Direct Thermal Desorption in the Analysis of Cheese Volatiles by Gas Chromatography and Gas Chromatography–Mass Spectrometry: Comparison with Simultaneous Distillation–Extraction and Dynamic Headspace

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Abstract

Direct thermal desorption (DTD) has been used as a technique for extracting volatile components of cheese as a preliminary step to their gas chromatographic (GC) analysis. In this study, it is applied to different cheese varieties: Camembert, blue, Chaumes, and La Serena. Volatiles are also extracted using other techniques such as simultaneous distillation–extraction and dynamic headspace. Separation and identification of the cheese components are carried out by GC–mass spectrometry. Approximately 100 compounds are detected in the examined cheeses. The described results show that DTD is fast, simple, and easy to automate; requires only a small amount of sample (approximately 50 mg); and affords quantitative information about the main groups of compounds present in cheeses.

Introduction

The commercial importance of various cheeses is related not only to their nutritive value, but also to their organoleptic characteristics. Their aroma is the result of a very complex process in which the milk origin, the cheese-making process, and the microflora that developed during ripening are involved. Cheeses may contain many volatile components that can differ quantitatively from one variety to another, their determination being important from both technological and economic points of view. Although gas chromatography (GC) and GC–mass spectrometry (MS) are the analytical techniques of choice, it is always necessary to include a prior step involving the extraction and preconcentration of the volatile fraction. Many different methods have been used for this purpose: high-vacuum distillation (1,2), simultaneous distillation–extraction (SDE) (3), static headspace (HS) (4), dynamic headspace (DHS) (5,6), and solid-phase microextraction (7).

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Thermal desorption is frequently used to transfer to the GC the volatiles previously trapped on an adsorbent. In direct thermal desorption (DTD), volatiles are desorbed from the sample and introduced in the GC by an online process, thus saving time, sample handling, and reagents. In a previous study, DTD was evaluated as a method for the GC determination of volatile components in plants (8). Reproducibility was found to be better than that achieved using SDE and solvent extraction, and the detection limit was between 10 and 50 pg for esters and methylketones (9). Nonetheless, DTD has been scarcely used for food analysis (10–14), perhaps because it can only be applied to solid and semisolid matrices. Some previous assays showed the possibility of extracting volatile components from cheese by using DTD (15). The aim of this work is to develop a DTD-based GC–MS method for cheese volatile analysis, extend it to several cheese varieties of different composition, and compare the results with those obtained by other well-known techniques such as SDE and DHS.

Experimental

Sampling methods

Commercial cheese samples representing different artisanal (La Serena, Camembert, Chaumes, Picón, and Cabrales) and blue

Table I. Thermal Desorption Conditions in the Three Extraction Methods

Extraction method	Temperature (°C)	Time (min)	Inlet split (mL/min)	Outlet split (mL/min)
DTD	65	30	8	8
DHS-TD	220	15	15	20
SDE–TD	220	15	15	20

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industrial (Asturian, Roquefort, and Edelpilz) cheese varieties were purchased, stored at –18°C for less than a month, and grated just before use. Each sample was extracted in triplicate. Three different extraction methods were employed. For DTD experiments, grated samples were mixed with sodium sulphate (1:6, w/w); 0.3 g aliquots of this mixture was introduced in Teflon-lined stainless steel tubes measuring 0.25- × 3.5-inch with Teflon caps (desorption cartridges, supplied by PerkinElmer, Norwalk, CT). For DHS, 15-g cheese samples were placed in glass vessels at 45°C and dynamically purged with 45 mL/min nitrogen for 120 min, as previously described (15). The stripped volatiles were trapped on 100 mg of a Tenax TA placed in a desorption cartridge. SDE was carried out as previously described (3) using 8 g of sample and pentane as the solvent, and 1 µL of the distillate was introduced into a desorption cartridge loosely packed with silanized glass wool by a microsyringe. All SDE extract, effluent of the DHS trap, and volatile compounds thermally desorbed by the experiment conditions are designated as "extracts" in this study.

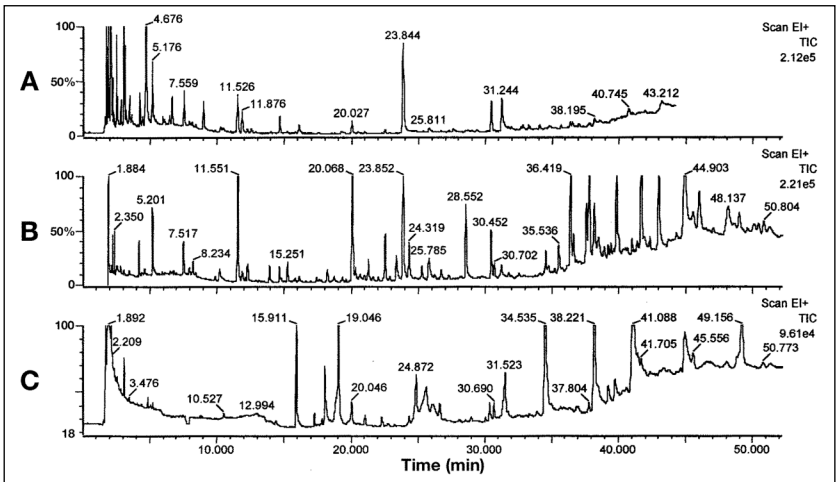

Figure 1. Reconstructed chromatographic traces from volatile components of a Camembert cheese sample extracted by (A) DTD, (B) SDE, and (C) DHS.

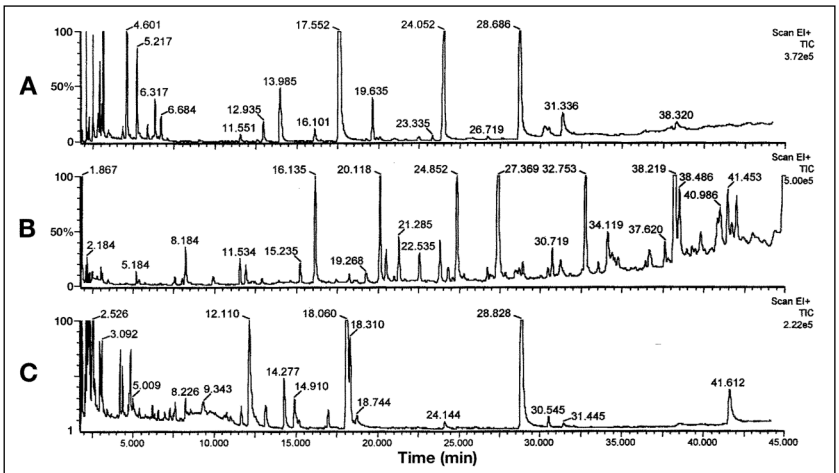

Figure 2. Reconstructed chromatographic traces from volatile components of a Chaumes cheese sample extracted by (A) DTD, (B) SDE, and (C) DHS.

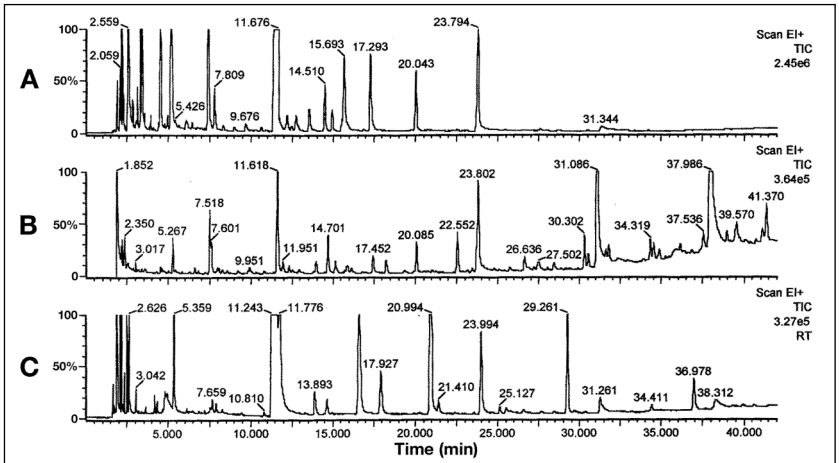

Figure 3. Reconstructed chromatographic traces from volatile components of a La Serena cheese sample extracted by (A) DTD, (B) SDE, and (C) DHS.

Thermal desorption

An automatic thermal desorption unit (ATD-400 from PerkinElmer) that was able to process up to 50 desorption cartridges automatically was used in these studies. The thermal desorption process was carried out by heating the cartridge to the desired temperature with 45 mL/min He as the carrier gas (primary desorption). The stripped volatiles were trapped on a Tenax GC cold trap (–30°C), which was later heated at a rate of 30°C/s up to 300°C (secondary desorption), thereby allowing a rapid transfer to the GC capillary column through a heated (225°C) fused-silica transfer line. The unit was equipped with two flow splitters—one (inlet split) placed between the desorption cartridge and the cold trap and the other (outlet split) between the cold trap and the transfer line. The primary desorption conditions were different for cheeses directly desorbed and for Tenax cartridges (detailed in Table I). Sample runs were always followed by blank runs in order to check for the complete transfer and carryover of volatiles through the system.

GC and GC–MS

The ATD-400 transfer line was connected to a Fisons 8000 GC (Fisons Instruments, Milan, Italy), which was equipped with a quadrupole mass detector (MD-800, Fisons, VG Masslab, Manchester, England) operating at 70 eV in the electron-ionization mode. Helium was used as the carrier gas. A homemade capillary column (25 m × 0.25 mm) coated with 0.25-µm FFAP/OV-1 (57:43, w/w) (16) was used. Temperature was held at 55°C for 10 min then programmed at 3°C/min to 180°C and held for 10 min. The injector pressure was 54 KPa.

Identification and quantitation

Most compounds in the analyzed samples were identified by the comparison of their retention

times and mass spectra with those of injected standards. The rest of the components were tentatively identified by comparing their spectra with those listed in the NIST Mass Spectral Library. Volatile percent composition was obtained directly from total ion current peak areas. Semiquantitative determinations (expressed as µg/g cheese) were carried out using a solution of methyl nonanoate in methanol, which was directly added by microsyringe to the desorption cartridges. Data were acquired and processed with a MassLab data system v1.18 (Fisons).

Results and Discussion

The DTD technique was compared with two methods of volatile fractionation: SDE, which has previously been applied in cheese analysis (17–20), and DHS using a technique developed for cheese volatiles in our laboratory (15). Because the extracts were introduced in the GC through the thermal desorption system in the three cases, the different results were related only to the extraction procedure itself (the eventual losses by adsorption in the transfer line and column being similar). Identical retention times were expected for the same components using this procedure. However, retention times obtained for some compounds by using DTD were slightly higher than those obtained when the same cheese was submitted to the other two extraction procedures. This delay was attributed to a solvent effect caused by water stripped from the sample. In order to confirm this hypothesis, different amounts of phosphorus pentoxide (Sicapent, Merck, Darmstadt, Germany) were placed at the cartridge outlet avoiding any contact with the sample. The amount of desiccant varied within 50 and 100 mg, and that of the sample was inversely reduced from 60 to 40 mg. The difference in retention times decreased markedly when the Sicapent amount was increased—

Table II. Volatile Composition* of a Camembert Cheese Sample Extracted by the Three Different Techniques

T_R	Compound	DHS	SDE	DTD	T_R	Compound	DHS	SDE	DTD
1.84	Carbon disulfide	6.536		0.800	12.15	Branched acid			0.926
2.04	2-Propanol	23.523			12.26	Nonenone	0.181		
2.18	Dichloromethane	0.554			12.30	$C_{10}H_{12}$			0.695
2.24	Heptane	0.282	0.200		14.65	2-Nonanol	1.064	0.557	
2.36	Benzene	0.111			15.70	2-Decanone		0.046	
2.49	2-Pentanone		0.267		16.09	Butyric acid	0.727		8.484
2.83	Methyl thioacetate	0.620			16.10	Decanal		0.329	1.256
3.08	Toluene	0.480	0.066	0.304	17.40	$C_{10}H_{14}O$			0.147
3.11	Dimethyldisulfide	1.904			17.53	Branched acid	0.119		2.397
3.23	Ethyl butyrate	0.220			18.05	2,3-Butanediol			12.736
3.47	Hexanal	0.371	0.209	0.313	19.24	Decanal	0.316		
3.49	2-Pentanol	1.065			20.07	2-Undecanone	0.921	5.443	0.042
3.64	1,2-Butanediol	0.329			20.30	Terpene		0.285	
4.46	C_8H_{10}	0.449			21.05	Furanone		0.305	
4.63	3-Methylbutanol	27.789	0.213	0.301	22.52	Alkane	0.315	2.120	0.903
5.20	2-Heptanone	2.571	1.814		22.84	2-Undecanol	0.071		
5.31	Heptanal	0.391		0.327	23.35	Decanol		1.211	
5.59	C_8H_{10}	0.071			23.85	2-Phenyl ethyl acetate	7.843	5.839	0.951
5.98	Acetoin	0.303	0.100		24.32	$C_{10}H_{14}O$		1.624	
6.09	Styrene	0.164	0.023		24.96	Hexanoic acid	7.184		
6.46	Dithiapentane	0.341			25.27	$C_{10}H_{14}O$		0.684	
6.53	Decanol		0.112		25.79	2-Phenylethanol	0.462	1.411	
7.52	$C_{10}H_{14}$		1.301		28.55	2-Tridecanone		3.488	0.686
7.56	2-Heptanol	1.805	0.100		30.45	BHT	2.226	1.866	3.123
7.91	C_9H_{12}	0.141	0.050		31.24	Octanoic acid	3.286		10.546
7.95	Limonene		0.253	0.225	31.79	Ethyl dodecanoate		0.241	
8.04	1-Hexanol	0.051			34.75	Nonanoic acid	0.100	0.215	0.586
8.14	Octanal	0.165	0.050	0.263	35.54	δ-Decalactone		1.588	1.404
8.23	$C_{10}H_{14}$		0.532		36.42	2-Pentadecanone		5.227	0.271
8.43	$C_{10}H_{14}$		0.127		37.80	Dodecanol	0.100	5.198	4.616
8.99	Dimethyltrisulfide	1.769			38.20	Decanoic acid	0.622		19.364
10.00	Acetic acid	0.431	0.300	9.112	39.25	Ethyl tetradecanoate		0.423	
10.46	Furancarboxaldehyde	0.539	0.031		39.87	Tridecanol		5.297	1.000
11.55	2-Nonanone	2.149	5.279	0.031	41.02	Phthalate			0.605
11.88	Nonanal	1.401	0.343	1.363	41.69	Unknown			5.340
12.00	Propanoic acid		0.228		44.90	Dodecanoic acid		8.993	

* Expressed as a percentage.

the only drawback being the necessity of reducing the sample quantity, because both are limited by the desorption cartridge volume. The small quantity of water released from the cheese during the desorption step did not show any deleterious effect on the column (which had a nonbonded stationary phase) after three months of use.

In order to compare their characteristics, the three extraction techniques were applied to eight different cheese varieties. The chromatographic results of each technique showed clear quantitative differences (as will be discussed for three artisanal cheese varieties).

Camembert is a well-known French variety made from cow's milk with a white layer of *P. candidum* on the surface. It has a strong, pleasant flavor and a high level of proteolysis. It contains a high number of volatile compounds: free fatty acids, methyl ketones, primary and secondary alcohols, lactones, esters, aldehydes, and sulfur compounds (1,21,22). Figure 1 shows the chromatographic traces obtained by the three methods, and the corresponding percent compositions appear in Table II. Free fatty acids were the main components (55%) in the DTD extract, followed by alcohols, aldehydes, and esters. DHS chromatograms were rich in alcohols (56%), followed by methyl ketones and esters (2-phenylethyl acetate especially). SDE afforded mainly apolar compounds such as methyl ketones and esters.

Chaumes is a cow's milk cheese from Dordogne (France) that is semisoft and has a strong, piquant flavor. No data about its volatile composition has been found in the literature. Figure 2 shows the chromatographic traces obtained by the three methods, and the

Table III. Volatile Composition* of a Chaumes Cheese Sample Extracted by Three Different Techniques

T_R	Compound	DHS	SDE	DTD	T_R	Compound	DHS	SDE	DTD
1.93	Carbon disulfide	0.125			9.03	Dimethyltrisulfide	0.056		0.093
1.94	2-Propanol			0.407	10.35	Acetic acid	0.045	0.64	6.028
2.12	Dichloromethane	1.781	0.256	13.88	11.05	Ethyl octanoate		0.082	
2.20	Heptane	0.066	0.291		11.55	2-Nonanone	0.301	0.984	0.176
2.27	3-Methylbutanal	0.358		10.12	11.90	Nonanal	0.018	0.873	
2.37	Benzene			1.116	14.22	Propanoic acid			1.993
2.52	Chloroform			14.31	14.91	Branched acid	3.836		1.361
2.52	2-Pentanone	1.656		5.277	15.24	Linalool		0.88	
2.62	Methyl butyrate			1.056	15.94	2-Decanone		0.051	
2.82	Methyl thioacetate	0.529			16.10	Butyric acid	0.683		0.599
2.92	3-Methyl-2-pentanone	1.021			16.14	Decanal		6.142	
3.05	Toluene	0.195	0.421	0.687	16.45	Phenyl acetate	0.048		
3.13	Dimethyldisulfide	3.021	0.151	0.051	17.55	Branched acid	41.47		14.12
3.22	Ethyl butyrate			0.043	18.55	Methyl dodecanoate		0.212	
3.45	2-Hexanone			0.151	19.64	Phenyl butyrate	1.726		
3.46	Hexanal		0.134		19.89	Benzyl methyl ketone	0.069		
3.47	2-Pentanol	0.305			20.12	α-Terpineol		7.289	
4.35	Methyl thiobutyrate	0.275			20.49	Undecanal		1.524	
4.35	3-Methylbutyl acetate			0.867	21.29	Carvone		2.212	
4.45	C_8H_{10}	0.020		0.917	21.67	Nonenol	0.073		
4.60	3-Methylbutanol	4.810		0.850	22.50	Phenyl hexanoate	0.291		
4.77	α-Pinene			0.244	22.54	Tetradecane		1.386	
5.22	2-Heptanone	2.394	0.339	0.329	23.34	2-Methylphenyl propanoate	0.248		
5.35	Heptanal	0.331	0.148	1.171	23.82	Caryophyllene			2.448
5.40	Methyl hexanoate			0.162	24.05	Hexanoic acid	11.44		0.428
5.75	β-Pinene			0.081	26.01	2-Phenylethyl alcohol			0.121
5.87	2-Heptanol	0.416	0.053		26.72	Branched acid	0.243		
6.17	Styrene	0.071			27.37	Valencene		21.59	
6.20	Decane			0.248	28.67	2-Tridecanone		0.433	
6.32	Methyl 3-methylthiobutyrate	1.093			28.69	Phenol	16.09	0.779	17.81
6.32	Myrcene			0.097	30.52	BHT	0.815		0.391
6.57	Phellandrene			0.171	31.34	*n*-Octanoic acid	2.635	1.486	0.252
6.95	Ethyl hexanoate			0.192	31.82	Indole		0.165	2.961
7.27	Limonene		0.208	0.265	32.75	$C_{15}H_{26}O$			8.067
7.50	1,8-Cineole			0.141	34.12	Decalactone		3.145	
7.59	C_9H_{12}			0.401	34.55	*n*-Nonanoic acid		0.416	0.100
8.18	Octanal		1.794		36.65	2-Pentadecanone		1.412	
8.20	Carene			0.327	38.32	*n*-Decanoic acid	1.288	16.94	
8.35	Hexanol			0.054	38.49	Sesquiterpene		2.969	
8.48	Methyl octanoate		0.144	0.578	40.99	Phthalate		3.238	

* Expressed as a percentage.

corresponding composition appears in Table III. This cheese seemed to be rich in components such as phenyl, ethyl and isoamyl esters, indol, sulfur compounds, and terpenes, which are less abundant in other varieties. Most likely, they are responsible in part for the peculiar flavor of this cheese.

La Serena is an artisanal cheese prepared in Extremadura (western Spain) with raw Merino sheep's milk coagulated with vegetable rennet. The rind is quite hard, but the paste, which undergoes extensive proteolysis, is fluid, very soft, and has a strong, nonacidic flavor. Some of their microbiological and physicochemical characteristics have been described (23–25), but their volatile composition has not been reported. Figure 3 compares the reconstructed chromatograms of volatile components from La Serena cheese that were obtained by DTD, DHS-TD, and SDE. The composition data appear in Table IV. Although fatty acids and methyl esters were the main components in DTD chromatograms, methyl ketones and alcohols were abundant in the DHS extract, and SDE showed, besides fatty acids and ketones, apolar compounds such as terpenes and hydrocarbons.

Each of the three chromatographic profiles in Figures 1–3 differed markedly, even though they contained many common peaks. Although SDE traces were richer in high-boiling apolar components, DHS traces' main components were high-volatility compounds and DTD traces showed an intermediate distribution.

Cabrales and Edelpilz cheeses (two blue varieties) were used to compare the extraction yield of the assayed fractionation techniques. Cheese samples were extracted by the three previously mentioned methods using methyl nonanoate as the internal standard. Although the results for the two cheeses were similar, only those corresponding to one of them (Edelpilz) are shown (Table V). Free fatty acids from acetic to decanoic acid were the most abundant components in DTD extracts, followed by methyl ketones from C5 to C15. 2-Alkanols, methyl, ethyl, and isoamyl esters also attained high levels. DHS gave the lowest extraction

Table IV. Volatile Composition* of a La Serena Cheese Sample Extracted by Three Different Techniques

T_R	Compound	DHS	SDE	DTD	T_R	Compound	DHS	SDE	DTD
1.87	Carbon disulfide	0.477			13.50	Branched acid	0.795		0.641
1.96	Methyl acetate			1.880	14.51	2-Nonanol	1.295		0.641
2.05	2-Propanol	0.460			14.96	Methyl octanoate	0.639	0.611	0.043
2.12	Dichloromethane	0.173		1.065	15.54	2-Decanone	0.362		
2.14	Ethyl acetate	0.057			15.69	Butyric acid	3.552	0.363	7.798
2.14	Heptane	3.552	0.042	2.692	16.15	Decanal			0.116
2.27	3-Methylbutanal	0.517	0.316	0.088	16.87	3-Methylbutyl hexanoate	0.109	0.116	
2.39	Benzene	0.028		0.365	17.31	Branched acid	2.746	1.020	2.522
2.49	Chloroform			3.150	20.04	2-Undecanone	1.862	1.540	0.048
2.55	2-Butanol	14.003	0.025	4.897	20.55	Phenyl acetate		0.050	
2.62	Methyl butyrate			1.580	20.76	Methyl decanoate	0.169		22.220
3.12	Toluene	0.759	0.037	0.428	21.27	Undecanal		0.051	
3.32	Ethyl butyrate	1.680	0.105	0.027	23.77	Hexanoic acid	4.105	5.750	4.710
3.47	2-Hexanone	0.220		0.029	23.78	Ethyl decanoate		1.000	0.063
3.92	Hexanal	0.292	0.222	0.012	24.69	Dimethylsulfone		0.024	
3.92	2-Pentanol	3.939			25.75	Phenylethyl alcohol		0.218	
4.05	Nonane	0.042		0.026	28.87	Benzothiazol		0.125	
4.16	C_8H_{10}			0.268	27.61	Branched acid	0.148	0.502	0.128
4.52	3-Methylbutanol	3.494			28.43	2-Tridecanone		0.475	0.097
5.19	2-Heptanone	11.701	0.991	2.000	28.69	2-Ethylphenyl acetate	0.058	1.240	
5.62	Methyl hexanoate	0.299	0.041	4.994	29.26	Methyl dodecanoate			4.540
6.80	Myrcene	0.031	0.308	0.061	30.30	BHT	0.080	1.236	0.060
7.00	2-Heptanol	2.483	2.160		31.37	Octanoic acid	0.714	16.860	1.710
7.42	Ethyl hexanoate	3.753	0.087	0.064	30.53	Alkane	0.036	0.284	
7.80	2-Octanone	1.201			32.36	Ethyl dodecanoate		0.254	0.060
8.10	Limonene		0.167	0.073	31.78	Branched acid		0.362	
8.34	*p*-Cymene	0.184	0.112		32.36	Phthalate		0.254	
8.99	Carene	0.143	0.176		34.31	Alkane		0.934	0.330
9.00	3-Methylbutyl butyrate	0.070			36.98	Methyl tetradecanoate			1.880
9.70	Acetic acid	0.334	0.516		37.98	2-Pentadecanone		0.167	
10.00	$C_{10}H_{16}$	0.021			37.97	Decanoic acid		39.713	1.570
11.90	Nonanal		0.297		38.97	Ethyl tetradecanoate		0.446	
11.60	2-Nonanone	30.716	7.510	0.177	38.89	Hexadecanol	0.048	1.870	
12.20	Nonenone	0.631	0.209		40.73	Phthalate		0.164	0.123
12.46	Propanoic acid			1.022	41.37	Unknown		3.514	
12.43	Methyl octanoate	0.180		9.054	44.26	Methyl hexadecanoate			0.670

* Expressed as a percentage.

Table V. Extraction Yield of Volatile Compounds* from an Edelpilz Sample Using Three Different Extraction Methods

Group of compounds	DTD	SDE	DHS
Free fatty acids	278	8.6	1.0
2-Alkanones	61.8	24.3	4.9
2-Alkanols	10.5	10.9	0.35
Methyl esters	9.4	8.1	0.29
Isoamyl esters	4.3	3.4	0.15
Ethyl esters	1.8	3.4	0.10

* Grouped by chemical families and expressed in µg/g.

yield for all the families, with methyl ketones being the most abundant compound. Extraction yield differences between DTD and SDE were high only for acids and ketones. All of the mentioned compounds have been previously described in blue cheeses as provenient from the intense lypolysis induced by the molds (26).

The origin of aromatic hydrocarbons (such as benzene, toluene, xylenes, and ethyl benzenes) and halogenated hydrocarbons (chloroform, dichloromethane, and others) in dairy products has not been satisfactorily explained, although they have been found in milk (27) and cheeses (22).

Conclusion

None of the assayed methods are capable of providing an exhaustive extraction of the cheese volatile components, which actually are not a well-defined group of compounds. Each method affords a unique pattern of volatiles that can be used to characterize cheese samples from their volatile composition.

SDE usually recovers a high percentage of apolar compounds but presents high losses of the polar ones such as free fatty acids. When a given cheese sample is repeatedly extracted by DHS, even for a long time, noticeable amounts of volatiles appear after several consecutive extraction steps, thus showing that their recovery is only partial. DTD has shown to be exhaustive for the extraction of high and medium volatility compounds in plants using desorption temperatures in the range of 180°C to 200°C (8), but under the milder conditions used in this work, desorption could not be complete, even using the maximum desorption time allowed by the equipment used (30 min).

From all of the presented data, it seems that DTD compares favorably with SDE and DHS because it affords the more-complete extraction yield and a volatile pattern with the lowest discrimination towards the main families of volatile compounds, such as fatty acids and methyl ketones. Besides this, for all the examined samples no artifacts related to thermal decomposition were detected. DTD appears to be a very attractive procedure because it is fast, simple, and automatic; however, when a sample poor in volatiles (i.e., a fresh cheese) is analyzed, the absolute quantity of substances arriving to the column is small because both the maximum sample amount (approximately 50 mg of cheese plus desiccant) and the maximum desorption time (30 min) are respectively limited by the desorption cartridge volume and operating parameters. Because every method has advantages and drawbacks, the choice should depend on the objectives and requirements of each particular analysis.

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