already been reported. Their concentration seems to vary during deep frozen storage of samples (19).

Table 2
Hydrocarbons

Peak[a] no.	*Compounds*	*LRI[b]*	*SPME[c]*	*Raclette 1 (peak height, arbitrary unit)*						*Raclette 2 (peak height, arbitrary unit)*						*Odour descriptor*	*Bibliographic references*
				grated (A)			*dispersed (B)*			*grated (A)*			*dispersed (B)*				
				outer	*inner*	*ratio*	*outer*	*inner*	*ratio*	*outer*	*inner*	*ratio*	*outer*	*inner*	*ratio*		
21	Benzene, 1,3- and 1,4-dimethyl	863	Y	603	708	0.9	n.d.	n.d.	n.d.	431	230	1.9	n.d.	n.d.	n.d.	dry, lightly pungent	(41)
27	1,6-Octadiene, 3,7-dimethyl- (T)	944	Y	n.i.	n.i.	n.i.	872	667	1.3	804	352	2.3	607	282	2.2	terpene, mint, harsh	–
30	Alkene	968	Y	n.i.	n.i.	n.i.	n.d.	n.d.	n.d.	791	449	1.8	n.d.	n.d.	n.d.		(41, 42)
31	Terpene	977	Y	n.i.	n.i.	n.i.	601	485	1.2	482	308	1.6	703	540	1.3	woody	(42)
32	Benzene, trimethyl-	991	Y	561	488	1.1	289	198	1.5	332	200	1.7	432	216	2.0	herbaceous	(42)
33	Heptane, 2,2,4,6,6-pentamethyl- (T)	1003	Y	498	395	1.3	n.d.	n.d.	n.d.	1145	566	2.0	n.d.	n.d.	n.d.		(43)
34	Limonene	1033	Y	193	147	1.3	n.d.	n.d.	n.d.	107	50	2.1	n.d.	n.d.	n.d.	green	(42, 44–46)

Caption: [a]=corresponding to fig. 2 and 3; [b]=linear retention index using a DB-1 column; [c]X=CAR/PDMS-fibre, Y=DVB/CAR/PDMS-fibre; (T)=tentatively identified; n.i.=not integrated due to peak overlapping; n.d.=not detected (below the detection limit: 50 arbitrary units).

Aldehydes

Aldehydes (table 3) are derived either from amino acids (20), methional (21) or from lipids (22). They are usually transitory compounds in cheeses because they are rapidly reduced to alcohols or oxidised to the corresponding carboxylic acids (23). In the current study, 3-methyl butanol and 2-methyl propanoic acid, derived from 3-methyl butanal and 2-methyl 2-propanal respectively, were e.g. detected.

Table 3
Aldehydes

Peak[a] no.	*Compounds*	*LRI[b]*	*SPME[c]*	*Raclette 1 (peak height, arbitrary unit)*						*Raclette 2 (peak height, arbitrary unit)*						*Odour descriptor*	*Bibliographic references*
				grated (A)			*dispersed (B)*			*grated (A)*			*dispersed (B)*				
				outer	*inner*	*ratio*	*outer*	*inner*	*ratio*	*outer*	*inner*	*ratio*	*outer*	*inner*	*ratio*		
1	2-Propenal	462	X	188	247	0.8	n.d.	n.d.	n.d.	194	446	0.4	n.d.	n.d.	n.d.		(47)
3	Propanal, 2-methyl-	532	X	114	267	0.4	n.d.	n.d.	n.d.	228	267	0.9	n.d.	n.d.	n.d.	bakery-like, malty	(42, 44, 48, 49)
8	Butanal, 3-methyl-	634	X	1578	2447	0.6	n.d.	n.d.	n.d.	2546	3321	0.8	n.d.	n.d.	n.d.	milky, chocolate	(34, 36, 49–51)
9	Pentanal	674	X	192	283	0.7	n.d.	n.d.	n.d.	242	457	0.5	n.d.	n.d.	n.d.	pungent, medicinal	(42, 44, 52)
26	Benzaldehyde	940	X	n.i.	n.i.	n.i.	n.d.	n.d.	n.d.	826	301	2.7	n.d.	n.d.	n.d.	aromatic, sweet	(42, 44, 53)

Caption: See table 2

Ketones

Ketones (table 4) are common constituents found in most cheese varieties. Moulds, such as *Geotrichum candidum*, are most important for their formation in mold-ripened cheeses. The precursors of ketones are mainly fatty acids which are transformed by β-oxidation and therefore originate from the cheese surface (20). In Raclette cheese, all ketones detected occurred in higher amounts in the outer than inner zones.

Table 4
Ketones

Peak[a] no.	*Compounds*	*LRI[b]*	*SPME[c]*	*Raclette 1 (peak height, arbitrary unit)*						*Raclette 2 (peak height, arbitrary unit)*						*Odour descriptor*	*Bibliographic references*
				grated (A)			*dispersed (B)*			*grated (A)*			*dispersed (B)*				
				outer	*inner*	*ratio*	*outer*	*inner*	*ratio*	*outer*	*inner*	*ratio*	*outer*	*inner*	*ratio*		
2	2-Propanone	468	X	4575	1415	3.2	n.d.	n.d.	n.d.	2424	1325	1.8	n.d.	n.d.	n.d.	slightly fruity	(46, 52)
4	2,3-Butanedione	558	X	9897	3878	2.6	n.d.	n.d.	n.d.	4922	2522	2.0	n.d.	n.d.	n.d.	buttery, creamy	(49, 50, 52)
5	2-Butanone	570	X	3545	1785	2.0	n.d.	n.d.	n.d.	1277	847	1.5	n.d.	n.d.	n.d.	slightly fruity	(41, 52, 54)
16	2-Pentanone, 3-methyl-	736	X	562	361	1.6	n.d.	n.d.	n.d.	349	200	1.7	n.d.	n.d.	n.d.		(41, 42, 44)
22	2-Heptanone	871	Y	4062	1971	2.1	n.d.	n.d.	n.d.	2589	1493	1.7	n.d.	n.d.	n.d.	fruity, musty, sweet	(36, 50, 51)
36	Ethanone, 1-phenyl-	1048	Y	1463	285	5.1	n.d.	n.d.	n.d.	349	75	4.7	n.d.	n.d.	n.d.	green	(41, 42, 44)
38	2-Nonanone	1074	Y	775	250	3.1	n.d.	n.d.	n.d.	351	247	1.4	n.d.	n.d.	n.d.	malty, cheese-like	(36, 49)
40	2-Undecanone	1278	Y	154	59	2.6	244	109	2.2	70	50	1.4	157	75	2.1	floral	(34, 36, 49)

Caption: See table 2

Alcohols

Alcohols (table 5) are considered to originate from the corresponding aldehydes by a reaction pathway involving alcohol dehydrogenases. With the exception of 1-hexanol, they were mainly located in the outer zone. As already mentioned before, 2-butanol could be a precursor of 2-butanone (24, 25). The presence of the branched-chain primary alcohols such as 2-methyl-1-butanol, 3-methyl-1-butanol and 1-phenyl ethanol indicates the conversion of the aldehydes produced from isoleucine, leucine and phenylalanine, respectively.

Table 5
Alcohols

Peak[a] no.	*Compounds*	*LRI[b]*	*SPME[c]*	*Raclette 1 (peak height, arbitrary unit)*						*Raclette 2 (peak height, arbitrary unit)*						*Odour descriptor*	*Bibliographic references*
				grated (A)			*dispersed (B)*			*grated (A)*			*dispersed (B)*				
				outer	*inner*	*ratio*	*outer*	*inner*	*ratio*	*outer*	*inner*	*ratio*	*outer*	*inner*	*ratio*		
6	2-Butanol	585	X	1399	957	1.5	n.d.	n.d.	n.d.	661	446	1.5	n.d.	n.d.	n.d.		(2, 42)
12	3-Buten-1-ol, 3-methyl-	716	X	822	562	1.5	n.d.	n.d.	n.d.	303	206	1.5	n.d.	n.d.	n.d.	slightly spicy	(44, 46)
13	1-Butanol, 3-methyl-	721	Y	1320	671	2.0	n.d.	n.d.	n.d.	763	470	1.6	n.d.	n.d.	n.d.		(36, 42, 44)
14	1-Butanol, 2-methyl-	726	X	657	354	1.9	n.d.	n.d.	n.d.	360	152	2.4	n.d.	n.d.	n.d.		(36)
20	1-Hexanol	853	Y	n.d.	n.d.	n.d.	965	1037	0.9	1107	531	2.1	1159	458	2.5	medicinal, fruity	(42, 44)
23	2-Heptanol	886	Y	1655	598	2.8	n.d.	n.d.	n.d.	1093	461	2.4	n.d.	n.d.	n.d.	slightly grassy	(35, 36, 49)
35	Ethanol, 1-phenyl-	1043	Y	367	152	2.4	n.d.	n.d.	n.d.	142	56	2.5	n.d.	n.d.	n.d.	aromatic, green	(44)
37	1-Octanol	1051	Y	n.d.	n.d.	n.d.	217	105	2.1	n.d.	n.d.	n.d.	159	50	3.2		(42, 44)

Caption: See table 2

Carboxylic acids

Table 6 shows the relative concentrations of carboxylic acids in the inner and outer zones of the Raclette cheese. The distribution of carboxylic acids was very heterogeneous, probably due to intensive changes in formation and degradation during ripening (4). In contrast to *S. equorum* subsp. *linens*, *S. succinus* subsp. *casei* ferments propionic acid (12). This fact could explain why the outer/inner ratios of propionic acid of cheese produced with *S. succinus* subsp. *casei* are lower than 1 in contrast to the cheese produced with *S. equorum* subsp. *linens*. All the carboxylic acids detected have also been found in Gruyère AOC (4).

Table 6
Carboxylic acids

Peak[a] no.	*Compounds*	*LRI[b]*	*SPME[c]*	*Raclette 1 (peak height, arbitrary unit)*						*Raclette 2 (peak height, arbitrary unit)*						*Odour descriptor*	*Bibliographic references*
				grated (A)			*dispersed (B)*			*grated (A)*			*dispersed (B)*				
				outer	*inner*	*ratio*	*outer*	*inner*	*ratio*	*outer*	*inner*	*ratio*	*outer*	*inner*	*ratio*		
7	Acetic acid	594	Y	n.d.	n.d.	n.d.	1291	1313	1.0	n.d.	n.d.	n.d.	1252	676	1.9	aluminium acetate	(34, 35, 49)
11	Propanoic acid	702	Y	5047	5730	0.9	n.d.	n.d.	n.d.	3293	1362	2.4	n.d.	n.d.	n.d.	sour milk-like, fruity	(37, 49, 51)
17	Propanoic acid, 2-methyl-	752	Y	1195	1604	0.7	548	1008	0.5	558	316	1.8	n.d.	n.d.	n.d.	acidic, cheese-like	(31, 34, 49)
18	Butanoic acid	780	Y	n.d.	n.d.	n.d.	3232	4660	0.7	n.d.	n.d.	n.d.	3451	2182	1.6	sweaty, rancid	(35, 37, 51)
19	Butanoic acid, 2-methyl-	839	Y	n.d.	n.d.	n.d.	2464	3584	0.7	n.d.	n.d.	n.d.	1924	614	3.1	sweaty, cheese-like	(37, 51, 55, 56)
25	Pentanoic acid, 4-methyl-	937	Y	18849	15071	1.3	n.d.	n.d.	n.d.	4811	1231	3.9	n.d.	n.d.	n.d.	cheese-like, pungent	(55)
29	Hexanoic acid	962	Y	4770	4778	1.0	n.d.	n.d.	n.d.	2648	1394	1.9	n.d.	n.d.	n.d.	cheesy, fatty, sweaty	(35, 37, 49, 51)

Caption: See table 2

Miscellaneous substances

Several esters are produced by yeasts (25) and staphylococci (26) which show a trend for esterifying butanoic acid with primary alcohols (27). The ester found in this study presented an outer/inner ratio >1. Moreover, Raclette cheeses made with *S. succinus* subsp. *casei* contained about twenty times more phenol than cheese produced with *S. equorum* subsp. *linens* (table 7). This compound could have been formed from benzene under oxygen-limited conditions beneath the cheese rind (28). The lack of benzene formation may be due to a high turnover of benzene or to a difference in the phenol degradation pathway between the staphylococci. It is however more probable that phenol was formed from shikimic acid (29) or from tyrosine (30, 31) from the biomass instead of benzene (32).

Table 7
Miscellaneous substances

Peak[a] no.	*Compounds*	*LRI[b]*	*SPME[c]*	*Raclette 1 (peak height, arbitrary unit)*						*Raclette 2 (peak height, arbitrary unit)*						*Odour descriptor*	*Bibliographic references*
				grated (A)			*dispersed (B)*			*grated (A)*			*dispersed (B)*				
				outer	*inner*	*ratio*	*outer*	*inner*	*ratio*	*outer*	*inner*	*ratio*	*outer*	*inner*	*ratio*		
10	Acetoin	680	Y	4418	2078	2.1	n.d.	n.d.	n.d.	5506	3603	1.5	n.d.	n.d.	n.d.	buttery	(2, 44, 52)
15	Disulfide, dimethyl	729	Y	4166	921	4.5	n.d.	n.d.	n.d.	1681	455	3.7	n.d.	n.d.	n.d.	cooked vegetables	(42, 44, 49, 51)
24	Ester	900	Y	107	88	1.2	n.d.	n.d.	n.d.	78	46	1.7	n.d.	n.d.	n.d.	bakery	(2, 42, 44)
28	Phenol	955	Y	26267	8216	3.2	n.d.	n.d.	n.d.	1158	458	2.5	n.d.	n.d.	n.d.	medicinal	(41, 42, 44)
39	Indol	1276	Y	186	71	2.6	n.d.	n.d.	n.d.	50	50	1.0	n.d.	n.d.	n.d.	moth ball, stable	(42, 44, 51)

Caption: See table 2

Methional was neither detected with GC-MS (its detection is very difficult) nor with olfactometry. This is unusual since methional can be produced by *Brevibacterium linens* and *Geotrichum candidum* (20) and has been detected in many other types of cheese (20, 33–37). Possibly it has been missed due to peakoverlapping with all three detectors used or because methional was below the detection or recognition threshold.

Microbiological analysis

Figures 4 and 5 show the development of microorganisms on the surface of Raclette 1 and 2. The colony forming units (cfu) isolated from the two batches did not differ significantly considering the non-staphylococcal microflora. After 6 weeks of ripening the genus *Corynebacterium* was dominant (10^8 cfu/cm^2) followed by *Arthrobacter* (10^7 cfu/cm^2) and *Staphylococcus* (10^7 cfu/cm^2 on Raclette 1 and 7×10^7 cfu/cm^2 on Raclette 2). In the case of these genera the results correspond with other studies (11, 38). The *Enterobacteriaceae* were at levels of about 10^6 cfu/cm^2 during the whole ripening period. These counts are about 10 fold higher than previously reported on Tilsiter (11).

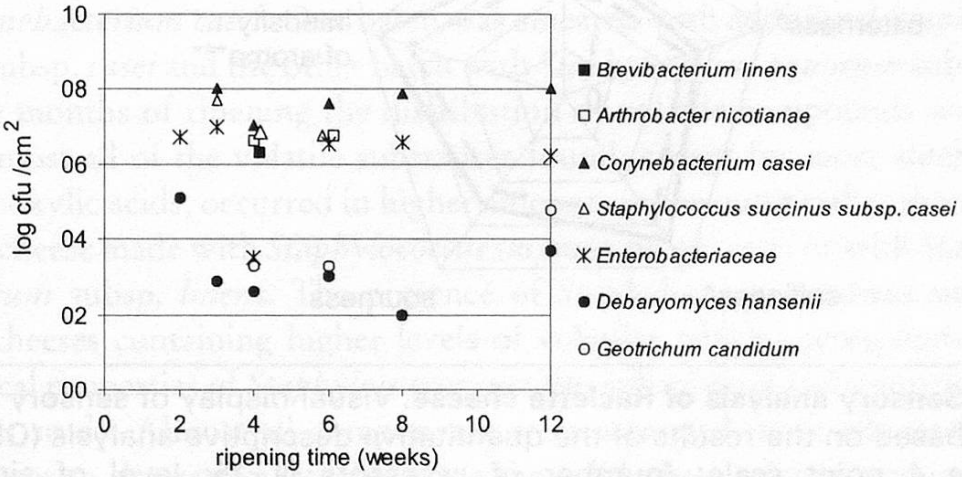

Figure 4 **Development of microorganisms of Raclette 1 which contained *Staphylococcus succinus* subsp. *casei* DSM 15096^T in the surface starter culture.**

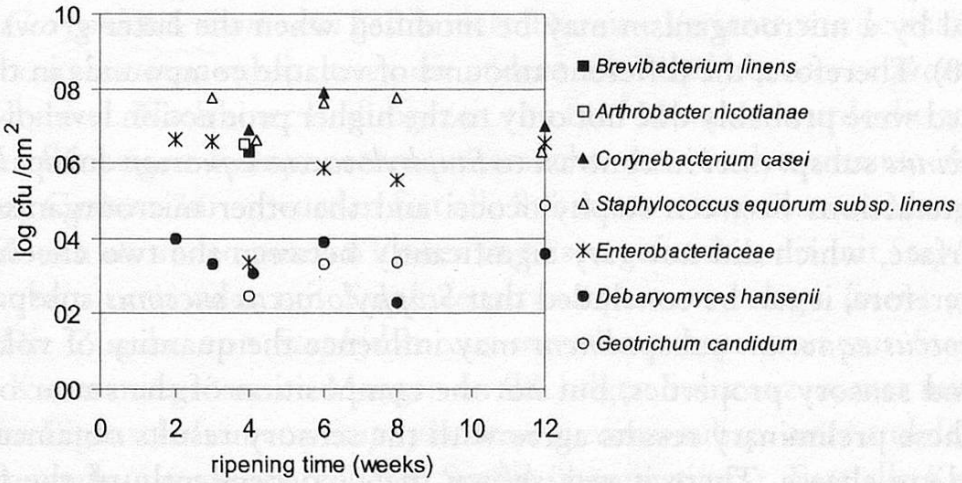

Figure 5 **Development of microorganisms of Raclette 2 which contained *Staphylococcus equorum* subsp. *linens* DSM 15097^T in the surface starter culture.**

Sensory analysis

The application of *S. equorum* subsp. *linens* resulted in a significantly milder product, with respect to all attributes tested, compared to cheeses produced with *S. succinus* subsp. *casei* DSM 15096^T (fig. 6). It has been shown previously that these results are independent of the factory (12).

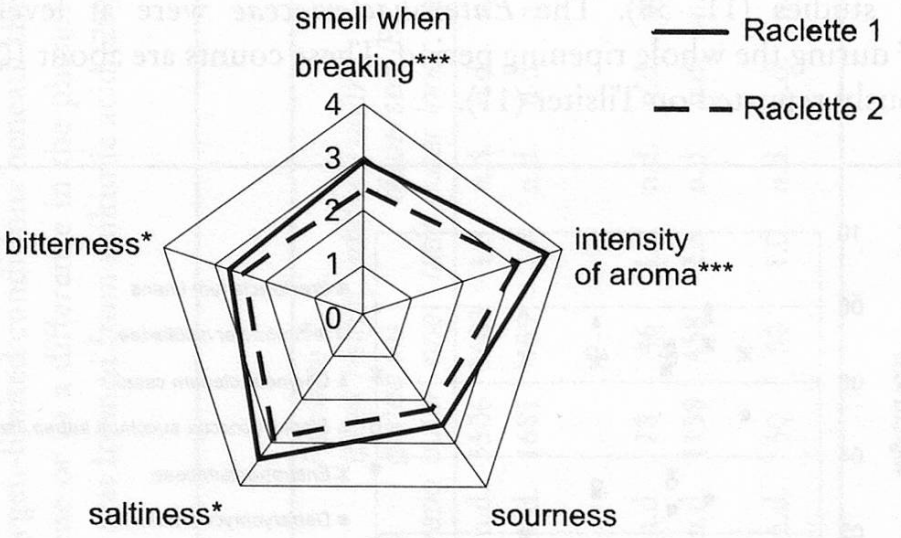

Figure 6 **Sensory analysis of Raclette cheese.** Visual display of sensory attributes based on the results of the quantitative descriptive analysis (QDA) using a 4 point scale; (number of assessors N=15; level of significance *=p<0.05, ***=p<0.001)(12).

Conclusion

It has been shown previously that staphylococci are able to produce different volatile compounds in pure culture (26, 27). Moreover, the production of a given compound by a microorganism may be modified when the latter grows in mixed cultures (8). Therefore, the different amounts of volatile compounds in the cheeses investigated were probably due not only to the higher production level of *Staphylococcus succinus* subsp. *casei* in contrast to *Staphylococcus equorum* subsp. *linens*, but also to interactions between staphylococci and the other microorganisms of the cheese surface, which did not vary significantly between the two cheeses investigated. Therefore, it can be concluded that *Staphylococcus succinus* subsp. *casei* and *Staphylococcus equorum* subsp. *linens* may influence the quantity of volatile compounds and sensory properties, but not the composition of the smear of Raclette cheese. These preliminary results agree with the sensory results obtained with the same Raclette cheese. There it was shown that, independently of the factory, in experimental Raclette cheese, the application of *Staphylococcus equorum* subsp. *linens* DSM 15097^T results in a significantly milder product compared to cheese produced with *S. succinus* subsp. *casei* DSM 15096^T.

The higher rate of biochemical reactions in *Staphylococcus succinus* subsp. *casei* compared to *Staphylococcus equorum* subsp. *linens* (12) could possibly explain the higher amounts of alcohols, hydrocarbons and ketones in the outer zone of the cheese produced with *Staphylococcus succinus* subsp. *casei*. Further work, however, is necessary with many more Raclette cheese loaves than the two loaves currently investigated to confirm these preliminary results. In addition, Raclette does not seem to be investigated in broad range reviews and databases on volatile flavour compounds (39, 40).

Summary

Raclette cheeses made from thermised milk were smeared with a starter culture containing *Debaryomyces hansenii, Geotrichum candidum, Arthrobacter nicotianae* and *Corynebacterium casei*. One batch was smeared with additional *Staphylococcus succinus* subsp. *casei* and the other batch with *Staphylococcus equorum* subsp. *linens*. After five months of ripening the distribution of volatile compounds was investigated. Almost all of the volatile substances found, except for most aldehydes and some carboxylic acids, occurred in higher amounts in the outer rather than the inner zones, in cheese made with *Staphylococcus succinus* subsp. *casei* or with *Staphylococcus equorum* subsp. *linens*. The presence of *Staphylococcus succinus* subsp. *casei* leads to cheeses containing higher levels of volatiles which corresponds with the biochemical properties of *Staphylococcus succinus* subsp. *casei* and sensory results of a previous study. Almost all components were extracted more efficiently by not adding water to the grated samples using the solid phase microextraction (SPME) technique.

Zusammenfassung

Aus thermisierter Milch hergestellter Raclette-Käse wurde mit Starterkulturen bestehend aus *Debaryomyces hansenii, Geotrichum candidum, Arthrobacter nicotianae* und *Corynebacterium casei* geschmiert. Die eine Variante wurde zusätzlich mit *Staphylococcus succinus* subsp. *casei*, die andere mit *Staphylococcus equorum* subsp. *linens* geschmiert. Nach einer Reifungszeit von fünf Monaten wurde die zonale Verteilung der flüchtigen Komponenten untersucht. Ausser den meisten Aldehyden und einigen Carbonsäuren wurden fast alle Substanzen in grösseren Mengen in den äusseren Zonen als in den inneren Zonen in Käsen gefunden, die mit *Staphylococcus succinus* subsp. *casei* oder mit *Staphylococcus equorum* subsp. *linens* geschmiert wurden. Die Zugabe von *Staphylococcus succinus* subsp. *casei* zur Starterkultur führte zu Käsen mit grösseren Mengen an flüchtigen Substanzen, was mit den biochemischen Eigenschaften von *Staphylococcus succinus* subsp. *casei* und den sensorischen Untersuchungen einer früheren Studie übereinstimmt. Fast alle Komponenten wurden effizienter extrahiert ohne Zugabe von Wasser zu den geriebenen Proben mittels SPME.

Résumé

Des fromages à raclette fabriqués avec du lait pasteurisé ont été emmorgés avec un levain contenant *Debaryomyces hansenii, Geotrichum candidum, Arthrobacter nicotianae* et *Corynebacterium casei*. L'une des charges a en outre été emmorgées avec *Staphylococcus succinus* subsp. *casei*, alors que l'autre l'a été avec *Staphylococcus equorum* subsp. *linens*. Après cinq mois d'affinage, la distribution zonale des composés volatils a été étudiée. A l'exception de la plupart des aldéhydes et de quelques acides carboxyliques, la plupart des composé volatils ont été trouvés en quantités plus élevées dans les zones extérieures que dans les zones intérieures des meules, qu'elles aient été emmorgées avec *Staphylococcus succinus* subsp. *casei* ou avec *Staphylococcus equorum* subsp. *linens*. L'ajout de *Staphylococcus succinus* subsp. *casei* au levain a conduit à des fromages contenant plus de composés volatils, ce qui est en accord avec les propriétés biochimiques de cette souche et avec les résultats sensoriels déjà publiés. Lors de leur analyse par SPME, la plupart des composés volatils ont été extraits à des taux plus élevés à partir d'échantillons râpés sans addition d'eau.

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Key words

Volatile compounds, Raclette cheese, concentration gradient, *Staphylococcus succinus* subsp. *casei, Staphylococcus equorum* subsp. *linens*.

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