

Gas Chromatography–Olfactometry (GC-O) and Proton Transfer Reaction–Mass Spectrometry (PTR-MS) Analysis of the Flavor Profile of Grana Padano, Parmigiano Reggiano, and Grana Trentino Cheeses

ELENA BOSCAINI,[†] SASKIA VAN RUTH,[‡] FRANCO BIASIOLI,[§] FLAVIA GASPERI,[§] AND
TILMANN D. MÄRK^{*,†,⊥}

Institut für Ionenphysik, Universität Innsbruck, Technikerstrasse 25, A-6020 Innsbruck, Austria,
Department of Food Science, Food Technology and Nutrition, University College Cork, Western
Road, Cork, Ireland, Istituto Agrario di S. Michele a/A, Via E. Mach, 2 38010 S. Michele, Italy, and
Department of Plasmaphysics, University of Bratislava, SK-84248 Bratislava, Slovak Republic

Gas chromatography–olfactometry (GC-O) and proton transfer reaction–mass spectrometry (PTR-MS) techniques were used to deduce the profile of odor-active and volatile compounds of three grana cheeses: Grana Padano (GP), Parmigiano Reggiano (PR), and Grana Trentino (GT). Samples for GC-O analysis were prepared by dynamic headspace extraction, while a direct analysis of the headspace formed over cheese was performed by PTR-MS. The major contributors to the odor profile were ethyl butanoate, 2-heptanone, and ethyl hexanoate, with fruity notes. A high concentration of mass 45, tentatively identified as acetaldehyde, was found by PTR-MS analysis. Low odor threshold compounds, e.g., methional and 1-octen-3-one, which contributed to the odor profile but were not detected by FID, were detected by PTR-MS. Principal component analysis on both GC-O and PTR-MS data separated the three cheese samples well and showed specific compounds related to each sample.

KEYWORDS: GC-O; PTR-MS; grana cheese; odor profile; volatile profile; PCA

1. INTRODUCTION

Grana cheese is a hard Italian cheese, known worldwide, appreciated for its fruity and creamy flavor. Cheese with protected designation of origin has a high commercial value; thus, quality control and certification must undergo severe and reliable controls. Currently, controls on maturation and aroma are still carried out by human experts, who check and judge the stage of ripeness of the moulds one by one. In particular, odor (consisting of the volatile compounds in the headspace above the food) gives a first flavor impression and influences the acceptability and judgment of the food. The use of an automatic technique which can reproduce the sensitivity of the human nose and can judge the quality of the cheese and link the cheese composition to its typical organoleptic features and original environment (*1*) could greatly reduce the time required to check the single moulds and probably reduce the costs of production. Two very interesting techniques with rather high sensitivity and/or speed which can be used for this purpose are

gas chromatography–olfactometry (GC-O) and proton transfer reaction–mass spectrometry (PTR-MS).

GC-O is a commonly used technique for analysis of odor-active flavor compounds, introduced in 1964 by Fuller et al. (*2*) and further developed in the following decades (*3*). Combining the capability of a capillary column to separate compounds with high sensitivity comparable to that of the human nose as a detector, GC-O turns out to be an optimal instrument to associate odor with eluting compounds. A description of the odor can be given for each retention time corresponding to an odor-active compound. Quantification can be carried out by a variety of methods, one of which is the detection frequency method, utilizing a group of assessors (*4–6*). The number of assessors detecting a specific odor-active compound at the sniffing port at the same time (the frequency of detection) is used as a measure of the intensity of a compound.

The PTR-MS technique, developed by Lindinger et al. (*7, 8*), is based on a novel chemical ionization cell allowing online measurements of trace gas components with concentrations as low as a few parts per trillion by volume. The method is based on reactions of H_3O^+ ions, which perform nondissociative proton transfer to many of the common volatile organic compounds (VOCs) but do not react with any of the major components present in clean air. The generation of the primary

* Address correspondence to this author at Universität Innsbruck (fax +43-512-507-2932, E-mail Tilmann.Märk@uibk.ac.at).

[†] Universität Innsbruck.

[‡] University College Cork.

[§] Istituto Agrario di S. Michele a/A.

[⊥] University of Bratislava.

H_3O^+ ion and the chemical ionization of the VOCs are individually controlled and temporally separated processes. One important consequence is that absolute concentrations can be calculated without calibration or use of standards. Another big advantage of PTR-MS is that the volatile compound samples do not need to be prepared before the measurement, e.g., involving preconcentration procedures; thus, a headspace sample over real food can be introduced directly into the reaction chamber consisting of a drift tube. The measured mass spectral profile closely reflects genuine headspace distribution and can be used to assess the authenticity of a product, monitor deviations in production from a reference sample, or classify products and raw materials (9). Interesting results were recently obtained in this sense, demonstrating that it is possible to distinguish different mozzarella cheeses on the basis of their volatile profile measured by PTR-MS (10). Similarly, PTR-MS headspace investigation allowed quantification of the effect of different stabilization treatments (heat versus pressure) applied to red orange juices (11).

In the present study, GC-O and PTR-MS techniques are applied to define the flavor profile of three certified grana cheeses: Parmigiano Reggiano and Grana Padano, the two well-known Italian PDO (protected designation of origin) cheeses, and Grana Trentino, a variety of Grana Padano with distinctive features only produced in Trentino, a mountainous province in northern Italy.

These Italian hard cheeses are made from raw bovine milk, partly skimmed by creaming, with the addition of a natural whey starter, cooked at 53–55 °C, and then usually ripened for about 2 years for the Parmigiano Reggiano, 14–16 months for the Grana Padano, and 18–20 months for the Grana Trentino (12). The place of origin, the cattle's feeding, and the manufacturing protocol are the main factors determining the uniqueness and peculiarity of the cheeses. Previous studies on the volatile profile exist for Parmigiano Reggiano (13–15) and Grana Padano (16).

Here, GC-O analysis is applied to identify the odor-active compounds, utilizing dynamic headspace extraction in order to define the odor profile. With the PTR-MS analysis, a real-time headspace profile is obtained and no preconcentration step is necessary; thus, no artifacts are introduced. Furthermore, the capability of the PTR-MS method to detect low odor threshold compounds that are perceived by the human nose but are not detected by FID or by any other instrumental detector has been investigated here.

The final goal of this work is to study the capability of GC-O and PTR-MS to distinguish between the three kinds of cheeses on the basis of their profiles of odor-active and volatile compounds.

2. MATERIALS AND METHODS

2.1. Cheese Samples. Five-kilogram samples of Parmigiano Reggiano (PR, 26 months), Grana Padano (GP, 24 months), and Grana Trentino (GT, 24 months) were obtained from a cheese supplier in Trento, Italy. For each cheese, 15 slices of 250–300 g were cut from the inner part of the mould and then vacuum-wrapped and stored at –20 °C until sampling. One day before the analysis, the samples were placed in a cold room to defrost at 4 °C. After the external surface was peeled off, the cheese slice was finely grated, homogenized, placed in vials, and stored at 4 °C.

2.2. GC-O Sample Preparation and Analysis. For the extraction of the volatile compounds for the GC-O analysis, a dynamic headspace method was used. Ten grams of the finely grated cheese was placed in a pear-shaped flask. The headspace was purged by purified nitrogen gas (100 mL min⁻¹) for 15 min at 37 °C, and the volatile compounds were trapped on Tenax TA (SGE, Kiln Farm, Milton Keynes, UK).

The compounds concentrated in the trap (kept during the purging at room temperature) were desorbed at 225 °C for 5 min by a thermal desorption device (SGE concentrator/headspace analysis injector, Kiln Farm). Cryogenic focusing was applied on the analytical column (SGE CTS.LCO2, Kiln Farm) to reduce band broadening. Gas chromatography was carried out on a Varian Star 3400 CX (JVA Analytical Ltd., Dublin, Ireland) equipped with a DB5 capillary column (60 m length, 0.32 mm i.d., and 1.0 μm film thickness; SGE, Kiln Farm). The standard oven temperature program used here started with an initial oven temperature of 40 °C for 4 min, followed by an increase rate of 2 °C min⁻¹ up to 90 °C, then by 4 °C min⁻¹ to 130 °C, and finally by 8 °C min⁻¹ to 250 °C. At the end of the capillary column, the effluent was split at a ratio of 40:30:30 for the flame ionization detector (FID; 275 °C), sniff port 1, and sniff port 2, respectively. Makeup gas (30 mL min⁻¹ He) was added at the splitter to increase the velocity. At the sniff port, humidified air was generally supplied at 300 mL min⁻¹.

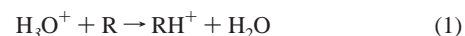
A group of six assessors (five women and one man, aged 20–30), most of them already experienced in sensory analysis, was used for GC-O analysis. To decrease the noise level, the assessors were trained on the technique of sniffing, initially with a mixture of known compounds and afterward with the extracts of the cheese samples.

Tenax tubes without absorbed volatile compounds were used as dummy sample for determining the noise level of the group of assessors. Assessors recorded the beginning and the end of an odor on laptop computers, choosing an odor descriptor after odor detection from a list of descriptors generated in preparatory sessions (rancid, cheesy, sweet, fruity, caramel, glue, nutty, cooked potatoes, earthy, mushroom, metal/sulfur, herbaceous, burnt/smoke, stocky, citrus). The detection frequency was calculated by determining the numbers of assessors detecting a specific compound.

The volatile compounds were identified by combined GC (Varian Star 3400 CX, JVA Analytical Ltd.) and ion trap mass spectrometry (Varian Saturn 3, JVA Analytical Ltd.), equipped with a Tekmar Purge and Trap 3000 concentrator (JVA Analytical Ltd.) and a Tekmar Cryofocusing Module (JVA Analytical Ltd.). The volatile compounds were thermally desorbed at 220 °C over 4 min, transferred via a heated line and focused at –120 °C, and finally injected for 2 min at 235 °C to the gas chromatograph. The capillary column and oven temperature program were the same as those used in the GC-O analysis. Mass spectra were obtained with 70 eV electron impact ionization, with the mass spectrometer scanning from *m/z* 40 to 400 (3 scans/s).

For a further confirmation of the identification, the linear retention indices (LRI) were calculated for each peak, using as reference the series of hydrocarbons C5–C17 (Merck). The LRI calculated for each peak was then compared with those of pure substances (from Sigma-Aldrich, Fluka, and Merck) analyzed on the same column under the same operating conditions.

2.3. Proton Transfer Reaction Mass Spectrometry (PTR-MS). PTR-MS has been described in detail elsewhere (7, 8); therefore, only a brief description of the key features will be given here. The instrument consists of four main components: an ion source, a drift tube, a mass analyzer (quadrupole), and an ion detector/amplifier. H_3O^+ ions are produced at high concentrations from pure water vapor within a hollow cathode ion source and pass via a Venturi-type inlet (17) into the drift tube. The air sample to be analyzed is introduced into the drift tube (close to its entrance) at a flow rate of ~11 mL min⁻¹, maintained at a pressure of ~2 mbar. Because of their low proton affinities, the major components of air undergo nonreactive collisions with H_3O^+ ions and therefore act as buffer gas. However, any collisions of H_3O^+ ions with the volatile organic compounds (VOCs) present in the gas flow possessing a greater proton affinity than water will result in a proton transfer reaction (eq 1). The resultant ions (both primary and product)



are mass-selected using a quadrupole mass analyzer and measured as count rates by an electron multiplier detector. Count rates of the RH^+ ions are subsequently converted to concentration (ppb_v) as described by Lindinger et al. (7, 8) and Hayward et al. (18).

Vials containing 2.6 g of grated cheese were placed in an oven at 37 °C, 1 h before performing the measurements, thus allowing equilibrium to be reached between the solid and gas phases. The headspace formed in these 40-mL silicon-septum closed glass vials (Supelco) was exchanged with ambient air with a continuous flow of 2 mL min⁻¹, diluted with purified nitrogen gas, and transferred through a heated capillary line directly into the reaction chamber. Five repeat measurements were performed for each cheese. The mass spectrometric data were collected over a mass range of m/z 20–260 amu using a dwell time of 0.2 s per mass (48 s per cycle). The maximum values from each measurement were considered to be a measure for the headspace concentration, and background values obtained by analyzing the air in an empty vial were subtracted. This vial sampling method has been recently tested for juice and cheese measurements; a more detailed description is given by Biasioli et al. (11).

2.4. Statistical Evaluation. The SPSS/PC+ program (SPSS Inc., Chicago, IL) was used for principal component analysis (PCA) of the data obtained from GC-O and PTR-MS analysis. Principal component analysis was used as the extraction method, and Varimax with Kaiser Normalization was used as the rotation method. In both cases, GC-O and PTR-MS data analysis, a rotation converged in three iterations.

3. RESULTS AND DISCUSSION

3.1. GC-O. A basic odor profile was obtained for each of the three cheeses analyzed. Fourteen compounds were present in the profile of Parmigiano Reggiano (PR) and of Grana Trentino (GT), while for Grana Padano (GP) there were 11 significant odor-active components. **Figure 1** represents the sniffing chromatograms of each of the three cheeses.

In total, 19 characteristic components have been found in the three cheeses. Thirteen of them have been identified by electron impact mass spectra and LRI by comparison with reference compounds and characterized by their FID peak areas and their odors described by assessors of the sniffing panel (**Table 1**). GC sniffing of dummy samples showed that detection of an odor at the sniffing port by one out of six assessors can be considered as “noise”. The standard deviation (SD) on repeated GC-O analyses was determined as 1; thus, a difference of 2 in the detection frequency between two cheeses was considered significant.

The three grana cheeses were characterized by fruity, buttery-caramel, sulfuric, cooked-potatoes, cheesy, and mushroom notes. Esters (ethyl butanoate, ethyl hexanoate, and 3-methylbutyl acetate), ketones (2-heptanone), aldehydes (3-methylbutanal), and an unknown compound (unknown 3) contributed to the fruity notes. The clear buttery-caramel note was associated with diacetyl, while methional gave the characteristic smell of cooked potatoes. A weak cheesy smell is attributed to 2-methylpropionic acid and the sulfur/cabbage odor to dimethyl sulfide. Although the mushroom note was not conclusively identified in this study, the authors believe the odor to be due to 1-octen-3-one rather than 1-octen-3-ol, as it was associated with mushroom odors in previous studies on grana cheeses (15, 16). In fact, 1-octen-3-one is a fermentation product, and it is found in cheese but not in milk. In contrast, 1-octen-3-ol, which gives a mushroom note as well, is found in raw and heated milk but only seldom in cheese (3).

All of these compounds have been previously associated with the volatile profile of PR (14) and, with the exception of diacetyl and dimethyl sulfide, also with the volatile profile of GP (16). Compounds such as methional, 1-octen-3-one, and the compound unknown 3 are present in quite low concentrations and, despite their low FID response, possessed considerable odor-active properties.

Most of the production of cheese flavor compounds derives from glycolysis, lipolysis, and proteolysis and the secondary

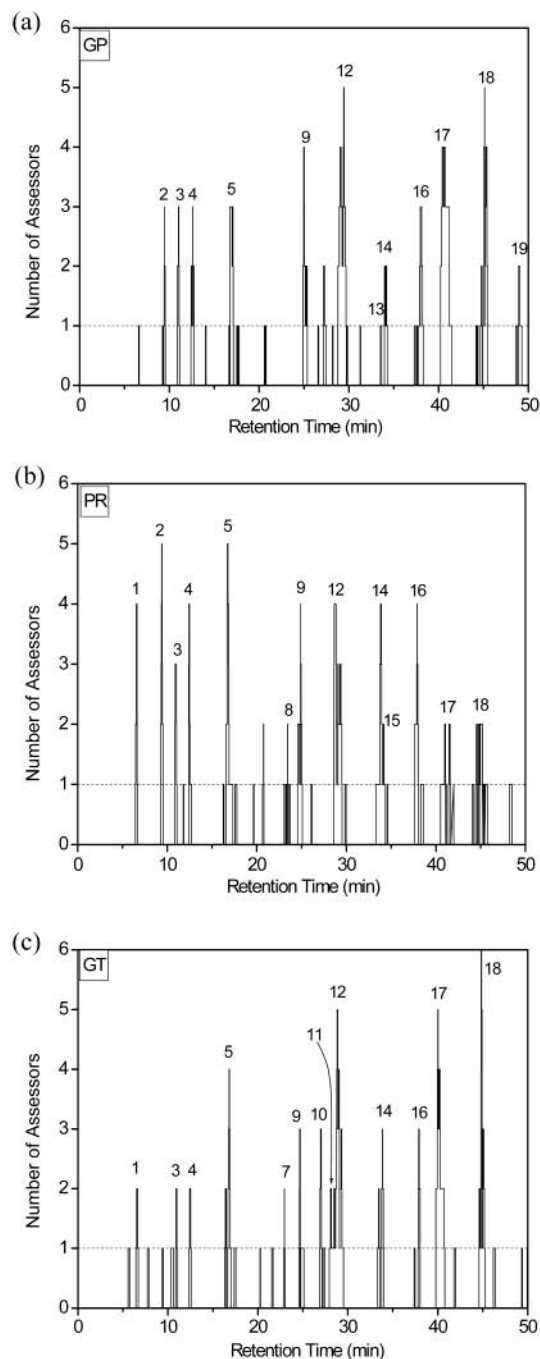


Figure 1. Sniffing chromatograms of volatile compounds of (a) Grana Padano (GP), (b) Parmigiano Reggiano (PR), and (c) Grana Trentino (GT) obtained by gas chromatography–olfactometry (GC-O) with the detection frequency method using a panel of six assessors. Numbers in the chromatograms refer to compounds in **Table 1**.

reactions involving their catabolic products (19–21) lactose/lactate/citrate, free fatty acids, and amino acids, respectively.

The presence of esters is probably linked to the esterase activity of lactic acid bacteria, as previously demonstrated by Hosono et al. (22).

The production of 2-alkanones follows a two-step process: the fatty acids are first oxidized to β -oxo acids, which are then decarboxylated to the corresponding 2-alkanones with one less carbon atom (16). Aldehydes may be produced by amino acid catabolism involving a decarboxylation to amines, followed by oxidation via Strecker degradation to aldehydes. 3-Methylbutanal and methional can be formed by this mechanism from

Table 1. Odor-Active Compounds Identified in the Three Grana Cheeses and Their Detection Frequencies (DF)

no.	compound	LRI	identification ^a	DF			odor descriptors (GCO)
				GP ^b	PR ^b	GT ^b	
1	unknown 1	457	ni		4	2	glue, smoke
2	dimethyl sulfide	526	MS, RI, GCO	3	6		sulfuric, earthy, rancid
3	2-methylpropionic acid	561	MS, RI, GCO	3	3	2	cheesy, earthy, caramel
4	diacetyl (t) ^c	603	RI, GCO	5	4	2	caramel, sweet
5	3-methylbutanal	665	MS, RI, GCO	4	6	6	cheesy, herbaceous, mushroom, caramel
6	2-pentanone	711	MS, RI, GCO		2		sweet
7	methyl butyrate	735	MS, RI, GCO			2	fruity
8	unknown 2	741	ni		2		glue, metal
9	unknown 3	759	ni	6	5	3	sweet, fruity
10	pentanol	781	MS, RI			3	earthy, smoke
11	unknown 4	790	ni			3	rancid
12	ethyl butanoate	798	MS, RI, GCO	6	6	6	fruity, citrus
13	unknown 5	849	ni			3	metal, sweet
14	3-methylbutyl acetate	855	MS, GCO	3	5	3	fruity, sweet, citrus
15	unknown 6	859	ni		2		herbaceous
16	2-heptanone	899	MS, RI, GCO	4	4	3	fruity, sweet
17	methional (t)	945	RI, GCO	5	2	6	cooked potatoes
18	1-octen-3-one (t), ethyl hexanoate	1001	MS, RI, GCO	5	6	6	fermented cabbage, earthy/mushroom, sweet
19	unknown 8	1068	ni	2			metal, smoke

^a Compound identified by GC-MS (MS) and/or by retention index on DB5 (RI) and/or by GC-olfactometry (GCO). ni, not identified. ^b GP, Grana Padano; PR, Parmigiano Reggiano; GT, Grana Trentino. ^c t, tentative.

valine and methionine, respectively (21). Moreover, aldehydes are the major secondary products of autoxidation of unsaturated fatty acids; intermediate products are hydroperoxides which undergo further degradation to hydrocarbon, alcohols, and carbonyl compounds (21).

Linear chain fatty acids can originate from lipolysis and branched chain fatty acids from deamination of amino acids (i.e., propioni bacteria generate 2-methylpropionic acid from the breakdown of valine) (16). Sulfur compounds such as dimethyl sulfide are produced from methionine degradation (3). Diacetyl may be a product of microbial action, involving lacto bacteria (15, 23) and citrate metabolism.

High- to medium-intensity compounds such as ethyl butanoate, ethyl hexanoate, 1-octen-1-one, 2-heptanone, and 2-methylpropionic acid appear to contribute to the odor profile of the three cheeses in a similar manner, with a preponderance of fruity/sweet notes; nevertheless, qualitative and quantitative differences existed between them. For instance, the fruity notes seem to be more preponderant in PR than in GP or GT. In fact, some of the fruity notes due to 3-methylbutanal, 3-methylbutyl acetate, and the unknown compound 3 are present at high intensity in PR but not in GP and GT. The sweet buttery-caramel note of diacetyl is much stronger in GP and PR than in GT. The characteristic cooked potatoes odor related to methional is very strong for the two Granas but quite low for Parmigiano. On the other hand, a quite strong sulfur/metal odor due to dimethyl sulfide is present for the latter one, is not present at all in GT, and has a low intensity in GP.

Some compounds, although at low intensities, appear to be characteristic only for one kind of cheese. The fruity note of 2-pentanone and the glue and herbaceous notes of two unknown compounds (unknowns 2 and 6, respectively) are present only in PR. Characteristic of GT are the fruity methyl butanoate and pentanol and the rancid and metal/mushroom notes of two unknown compounds (unknown 4 and 5), respectively. The only unique note for GP is a burnt odor from an unknown compound with an LRI of 1068 (unknown 8).

PCA was performed on the combined sniffing chromatogram to better illustrate the separation between the three cheeses and the related compounds for each sample (Figure 2). The first two principal components explained 62.56% and 37.44% of the

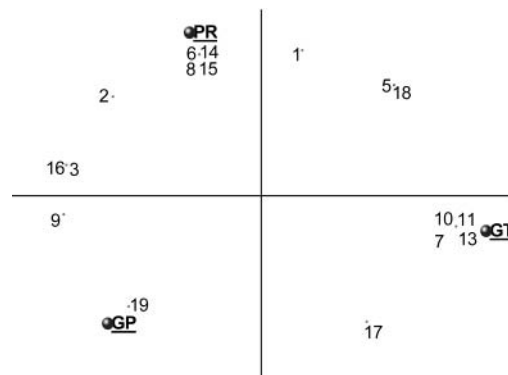


Figure 2. PCA on GC-O data. Scores of cheese samples (GP, Grana Padano; PR, Parmigiano Reggiano; and GT, Grana Trentino) and loadings of odor-active compounds are given on the first (horizontal) and the second (vertical) principal component axes. Numbers refer to compounds in Table 1.

total variance in the data sets. Cheese scores in the PCA map show that Grana Trentino (GT) was separated from Parmigiano Reggiano (PR) and Grana Padano (GP) along the first component. PR and GP were separated along the second components. The diagram reveals three sites at which cheese samples and odor-active compounds are closely related to each other. In the following, the numbers refer to the compounds listed in Table 1. Sweet (compounds 6 and 14), herbaceous (compound 15), and glue/metal (compound 8) odors showed high positive loadings on the second principal components and low negative loadings on the first components and correlated well with PR. Earthy/smoke, rancid, fruity/citrus, and metal/sweet (compounds 7, 10, 11, and 13, respectively) odors showed high positive loadings on the first principal components and low negative loadings on the second components and correlated well with GT. Component 19 (metal/smoke) correlated well with GP.

In previous GC-O studies on GP conducted by Moio and Addeo (16), a more complex aroma with ~31 components was defined for this cheese. In that study, the volatile concentrates from GP were obtained by vacuum distillation followed by a liquid-liquid extraction. In this way, not only the most volatile compounds but also high-boiling-point compounds were ex-

Table 2. Headspace (HS) Composition of the Three Grana Cheeses ($n = 5$)^a

mass (amu) ^b	compound(s) contributing to PTR-MS mass intensities	headspace concentration (ppb _v) ± standard deviation		
		GP	PR	GT
27	fragment ethanol	28.2 ± 3.4	39.7 ± 12.5	18.4 ± 11.7
29	fragment ethanol, propanoic acid	671 ± 27	608 ± 69	657 ± 74
31	formaldehyde, fragment of aldehydes	31.9 ± 5.6	40.4 ± 4.8	38.9 ± 8.3
33	methanol	296 ± 31	629 ± 82	439 ± 16
37	(H ₃ O ⁺)(H ₂ O) ^c	35420 ± 1473	28857 ± 2109	36865 ± 5346
41	fragment various compounds	619 ± 347	1071 ± 77	474 ± 69
43	fragment various compounds	4314 ± 541	4758 ± 854	5106 ± 930
45	acetaldehyde	5780 ± 2595	18423 ± 2066	89083 ± 3677
47	ethanol	9146 ± 4576	7193 ± 1338	8076 ± 796
49	methanethiol	391 ± 125	212 ± 21	512 ± 52
51	methanol(H ₂ O) ^c	60.9 ± 19.5	52.8 ± 8.3	80.7 ± 16.5
53	ni	10.9 ± 4.4	12.1 ± 5.6	11.5 ± 5.1
55	(H ₃ O ⁺)(H ₂ O) ₂ ^c	68 ± 17	64 ± 16	90 ± 30
57	(1- or 2-butanol+H ⁺)–H ₂ O, fragment propionic acid, aldehydes and alcohols	625 ± 202	212 ± 37	409 ± 35
59	acetone, propanal	5710 ± 869	13674 ± 2361	8314 ± 559
61	acetic acid, 1-propanol	5443 ± 1061	5701 ± 241	7768 ± 1462
63	dimethyl sulfide	133 ± 21	176 ± 40	246 ± 18
65	ethanol(H ₂ O) ^c	98 ± 17	74 ± 31	80 ± 18
67	(2-pentenal+H ⁺)–H ₂ O ^d	8.2 ± 4.2	3.9 ± 1.8	11.7 ± 5.8
69	fragment aldehydes	229 ± 39	221 ± 24	282 ± 55
71	(2-pentanol+H ⁺)–H ₂ O, ^d (Z)-2-butenal, (butanoic acid+H ⁺)–H ₂ O ^d	177 ± 27	172 ± 19	277 ± 57
73	2-butanone, tetrahydrofuran, 4C aldehydes	664 ± 55	739 ± 130	874 ± 67
75	propionic acid, methyl acetate	471 ± 83	78 ± 14	973 ± 178
77	ni	5.1 ± 2.8	3.7 ± 2.2	8.9 ± 5.0
79	ni	13.2 ± 4.0	12.8 ± 3.3	19.0 ± 5.5
81	pyrazine	7.7 ± 2.8	6.2 ± 1.7	11.9 ± 2.5
83	(1,3- or 2,3-butanediol+H ⁺)–H ₂ O, ^d 3-penten-2-one, other 5C carbonyl compounds	13.5 ± 7.8	13.1 ± 4.4	17.4 ± 3.5
85	(1-hexanol+H ⁺)–H ₂ O, ^d 2-pentenal	16.3 ± 2.0	14.2 ± 5.5	28.9 ± 8.7
87	2-pentanone, 3-methylbutanal, diacetyl	761 ± 110	1559 ± 140	939 ± 40
89	2-methylpropionic acid, butyric acid, acetoin	1466 ± 329	1658 ± 339	2364 ± 428
91	ni	12.2 ± 4.5	11.4 ± 2.9	14.8 ± 4.0
93	toluene	26.0 ± 5.7	15.2 ± 5.1	19.1 ± 2.7
95	dimethyl disulfide	14.4 ± 4.6	12.8 ± 4.1	21.9 ± 11.2
97	2-vinyl-2-butenal, 2-ethylfuran	10.1 ± 4.4	10.2 ± 3.8	13.9 ± 5.6
99	2-furylmethanol	16.5 ± 4.7	12.6 ± 3.0	25.4 ± 9.3
101	2-hexanone	23.6 ± 9.3	20.2 ± 3.4	31.5 ± 6.8
103	2,3-methylbutanoic acid, methylbutanoate, ethyl propanoate	53.1 ± 12.8	20.1 ± 4.5	38.3 ± 4.3
105	methional	1.2 ± 0.7	2.8 ± 2.4	1.7 ± 0.8
107	<i>o,p,m</i> -xylene, ethyl benzene, benzaldehyde	4.5 ± 1.2	8.0 ± 2.2	6.0 ± 2.7
115	2-heptanone	85 ± 12	81 ± 12	102 ± 13
117	ethyl butanoate/isobutanoate, 2-heptanol, hexanoic acid	612 ± 140	578 ± 78	477 ± 53
119	2-butoxyethanol	3.4 ± 1.5	3.4 ± 1.5	4.6 ± 3.8
121	isopropylbenzene	6.2 ± 1.8	2.7 ± 2.2	7.1 ± 5.1
123	3,4-dimethylphenol, 2-phenylethanol	4.9 ± 2.4	1.7 ± 0.8	4.3 ± 2.0
127	1-octen-3-one	0.0	0.0	0.85 ± 0.64
131	3-methylbutyl acetate, methyl hexanoate	5.7 ± 2.1	5.0 ± 2.9	7.7 ± 4.0
143	nonanal, 2-nonanone, 2-methylnaphthalene	6.8 ± 3.7	9.1 ± 4.3	6.9 ± 2.8
145	ethyl hexanoate, butyl butanoate	93 ± 11	92 ± 8.1	88 ± 20
157	2-decanone, decanal	1.4 ± 1.2	4.1 ± 1.0	1.4 ± 1.6
162	ni	2.4 ± 0.5	2.4 ± 2.3	3.5 ± 2.1
173	ethyl octanoate, butyl, isobutyl hexanoate	4.3 ± 1.9	1.7 ± 1.6	3.1 ± 2.3

^a Chemical assignment of proton transfer reaction–mass spectrometry HS profiles of Grana Padano (GP), Parmigiano Reggiano (PR), and Grana Trentino (GT) based on literature data (13–16, 19, 20). Taking into account that the compounds are identified from their mass and literature, these assignments have to be considered as tentative. ^b The indicated masses correspond to the protonated molecular masses (parent ions) and product ions. ^c Molecules with strong permanent dipole moments (H₂O and small alcohols) tend to form clusters with water molecules in the drift tube. Due to their relatively strong intermolecular forces, these clusters leave the drift tube intact and are detected concomitantly with the nonclustered parent and product ions. The concentration of (VOC–H⁺)(H₂O) is generally below 1% of the concentration of parent and product ions. ^d Protonated alcohols (except methanol), carboxylic acids, and aldehydes can fragment via loss of an H₂O unit. The most intense peaks for alcohols and aldehydes are generally at the mass (VOC–H⁺) – 18.

tracted. The dynamic headspace technique used here favors, instead, the extraction only of low-boiling compounds, thus yielding an extract with fewer components in comparison with the distillation technique; however, it is closer to the genuine odor profile.

3.2. PTR-MS. The real-time headspace analysis of the three grana cheeses conducted by PTR-MS yielded a volatile profile

defined by 50 masses, the intensities of which, ranging between 1 ppb and 90 ppm, showed a significant increase compared to the values in a blank sample. Those masses, including parent (protonated parent) and product ions, ranged from 27 to 173 amu (Table 2). The mean standard deviation of headspace concentrations for the major masses was calculated to be around 10–15%, while, as expected, masses with low intensity have a

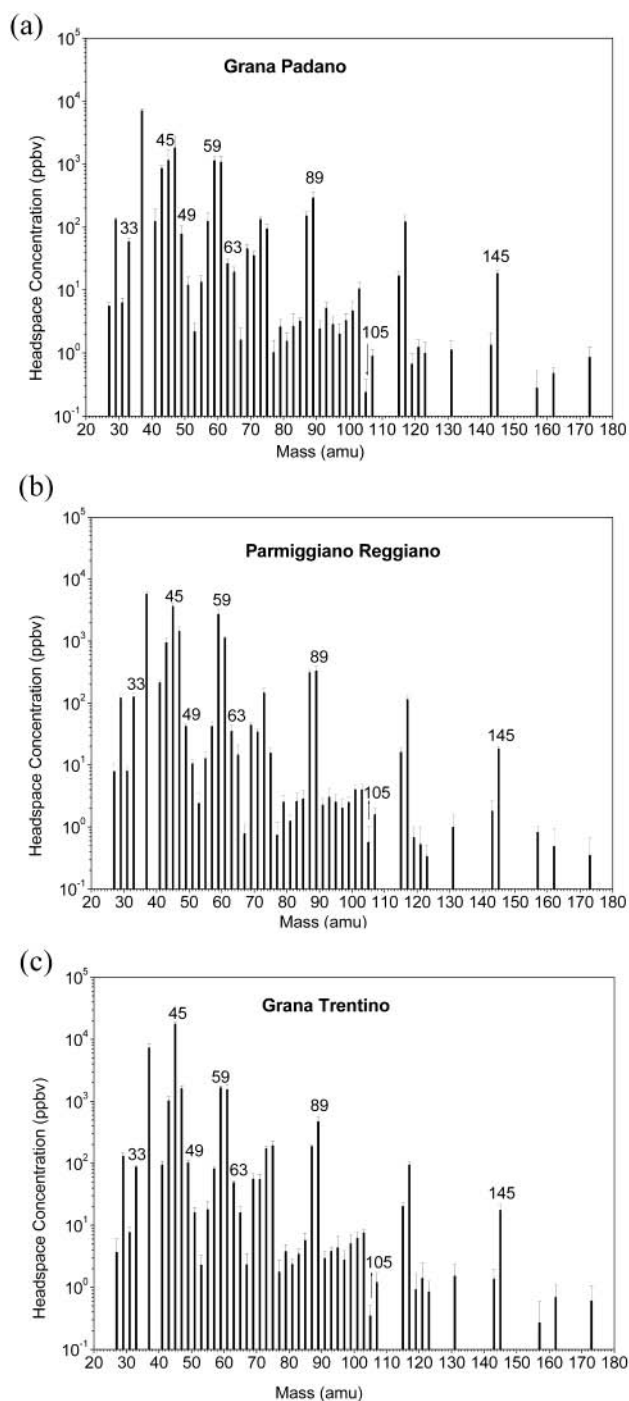


Figure 3. Headspace mass spectra (average value over five repeated measurements) of (a) Grana Padano (GP), (b) Parmigiano Reggiano (PR), and (c) Grana Trentino (GT) obtained by proton transfer reaction–mass spectrometry (PTR-MS).

bigger error due to the signal-to-noise ratio. To obtain better quantitative results, longer dwell times should be applied for those masses. However, a longer integration would not allow the detection of any changes in concentration that occur on short time scales. Mass spectra resulting from the average of the repeated measurements are shown in **Figure 3**.

Seven out of these 50 masses selected have a concentration higher than 1 ppm. Mass 45 (acetaldehyde, fragment of aldehydes) has the highest concentration, followed by masses 59 (acetone, propanal), 47 (ethanol), 61 (acetic acid, fragment ethyl acetate), 43 (common fragment of many compounds), and 89 (butyric acid, acetoin, 2-methylpropionic acid). Only for

Parmigiano Reggiano, masses 87 and 41 have a value higher than 1 ppm. Approximately 2 times more masses fall into the concentration range between 1 and 100 ppb, including masses 75 (propionic acid, methyl acetate), 87 (diacetyl, 2-/3-methylbutanal, 2-pentanone, 3-methyl-3-buten-1-ol), other 5C carbonyl compounds), 73 (4C aldehydes, 2-butanone), 29, 49 (methanliol), 117 (ethyl butanoate and isobutanoate, hexanoic acid), 41 (common fragment of many compounds), 33 (methanol), 57 (fragment of aldehydes, 1-/2-butanol, propionic acid and alcohols), 69 (fragment nonanal), 71, 63 (dimethyl sulfide), 39, and 115 (2-heptanone). One of the most intense masses within this range is 145, which is attributed to ethyl hexanoate, a compound that gives one of the two most intense fruity notes in grana cheese. Other masses attributed to compounds that play an important role in the grana volatile profile are 93 (toluene), 143 (nonanal, 2-nonanone), 173 (ethyl octanoate), and 105 (methional). As expected, compounds with low mass (<100 amu) are found to be the most abundant in the headspace due to their higher vapor pressure. In fact, lipids, which are present in high concentrations in the grana cheeses, significantly reduce headspace concentrations of larger, more hydrophobic compounds and hence perceived flavor intensity. In particular, it was found that the air–oil partition coefficients determined for several chemical classes of compounds decrease as the molecular weight of the flavoring compound increases (24).

Although there exists a common volatile profile for the three cheeses, nevertheless at least 16 masses are predominant in only one of them. For instance, mass 45 (attributed to acetaldehyde) has in Grana Trentino a nearly 5 times higher concentration than in Parmigiano Reggiano and 15 times higher than in Grana Padano. Masses 89 (2-methylpropionic acid, butyric acid, acetoin), 75 (propionic acid, methyl acetate), 49 (methanliol), 63 (dimethyl sulfide), 69 and 71 (attributed to fragments of various compounds) also show a higher headspace concentration for Grana Trentino. Masses showing relevant differences between the three cheeses analyzed or with high intensity are reported in **Figure 4**. Most of the cheese aroma compounds are formed during milk fermentation by microorganisms (the production of which has been discussed in the GC-O section). Besides the fermentation production by microorganisms (lactose metabolism) (25), acetaldehyde can derive from threonine degradation, a process that could be of importance during cheese ripening (26). Thus, besides being, together with diacetyl, one of the most characteristic odors of cultured milk and fresh cheeses (25), acetaldehyde was found to increase during ripening in Swiss Emmental (27) and Pecorino Sardo (1). Moreover, it was found in other two hard cheeses, Gruyere (28) and Parmigiano Reggiano (15). These previous results might validate the high concentration of acetaldehyde resulting from PTR-MS analysis, confirmed by further measurements on samples of different origins.

Methanol (mass 33), acetone (mass 59), mass 87 (diacetyl, 2-methylbutanal, 2-pentanone, 3-methyl-3-buten-1-ol), and mass 41 (attributed to fragments of various compounds) showed in Parmigiano Reggiano concentrations nearly twice as high as those in Grana Padano and Grana Trentino. Masses 157 (2-decanone, decanal) and 27 (attributed to fragments of various compounds) were also predominant in Parmigiano Reggiano.

The volatile profile of Grana Padano seems to exhibit lower concentrations than the other two cheeses. Nevertheless, masses 57 (1,2-butanol, fragment propionic acid, aldehydes, and alcohols), 93 (toluene), and 103 (methyl butanoate, 2,3-methylbutanoic acid) are clearly predominant for Grana Padano.

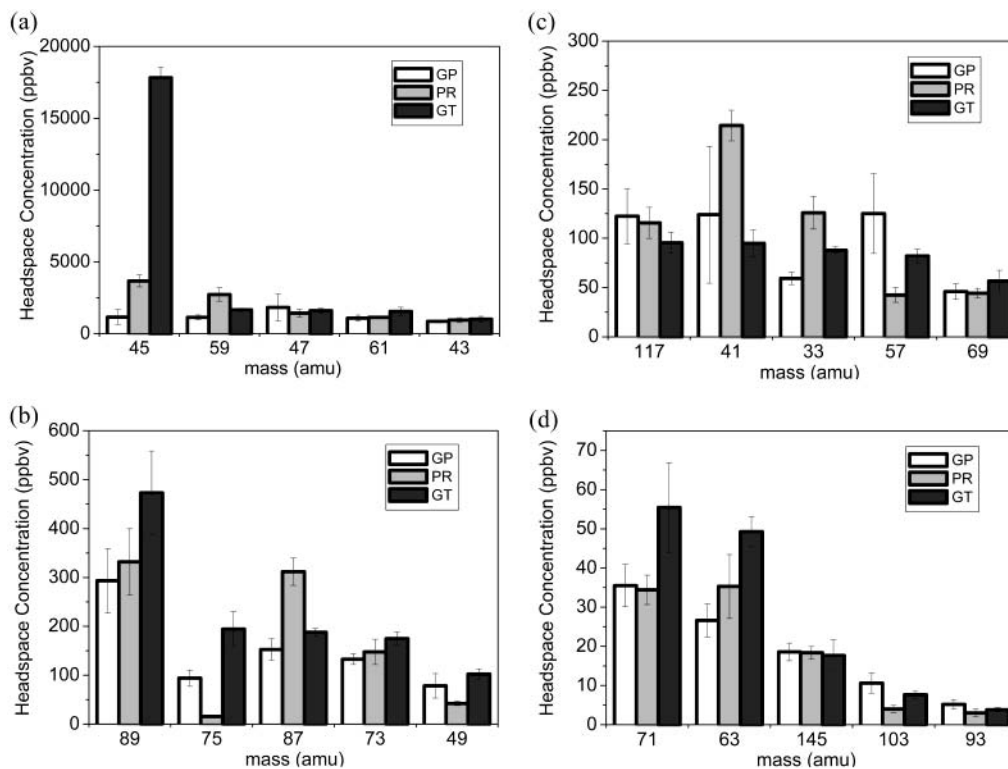


Figure 4. Relevant masses in the headspace of grana cheeses analyzed by proton transfer reaction–mass spectrometry are reported on different y-axes for a better evaluation of differences and similarities between Grana Padano (GP), Parmigiano Reggiano (PR), and Grana Trentino (GT). Masses are reported in graphs a–d with decreasing concentration.

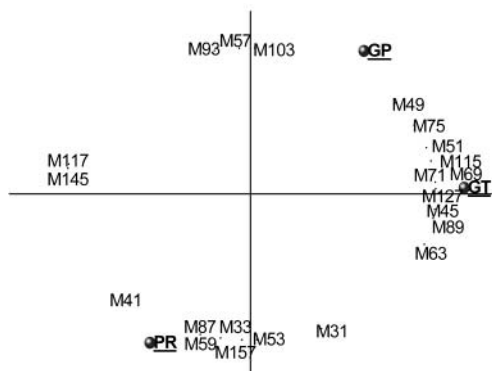


Figure 5. PCA on PTR-MS data. Scores of cheese samples (GP, Grana Padano; PR, Parmigiano Reggiano; and GT, Grana Trentino) and loadings of masses are given on the first (horizontal) and the second (vertical) principal component axes. Numbers with M prefix refer to masses in Table 2.

For masses below 30 ppb, increased relative experimental errors result in nonsignificant differences between the cheeses.

PCA of the quantitative mass spectra confirms that the three cheese samples are well separated and correlate with specific volatile compounds (Figure 5). The first two principal components explained 59.76% and 40.24% of the variance in the data set. Cheese scores in the PCA map show that Parmigiano Reggiano (PR) was separated from Grana Trentino (GT) and Grana Padano (GP) along the first component. GT and GP were separated along the second component. The diagram reveals three sites in which cheese samples and volatile compounds are closely related to each other. The following numbers, with the M prefix, refer to the masses in Table 2, as detected by PTR-MS. Masses 75 (M75), 51 (M51), 115 (M115), 69 (M69), 71 (M71), 127 (M127), 45 (M45), 89 (M89), and 63 (M63)

showed high positive loadings on the first components and low loadings on the second components and correlated well with GT. PR correlated well with M41, M87, M59, M33, and M157 and GP with M93, M57, and M103.

The chemical identification of PTR-MS headspace profiles of Grana Padano, Parmigiano Reggiano, and Grana Trentino was based on literature data (12–15, 19, 28), on the breakup pattern of pure standards, and on comparison with compounds identified by GC-O and GC-MS analysis in the present work. Because PTR-MS is not a separation technique, and because all the compounds are analyzed at the same moment, the identification of the masses is, without further validation, tentative.

3.3. Comparison of GC-O and PTR-MS Results. The aim of this study was to understand the kind of information GC-O and PTR-MS analysis could provide on the volatile profile of grana cheeses and to compare these two instrumental techniques.

The GC-O analysis showed that the basic odor profile of grana cheese is a blend of 11–14 odor-active compounds. The volatile profile of the same cheeses obtained from PTR-MS analysis is, as expected, more complex: 50 masses (parent and fragment ions) gave a significant contribution, with at least 60 compounds tentatively identified. This shows that not all the volatile compounds in the headspace contribute to the odor profile of cheese as detected by the human sensors. In contrast, all the identified odor-active compounds were detected also with the PTR-MS method, demonstrating its high sensitivity.

A direct comparison between odor intensity (measured as detection frequency) and concentration in the headspace (measured with PTR-MS) of the odor-active compounds is not always possible because several compounds have the same molecular weight (parent ion mass) and product ions. For example, diacetyl, 3-methylbutanal, and 2-pentanone all have the parent

ion at mass 87, and their main product ions at masses 41, 43, and 69 are present in the breakup pattern of other compounds identified in the PTR-MS mass spectrum. Moreover, odor-active and non-odor-active compounds could be associated with the same mass in the PTR-MS spectrum. Nevertheless, some masses are associated with only one compound. For instance, good agreement has been found between odor intensity and headspace concentration for ethyl butanoate, 2-heptanone, and ethyl hexanoate, associated respectively with masses 117, 115, and 145. The detection frequencies of these three compounds were the same in the three cheeses; similarly their headspace concentrations gave comparable values within the measurement error.

Moreover, methional and 1-octen-3-one possess a rather low odor threshold value in air (30); i.e., those compounds can be sensed with the human nose even at very low concentrations in air. Thus, although contributing to the odor profile of cheese, they have either a low or no FID response. Instead, PTR-MS was able to detect those two compounds even at very low concentration: mass 105 (methional) between 1 and 2 ppb and mass 127 (1-octen-3-one) at 0.8 ppb. As explained previously in this work, experimental precision can be increased for volatile compounds at low concentrations by adopting a longer dwell time for those masses; however, this reduces the temporal resolution between successive measurements. Nevertheless, the present results demonstrate the low detection limit of PTR-MS for certain volatile flavor compounds.

In conclusion, the capability of GC-O and PTR-MS to distinguish between the three kinds of Grana cheese on the basis of their odor and volatile profile has been proven in this work, and it is clearly shown in the two PCA maps. Moreover, these techniques showed high sensitivity toward low-odor-threshold components, e.g., methional or 1-octen-3-one, that are difficult to detect with other instrumental techniques. GC-O was confirmed to be an optimal tool for the selection of odor-active compounds, while PTR-MS is a good tool for quantification of compounds in the volatile profile of cheese, where concentrations are not affected by any sample preparation.

ACKNOWLEDGMENT

We gratefully acknowledge Dr. C. Delahunty for useful discussion, and all the colleagues in Cork who participated in the sniffing panel.

LITERATURE CITED

- Larrayoz, P.; Addis, M.; Gauch, R.; Bosset, J. O. Comparison of dynamic headspace and simultaneous distillation extraction techniques used for the analysis of the volatile components in three European PDO ewes' milk cheeses. *Int. Dairy J.* **2001**, *11*, 911–926.
- Fuller, G. H.; Steltenkamp, R.; Tisserand, G. A. *Ann. N.Y. Acad. Sci.* **1964**, 116–711.
- Friedrich, J. E.; Acree, T. E. Gas Chromatography (GC/O) of dairy products. *Int. Dairy J.* **1998**, *8*, 235–241.
- Pollien, P.; Ott, A.; Montingon, F.; Baumgartner, M.; Munoz-Box, R.; Chaintreau, A. Hyphenated Headspace–Gas Chromatography–Sniffing Technique: Screening of Impact Odorants and Quantitative Aromagram Comparison. *J. Agric. Food Chem.* **1997**, *45*, 2630–2637.
- van Ruth, S. M.; O'Connor, C. H. Influence of assessors' qualities and analytical conditions on gas chromatography–olfactometry analysis. *Eur. Food Res. Technol.* **2001**, *213*, 77–82.
- van Ruth, S. M. Methods for gas chromatography–olfactometry: a review. *Biomol. Eng.* **2001**, *17*, 121–128.
- Hansel, A.; Jordan, A.; Holzinger, R.; Prazeller, P.; Vogel, W.; Lindinger, W. Proton transfer reaction mass spectrometry: on-line trace gas analysis at ppb level. *Int. J. Mass Spectrom. Ion Processes* **1995**, *149/150*, 609–619.
- Lindinger, W.; Hansel, A.; Jordan, A. On-line monitoring of volatile organic compounds at pptv levels by means of Proton Transfer-Reaction Mass Spectrometry (PTR-MS). Medical applications, food control and environmental research. *Int. J. Mass Spectrom. Ion Processes* **1998**, *173*, 191–241.
- Yeretzian, C.; Jordan, A.; Brevard, H.; Lindinger, W. Time-resolved headspace analysis by Proton-Transfer-Reaction Mass Spectrometry. In *Flavour Release*; Roberts, D., Taylor, A., Eds.; ACS Symposium Series 763; American Chemical Society: Washington, DC, 2000; Chapter 6, pp 58–72.
- Gasperi, F.; Gallerani, G.; Boschetti, A.; Biasioli, F.; Monetti, A.; Boscaini, E.; Jordan, A.; Lindinger, W.; Iannotta, S. The mozzarella cheese flavor profile: a comparison between judge panel analysis and proton transfer reaction mass spectrometry. *J. Sci. Food Agric.* **2000**, *81*, 357–363.
- Biasioli, F.; Gasperi, F.; Aprea, E.; Boscaini, E.; Colato, L.; Märk, T. D. Fingerprinting mass spectrometry by PTR-MS: heat treatment versus pressure treatment of red orange juice—a case study. *Int. J. Mass Spectrom.* (Werner Lindinger special issue), **2003**, *223/224*, 343–353.
- Battistotti, B.; Corradini, C. Italian cheese. In *Cheese, chemistry, physics and microbiology*, 2nd ed.; Fox P. F., Ed.; Chapman & Hall: London, 1993; pp 221–223.
- Dumont, J.-P.; Roger, S.; Adda, J. Volatile compounds in whole cheese and grated cheese: Parmesan. *Lait* **1974**, *54*, 386–396.
- Meinhart, E.; Schreier, P. Study of flavour compounds from Parmigiano-Reggiano cheese. *Milchwissenschaft* **1986**, *41*, 689–691.
- Barbieri, G.; Bolzoni, L.; Careri, M.; Mangia, A.; Parolari, G.; Spagnoli, S.; Virgili, R. Study of the volatile fraction of Parmesan cheese. *J. Sci. Food Agric.* **1994**, *42*, 1170–1176.
- Moio, L.; Addeo, F. Grana Padano cheese aroma. *J. Dairy Res.* **1998**, *65*, 317–333.
- Lindinger, W. In *Gaseous Ion Chemistry and Mass Spectrometry*; Furtrell, J. H., Ed.; Wiley and Sons: New York, 1986; pp 141–154.
- Hayward, S.; Hewitt, C. N.; Sartin, J. H.; Owen, S. M. Performance characteristic and applications of a Proton Transfer Reaction-Mass Spectrometer for measuring volatile organic compounds in ambient air. *Environ. Sci. Technol.* **2002**, *36*, 1554–1560.
- Thierry, A.; Maillard, M. B. Production of cheese flavor compounds derived from amino acid catabolism by *Propionibacterium freudenreichii*. *Lait* **2002**, *82*, 17–32.
- McSweeney, P. L. H.; Nursten, H. E.; Urbach, G. Flavours and off-flavours in milk and dairy products. In *Advanced Dairy Chemistry, Lactose, Water, Salts and Vitamins*; Fox P. F., Ed.; Chapman & Hall: London, 1997; pp 403–468.
- McSweeney, P. L. H.; Sousa, M. J. Biochemical pathways for the production of flavour compounds in cheese during ripening: A review. *Lait* **2000**, *80*, 293–324.
- Hosono, A.; Elliott, J. A.; McGugan, W. A. Production of ethyl esters by some lactic acid and psychrotropic bacteria. *J. Dairy Sci.* **1974**, *57*, 535–539.
- Lawless, H. T.; Antinone, M. J.; Ledford, R. A.; Johnston, M. Olfactory responsiveness to diacetyl. *J. Sens. Stud.* **1994**, *9*, 47–56.
- Pirapez, G.; Hérent, M.-F.; Collin, S. Flavor retention by lipids measured in a fresh cheese matrix. *Food Chem.* **1998**, *61*, 119–125.
- Ott, A.; Germond, J. E.; Chaintreau, A. Origin of acetaldehyde during milk fermentation using ¹³C-labeled precursors. *J. Agric. Food Chem.* **2000**, *48*, 1512–1517.
- Engels, W. J. M.; Dekker, R.; de Jong, C.; Neeter, R.; Visser, S. A comparative study of volatile compounds in the water-soluble fraction of various types of ripened cheese. *Int. Dairy J.* **1997**, *4*, 255–263.

- (27) Bosset, J. O.; Buetikofer, U.; Gauch, R.; Sieber, R. Reifungsverlauf von in Folien verpacktem Emmentaler Käse, mit und ohne Zusatz von *Lactobacillus casei* subsp. *casei* II. Gaschromatographische Untersuchung einiger flüchtiger, neutraler Verbindungen mit Hilfe einer dynamischen Dampfdruckanalyse. *Lebensm. Wiss. Technol.* **1997**, *30*, 464–479.
- (28) Rychlik, M.; Bosset, J. O. Flavour and off-flavour compounds of Swiss Gruyere cheese. *Int. Dairy J.* **2001**, *11*, 903–910.
- (29) Manning, D. J.; Moore, C. Headspace analysis of hard cheese. *J. Dairy Res.* **1979**, *46*, 539–545.
- (30) Odor threshold, Leffingwell & Leffingwell, 2002, <http://www.leffingwell.com/odorthre.htm>.

Received for review September 3, 2002. Revised manuscript received November 30, 2002. Accepted December 7, 2002. This work was partially supported by the FWF, Wien, Austria, through project 14130, by the European Commission, Brussels, through a Marie Curie fellowship to E.B. and the COST action 921, and by the PAT-CNR project AGRIND.

JF020922G