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Comparison of Various Sample Treatments for the Analysis of Volatile Compounds by GC-MS: Application to the Swiss Emmental Cheese

Key words: Volatile compounds, Cheese, Comparison of methods, GC-MS analysis

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Introduction

Numerous techniques have already been proposed for the extraction, concentration and injection of volatile components of foods for gas chromatographic (GC) analysis, especially of dairy products. In this context, some important review articles should be mentioned (1, 2). The most frequently used methods may be summarised as follows:

Headspace techniques, in which the sample analysed is an aliquot of the gas phase containing volatiles released by the condensed phase. In their classical *static* form (3, 4), the use of these techniques is restricted to the most volatile components. The detection of flavour compounds depends mainly on their concentration and vapour pressure, as well as on the temperature and the matrix of the food product. To obtain more concentrated extracts, *dynamic headspace methods* (5, 6) have been developed, in which the volatile components of the gas phase are continuously removed and concentrated in a cold trap or adsorbed on an inert support, and then recovered either by thermal desorption or by elution with a suitable solvent.

Steam distillation techniques, in which an aqueous extract is concentrated by liquid/liquid partitioning or by cryoconcentration. Although widely used, these very popular techniques present several problems. For example very volatile components may cause low recoveries and/or may be overlapped by the chromatographic peak of the solvent, i.e. the solvent may contaminate the sample. In addition heat labile compounds may decompose thermally if the steam distillation and solvent extraction are not carried out under reduced pressure. Despite those difficulties, the technique has been largely applied to cheese varieties such as Emmental (7), Limburger (8), Fontina (9), Gruyère (10), Cheddar (11) as well as to yogurt (12), milk (13, 14), butter (15), ghee (16, 17), butter oil (18) and milk powder (19).

High-vacuum distillation techniques, produce small volumes of concentrated aqueous extracts which are in turn extracted with organic solvents such as methylene chloride, freon, and diethyl ether. High-vacuum techniques have been applied to solid fat after ultracentrifugation at 25000 g (20, 21), liquid fat (22) from a homogeneous suspension of fat in water (8, 23) or even from fat/water/isopentane mixtures (24). Sour cream, as an aqueous suspension may also be distilled under high-vacuum (25). *Moinas* (26) used a «gas stripping» technique combined with a high-vacuum distillation by purging the volatiles with inert gas at low temperatures (27–29). The alkaline volatile components of Swiss Gruyère cheese have also been isolated by applying high-vacuum gas stripping to grated cheese samples at room temperature followed by ether extraction of the alkalinised distillates (30).

Molecular distillation techniques, similar to high-vacuum distillation, involve the direct transfer of volatile compounds from the food matrix to a cold condenser. This technique requires very short distances between the condenser and food sample as well as the use of high-vacuum ($< 10^{-5}$ mbar) pumping systems. Such techniques have been used to isolate volatiles from fats and oils, and require water free samples. *Libbey* et al., calling this technique «cold-finger molecular distillation» (31, 32), applied it to analyse cheese flavour (Cheddar), *Urbach* for milk fat (33), and butter oil (34). *Dumont* and *Adda* (35) have described this technique as «distillation sous vide poussé» (translated as «high-vacuum distillation»). Molecular distillation has also been carried out by *Guichard* (36) on Emmental, Comté, Beaufort and by *Guichard* et al. (37) on Comté cheese.

Direct extraction techniques, in which an extract is obtained by liquid/liquid or liquid/solid partitioning. While these techniques are generally rapid and efficient, they should only be applied to samples with a very low fat content in order to prevent simultaneous fat extraction. Due to its inability to dissolve triglycerides, acetonitrile may be used to extract cheese flavors. The relatively high boiling point of this solvent (82 °C), however, causes a loss of volatile components during the concentration step. Several peaks in the chromatogram will be masked by this solvent (38, 39). *Preiniger* et al. (40) also treated grated Swiss cheese directly with diethyl ether, filtered the insoluble material and finally, under high-vacuum, they distilled the volatile compounds from the non-volatile material (i.e. fat) dissolved in this solvent. In order to separate the neutral and alkaline fraction from the acidic fraction, the distillate which smelled intensively like Emmental cheese, was treated with aqueous sodium bicarbonate or aqueous hydrochloric acid respectively.

Supercritical fluid extraction methods (SFE), using carbon dioxide as solvent, which avoid the problems of concentrating the extracts. Because of the simultaneous extraction of mono-, di- and triglycerides and the volatile compounds, the application of these techniques is limited to samples with a low fat content. Currently in the case of cheese samples, SFE-SFC (= supercritical fluid chromatography) coupling seems to be the only possible way to make use of this promising technique (41) which is already used on a preparative scale.

Simultaneous (steam) distillation extraction methods (SDE), which were first developed by *Likens* and *Nickerson* (42), use only very low boiling solvents such as pentane for the concentration of the the aroma volatiles. In a continuous process, the condensing water vapour is extracted by the condensing solvent vapour, yielding a high extraction rate. Both the water and the solvent are recirculated. A micro-scale modification, proposed by *Godefroot* (43) has recently been applied to cheese volatiles (44). *Maignial* et al. (45) have proposed a system working under reduced pressure and at low temperature (20–40 °C) to prevent thermal generation of artifacts.

Dialysis techniques which separate volatiles according to their ability to diffuse through a membrane as a result of a concentration gradient. Although a high yield is obtained, the sample preparation and the dialysis are very time consuming.This technique was developed by *Benkler* and *Reineccius* (46), modified by *Chang* (47), and compared with other techniques by *Vandeweghe* and *Reineccius* (9).

Solid-phase extraction (SPE) was used for the selective separation and concentration of analytes from liquid samples. Extraction of the analytes is based on the distribution of dissolved substances (i.e. volatiles) between a solid-phase surface and the liquid sample. Separation may be a result of differences in polarity, molecular size, or even differences with respect to ion-exchange capacity. SPE or even micro-solid-phase extraction (SPME) (48) techniques are relatively new for isolating volatiles. *Takacs* reported that flavour components in UHT processed milk could be extracted with C18Sep-Pak materials and eluted subsequently with methylene chloride (49). A rapid and sensitive solid phase method was developed by *Coulibaly* and *Jeon* for the extraction of lactones at µg/kg levels (50).

Among the various techniques reviewed briefly above, four have been developed or improved and applied in the author's laboratories: a high-vacuum distillation, a steam distillation and two dynamic headspace extractions (with and without cryofocusing), using different commercially available equipments (from Rektorik and Tekmar company). The present work, partially presented in a preliminary report (51), compares the performances of these techniques applied to Swiss Emmental cheese. It also cumulatively lists the 158 volatiles identified using one or several of these techniques prior to GC-MS analysis. In addition, this paper also cites the components already found by other authors in different cheese varieties.

Materials and Methods

Samples and sampling procedures

Samples were obtained from a single ripe Swiss Emmental cheese wheel (approximately 1 year old), divided into two parts: the external zone consisting of the rind and 1 cm of cheese beneath it, and the central zone consisting of the remaining cheese wheel. Both samples were finely grated, mixed thoroughly and kept deep frozen at –20 °C until use.

Extraction, concentration and injection techniques

Four techniques were used as previously described in the original papers, with the following modifications:

a) High-vacuum distillation (abbreviated «Tower», author's construction) (30), using 1.8 kg of grated cheese. The aqueous distillate was adjusted to pH 10.5, extracted with ether, concentrated on a Dufton column and injected in the split mode.
b) Steam distillation (abbreviated «Rotavapor», Büchi, CH-9230 Flawil) (10) with 250 g of grated cheese dispersed in 250 ml of distilled water. The trap corresponding to position 9 of figure 1 in the original paper was empty and the resulting condensate was treated as described under a).
c) Dynamic headspace extraction (abbreviated «Rektorik» CH-1202 Genève) (52), using the MWS-1® system which traps the volatile compounds on graphite powder and desorbs them by ultra-fast microwave heating. In this technique, 25 g of cheese were suspended in 50 ml distilled water, and the pH was adjusted to 7.5. The headspace volume aspirated through the graphite trap was 40 x 50 ml.
d) Dynamic headspace extraction followed by cryofocussing (abbreviated «Purge & trap») (53), using a LSC-2000® equipment (Tekmar, Cincinnati, Ohio USA), which includes a purge & trap system and a cryofocussing unit. About 3.5 g of grated cheese was suspended in 3.5 ml distilled water and the pH-value adjusted to 12. The resulting slurry was poured in a 25 ml fritted disk sparger. Operating conditions were the following: purge gas: He at 20 ml/min, trap Supelco # 2-0295 (1 cm 3% SP-2100 on Chromosorb + 23 cm Tenax TA); 0.75 min prepurge; 1 min preheat at 60 °C; 15 min purge at 60 °C; 11 min dry purge; 1min inject from –100 °C to +200 °C, 10 min bake at 210 °C.

Gas chromatography and mass spectrometric detection

The concentrated mixture of the flavour compounds obtained after extraction was analysed under the following conditions:

Gas chromatography

Hewlett-Packard 5890. Column: J&W DB-WAX 60 m x 0.25 mm i.d., df = 0.25 µm. Carrier gas flow: 0.71 ml He/min; inlet pressure 150 kPa. Injected volumes: 1 µl, split ratio 1:27 (for a, b & c). Injector temperature: 200 °C (for a, b & c). Temperature program: 13 min at 45 °C, heating to 220 °C at 5 °C/min, and 10 min at 220 °C.

Detection

Hewlett-Packard mass-sensitive detector (MSD model 5970), working in the scan mode from 19 to 250 atomic mass unit at 1.85 scan/s; ionization by EI at 70 eV and 0.8 mA. This detector was used in open-split mode (for a, b & c) and with a direct interface (for d).

Results and discussion

Figure 1 shows the chromatograms obtained under identical conditions for the four methods investigated, with ordinate expansion chosen to obtain the same baseline noise. For the first three methods (a, b, c), an open-split interface was used to connect the capillary column and the MSD. For the fourth method (d) (running on another GC) a direct inlet was mounted instead of this open split, which probably explains the higher sensitivity (i.e. higher intensity and higher number of peaks) of the determination. Otherwise the other visible differences between these 4 chromatograms are mainly due to the different extraction and concentration rates of the methods, especially during sample treatment.

A total of 158 volatile components were identified, reflecting the extreme complexity of the cheese biochemistry: 31 hydrocarbons (table 1), 26 alcohols (table 2), 21 aldehydes (table 3), 27 ketones (table 4), 5 ethers (table 5), 6 carboxylic acids and 1 phenol (table 6), 22 esters (table 7), 4 lactones (table 8), 6 nitrogen compounds (pyrazines), and 2 sulfur containing compounds (table 9), as well as 7 various contaminants (table 10).

Tables 1 to 10 do not indicate significant differences between the chemical composition of the central and external zone. This similarity of chemical composition could be due to a smearless cheese ripening.

The yield of extraction, the concentration and the recovery of the various techniques investigated in this work depended strongly on physico-chemical properties of these volatile components, such as polarity, vapor pressure, solubility and partition coefficients for the various phases coexisting during the differents steps of the procedure. Figure 2 shows the distribution of the compounds identified as a function of their (chemical) functional group and the analytical method used. The high-vacuum distillation and the dynamic headspace techniques using the Tekmar LSC-2000® equipment are more efficient than steam distillation and the dynamic headspace technique using Rektorik MWS®1 equipment. Generally hydrocarbons, alcohols, ketones and esters seem to be better recovered under high-vacuum distillation. Inversely aldehydes, ethers, lactones and acids seem to be better extracted using the Purge & Trap (abbreviated P&T) equipment. Steam distillation using the «Rotavap®» equipment and dynamic headspace with the «Rektorik» system are the least efficient techniques.

The two key factors responsible for the distributions indicated in table 11 and figure 2 were: the extraction rate and the loss of the compounds during the

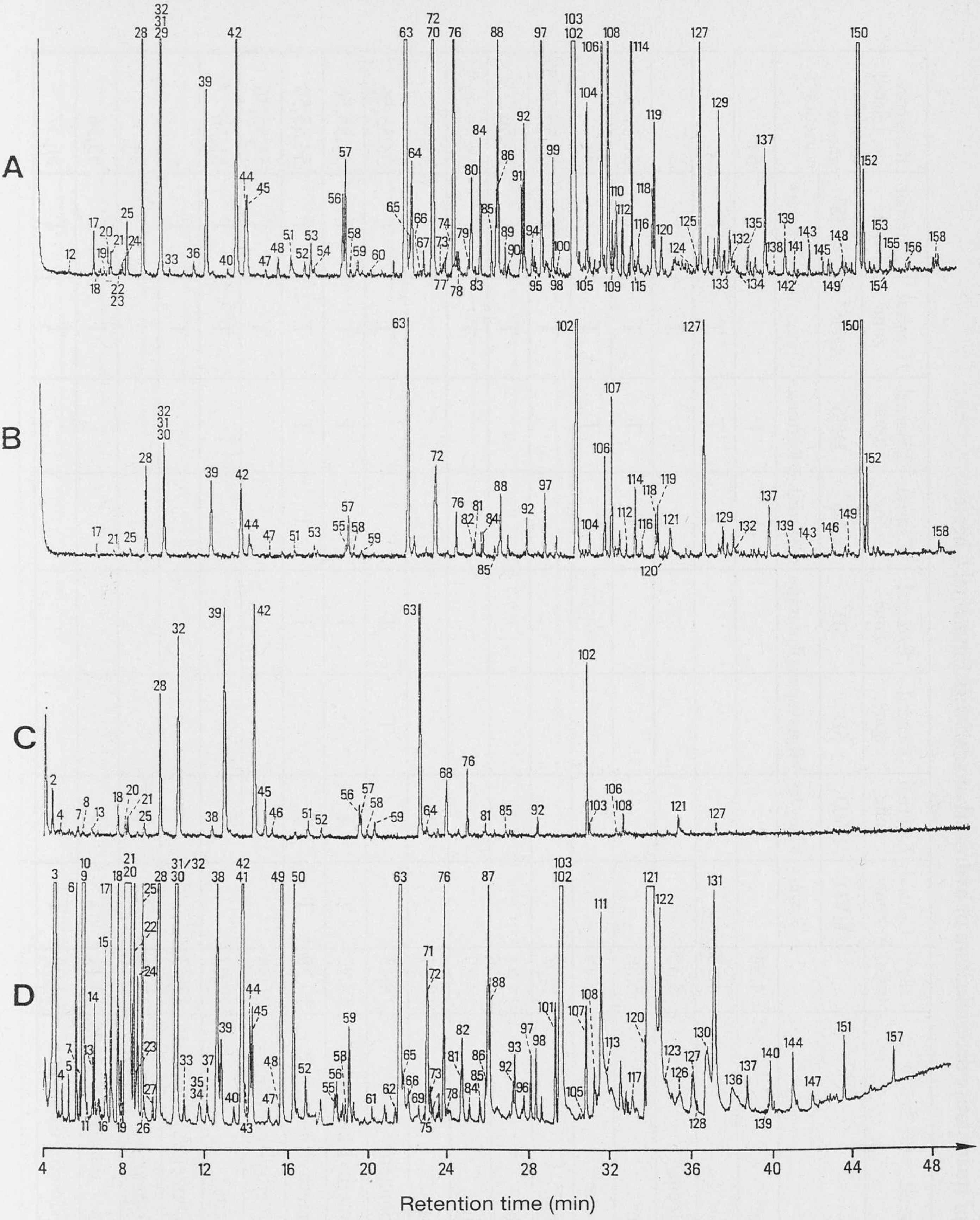

Fig. 1. GC-MS fingerprints of volatile flavour components of Swiss Emmental cheese using four different extraction methods: A = High-vacuum distillation; B = Steam distillation; C = Dynamic headspace (Rektorik MWS-1® equipment); D = Dynamic headspace (Tekmar LSC-2000® equipment)

Table 1. Semi-quantitative determination of hydrocarbons in Swiss Emmental Cheese

Peak Nr.	Compound	Rt (min)	Central zone	External zone	Central zone	External zone	Central zone	External zone	Central zone	External zone	Reference no. related to other cheese varieties**
			HVD[1]	HVD[1]	SD[2]	SD[2]	DHS[3]	DHS[3]	DHS[4]	DHS[4]	
			«Tower»	«Tower»	«Rotavap»	«Rotavap»	«Rektorik»	«Rektorik»	«Tekmar»	«Tekmar»	
2	Hexane*	4.54					L	L			54
4	Heptane*	4.98					T	T	T	T	–
5	Hept-1-ene*	5.30							T	T	–
7	Octane*	5.86					T	T	T	T	54,55,56
13	Oct-2-ene*	6.49					T	T	T	T	54
26	Benzene*	9.07							T	T	54,55,56,57
34	Decane*	11.71							T	T	55,56
41	Toluene*	13.79							T	T	54,55,56,57
54	Undecane*	17.31		T							55,56
55	Ethyl benzene*	18.43			T	T			T	T	54,55,56
58	1,4-Dimethyl-benzene*	18.90	T	T		T		T	T	T	54,55,56
59	1,3-Dimethyl-benzene*	19.29	T	T		T	T	T	T	T	54,55,56
62	1,2-Dimethyl-benzene*	21.36							T	T	54,55,56
66	Dodecane*	22.02		T					T	T	55,56
69	Alkyl benzene	22.53							T	T	–
70	Methyl-hexene	22.74	H	T							54
73	1-Ethyl-3-methyl-benzene	23.11	T	T					T	T	55,56
78	1,3,5-Trimethyl-benzene*	24.04	T	T					T	T	56,57

81	Styrene*	24.62			L	T		T	T	T	55
83	1-Ethyl-4-methyl-benzene	24.71	T	T				T			56
85	1,2,4-Trimethyl-benzene*	25.56	T	T		T	T	T	T	T	56
89	Tridecane*	26.14	T	T							55,56
95	1,2,3-Trimethyl-benzene*	27.55	T	T							56
100	Ethyl-dimethyl-benzene	28.64		T							55
124	Hexadecane*	35.23	T	T							–
133	Propyl-benzene*	37.21	T	T							–
139	Naphtalene*	39.61	T	T		T			T	T	54,55,56,57
141	1-Dodecene	40.12	T	T							–
142	Octadecane*	40.30	T	T							–
153	Cyclododecane	44.39	T	T							–
154	Eicosane*	44.89	T	T							–

* MS-Identification confirmed by comparison of Rt's of authentic compound
[1] HVD = High Vacuum Distillation («Tower»)
[2] SD = Steam distillation («Rotavap»)
[3] DHS = Dynamic Headspace («Rektorik»)
[4] DHS = Dynamic Headspace («Tekmar»)
Highest Peak of the chromatogram = 100%
T = Traces (< 10% of the Highest Peak)
L = Low (10% of the Highest Peak < L < 60% of the Highest Peak)
H = High (> 60% of the Highest Peak)
** Ref. no 54 related to Parmesan Cheese
Ref. no 55 related to Beaufort Cheese
Ref. no 56 related to Comté Cheese
Ref. no 57 related to Gruyère Cheese of Switzerland
Ref. no 58 related to Swiss type Cheese (Emmental of Allgäu/Germany) – no reference found

Table 2. Semi-quantitative determination of alcohols in Swiss Emmental Cheese

Peak Nr.	Compound	Rt (min)	Central zone	External zone	Central zone	External zone	Central zone	External zone	Central zone	External zone	Reference no. related to other cheese varieties**
			HVD[1]	HVD[1]	SD[2]	SD[2]	DHS[3]	DHS[3]	DHS[4]	DHS[4]	
			«Tower»	«Tower»	«Rotavap»	«Rotavap»	«Rektorik»	«Rektorik»	«Tekmar»	«Tekmar»	
24	Propan-2-ol*	8.73	T	T					L	T	57
25	Ethanol*	8.95	T	T	T	T	T	T	L	L	54,55,57
40	Butan-2-ol*	13.47	T	T					T	T	54,55,56,57
44	Propan-1-ol*	14.25	L	T	L	T			L	T	54,55,56,57
53	2-Methyl-propan-1-ol*	17.15	T	T		T					54,55,56,57
57	Pentan-2-ol*	18.80	T	T	L	T	L	L			54,55,56,57
60	Butan-1-ol*	19.88		T							54,55,56,57
67	4-Methyl-pentan-2-ol	22.32	T	T							55
68	Hexan-2-ol*	22.54					L	L			55,56
71	2-Methyl-butan-1-ol*	22.92							L	T	–
72	3-Methyl-butan-1-ol*	22.99		T	H	L			L	T	54,55,57
79	2- or 3-Pentene-1-ol	24.41	T	T							57
82	Pentan-1-ol*	24.71			T	T	T		T	T	54,55,57
92	Heptan-2-ol*	27.21	T	T	L	T	T	T	T	T	54,55,56,57
97	Pentan-3-ol	28.06	L	T	H	T			T	T	–
98	Hexan-1-ol*	28.35	T	T					T	T	54,55,56,57
99	Butan-2,3-diol	28.44	L	T							54
105	Octan-2-ol*	30.68	T	T					T	T	55,56

111	Heptan-1-ol*	31.57							T	T	55,57
115	2-Ethyl-hexan-1-ol*	32.40	T	T							54
118	Nonan-2-ol*	33.25		T		T					55,56,57
122	Octan-1-ol*	34.47							T	L	54,55,56,57
126	Methyl-heptanol	35.40							T	T	56
132	Furfurol*	37.12	T	T	L	T					54,55,57
148	Methyl-butene-ol	42.50	T	T							–
149	Phenyl-methanol*	42.68	T	T	T	T					57
152	2-Phenyl-ethanol*	43.55	T	T	T	T					55,56,57

See also footnotes to table 1

Table 3. Semi-quantitative determination of aldehydes in Swiss Emmental Cheese

Peak Nr.	Compound	Rt (min)	Central zone	External zone	Central zone	External zone	Central zone	External zone	Central zone	External zone	Reference no. related to other cheese varieties**
			HVD[1]	HVD[1]	SD[2]	SD[2]	DHS[3]	DHS[3]	DHS[4]	DHS[4]	
			«Tower»	«Tower»	«Rotavap»	«Rotavap»	«Rektorik»	«Rektorik»	«Tekmar»	«Tekmar»	
3	Ethanal*	4.60							H	H	–
6	Propanal*	5.66							T	L	–
9	3-Methyl-propanal*	6.02							L	L	56,57
15	Butanal*	7.13							T	T	56
20	2-Methyl-butanal*	8.14	T	T	T		L	T	L	H	54,55,56
21	3-Methyl-butanal*	8.30	T	T	T	T	T	T	L	H	54,55,56,57,58
31	Pentanal*	10.62	L	L	H	L	H	L	L	L	55,56,57

Peak Nr.	Compound	Rt (min)	Central zone HVD[1] «Tower»	External zone HVD[1] «Tower»	Central zone SD[2] «Rotavap»	External zone SD[2] «Rotavap»	Central zone DHS[3] «Rektorik»	External zone DHS[3] «Rektorik»	Central zone DHS[4] «Tekmar»	External zone DHS[4] «Tekmar»	Reference no. related to other cheese varieties**
43	2-Butenal*	14.20							T	T	54
50	Hexanal*	16.39							T	L	55,56
52	2-Pentenal*	16.97	T	T			T	T	T	T	54
74	2-Methyl-hexanal	23.31	T	T							–
87	Octanal*	25.96							L	L	55,56,57
93	2-Heptenal*	27.31							T	T	56
103	Nonanal*	29.62	H					T	L	L	55,57
107	2-Octenal*	30.83							T	T	–
117	Decanal*	33.14							T	T	–
120	Benzaldehyde*	33.73	T	T	L	T	T	T	T	T	54,55,56
123	2-Nonenal*	34.69							T	T	58
128	Undecanal*	36.10							T	T	–
131	2-Decenal*	37.09							T	T	–
140	2-Undecenal*	39.81							L	T	–

See also footnotes to table 1

Table 4. Semi-quantitative determination of ketones in Swiss Emmental Cheese

Peak Nr.	Compound	Rt (min)	Central zone HVD[1] «Tower»	External zone HVD[1] «Tower»	Central zone SD[2] «Rotavap»	External zone SD[2] «Rotavap»	Central zone DHS[3] «Rektorik»	External zone DHS[3] «Rektorik»	Central zone DHS[4] «Tekmar»	External zone DHS[4] «Tekmar»	Reference no. related to other cheese varieties**
10	Propanone*	6.04					T	T	L	H	54,55
18	Butan-2-one*	7.75	T	T			L	T	T	L	54,56,57
23	4-Methyl-butan-2-one	8.64		T					T	T	–
29	3-Methyl-butan-2-one*	10.41	L	L							54
30	Butan-2,3-dione (Diacetyle)*	10.62			H	L			L	L	54,58
32	Pentan-2-one*	10.62	L	L	H	L	H	L	L	L	54,55,56,57
36	Methyl-hexanone	11.87	T	T							–
38	4-Methyl-pentan-2-one*	12.48					T	T	T	T	57
39	3-Methyl-pentan-2-one*	12.63	T	T	L	T	L	H	T	L	55,56
46	Hexan-3-one*	14.80						T			54
47	Pentan-2,3-dione*	15.17	T	T	T		T		T	T	–
51	Hexan-2-one*	16.40	T	T		T	T	L			55,56,57
63	Heptan-2-one*	21.58	H	L	H	L	H	H	L	L	54,55,56,57,58
77	6-Methyl-heptan-2-one	23.86	T	T							57
80	5-Methyl-heptan-2-one	24.54	L	T							–
86	Octan-2-one*	25.86		T					T	T	54,55,56,57
88	3-Hydroxy-butan-2-one (Acetoine)*	26.06	L	T	L	T			L	T	54

90	Phenyl-butanone	26.29	T	T							57
102	Nonan-2-one*	29.54	H	H	H	H	L	L	T	L	54,55,56,57,58
108	Non-8-ene-2-one	31.22	T	L	L	L	T	T	T	T	55,56
116	Decan-2-one*	32.62	T	T		T					55,56,57
127	Undecan-2-one*	36.02	L	L	L	L	T	T	T	T	54,55,56
134	Alcan-2-one	37.38	T	T							–
138	1-Phenyl-propan-2-one*	39.15		T							57
143	Tridecan-2-one*	40.85	T	T	T	T					54,55,56,57
147	Dimethyl-undecadienone	41.93							T	T	–
157	Alcan-2-one	45.70	T	T							–

See also footnotes to table 1

Table 5. Semi-quantitative determination of ethers in Swiss Emmental Cheese

Peak Nr.	Compound	Rt (min)	Central zone	External zone	Central zone	External zone	Central zone	External zone	Central zone	External zone	Reference no. related to other cheese varieties**
			HVD[1]	HVD[1]	SD[2]	SD[2]	DHS[3]	DHS[3]	DHS[4]	DHS[4]	
			«Tower»	«Tower»	«Rotavap»	«Rotavap»	«Rektorik»	«Rektorik»	«Tekmar»	«Tekmar»	
27	2-Ethyl-furane*	9.45							T	T	54
61	2-Butyl-furane*	20.18							T	T	–
75	2-Pentyl-furane	23.55							T	T	–
96	2-Hexyl-furane	27.71							T	T	–

See also footnotes to table 1

Table 6. Semi-quantitative determination of acids in Swiss Emmental Cheese

Peak Nr.	Compound	Rt (min)	Central zone	External zone	Central zone	External zone	Central zone	External zone	Central zone	External zone	Reference no. related to other cheese varieties**
			HVD[1]	HVD[1]	SD[2]	SD[2]	DHS[3]	DHS[3]	DHS[4]	DHS[4]	
			«Tower»	«Tower»	«Rotavap»	«Rotavap»	«Rektorik»	«Rektorik»	«Tekmar»	«Tekmar»	
113	Acetic acid*	31.86							T	L	54,58
121	Propanoic (Propionic) acid*	33.85			L	T	L	T	H	H	54,58
130	Butanoic (Butyric) acid*	36.69							L	T	54,58
136	3-Methyl-butanoic (Isolvaleric) acid*	37.94					T		T	T	54,58
146	Hexanoic (Caproic) acid*	41.86				T					54,58
158	4-Methyl-phenol*	47.21	T	T	L	T					–
159	Octanoic (Caprylic) acid	51.81			T	T					54,58

See also footnotes to table 1

Table 7. Semi-quantitative determination of esters in Swiss Emmental Cheese

Peak Nr.	Compound	Rt (min)	Central zone	External zone	Central zone	External zone	Central zone	External zone	Central zone	External zone	Reference no. related to other cheese varieties**
			HVD[1]	HVD[1]	SD[2]	SD[2]	DHS[3]	DHS[3]	DHS[4]	DHS[4]	
			«Tower»	«Tower»	«Rotavap»	«Rotavap»	«Rektorik»	«Rektorik»	«Tekmar»	«Tekmar»	
11	Ethyl formate*	6.2–6.3							T	T	–
12	Methyl formate*	6.2–6.3	T								–
17	Ethyl acetate*	7.41	T	T	T	T			T	L	54,55,56
19	Methyl propanoate	7.92	T	T					T	T	–
28	Ethyl propanoate*	9.75	L	T	L	T	H	L	L	L	54,55,56
33	Methyl butanoate*	10.99	T	T					T	T	54
42	Ethyl butanoate*	13.91	L	L	L	L	L	H	T	L	55,57,58
45	Propyl propanoate*	14.39	L	T			L	L	T	T	55,56
48	Ethyl isopentanoate*	15.77	T	T					T	T	–
56	Propyl butanoate*	18.55	T	T					T	T	55,56
64	Isopentyl propanoate*	21.76	L	T			T	T			–
65	Methyl hexanoate*	21.80		T					T	T	55,56
76	Ethyl hexanoate*	23.79	L	L	L	T	T	L	T	L	56,57
84	2-Methyl-propanoic acid, 2-methyl-butyl ester	25.05	T	T	T	T			T	T	58
91	Propyl hexanoate*	26.92	T	T							56
106	Ethyl octanoate*	30.79	L	L	L	L	T	T			55,56,57,58
110	3-Methyl-butyl hexanoate	31.48	T	T							–

125	Methyl decanoate*	35.36	T	T							55,56
129	Ethyl decanoate*	36.52	T	T	L	T					55,56
135	Ethyl dec-9-enoate	37.84	T	T							56
145	Ethyl dodecanoate	41.57	T	T							56
155	Octyl prop-2-enoate	45.03	T	T							–

See also footnotes to table 1

Table 8. Semi-quantitative determination of lactones in Swiss Emmental Cheese

Peak Nr.	Compound	Rt (min)	Central zone	External zone	Central zone	External zone	Central zone	External zone	Central zone	External zone	Reference no. related to other cheese varieties**
			HVD[1]	HVD[1]	SD[2]	SD[2]	DHS[3]	DHS[3]	DHS[4]	DHS[4]	
			«Tower»	«Tower»	«Rotavap»	«Rotavap»	«Rektorik»	«Rektorik»	«Tekmar»	«Tekmar»	
137	5-Ethyl-2(3H)-furanone	38.68	T	T	L	T			T	T	–
151	5-Propyl-2(3H)-furanone	40.93							T	T	–
144	5-Butyl-2(3H)-furanone	43.52							T	T	–
157	5-Pentyl-2(3H)-furanone	45.96							T	T	–

See also footnotes to table 1

Table 9. Semi-quantitative determination of sulfur- and nitrogen containing compounds in Swiss Emmental Cheese

Peak Nr.	Compound	Rt (min)	Central zone HVD[1] «Tower»	External zone HVD[1] «Tower»	Central zone SD[2] «Rotavap»	External zone SD[2] «Rotavap»	Central zone DHS[3] «Rektorik»	External zone DHS[3] «Rektorik»	Central zone DHS[4] «Tekmar»	External zone DHS[4] «Tekmar»	Reference no. related to other cheese varieties**
49	Dimethyl disulfide*	15.80							L	L	57
94	2,6-Dimethyl-pyrazine*	27.43	T	T							55,57
101	Dimethyl trisulfide*	29.31							T	T	–
104	2,3,5-Trimethyl-pyrazine*	30.05	L	T	L	T					56
109	Ethyl-dimethyl-pyrazine	31.29	T	T							56
112	Ethyl-dimethyl-pyrazine	31.80	T	T							56
114	Tetramethyl-pyrazine*	32.30	L	T	L	T					56,57
119	3,5-Diethyl-2-methyl-pyrazine	33.36	L	T	L						–

See also footnotes to table 1

Table 10. Semi-quantitative determination of various contaminants in Swiss Emmental Cheese

Peak Nr.	Compound	Rt (min)	Central zone	External zone	Central zone	External zone	Central zone	External zone	Central zone	External zone	Reference no. related to other cheese varieties**
			HVD[1]	HVD[1]	SD[2]	SD[2]	DHS[3]	DHS[3]	DHS[4]	DHS[4]	
			«Tower»	«Tower»	«Rotavap»	«Rotavap»	«Rektorik»	«Rektorik»	«Tekmar»	«Tekmar»	
1	Carbon dioxyde	4.20					L	L			–
14	Hexamethylcyclotrisiloxane	6.59							T	T	–
16	1,1,1-Trichlorethane*	7.22							T	T	–
22	Dichloromethane*	8.55	T	T					L	L	–
35	Polymethyl-siloxane	11.75							T	T	–
37	Trichloromethane*	12.14	T	T					T	T	–
150	Butyl-hydroxy-toluene	43.30	H	H	H	H					–

See also footnotes to table 1

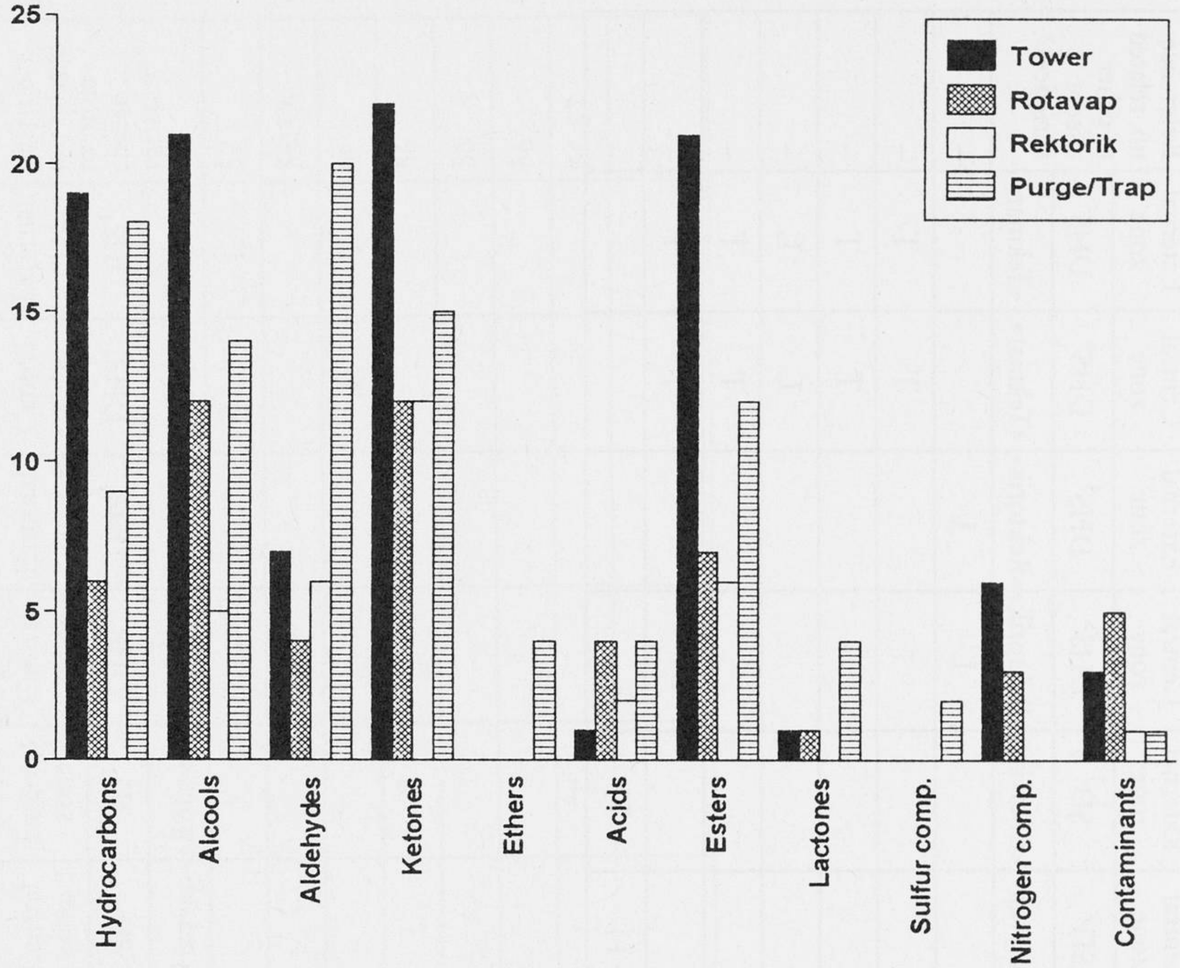

Fig. 2. Distribution of the compounds identified as a function of their (chemical) functional group and the analytical method used

concentration step. A study of the distribution of the compounds detected in terms of retention times or volatility ranges (low, middle and high) on table 11 leads to the following main trends:

Among the *hydrocarbons*, the same number of compounds was obtained using the first two above mentioned methods. The P&T equipment allowed the detection of the most volatile compounds whereas the high-vacuum distillation was more suitable to isolate the less volatile ones.

Among the *alcohols*, a total of 21 compounds were identified. All were found using the high-vacuum technique, while only 14 components, having rather low and middle retention times, were determined using the P&T equipment of Tekmar. The performance of the rotavap steam distillation was similar to that of the P&T equipment. The working procedure according to Rektorik method was the less efficient.

Among the *aldehydes*, 20 compounds ranging from C2 to C11 were found using the P&T equipment versus 7 using the high-vacuum distillation, 4 using the steam distillation and 6 using the Rektorik equipment. The high efficiency of the Tekmar equipment demonstrates that mild conditions (low temperature, and inert gas purging) prevent or at least reduce the loss of highly volatile compounds.

Table 11. Distribution of the compounds identified as a function of their (chemical) functional group, the analytical method used and their retention time

Chemical functionality	Volatility range	Retention time (min)	High-vacuum distillation Tower	Steam distillation Rotavap	Dynamic headspace Rektorik	Dynamic headspace Purge & Trap
Hydrocarbons	High	0–18	1	1	5	8
	Middle	19–35	12	4	0	9
	Low	36–48	6	1	4	1
	Total	0–48	19	6	9	18
Alcohols	High	0–18	6	4	2	4
	Middle	19–35	11	5	3	10
	Low	36–48	4	3	0	0
	Total	0–48	21	12	5	14
Aldehydes	High	0–18	4	3	4	10
	Middle	19–35	3	1	2	7
	Low	36–48	0	0	0	3
	Total	0–48	7	4	6	20
Ketones	High	0–18	8	5	8	8
	Middle	19–35	9	5	3	5
	Low	36–48	5	2	1	2
	Total	0–48	22	12	12	15
Ethers	High	0–18	0	0	0	1
	Middle	19–35	0	0	0	3
	Low	36–48	0	0	0	0
	Total	0–48	0	0	0	4
Acids	High	0–18	0	0	0	0
	Middle	19–35	0	1	1	2
	Low	36–48	1	3	1	2
	Total	0–48	1	4	2	4
Esters	High	0–18	9	3	3	9
	Middle	19–35	8	3	3	3
	Low	36–48	4	1	0	0
	Total	0–48	21	7	6	12
Lactones	Low	36–48	1	1	0	4
Sulfur comp.	Middle	19–35	0	0	0	2
Nitrogen comp.	High	36–48	6	3	0	0
Contaminants	High	0–18	2	0	1	0
	Middle	19–35	0	5	0	0
	High	36–48	1	0	0	1
	Total	0–48	3	1	1	5

Because *ketones* are thermally more stable than aldehydes, they may be better extracted and isolated using all four techniques. However once again, high-vacuum distillation was the most efficient in isolating the less volatile compounds.

Similar behaviour was observed with the *esters* using the high-vacuum technique with the high molecular (i.e. less volatiles) compounds better isolated than the low molecular ones.

Sulfur containing compounds, acids, ethers and lactones were mainly found with the P&T equipment. In contrast, most *pyrazines* were easier to extract under high-vacuum or with a steam distillation under reduced pressure.

The various *contaminants* included carbon dioxide detected with the «Rektorik» equipment. This compound resulted from the partial combustion of charcoal contained in the traps.Traces of siloxane derivatives were probably due to the Tenax® traps in the P&T equipment. *Chlorinated pollutants,* ubiquitous contaminants from the environment (59), were detected by the «Tower» and the P&T equipment. Finally, butylhydroxytoluene was detected as a contaminant of the diethyl ether used as organic solvent in high-vacuum distillation and Rotavap methods.

Conclusions

Two conclusions can be drawn from this study: the first one concerns the choice of the analytical method, the second concerns the composition of the volatile flavour components of Swiss Emmental cheese.

There is no ideal method for the extraction, concentration and injection of the volatile flavour components of cheese: each method investigated has advantages and drawbacks. In general, however, the dynamic headspace analysis using the Purge & Trap LSC-2000® of Tekmar equipment is the most promising technique, due to the small sample size (3.5 g), the short analysis time (90 min, including sample preparation) and the large number of compounds obtainable (98 peaks). Such a technique is suitable both for routine and research work. The mild low-temperature, high-vacuum extraction technique using the «tower» procedure resulted in the detection of slightly more compounds (102 peaks), but required a very large sample size (1.8 kg) and was very time consuming (2–3 days). It is therefore most suitable to research work. Steam distillation using the Rotavapor® equipment is an effective compromise with respect to sample size (250 g) and analysis time (3–4 hours), and may be used as a routine tool, especially if the compounds to analyse have relatively low polarity and low volatility. The dynamic headspace analysis using the Rektorik MSW-1® equipment is efficient under very drastic desorption conditions ($T = 400\ ^{\circ}C$) but is limited to graphite and activated charcoal as adsorbents. Moreover, it does not include a focusing step between the desorption and the injection. Its only advantage is that the traps can be easily loaded in a remote location before analysis in the laboratory. While it may be used as a routine tool, it only detects a few compounds, mostly those with high volatilities.

Table 12. List of compounds not identified in other cheese varieties (see Table 1, footnote**)

Hydrocarbons	Alcohols	Aldehydes	Ketones	Ethers
Heptane Hept-1ene Hexadecane Propyl-benzene 1-Dodecene Octadecane Cyclododecane Eicosane	2-Methyl-butan-1-ol Pentan-3-ol Methyl-butene-ol	Ethanal Propanal 2-Methyl-hexanal 2-Octenal Decanal Undecanal 2-Decenal 2-Undecenal	4-Methyl-butan-2-one Methyl-hexanone Pentan-2,3-dione 5-Methyl-heptan-2-one Alcan-2-one Dimethyl-undecadienone	2-Butyl-furane 2-Pentyl-furane 2-Hexyl-furane
Acids	Esters	Lactones	Sulfur compounds	Nitrogen compounds
4-Methyl-phenol	Ethyl formate Methyl formate Methyl propanoate Ethyl isopentanoate Isopentyl-propanoate 3-Methyl-butyl-hexanoate Octyl prop-2-enoate	5-Ethyl-2(3H)-furanone 5-Propyl-2(3H)-furanone 5-Butyl-2(3H)-furanone 5-Pentyl-2(3H)-furanone	Dimethyltrisulfide	3,5-Diethyl-2-methyl-pyrazine

None of the methods investigated was able to extract extremely high polar or low volatile substances such as hydroxyfuranone derivatives (40, 58) or α-hydroxycarboxylic acids (60).

A comparison of the volatiles found in this study, using one or several of the above mentioned analytical techniques, with those already identified in other cheese varieties indicates only a few new components (table 12). For most components several authors have already reported their occurence in cheese varieties such as Italian Parmesan (54), French Beaufort (55), Swiss Gruyère (57) and French Comté (56) as well as German «Swiss type cheese» (58). This observation confirms that the «harmonic balance» (61, 62) between the different components make up the flavour of every cheese variety, is analogous to an orchestra in which each instrument, by means of its own specific characteristics, contributes to the complex sound perceived in a symphony. However, in the case of cheese, it is very difficult to establish the relative contribution of each compound to overall flavour. Nevertheless, the follow-up to this study will attempt to identify and quantify the most odorant potent volatiles of Swiss Emmental cheese.

Summary

The extraction, concentration and injection by the GC-MS analysis of the volatile components of ripe Swiss Emmental cheese was carried out using four different techniques: a high-vacuum distillation, a steam distillation using a Rotavapor, and two systems performing dynamic headspace analysis («MWS»-1® and «Purge and Trap®»). A total of 158 compounds were identified within the two sampling zones, i.e. the external and central zones of the cheese wheel. These techniques were used to investigate the high, medium and low volatile components of this cheese variety. Advantages and drawbacks of the different extraction and concentration methods are briefly discussed.

Zusammenfassung

Vier verschiedene Verfahren wurden zur Extraktion, Aufkonzentrierung und Einspritzung der flüchtigen Komponenten eines (schweizerischen) reifen Emmentalers für die GC-MS-Analyse verwendet: Destillation unter Hochvakuum, Wasserdampf-Destillation mit Hilfe eines Rotavapors und zwei Systeme der dynamischen Dampfraum-Analyse («MWS»-1® und «Purge and Trap®»). Insgesamt wurden 158 Verbindungen in den beiden untersuchten Zonen des Käses (Mitte und Rand) identifiziert. Diese Verfahren wurden zum Studium der hoch-, mittel- und leichtflüchtigen Komponenten dieser Käsesorte herbeigezogen. Vor- und Nachteile der verschiedenen Extraktions- und Konzentrationsmethoden sind zusammengefasst.

Résumé

Quatre différentes techniques ont été utilisées pour l'extraction, la concentration et l'injection des composants volatils d'un fromage d'emmental (suisse) mûr analysé par GC-

MS: une distillation sous vide poussé, une distillation (ou entraînement) à la vapeur à l'aide d'un Rotavapor et deux systèmes d'analyse dynamique d'effluves (un «MWS»-1® et un «Purge and Trap®»). Au total, 158 composés ont été identifiés dans les deux zones de la meule de fromage considérées: une zone externe et une zone interne. Ces techniques ont été utilisées pour étudier les composants très volatils, moyennement volatils et faiblement volatils de cette sorte de fromage. Les avantages et les inconvénients des différentes méthodes d'extraction et de concentration font l'objet d'une brève discussion.

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