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Use of preconcentration techniques applied to a MS-based "Electronic Nose"

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Abstract. Four Swiss Emmental cheeses from four different factories were analysed with an "electronic nose" based on a mass spectrometer detector, the SMart Nose™. Three sampling methods, *i.e.* non-preconcentrated static headspace, Purge-and-Trap and solid phase microextraction (SPME), were compared in order to discriminate the cheese ripening ages. The use of a preconcentration technique was found to be helpful for this application due to the possibility to extract volatile compounds with higher molecular masses. From the two systems tested, the SPME was considered from far the best method because of its better repeatability, its simplicity and its compatibility with an autosampler.

Keywords. MS – electronic nose – static headspace – SPME – Purge-and-Trap – cheese.

Introduction

"Electronic nose" systems have raised a lot of interest since they appeared on the market at the beginning of the nineties. Since then, many searchers and industrials have tested different systems, and the limits of these instruments were revealed. One of the most important limitations is the lack of sensibility of "electronic noses" to some volatile compounds. The use of a preconcentration technique instead of the non-preconcentrated static headspace commonly employed with such "electronic nose" systems could cross this drawback. The Purge-and-Trap technique has already been used to improve the selectivity of the sensors. This method was successfully employed as a filter for ethanol [1,2], *i.e.* the ethanol contained in the samples was not adsorbed by the porous polymer material, and therefore, was not delivered to the sensors. Consequently, the sensors were not blinded by the ethanol content, and could response to other components. The same Purge-and-Trap technique was used by Aishima [3] as a preconcentration method for coffee aroma with his laboratory-made instrument based on MOS sensors. Marsili [4] has worked with milk samples using a Solid Phase MicroExtraction (SPME)-MS system as an "electronic nose", and has made a comparison to static and dynamic headspace methods.

The present work studies the potential use of two preconcentration techniques, *i.e.* Purge-and-Trap and SPME, coupled to a MS-based "electronic nose", as well as the comparison of both techniques *vs.* non-preconcentrated static headspace. The aim of the tests with the different methods was to classify Swiss Emmental cheeses at four different ripening grades.

Material and methods

Samples

Four Swiss emmental cheese loaves were manufactured on the same day in four different cheese factories. The cheese loaves were than collected and ripened in the same cellar. Samples were taken on the same cheese in the middle of the loaf at four different ripening grades, *i.e.* after 1, 21, 98 and 180 days of ripening. For the "electronic nose" analyses, four samples from each cheese at each ripening grade were grated, which gives a total of 192 samples (64 for each extraction method).

Extraction methods and measurement of the volatile components

Non-preconcentrated static headspace

The non-preconcentrated static headspace was generated with a CTC Combi PAL (CTC Analytics AG, Zwingen, CH) autosampler containing an oven and 2 trays of 32 20-mL vials each. The measurements were performed with the following setting parameters: sample: 2.00 ± 0.002 g of grated cheeses; vial: 20 mL sealed with silicon/Teflon septa and magnetic caps; incubation: 10 min at 60 °C; syringe: 2.5 mL; syringe temperature: 100 °C; syringe purge time: 7 min; filling speed: 200; pull-up delay: 100 ms; injection speed: 70; pre-injection delay: 100 ms; post-injection delay: 100 ms; Agitator: speed: 300 rpm; agi on time: 5 s; agi off time: 2 s.

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Purge-and-Trap

The instrument used was a Tekmar LSC 2000 (Cincinnati, OH, USA) equipped with a trap n° 8, containing a mixture of Carbosieve SIII (0.05 g) and Carbopack B60/80 (0.2 g).

The Purge-and-Trap extraction for the SMart Nose system was performed with the following setting parameters: sample: 1.00 ± 0.002 g of grated cheeses; vial: 10 mL sealed with silicon/Teflon septa and magnetic caps; purge gas: nitrogen 99.95 %; purge: 10 min at 40 °C; purge flow rate: 20 mL/min; desorb preheat: 200 °C; desorb: 2 min at 220 °C; desorb flow rate: 4 mL/min during 15 s; bake: 8 min at 260 °C; valve: 150 °C; transfer line: 150 °C; mount: 50 °C.

The Purge-and-Trap extraction for the gas chromatography-mass spectrometer (GC-MS) system was performed with the following parameters: sample: 2 g of grated cheese finely dispersed in 4 g water; sparger: 25 mL; purge gas: nitrogen 99.95 %; prepurge: 1 min; purge: 15 min at 45 °C; purge flow rate: 30 mL/min; desorb preheat: 210 °C; desorb: 4 min at 220 °C; bake: 5 min at 260 °C; valve: 150 °C; line: 150 °C; mount: 60 °C.

Solid Phase Microextraction

The SPME fiber was a 65 µm CW/DVB from Supelco (Bellefonte, PA, USA). The measurements were performed with the following setting parameters: sample: 1.00 ± 0.002 g of grated cheeses; vial: 10 mL sealed with silicon/Teflon septa and magnetic caps; incubation: 10 min at 40 °C; extraction: 10 min at 40 °C; desorption in the split/splitless injector of the GC: 3 min at 150 °C.

SMart Nose™

The "electronic nose" system used was a SMart Nose™ (SMart Nose, Marin-Epagnier, Switzerland). This instrument is based on mass spectrometer detection. The measurements were carried out with the setting parameters indicated in table I.

The PCA multivariate statistical analysis was performed with the SMart Nose software version 1.51. This software allows a choice of the relevant masses by studying the intergroup variance for each measured mass. The redundant masses were then eliminated. The masses chosen for the statistical analysis are tabulated in table II.

Table I. List of setting parameters for the measurements with the SMart Nose^{TM} system.

Extraction method	*Autosampler*	*Purge-and-Trap*	*SPME*
Mode	BG 10-120 (1 s)	BG 10-120 (1 s)	BG 10-120 (0.5 s)
Ion detection	SEM 1000	SEM 1000	SEM 1000
Acquisition time	330 s	330 s	165 s
Dead time	40 s	80 s	30 s
Injector temperature	120 °C	160 °C	150 °C
Injector clean-up	7 min	5 min	5 min
Clean-up flow rate	220 mL/min	220 mL/min	220 mL/min
Total analysis time	13.5 min	25 min	20 min

Table II. List of the masses chosen for the statistical analyses.

Extraction method	*Headspace*	*Purge-and-Trap*	*SPME*
Masses	41, 42, 43, 44, 46, 47, 49, 58, 59, 73, 74, 86	37, 45, 46, 58, 59, 67, 69, 70, 71, 73, 79, 81, 82, 105, 120	37, 45, 46, 58, 59, 67, 69, 70, 71, 73, 79, 81, 82, 105, 120

Gas chromatography

GC-MS

The gas chromatograph (GC) was a Hewlett-Packard 5890, Series II used with the following setting parameters: column: SPB-1 sulfur (Supelco) 30 m × 0.32 mm id., film thickness: 4 µm; carrier gas: helium; gas flow: 1.6 mL/min; pressure: 40 kPa; injection temperature: 45 °C; temperature program: 13 min at 45 °C, heating rate 5 °C/min to 240 °C, 5 min at 240 °C [6].

The mass spectrometer (MS) detector was a HP 5972 operating in the scan mode (TIC) from 19 to 250 amu at 2.9 scan/s, ionisation by EI at 70 eV by autotuning; MS-Scan after 4.0 min [6].

GC-FID

The gas chromatography (GC) instrument was a Hewlett-Packard 5890, Series II with the following setting parameters: column: DB-1 (Supelco) 60 m × 0.32 mm id., film thickness: 1 µm; carrier gas: helium; gas flow: 1.6 mL/min; pressure: 140 kPa; injection temperature: 220 °C; temperature program: 2 min at 35 °C, heating rate 11 °C/min to 180 °C, heating rate 5 °C/min to 275 °C, 5 min at 275 °C. The detection was done with a FID detector at 300 °C.

Results and discussion

Electronic nose

For greater clarity in the following text, the non-preconcentrated static headspace is referred as "static headspace".

When "static headspace", SPME and Purge-and-Trap techniques are compared together (Fig. 1), measurements performed with "static headspace" show comparable intensities than those done with the two preconcentration methods for small molecular masses, *i.e.* approx. until mass 45. When the small molecular masses, *i.e.* under 45, are taken away (Fig. 2), the "static headspace" exhibits very small responses in comparison with those obtained with the two other techniques. From the two systems tested, the SPME method shows the highest responses.

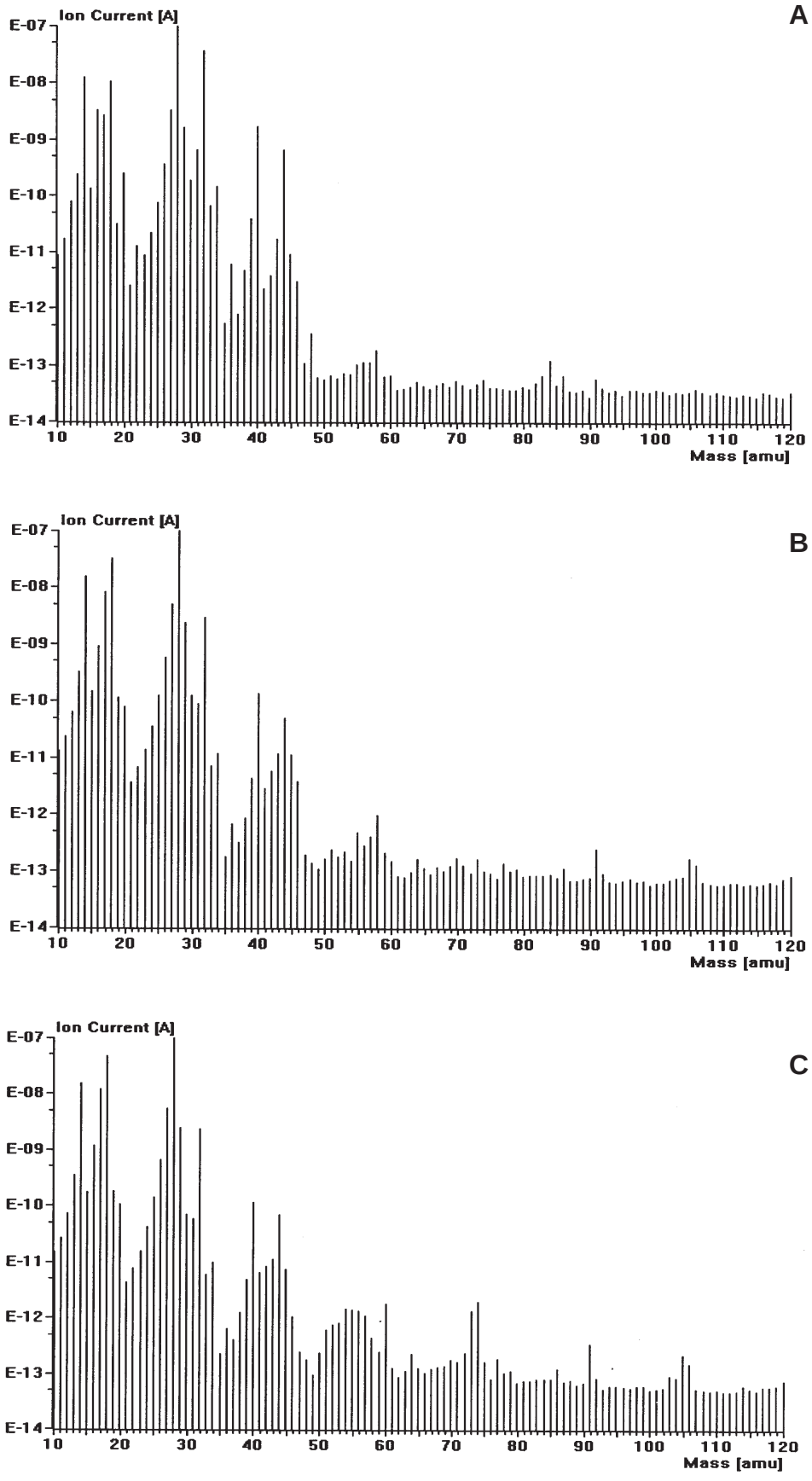

Figure 1. Mass spectra of the same 180-day old Swiss Emmental cheese with three extraction methods: non-preconcentrated static headspace (A), Purge-and-Trap (B) and SPME (C). **Caption:** The *y* scale is in logarithmic units.

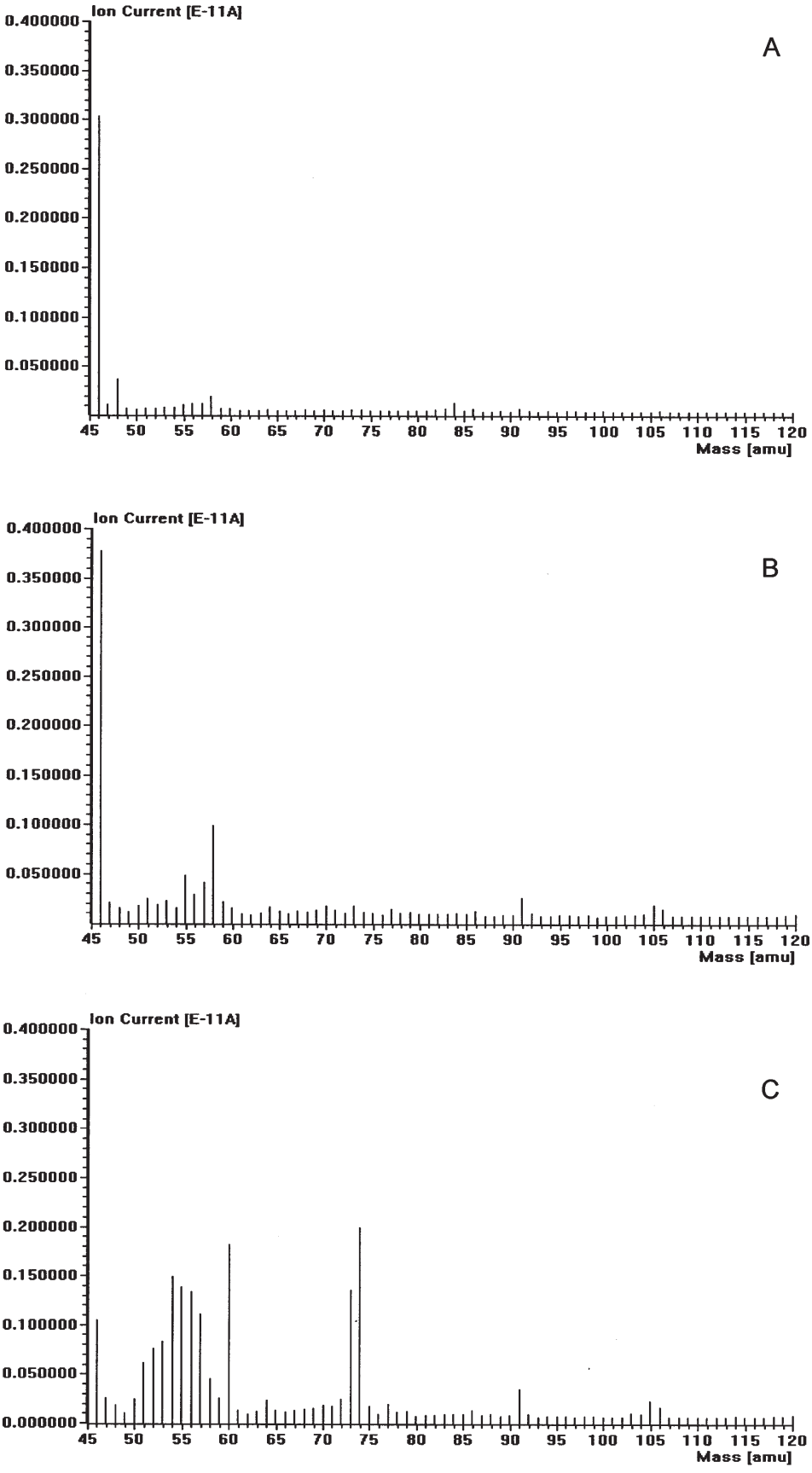

Figure 2. Mass spectra of the same 180-day old Swiss Emmental cheese with three extraction methods: non-preconcentrated static headspace (A), Purge-and-Trap (B) and SPME (C). Only the highest molecular masses from 45 up are represented. **Caption:** The *y* scale is in linear units.

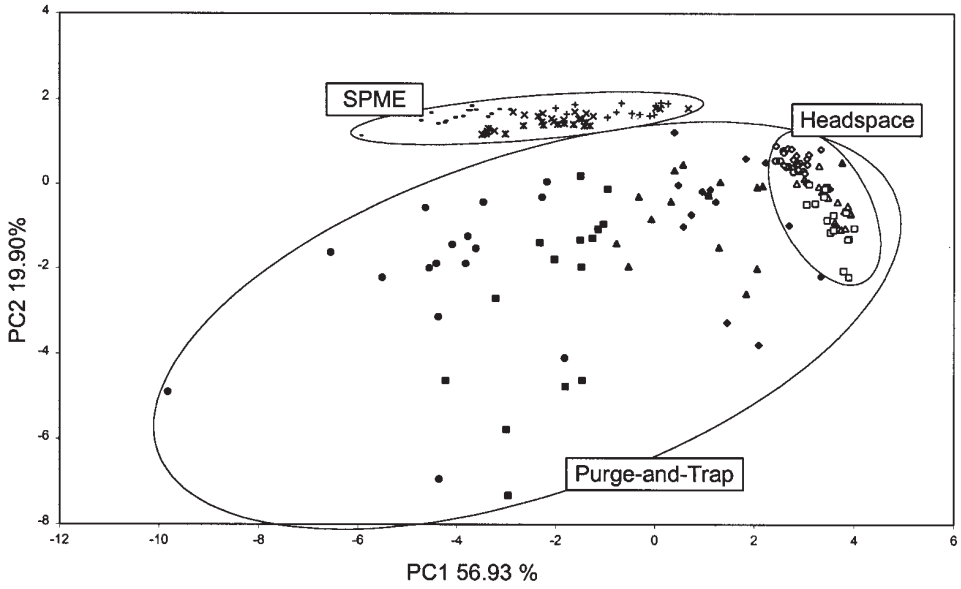

Figure 3. Principal component analysis of all Swiss emmental cheese measurements with the three extraction methods: non-preconcentrated static headspace, Purge-and-Trap and SPME. **Caption:** SPME: 1(–), 21(+), 98(×) and 180(∗) days; Purge-and-Trap: 1(■), 21(◆), 98(▲) and 180(●) days; non-preconcentrated static headspace: 1(□), 21(◇), 98(△) and 180(○) days.

The repeatability of measurements using the "static headspace" and SPME techniques was comparable. In contrary, a very poor repeatability was observed for measurements performed with the Purge-and-Trap instrument (Fig. 3). This poor repeatability can be partly related to the dynamic injection of this system. This observation is in agreement with that of Roussel *et al.* [5], who found that a dynamic injection gives less repeatable measurements than static one. They also consider the Purge-and-Trap system as "complex and difficult to master". Marsili [4] found also a better "precision of replicates" with the SPME compared to that of a dynamic headspace method. In the same paper, he reported two additional advantages of the SMPE: "i) no carry-over peaks from sample to sample, and ii) no background peaks". Inversely to the SPME technique, which is already used as a fully automated system, the Purge-and-Trap method would need numerous technical modifications to be adapted as an automatic preconcentration technique to the SMart Nose™ system.

Normalisation of the data was done by dividing all mass intensities by another constant one, which was found by the SMart Nose software as non-discriminant. "Static headspace" was normalised with molecular mass 48 and SPME with molecular mass 86. After this treatment, the principal component analysis shows three groups for both the "static headspace" and the SPME measurements. The groups were however different, *i.e.* 1 + 21 and 98, 180 days for the "static headspace" method (Fig. 4), and 1, 21 and 98 + 180 days for the SPME extraction (Fig. 5). An automation of the SPME technique would probably lead to a separation of the group 98 and 180 days. The Purge-and-Trap measurements were so unrepeatable that, even after normalisation any kind of separation was not possible with multivariate statistical analysis.

Purge-and-Trap/GC-MS

The measurements were carried out using the Purge-and-Trap preconcentration technique according to the method commonly used at the Swiss Federal Dairy Station. Figure 6 shows the GC-MS chromatogram of a 6-month old cheese and table III a list of the volatile compounds identified with the Wiley library including the retention time of the peaks. All components were confirmed with authentic reference compounds.

SPME/GC-FID

The measurements were done using the SPME preconcentration technique with the same fibre as that used for the SMart Nose analysis. Figure 7 shows the GC-FID chromatogram of the same 6-month old cheese. The broad peaks around 9.5 min are due essentially to propionic and butyric acids. The two chromatograms (Fig. 6 and 7) are very different, although they come from the same cheese sample. Consequently, the Purge-and-Trap and SPME techniques did not extract exactly the same volatile compounds or to a different ratio.

Conclusion

The non-preconcentrated static headspace is particularly useful for extraction of high volatile compounds. Due to their enrichment ability, Purge-and-Trap and SPME techniques are less efficient towards small molecular masses but can also extract compounds with higher molecular masses. Both techniques extract approximately the same class of compounds but the SPME technique seems to be more favourable for measurements with the SMart Nose since it can extract more compounds in a higher concentration. It is

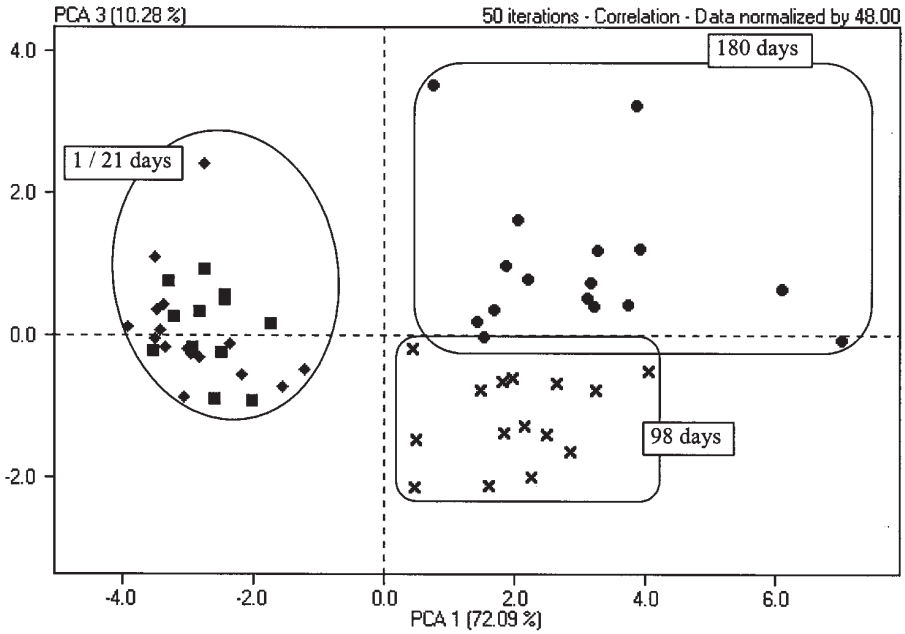

Figure 4. Principal component analysis of Swiss Emmental cheese at four different ripening grades, i.e. 1(■), 21(◆), 98(×) and 180(●) days. **Caption:** Measurements were done with non-preconcentrated static headspace technique.

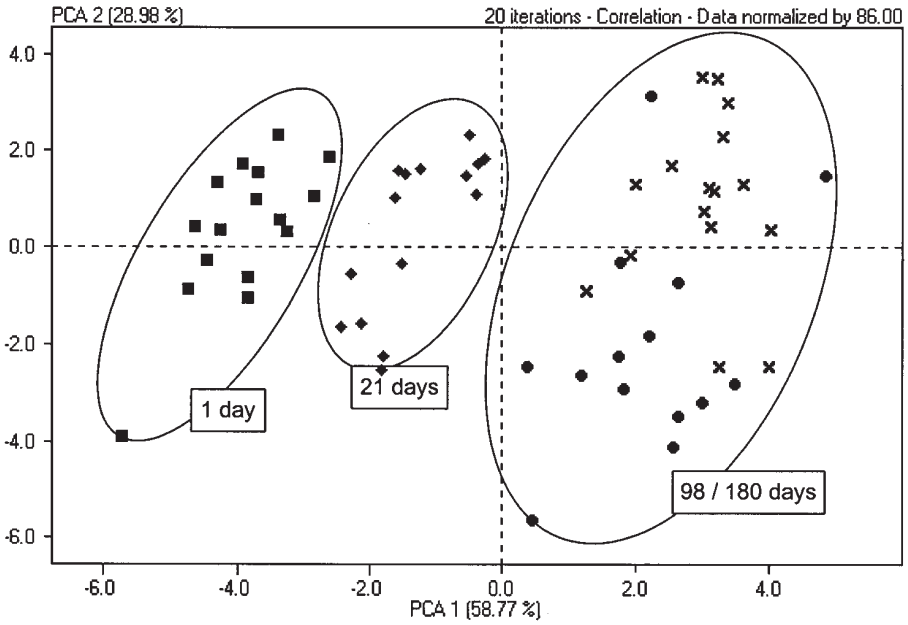

Figure 5. Principal component analysis of Swiss Emmental cheese at four different ripening grades, i.e. 1(■), 21(◆), 98(×) and 180(●) days. **Caption:** Measurements were done with solid phase microextraction technique (SPME).

more repeatable, smaller and easier to use than the Purge-and-Trap technique. Moreover, the SPME is quite compatible with an autosampler, *i.e.* a CTC system, what is hardly possible using the Purge-and-Trap system.

The use of a preconcentration technique for MS-based "electronic nose" systems can be recommended for analyses of samples differing mainly in volatile compounds with middle up to high molecular masses. For samples differing only in small molecular masses, the non-preconcentrated static headspace would be preferable. This preliminary study demonstrated that the SPME technique significantly improves the sensibility of the SMart Nose system.

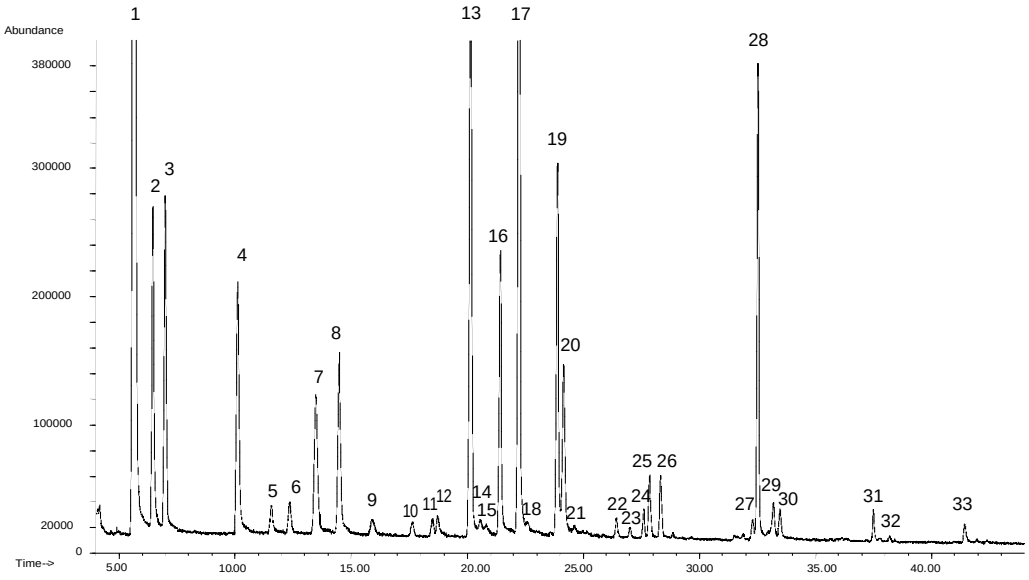

Figure 6. GC-MS chromatogram of Swiss Emmental cheese ripened for 180 days with the Purge-and-Trap preconcentration technique.

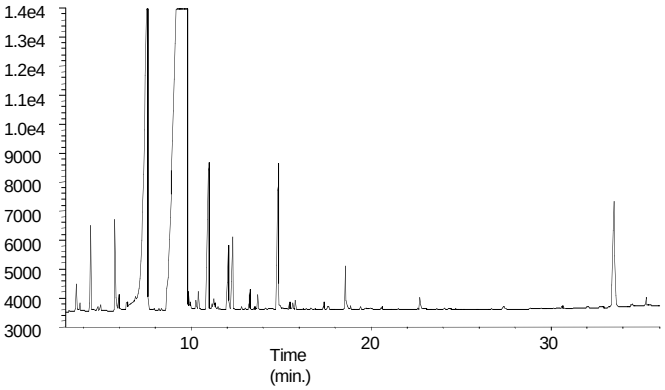

Figure 7. GC-FID chromatogram of Swiss Emmental cheese ripened for 180 days with the solid phase microextraction preconcentration (SPME) technique.

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Table III. List of components corresponding to the peaks represented in figure 6.

Peak number	Retention Time [min]	Name
1	5.66	Ethanol
2	6.47	2-Propanone
3	6.98	2-Propanol
4	10.09	1-Propanol
5	11.55	2,3-Butanedione
6	12.33	2-Butanone
7	13.44	2-Butanol
8	14.44	Acetic acid, ethyl ester
9	15.85	1-Propanol, 2-methyl-
10	17.61	Butanal, 3-methyl-
11	18.48	Butanal, 2-methyl-
12	18.71	1-Butanol
13	20.12	2-Pentanone
14	20.52	2,3-Pentanedione
15	20.78	Pentanal
16	21.42	2-Pentanol
17	22.23	Propanoic acid, ethyl ester
18	22.58	Heptane
19	23.91	1-Butanol, 3-methyl-
20	24.17	1-Butanol, 2-methyl-
21	24.61	Disulfide, dimethyl
22	26.43	Benzene, methyl-
23	27.00	2-Hexanone
24	27.60	Hexanal
25	27.85	Butanoic acid, ethyl ester
26	28.32	Propanoic acid, propyl ester
27	32.29	Benzene, 1,3-dimethyl-
28	32.52	2-Heptanone
29	33.19	2-Heptanol
30	33.43	Benzene, 1,2-dimethyl-
31	37.50	Hexanoic acid, ethyl ester
32	38.21	Benzene, 1,3,5-trimethyl-
33	41.43	2-Nonanone