Research Article

Received: 10 June 2010 Revised: 2 August 2010 Accepted: 23 September 2010 Published online in Wiley Online Library:

(wileyonlinelibrary.com) DOI 10.1002/jsfa.4198

Composition and volatile profiles of commercial Argentinean blue cheeses

Irma V Wolf,* María C Perotti and Carlos A Zalazar

Abstract

BACKGROUND: A first approach was made to acquire knowledge of the global composition, proteolysis, lipolysis and volatile profile of Argentinean blue cheeses. A total of 20 samples belonging to six leading commercial brands were analysed. A comparison of the results with bibliographical data on other blue cheeses was performed and the variability among and within dairy plants was also evaluated.

RESULTS: Values of global composition were intermediate in relation to those reported for mould-ripened cheeses. Levels of proteolysis and lipolysis were lower than those of other blue cheeses. Volatile compound profiles were characterised mainly by short-chain fatty acids, methyl ketones and secondary alcohols, as reported previously for blue cheeses. Wide-ranging values of physicochemical parameters, lipolysis and proteolysis levels as well as volatile compound areas of cheeses produced by each dairy plant were observed. Owing to this high variability in the chemistry and volatile profile of cheeses, principal component analysis of the data did not show groupings by commercial brands.

CONCLUSION: On the whole, Argentinean blue cheeses were characterised both by gross intermediate composition values and by proteolysis and lipolysis levels lower than those of blue cheeses of different origins. A typical volatile compound profile of blue cheeses was observed. The high variability found within each commercial brand could reflect the lack of standardisation of the technological processes used in blue cheese manufacturing in Argentina.

Keywords: Argentinean blue cheeses; volatile compounds; proteolysis; lipolysis; global composition

INTRODUCTION

Blue cheese is a type of soft cheese characterised by the growth of *Penicillium roqueforti* in fissures throughout the matrix. During ripening, blue cheeses undergo extensive proteolysis and lipolysis due to the action of enzymes produced by the microflora. These biochemical processes lead to important changes in texture and flavour,[1,2] and some studies indicate that they are more pronounced than in other varieties of cheese.[1,3] Several blue cheese varieties are known and have become popular worldwide, e.g. Gorgonzola (Italy), Roquefort and Bleu d'Auvergne (France), Danish Blue (Denmark), Stilton, Hunstman and Blue Shropshire (England) and Cashel Blue (Ireland) among others.

Blue cheeses have been widely studied in relation to gross composition, proteolysis, lipolysis and the volatile compounds involved in their typical flavour.[4] In Argentina, blue cheeses account for only a small proportion of total cheese production, reaching about 440 t annually, of which over 190 t are exported (about 50% to the Mercosur region; www.quesosargentinos.gov.ar). However, owing to their special characteristics, they are much appreciated by specific sectors of the market, principally by consumers who prefer a piquant and strong taste. These cheeses are made with pasteurised and fat-standardised (3.2–3.3%) cow's milk obtained from skimmed milk and homogenised 12% cream. Lactic acid bacteria and mould spores are added to the milk prior to coagulation. The cheese milk is clotted principally with liquid adult bovine rennet. The clot is then cut and the whey is drained off. Finally the clot is put in moulds and, when the desired shape has been obtained, a dry-salting procedure is applied (*ca* 500 g of NaCl per mould). The cheese is acidified in the moulds until a pH of about 5.1. After 12 days of production the cheeses are pierced. Their average weight ranges from 2 to 4 kg. According to the *Código Alimentario Argentino*,[5] a minimum ripening time of 35 days is necessary, but in practice it is common to ripen the cheeses for 60 days. Among their sensory properties can be mentioned a white or white/yellow colour with blue or green veins, a piquant, salty taste and a very intense odour somewhat resembling ammonia. The cheeses have a semi-soft crumbly texture with a uniform distribution of the P. roqueforti.

In spite of the fact that most cheeses in Argentina are produced in the central area of the country, this area is very large and the weather is highly variable. As a consequence, cows produce milk of different characteristics. In addition, each dairy plant uses different milk coagulants and strains of *P. roqueforti*, and there are also variations in the technological processes employed. Taking this situation into account, it is not surprising to find Argentinean blue cheeses with different sensory characteristics on the market.

* *Correspondence to: Irma V Wolf, Instituto de Lactología Industrial, Facultad de Ingeniería Química, Universidad Nacional del Litoral – CONICET (Consejo Nacional de Investigaciones Científicas y Técnicas), Santiago del Estero 2829, S3000AOM, Santa Fe, Argentina. E-mail: vwolf@fiq.unl.edu.ar*

Instituto de Lactología Industrial, Facultad de Ingeniería Química, Universidad Nacional del Litoral – CONICET (Consejo Nacional de Investigaciones Científicas y Técnicas), Santiago del Estero 2829, S3000AOM, Santa Fe, Argentina

Today in Argentina, few data are available on physicochemical parameters, proteolysis and lipolysis, and no studies have been performed on volatile compound profiles. Therefore the objective of the present study was to investigate the chemical characteristics and identify the volatile compounds of this type of cheese by analysing samples from the most important producers in the region. Comparisons of sample composition among and within dairy industries as well as with bibliographical data on blue cheeses of different origins are also discussed. The results obtained will be used as a starting point for further studies on this product.

MATERIALS AND METHODS

Cheeses and sampling

A total of 20 samples were analysed. The samples belonged to six leading commercial brands of blue cheese (A, B, C, D, E, F) and were purchased at the end of ripening (*ca* 2 months) from local markets in Santa Fe city on four different occasions (I, II, III, IV). All brands were produced by dairy plants in the Santa Fe (Argentina) area.

For physicochemical, proteolytic and lipolytic analyses, cheeses were sampled according to the IDF standard method[6] and kept at −18 °C until analysis.

For volatile compound determination, cheese samples were sliced into wedges, wrapped in aluminium foil and stored at −18 °C. Prior to analysis, samples were cut into cubes, finely grated and homogenised in a 600 W food processor.

Gross composition

The following parameters of cheese samples were determined according to standard methods: pH with a pH meter (Horiba, Kyoto, Japan),[7] moisture content by oven drying at 102 °C,[8] protein content by the macro-Kjeldahl method,[9] fat content by the Gerber–Van Gulick method[10] and salt content by the atomic absorption spectrophotometric method.[11]

All physicochemical analyses were carried out in duplicate.

Proteolysis

Proteolysis was assessed by determination of soluble nitrogen at pH 4.6 (SN-pH4.6), soluble nitrogen in 120 mL L^{-1} trichloroacetic acid (SN-TCA) and soluble nitrogen in 25 mL L^{-1} phosphotungstic acid (SN-PTA) according to the method of Gripon *et al.*[12] Nitrogen content was determined by the macro-Kjeldahl method.[9] Values were expressed as a percentage of total nitrogen (TN).

All analyses were performed in duplicate.

Lipolysis

Extraction of cheese lipids, isolation of free fatty acids (FFAs), derivatisation to ethyl esters and determination of their concentrations by gas/liquid chromatography were performed according to Perotti *et al.*[13] with some modifications. A gas chromatograph (GC-9000, Perkin Elmer Corp., Waltham, MA, USA) equipped with a flame ionisation detector (FID) and a split/splitless injector was used. FFAs were separated on a fused silica capillary column (PE-Wax, 60 m × 0.25 mm) coated with a bonded polyethylene glycol stationary phase (0.25 mm layer thickness) (Perkin Elmer Corp., Norwalk, USA). The temperature programme was as follows: 75 °C (1.5 min); 8 °C min^{-1} to 150 °C (3 min); 10 °C min^{-1} to 230 °C (15 min). The carrier gas (nitrogen) flow rate was set at 3 mL min^{-1} and the split ratio at 1 : 50. The injector and detector temperatures were 250 and 300 °C respectively. Quantification ($C_{4:0}$ to $C_{18:2}$) was performed using the internal standard method with enantic ($C_{7:0}$) and margaric ($C_{17:0}$) fatty acids (Sigma Aldrich, St Louis, MO, USA) as standards. Calibration curves were prepared by combining increasing concentrations of a mixture of butyric ($C_{4:0}$), caproic ($C_{6:0}$), caprylic ($C_{8:0}$), capric ($C_{10:0}$), lauric ($C_{12:0}$), myristic ($C_{14:0}$), palmitic ($C_{16:0}$), stearic ($C_{18:0}$), oleic ($C_{18:1}$) and linoleic ($C_{18:2}$) fatty acids with fixed concentrations of enantic ($C_{7:0}$) and margaric ($C_{17:0}$) fatty acids. Both standards were added to the cheese sample before starting the extraction step. The FID out-signal was recorded and the chromatogram was processed using Turbochrom Version 4 software (Perkin Elmer Corp.). Results were expressed as g FFA kg^{-1} cheese.

Volatile fraction composition

A manual solid phase microextraction (SPME) holder equipped with a 1 cm × 50/30 μm DVB/CAR/PDMS Stable-Flex fibre (Supelco, Bellafonte, PA, USA) was used to isolate and concentrate the volatile compounds. A 5 g portion of grated cheese was placed in a 30 mL glass vial and hermetically sealed with an aluminium seal and butylteflon septum. The vial was thermostatted at 40 ± 1 °C for 10 min, then the fibre was exposed to the headspace for 5 min. Volatile compounds adsorbed on the fibre were immediately desorbed thermally in the GC injector port at 250 °C for 5 min (splitless mode).

Optimisation of conditions of SPME analysis such as extraction time (1–60 min), extraction temperature (40 and 60 °C) and desorption time (5 and 10 min) was performed on a total of nine cheese samples. In particular, the fibre exposure time was selected so as to avoid analyte competition and/or displacement phenomena on the fibre (results not shown).

A GC-9000 (Perkin Elmer Corp.) equipped with an FID was used to obtain information on peak areas (arbitrary units) and to compare the profiles of different cheeses. The volatile compounds were separated on the same column as in the FFA analysis (PE-Wax), with the following temperature programme: 45 °C (4 min); 8 °C min^{-1} to 150 °C (3 min); 10 °C min^{-1} to 250 °C (5 min). Nitrogen was used as carrier gas at a flow rate of 2 mL min^{-1}.

A GC-17A gas chromatograph (Shimadzu Corp., Japan) coupled to an ion trap mass spectrometer (QP-5000, Shimadzu Corp.) was employed to identify the compounds under the same chromatographic conditions and on the same column as in the GC-FID analysis. Spectrometric detection was performed under electron impact (EI) ionisation conditions at 70 eV. Mass spectra were obtained in scan mode within a mass range of *m/z* 72–300 at 250 amu per second. Identification of volatile compounds was performed by matching their mass spectral data with those of known compounds in the NIST-62 library of standard compounds.

Both mass spectrometric identifications and chromatographic peaks of FID were further confirmed by comparing retention times with reference standards (Sigma Aldrich, Milan, Italy) (when available or bibliographical data[14–16] (elution order of compounds on similar polarity columns).

All analyses were conducted in duplicate.

Statistical analysis

Analysis of variance and a multiple comparison test (least significant difference) were carried out on data obtained from physicochemical, proteolytic, lipolytic and volatile profile analyses using Statistix 7 (Analytical Software, Tallahassee, FL, USA) in order to observe statistically significant differences among commercial brands of cheese.

Table 1. Mean values ± standard deviations for physicochemical parameters, nitrogen fractions and global lipolysis in Argentinean blue cheeses grouped according to commercial brands

	Commercial brands of Argentinean blue cheese (*n*, number of samples)					
Parameter	A ($n = 4$)	B ($n = 3$)	C ($n = 4$)	D ($n = 3$)	E ($n = 3$)	F ($n = 3$)
pH	6.3 ± 0.1a	5.5 ± 0.3bc	5.9 ± 0.3c	5.2 ± 0.1b	5.5 ± 0.4bc	5.6 ± 0.1c
Moisture (g kg^{-1})	416 ± 11a	445 ± 40a	417 ± 37a	433 ± 8a	397 ± 22a	407 ± 13a
NaCl (S/M) (g kg^{-1})	76 ± 16a	61 ± 4a	54 ± 12a	61 ± 13a	63 ± 13a	65 ± 6a
Fat (DM) (g kg^{-1})	507 ± 6a	547 ± 43a	593 ± 43b	519 ± 14a	547 ± 15a	548 ± 14ab
Protein (DM) (g kg^{-1})	371 ± 18a	337 ± 33a	334 ± 19a	350 ± 6a	359 ± 7a	358 ± 5a
SN-pH4.6/TN (%)	37.9 ± 4.6a	42.8 ± 13.6a	43.7 ± 4.9a	38.7 ± 12.8a	37.7 ± 2.7a	33.2 ± 0.4a
SN-TCA/TN (%)	30.5 ± 2.8a	39.6 ± 12.4a	39.1 ± 5.3a	42.0 ± 18.7a	32.6 ± 3.2a	29.8 ± 1.0a
SN-PTA/TN (%)	9.1 ± 2.3a	18.9 ± 10.6a	16.3 ± 5.4a	13.8 ± 5.8a	12.7 ± 2.7a	12.7 ± 0.5a
FFAs (g kg^{-1})	13.8 ± 8.9a	19.4 ± 20.4a	39.4 ± 11.6b	18.6 ± 5.3a	17.3 ± 9.0a	8.8 ± 2.1a

Means in the same row followed by different letters are significantly different among commercial brands ($P \leq 0.05$).
S/M, salt in moisture; DM, dry matter.

Multivariate statistical analysis of the results obtained from all experimental measurements was perfomed by principal component analysis (SPSS Version 10.0, SPSS Inc., Chicago, IL, USA) using the Pearson correlation matrix.

RESULTS AND DISCUSSION

Global composition

Average moisture content, pH, NaCl content (salt in moisture), fat content (dry matter) and protein content (dry matter) values of blue cheese samples ($n = 20$) were 419 ± 28 g kg^{-1}, 5.7 ± 0.4, 63 ± 13 g kg^{-1}, 540 ± 56 g kg^{-1} and 352 ± 22 g kg^{-1} respectively. Except for pH, all values were intermediate or similar to those reported for European blue cheeses. In relation to pH, the mean value was generally lower than those found in other studies on mould-ripened cheeses.[2,17–20]

The analysis of variance indicated significant differences in pH and fat content ($P \leq 0.05$) among dairy factories (Table 1). Within some commercial brands a noticeable variability (according to standard error values) in pH (B, C, E), moisture content (B, C), fat content (B, C), protein content (A, B, C) and salt content (A, C, D, E) was observed.

In particular, salt concentration is a significant parameter for all microbiological and physicochemical characteristics of cheeses, since it regulates water activity, microbial flora[21] and therefore enzymatic activities, especially proteolytic activity.[22] Important differences in salt levels have also been observed in mould-ripened cheeses,[23] which could be associated with differences between salting methods. Fat content is another parameter that shows high variability in cheeses. According to Prieto *et al*.,[19] the differences in fat and protein contents in mould-ripened cheeses are mainly due to differences in the fat/casein ratio in the milk used in cheesemaking.

Proteolysis

Proteolysis in blue cheeses is mainly due to the action of proteinases and peptidases from *P. roqueforti*[17,24] and results in a gradual softening of the cheese body and the production of peptides and free amino acids.[2] Levels of SN-pH4.6 and SN-TCA in mould ripened-cheeses are higher than those reported for bacterially ripened cheeses,[23] and these fractions increase notably throughout ripening.[17,19] Mould strain and ripening time are the main factors that affect the degree of proteolysis.[2] The proteolytic activity of the strain is important for the development of a cheese with optimal texture and flavour.[24] A study carried out on the proteolytic activity of *P. roqueforti* strains isolated from Argentinean blue cheeses indicated that only a few strains showed proteolytic activity on casein agar.[21]

The average values of SN-pH4.6, SN-TCA and SN-PTA as a percentage of TN were 39.4 ± 7.8, 35.0 ± 9.0 and 13.9 ± 6.0% respectively, which were lower than those found in European blue cheeses.[2,19,20] Values of proteolysis lower than those for Argentinean blue cheeses have been reported only in Kuflu[23] and Gorgonzola[17] cheeses.

Statistically significant differences in the values of nitrogen fractions among dairy plants were not detected (Table 1), which is a consequence of the important variability observed in the individual values of samples within some commercial brands (mainly B and D).

Lipolysis

Lipolysis and subsequent FFA catabolism are the main biochemical processes related to the production of aroma in blue cheeses.[25,26] Mould-ripened cheeses have the highest lipolysis rate, and the hydrolysis of fat matter can reach up to 10–20%, which would correspond to over 300 mmol FFA kg^{-1} cheese.[1,27] Differences in lipolytic as well as in oxidative metabolic activity on some FFAs among strains of *P. roqueforti* were reported.[28,29] These results had an important influence on the characteristics of the blue cheeses produced.[29] *Penicillium roquerti* strains used in the manufacture of Argentinean blue cheeses have been demonstrated to degrade tributyrin, with some minor differences in relative activity.[21]

In the present work a wide variability in the individual concentrations of each FFA and in the total FFA content (sum of individual FFAs) among the different samples of blue cheeses was observed. The average value was 20.3 ± 14.1 g kg^{-1} cheese, with individual values ranging from 6.1 to 49.2 g kg^{-1} cheese. In a study carried out on commercial Argentinean blue cheeses, Bernal *et al*.[30] also observed a wide difference in overall lipolysis values between samples. This variability has been commonly reported in blue cheeses by other authors. For example, in Gorgonzola cheese the lipolysis values ranged from 6.0 to 40.0 g kg^{-1} cheese at 60 days of ripening.[17,31,32] In Roquefort and commercial blue cheeses the lipolysis levels were 31 and 34 g kg^{-1} cheese respectively.[3]

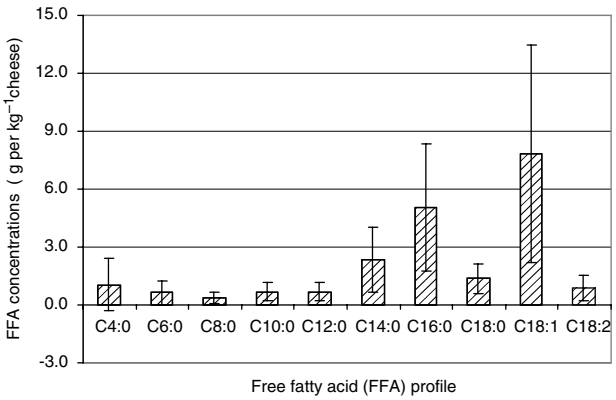

Figure 1. Levels of individual free fatty acids from $C_{4:0}$ to $C_{18:2}$ in Argentinean blue cheeses. Values are mean ± standard deviation of 20 samples.

Figure 1 shows the FFA profile for Argentinean blue cheeses (average value of individual FFAs measured in 20 samples from $C_{4:0}$ to $C_{18:2}$). Oleic ($C_{18:1}$), palmitic ($C_{16:0}$) and myristic ($C_{14:0}$) acids were the main FFAs. A similar profile of FFAs was observed in blue-veined cheeses by Bernal *et al.*,[30] Madkor *et al.*[33] and Prieto *et al.*[19] During ripening, the amount of FFAs released is determined by both esterase and lipase activity, and a large release of FFAs is dependent on the ability of the strain to hydrolyse short- and long-chain fatty acids.[29] *Penicillium roqueforti* strains have diverse activity on both groups of fatty acids.[27,29,32,34]

The analysis of variance indicated significant differences in lipolysis level ($P \leq 0.05$) according to the dairy plant (Table 1). Sample cheeses from dairy plant C had the highest FFA concentration. In addition, variation coefficients above 50% were found for commercial brands A, B and E.

Volatile compound profile

A total of 50 volatile compounds were identified in the samples of blue cheese, which were classified by chemical family (alcohols, esters, ketones, fatty acids, etc.). Important differences in volatile profiles of the samples analysed, taking into account the values of peak areas, were observed. Table 2 lists the identified compounds, showing the mean value ($n = 20$ samples) and standard deviation, the range of values (minimum–maximum) and the frequency of occurrence for each compound. In agreement with the high variability observed for gross composition, proteolysis and lipolysis of the samples belonging to each dairy plant, the volatile profiles also presented wide differences.

A typical chromatogram of the volatile fraction of Argentinean blue cheese by SPME-GC-FID is shown in Fig. 2.

Ketones

A total of ten ketones were identified, which, according to the higher area values, would be very important quantitatively. The broad range of area values indicated great variability of these compounds in individual samples. This high variability agrees with data contributed by Schwartz and Parks[35,36] on Roquefort and commercial blue cheeses. Dartey and Kinsella[37] observed fluctuations in the relative concentrations of methyl ketones during ripening as a consequence of interconversions from their corresponding secondary alcohols.

Methyl ketones with odd-numbered carbon atoms from C_3 to C_9 were present in all samples. The predominant methyl ketones were 2-pentanone, 2-heptanone and 2-nonanone. Concerning unsaturated ketones, 8-nonen-2-one was detected in all cheeses. On the other hand, ketones with an even number of carbon atoms represented a minority group and some of them were not detected in all cheeses. In particular, sample C-I had the highest areas for 2-hexanone, 2-heptanone, 2-octanone, 2-nonanone, 8-nonen-2-one and 2-decanone, whereas sample E-III showed a higher content of 2-propanone and 2-pentanone.

All ketones identified in this work as well as the majority presence of methyl ketones have been reported previously in different blue cheeses.[2,23,32,33,36,38–41]

Methyl ketones with an odd number of carbon atoms, mainly 2-heptanone and 2-nonanone, are considered the main compounds responsible for the unique flavour of blue cheeses.[4,38,42–45]

The formation of methyl ketones from fatty acids in blue cheeses is a result of β-oxidation by the enzymes produced by *P. roqueforti*.[42] Factors such as mycelium physiological stage, pH, salt content and concentration of fatty acids play an important role in the production of these compounds.[1,46]

Alcohols

A wide range of alcohols (17) were identified in the different samples evaluated. Among this group, primary, secondary, linear-chain and branched-chain alcohols were found. Samples such as B-II, D-I and F-IV presented the highest alcohol levels according to their area values.

2-Alkanols with odd-numbered carbon atoms from C_3 to C_9 were identified in most cheeses, with 2-pentanol and 2-heptanol having high area values. Some secondary alcohols such as 2-heptanol contribute markedly to the typical flavour.[40,43] These compounds are formed by enzymatic reduction of methyl ketones, a step considered to be reversible under aerobic conditions.

Table 2. Volatile compounds identified in Argentinean blue cheeses

Peak no.[a]	Compound	Mean ± SD[b]	Range[b]	*n*[b]	Identification[c]
	Ketones				
3	2-Propanone	1479 ± 1231	85–3646	20	MS, ST, R
5	2-Butanone	116 ± 68	27–306	20	MS, ST, R
9	2-Pentanone	3996 ± 2981	86–9533	20	MS, ST, R
14	2-Hexanone	127 ± 154	ND–610	17	MS, ST, R
18	2-Heptanone	4996 ± 3808	614–13232	20	MS, ST, R
25	2-Octanone	129 ± 101	ND–359	18	MS, ST, R
28	2-Nonanone	1539 ± 1527	239–5750	20	MS, ST, R

Table 2. (*Continued*)

Peak no.[a]	Compound	Mean ± SD[b]	Range[b]	*n*[b]	Identification[c]
32	8-Nonen-2-one	504 ± 338	99–1319	20	MS, R
35	2-Decanone	2 ± 4	ND–13	5	MS, ST, R
39	2-Undecanone	37 ± 37	ND–156	18	MS, ST, R
	Alcohols				
7	2-Propanol	188 ± 195	ND–714	18	MS, ST, R
8	Ethanol	513 ± 752	14–2925	20	MS, ST, R
11	2-Butanol	2 ± 4	ND–14	6	MS, ST, R
13	1-Propanol	13 ± 45	ND–192	6	MS, ST, R
15	2-Methyl-1-propanol	87 ± 71	ND–233	18	MS, ST, R
16	2-Pentanol	686 ± 673	17–2038	20	MS, ST, R
17	1-Butanol	21 ± 46	ND–193	10	MS, ST, R
20	3-Methyl-1-butanol	981 ± 791	65–2867	20	MS, ST, R
21	2-Hexanol	20 ± 25	ND–96	11	MS, ST, R
23	1-Pentanol	5 ± 10	ND–31	6	MS, ST, R
26	2-Heptanol	569 ± 565	11–1903	20	MS, ST, R
27	1-Hexanol	5 ± 7	ND–21	6	MS, ST, R
30	2-Octanol	4 ± 11	ND–47	5	MS, ST, R
34	2-Ethyl-1-hexanol	5 ± 7	ND–16	6	MS, ST, R
36	2-Nonanol	51 ± 54	ND–189	19	MS, ST, R
37	2,3-Butanediol	66 ± 84	ND–330	16	MS, R
45	2-Phenylethanol	4 ± 5	ND–15	8	MS, R
	Esters				
4	Ethyl acetate	22 ± 85	ND–359	3	MS, ST, R
10	Methyl butanoate	103 ± 218	ND–733	6	MS, ST, R
12	Ethyl butanoate	199 ± 351	5–1370	20	MS, ST, R
19	Methyl hexanoate	193 ± 433	ND–1397	6	MS, ST, R
22	Ethyl hexanoate	225 ± 474	ND–1738	16	MS, ST, R
24	Isoamyl butanoate	80 ± 66	15–296	20	MS, ST, R
29	Isopropyl hexanoate	7 ± 20	ND–78	4	MS, R
31	Ethyl octanoate	29 ± 36	ND–151	18	MS, ST, R
33	Isoamyl hexanoate	13 ± 20	ND–84	10	MS, R
	Acids				
38	2-Methylpropanoic acid	30 ± 26	ND–85	16	MS, ST, R
40	Butanoic acid	2436 ± 2095	84–6640	20	MS, ST, R
41	3-Methylbutanoic acid	129 ± 143	22–652	20	MS, ST, R
43	Pentanoic acid	16 ± 19	ND–59	11	MS, ST, R
44	Hexanoic acid	1567 ± 1519	37–4703	20	MS, ST, R
46	Heptanoic acid	8 ± 9	ND–27	11	MS, ST, R
47	Octanoic acid	232 ± 214	9–723	20	MS, ST, R
49	Decanoic acid	65 ± 53	5–192	20	MS, ST, R
50	Dodecanoic acid	21 ± 29	ND–116	14	MS, ST, R
	Other compounds				
1	1,3-Pentadiene	2496 ± 2887	ND–8938	15	MS, R
2	Acetaldehyde	7 ± 12	ND–36	7	MS, R
6	3-Methylbutanal	137 ± 254	ND–1068	18	MS, ST, R
42	γ-Hexanolactone	5 ± 8	ND–22	6	MS, ST, R
48	*p*-Cresol	3 ± 6	ND–20	4	MS, R

[a] Peak numbers are the same as in Fig. 1.
[b] Average peak area ($\times 10^3$, arbitrary units) ± standard deviation, range of individual values (ND, not detected) and occurrence of compounds (*n*, number of samples in which component was detected).
[c] Methods of identification: MS, mass spectral comparison with NIST-62 library; ST, comparison with retention times and mass spectra of authentic substances; R, comparison with bibliographic data (elution order on similar polarity columns).[14–16]

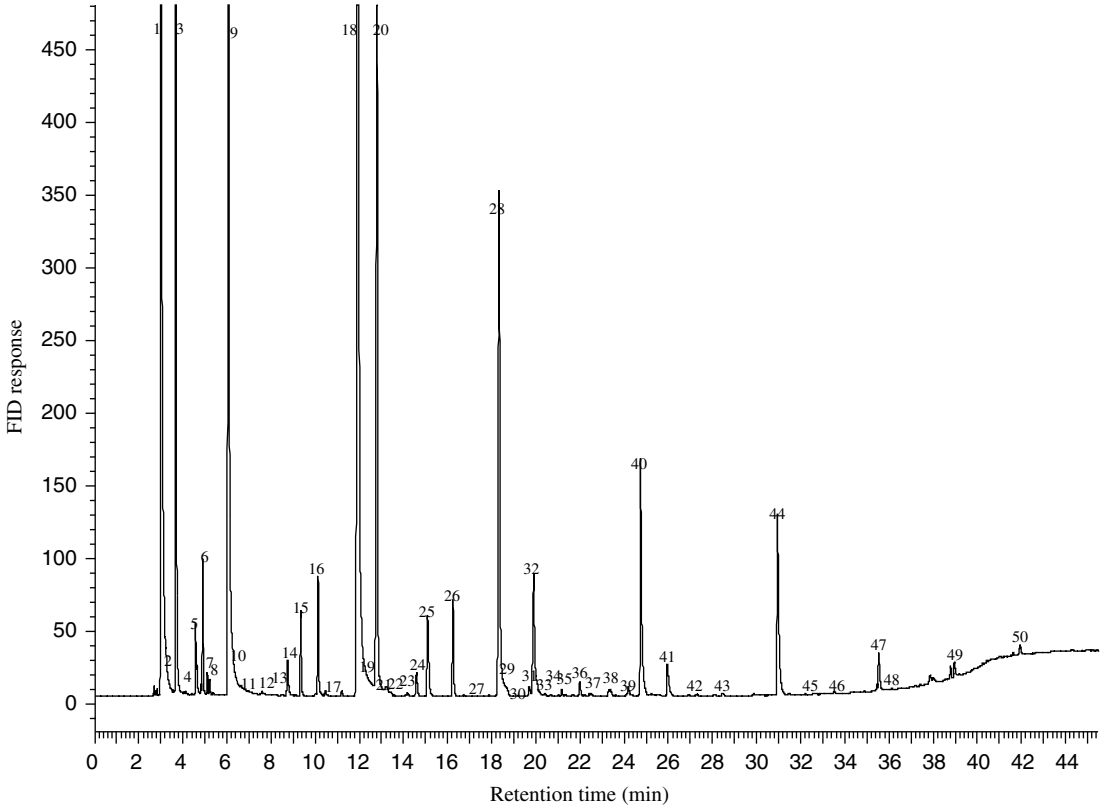

Figure 2. Typical chromatogram of volatile fraction of Argentinean blue cheese by SPME-GC-FID. For peak identification see Table 2.

This reaction is attributed to *Penicillium* metabolism,[47] being a detoxifying pathway to protect micro-organisms.[48]

Linear-chain primary alcohols were detected only in some samples and may impart sweet, fruity and nutty notes to the cheese flavour.[38] Primary alcohols are produced mainly by reduction of the respective aldehydes derived from amino acids and free fatty acids.[25,49] Among them, ethanol was the most abundant and was present in all samples. Ethanol has a limited role in cheese aroma despite its high levels, but it contributes to the formation of esters.[50]

Branched-chain alcohols such as 2-methyl-1-propanol, 3-methyl-1-butanol and 2-ethyl-1-hexanol were identified, with 3-methyl-1-butanol being the most important. Both 2-methyl-1-propanol and 3-methyl-1-butanol are products of aldehyde reduction and are derived from valine and leucine respectively.[25,51]

The aromatic alcohol 2-phenylethanol was detected in a few samples. This compound is considered an odour-active alcohol in blue cheese,[45] contributing rose flower notes, and its production from phenylalanine seems to be essentially achieved by yeasts.[48]

Both the alcohols identified in this work and the majority presence of linear-chain secondary alcohols such as 2-pentanol, 2-heptanol and 2-nonanol and branched-chain primary alcohols such as 3-methyl-1-butanol have been reported in other studies on blue cheeses.[2,23,32,38–41]

Esters

Nine esters were detected in the analysed samples, and, according to the lower area values, they represented a minority fraction, which agrees with the results observed for Gorgonzola[40] and French Blue[38] cheeses. However, owing to their low perception thresholds,[48] some esters contribute in a synergistic way to the fruity aroma.[52] Moreover, the role of esters in blue cheeses is very important, because they may attenuate the typical pungent flavour of methyl ketones.[40]

In this study, esters formed by linear-chain acids of an even number of carbon atoms (butanoic, hexanoic, etc.) with linear-chain primary alcohols (methanol, ethanol) and branched-chain primary alcohols (2-methyl-1-propanol, 3-methyl-1-butanol) were identified. Ethyl butanoate and isoamyl butanoate were found in all cheeses and presented high area values, together with methyl hexanoate and ethyl hexanoate in some samples, principally B-II and D-II.

Ethyl esters, mainly ethyl butanoate and ethyl hexanoate, have been reported as impact compounds in blue cheeses.[40,44,45]

Esterification reactions in cheeses between short- and medium-chain fatty acids and alcohols occur by an enzymatic or a chemical pathway,[46] though other mechanisms of ester production such as alcoholysis have also been suggested.[53] The enzymology of ester biosynthesis by moulds has not been investigated. Sporadic evidence suggests that lipases from dairy moulds synthesise esters via esterification.[53] Usually, alcohol concentration is the limiting factor in the formation of these compounds.[54]

Fatty acids

These compounds are important in the aroma of many types of cheese. In blue cheeses they are not only flavour compounds *per se* but are also precursors of methyl ketones, alcohols, lactones and esters.[48] In spite of the important lipolysis in blue cheeses, the impact of free fatty acids on their flavour is less than in hard Italian cheeses, probably owing to the neutralisation of pH throughout ripening and the influence of methyl ketones on flavour.[25]

Nine fatty acids were found in the volatile profile of Argentinean blue cheeses. Saturated fatty acids with even-numbered carbon atoms from C_4 to C_{12} were the most abundant. Butanoic and hexanoic acids presented the highest area values, especially in samples such as B-II, C-III and D-III. Both compounds are characteristic flavour components of blue cheeses.[40,44,45] Butanoic acid has a rancid and cheesy odour, while hexanoic acid has a pungent and blue cheese flavour note.[46,48] Their presence is associated with the lipolytic activity of the microflora.[49]

Linear-chain fatty acids with an odd number of carbon atoms represented a minority fraction. Among branched-chain fatty acids, only 2-methylpropanoic and 3-methylbutanoic acids were found, with the latter being more abundant. Proteolytic enzymes are responsible for their formation from the catabolism of valine, leucine and isoleucine.[49,51,55] The majority presence of short-chain fatty acids and 3-methylbutanoic acid agreed with data reported for other varieties of blue cheese.[2,23,32,39–41,45,50]

Aldehydes and other compounds

Only acetaldehyde and 3-methylbutanal were identified among the aldehyde group. The latter was present in the majority of samples and has been reported as an important compound in blue cheeses.[23,31,40,41] The importance of aldehydes in the flavour of blue cheeses has not yet been elucidated.[38] However, 3-methylbutanal has been reported as an odour-active compound,[43,44] and the proteolytic activity of *P. roqueforti* would be responsible for its formation[31] from leucine catabolism by Strecker degradation.[25,51,55] This compound is characterised by green and malty notes.[4]

γ-Hexanolactone and *p*-cresol (4-methylphenol) were also identified in some samples of blue cheeses analysed. In particular, *p*-cresol was reported in Danish Blue[41] and Kuflu[23] cheeses and contributes barnyard and phenolic notes. The catabolism of tyrosine produces *p*-cresol by atypical Strecker degradation. Non-starter lactobacilli are thought to be responsible for its production.[25]

Statistical analysis

The physicochemical parameters, proteolysis, lipolysis and volatile compound profile of cheeses are aspects closely related to the technology, raw material and starters that each dairy industry uses. As stated in the 'Introduction', it is common that each dairy plant adopts its own scheme of production and uses specific strains of *Penicillium*. In addition, other explanations as to why which the results show great variability must be considered. Cheeses are non-homogeneous systems and their composition in terms of volatile components varies considerably from the rind to the interior, making it difficult to obtain a representative sample. Blue cheeses have additional non-homogeneity due to the presence of blue and white zones. The nature and concentration of the volatile components in these cheese micro-regions are different,[56] and mixtures of all cheese zones cannot lead to homogeneous samples. Thus, in order to have a correct comparison between the volatile components of blue cheeses, blue and white zones of specific cheese regions should be taken from each cheese and studied separately.

For this reason, in spite of the limited number of samples, it was of interest to compare the six dairy plants by principal component analysis. This method analyses all parameters simultaneously, allowing one to detect the most important variables and to establish differences among commercial brands. The data matrix consisted of 20 cheese samples and 41 input variables (loadings ≥ 0.6).

Data analysis by principal components did not lead to interesting results. The samples were not differentiated by commercial brands of cheese and thus the results are not included here. This fact is not surprising owing to the high variability observed within commercial brands.

Taking into account that the lipolysis level in C cheeses was statistically higher ($P \leq 0.05$) than in the other commercial brands, it was of interest to examine whether the volatile compounds derived from milk fat reached the highest values in this brand.

Table 3 lists the main volatile compounds associated with lipolysis and FFA catabolism, showing the mean values and standard deviations for each commercial brand. Statistical differences were detected only for some compounds such as 2-pentanone, 2-heptanone, 2-pentanol, 2-heptanol and isoamyl butanoate. As can be seen, although commercial brand C presented the highest levels of lipolysis among the dairy plants, this fact was not reflected in higher contents of FFAs, methyl ketones or secondary alcohols.

Table 3. Main volatile compounds associated with milk fat degradation in Argentinean blue cheeses

	Commercial brands of Argentinean blue cheese (*n*, number of samples)					
Compound	A ($n = 4$)	B ($n = 3$)	C ($n = 4$)	D ($n = 3$)	E ($n = 3$)	F ($n = 3$)
2-Pentanone	171 ± 119a	4803 ± 338b	5238 ± 2063b	4866 ± 1276b	4415 ± 4613b	6043 ± 2587b
2-Heptanone	817 ± 296a	6644 ± 2667b	7381 ± 4107b	7709 ± 1468b	4187 ± 4812ab	4594 ± 2372ab
2-Nonanone	448 ± 230a	1710 ± 1114a	2525 ± 2173a	2344 ± 1427a	1317 ± 1773a	726 ± 377a
2-Pentanol	22 ± 6a	962 ± 863bc	812 ± 438b	1680 ± 403c	288 ± 159ab	991 ± 603bc
2-Heptanol	25 ± 11a	889 ± 539c	540 ± 194bc	1745 ± 270d	199 ± 36ab	786 ± 399c
2-Nonanol	7 ± 5a	71 ± 78a	58 ± 36a	118 ± 88a	31 ± 12a	73 ± 24a
Ethyl butanoate	27 ± 18a	319 ± 431a	149 ± 188a	133 ± 24a	502 ± 752a	64 ± 19a
Ethyl hexanoate	2 ± 3a	598 ± 988a	122 ± 128a	100 ± 37a	463 ± 676a	103 ± 33a
Isoamyl butanoate	52 ± 34a	106 ± 55ab	59 ± 14a	196 ± 111b	60 ± 51a	76 ± 43a
Butanoic acid	594 ± 659a	2137 ± 2370a	3477 ± 2348a	2021 ± 616a	4176 ± 2808a	2442 ± 1044a
Hexanoic acid	274 ± 284a	1582 ± 2192a	2648 ± 1745a	1360 ± 630a	2315 ± 1662a	1096 ± 383a

Values are average peak area ($\times 10^3$, arbitrary units) ± standard deviation for samples from each dairy plant (A–F). Means in the same row followed by different letters are significantly different among commercial brands ($P \leq 0.05$).

FFA catabolism is conditioned by numerous factors, with the enzymatic activities of moulds playing an important role. Besides, the SPME method is strongly affected by the physicochemical parameters of cheese such as pH and fat content, which could have an effect on the headspace composition and on the quantities adsorbed onto the fibre.[45] In the case of fatty acids, pH is a critical parameter owing to the equilibrium between dissociated and non-dissociated forms. The higher pH values of samples belonging to dairy plant C could explain the lower liberation of FFAs such as butanoic acid and hexanoic acid into the headspace and consequently their lower adsorption onto the fibre.

CONCLUSIONS

The present work presents a first analysis of the global composition, proteolysis level, lipolysis degree and characteristic volatile compounds of commercial Argentinean blue cheeses. The global composition parameters were intermediate with respect to other blue cheeses. Proteolysis and lipolysis levels were generally lower than those reported for this variety.

The main groups of characteristic compounds of blue cheeses such as ketones, alcohols, esters and acids were found in our study. Among these groups, 2-pentanone, 2-heptanone, 2-nonanone, 2-pentanol, 2-heptanol, 3-methyl-1-butanol, ethyl butanoate, methyl hexanoate, ethyl hexanoate, butanoic acid and hexanoic acid had high area values.

The high variability observed within the commercial brands for the different chemical parameters could be associated, among other factors, with the milk quality, the use of different strains of *Penicillium* in the manufacture of cheeses and the slight differences in the technological process. This situation is common in the Argentinean dairy industry and evidences the necessity to work on the standardisation of methods and raw materials. In addition, as mentioned, another source of variability would be associated with the heterogeneity of the samples.

ACKNOWLEDGEMENTS

The authors thank Instituto de Desarrollo Tecnológico para la Industria Química (INTEC) and particularly Dr Orlando Alfano for allowing the use of a gas chromatograph coupled to a mass spectrometer. The technical assistance by Dr Cristina Zalazar and Antonio Negro (INTEC) and the collaboration of Lic. Susana Palma (INLAIN) in the analyses of samples are greatly appreciated. This work was supported by Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET).

REFERENCES

1 Gripon JC, Mould-ripened cheeses, in *Cheese: Chemistry, Physics and Microbiology*, ed. by Fox PF. Chapman and Hall, London, pp. 111–136 (1993).
2 Lawlor JB, Delahunty CM, Sheehan J and Wilkinson MG, Relationships between sensory attributes and the volatile compounds, non-volatile and gross compositional constituents of six blue-type cheeses. *Int Dairy J* **13**:481–494 (2003).
3 Woo AH, Kollodge S and Lindsay RC, Quantification of major free fatty acids in several cheese varieties. *J Dairy Sci* **67**:874–878 (1984).
4 Sablé S and Cottenceau G, Current knowledge of soft cheeses flavor and related compounds. *J Agric Food Chem* **47**:4825–4836 (1999).
5 *Código Alimentario Argentino* [Online]. (2009). Available: http://www.anmat.gov.ar/codigoa/caa1.asp [10 April 2010].
6 FIL-IDF, *Lait et Produits Laitiers. Guide de l'Echantillonnange. Standard 50C*. International Dairy Federation, Brussels (1995).
7 Bradley RL, Arnold E, Barbano DM, Semerad RG, Smith DE and Vines BK, Chemical and physical methods, in *Standard Methods for the Examination of Dairy Products*, ed. by Marshall R. American Public Health Association, Washington, DC, pp. 433–531 (1993).
8 FIL-IDF, *Formaggio e Formaggio Fuso. Determinazione della Materia Secca. Standard 4A*. International Dairy Federation, Brussels (1982).
9 FIL-IDF, *Latte. Determinazione del Tenore in Azoto. Standard 20B*. International Dairy Federation, Brussels (1993).
10 ISO, *Cheese. Determination of Fat Content. Van Gulik. Standard 3433*. International Organization for Standardization, Switzerland (1975).
11 AOAC, *Minerals in Ready-to-feed Milk-based Infant Formula. Atomic Absorption Spectrophotometric Method 985.35*. Association of Official Analytical Chemists, Arlington, VA (1990).
12 Gripon J, Desmazeaud MJ, Le Bars D and Bergère JL, Etude du rôle des micro-organismes et des enzymes au cours de la maturation des fromages. II. Influence de la présure commérciale. *Lait* **55**:502–516 (1975).
13 Perotti M, Bernal S, Meinardi C and Zalazar C, Free fatty acids profiles of Reggianito Argentino cheese produced with different starters. *Int Dairy J* **15**:1150–1155 (2005).
14 Mallia S, Fernández-García E and Bosset J, Comparison of purge and trap and solid phase microextraction techniques for studying the volatile aroma compounds of three European PDO hard cheeses. *Int Dairy J* **15**:741–758 (2005).
15 Povolo M, Contarini F, Mele M and Secchiari P, Study on the influence of pasture on volatile fraction of ewes' dairy products by solid-phase microextraction and gas chromatography–mass spectrometry. *J Dairy Sci* **90**:556–569 (2007).
16 Ziino M, Condurso C, Romeo V, Giuffrida D and Verzera A, Characterization of 'Provola dei Nebrodi', a typical Sicilian cheese, by volatiles analysis using SPME-GC/MS. *Int Dairy J* **15**:585–593 (2005).
17 Gobbetti M, Burzigotti R, Smacchi E, Corsetti A and De Angelis M, Microbiology and biochemistry of Gorgonzola cheese during ripening. *Int Dairy J* **7**:519–529 (1997).
18 Cattaneo T, Toppino P and Civardi G, Proteolysis in Gorgonzola cheese during ripening. *Sci Tecn Latt-Cas* **48**:261–273 (1997).
19 Prieto B, Franco I, Fresno J, Bernardo A and Carballo J, Picón Bejes-Tresviso blue cheese: an overall biochemical survey throughout the ripening process. *Int Dairy J* **10**:159–167 (2000).
20 Fernández-Salguero J, Internal mould-ripened cheeses: characteristics, composition and proteolysis of the main European blue vein varieties. *Ital J Food Sci* **16**:437–445 (2004).
21 Pose G, Ludemann A, Gómez Z and Segura J, Comparison of growth characteristics and roquefortin C production of *Penicillium roqueforti* from blue-veined cheese. *Mycotoxin Res* **23**:122–126 (2007).
22 Malacarne M, Summer A, Panari G, Pecorari M and Mariani P, Caratterizzazione chimico-fisica della maturazione del Parmigiano-Reggiano. *Sci Tecn Latt-Cas* **57**:215–228 (2006).
23 Hayaloglu AA, Brechany EY, Deegan KC and McSweeney PLH, Characterization of the chemistry, biochemistry and volatile profile of Kuflu cheese, a mould-ripened variety. *Lebensm Wiss Technol* **41**:1323–1334 (2008).
24 Larsen MD, Kristiansen KR and Hansen TK, Characterization of the proteolytic activity of starter cultures of *Penicillium roqueforti* for production of blue veined cheeses. *Int J Food Microbiol* **43**:215–221 (1998).
25 McSweeney PLH and Sousa MJ, Biochemical pathways for the production of flavour compounds in cheeses during ripening: a review. *Lait* **80**:293–324 (2000).
26 Alewijn M, Sliwinski EL and Wouters JTM, A fast and simple method for quantitative determination of fat-derived medium and low-volatile compounds in cheese. *Int Dairy J* **13**:733–741 (2003).
27 Svensson I, Hernández I, Virto M and de Renobales M, Determination of lipase activity in cheese using trivalerin as substrate. *Int Dairy J* **16**:423–430 (2006).
28 Ghitti C, Bianchi Salvadori B and Rottigni C, Gorgonzola cheese III. *Sci Tecn Latt-Cas* **47**:195–216 (1996).
29 Larsen MD and Jensen K, The effects of environmental conditions on the lipolytic activity of strains of *Penicillium roqueforti*. *Int J Food Microbiol* **46**:159–166 (1999).
30 Bernal S, Perotti M, Zalazar MC, Cardell D and Zalazar CA, Evaluación de ácidos grasos libres en quesos argentinos. *Rev Argent Lactol* **17**:35–48 (1998).
31 Contarini G and Toppino PM, Lipolysis in Gorgonzola cheese during ripening. *Int Dairy J* **5**:141–155 (1995).

32 Ghitti C, Bianchi Salvadori B and Rottigni C, Il formaggio Gorgonzola XI. *Sci Tecn Latt-Cas* **50**:231–240 (1999).
33 Madkor S, Fox PF, Shalabi SI and Metwalli NH, Studies on the ripening of Stilton cheese: lipolysis. *Food Chem* **25**:93–109 (1987).
34 Ha J and Lindsay R, Release of volatile branched-chain and other fatty acids from ruminant milk fats by various lipases. *J Dairy Sci* **76**:677–690 (1993).
35 Schwartz DP and Parks OW, Quantitative analysis of methyl ketones in blue cheese fat. *J Dairy Sci* **46**:989–990 (1963).
36 Schwartz DP and Parks OW, Methyl ketones in Roquefort cheese. *J Dairy Sci* **46**:1422–1423 (1963).
37 Dartey CK and Kinsella JE, Rate of formation of methyl ketones during blue cheese ripening. *J Agric Food Chem* **19**:771–774 (1971).
38 Gallois A and Langlois D, New results in the volatile odorous compounds of French cheeses. *Lait* **70**:89–106 (1990).
39 Gonzalez de Llano D, Ramos M, Polo C, Sanz J and Martinez-Castro I, Evolution of the volatile components of an artisanal blue cheese during ripening. *J Dairy Sci* **73**:1676–1683 (1990).
40 Moio L, Piombino P and Addeo F, Odour-impact compounds of Gorgonzola cheese. *J Dairy Res* **67**:273–285 (2000).
41 Trihaas J, Vognsen L and Nielsen PV, Electronic nose: new tool in modelling the ripening of Danish Blue cheese. *Int Dairy J* **15**:679–691 (2005).
42 Patton S, The methyl ketones of blue cheese and their relation to its flavour. *J Dairy Sci* **33**:680–684 (1950).
43 Curioni PMG and Bosset JO, Key odorants in various cheese types as determined by gas chromatography–olfactometry. Review. *Int Dairy J* **12**:959–984 (2002).
44 Qian M, Nelson C and Bloomer S, Evaluation of fat-derived aroma compounds in blue cheese by dynamic headspace GC/olfactometry-MS. *J Am Oil Chem Soc* **79**:663–667 (2002).
45 Frank DC, Owen CM and Patterson J, Solid phase microextraction (SPME) combined with gas-chromatography and olfactometry-mass spectrometry for characterization of cheese aroma compounds. *Lebensm Wiss Technol* **37**:139–154 (2004).
46 Collins YF, McSweeney PLH and Wilkinson MG, Lipolysis and free fatty acid catabolism in cheese: a review of current knowledge. *Int Dairy J* **13**:841–866 (2003).
47 Jackson HW and Hussong RV, Secondary alcohols in blue cheese and their relation to methyl ketones. *J Dairy Sci* **41**:920–924 (1958).
48 Molimard P and Spinnler HE, Review. Compounds involved in the flavour of surface mold-ripened cheeses: origins and properties. *J Dairy Sci* **79**:169–184 (1996).
49 Barbieri G, Bolzoni L, Careri M, Mangia A, Parolari G, Spagnoli S, *et al*, Study of the volatile fraction of Parmesan cheese. *J Agric Food Chem* **42**:1170–1176 (1994).
50 Vítová E, Loupancová B, Zemanová J, Stoudkova H, Brezina P and Babák L, Solid-phase microextraction for analysis of mould cheese aroma. *Czech J Food Sci* **24**:268–274 (2006).
51 Urbach G, Contribution of lactic acid bacteria to flavour compound formation in dairy products. *Int Dairy J* **5**:877–903 (1995).
52 Di Cagno R, Banks J, Sheehan L, Fox PF, Brechany EY, Corsetti A, *et al*, Comparison of the microbiological, compositional, biochemical, volatile profile and sensory characteristics of three Italian PDO ewes' milk cheeses. *Int Dairy J* **13**:961–972 (2003).
53 Liu S-Q, Holland R and Crow VL, Esters and their biosynthesis in fermented dairy products: a review. *Int Dairy J* **14**:923–945 (2004).
54 Holland R, Liu S-Q, Crow VL, Delabre M-L, Lubbers M, Bennett M, *et al*, Esterases of lactic acid bacteria and cheese flavour: milk fat hydrolysis, alcoholysis and esterification. Review. *Int Dairy J* **15**:711–718 (2005).
55 Marilley L and Casey MG, Flavours of cheese products: metabolic pathways, analytical tools and identification of producing strains. *Int J Food Microbiol* **90**:139–159 (2004).
56 Gkatzionis K, Linforth RS and Dodd CE, Volatile profile of Stilton cheeses: differences between zones within a cheese and dairies. *Food Chem* **113**:506–512 (2009).

