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Volatile components of Grana Parmigiano-Reggiano type hard cheese

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Abstract

GC–MS analysis of volatile components of Grana Parmigiano-Reggiano type Italian hard cheese was performed by solid phase micro extraction (SPME) and Purge & Trap (PT) methods. Half of the 24 samples analysed were produced in flat land (<90 m over sea level), the other half in mountain (90–600 m) regions, by small to medium cheese factories. The composition of the volatile components reveals more dependence on the individual factories than on the geographic location. Ripening increases the difference among samples.

Keywords: Parmigiano-Reggiano cheese; Flavour; PCA analysis; SPME; GC–MS

1. Introduction

One of the most typical products of the Italian agricultural/food industry is the grana (also known as Parmesan) type hard cheese (Belitz & Grosch, 1999; Scott, 1981). Two principal kinds are known as Grana Padano and Grana Parmigiano-Reggiano (these names are also registered trademarks). The latter is produced in the southern lowlands of the Po valley and the northern Appennines (Stein & Imhof, 1978) (provinces of Parma, Reggio Emilia, Modena and, partly, of Bologna and Mantova). In this paper we report identification of the volatile components of Parmigiano-Reggiano, as the first part of a broader project aiming at the instrumental and chemical characterization of this class of hard cheeses.

Parmigiano Reggiano is produced by a Consortium of small and medium size cheese factories, mainly cooperatives, exclusively from cows' milk. The producers must obey very rigorously controlled rules of the Consortium (D.M. 17/06/1956), starting from the alimentation of the cows and ranging to rules about production and ripening processes. These rules are partly based on secular experience, as well as on some easily measurable parameters (e.g. density, pH, temperature) (Secchi, 1979) and Italian legislation regarding dairy industry (Italian Law DPR 30/10/1965 no 1269; EU Regulations no 2081/82 and 1107/96). The principal aim of the present project is to add to these parameters some new molecular level features using modern industrial analytical chemistry (Carieri, Mangia, Mori, & Muci, 1999; Foissi, 1994; Mayer, 1996; Mayer & Rockenbauer, 1998; McGoldrick & Fox, 1999).

The traditional elements in the technology used for Parmigiano-Reggiano represent one of its most attractive features from both commercial and nourishmental points of view. On the other hand, a considerable microbial content diversity (Robinson, 1999) results from the the normal production course which is as follows: the milk is left to rest overnight in large-surface steel containers, skimmed and placed, together with the other morning portion, in the cauldron. Then the process is continued by inoculating the milk with the microbial culture or the previous day production and (exclusively calf) rennet. An additional element of

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individuality arises from tradition: beyond the rigorous application of the above mentioned rules, the so-called cheese-masters (casaro, generally one in each factory) have liberty in using "know-how", which influences the taste and flavour of the cheese, enabling experts even to identify the factory on this basis.

From the consumers' point of view, one of the first features examined in making cheese is the flavour sensed in the product. In spite of important earlier publications (Chianese et al., 1987; Curtis de Angelis et al., 1999; Ferranti et al., 1997; Gabelli, Belloni, Ingrao, Pizzoferrato, & Santaroni, 1999; Marchelli et al., 1979; Moio, & Addeo, 1998; Panfini, Manzi, & Pizzoferrato, 1998; Rampilli, Toppino, Raia, & Dagletta, 1997), knowledge seems to be insufficient. These considerations prompted us to start a systematic study for obtaining molecular level data on Parmigiano-Reggiano cheese. The first results of these efforts, obtained with volatile organic compounds, are reported here.

2. Materials and methods

2.1. Sampling

Samples were taken from 11 cheese factories located in flat land (<90 m) and hilly (90–600 m) regions of the Province of Modena. Two identical samples were collected from the same geometric positions in the cheese forms (standard cylindrical size: diameter ~70 cm, height ~30 cm) to minimize effects of difference in oxygen diffusion into the cheese bulk and consistency of the "paste". One set of samples entered an organoleptic panel test (Puisais & Durand, 2001), while a second was stored at −2 °C in closed glass recipients. The volatiles were transferred for analysis by both SPME (Bellesia, Bianchi, Pinetti, & Tirillini, 1996, 1997, 1998) and PT (Bellesia et al., 1998) techniques.

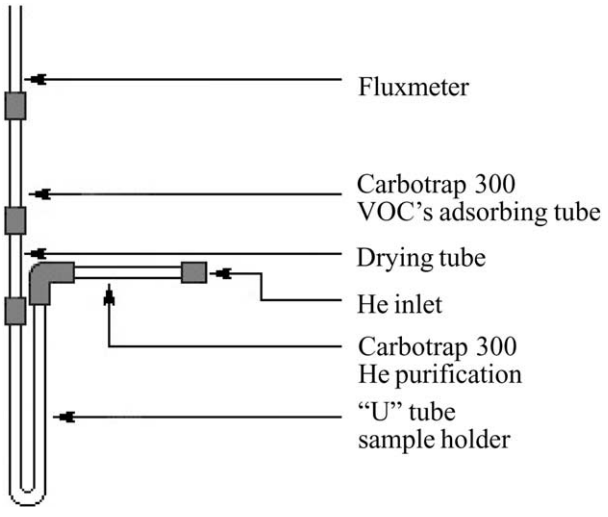

Fig. 1. P&T sampling device.

2.2. SPME analysis

A sample of grana Parmigiano-Reggiano (~5 g) was finely grated and put into a glass sample container fitted with a self-sealing septum at its top, through which the SPME syringe needle (bearing a 2-cm fibre coated with 50/30 mm divinylbenzene/Carboxen on poly-dimethylsiloxane bonded to a flexible fused silica core, Supelco, Bellefonte, PA, USA) was introduced. The container was then thermostatted at 80 °C for 30–35 min. The absorbed volatile analytes were then analysed by GC–MS (Dirinck & De Winne, 1999) (Hewlett-Packard G-1800C, glass capillary column Se54, 25 m, 0.2 mm i.d.; oven temperature programme: 35 °C for 3 min, 5 °C/min to 110 °C, then 10 °C/min to 240 °C and 240 °C for 10 min; He carrier gas: 1.5 ml/min; injector temperature 280 °C; detector temperature 250 °C). The eluted compounds were identified by comparison with mass spectral database (HP-PBM).

2.3. P&T analysis

A finely grated sample (100–150 mg) was introduced into the glass sampling apparatus shown in Fig. 1, with the U-shaped sample holder (length 15 cm, i.d. 3 mm) connected to a drying tube filled with anhydrous cal-

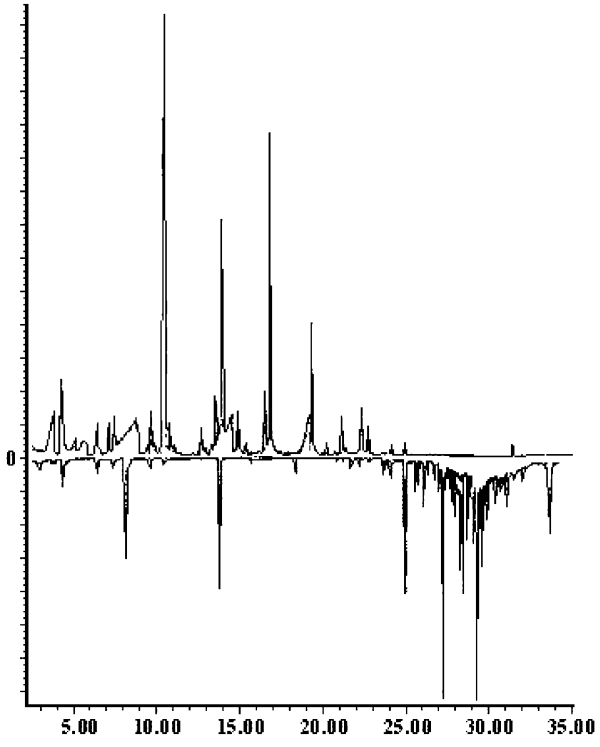

Fig. 2. Comparison between GC profiles of VOC's collected by SPME (upper part) and P&T methods.

cium chloride, and with a tube filled with Carbotrap 300 adsorbent. The device was thermostatted at 50 °C for 30 min and flushed with helium (2 ml/min). The Carbotrap 300 tube was then placed into a Chrompack P&T injector and directly desorbed into the GC–MS system (chromatographic conditions as described earlier).

3. Results and discussion

A comparison between the two methods is shown in Fig. 2. The results show: (i) an excellent complementarity of the SPME to the P&T method, as well as (ii) a broad chemical diversity in the most volatile compounds (with the SPME technique) of the various samples. Despite this variability, most of the 81 detected compounds (73 identified) are present in each sample, although in fairly varying quantities. Another remarkable feature is the ratio (~3%) of the most volatile compounds to the less volatile ones (mainly detected using the P&T method), that remains roughly the same. The less volatile compounds GC profiles of all the examined samples are nearly superimposable, and result from a mixture of long chain hydrocarbons, free fatty acids and high molecular weight linear ketones, as well as some modified steroids. This mixture could constitute the "greasy" base of the flavour.

Because of their variability and the particular "note" that they can give to the flavour, our attention has been focussed on the most volatile compounds.

The data obtained are shown in Tables 1 and 2, ordered according to the geographic locations of

Table 1
Volatile organic compounds in Parmigiano-Reggiano cheese samples from flat land[a]

Rt	Compounds													Group
	Sample No.	1	2	3	4	5	6	7	8	9	10	11	12	
	Ageing (months)	21	24	21	24	24	24	27	24	18	18	18	18	
3.01	Acetic acid	11.84	10.05	10.2	7.29	12.8	12.96	11.6	3.48	6.88	1.16	2.05	1.70	B
3.16	2-Butenal	–	–	–	2.02	–	–	0.53	0.69	–	–	1.39	t	A
3.45	3-Methylbutanal	3.06	t	1.64	–	1.93	–	–	–	t	1.68	–	0.50	A
3.59	2-Methylbutanal	2.09	–	1.10	–	1.03	–	–	–	t	1.33	–	–	A
4.15	2-Pentanone	1.96	t	4.66	1.48	15.0	2.51	t	t	1.39	6.82	–	t	E
4.42	2-Pentanol	t	t	t	1.64	0.58	t	t	t	11.0	–	t	t	C
4.46	3-Methyl-2-butanol	–	–	–	–	–	–	–	–	–	1.12	–	–	C
4.81	Ethyl propanoate	–	–	–	–	–	–	–	–	1.37	–	–	–	D
4.92	Propanoic acid	–	–	–	–	–	–	–	–	3.38	–	–	–	B
5.40	3- Methylbutanol	t	t	t	–	t	–	–	–	–	0.56	–	–	C
6.38	Toluene	t	t	t	t	0.61	t	t	t	t	1.54	t	t	F
6.88	2.3-Butandiol	0.63	–	0.51	t	t	t	–	–	t	t	–	t	C
7.19	1.3-Butandiol	1.17	t	0.57	t	t	t	–	–	t	0.68	–	t	C
7.44	Ethyl butanoate	t	2.30	3.48	1.81	1.16	1.81	2.55	t	2.57	0.68	1.01	0.64	D
9.19	Butanoic acid	13.2	21.0	15.9	14.5	9.37	17.8	17.9	10.6	22.7	6.27	13.5	15.0	B
10.48	2-Heptanone	9.18	6.10	6.92	10.4	–	4.87	–	3.85	8.23	14.8	1.20	3.06	E
10.80	2-Heptanol	t	t	–	1.28	t	t	t	t	–	0.67	t	–	C
11.23	2.6-Dimethylpyrazine	0.64	t	t	t	t	–	–	–	–	1.33	–	–	G
11.23	Pentyl acetate	–	–	–	–	0.52	–	–	–	–	–	–	–	D
12.82	Benzaldehyde	1.36	t	t	t	t	t	t	t	t	1.28	t	t	F
13.80[b]	2-Octanone	–	–	–	–	0.68	–	–	–	–	–	–	–	E
13.81	RH 142b[b]	1.78	2.70	0.63	1.42	–	–	–	t	1.08	0.94	–	–	H
14.08	Ethyl hexanoate	2.20	13.63	15.69	10.23	3.34	9.11	14.4	6.18	4.41	2.66	9.79	4.58	D
15.29	Hexanoic acid	17.6	16.8	14.9	22.0	11.2	26.9	33.5	t	20.6	10.1	32.9	35.0	B
15.59	Phenylacetaldehyde	0.66	–	t	–	t	–	–	–	–	–	–	–	F
16.81	8-Nonen-2-one	0.69	t	t	0.57	0.55	t	–	t	t	1.20	–	t	E
16.93	Heptanoic acid	–	–	–	t	–	–	1.74	–	–	t	0.70	0.50	B
17.14	2-Nonanone	7.22	2.01	2.30	3.29	7.41	1.68	–	2.52	1.72	10.7	t	0.97	E
17.32	2-Nonanol	–	–	–	–	–	–	–	–	–	0.51	–	–	C
17.34	Nonanal	t	t	–	–	t	t	–	–	–	0.51	–	–	A
19.73	Octanoic acid	7.86	5.30	5.20	8.86	3.16	10.5	14.1	17.3	9.50	3.85	6.68	18.5	B
19.76	Ethyl octanoate	3.51	2.56	2.91	2.95	0.58	1.99	2.22	3.85	1.02	0.50	3.09	1.62	D
21.59	2-Undecanone	0.60	t	t	t	0.53	t	t	t	t	1.76	0.53	t	E
22.88	Decanoic acid	3.16	2.35	2.01	3.49	1.45	3.75	5.10	11.30	2.96	1.56	7.60	9.64	B
25.44	Dodecanoic acid	t	t	t	t	–	t	t	0.50	t	t	t	t	B

Simplified as follows: compounds less than 0.1% are not included or indicated by –. Values from 0.5 to 0.1 are indicated by t (traces).

[a] The data shown (%) are obtained as mean values (±10%) with three parallel GC–MS runs on each sample.

[b] Unidentified branched hydrocarbon with MW = 142.

Table 2
Volatile organic compounds in Parmigiano-Reggiano cheese samples from mountain regions[a]

Rt	Compounds													Group
	Sample No.	13	14	15	16	17	18	19	20	21	22	23	24	
	Ageing (months)	18	22	24	18	15	15	15	15	13	16	16	15	
3.01	Acetic acid	2.12	1.48	1.62	3.13	2.84	1.37	4.79	4.52	0.60	5.46	5.30	5.04	B
3.45	3-Methylbutanal	t	–	–	1.21	–	–	–	–	0.52	–	–	–	A
3.59	2-Methylbutanal	–	–	–	0.59	–	–	t	–	0.57	–	–	–	A
4.15	2-Pentanone	2.53	7.48	8.60	21.5	7.46	4.00	5.84	4.14	1.93	–	–	–	E
4.42	2-Pentanol	0.67	–	6.41	5.33	0.60	–	–	–	1.90	–	t	–	C
4.46	3-Methyl-2-butanol	–	1.03	–	–	–	1.05	1.05	t	t	–	–	–	C
4.92	Propanoic acid	–	–	–	–	–	–	–	0.63	–	–	–	–	B
5.40	3-Methylbutanol	–	–	–	0.51	–	–	–	t	t	–	–	–	C
6.38	Toluene	t	0.65	–	–	t	t	0.51	1.40	2.04	–	–	–	F
6.88	2.3-Butandiol	–	t	–	t	–	–	t	0.67	t	–	–	–	C
7.04	2-Hexanone	t	0.60	0.51	t	0.70	t	–	–	t	–	–	–	E
7.44	Ethyl butanoate	1.72	1.56	3.37	2.32	1.92	t	0.93	0.68	t	–	–	–	D
9.19	Butanoic acid	12.9	6.10	13.5	3.51	12.1	10.4	15.0	9.14	7.10	6.61	5.35	6.20	B
10.48	2-Heptanone	15.4	20.8	25.6	–	13.7	16.5	5.31	22.87	21.1	6.53	4.32	t	E
10.80	2-Heptanol	1.37	0.52	0.68	–	t	0.58	1.11	0.64	1.57	2.01	0.67	–	C
11.23	2.6-Dimethylpyrazine	t	–	t	–	t	0.53	–	t	1.50	–	–	–	G
11.23	Pentyl acetate	–	1.12	–	–	–	–	t	–	–	–	–	–	D
12.82	Benzaldehyde	t	0.60	t	t	t	t	t	1.59	1.58	t	t	2.79	F
13.41	1-Octen-3-ol	–	t	–	–	–	–	t	–	–	–	–	0.53	C
13.80	2-Octanone	t	0.91	–	–	1.00	t	t	1.40	0.86	t	–	–	E
14.08	Ethyl hexanoate	7.75	5.75	9.57	3.16	4.38	2.02	4.18	5.46	1.68	5.67	2.35	1.74	D
15.11	Limonen	–	–	–	–	–	–	–	1.14	t	0.58	t	1.61	H
15.29	Hexanoic acid	21.6	10.0	15.8	14.1	18.9	22.3	26.5	18.79	11.8	6.66	17.1	13.2	B
15.59	Phenylacetaldehyde	–	t	–	–	–	–	t	t	0.50	t	t	1.12	F
16.81	8-Nonen-2-one	1.75	3.29	1.48	1.06	1.58	1.88	0.63	1.56	2.68	2.45	1.00	–	E
17.14	2-Nonanone	6.93	16.9	7.49	6.39	9.17	8.38	5.77	9.82	18.4	13.1	6.44	1.25	E
17.32	2-Nonanol	t	–	–	t	–	–	t	–	t	0.53	t	–	C
17.34	Nonanal	t	–	–	–	–	–	–	–	–	–	–	1.92	A
17.79	2-Phenylethanol	–	–	–	t	–	–	–	–	t	–	0.92	–	F
19.65	2-Decanone	–	–	–	–	–	–	–	1.10	t	–	–	–	E
19.73	Octanoic acid	9.12	6.24	7.88	2.44	12.1	15.9	12.1	3.88	6.59	13.8	19.2	16.8	B
19.76	Ethyl octanoate	2.58	2.78	0.88	1.32	1.92	0.64	1.40	2.63	t	3.61	2.10	2.80	E
21.59	2-Undecanone	t	1.77	t	t	1.03	0.64	0.52	t	1.52	1.98	1.99	1.69	E
22.88	Decanoic acid	4.31	2.93	2.51	0.58	8.19	7.06	5.67	2.03	2.99	7.20	17.3	15.3	B
23.17	Ethyl decanoate	t	t	t	t	t	t	t	t	t	1.44	0.66	1.87	D
25.44	Dodecanoic acid	t	t	t	–	0.50	t	t	t	t	1.14	3.72	4.66	B

Simplified as follows: compounds less than 0.1% are not included or indicated by –. Values from 0.5 to 0.1 are indicated by t (traces).
[a] The data shown (%) are obtained as mean values (±10%) with three parallel GC–MS runs on each sample.

producing factories. These results provide a very important proof for the expectation that the rules of production were de facto meticulously respected in all of the 24 (casually chosen) cheese factories involved in this project. Beyond the qualitative result, the observed diversity might reflect the individuality of the samples. This aspect was analysed by subjecting the data to principal component analysis (PCA) (Bucci, Magri, Magri, Marini, & Marini, 2002; Charlton, Farrington, & Brereton, 2002; Duarte et al., 2002) using the following approximations:

(i) the value of 0.02 was assigned to components present in amounts $<0.04\%$;
(ii) the absence of a component was indicated as 0.00%;
(iii) the components present in traces in not more than two samples were not taken into account.

Under these conditions, PCA analysis did not indicate important differences in term of geographic location or ripening, in accordance with the sensorial data obtained for the analogous set of samples.

In another attempt at rationalisation of the data, the volatile compounds were grouped (Tables 1 and 2, last columns) according to their (possible) formation. As far as *aldehydes* (group A) are concerned, the linear ones (saturated or unsaturated) can be derived from lipidic oxidation (Assaf, Hadar, & Dosoretz, 1997; Bisakowski, Perraud, & Kermasha, 1997; Mau, Chyau, Li, & Tseng, 1997), while the branched ones can be deduced from Strecker degradation (Ager & Fotheringham, 2001;

Table 3
Volatile organic compounds grouped according to their supposed formation[a]

	Group	Sample											
		1	2	3	4	5	6	7	8	9	10	11	12
Aldehydes	A	8.09	1.06	3.37	2.38	3.80	0.38	0.87	0.97	0.76	4.87	1.74	1.08
Acids	B	54.1	55.6	48.3	5.49	38.1	72.2	84.2	43.5	66.2	23.3	64.2	80.8
Alcohols	C	3.11	1.37	1.71	3.34	2.17	1.10	0.43	0.72	11.54	3.90	0.51	0.32
Esters	D	2.70	19.3	22.7	15.7	5.91	13.4	20.0	10.8	9.63	4.03	15.2	7.57
Ketones	E	20.3	9.29	15.2	16.3	25.2	9.60	0.54	7.35	11.6	36.1	2.51	4.60
Aromatics	F	1.50	0.40	0.50	0.47	0.33	0.24	0.34	0.26	0.17	1.46	0.45	0.37
Maillard products	G	1.13	0.48	0.77	0.26	0.39	0.24	0.10	0.25	0.08	1.54	0.10	0.12
Hydrocarbons	H	2.25	3.52	1.01	1.67	0.73	0.24	0.31	0.62	1.45	2.97	0.28	0.26
		13	14	15	16	17	18	19	20	21	22	23	24
Aldehydes	A	0.66	0.99	0.46	2.13	0.44	0.59	0.77	1.80	3.16	0.73	0.62	6.22
Acids	B	50.4	27.0	41.6	23.83	55.1	57.8	64.5	39.2	29.4	50.9	68.4	61.8
Alcohols	C	2.36	1.92	7.20	7.07	1.16	1.70	3.52	2.67	5.65	2.65	0.97	0.57
Esters	D	13.4	11.8	14.3	7.04	8.84	3.49	7.28	9.67	2.68	11.4	5.27	6.86
Ketones	E	27.4	51.8	43.9	29.9	34.8	32.0	18.5	42.0	47.6	24.8	14.1	3.67
Aromatics	F	0.58	0.80	0.42	0.46	0.38	0.53	0.33	1.65	2.04	0.18	0.27	2.83
Maillard products	G	0.21	0.10	0.46	0.08	0.46	0.71	0.32	0.49	1.79	0.37	0.71	0.75
Hydrocarbons	H	0.37	0.67	0.68	3.10	0.35	0.31	0.57	2.73	2.50	0.60	0.76	2.05

[a] Values are comprehensive of the ones of compounds not included in Tables 1 and 2.

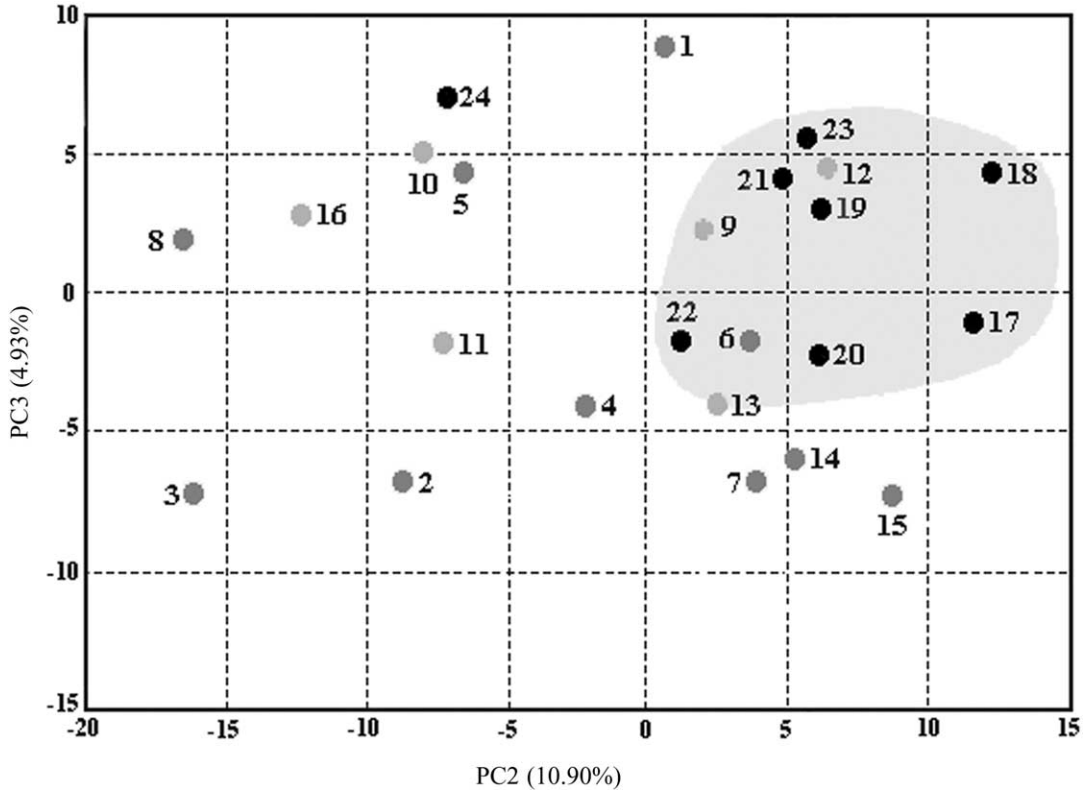

Fig. 3. PCA analysis of data in Table 3. PC2 vs PC3. Black, 13–16 months aged cheeses; Light grey, 17–20 months aged cheeses; Grey, 21–27 months aged cheeses.

Counet, Callemien, Ouwerx, & Collin, 2002; Hofmann, Munch, & Schieberle, 2000; Martin & Ames, 2001; Ruiz, Ventanas, & Cava, 2001) of the amino acids. *Carboxylic acids* (group B) are regarded as oxidation products (not necessarily enzymatic) of the corresponding aldehydes (Henehan, Kenyon, & Oppenheimer, 1993; Olson, Luo, Almarsson, & Bruce, 1996; Oppenheimer & Henehan, 1995). *Alcohols* (group C) can derive from fermentation processes (mainly linked to reducing NADH-dependent enzymes) (Fontaine et al.,

2002; Gabelli et al., 1999; Hadwiger, Mayr, Tauss, Stutz, & Nidetzky, 1996; Nidetzky, Klimacek, & Mayr, 2001). *Esters* (group D) can be assigned to enzymatic activity, most frequently by lipases (Alston & Freedman, 2002; Bojarski, Oxelbark, Andersson, & Allenmark, 1993). *Ketones* (group E), with even or uneven carbon atom numbers, are products of changes in the usual lipidic degradative metabolic microbal pathways (Lindsy, 1996; chap. 11). *Aromatic* (group F) compounds principally arise from degradation of amino acids (mainly phenyl-alanine and tyrosine) (Chen & Pillips, 1993; Gao et al., 1997; Pletnev et al., 1996; Smith & Somerville, 1997). *Heterocyclic* (group G) compounds are formed during the thermal treatments (Maillard reaction) (Ahmed, Dunn, Wall, Thorpe, & Baines, 1988; Cuzzoni, Stoppini, Gazzani, & Mazza, 1988; Horvat, Varga-Defterdarovic, Rosic, & Horvat, 1998; Skog, Johansson, & Jagerstad, 1989). The *hydrocarbon* group (H) includes compounds from well known biogenetic pathways as well as volatiles of undefined structure. These sums are listed in Table 3, and were again submitted to PCA analysis. No significant correlation for the first three most abundant components was detected. Only the PCA treatment of the data for the second and third principal components, accounting for 10.90% and 4.93% of the total variance, respectively, show some division (Fig. 3) indicating a separation of the data for cheeses 13–16 months old from those of the 17–20 and 21–27 month-old ones.

4. Conclusions

The volatile component analysis of the aroma of Grana Parmigiano-Reggiano cheeses showed:

(i) the standardisation effort of the Consorzio Parmigiano-Reggiano seems to be efficient in terms of main features of distribution of molecularly characterised volatiles;
(ii) the well known individuality of the small and medium size cheese factories is also reflected in the VOC's analysis;
(iii) seasoning enhances the differences, thus amplifying the spectrum of the market offer.

A deeper analysis requires a much broader data base, which, in turn, might be compared with analyses of non-volatile compounds from the same samples. Comparative studies of this kind are prospected within the next steps of the present project.

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References

Ager, D. J., & Fotheringham, I. G. (2001). *Current Opinion in Drug Discovery and Development*, *4*(6), 800–807.
Ahmed, M. U., Dunn, J. A., Walla, M. D., Thorpe, S. R., & Baines, J. W. (1988). *Journal of Biological Chemistry*, *263*, 8816–8824.
Alston, M. J., & Freedman, R. B. (2002). *Biotechnology and Bioengineering*, *77*, 641–650.
Assaf, S., Hadar, Y., & Dosoretz, C. G. (1997). *Enzyme and Microbial Technology*, *21*, 484–494.
Belitz, H.-D., & Grosch, W. (1999). *Food chemistry* (2nd ed). Berlin: Springer.
Bellesia, F., Bianchi, A., Pinetti, A., & Tirillini, B. (1996). *Flavour and Fragrance Journal*, *11*, 239–242.
Bellesia, F., Bianchi, A., Pinetti, A., & Tirillini, B. (1997). *Rivista Italiana E.P.P.O.S.*, *23*, 41–48.
Bellesia, F., Bianchi, A., Pinetti, A., & Tirillini, B. (1996). *Flavour and Fragrance Journal*, *13*, 56–60 *Journal of Essential Oil Research*, *10*, 1998, 483–487.
Bisakowski, B., Perraud, X., & Kermasha, S. (1997). *Bioscience, Biotechnology and Biochemistry*, *61*, 1262–1270.
Bojarski, J., Oxelbark, J., Andersson, C., & Allenmark, S. (1993). *Chirality*, *5*, 154–158.
Bucci, R., Magri, A. D., Magri, A. L., Marini, D., & Marini, F. (2002). *Journal of Agricultural and Food Chemistry*, *50*, 413–418.
Carieri, M., Mangia, A., Mori, G., & Musci, M. (1999). *Analytica Chimica Acta*, *386*, 169–180.
Charlton, A. J., Farrington, W. H. H., & Brereton, P. (2002). *Journal of Agricultural and Food Chemistry*, *50*, 3098–3103.
Chen, H., & Pillips, R. S. (1993). *Biochemistry*, *32*, 11591–11602.
Chianese, L., Caira, S., Ferranti, P., Laezza, P., Malori, A., Mucchietti, G., Garro, G., & Addeo, F. (1987). *Lait*, *77*, 699–715.
Counet, C., Callemien, D., Ouwerx, C., & Collin, S. (2002). *Journal of Agricultural and Food Chemistry*, *50*, 2385–2391.
Curtis De Angelis, S., Cuttini, R., D'Ascenzo, G., Sagone, F., Fachin, S., & Bocca, A. (1999). *Food Chemistry*, *66*, 375–380.
Cuzzoni, M. T., Stoppini, G., Gazzani, G., & Mazza, P. (1988). *Food and Chemical Toxicology*, *35*, 815–819.
Dirinck, P., & De Winne, A. (1999). *Journal of Chromatography*, *847*, 203–210.
Duarte, I., Barros, A., Belton, P. S., Righelato, R., Spraul, M., Humpfer, E., & Gil, A. M. (2002). *Journal of Agricultural and Food Chemistry*, *50*, 2475–2481.
Ferranti, P., Barone, F., Chianese, L., Addeo, F., Scaloni, A., Pellegrino, L., & Resini, P. (1997). *Journal of Dairy Research*, *64*, 601–615.
Foissi, H. (1994). *Österreichische Milchwirtschaft*, *11*, 15–19.
Fontaine, L., Meynial-Salles, I., Girbal, L., Yang, X., Croux, C., & Soucaille, P. (2002). *Journal of Bacteriology*, *184*, 821–830.
Gabelli, L., Belloni, P., Ingrao, G., Pizzoferrato, L., & Santaroni, G. P. (1999). *Journal of Food Composition and Analysis*, *12*, 27–35.
Gao, S., Oh, D.-H., Broadbent, J., Johnson, M., Weimer, B., & Steele, J. (1997). *Lait*, *77*, 371–383.
Hadwiger, P., Mayr, P., Tauss, A., Stutz, A. E., & Nidetzky, B. (1996). *Bioorganic and Medicinal Chemistry Letters*, *9*, 1683–1686.
Henehan, G. T., Kenyon, G. L., & Oppenheimer, N. J. (1993). *Advances in Experimental Medicine and Biology*, *328*, 481–491.

Hofmann, T., Munch, P., & Schieberle, P. (2000). *Journal of Agricultural and Food Chemistry*, *48*, 434–440.
Horvat, S., Varga-Defterdarovic, L., Rösic, M., Horvat, J. (1998). *Journal of the Chemical Society: Chemical Communications*, 1663–1664.
Lindsy, R. C. (1996). Flavours. In O. R. Fennema (Ed.), *Food chemistry* (3rd ed.). NY: Marcel Dekker.
Marchelli, R., Palla, G., Dossena, A., Galaverna, G., Corradini, R., & Clementi, S. (1979). *Scienze e Tecnica Lattiero Casearia*, *48*, 21–32.
Martin, F. L., & Ames, J. M. (2001). *Journal of Agricultural and Food Chemistry*, *49*, 3885–3892.
Mau, J. L., Chyau, C. C., Li, J. L., & Tseng, Y. H. (1997). *Journal of Agricultural and Food Chemistry*, *45*, 4726–4731.
Mayer, H. K. (1996). *Zeitschrift fur Lebensmittel Untersuchung und Forschung (A)*, *202*, 465–470.
Mayer, H. K., & Rockenbauer, C. (1998). *Lait*, *78*, 425–438.
McGoldrick, M., & Fox, P. F. (1999). *Zeitschrift fur Lebensmittel Untersuchung und Forschung (A)*, *208*, 90–99.
Moio, L., & Addeo, F. (1998). *Journal of Dairy Research*, *65*, 317–333.
Nidetzky, B., Klimacek, M., & Mayr, P. (2001). *Biochemistry*, *40*, 10371–10381.
Olson, L. P., Luo, J., Almarsson, O., & Bruice, T. C. (1996). *Biochemistry*, *35*, 9782–9791.
Oppenheimer, N. J., & Henehan, G. T. (1995). *Advances in Experimental Medicine and Biology*, *372*, 407–415.
Panfini, G., Manzi, P., & Pizzoferrato, L. (1998). *Journal of Dairy Resarch*, *65*, 253–260.
Pletnev, S. V., Isupov, M. N., Dauter, Z., Wilson, K. S., Faleev, N. G., Harutyunyan, E. G., & Demidkina, T. V. (1996). *Biochemistry and Molecular Internation*, *38*, 37–45.
Puisais, J., & Durand, M. P. (2001). *Bulletin de l'Academie Nationale de Medecine*, *185*, 323–327.
Rampilli, M., Toppino, P. M., Raia, V., & Dagletta, A. (1997). *Scienze e Tecnica Lattiero Casearia*, *48*, 33–42.
Robinson, R. K. (Ed.). (1999). *Encyclopediae of food microbiology*. London: Academic Press.
Ruiz, J., Ventanas, J., & Cava, R. (2001). *Journal of Agricultural and Food Chemistry*, *49*, 5115–5121.
Scott, R. (1981). *Cheese making practice*. London: Appl. Sci. Publishers.
Secchi, G. (1979). I nostri alimenti. In H. Hoepli (Ed.). Milano.
Skog, K. I., Johansson, M. A., & Jagerstad, M. I. (1989). *Food and Chemical Toxicology*, *36*, 879–888.
Smith, H. Q., & Somerville, R. L. (1997). *Journal of Bacteriology*, *179*, 5914–5921.
Stein, J. L., & Imhof, K. (1978). *Ullmanns Enz. Techn. Chem.*, *16*, 689.