Eur Food Res Technol (2003) 216:179–183
DOI 10.1007/s00217-002-0629-4

ORIGINAL PAPER

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Analytical methods for the determination of the geographic origin of Emmental cheese: volatile compounds by GC/MS-FID and electronic nose

Received: 29 May 2002 / Revised: 18 September 2002 / Published online: 26 November 2002

Abstract The volatile compounds of Emmental cheese of different origins were investigated to check their suitability as markers of geographic origin. A total of 20 Emmental cheese samples from Switzerland, Allgäu (D), Bretagne (F), Savoie (F), Vorarlberg (A) and Finland (produced in winter with a ripening time according to that sold in the corresponding regions) were analysed using dynamic headspace gas chromatography followed by flame ionisation and mass spectrometry. All regions were easily differentiated by using compounds occurring only in the corresponding region or by combining a few compounds by principal component analysis (PCA). Further analyses were carried out using a mass spectrometry-based electronic nose. PCA achieved 90 and 91% of correct classifications for the Emmental from Switzerland and other regions, respectively.

Keywords Emmental cheese · Authenticity · Traceability · Volatile compound · Gas chromatography · GC-MS · Electronic nose

Introduction

The origins of volatile compounds found in cheese are diverse and can be classified into two groups: the first one contains native volatile compounds already present in milk which are not transformed during cheese manufacturing while the second group includes components produced in the cheese itself during manufacture or maturation. Forage is an important factor influencing the composition of the volatiles of the first group. Feeding cows with e.g. highland grass (rich in dicotyledones) during mountain pasture leads to higher concentrations of terpenes, sesquiterpenes and esters, as described in literature for different cheese types [1, 2, 3, 4]. This information may be useful for recognising a typical highland cheese (e.g. protected denomination of origin) from a lowland cheese. However, they are not adequate as geographic markers for a given cheese type produced in different countries. Moreover, the composition of forage undergoes dynamic changes over the year and so will any parameter strongly related to it [5]. Local manufacturing practices can also modify the composition of volatiles in milk. When curd is cooked on an open log fire, significantly higher amounts of polycyclic aromatic hydrocarbons are found in cheese [6]. Once again, such markers are restricted to very specific products within a small scale production.

The volatile compounds of the second group are formed during manufacture and ripening of cheese by microbial, enzymatic and (bio)chemical transformations. Among these, proteolysis, lipolysis and lactose fermentation are major biochemical events. Degradation of amino acids leads to amines, aldehydes, alcohols, acids and sulphur compounds. Breakdown of fatty acids produces esters, methyl ketones and secondary alcohols [7, 8, 9]. The occurrence of the various pathways depends on the manufacturing technology, including the choice of the starter and non-starter bacteria used. A manufacturing process is often typical of a defined region and, therefore, the resulting composition of volatile compounds could be an interesting indicator of Emmental origin.

Different techniques and types of apparatus [10] as well as preconcentration methods [11] have been proposed for analysis of food volatiles. GC-MS with purge & trap technique is one of the most widely used techniques and represents the method of choice for the current work because of its high reproducibility. This technique is, however, expensive and very time-consuming. A promising alternative is the use of electronic noses because of their rapid screening capacity. In recent years, electronic noses have been gaining attention as useful tools, especially for quality control in the food industry

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[12] as they are ideally suited for rapid discrimination. An electronic nose based on mass spectrometry has already been successfully used to differentiate and recognise four different processed cheeses [13] in our laboratory.

The present investigation forms part of a broad preliminary screening test of discriminatory analytical tools within a 3-year study on the authenticity of Emmental cheese and its geographic traceability [14,15]. This study is especially concerned with distinguishing cheeses produced in Switzerland from those produced in other countries. At this point in the investigation, a great number of analytical methods already have been tested with respect to their discriminating potential, using three cheese samples from the regions Allgäu, Vorarlberg, Savoie, Bretagne, two from Finland and six from Switzerland [5, 15, 16, 17]. It is therefore obvious that the analytical results obtained from such a modest number of cheese samples per region can only give trends that will have to be confirmed later by tests performed on a larger number of samples. The objective of the present investigation was to check the possibility of using volatile compounds to discriminate Emmental cheese from different European countries, especially the Emmentaler Switzerland, by using two different analytical techniques: a dynamic headspace GC-MS plus FID and an MS-based electronic nose.

Materials and methods

Origin and selection of the cheese samples

The main framework of this study and the sampling procedure have been described in detail earlier [15]. Table 1 summarises the origin, the date of manufacture and the ripening time of the samples. They were chosen with different ripening time according to the reality of the market. Each region produces a cheese with typical characteristics, one of which is the ripening time, which can vary from 6 weeks to several months. At the end of the ripening time, all samples were stored deep frozen (–20 °C) until analysis.

Purge & trap analysis

Grated sample (20 g) and 80 g of boiled Milli-Q water were homogenised with a Polytron PT3000 running at 10 000 rpm for 1 min. An amount of 10 g of the suspension obtained was introduced into a 25-mL non-fritted glass sparger immersed in a water bath at 45 ± 0.5°C. The purge & trap system 3100 (Tekmar, Cincinnati, OH, USA) including a Tenax trap was op erated under the following conditions: prepurge time of 1 min; nitrogen as purge gas; purge flow of 30 mL/min; purge pressure of 150 kPa; purge time of 15 min, dry purge time of 6 min; desorb preheat at 220 °C; desorption at 225 °C for 4 min; cryofocus temperature at –145 °C; injection temperature program: within 1 min from –125 to 225 °C; bake 7 min at 230 °C; 6-port valve at 150 °C; line at 150 °C; transfer line from P&T to GC at 150 °C; mount temperature at 60 °C.

A Hewlett-Packard (HP) 5890 GC, series II was used. Separations were performed on a 30 m×0.32 mm i.d.×4 µm SPB1 sulphur column (Supelco). Helium was employed as carrier gas with an inlet pressure of 50 kPa. Following sample transfer, the oven temperature was maintained at 45 °C for 13 min and then heated at 5 °C/min to 240 °C, which was hold for 5 min.

Two detectors were mounted in parallel by splitting the flow at the end of the capillary column; one stream (0.99 mL/min at 45 °C) led to a flame ionisation detector (FID), the other (1.05 mL/min at 45 °C) to a mass-selective detector (MSD model HP 5972). The latter operated in the scan mode (TIC) from 26 to 250 amu at 1.1 scan/s, ionisation was by EI at 70 eV by autotuning.

The FID signal was used for the semi-quantitative determination of the peak height. Only compounds with a peak height greater than the value of 3.6 arbitrary units (selected detection threshold) have been considered for this study. This value corresponds approximately to an MSD signal-noise ratio of five. Peaks suffering from overlapping (asymmetric shape, poor resolution) have been excluded.

Statistical methods (static headspace)

The averages and standard deviations were calculated for each value. The pair-wise comparison of mean values with Fisher's LSD test as well as the principal component analysis were performed using Systat for Windows version 9.0 (SPSS Inc., Chicago, IL).

Electronic nose

Aliquots of 4 g per sample were grated and filled into 10-mL vials and closed with a butyl/PTFE septum and a cap. A Smart Nose (LDZ, Marin, Switzerland) equipped with a Combi PAL autosampler (CTC Analytics AG, Zwingen, Switzerland) was used. Main operating conditions were as follow: incubation temp at 90 °C, incubation time of 30 min, injection volume of 2.5 mL, syringe temp at 110 °C, injector temp at 170 °C, nitrogen as purge gas, with a purge flow of 350 mL/min; syringe purge time of 2 min, mass spectrometer scan speed of 0.5 s/amu, mass range of 10–110 amu, ionisation at 45 eV. The total acquisition time was set to 150 s so that three cycles were measured for each injection.

Generally, four replicates were measured for each sample. The analyses occurred in a randomised order. By introducing each sample into the incubator exactly 30 min (incubation time) before its programmed injection time, the effective measuring/sampling time was reduced to 4.5 min per vial. This was possible due to the programming capabilities of the auto-sampler and its capacity to incubate six vials at a time. Data were treated using the software supplied with the Smart Nose. For more details see [13].

Table 1 Origin, date of manufacture and ripening time of the 20 cheese samples investigated

Abbreviation	Region (country)	Number of samples	Date of manufacture	Ripening time (months)
AL	Allgäu (D)	3	25 Dec 2000	4
BR	Bretagne (F)	3	20 Feb 2001	2.5
CH	Switzerland (CH)	6	26 Dec 2000	4
FI	Middle Finland (FI)	2	04 Feb 2001	3
SA	Savoie (F)	3	05 Feb 2001	3
VO	Vorarlberg (A)	3	02 Feb 2001	3

Table 2 Concentration of volatile compounds showing significant differences (ANOVA, difference test on mean values) using purge & trap gas chromatography (peak height given in arbitrary units)

Peak no. (Fig.1)	Analytes	Retention index[1]	ANOVA[2]	Region (n)											
				AL (3)		BR (3)		CH (6)		FI (2)		SA (3)		VO (3)	
				x	s_x	x	s_x	x	s_x	x	s_x	x	s_x	x	s_x
	Ketones and aldehydes														
1	Propan-2-one[a]	466	*	78^{B}	29	341^{AB}	230	572^{A}	260	552^{AB}	200	280^{AB}	370	89^{B}	52
5	Butan-2,3-dione[a]	556	*	20^{AB}	10	60^{A}	29	35^{AB}	17	31^{AB}	7	30^{AB}	7	12^{B}	3
6	Butan-2-one[a]	567	***	44^{BC}	27	41^{BC}	9	21^{C}	7	118^{A}	68	74^{AB}	13	21^{BC}	10
10	3-Hydroxybutanone[b]	683	***	u.d.l.^{B}	–	3.4^{B}	2.6	18.7^{A}	7.9	u.d.l.^{B}	–	u.d.l.^{B}	–	u.d.l.^{B}	–
18	Hexanal[a]	780	***	24^{AB}	10	4.8^{C}	1.6	26.9^{A}	8.2	30.4^{A}	2.7	7.3^{C}	2.4	13.2^{BC}	1.9
	Alcohols														
2	Propan-2-ol[a]	481	***	131^{BC}	64	167^{B}	80	62^{C}	35	38^{C}	19	291^{A}	56	66^{BC}	11
4	Propan-1-ol[a]	535	*	530^{A}	310	148^{B}	42	143^{B}	94	115^{B}	51	271^{B}	140	144^{AB}	120
7	Butan-2-ol[a]	583	***	83^{AB}	53	21^{BC}	20	u.d.l.^{C}	–	7^{BC}	6	116^{A}	55	18^{BC}	23
8	2-Methylpropanol[a]	616	*	11.8^{AB}	2.5	4.3^{B}	2.5	7.8^{AB}	3.9	7.1^{AB}	2.4	13.5^{A}	2.1	6.5^{AB}	3.8
9	Butan-1-ol[a]	652	**	33^{B}	18	143^{A}	61	19^{B}	21	52^{B}	62	18^{B}	4	24^{B}	32
11	Pentan-2-ol[a]	685	***	100^{B}	75	45^{B}	38	11^{B}	7	u.d.l.^{B}	–	291^{A}	92	23^{B}	7
14	2-Methylbutanol[a]	725	*	77^{AB}	12	61^{AB}	9	61^{B}	25	100^{AB}	51	129^{A}	58	43^{B}	13
24	Hexan-1-ol[a]	852	***	8.6^{A}	3.7	u.d.l.^{B}	–	u.d.l.^{B}	–	u.d.l.^{B}	–	u.d.l.^{B}	–	u.d.l.^{B}	–
	Esters														
12	Butanoic acid ethyl ester[a]	695	**	54^{BC}	30	23^{C}	10	169^{AB}	66	122^{ABC}	30	216^{A}	110	82^{ABC}	25
13	Acetic acid propyl ester [a]	697	***	25^{A}	14	u.d.l.^{B}	–	u.d.l.^{B}	–	u.d.l.^{B}	–	u.d.l.^{B}	–	u.d.l.^{B}	–
25	Acetic acid 3-methylbutylester[b]	859	**	5.1^{A}	2.6	u.d.l.^{B}	–	u.d.l.^{B}	–	u.d.l.^{B}	–	3.3^{AB}	1.1	u.d.l.^{B}	–
	Hydrocarbons														
3	Pentane[a]	499	**	14.9^{A}	1.7	9.2^{B}	4.5	8.2^{B}	0.9	9.6^{AB}	2.1	10.8^{AB}	2.1	6.9^{B}	1.0
15	2,3,4-trimethylpentane[b]	756	**	u.d.l.^{B}	–	u.d.l.^{B}	–	u.d.l.^{B}	–	9^{AB}	11	u.d.l.^{B}	–	12.0^{A}	5.7
16	Toluene[a]	761	**	12.6^{B}	2.3	12.0^{B}	2.8	10.6^{B}	3.7	25^{AB}	22	11.0^{B}	3.6	37^{A}	13
17	3-Methylheptane[a]	776	*	u.d.l.^{B}	–	u.d.l.^{B}	–	6.0^{A}	2.7	6.6^{A}	8.0	u.d.l.^{B}	–	u.d.l.^{B}	–
19	2,2-Dimethylheptane[b]	787	**	u.d.l.^{B}	–	u.d.l.^{B}	–	u.d.l.^{B}	–	7.9^{AB}	9.7	u.d.l.^{B}	–	10.9^{A}	4.9
20	Oct-1-ene[b]	790	***	u.d.l.^{B}	–	u.d.l.^{B}	–	u.d.l.^{B}	–	u.d.l.^{B}	–	u.d.l.^{B}	–	22.1^{A}	9.4
21	Oct-?-ene[b]	803	*	u.d.l.^{B}	–	u.d.l.^{B}	–	u.d.l.^{B}	–	7.3^{A}	8.9	u.d.l.^{B}	–	5.8^{AB}	2.5
22	Alcene (C8H16)[b]	808	***	u.d.l.^{B}	–	u.d.l.^{B}	–	u.d.l.^{B}	–	u.d.l.^{B}	–	u.d.l.^{B}	–	5.5^{A}	2.2
23	Oct-?-ene[b]	813	*	u.d.l.^{B}	–	u.d.l.^{B}	–	u.d.l.^{B}	–	5.2^{A}	5.9	u.d.l.^{B}	–	4.9^{A}	2.1

Mean values within same row with at least one identical letter in the superscript are not significantly different. A>B>C>D (= significantly different contents) by using an univariate discriminant analysis

x mean value, s_x standard deviation, u.d.l. under the detection limit

AL Allgäu, *BR* Bretagne, *CH* Switzerland, *FI* Finland, *SA* Savoie, *VO* Vorarlberg

[a] Confirmed by comparing retention indices

[b] Identification with MassLib

[1] SPB1 chromatographic column; [2]* $p \leq 0.5$, ** $p \leq 0.1$, *** $p \leq 0.01$

Results and discussions

As the number of samples analysed was very limited (20 in total from six regions), we restricted the discussion of the results to tests of mean value differences and untrained classification techniques such as principal component analysis (PCA). In this technique, a linear combination of n parameters is calculated to maximise the distance between the points or samples in the n-dimensional space created. PCA shows natural group tendencies as the system does not know how to build the groups (untrained). PCA is less subject to over-fitting than trained classification techniques such as linear discriminant analysis, which would require a larger data set to be reliable.

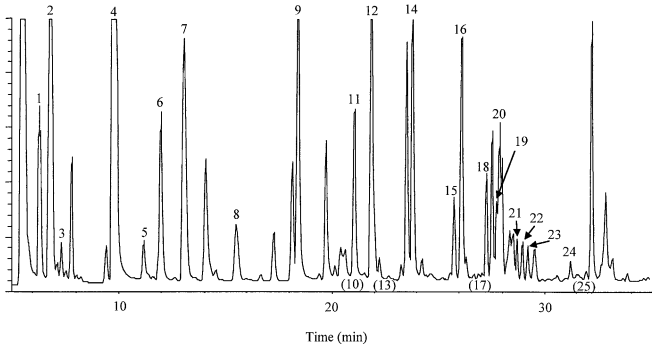

Fig. 1 Typical GC-FID of the volatile fraction of Emmental cheese (the peak numbering is listed in Table 2). Peaks 10, 13, 17 and 25 are not visible on this chromatogram

Purge & trap GC/MS-FID system

Figure 1 shows a typical chromatogram. The corresponding peak numbering is indicated in Table 2. For the Emmentaler Switzerland, four compounds showed extreme concentrations. The value of 3-hydroxybutanone was significantly higher in "Switzerland" than in all other regions. The propan-2-one concentration was the

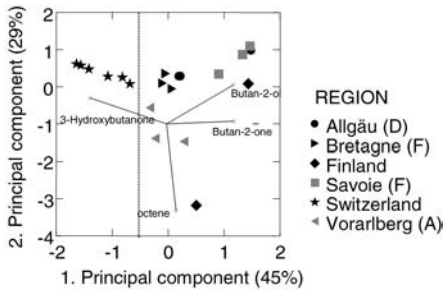

Fig. 2 Principal component analysis using the concentrations of butan-2-one, 3-hydroxybutanone, butan-2-ol and octene (RI=803). 100% separation of the Emmentaler Switzerland

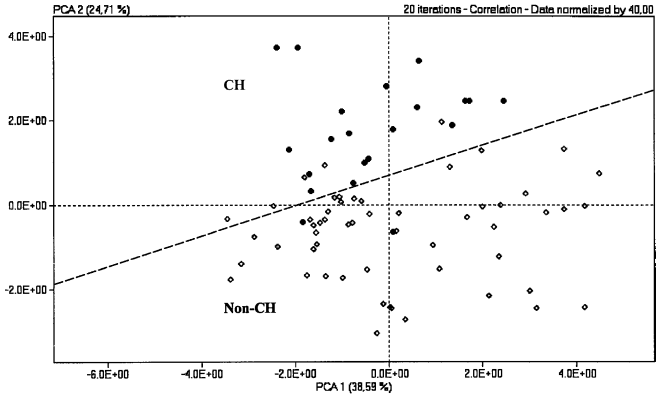

Fig. 3 Principal component analysis using an electronic nose. 90% correct classification for the Emmentaler Switzerland (*CH*) and 91% for the other samples grouped as one region (*non-CH*).

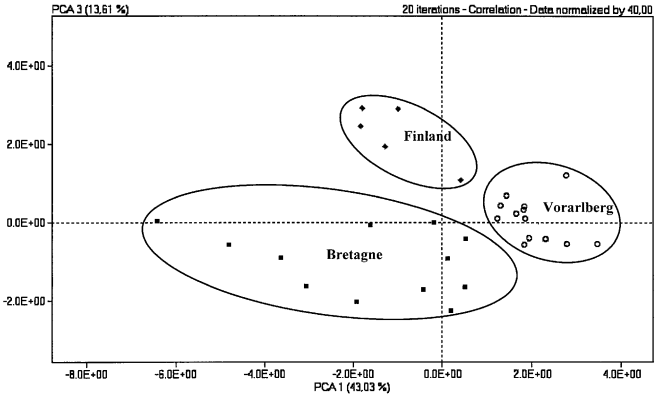

Fig. 4 Principal component analysis using an electronic nose. The three regions *Finland*, *Bretagne* and *Vorarlberg* are well separated

highest in "Switzerland;" however, not significantly different from "Bretagne," "Finland" and "Savoie." The concentrations of butan-2-one and butan-2-ol were the lowest in "Switzerland." The former was significantly lower than in "Finland" and "Savoie," the latter significantly lower than in "Allgäu" and "Savoie." 3-Methylheptane was found only in "Finland" and in "Switzerland" and at similar concentrations. These semi-quantitative results can unfortunately not be compared with those from previous studies due to the different traps used for preconcentration (e.g. Tenax vs. Carbosieve SIII/Carbopack B60/80 in [18]).

Certain compounds seemed highly specific to a given region. Hexan-1-ol and acetic acid propyl ester were present only in "Allgäu." 3-Methylbutylester of acetic acid occurred only in "Allgäu" and "Savoie." "Savoie" showed significantly higher concentrations of propan-2-ol and pentan-2-ol than in the cheeses from the other regions. "Finland" and "Vorarlberg" showed some similarities: 2,3,4-trimethylpentane, 2,2-dimethylheptane as well as two different octenes were detected only in cheeses from these two regions. It must be pointed out that only the sample FI1, and not FI2, contained the latter compounds. Furthermore, "Vorarlberg" was the only region where oct-1-ene and a further alkene were found. It was therefore very easy to separate "Allgäu," "Vorarlberg," "Savoie" and "Finland" one by one from the other regions using their specific compounds. The concentration of butan-1-ol was significantly higher in "Bretagne." This region may be separated from the others using the concentrations of butan-1-ol and hexanal. "Switzerland" was the single region where butan-2-ol was not detected in the samples. Combining the concentration of butan-2-ol, butan-2-one, 3-hydroxybutanone and octene (RI=803) by PCA, Switzerland" formed a separated group (Fig. 2). A tendency of grouping by the other regions (except "Finland") was also observed on the latter figure. These preliminary results will naturally have to be confirmed with a larger number of samples.

Electronic nose

The statistical analyses were carried out using the Software supplied with the Smart Nose. Though PCA is not a classification method, the program gives the possibility of making a group assignment by Euclidean distances in the multidimensional space created by the PCA. For each separation pattern, a new set of parameters was chosen to calculate the principal component scores.

A PCA on 76 analyses (in general 4 replicates of the 20 samples) showed a good classification of cheese samples as Swiss and non-Swiss (Fig. 3). Correct classification was achieved for 90% of the Swiss replicates and 91% of the non-Swiss replicates. The analysis of the results showed that out of the 21 Swiss replicates, two belonging to two different samples were misclassified. This result is out-weighted by the fact that the other 3 or 4 replicates of the respective samples were correctly classified. The same considerations were valid for the misclassified non-Swiss samples, where one replicate each from Finland, Vorarlberg and Savoie, and two from Germany were misclassified.

As expected, performing the statistical analysis with a limited number of regions enhances the quality of the classification. For instance, considering only samples from Bretagne, Vorarlberg and Finland, 100% correct classification was achieved (Fig. 4). Table 3 gives the

Table 3 Classification results from a PCA using an electronic nose

	Region	% Correct classification	
Switzerland vs	Other regions	CH: 90	non-CH: 91
Switzerland vs	Allgäu	CH: 100	AL: 92
Switzerland vs	Bretagne	CH: 95	BR: 100
Switzerland vs	Finland	CH: 90	FI: 100
Switzerland vs	Savoie	CH: 90	SA: 83
Switzerland vs	Vorarlberg	CH: 95	VO: 100

percentage of correctly classified cheese samples from Switzerland versus one of the other regions, one at a time. In this region-by-region analysis, Swiss replicates were at least 90% correctly classified. As for the rest of the regions, only "Allgäu" and "Savoie" showed less than 100% correct classification (92 and 83%, respectively).

Conclusion

The volatile compounds of Emmental cheese samples from different European regions were investigated by GC-MS. Each region could be separated from the others using compounds which were more or less specific to one or two regions. For instance, the concentrations of butan-2-one, 3-hydroxybutanone, butan-2-ol and octene made it possible to separate "Switzerland" from the other cheeses. These investigations showed the potential of volatile compounds to discriminate cheese samples with different origins. However, this analytical tool is very expensive and time-consuming and a less costly method would be preferable.

The use of an MS-based electronic nose turned out to be an interesting alternative to the GC-MS with purge & trap technique whose main advantage consists in a clear identification of the discriminating compounds. By separating Swiss and non-Swiss samples using PCA, 90% of "Switzerland" and 91% of the remaining samples were correctly classified via the Euclidean distances. By discriminating each non-Swiss region one at a time from "Switzerland", correct classification rates of 90–100% were obtained for "Switzerland" and 83–100% for the other regions. The use of a trained classification technique, such as linear discriminant analysis or differential factor analysis, in combination with a larger database, should improve the discrimination. Moreover, the preconcentration of the headspace before injecting it into the MS detector should enhance the sensivity of the analysis and hence the potential of this analytical technique.

Acknowledgement The authors thank the Swiss Federal Office of Public Health for its financial and logistical support to the project, G. Urbach and G. Gremaud for their reviewing of the publication, R. Gauch for his technical support as well as J.F. Chamba (ITFF, France), M. Carl (MUVA Germany), H. Spring (Emmentaler Switzerland), H. Jatila (Valio Ltd, Finland) and H. Schmallegger (Alma GmbH, Austria) for collecting the samples.

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