Study of the Volatile Fraction of Parmesan Cheese

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The aroma fraction of Parmesan cheese was studied on 21 samples of certified origin and aging. Volatile compounds were isolated using the dynamic headspace and simultaneous distillation–extraction techniques and analyzed by means of GC and GC–MS. A total of 167 compounds were identified: hydrocarbons (23), aldehydes (19), ketones (19), alcohols (29), esters (24), and acids (25) are the prevalent classes of substances. With the headspace technique the most volatile compounds were sampled, whereas the distillation–extraction technique was suitable even for long-chain carbonyl derivatives, acids, esters, and lactones, which are known to be important components of the flavor. In addition, free fatty acids and the volatile products of the lactic fermentation (ethanol, acetaldehyde, diacetyl, and acetoin) were determined.

INTRODUCTION

Parmesan cheese is one of the most typical Italian foods, having considerable commercial importance and particular features: a strictly defined area of origin, a traditionally established production technology, and a long period of aging (16–24 months). Composition, ripening mechanism, and microbiological aspects concerning the nonvolatile fraction of this product are well-known; however, the flavor of Parmesan cheese has not been systematically studied, at least on a significant number of samples of certified origin and aging. In fact, few studies have dealt with the flavor components of Parmesan and have taken into account a limited number of samples.

In a comparative study of the volatile constituents of seven cheeses, Groux and Moinas (1974) used vacuum distillation as a stripping technique, followed by extraction of the volatiles from the aqueous distilled phase with diethyl ether and analysis of the concentrated extract by GLC. They found small quantities of volatile compounds in Parmesan compared with other cheeses: only primary alcohols were present in appreciable amounts. Dumont et al. (1974) studied the volatile substances responsible for the aroma of both block and grated Parmesan cheese by using the same procedure reported by Groux and Moinas but with smaller quantities of samples. The result of their work from GC–MS analysis is that ethyl esters were not present in the grated cheese, which lacked the fruity flavor typical of the block product. Manning and Moore (1979) described a method for the chromatographic analysis of the headspace of Parmesan and of other hard cheeses of unspecified age. These authors considered in particular sulfur compounds and obtained similar chromatograms from a wide variety of commercial cheeses, finding some differences concerning the content of H_2S and CH_3SH, which was very low in Parmesan cheese.

The free fatty acid profile in Parmesan, among other Italian cheeses, has been investigated by Lindsay (1983); the GLC analysis was performed after the removal of lactic acid and concentration of the fatty acids on an alkaline arrestant column, showing a high content of short-chain FFA in Parmesan. Also, branched short-chain acids were considered important in the flavor of ripened Italian cheeses. Individual FFA concentrations of four Italian cheese varieties of known ages have been measured by Woo and Lindsay (1984) with a gas chromatographic method; they collected FFA data for three Parmesan samples during aging (2, 6, and 18 months at the beginning of the study and 6 additional months of maturation at 7 °C for each sample). It was found that the concentrations of major free fatty acids in pleasantly flavored Parmesan cheeses increased only moderately during maturation.

Meinhart and Schreier (1986) studied neutral and acidic volatile constituents of Parmesan cheese; the aroma compounds have been isolated by high-vacuum distillation–solvent extraction; the separation of acids was obtained by alkaline treatment and derivatization to their methyl esters. Among the volatile substances identified by HRGC–MS, ethyl hexanoate, 2-heptanone, and 2-pentanol quantitatively predominated.

The analysis of aroma of a Parma-type cheese produced in Spain was performed by Rafecas et al. (1986) by the stripping of volatile components with nitrogen and adsorption on activated charcoal: esters, secondary alcohols, and methyl ketones were found to be the most abundant compounds.

The present work was undertaken to improve the knowledge of the flavor of Parmesan cheese, taking into account an adequate number of selected aged (24 months) samples, coming from different zones of the typical production area and representative of all seasonal productions. Aroma compounds of Parmesan cheese were extracted using the dynamic headspace and the simultaneous distillation–extraction techniques; the analysis was carried out by gas chromatography and gas chromatography–mass spectrometry. In addition, free fatty acids (FFA) and the volatile components produced by lactic acid bacteria (ethanol, acetaldehyde, diacetyl, and acetoin) were analyzed.

MATERIALS AND METHODS

Samples. Twenty-one samples of aged Parmesan cheese of different, but always acceptable, quality (24 months old) were obtained from producers in different geographical zones of the production area (13 products were from the plain and 8 from the mountain zone). Among the samples examined, 6 cheeses were produced during the winter and 5 during each other season in

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such a way that all of the samples were at the same maturation level; the analysis was carried out over a period of 1 year.

The cheese was cut in 1-kg portions to be used for the different analyses. Each part was vacuum-packaged in sealed polyethylene bags and maintained at 4 °C until analysis. For each determination the samples were taken from the 1-kg cheese block at least 4 cm from the rind and 3 cm from the center.

Aroma Sampling Techniques. Volatile components were isolated by means of two different techniques: simultaneous distillation and extraction (SDE technique), using a modified Likens–Nickerson microapparatus (Chrompack, Middelburg, The Netherlands) and dichloromethane as extraction solvent, and dynamic headspace with adsorption on Tenax traps and thermal desorption with cryofocusing of the aroma volatiles into the GC capillary column.

The aromatic profiles of the samples were obtained by means of capillary gas chromatography. The whole analytical procedure, including cheese headspace sampling and thermal desorption, isolation of volatiles by distillation–extraction technique, and GC analysis, was checked for reproducibility. All 21 cheeses were analyzed six times by dynamic headspace technique and four times by the SDE method, and the obtained area values were averaged. Coefficients of variation for relative peak areas were determined for components having relative area greater than 0.02%; compounds that were difficult to measure accurately because peak areas were small were not included in these calculations.

The identification was performed by gas chromatography–mass spectrometry; the mass spectrometric identifications were confirmed by comparing the gas chromatographic retention times and the components' mass spectra with those of authentic reference standards (66 and 69 standards in the case of the headspace and SDE techniques, respectively). Experimental details of sampling and chromatographic conditions are reported elsewhere (Careri et al., 1994).

Analysis of Free Fatty Acids. Determinations of the free fatty acids in the Parmesan cheese were performed according to the method reported by de Jong and Badings (1990). This procedure was modified in the following way: four steps of lipid extraction with 3 mL of diethyl ether/heptane (1:1 v/v), isolation of the FFA using alumina, and subsequent desorption with 5 mL of ether containing 6% formic acid. The separation of FFA with chain lengths from $C_{2:0}$ to $C_{18:1}$ was performed by means of high-resolution gas chromatography using a fused silica capillary column FFAP-CB (25 m × 0.32 mm, 0.3-μm film thickness) (Chrompack). An HRGC 5160 gas chromatograph (Carlo Erba, Milan, Italy), equipped with a data acquisition system (Maxima, Waters, Millipore, Milford, MA) was utilized. The chromatograph was fitted with a FID kept at 300 °C and a split/splitless injector used in the split mode (15:1 split ratio) and maintained at 270 °C. Oven temperature was programmed from 65 to 240 °C at 10 °C/min and held for 24 min. The carrier gas was helium at 2 mL/min. Two independent determinations were carried out for each sample.

Analysis of Volatile Components Produced by Lactic Acid Bacteria. As fermentation products of homofermentative lactic acid bacteria, ethanol, acetaldehyde, diacetyl, and acetoin were considered.

Ethanol and acetaldehyde were analyzed by enzymatic determinations: In a 100-mL flask, 10 g of grated cheese was extracted with 50 mL of water at 50 °C for 15 min, under stirring. After cooling, the mixture was treated with the Carrez reagent and with 10 mL of 0.1 M NaOH and diluted with water to 100 mL. The filtered solution was analyzed for ethanol by using the enzymatic method (Boehringer-Mannheim kit no. 176 290, Boehringer-Mannheim Italia, Milan). Acetaldehyde was determined in the same way, using the enzymatic procedure (Boehringer-Mannheim no. 668 613). In this case, to ensure that the test solution had an acetaldehyde concentration between 0.008 and 0.05 g/L, as required by the method, the amount of grated cheese analyzed was increased to 50 g.

Diacetyl and acetoin were determined by means of a colorimetric method (Brandon, 1964; Murdock, 1967; Lees and Jago, 1970; La Placa, 1979; Gierschner and Herbst, 1981). For the determination of diacetyl, 20 g of grated cheese was added to 200 mL of water and steam distilled. In the distilled solution, diacetyl was made to react with creatine in the presence of α-naphthol with formation of a red compound. The absorbance was measured at λ = 545 nm, using an Uvikon 860 spectrophotometer (Kontron Instruments, Schlieren, Switzerland). For the determination of acetoin, before the distillation step the sample was oxidized using $FeCl_3$ (50 mmol for a 20-g sample) to transform acetoin into diacetyl. In this way the total concentration of diacetyl was determined; the acetoin concentration was calculated by subtracting from the total diacetyl concentration the value obtained without oxidation. A replication of these determinations was performed.

The significance of the differences among the results was checked using the Duncan multiple test.

RESULTS AND DISCUSSION

The volatile components identified in the headspace or in the SDE extracts of the 21 samples of aged Parmesan cheese using HRGC–MS are listed in Table 1; for each sampling technique, relative average peak area, coefficient of variation, and occurrence of the volatiles are reported. A total of 167 substances were detected, 69 for the first time in this work.

In the headspace of the examined samples 110 substances were identified, 39 of which were found for the first time as components of Parmesan cheese. These substances included 20 hydrocarbons, 23 alcohols, 8 aldehydes, 13 ketones, 11 acids, 20 esters, 5 sulfur compounds, 1 pyrazine, 4 furans, and 5 miscellaneous compounds. Among these components, 2-pentanone, ethyl acetate, and butanoic acid quantitatively predominated.

With regard to the components isolated by the simultaneous distillation and extraction technique (SDE), in the chromatograms of the 21 samples of Parmesan cheese 105 compounds were detected, 30 of which were newly reported substances. The extracts made up of 7 hydrocarbons, 16 alcohols, 14 aldehydes, 13 ketones, 23 acids, 14 esters, 6 lactones, 2 sulfur compounds, 2 pyrazine, 3 furans, and 5 miscellaneous compounds; fatty acids and 2-alkanones were found to be the most abundant compounds, hexanoic acid dominating.

Many of the identified compounds are reported for the first time as constituents of Parmesan aroma, e.g., 2-methylpropanal, 2-methylbutanal, dimethyl disulfide and trisulfide, limonene, 1-hydroxy-2-propanone, 3-(methylthio)propanal, hexadecanal, tridecanoic acid, 9-tetradecenoic acid, 9-hexadecenoic acid, and δ-tetradecalactone.

On examination of the different classes of substances detected with the two sampling procedures, 23 hydrocarbons were detected as components of the Parmesan volatile fraction, 11 of which were found for the first time in this study. Hydrocarbons are secondary products of lipid autoxidation, as previously discussed (Barbieri et al., 1992).

Aldehydes accounted for 2.0% of the relative chromatogram area both in the headspace and in samples prepared by steam distillation–extraction. The headspace fraction contained linear and branched-chain saturated aldehydes and two aromatic derivatives, whereas linear unsaturated aldehydes were not found. These compounds were isolated using the SDE technique together with the less volatile aldehydes such as tetradecanal, pentadecanal, and hexadecanal. The occurrence of the sulfur-containing aldehyde, 3-(methylthio)propanal, is reported for the first time in Parmesan cheese. Although a low concentration methional can be a positive aroma contributor, when too high or out of balance, it forms a boiled sweetcorn-like odor (Vercellotti et al., 1989).

Aldehydes may derive from amino acids during cheese ripening (Strecker degradation); at the beginning of cheese curing, with a lower pH, amino acids are decarboxylated

Table 1. Volatile Components of Parmesan Cheese

	compound	rel percentage area				occurrence[b]		
		headspace	CV (%)	SDE	CV (%)		headspace	SDE
			Hydrocarbons					
1	*n*-hexane	8.44	20.5			a*	21	
2	2-methylhexane	0.66	25.6			a*	21	
3	*n*-heptane	1.80	13.4			a*	21	
4	methylcyclohexane	0.11	20.5			a*	18	
5	*n*-octane	0.29	26.9			a*	10	
6	1-octene	0.20	16.8			a*	4	
7	(?)-octene	0.34	23.2			a*	21	
8	benzene	0.29	17.2			a	21	
9	2,2-dimethyldecane	0.06	24.2			a*	5	
10	toluene	0.29	16.4	0.17	19.4	a, b	21	21
11	ethylbenzene	0.08	18.0	<0.02		a, b	12	12
12	isopropylbenzene	<0.02				a*	13	
13	*p*-xylene	0.04	14.7			a	19	
14	*m*-xylene	0.04	21.1	0.02	7.8	a, b	17	17
15	*o*-xylene	0.05	23.3			a	5	
16	limonene	0.05	14.7	0.03	25.8	a, b	16	16
17	4-methyl-1-hexene	<0.02				a*	7	
18	1-methylstyrene	0.16	15.0			a	19	
19	*n*-heptadecane			0.08	15.8	b		21
20	naphthalene	<0.02				a	18	
21	1-methylnaphthalene	0.06	29.2			a	3	
22	(2,6,10,14-tetramethylhexadecane)			0.03	27.4	b*		10
23	*n*-octadecane			0.22	26.4	b		18
			Aldehydes					
1	2-methylpropanal	0.16	26.9			a*	21	
2	2-methylbutanal	0.83	11.5			a*	21	
3	3-methylbutanal	0.80	13.2			a	21	
4	pentanal	<0.02				a	1	
5	(*E*)-2-butenal			0.94	15.7	b*		21
6	(*Z*)-2-butenal			0.05	16.2	b*		19
7	hexanal	0.06	19.9	0.03	18.7	a, b	16	16
8	2-pentenal			<0.02		b*		19
9	2-vinyl-2-butenal			0.04	17.2	b*		10
10	(*Z*)-2-heptenal			<0.02		b*		6
11	nonanal	0.03	12.6	<0.02		a, b	9	9
12	2,4-hexadienal			0.05	14.8	b*		6
13	3-(methylthio)propanal			0.07	22.1	b*		21
14	benzaldehyde	0.05	19.5	0.07	17.5	a, b	21	21
15	2-hydroxybenzaldehyde	<0.02				a*	3	
16	phenylacetaldehyde			0.09	18.2	b		21
17	tetradecanal			0.11	23.8	b*		17
18	pentadecanal			0.04	13.4	b*		14
19	hexadecanal			0.17	12.1	b*		14
			Ketones					
1	acetone	0.18	26.9			a	21	
2	2-butanone	3.62	17.2			a	21	
3	3-methyl-2-butanone	0.33	15.7			a*	21	
4	2-pentanone	16.22	9.8	1.30	33.7	a, b	21	21
5	diacetyl			0.45	33.7	b*		21
6	3-hexanone			0.04	14.7	b		20
7	2-hexanone	0.20	14.0	0.04	18.7	a, b	21	21
8	3-penten-2-one			0.03	17.6	b*		16
9	2-heptanone	3.02	10.4	2.39	13.0	a, b	21	21
10	4-hydroxy-3-propyl-2-hexanone	<0.02				a*	3	
11	acetoin	0.24	16.1	0.21	21.4	a, b	21	21
12	2-octanone			0.02	5.1	b		21
13	1-hydroxy-2-propanone	0.04	40.6	0.03	14.7	a, b	16	21
14	2-nonanone	0.23	12.6	1.29	14.1	a, b	21	21
15	2-undecanone	0.05	23.3	0.95	18.3	a, b	21	21
16	acetophenone	0.04	33.9			a	20	
17	2-tridecanone			0.54	19.5	b		21
18	geranyl acetone	<0.02				a*	9	
19	2-pentadecanone			0.42	21.5	b		21
			Alcohols					
1	ethanol	0.88	37.9			a	21	
2	2-butanol	7.06	12.8			a	21	
3	1-propanol	0.80	21.7	0.08	14.9	a, b	18	18
4	2-methyl-1-propanol	0.63	10.6	0.04	17.5	a, b	21	21
5	2-pentanol	4.22	10.8	0.38	15.6	a, b	21	21
6	1-butanol	3.02	11.4	0.20	15.2	a, b	21	21
7	3-penten-2-ol			0.03	20.4	b*		15
8	3-methyl-1-butanol	0.88	11.9	0.11	15.1	a, b	21	21
9	3-methyl-3-buten-1-ol	0.41	11.3	0.06	14.5	a, b	21	21

Table 1 (Continued)

	compound	rel percentage area					occurrence[b]	
		headspace	CV (%)	SDE	CV (%)		headspace	SDE
			Alcohols					
10	1-pentanol	0.05	15.1	<0.02		a, b	18	18
11	2-heptanol	0.03	32.3	0.07	14.1	a, b	19	19
12	1,3-butanediol	0.06	17.8	0.02	10.2	a, b	10	17
13	1-hexanol	0.08	20.5	0.03	13.4	a, b	20	20
14	2-butoxyethanol	0.09	33.2			a*	20	
15	2-ethyl-1-hexanol	0.03	39.7			a	12	
16	1-hepten-4-ol			0.03	15.4	b*		14
17	1-octanol	<0.02				a	2	
18	2,3-butanediol	<0.02				a*	6	
19	2-propoxyethanol	<0.02				a*	2	
20	1-methoxy-2-propanol	<0.02				a*	6	
21	1,2-propanediol	<0.02				a*	3	
22	2-phenyl-2-propanol	<0.02				a*	4	
23	2-(2-butoxy)ethoxyethanol	<0.02				a*	9	
24	1-decanol			0.05	21.1	b		19
25	1-undecanol			0.09	18.7	b*		21
26	2,6-bis(*tert*-butyl)-4-methylphenol			0.05	19.3	b*		12
27	1-dodecanol	<0.02				a	10	
28	phenol	0.05	36.3			a	19	
29	1-hexadecanol			0.06	17.5	b		12
			Esters					
1	methyl acetate	0.06	32.6			a*	8	
2	ethyl acetate	8.44	17.2			a	21	21
3	ethyl propanoate	0.36	14.9			a	5	
4	methyl butanoate	1.02	14.6	0.16	16.3	a, b	21	21
5	isobutyl acetate	0.28	21.6			a*	1	
6	ethyl butanoate	3.02	12.8	0.40	15.7	a, b	21	21
7	isopropenyl acetate	<0.02				a*	1	
8	butyl acetate	<0.02				a	1	
9	propyl butanoate	0.02	29.1	<0.02		a, b	17	17
10	ethyl pentanoate	0.05	17.7			a	20	
11	methyl hexanoate	0.27	14.5	0.02	19.9	a, b	19	19
12	butyl butanoate	0.02	20.5	0.03	17.1	a, b	13	13
13	ethyl hexanoate	2.91	11.1	1.10	13.4	a, b	21	21
14	methyl heptanoate			0.02	15.5	b		6
15	propyl hexanoate	0.11	17.3	<0.02		a, b	13	13
16	ethyl heptanoate	0.04	19.1	0.04	14.6	a, b	18	18
17	methyl octanoate	0.03	12.6			a	5	
18	isobutyl hexanoate			0.02	19.6	b*		6
19	ethyl octanoate	2.32	29.7	0.55	16.2	a, b	21	21
20	ethyl nonanoate	<0.02				a*	1	
21	methyl decanoate	<0.02				a	3	
22	ethyl decanoate	0.05	33.1	0.72	18.2	a, b	21	21
23	ethyl tetradecanoate			0.04	20.3	b		18
24	ethyl hexadecanoate			0.04	15.4	b		18
			Acids					
1	acetic acid	1.25	29.7	0.07	16.1	a, b	21	21
2	propanoic acid	0.62	27.2	0.11	19.8	a, b	17	21
3	2-methylpropanoic acid	0.03	20.6			a	8	
4	butanoic acid	8.21	34.6	9.32	31.4	a, b	21	21
5	3-methylbutanoic acid	0.05	27.2	0.12	32.9	a, b	21	21
6	(?-dimethylbutanoic acid)			<0.02		b*		21
7	pentanoic acid	0.04	24.2	0.17	24.1	a, b	21	21
8	hexanoic acid	2.69	23.4	18.42	15.6	a, b	21	21
9	2-ethylhexanoic acid	<0.02				a	5	
10	heptanoic acid	<0.02		0.48	17.7	a, b	21	21
11	octanoic acid	0.39	28.6	16.54	16.3	a, b	17	17
12	nonanoic acid	<0.02		0.37	17.5	a, b	18	21
13	decanoic acid			14.34	15.5	b		21
14	9-decenoic acid			2.02	19.1	b		21
15	undecanoic acid			0.13	19.0	b		20
16	dodecanoic acid			7.59	20.2	b		21
17	tridecanoic acid			0.14	25.8	b*		21
18	(?-methyltridecanoic acid)			0.18	21.7	b*		18
19	tetradecanoic acid			7.83	24.7	b		21
20	9-tetradecenoic acid			0.53	21.6	b*		21
21	(?-methyltetradecanoic acid)			0.16	19.2	b*		21
22	(?-methyltetradecanoic acid)			0.23	21.1	b*		21
23	pentadecanoic acid			0.45	24.4	b		21
24	hexadecanoic acid			3.89	36.7	b		21
25	9-hexadecenoic acid			0.26	28.7	b*		20

Table 1 (Continued)

	compound	rel percentage area: headspace	CV (%)	SDE	CV (%)		occurrence[b]: headspace	SDE
			Lactones					
1	δ-octalactone			0.02	12.4	b		11
2	γ-decalactone			0.02	22.1	b		11
3	δ-decalactone			0.23	19.3	b		21
4	γ-dodecalactone			0.18	18.7	b		21
5	δ-dodecalactone			0.28	19.0	b		21
6	δ-tetradecalactone			0.16	24.3	b*		21
			Sulfur Compounds					
1	dimethyl disulfide	0.34	20.0	0.02	24.8	a, b*	21	21
2	dimethyl trisulfide	<0.02		0.03	13.7	a, b*	21	21
3	dimethyl tetrasulfide	<0.02				a*	16	
4	tetramethyl thiourea	<0.02				a*	9	
5	benzothiazole	<0.02				a	15	
			Pyrazines					
1	2,6-dimethylpyrazine	0.01	32.3	0.07	15.3	a, b	21	21
2	3-ethyl-2,5-dimethylpyrazine			<0.02		b		6
			Furans					
1	tetrahydrofuran	9.38	17.9			a*	21	
2	2-ethylfuran	0.05	40.2			a*	21	
3	2-(chloromethyl)furan			0.03	14.7	b*		5
4	furfural			0.03	13.0	b		20
5	furfuryl alcohol	0.08	21.0	0.64	21.9	a, b	21	21
6	3-methyl-2-(5*H*)-furanone	0.03	33.2			a*	6	
			Miscellaneous Compounds					
1	tetrachloromethane	0.40	22.4			a	15	
2	chloroform	0.04	17.8	0.17	19.4	a, b	21	21
3	tetramethylurea	<0.02				a	4	
4	benzonitrile	<0.02				a	10	
5	unknown			0.03	15.0	b		3
6	acetamide	<0.02				a	11	
7	(long-chain carbonyl compound)			0.14	18.7	b		21
8	(long-chain carbonyl compound)			0.06	17.9	b		15
9	(long-chain carbonyl compound)			0.11	23.4	b		17

[a] Method of identification: a, detected in headspace samples; b, detected in SDE samples; *, not previously found in Parmesan cheese. [b] Number of samples (of 21) in which the component was detected. [c] Tentative identifications are enclosed in parentheses.

to amines, and then, in later phases, oxidation reactions take place at a higher pH (Belitz and Grosch, 1987a).

Aldehydes, together with ketones, are major secondary products of autoxidation of unsaturated fatty acids: the primary products of this autoxidation are hydroperoxides, which undergo further degradation to hydrocarbons, alcohols, and carbonyl compounds (de Man, 1990a).

Acetaldehyde, 2-methylpropanal, 2- and 3-methylbutanal, and phenylacetaldehyde represent some aldehydes found in Parmesan cheese; in particular, acetaldehyde is considered to provide a sharp, penetrating, and fruity note to the food.

Long-chain aldehydes (C_{13}–C_{17}) are formed from fatty acids by an α-oxidation mechanism (Belitz and Grosch, 1987b).

Ketones were found to be the most abundant components of the Parmesan headspace (26.2% of the total area), 2-pentanone and 2-heptanone prevailing; the relative percentage area in the 21 extracts obtained after distillation–extraction was lower (7.7%) because of the predominance of the fatty acids. A lot of methyl ketones were detected with both the techniques, some of which have not previously been reported in Parmesan cheese: diacetyl, 3-penten-2-one, 1-hydroxy-2-propanone, 3-methyl-2-butanone, and 4-hydroxy-3-propyl-2-hexanone. Methyl ketones are considered to derive from acyl lipids through a microbial degradation, during the which the triacylglycerols are enzymatically hydrolyzed and then the corresponding free fatty acids <C_{14} degraded to 2-alkanones by a β-oxidation pathway mechanism. Some of the 2-alkanones are reduced to the corresponding 2-alkanols by the oxidoreductase enzyme (Belitz and Grosch, 1987c). Because of their sensory properties, methyl ketones act as important flavor constituents, especially in the case of semisoft cheeses.

Table 2. Concentrations (Average Values, Milligrams per 100 g) of Compounds Deriving from Lactobacterial Activity in Parmesan Cheese Samples Grouped According to Season and Zone of Production[a]

	acetaldehyde	ethanol	diacetyl	acetoin
c_{total}	0.57	5.37	0.06	0.30
SD	0.30	1.50	0.03	0.21
c_{spring}	0.52[a,b]	4.12	0.06	0.40
SD	0.47	0.78	0.05	0.29
c_{summer}	0.34[b]	5.36	0.06	0.26
SD	0.21	1.33	0.02	0.14
c_{autumn}	0.62[a,b]	5.30	0.06	0.18
SD	0.13	2.08	0.03	0.07
c_{winter}	0.76[a]	6.48	0.06	0.34
SD	0.19	0.79	0.03	0.25
$c_{mountain}$	0.44	4.90	0.04[a]	0.16[a]
SD	0.23	0.84	0.02	0.05
c_{plain}	0.65	5.66	0.07[b]	0.38[b]
SD	0.32	1.76	0.03	0.23

[a] Different letters on the concentration values denote significant differences ($P < 0.05$). Data not labeled are not significantly different.

With regard to the compounds deriving from lactobacterial activity, in Table 2 the results of the determinations of acetaldehyde, ethanol, diacetyl, and acetoin are reported. The average concentrations in the samples grouped according to season and zone of production were calculated. Significant differences resulted in the concentrations of acetaldehyde in samples produced in winter with respect to those of summer production. In contrast, in the case

Table 3. FFA Concentrations (Average Values, Milligrams per 100 g) in Parmesan Cheese Samples Grouped According to Season and Zone of Production[a]

	$C_{4:0}$	$C_{6:0}$	$C_{8:0}$	$C_{10:0}$	$C_{12:0}$	$C_{14:0}$	$C_{16:0}$	$C_{18:0}$	$C_{18:1}$
c_{total}	11.4	7.2	4.4	8.3	9.6	48.0	151.2	50.3	153.3
SD	5.6	3.7	2.0	4.8	4.9	28.3	90.6	25.5	62.5
c_{spring}	8.8[a]	8.2	4.3	6.2[a]	8.3[a]	44.9[a]	119.9[a]	54.0[a]	145.5
SD	2.0	4.0	1.7	2.1	1.9	8.8	38.5	21.2	47.0
c_{summer}	11.0[a]	6.6	4.4	7.0[a]	7.9[a]	36.5[a]	109.0[a]	40.8[a]	134.2
SD	4.4	3.0	1.9	2.5	2.5	13.3	24.7	7.4	30.9
c_{autumn}	8.3[a]	4.4	3.6	5.5[a]	6.8[a]	24.7[a]	86.6[a]	30.4[a]	117.1
SD	2.2	2.2	1.7	1.7	2.4	14.2	48.4	18.0	59.9
c_{winter}	16.6[b]	9.1	5.2	13.5[b]	14.3[b]	79.4[b]	261.6[b]	71.9[b]	205.8
SD	7.4	4.3	2.5	5.9	6.6	31.2	89.2	29.5	71.9
$c_{mountain}$	11.8	7.9	4.2	7.6	7.9	48.1	139.7	48.5	144.8
SD	7.5	5.2	2.6	5.6	5.3	34.2	100.3	32.9	84.1
c_{plain}	11.2	6.7	4.5	8.8	10.5	42.1	158.3	51.4	158.5
SD	4.4	2.7	1.6	4.5	4.5	25.1	87.5	21.1	48.0

[a] Different letters on the concentration values denote significant differences ($P < 0.05$). Data not labeled are not significantly different.

of acetoin and diacetyl the differences are between the samples of different production zones (plain or mountain); both compounds have the amino acids valine and leucine as aroma precursors.

Numerous alcohols were found both in Parmesan cheese headspace in the samples obtained with the SDE method: 14 primary alcohols, 6 secondary alcohols, 2 phenols, and 7 alcohols with different structures. Ethanol does not represent the main volatile constituent of Parmesan cheese, as indicated by Groux and Moinas (1974). The formation pathway mechanism of 2-alkanols as byproducts of the β-oxidation of fatty acids was discussed above in connection with 2-alkanones. Primary alcohols are considered to originate from the corresponding aldehydes produced from fatty acid and amino acid metabolism, following a reaction pathway previously described (Barbieri et al., 1992).

Several fatty acids were isolated and detected, especially in the extracts obtained with the SDE method (81.4% of the total area instead of 10.7% for the headspace sampling). As indicated in Table 1, the SDE technique confirmed the presence of nearly all the acids identified in the Parmesan headspace (except 2-methylpropanoic and 2-ethylhexanoic acid) and revealed many additional compounds; in fact, the method was more effective in isolating long-chain fatty acids ($C_{10:0}$–$C_{16:1}$). In all 21 cheese extracts examined, the presence of the unsaturated 9-decenoic and 9-tetradecenoic acids was observed. Moreover, the volatile fraction of 20 samples contained 9-hexadecenoic acid; myristoleic ($C_{14:1}$) and palmitoleic ($C_{16:1}$) acids were described for the first time as constituents of the Parmesan aroma. On the other hand, it is known that monoenoic fatty acids from $C_{10:1}$ to $C_{26:1}$, except $C_{11:1}$, are present in cow's milk fat (de Man, 1990b). In addition to the main even-numbered straight-chain fatty acids, small amounts of odd-C-number branched-chain fatty acids were found in the analysis of the volatile fraction of Parmesan cheese. Pentanoic and heptanoic acids are found in food only in small amounts; pentadecanoic and heptadecanoic acids are present in milk and in some plant oils (Belitz and Grosch, 1987d).

During the ripening of cheese, free fatty acids can originate from three main biochemical pathways: lipolysis, proteolysis, and lactose fermentation. Enzymes with lipolytic activity (esterases, lipases) may cause the release of linear-chain fatty acids, whereas proteolytic enzymes are responsible for the formation of branched-chain fatty acids (i.e., isobutanoic and isovaleric acids) owing to the deamination of amino acids such as valine, leucine, and isoleucine (Berdagué et al., 1987; Kuzdzal-Savoie, 1980). From lactose fermentation some short-chain organic acids such as acetic, propionic, and butanoic acids may form (Belitz and Grosch, 1987e; Kuzdzal-Savoie, 1980). Since free fatty acids are recognized as compounds contributing to the aroma of aged cheeses, concentrations of major FFA deriving from the lipolytic process were measured in the 21 samples of Parmesan cheese. The average values of the concentrations of compounds, grouped on the basis of season and zone of production, are quoted in Table 3. Significant differences were observed for some acids, according to the production season. Free fatty acids originating from lipolysis are represented by the homologs from C_4 to C_{18}. Concentrations of FFA in Parmesan cheese compared to other cheeses (Woo and Lindsay, 1984) are in general moderate: this fact indicates a low rate of lipolysis as a consequence of the technology of this product, which does not favor lipase activity. The highest concentrations were observed for longer-chain C_{14}–$C_{18:1}$ FFA and, in particular, for palmitic acid (minimum value 2290 mg/kg; maximum value 3760 mg/kg). However, because of the high variability of the FFA values in the examined samples, in this case lipolysis cannot be considered as maturation rating. The determination of free fatty acids of cheese allows the detection of defective samples owing to microbial fermentations. Low values of butanoic acid were found in all samples; a high content of this acid indicates a butyric fermentation, responsible for the swelling of Parmesan cheese (Scolari et al., 1985).

In accordance with the literature, the aroma of the examined Parmesan cheese samples contains relatively more ethyl esters of even-number fatty acids from C_2 to C_{16} and fewer methyl, propyl, and butyl esters. Ethyl esters play an important role in the formation of the fruity character of this type of cheese. Ethyl nonanoate, isobutyl acetate, and isopropenyl acetate were identified in Parmesan cheese for the first time in this study.

Six lactones were isolated by using the distillation–extraction technique, whereas the absence of these scarcely volatile compounds in the headspace was not unexpected. Since the headspace technique fails to isolate these compounds and, on the other hand, these compounds are known to be relevant for the aromatic profile of a cheese, the efficiency of the SDE method was checked by determining the recovery of some lactones in Parmesan cheese. Components were selected on the basis of preliminary investigations of Parmesan cheese aroma. Their concentrations (0.4 mg/kg) were similar to that reported for γ-octalactone in this product (Meinhart and Schreier, 1986). The considered lactones ranged from γ-hexalactone to γ- and δ-dodecalactone; recovery values were averaged on four replicates and varied between 70% in the case of γ-hexalactone and 91% in the case of

γ-octalactone. Coefficients of variation were between 8.5% for δ-dodecalactone and 15.0% for γ-hexalactone.

Long-chain lactones (δ-decalactone and δ-dodecalactone) predominate quantitatively among those identified; the occurrence of δ-tetradecalactone has not previously been reported as an aroma contituent of Parmesan cheese. Long-chain lactones, which are commonly found in fat-containing food (i.e., milk fat, meat) and in some fruits such as apricots and coconut, are formed from the corresponding hydroxycarboxylic acids (C_8–C_{16}) by the elimination of water (Belitz and Grosch, 1987f).

Concerning the sulfur compounds, the presence of the potent odorants dimethyl disulfide and dimethyl trisulfide is reported for the first time in Parmesan cheese. Dimethyl disulfide is formed as endproduct by the Strecker degradation of methionine (Belitz and Grosch, 1987g); in the first step of this reaction the already mentioned 3-(methylthio)propanal is obtained. Dimethyl trisulfide is considered to be an important contributor to the flavor of cooked white cabbage, broccoli, and cauliflower (Buttery et al., 1976). The occurrence of the methionine-related sulfur compound benzothiazole in Parmesan aroma has already been reported (Meinhart and Schreier, 1986).

Two alkylpyrazines and six furans were detected among the heterocyclic volatile compounds of aged Parmesan cheese; 2,6-dimethylpyrazine was identified both in the headspace and in the samples prepared by simultaneous distillation–extraction. Alkylpyrazines have been recognized as important trace flavor components of a large number of heated foods and are believed to form as a result of the Maillard nonenzymatic browning reaction (Hodge, 1953). Heterocyclic compounds may also originate enzymatically in fruits and vegetables and during the ripening of cheese. Pyrazines appear to be present in unprocessed as well as in heated foods as natural aroma components (de Man, 1990). 3-Ethyl-2,5-dimethylpyrazine, identified in the extracts of six samples, has been already isolated in Parmesan cheese (Meinhart and Schreier, 1986) and also from chocolate aroma together with other alkyl-substituted pyrazines (Rizzi, 1967).

CONCLUSIONS

The combined use of the two sampling techniques on a relevant number of certified samples allowed us to achieve a typical aromatic profile and a relative composition of the volatile fraction of aged Parmesan cheese. These results can represent a useful reference for characterization of the product.

Determination of free fatty acids and the compounds derived from lactobacterial activity permitted us to observe significant differences based on the zone or the season of production of Parmesan cheese.

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LITERATURE CITED

Barbieri, G.; Bolzoni, L.; Parolari, G.; Virgili, R.; Buttini, R.; Careri, M.; Mangia, A. Flavor compounds of dry-cured ham. *J. Agric. Food Chem.* **1992**, *40*, 2389–2394.

Belitz, H. D.; Grosch, W. In *Food Chemistry*; Springer: Berlin, 1987; (a) p 399; (b) pp 280–281; (c) pp 181–182; (d) p 189; (e) p 397; (f) p 131; (g) p 272.

Berdagué, J. L.; Jeunet, R.; Grappin, R. Ripening and quality of Gruyére de Comté cheese. III: Lactic acid fermentation and levels of volatile fatty acids in Comté cheese. *Lait* **1987**, *67*, 249–263.

Brandon, A. L. Semiautomated procedure for the determination of diacetyl and acetoin in beer and wort. Presented at the Technicon International Symposium, New York, 1964.

Buttery, R. G.; Guadagni, D. G.; Ling, L. C.; Seifert, R. N.; Lipton, W. Additional volatile components of cabbage, broccoli and cauliflower. *J. Agric. Food Chem.* **1976**, *24*, 829.

Careri, M.; Manini, P.; Spagnoli, S.; Barbieri, G.; Bolzoni, L. Use of Simultaneous Distillation-Extraction and Dynamic Headspace Methods in the Gas Chromatographic Analysis of Parmesan Cheese Volatile Components. *Chromatographia* **1994**, *38*, 386–394.

de Jong, C.; Badings, H. T. Determination of free fatty acids in milk and cheese. Procedures for extraction, clean up, and capillary gas chromatographic analysis. *J. High Resolut. Chromatogr.* **1990**, *13*, 94–98.

de Man, J. N. In *Principles of Food Chemistry*; Van Nostrand Reinhold: New York, 1990; (a) pp 57–68; (b) p 42.

Dumont, J. P.; Rogier, S.; Adda, J. Volatile compounds of whole and grated cheese, as illustrated by Parmesan. *Lait* **1974**, *54*, 386–396.

Gierschner, K.; Herbst, R. Determination of chemical parameters used in the evaluation of the microbiological and hygienic condition of fruit juices and similar products. II. *Dtsch. Lebensm. Rundsch.* **1981**, *77*, 178–186.

Groux, M.; Moinas, M. Cheese flavor. II. Comparison of the volatile fraction of various cheeses. *Lait* **1974**, *54*, 44–52.

Hodge, J. E. Chemistry of browning reactions in model systems. *J. Agric. Food Chem.* **1953**, *1*, 928–953.

Kuzdzal-Savoie, S. Determination of free fatty acids in milk and milk products. *Int. Dairy Fed. Bull.* **1980**, *118*, 53–66.

La Placa, M. In *Principi di Microbiologia Medica*; Soc. Ed. Esculapio: Bologna, Italy, 1979; pp 477–512.

Lees, G. I.; Jago, G. R. The estimation of diacetyl in the presence of other carbonyl compounds. *J. Dairy Res.* **1970**, *37*, 129–132.

Lindsay, R. C. Analysis of free fatty acids in Italian cheese. *Dairy Field* **1983**, *166*, 20–21.

Manning, D. J.; Moore, C. Headspace analysis of hard cheese. *J. Dairy Res.* **1979**, *46*, 539–545.

Meinhart, E.; Schreier, P. Study of flavour compounds from Parmigiano Reggiano cheese. *Milchwissenschaft* **1986**, *41*, 689–691.

Murdock, D. I. Methods employed by the citrus concentrate industry for detecting diacetyl aand acetylmethylcarbinol. *Food Technol.* **1967**, *4*, 157–161.

Rafecas, M.; Boatella, J.; de la Torre, M. C. Analysis of volatile compounds from a Parmesan type cheese by adsorption with activated charcoal. *Rev. Agroquim. Tecnol. Aliment.* **1986**, *26*, 597–601.

Rizzi, G. P. The occurrence of simple alkylpyrazines in cocoa butter. *J. Agric. Food Chem.* **1967**, *15*, 549–551.

Scolari, G.; Bottazzi, V.; Brambilla, E. Evaluation of volatile metabolic products for the characterization of homofermentative lactic acid bacteria cultures. *Sci. Tecn. Lattiero-Casearia* **1985**, *36*, 593–602.

Vercellotti, J. R.; Kuan, J. W.; Spanier, A. M.; St. Angelo, A. J. Thermal generation of sulfur-containing flavor compounds in beef. In *Thermal Generation of Aromas*; Parliment, T. H., McGorrin, R. J., Ho, C.-T., Eds.; ACS Symposium Series 409; American Chemical Society: Washington, DC, 1989; pp 452–459.

Woo, A. H.; Lindsay, R. C. Concentration of major free fatty acids and flavor development in Italian cheese varieties. *J. Dairy Sci.* **1984**, *67*, 960–968.

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