Long Term Study of Volatile Compounds from Deep Frozen Canned Processed Cheeses Proposed as Control Standards

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Introduction

When using dynamic headspace gas chromatographic (DH-GC) analysis of volatile compounds in food, a very old problem is the lack of simple and stable materials usable for calibration in (semi-)quantitative analysis. The four main calibration methods used in GC have been described in a comprehensive paper by *Lord* and *Pawliszyn* * (1).

The first is the so called standard addition. It is a widely used calibration method but it is extremely time consuming and laborious. To obtain a precision in the range of 2–5 %, the recommendation is to analyse three standard addition samples in triplicate. Further authors even propose the preparation of 7–8 standard addition samples to check the dynamic or linear range of the signal and to estimate the uncertainty of the measurements (2). Also the method becomes overly cumbersome if more than a few volatile compounds are to be quantified.

The second method is the spiking of samples with an internal standard. The major problem here is to get equilibrium of the internal standard in the matrix, specially if the standard is a highly volatile compound and the matrix a solid or viscous food sample. Furthermore, the behaviour of the internal standard during the various steps of the analysis is unpredictable and may be different from the analytes of interest, even if they are chemically very close.

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* Their description focuses on SPME applications but the calibration methods remains valid for purge & trap

The third technique is the use of isotopically labelled standards. This is by far the most accurate method but is only usable with a mass sensitive detector (MS). Unfortunately these standards are very expensive and not always commercially available. Moreover the same problem of equilibrium occurs as with internal standards.

The fourth and final quantification method available is external calibration. Usually three different concentrations of standard solutions are measured in triplicate, leading to a calibration line. However, these solutions present several problems: if the standards are dissolved in a pure organic solvent, the latter will be over-abundant, saturate the trap in DH-GC and mask the other peaks by overlapping; if the standards are dissolved in water (which is not always possible due to their lipophilic character), the risk of artifact formation due to the high amount of water vapour renders the use of standards problematic (3). Another way is to inject a known volume of standard in a flask or a bag and allow the system to equilibrate. A known volume of air-standard mix is then withdrawn with a syringe and injected into the GC-port. In the case of a purge & trap system, the introduction of the syringe content into the latter is not possible without a technical modification of the instrument. The last solution would be the addition of standards to an adequate matrix such as a mixture of triglycerides. Once again, the equilibrium between the added standard and the matrix presents a major problem. Furthermore, the matrix should in principle have a composition very close to the investigated samples to simulate the reactions and the interactions in the matrix and between the compounds themselves during the analysis. This makes it possible to take into account the alteration of the ad(ab)sorbent material used for preconcentration as well as possible displacement effects, breakthrough, etc. Such stable and homogeneous matrices have however never been related for volatile compounds analysis in cheeses. As no easy calibration method is available for a GC system with a preconcentration step, a simple control standard would be very useful in a first step.

The main objective of the current paper was to follow volatile compounds from four different deep frozen processed cheeses to check their ability to be used as native control standards. The measurements were carried out on both a Tekmar LSC 2000 and a Tekmar 3100 over approx. one year each. The stability of the FID signal for the volatile compounds previously identified with MS was studied, with the aim of selecting a few of them for cheese flavour research. Applications of such stable, homogeneous and reproducible standards are e.g.: comparison of different types of analytical equipment or quality control charts of gas chromatographs (combined with purge & trap or solid phase microextraction) and electronic noses. These materials have already been successfully used within a data transferability test between two electronic noses (4).

In addition to the main objective, it was possible to make some comparison between the two purge & trap concentrators used.

Experimental

Materials

Within the eleven processed cheese types already investigated for their volatile compounds (5), four varieties were chosen for their interesting chromatographic profiles. They were supplied by Tiger Käse AG (CH-3550 Langnau): ¼ fett, Emmentaler, Glarissa (with herbs) and Salami (with small salami pieces). They were all conditioned in gas-tight polymer coated aluminium cans (approximately 25 g). All cans of the same variety originated from the same production batch ensuring the needed homogeneity/reproducibility of the samples. All samples were stored at –20° C and left at +4° C overnight prior to sample preparation.

Sample preparation

Processed cheese samples were manually grated using a domestic rasp. Ten g of grated cheese were placed in the 25 ml non-fritted glass sparger of the Tekmar instrument. The bath was kept at 30° C, slightly below the melting point of the cheese. Table 1 summarises the plan of the analyses. Each repetition of an analysis was carried out using a fresh cheese can.

Table 1
Day and number of repetitions of the analyses

	Days	0	10	15	27	32	93	136	210	281	350
"2000" series	Repetitions (n=)	1	1	1	2	2	2	2	2	2	2
	Days	0	69	119	187	285	341	416			
"3100" series	Repetitions (n=)	6	2	2	2	2	2	2			

Dynamic headspace analyses

The Purge & Trap systems were successively the following: i) Tekmar LSC 2000 and ii) Tekmar 3100 sample concentrator (Tekmar, Cincinnati, OH, USA) over one year each, with a one-year break between these two periods. They included a slightly modified 25 ml non-fritted sparger (Schmidlin Co, part no. 14-2333-4SL, CH-6345 Neuheim), a trap (no. 8, containing a mixture of Carbosieve SIII (0.05 g) and Carbopack B60/80 (0.2 g)) as well as a cryofocusing unit. The modification allowed the glass sparger to be placed in a water bath for better temperature control (±0.1° C).

The Moisture Control Module (MCM) was removed from the 2000 series. The corresponding device on the 3100 series, the Moisture Control System (MCS), was set at a temperature of 150° C.

Operating conditions were as follows (values in brackets are specific for the "3100" series): prepurge time, 1 min; purge gas, nitrogen; purge flow, 30 ml/min;

purge pressure, 150 kPa; purge time, 10 min, dry purge time, 10 min; desorb pre-heat, 210°C (240°C); desorption at 220°C (240°C) for 4 min; cryofocus temperature, −125°C (−140°C); injection temperature program, within 1.5 min (1 min) from −125 to 200°C (225°C); bake, 5 min at 260°C; 6-port valve, 150°C; line, 150°C; transfer line from P & T to GC, 150°C; mount temperature, 60°C.

A Hewlett-Packard (HP) 5890 GC, Serie 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 40 kPa (50 kPa). Following sample transfer, the oven temperature was maintained at 45°C for 13 min and then programmed at 5°C/min to 240°C which was held for 5 min.

Detection

Two detectors were mounted in parallel by splitting the flow at the end of the capillary column; one stream (0.79 ml/min at 45°C) led to a flame ionisation detector (FID), the other (0.86 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 MSD was used for the identification of the volatile compounds. In addition, the identity was confirmed by comparison of retention times of authentic reference compounds. 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 360 arbitrary units (fixed threshold) have been considered for this study. This value corresponds approximately to a MSD signal-to-noise ratio of 5. Peaks suffering from peak tailing or having a high base line, a poor resolution or an asymmetric shape have been excluded. For these reasons, the whole range from compounds no. 20 to 26 in "Emmental" and from compounds no. 14 to 29 in "Glarissa" have been excluded. The presence of volatile fatty acids within these ranges is responsible for the poor resolution.

Results and discussion

The volatile compounds found in the four processed cheese types are listed in tables 2 and 3. Their chromatograms are shown in figure 1. Three criteria have been applied to the selection of the volatile compounds most appropriate as standards for GC: i) a relative standard deviation lower than 6%, ii) no more than one outlier over the period of the trial and iii) an average peak height of at least 1000 arbitrary units (corresponds approximately to the height of peak no. 12 from "Glarissa" in fig. 1). The volatile compounds fulfilling these requirements appear in italic and underlined characters in tables 2 and 3.

Table 2

Occurrence of the volatile compounds meeting the conditions of detection using the Tekmar LSC 2000 series. Italic and underlined characters indicate compounds with less than 6% RSD, a maximum of one outlier and an average peak height of at least 1000 arbitrary units

Functional Group	Peak No.	Compound	Retention Index[a]	¼ fett RSD	Outliers	Processed cheeses Emmentaler RSD	Outliers	Glarissa RSD	Outliers	Salami RSD	Outliers
Alcohol	1	Ethanol	443	0.081	1	0.139	0	0.069	0	0.090	0
	3	Propan-2-ol	482	0.064	1	0.140	0	0.084	1	0.067	0
	5	Propan-1-ol	536	0.082	0	0.095	0	*0.050*	0	0.066	1
	10	Butan-2-ol	583			0.154	0	0.090	0	0.135	0
	12	2-Methylpropanol	616			0.149	0	0.220	0	0.149	0
	18	Pentan-2-ol	685							0.233	0
	21	3-Methylbutanol	720	0.115	0					0.155	0
	23	2-Methylbutanol	724							0.172	0
	31	2-Furanmethanol	830					0.312	0		
	34	Heptan-2-ol	884			0.249	0				
Ketone	2	Propan-2-one	466	0.097	0	0.066	0	0.077	0	*0.058*	0
	7	Butane-2,3-dione	557	0.096	1	0.142	0	0.086	0	0.083	1
	9	Butan-2-one	568	0.089	0	*0.058*	0	*0.049*	0	*0.051*	0
	15	Pentan-2-one	669	0.072	0	*0.049*	0			*0.043*	0
	16	Pentane-2,3-dione	674	0.194	0					0.128	0
	22	4-Methylpentan-2-one	723	0.080	0						
	26	Hexan-2-one	770			0.072	0				
	32	Heptan-2-one	871	0.061	0	*0.055*	0	*0.042*	0	*0.035*	0
	47	Nonan-2-one	1075	0.111	0	0.076	0	0.070	0	0.071	0
Aldehyde	4	2-Methylpropanal	531	0.257	0	0.203	0	0.239	0	0.176	0
	6	2-Butenal[†]	542	0.164	0	0.134	0			0.118	1
	8	Butanal	563					0.145	0		
	13	3-Methylbutanal	638	0.079	0	0.100	0	0.066	0	0.108	0
	14	2-Methylbutanal	648	0.066	1	*0.057*	0			0.081	0
	17	Pentanal	677	0.131	1					0.072	0
	27	Hexanal	779							0.115	0

Functional Group	Peak No.	Compound	Retention Index[a]	1/4 fett RSD	1/4 fett Outliers	Emmentaler RSD	Emmentaler Outliers	Glarissa RSD	Glarissa Outliers	Salami RSD	Salami Outliers
							Processed cheeses				
Ester	11	Acetic acid ethyl ester	598	0.199	0	0.082	0	0.201	0	0.063	0
	19	Propanoic acid ethyl ester	695			_0.050_	0			_0.056_	0
	28	Butanoic acid ethyl ester	784			_0.055_	0	0.267	1	0.052	0
	29	Acetic acid butyl ester	795					_0.045_	0		
	33	Butanoic acid propyl ester	880					_0.044_	0		
	35	Propanoic acid butyl ester	888					0.067	0		
	38	Butanoic acid butyl ester	976					0.070	0		
Terpene	36	Alpha-thujene	934							0.106	2
	37	Alpha-pinene	946							0.106	1
	39	Sabinene	980							0.149	1
	40	Beta-myrcene	985							0.068	1
	41	Beta-pinene	990							0.085	1
	43	Alpha-phellandrene	1011							0.071	1
	44	Delta-3-carene	1021							_0.059_	1
	45	Limonene	1037							_0.050_	1
	46	Gamma-terpinene	1062							0.129	1
Other	20	Heptane	700			0.092	0				
	24	Dimethyldisulfide	732	_0.049_	0						
	25	Toluene	761	0.321	0	0.161	0			1.004	0
	30	Octane	800			_0.060_	0	_0.041_	0	0.111	0
	42	2,2,4,6,6-pentamethylheptane	1006							0.067	0
Number of compounds considered				20		23		21		36	
Number of compounds usable as control standards				1		7		6		7	

[†]=tentatively identified by MS (86%)

[a]=SPB1 chromatographic column

Table 3

Occurrence of the volatile compounds meeting the conditions of detection using the Tekmar 3100 series. Italic and underlined characters indicate compounds with less than 6% RSD, a maximum of one outlier and an average peak height of at least 1000 arbitrary units

Functional Group	Peak No.	Compound	Retention Index[a]	¼ fett RSD	¼ fett Outliers	Emmentaler RSD	Emmentaler Outliers	Glarissa RSD	Glarissa Outliers	Salami RSD	Salami Outliers
						Processed cheeses					
Alcohol	1	Ethanol	443	0.226	0	0.223	0	0.138	0	0.360	0
	3	Propan-2-ol	482	0.199	0	0.235	0	0.161	0	0.112	0
	5	Propan-1-ol	536	0.223	0	0.182	0	0.127	0	0.157	0
	10	Butan-2-ol	583			0.177	0	0.130	0	0.128	0
	12	2-Methylpropanol	616			0.125	2	0.545	0	0.143	0
	18	Pentan-2-ol	685							0.168	0
	21	3-Methylbutanol	720	0.152	0					0.111	0
	23	2-Methylbutanol	724							0.108	0
	31	2-Furanmethanol	830					0.764	0		
	34	Heptan-2-ol	884			0.131	0				
Ketone	2	Propan-2-one	466	0.114	1	0.075	0	0.061	2	*0.042*	0
	7	Butane-2,3-dione	557	0.176	0	0.263	0	0.260	0	0.139	0
	9	Butan-2-one	568	0.088	1	0.067	0	*0.047*	0	0.064	0
	15	Pentan-2-one	669	0.065	1	*0.059*	0			*0.058*	0
	16	Pentane-2,3-dione	674	n.d.	–					0.157	1
	22	4-Methylpentan-2-one	723	0.073	1						
	26	Hexan-2-one	770			0.078	0				
	32	Heptan-2-one	871	*0.046*	1	0.069	0	0.038	2	*0.056*	0
	47	Nonan-2-one	1075	n.d.	–	0.098	0	0.076	2	0.103	0
Aldehyde	4	2-Methylpropanal	531	0.509	0	0.355	0	0.412	2	0.253	0
	6	2-Butenal†	542	n.d.	–	0.156	2			n.d.	–
	8	Butanal	563					0.458	0		
	13	3-Methylbutanal	638	*0.050*	1	*0.051*	0	0.056	2	*0.038*	0
	14	2-Methylbutanal	648	0.095	1	*0.057*	0			*0.034*	0
	17	Pentanal	677	n.d.	–					0.080	0
	27	Hexanal	779							0.109	0

Functional Group	Peak No.	Compound	Retention Index[a]	¼ fett RSD	¼ fett Outliers	Processed cheeses Emmentaler RSD	Emmentaler Outliers	Glarissa RSD	Glarissa Outliers	Salami RSD	Salami Outliers
Ester	11	Acetic acid ethyl ester	598	0.242	1	0.066	1	0.308	0	0.062	1
	19	Propanoic acid ethyl ester	695			0.056	0			0.052	0
	28	Butanoic acid ethyl ester	784			0.065	0	n.d.	–	0.079	0
	29	Acetic acid butyl ester	795					n.d.	–		
	33	Butanoic acid propyl ester	880					0.039	2		
	35	Propanoic acid butyl ester	888					0.068	0		
	38	Butanoic acid butyl ester	976					0.076	2		
Terpene	36	Alpha-thujene	934							0.196	1
	37	Alpha-pinene	946							0.195	0
	39	Sabinene	980							0.439	0
	40	Beta-myrcene	985							n.d.	–
	41	Beta-pinene	990							0.184	1
	43	Alpha-phellandrene	1011							n. d.	–
	44	Delta-3-carene	1021							0.064	1
	45	Limonene	1037							0.076	1
	46	Gamma-terpinene	1062							0.344	0
Other	20	Heptane	700			0.086	0				
	24	Dimethyldisulfide	732	0.039	1						
	25	Toluene	761	0.366	0	0.127	0			0.141	3
	30	Octane	800			0.059	0	0.032	2	n.d.	–
	42	2,2,4,6,6-pentamethylheptane	1006							n.d.	–
Number of compounds considered				20		23		21		36	
Number of compounds usable as control standards				3		5		1		5	

[†] = tentatively identified by MS (86%)

[a] = SPB1 chromatographic column

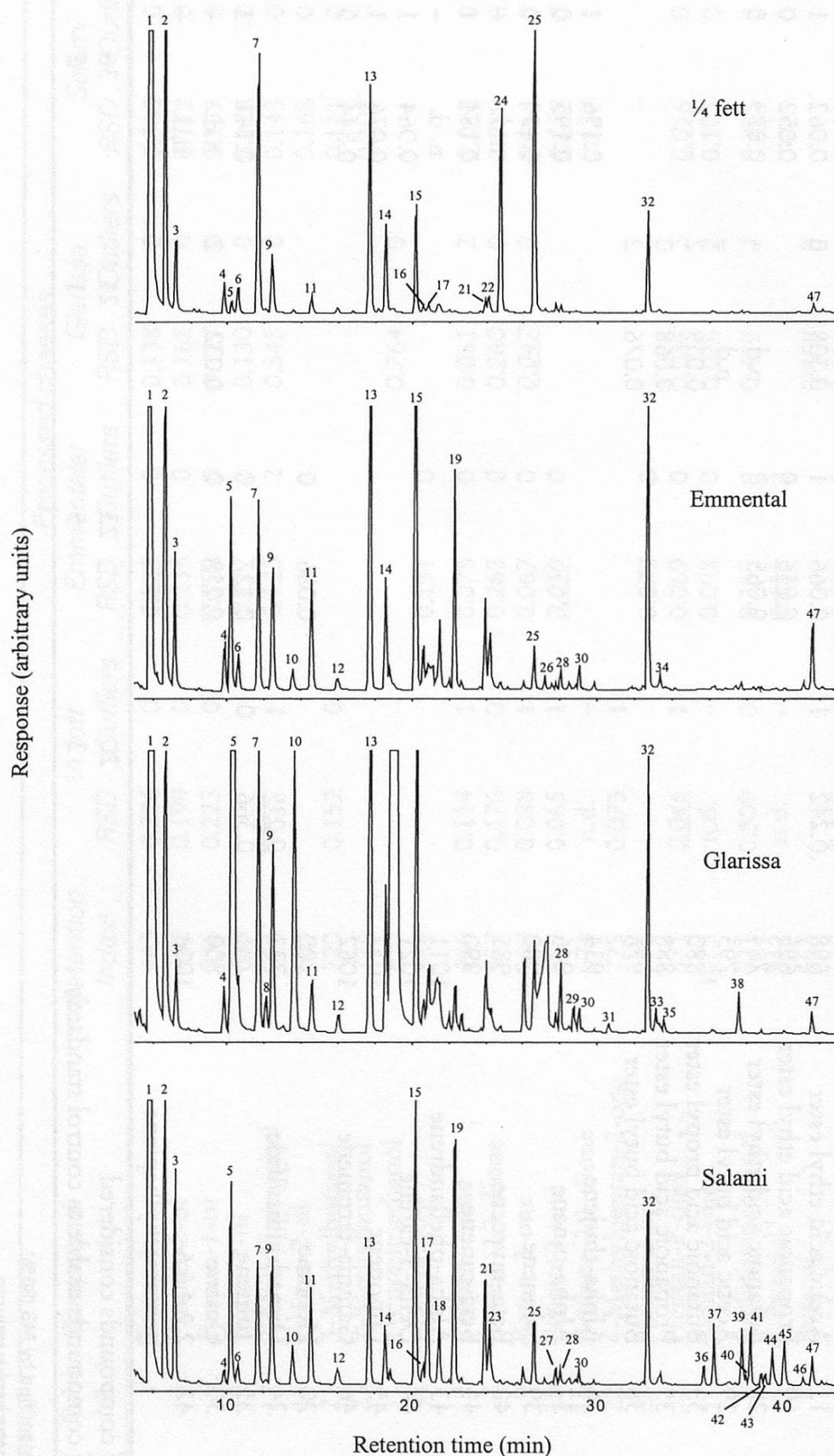n.d. = below the detection limits of Tekmar 3100 but detected using LSC 2000

Figure 1 **Typical GC/FID chromatogram of volatile compounds found in the four cheese types.** Caption: see corresponding compounds listed in table 2

Stable compounds for the Tekmar LSC 2000 series

A single compound met the conditions in the "¼ fett" type cheese, seven compounds in "Emmentaler", six in "Glarissa" and seven in "Salami" (table 2). All these compounds would in practice be stable enough to be used as control standards.

The most interesting processed cheese type is Salami. The stability of the selected compounds is illustrated in figure 2. "Salami" provided stable compounds in three different chemical groups which were spread over the whole chromatogram (fig. 1 peaks no. 2, 9, 15, 19, 32, 44, 45). Two of them, Δ-3-carene and limonene, were terpenes from the spices added. This could be of particular interest when the purge & trap/GC-FID system has to be controlled for the analysis of cheese samples containing terpenes.
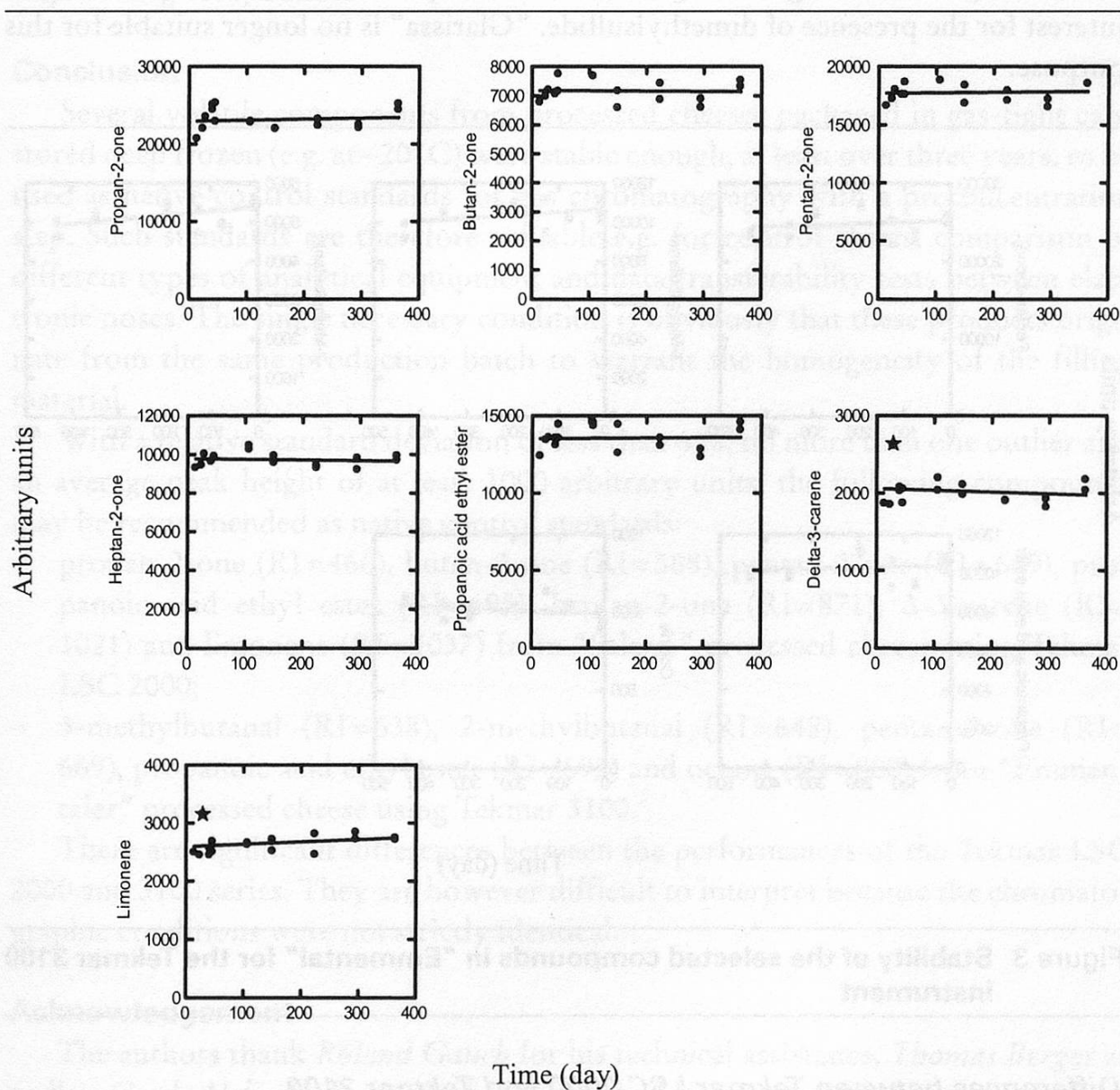
Figure 2 **Stability of the selected compounds in "Salami" for the Tekmar LSC 2000 instrument**

"Emmentaler" and "Glarissa" both provided stable compounds in four different chemical groups. "Emmentaler" even possessed a stable compound within the aldehyde group and "Glarissa" within the alcohol group; "¼ fett" may be, considered when dimethylsulfide is to be estimated in an unknown sample.

Stable compounds for the Tekmar 3100 series

Three compounds met the conditions mentioned above in the "¼ fett" type cheese, five in "Emmentaler", one in "Glarissa" and five in "Salami" (table 3). Except for the presence of three ketones, "Salami" is no longer of special interest. The most appropriate cheese here would be "Emmentaler", with stable compounds in four different chemical groups. The stability of the selected compounds is illustrated in figure 3. Though having three stable compounds, "¼ fett" is still only of interest for the presence of dimethylsulfide. "Glarissa" is no longer suitable for this purpose.
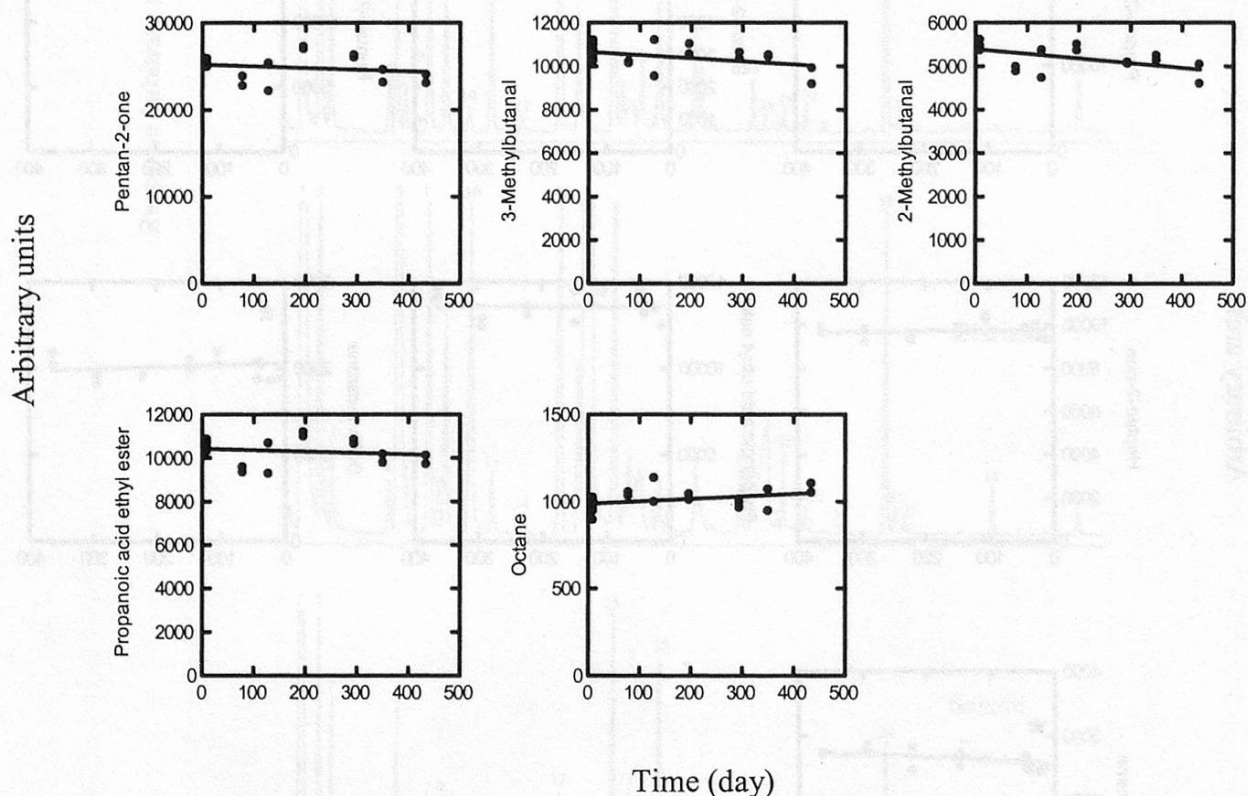
Figure 3 **Stability of the selected compounds in "Emmental" for the Tekmar 3100 instrument**

Differences between Tekmar LSC 2000 and Tekmar 3100

There were two general trends which differentiated the two series of analysers. The system with the concentrator Tekmar 3100 showed a poorer repeatability (higher RSD) and a poorer sensivity (some compounds found with the other system

are below the detection limit). This may be due to aging of the column, deterioration of the cheese samples or also to the concentrator itself.

There are three main modifications of design which differentiate the two concentrators compared, which could explain the differences in sensivity and reproducibility observed. Firstly, the trap of the 3100 series works differently from that of the older version. In the 3100 series there is a back pressure on the trap, which increases the total pressure in the cartridge and shifts the partitioning of the volatile analytes from the gas phase to the adsorbent. Secondly, the Moisture Control Module (MCM) of the 2000 series was removed and the one from the 3100 series was only kept as a "by pass" at the highest possible temperature, namely 150°C. Thirdly, the glass line is longer in the "3100", increasing the risk of condensation for high boiling compounds.

Conclusion

Several volatile components from processed cheeses packaged in gas-tight cans stored deep frozen (e.g. at –20°C) were stable enough, at least over three years, to be used as native control standards for gas chromatography with a preconcentration step. Such standards are therefore valuable e.g. for control charts, comparison of different types of analytical equipment and data transferability tests between electronic noses. The single necessary condition is obviously that these products originate from the same production batch to warrant the homogeneity of the filling material.

With a relative standard deviation of less than 6 %, no more than one outlier and an average peak height of at least 1000 arbitrary units, the following compounds may be recommended as native control standards:

– propan-2-one (RI=466), butan-2-one (RI=568), pentan-2-one (RI=669), propanoic acid ethyl ester (RI=695), heptan-2-one (RI=871), Δ-3-carene (RI= 1021) and limonene (RI=1037) from "Salami" processed cheese using Tekmar LSC 2000;
– 3-methylbutanal (RI=638), 2-methylbutanal (RI=648), pentan-2-one (RI= 669), propanoic acid ethyl ester (RI=695) and octane (RI=800) from "Emmentaler" processed cheese using Tekmar 3100.

There are significant differences between the performances of the Tekmar LSC 2000 and 3100 series. They are however difficult to interpret because the chromatographic conditions were not strictly identical.

Acknowledgement

Summary

For the analysis of food volatiles using analytical techniques where no simple calibration method exists, standards are necessary for control charts, comparison of different types of equipment and stability control of the latter. For this purpose, volatile components from four industrial processed cheese types (¼ fat, Emmental, Glarissa and Salami), stored in gas-tight aluminium cans at –20° C, were investigated by dynamic headspace gas chromatography using two concentrators, Tekmar LSC 2000 and Tekmar 3100, over one year each, with a one-year break between the two periods. On the basis of their relative standard deviation (<6%), repeatability (≤1 outlier) and signal intensity (>1000 arbitrary units), seven compounds in the "Salami"-type for the Tekmar LSC 2000 and five in the "Emmentaler"-type for the Tekmar 3100 respectively were selected as potential control standards with very different retention times. Moreover some differences of performance were found between the two purge & trap concentrators used.

Zusammenfassung

Bei der Bestimmung flüchtiger Verbindungen in Lebensmitteln, bei der keine einfachen Kalibrierungsmethoden existieren, sind Standards für Regelkarten, für den Vergleich verschiedener Analysengeräte und für ihre Stabilitätskontrolle notwendig. Deshalb wurden flüchtige Verbindungen von vier industriellen, in gas-dichten Aludosen bei –20° C gelagerten Schmelzkäsesorten (¼ fett, Emmentaler, Glarissa und Salami) im Laufe eines Jahres mit Hilfe einer dynamischen Headspace-Gaschromatographie mit dem Aufkonzentrierungsgerät Tekmar LSC 2000 untersucht. Nach einem Jahr Unterbruch wurden diese Untersuchungen mit dem Tekmar 3100 wiederholt. Auf der Basis ihrer relativen Standardabweichung (<6%), Wiederholbarkeit (≤1 Ausreisser) und Signalintensität (>1000 willkürliche Einheiten) wurden sieben Verbindungen in der Sorte «Salami» mit dem Tekmar 2000, respektive fünf in der Sorte «Emmentaler» mit dem Tekmar 3100, als potentielle Standards mit sehr unterschiedlichen Retentionszeiten ausgewählt. Einige Unterschiede in der Leistung der beiden verwendeten Purge & Trap-Apparaturen wurden dabei festgestellt.

Résumé

Pour l'analyse des composés volatils d'aliments où l'on ne dispose d'aucune méthode simple de calibrage, des standards sont nécessaires pour des applications telles que cartes de contrôle, comparaisons de différents types d'analyseurs et contrôles de leur stabilité. A cette fin, les composés volatils de quatre sortes de fromages fondus industriels (¼ gras, Emmental, Glarissa et Salami), stockés dans des boîtes étanches en aluminium à –20° C, ont été analysés par GC avec une préconcentration par espace de tête dynamique. Les analyses ont été effectuées consécutivement avec deux types d'appareils (Tekmar LSC 2000 & Tekmar 3100), durant une année chacun, avec une interruption d'une année entre les deux périodes d'essai. Sur

la base de leur déviation standard relative (<6%), de leur répétabilité (≤1 valeur aberrante) et l'intensité de leur signal (>1000 unités arbitraires), sept composés dans le type «Salami» pour le Tekmar LSC 2000 et cinq dans le type «Emmentaler» pour le Tekmar 3100 respectivement ont été sélectionnés comme standards de contrôle potentiels avec des temps de rétention très différents. Les deux appareils purge & trap utilisés présentent quelques différences de performance.

Key words

Volatile compound, Gas chromatography, Processed cheese, Control standard

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