J. Dairy Sci. 100:1679–1687
https://doi.org/10.3168/jds.2016-11781

Composition, proteolysis, and volatile profile of Strachitunt cheese

F. Masotti,*[1] S. Cattaneo,* M. Stuknytė,* G. Battelli,† L. Vallone,‡ and I. De Noni*
*Dipartimento di Scienze per gli Alimenti, la Nutrizione e l'Ambiente, Università degli Studi di Milano, via G. Celoria 2, 20133 Milan, Italy
†Istituto di Scienze delle Produzioni Alimentari, Consiglio Nazionale delle Ricerche, via G. Celoria 2, 20133 Milan, Italy
‡Dipartimento di Scienze Veterinarie per la Salute, la Produzione Animale e la Sicurezza Alimentare, Università degli Studi di Milano, via G. Celoria 10, 20133 Milan, Italy

ABSTRACT

Strachitunt, a blue-veined Italian cheese, received the Protected Designation of Origin (PDO) label in 2014. Its unique technological feature is represented by the dual-curd method of production. Strachitunt is produced from raw bovine milk with or without the inoculation of natural starter cultures of lactic acid bacteria, and the addition of secondary cultures of mold spores is not permitted by the product specification. Physicochemical properties, proteolysis, and volatile profile of Strachitunt were investigated in 10 cheese samples (ripened for 75 d) made throughout spring 2015 and provided by the main cheese maker. Overall, composition parameters showed a large variability among samples. Cheese was characterized by an acid paste (pH 5.46) and a lower extent of proteolysis compared with other blue-veined varieties. The main chemical groups of volatile organic compounds were alcohols and esters, whereas ketones represented only a minor component. The erratic adventitious contamination by mold spores of the cheese milk, the unique dual-curd method of cheese-making, and the large time variability between the piercing time and the end of ripening could be highlighted as the main causes of both the distinctive analytical fingerprint and the scarce standardization of this blue-veined cheese.
Key words: Strachitunt cheese, proteolysis, volatile organic compounds, dual-curd method of production

INTRODUCTION

Strachitunt, an Italian blue-veined cheese made in Valtaleggio, a restricted area of Lombardy region, was included in 2000 among the most valuable traditional products made in this region, and in 2014 it was given the Protected Designation of Origin (**PDO**) label (European Commission, 2014). This artisanal PDO cheese, produced using a traditional method and complying with regulatory manufacturing standards, provides an annual production around 50 tons. Raw cow milk of Italian Brown breed is used according to the product specification (European Commission, 2016). Bacteria promoting acid development in Strachitunt curd can be present as "wild" microflora or added as autochthonous natural starters (Figure 1). The use of natural starters is adopted only in the hot periods of the year, and it is intended to (1) achieve a uniform rate of milk acidification, (2) control unwanted bacteria, (3) balance the variability of microbial population, (4) positively affect curd syneresis and cheese rheology, and (5) favor rennet action. Milk in vat is heated to 36 to 37°C, and calf rennet is added. Coagulation takes place at 33 to 38°C for 20 to 30 min. The coagulum after 2 successive cuttings is reduced to dimensions of 10 × 10 × 10 mm. The technological feature of Strachitunt is the so-called dual-curd method of production, based on the mix of a 12-h-old curd, called "cold curd," with a fresh curd, called "hot curd" (in a 1:10 ratio, respectively), to make the body of the final cheese. The cold curd is kept at a temperature above 10°C and relative humidity of 80 to 90%. Cold and hot curds are manually mixed in layers and molded (Figure 1). With exception of Beacon Fell Traditional Lancashire, a crumbly hard cheese (Commission Regulation, 1996), Strachitunt is the only PDO cheese produced by this method. No addition of mold spores is permitted during Strachitunt cheese-making. The dual-curd mixing, promoting the formation of cavities entrapping air in the paste, coupled with the cheese piercing is known to favor the natural growth of molds. The cylindrical cheese weighs between 4 and 6 kg and ripens for at least 75 d in chambers at 4 to 8°C and relative humidity between 50 and 80%. The compact and marbled texture shows uneven creamy streaks and green-blue veins ascribed to the mold formation. In general, the degree of marbling of blue cheeses matrix varies according to the number of spores naturally present in the milk, their capacity to develop, and the local sporulation of the mold in a fraction of the total mass (Gkatzionis et al., 2009). In case of Strachitunt cheese

Received July 26, 2016.
Accepted October 29, 2016.
[1]Corresponding author: fabio.masotti@unimi.it

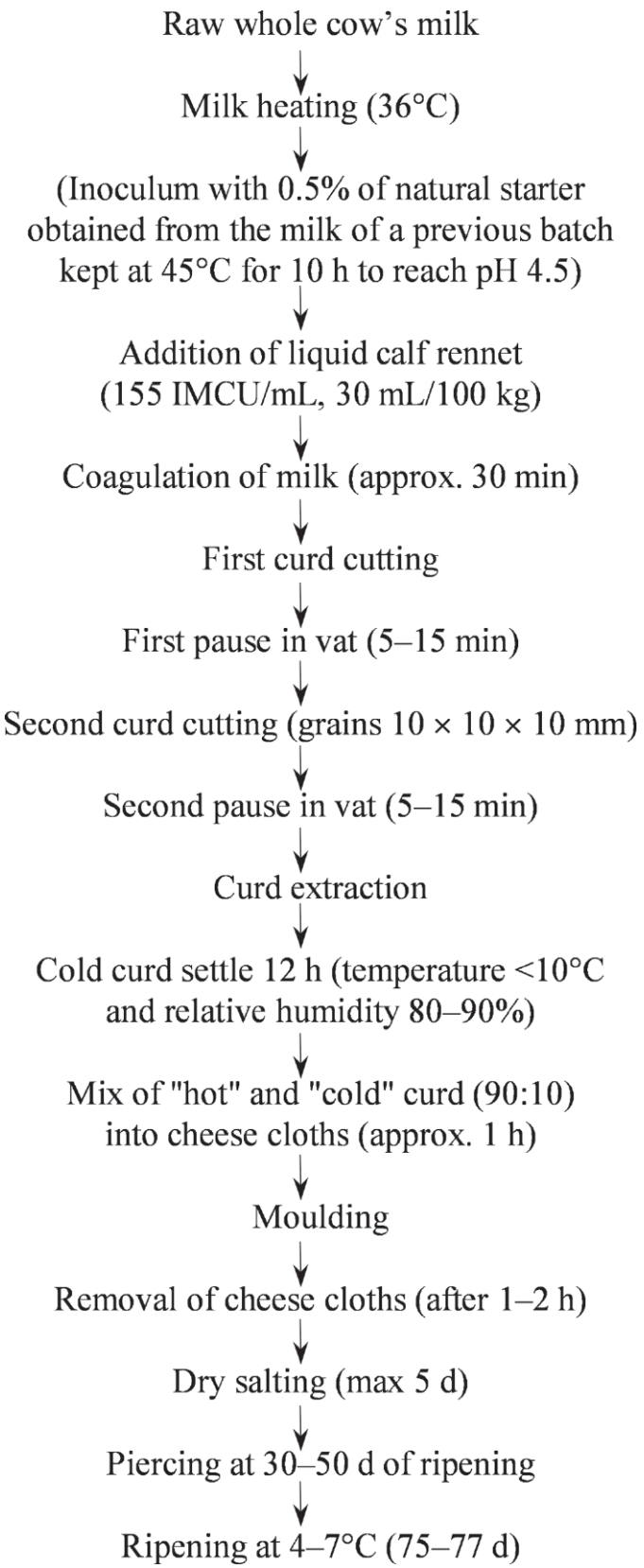

Figure 1. Flowchart for Strachitunt cheese-making. IMCU = international milk-clotting unit.

an additional feature is the variable piercing time from the 30th to the 50th d of ripening.

Previous research (Belotti et al., 2003) described the microbiological evolution in Strachitunt during ripening (from 1 to 3 mo). The lactic acid bacterial microflora of Strachitunt consisted of micrococci, enterococci, thermophilic, and mesophilic lactobacilli. From the first/second month to the second/third month of ripening these authors measured a slight decrease of lactic acid bacteria (cocci from 7.7 to 7.5 log cfu/g; lactobacilli from 7.3 to 7.2 log cfu/g), an almost equal level of molds and the yeasts (6.7 log cfu/g and 4.0 log cfu/g, respectively) and the increase of heterofermentative lactobacilli (4.1 log cfu/g and 5.9 log cfu/g, respectively). Heterofermentative lactobacilli produced micro holes in the paste promoting the growth of molds. Generally, in blue-veined cheeses, the production of lactic acid by thermophilic bacteria promotes the rapid growth of *Penicillium roqueforti*, which is the prevailing mold of these varieties. *Penicillium roqueforti* is the main donor of proteolytic enzymes, released when the mold dies and lyses, leading to the formation of water-soluble nitrogen and free amino acids acting as precursors of volatile compounds (Lawlor et al., 2003). In addition, *Penicillium* spp. are also the main lipolytic microbiota in these cheese varieties. The lipid fraction is the major contributor to the development of taste and aroma profiles in blue-veined cheeses (Preedy et al., 2013). Enzymatic reactions produce a large number of volatile organic compounds (**VOC**) contributing to cheese aroma. In particular, in blue-veined cheeses the main contribution to flavor is ascribed to methyl ketones produced by the β-oxidation of free fatty acids followed by a decarboxylation reaction (Voigt et al., 2010).

The scope of the present study was to provide a chemical portrait of PDO Strachitunt cheese. For this purpose, we surveyed the physico-chemical characteristics, proteolysis, and the volatile profile of the 10 cheese samples. In the sampling scheme we also took into consideration the procedure adopted by cheese makers to add a natural starter culture only in the hottest period of the year.

MATERIALS AND METHODS

Cheese-Making and Sampling

Ten samples of Strachitunt cheeses were made consecutively on a weekly basis during the spring of 2015 and ripened 75 to 77 d. These no commercial cheese samples were provided by the main cheese maker belonging to the "Consorzio per la tutela dello Strachitunt Valtaleggio" (protection body for PDO Strachitunt). Cheese samples from A to H (Table 1) were obtained by

milk renneted without starter inoculation. In contrast, the milk-in-vat of samples I and L was added with a starter culture produced through backslopping (Figure 1). This sampling scheme was unbalanced but reflected the cheese-making practice adopted to guarantee a better standardization of the cheese during the hot period of the year. Each sample consisted of a wheel of cheese. After rind removal (10 mm), the cheese matrix was homogenized and sampled for analyses.

Compositional Analysis and Assessment of Proteolysis

The following analyses were carried out on Strachitunt cheese samples: total solids by drying at 102°C (ISO, 2004a), water activity by dew-point measurement (ISO, 2012) with an Aqua Lab 3TE Water Activity System apparatus (Decagon Devices Inc., Pullman, WA), pH by pH-meter pH25 (Crison Instruments SA, Barcelona, Spain) with a puncture electrode (Crison Instruments SA), fat by gravimetric method (ISO, 2004b), and total nitrogen by the Kjeldahl method (ISO, 2008).

Proteolysis was evaluated by measuring the nitrogenous fractions by Kjeldahl method according to an ISO Standard (ISO, 2011). The procedure consisted in the separation of the pH 4.4-soluble nitrogen (**pH 4.4-SN**) from a citrate solution of the cheese. This fraction was further extracted with 12% trichloroacetic acid (**TCA**) or phosphotungstic acid (**PTA**) to obtain TCA-soluble nitrogen (**TCA-SN**) or PTA-soluble nitrogen (**PTA-SN**), respectively.

Urea PAGE

The pH-4.4 insoluble fractions of the cheeses were analyzed by urea PAGE according to the method of Andrews (1983) with modifications of Veloso et al. (2004) on a 10% resolving gel. Electrophoresis was performed on a Mini vertical electrophoresis unit (SE250, Hoefer, Holliston, MA) at a constant voltage of 60 V, with a power supply EPS 500/400 (GE Healthcare, Uppsala, Sweden). Gels were stained with Coomassie Brilliant Blue G-250. Band identification was performed by comparison with those of a commercial sample of sodium caseinate (Fonterra, Auckland, New Zealand) and with the electropherograms of Bertolino et al. (2011).

Volatile Organic Compound Analysis

Volatile organic compounds were extracted from the headspace of cheese by solid-phase microextraction and analyzed by a GC-MS procedure according to Cornelli et al. (2015), slightly modified for better evaluation of the acidic fraction. Briefly, 1 mL of a 0.12 M H_2SO_4 so-

Table 1. Composition and physico-chemical parameters of Strachitunt Protected Designation of Origin cheeses made during springtime and ripened for 75 to 77 d[1]

	Starter-free samples								Samples made with LAB[3] starter			
Parameter[2]	A	B	C	D	E	F	G	H	I	L	Mean	SD
DM (g/kg)	522.1^{f}	538.6^{d}	528.6^{e}	547.3^{c}	541.2^{d}	546.3^{c}	538.9^{d}	568.3^{a}	562.2^{b}	536.9^{d}	543.0	14.0
Fat (g/kg of DM)	487.3^{de}	504.5^{bc}	512.8^{b}	498.6^{cd}	497.2^{cd}	495.1^{cd}	515.5^{b}	491.5^{de}	481.8^{e}	534.7^{a}	501.9	15.6
Protein (g/kg of DM)	401.6^{ab}	392.2^{bc}	400.4^{ab}	399.5^{ab}	401.9^{ab}	409.4^{a}	383.8^{c}	384.8^{c}	399.0^{ab}	399.7^{ab}	397.2	8.0
Water activity (a_w)	0.960^{b}	0.960^{b}	0.975^{a}	0.957^{bc}	0.964^{b}	0.947^{c}	0.957^{bc}	0.963^{ab}	0.958^{b}	0.956^{bc}	0.960	0.007
pH	5.22^{e}	5.70^{ab}	5.62^{b}	5.39^{d}	5.49^{e}	5.48^{c}	5.47^{c}	5.75^{a}	5.08^{f}	5.24^{e}	5.46	0.22
pH 4.4-SN (% of TN)	20.26^{a}	19.02^{b}	16.26^{e}	13.38^{i}	14.84^{h}	16.71^{d}	15.24^{g}	17.16^{c}	15.78^{f}	20.26^{a}	16.89	2.31
TCA-SN (% of TN)	10.93^{b}	10.57^{bc}	8.86^{d}	7.87^{e}	8.11^{e}	8.70^{d}	9.00^{d}	8.97^{d}	10.46^{c}	11.59^{a}	9.51	1.28
PTA-SN (% of TN)	4.04^{a}	3.50^{b}	2.61^{c}	2.02^{fg}	1.88^{g}	2.31^{de}	2.51^{d}	2.12^{ef}	4.20^{a}	3.47^{b}	2.87	0.86

$^{a-i}$Values with different letters in the same row are statistically different ($P < 0.05$).

[1]Each value is the mean of 2 determinations.

[2]Protein = N × 6.38; SN = soluble nitrogen; TN = total nitrogen; TCA-SN = soluble N in trichloroacetic acid; PTA-SN = soluble N in phosphotungstic acid.

[3]LAB = lactic acid bacteria.

lution was added to 5 g of cheese along with 2 g of NaCl immediately before volatile extraction, all other parameters being the same previously described. Semiquantitative evaluation of VOC was carried out by integrating the peak area of the characteristic ion (Q_{ion}) using the MS-Chemstation software (Agilent Technologies, Santa Clara, CA). Data are the mean values of 2 replicates.

Statistical Analysis

The physico-chemical parameters and the nitrogen fractions of the Strachitunt cheese samples were submitted to one-way ANOVA followed by Tukey post hoc test, taking 0.05 as the limit of significance. Data analyses were carried out with Minitab software (Release 17, 2016, State College, PA). Principal component analysis (**PCA**) was performed on VOC, classified as chemical classes. Results were graphically represented by the projection of the first 2 principal components (**PC**).

RESULTS AND DISCUSSION

Compositional Analysis

The main physico-chemical characteristics of Strachitunt cheese samples are reported in Table 1. Mean DM content was 543.0 g/kg (ranging from 522.1 to 568.3 g/kg), a value similar to that reported for Gorgonzola (Gobbetti et al., 1997) and within limits of other internal mold-ripened cheeses (Fernández-Salguero, 2004; Hayaloglu et al., 2008). The measured water activity was 0.960, a value in accordance with that of Gorgonzola and Bleu Bresse (Fernández-Salguero, 2004). The mean protein content was 397.2 g/kg expressed on DM, and it varied from 383.8 to 409.4 g/kg, being in the range of the data reported in literature (Fernández-Salguero, 2004). In addition, the fat level (501.9 g/kg on DM, on average) was similar to the contents of other blue-veined varieties (Fernández-Salguero, 2004). The pH proved to be a distinguishing parameter of Strachitunt in comparison with other blue cheeses. Values were included between 5.08 and 5.78, and batches with starter (I and L) positioned in the lower side of this interval. Typically, the pH of blue-veined cheeses increases throughout ripening, reaching the level 6.5 to 6.8 at 90 d ripening (Prieto et al., 2000). The consumption of lactic acid by molds and the deamination of free amino acids are responsible for such a high pH level (Fox et al., 2000). The significantly lower pH values observed in Strachitunt in relation to other blue varieties were attributed to the less extensive proteolytic phenomena, because of the lack of inoculation of mold spores.

Proteolysis Assessment

The growth of *P. roqueforti* throughout the matrix of blue-veined varieties sensitively enhances the proteolysis affecting both flavor and texture of the mature cheese (Fox et al., 2000). Strachitunt is pierced after 30 to 50 d of ripening. On this basis, the main biochemical processes, proteolysis and lipolysis, are expected to be slowed. A distinctive phenomenon in the blue-veined cheeses is the extensive proteolysis caused by lactic acid bacteria, the coagulant enzyme (chymosin) and mainly by *Penicillium* spp. (Prieto et al., 2000; Cantor et al., 2004)

We first evaluated the proteolysis of Strachitunt samples by measuring the nitrogenous fractions. The relative distribution of nitrogen in the pH 4.4-SN, TCA-SN, and PTA-SN fractions is shown in Table 1. The pH 4.4-SN is a heterogeneous fraction representing an accepted index of the degree of proteolysis (Diezhandino et al., 2015). This fraction was on average 16.89% on total nitrogen (**TN**) in the 10 cheese samples. No significant ($P > 0.05$) effects were observed between the samples with or without the inoculation of starter cultures. This nitrogen level indicated the reduced occurrence of proteolytic phenomena in comparison with other mold-ripened cheeses (Prieto et al., 2000; Diezhandino et al., 2015). A survey on the characteristics on the main European blue-veined cheeses reported values of pH 4.4-SN more than 2-fold higher, ranging from 55.9% for Cabrales to 72.6% (on TN) for Roquefort (Fernández-Salguero, 2004).

The TCA-SN consists of small peptides (2–20 residues) and free amino acids deriving from the hydrolysis of intermediate peptides (Sousa et al., 2001). This nitrogen fraction reached 9.51% expressed on TN (Table 1) ranging from 7.87 to 11.59%. The TCA-SN content expressed as a percentage of pH 4.4-SN (56.3%) was low in relation to that reported in literature for other blue-veined varieties (Gobbetti et al., 1997; Zarmpoutis et al., 1997; Diezhandino et al., 2015), proving the lower extent of proteolysis in Strachitunt cheese.

The PTA-SN, consisting of small peptides (<600 Da) and free amino acids, was 2.86% expressed on TN and one-tenth of the levels recorded in other blue-veined cheeses (Zarmpoutis et al., 1997; Diezhandino et al., 2015). In addition, this nitrogen fraction did not differ significantly between the batches without or with starter ($P > 0.05$). Overall, amounts of the different nitrogen fractions of Strachitunt indicate that this cheese, when ready for consumption (75 d), is subjected only to limited proteolytic phenomena.

Subsequently, we evaluated the proteolysis of Strachitunt by urea PAGE. The electropherograms of the pH

4.4-insoluble fractions of the cheese samples showed appreciable differences in number and intensity of bands (Figure 2). Some profiles almost overlapped, in particular A versus B and D versus I. In blue-veined cheeses, both α_S-CN and β-CN are expected to be completely hydrolyzed at the end of ripening (Fox et al., 2000). In our work, intact β-CN was present in all samples, suggesting that its degradation by action of plasmin was only partial, likely because the pH value of the cheeses (on average 5.46) was lower than the optimal for this enzyme (7.5). Accordingly, in all samples (Figure 2), the occurrence of low mobility products corresponding to γ-CN, formed by plasmin from β-CN, was less pronounced than that reported in literature for other varieties of blue-veined cheeses (Diezhandino et al., 2015). Indeed, the presence of γ-CN in blue-veined cheeses such as Stilton, Danablu, Cashel, Chetwynd, and Gorgonzola was attributed to a high level of plasmin activity, in view of the high pH of these cheeses (Zarmpoutis et al., 1997).

The α_{S1}-CN band was evident in samples C to L, but less so in A and B. The first hydrolysis product of this CN fraction by chymosin, represented by α_{S1}-CN f(24–199) (α_{S1}-I-CN), was revealed in samples C to L. The electrophoretic patterns A and B lacked α_{S1}-I-CN band, but they showed other low molecular degradation products migrating faster and probably corresponding to the further degradation of this peptide. In blue-veined cheeses, after sporulation, enzymes from *P. roqueforti* also hydrolyze α_{S1}-I-CN, changing the peptide profile (Fox et al., 2000). The distinguishing patterns of samples A and B were paralleled by the highest levels of the 3 nitrogen fractions in relation to that of samples C to L (Table 1). Such results were consistent with those of Zarmpoutis et al. (1997) who coupled the low extent of CN hydrolysis with the low values for SN, TCA-SN, and PTA-SN in some Irish blue cheeses in comparison with Danablu, Stilton, and Gorgonzola.

A qualitative evaluation of the electrophoretic profiles (Figure 2) suggested that Strachitunt samples were characterized by noticeable heterogeneity in the proteolytic phenomena, and no differences were observed between the batches with or without inoculation of starter culture. Likely, the adventitious secondary microflora developed differently as a result of variable selective conditions (pH, water activity, temperature, time of piercing, and development of environmental molds).

Analysis of VOC

Table 2 summarizes the mean percentages of the main chemical classes of compounds as well as the individual volatile components and their relative abundance in the cheese samples. The 29 compounds identified in Strachitunt cheeses comprised 1 aldehyde, 5 alcohols, 10 esters, 6 ketones, and 7 acids. Other components present at trace levels were not considered.

3-Methyl butanal was the only aldehyde detected, with a mean area of 55×10^3 arbitrary units. The branched-chain aldehyde, considered in different cheese varieties as an important odor-active substance, is formed by *P. roqueforti* from leucine via Strecker degradation (Fox et al., 2000). Generally, aldehydes are not expected to accumulate in the ripened cheese, with their levels depending on the balance between production and degradation, which is linked to the degree of maturity of the cheese (Fox et al., 2004). Because of the low oxidation–reduction potential of the cheese, it is

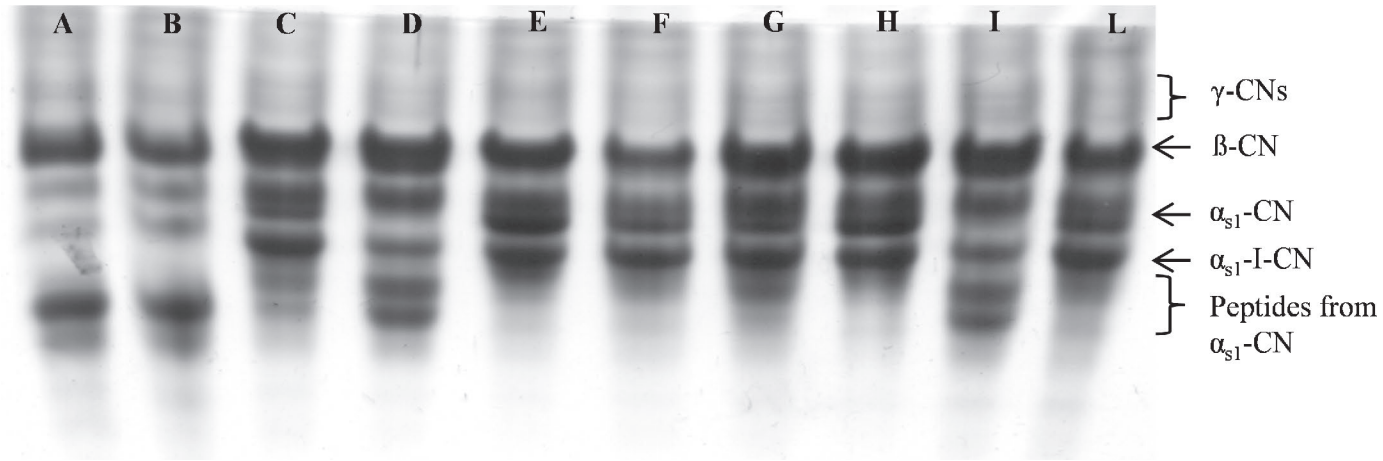

Figure 2. Urea-PAGE patterns of the pH 4.4-insoluble fraction of Strachitunt cheeses ripened 75 d. Lanes A to H = starter-free samples; lanes I and L = samples made with lactic acid bacteria starter inoculation.

Table 2. Volatile organic compounds (VOC) of Strachitunt Protected Designation of Origin cheeses made during springtime and ripened 75 d[1]

	Starter-free samples								Samples made with LAB[2] starter			
Chemical class	A	B	C	D	E	F	G	H	I	L	Mean	SD
Aldehydes (0.1% of total VOC)												
3-Methyl butanal (41)	28^{bc}	42^{b}	210^{a}	195^{a}	27^{bc}	28^{bc}	12^{bc}	11^{bc}	6^{bc}	4^{c}	55	76
Alcohols (9.5% of total VOC)												
Ethanol (45)	2,529^{b}	804de	2,431^{b}	9,713^{a}	1,990^{bc}	1,302^{cd}	1,960^{bc}	41^{e}	1,087^{d}	1,309^{cd}	2,317	2,708
2-Methyl 1-propanol (43)	701^{a}	274^{c}	462^{b}	414^{b}	164^{d}	126de	53^{ef}	102^{def}	72^{ef}	45^{f}	241	219
3-Methyl 1-butanol (55)	8,746^{a}	4,682^{bc}	9,120^{a}	7,676^{a}	4,683^{bc}	3,555^{cd}	884^{ef}	5,517^{b}	2,344^{de}	778^{f}	4,798	3,021
2-Heptanol (45)	51^{d}	223^{bc}	160^{bcd}	247^{b}	159^{bcd}	220^{bc}	56^{d}	650^{a}	103^{cd}	245^{b}	212	171
2-Phenyl ethanol (91)	992de	1,982^{d}	3,172^{c}	1,283^{de}	5,875^{a}	4,798^{b}	753^{e}	1,196^{de}	1,004^{de}	782^{e}	2,183	1,829
Esters (21.7% of total VOC)												
Ethyl acetate (43)	35,379^{a}	2,243^{d}	11,658^{b}	5,168^{c}	1,385^{d}	875^{d}	1,395^{d}	2^{d}	656^{d}	180^{d}	5,894	10,939
Ethyl butanoate (71)	50^{e}	9^{e}	56^{e}	777^{b}	262de	204de	1267^{a}	<1^{e}	347^{cd}	554^{bc}	347	523
Ethyl hexanoate (88)	122^{b}	50^{b}	282^{b}	250^{b}	1220^{b}	1543^{b}	23610^{a}	3660^{b}	102^{b}	1779^{b}	2894	7309
Isobutyl acetate (43)	3,329^{a}	2,515^{b}	2,520^{b}	147^{d}	50^{d}	24^{d}	675^{c}	239^{cd}	11^{d}	5^{d}	951	1,301
Isoamyl acetate (43)	13,745^{b}	18,945^{a}	20,271^{a}	2,793^{d}	888de	572de	7,411^{c}	1,010^{de}	188^{e}	151^{e}	6597	8,086
Isoamyl propionate (57)	310^{c}	942^{a}	192^{cd}	106^{def}	49^{def}	40^{ef}	138de	619^{b}	3^{ef}	<1^{f}	240	309
Isoamyl butanoate (71)	112de	79de	33^{e}	88de	1,231^{c}	1,599^{b}	2,042^{a}	66de	7^{e}	226^{d}	548	769
Isoamyl hexanoate (70)	19^{cd}	10^{d}	10^{d}	15^{cd}	210^{bc}	275^{b}	1,028^{a}	13^{cd}	1^{d}	38^{cd}	162	319
2-Phenylethyl acetate (104)	10,053^{a}	5,352^{bcd}	7,198^{ab}	3,139^{cde}	978^{e}	728^{e}	6,648^{abc}	2,306^{de}	65^{e}	76^{e}	3654	3,478
2-Phenylethyl isobutanoate	20^{b}	86^{b}	122^{b}	291^{b}	4,070^{a}	3,716^{a}	261^{b}	262^{b}	11^{b}	10^{b}	885	1,591
Ketones (9.0% of total VOC)												
Acetone (43)	413^{b}	354^{bc}	437^{b}	184^{bc}	321^{bc}	341^{bc}	195^{bc}	759^{a}	123^{c}	92^{c}	322	195
2-Butanone (43)	609^{b}	107^{b}	205^{b}	87^{b}	284^{b}	212^{b}	33^{b}	12,836^{a}	417^{b}	20^{b}	1,481	3,994
3-Hydroxy 2-butanone (45)	180^{b}	168^{b}	106^{bc}	101^{bc}	119^{bc}	100^{bc}	95^{bc}	106^{bc}	296^{a}	64^{c}	133	67
2-Pentanone (43)	408^{b}	350^{b}	266^{b}	984^{b}	1,119^{b}	1,707^{b}	1,634^{b}	9,440^{a}	1,823^{b}	249^{b}	1,798	2,755
2-Heptanone (43)	476^{d}	1,497^{cd}	640^{d}	3,202^{bc}	3,155^{bc}	4,994^{b}	1,604^{cd}	20,767^{a}	1,593^{cd}	884^{cd}	3872	6,069
2-Nonanone (43)	111^{d}	2,110^{b}	184^{d}	1,702^{bc}	852^{cd}	1,320^{bcd}	703^{cd}	8,410^{a}	166^{d}	576^{cd}	1,613	2,481
Acids (59.7% of total VOC)												
Acetic acid (60)	1,634^{d}	1,468^{d}	1,045^{d}	833^{d}	2,100^{d}	2,406^{bcd}	3,935^{ab}	2,243^{cd}	4,304^{a}	3,658^{abc}	2,383	1,246
2-Methyl propanoic acid (43)	461^{c}	427^{c}	1,022^{bc}	772^{bc}	2,981^{a}	2,801^{a}	748^{bc}	2,525^{a}	1,563^{b}	559^{c}	1,386	1,014
Butanoic acid (60)	10,030^{f}	19,748^{de}	3,498^{g}	18,320^{e}	25,058^{cd}	30,558^{bc}	55,969^{a}	31,392^{b}	20,749^{de}	32,465^{b}	24,726	14,356
3-Methyl butanoic acid (60)	3,122^{d}	4,181^{d}	9,538^{bc}	8,810^{bc}	20,025^{a}	17,947^{a}	4,048^{d}	10,027^{b}	7,955^{bc}	7,158^{c}	9,290	5,663
Hexanoic acid (60)	11,139^{de}	14,582^{d}	2,489^{f}	9,184^{def}	23,087^{bc}	27,656^{b}	50,178^{a}	27,690^{b}	4,560^{ef}	20,950^{c}	19,094	14,141
Octanoic acid (60)	1,171^{b}	1,573^{ab}	234^{b}	1,385^{b}	3,171^{ab}	4,520^{ab}	14,215^{a}	7,758^{ab}	430^{b}	4,998^{ab}	3,928	4,317
Decanoic acid (60)	240^{b}	181^{b}	36^{b}	89^{b}	421^{b}	629^{b}	2,664^{a}	89^{b}	106^{b}	724^{b}	518	791

[a–g]Values with different letters in the same row are statistically different ($P < 0.05$).

[1]Data expressed as arbitrary units ($\times 10^3$) of the peak area of the characteristic ion (Q_{ion}, in parentheses beside each VOC). Each value is the mean of 2 determinations.

[2]LAB = lactic acid bacteria.

likely that aldehydes were reduced to the correspondent alcohols.

Alcohols represented 9.5% of all VOC, the majority being primary alcohols. Ethanol was the only linear-chain alcohol detected. It was the component with the largest fluctuations (CV: 117%) among this chemical group. Ethanol is formed during lactose fermentation by starter bacteria and yeasts, and it is reported to have a role as a precursor of ethyl esters (Corrëa Lelles Nogueira et al., 2005). Two methyl alcohols were identified, 2-methyl-1-propanol and 3-methyl-1-buthanol, with the latter being the most abundant, in agreement with results reported in literature for blue cheeses (Gallois and Langlois, 1990; Moio et al., 2000). These branched-chain alcohols are formed by reduction of the corresponding aldehydes, which derive from the metabolism of specific amino acids (Corrëa Lelles Nogueira et al., 2005). A minor component was 2-heptanol, a secondary alcohol, formed through enzymatic reduction of methyl ketones by *P. roqueforti* metabolism (Cantor et al., 2004). We did not find 2-alkanols from 2-propanol to 2-nonanol, typical components of the flavor of blue cheeses (Engels et al., 1997). All samples of Strachitunt showed a distinctive abundance of 2-phenyl ethanol (2.2×10^6 arbitrary units). This aromatic alcohol formed by the metabolism of amino acids essentially by yeasts is reported as an important odor compound in blue cheeses (Gallois and Langlois, 1990). Moio et al. (2000) reported that alcohols in Gorgonzola represented more than 30% of the neutral volatiles, a value similar to that measured for Strachitunt (25%). However, in our study a different profile of alcohols was observed.

A prominent group of volatiles in Strachitunt consisted of esters. Among the 10 compounds identified, the major ones (82.3%) were both methyl and ethyl esters of free fatty acids. The occurrence and variety of ethyl esters in Strachitunt could be related to the abundant presence of the precursor molecule, ethanol. The ester profile of Strachitunt was also characterized by 2-phenyl-ethyl acetate and 2-phenyl-ethyl isobutanoate. In this case, their presence was related to that of the aromatic alcohol 2-phenyl ethanol. Esters, accounting for 53.8% of neutral volatiles in Strachitunt, provide fruity notes and likely could be responsible for the aromatic note of Strachitunt. A large amount of esters was revealed also in Roquefort, but in Gorgonzola and other blue-veined cheeses, esters represented only a minor fraction (Gallois and Langlois, 1990; Moio et al., 2000; Wolf et al., 2011).

Ketones consisted of 6 components and represented 9.0% of Strachitunt VOC (Table 2), but this percentage was exactly halved when cheese sample H was not taken into consideration, resulting as outlier for most ketones for Grubbs' test ($P < 0.05$). In this last case, the GC-MS total peak area of this chemical group ranged from 2.1% (sample A) to 8.8% (sample I) of all volatiles. Ketones are known as the most abundant volatiles in blue-veined cheeses (Fox et al., 2000). Their production is affected by the physiological state of mycelium, pH, salt content, and concentration of fatty acids (Wolf et al., 2011). Gkatzionis et al. (2009) reported the predominance of ketones and alcohols in Stilton's aroma profile. These authors, in a stratigraphic study, found the highest amount of volatiles (mainly ketones) in the blue part, while the proportion of alcohols in the white core of Stilton was 3 times higher than that in the volatile profile of the outer crust. The ketones more abundant in Strachitunt were 2-alkanones (methyl ketones) with an odd number of carbon atoms (from C_5 to C_9). In particular, the area of 2-heptanone was 42.0% of all ketones in accordance with the predominance of this compound reported in blue cheeses (Fox et al., 2000). Both 2-butanone and acetone were present in similarly low amounts (219×10^3 and 273×10^3 arbitrary units, respectively) when sample H was excluded. Acetoin was the least abundant compound (133×10^3 arbitrary units) among ketones. Methyl ketones in Strachitunt represented the minor contributors (22.1%) among neutral volatile constituents with the exception of aldehydes, probably because of their conversion to secondary alcohols. This percentage further decreased (12.2%) when cheese sample H was not considered. As a comparison, this chemical group represented 50 to 70% of neutral volatiles in French blue cheeses such as Roquefort, Bleu des Causses, and Bleu d'Auvergne and in Gorgonzola (Gallois and Langlois, 1990; Moio et al., 2000).

Generally, extensive lipolytic phenomena characterize blue cheeses. In Strachitunt acids accounted for 59.8% of VOC. We observed large variations among samples, with peak areas ranging from 27×10^6 to 132×10^6 arbitrary units (samples A and G, respectively). Among the 7 identified acids, butanoic and hexanoic acids were the most abundant. Meanwhile, we measured lower amounts of acetic, octanoic, and decanoic acids. In addition to n-acids, we also found branched-chain acids (2-methyl propanoic acid and 3-methyl butanoic acid) in high amounts, deriving from amino acid metabolism (valine and leucine, respectively). In comparison with Gorgonzola (Moio et al., 2000), Strachitunt showed a high relative level of butanoic acid and 3-methyl-butanoic acid, while octanoic acid and decanoic acid were present in low amounts.

In summary, the differences in the VOC composition of the 10 samples of Strachitunt were likely attributable to the uneven growth and development of the adventitious microflora expected in an artisanal environment (Williams and Withers, 2010). Molds are known as the

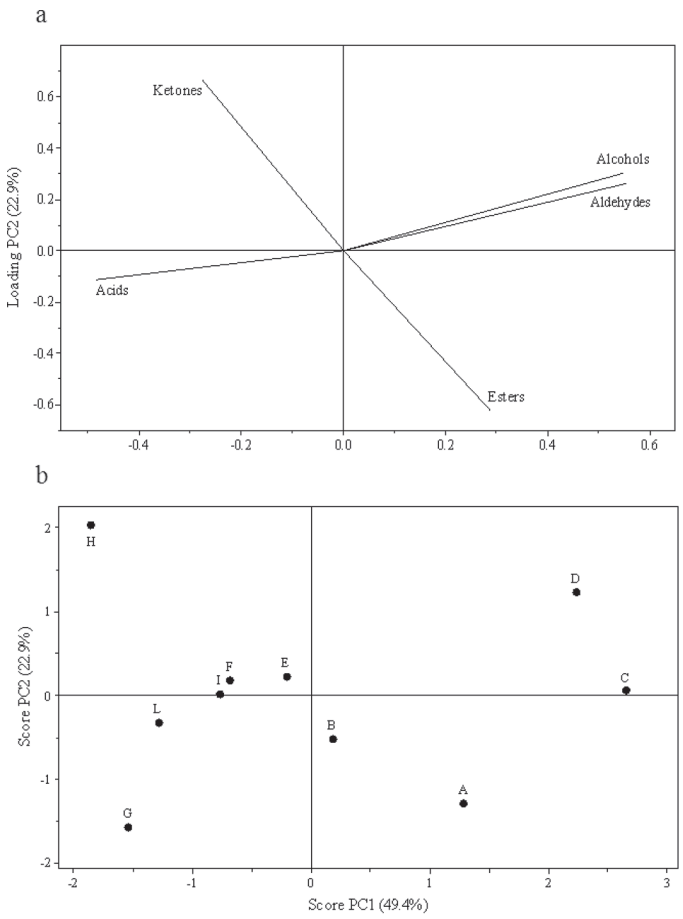

Figure 3. Loading (a) and score (b) plots of the principal components (PC) 1 and 2 describing the variations among chemical classes of volatile organic compounds and among Strachitunt cheese samples, respectively. Samples A to H are starter-free samples; samples I and L were made with lactic acid bacteria starter inoculation.

major source of volatiles in blue-veined cheeses (Fox et al., 2000). The VOC balance differed from that of the other known Italian blue-veined cheeses such as Gorgonzola. This latter cheese has a typical piquant note promoted by the prevailing presence of methylketones (Moio et al., 2000), while esters and alcohols (responsible for a mild flavor) prevailed in Strachitunt. Among these latter components, branched-chain and aromatic compounds deriving from amino acid catabolism were the most relevant. These data suggested that metabolic pathways other than lipolysis contributed to VOC formation. We noticed that cheese samples I and L obtained by natural starter inoculation were less rich in volatiles than samples A to H, although the differences were not significant ($P > 0.05$).

We adopted PCA as a means to visualize any relation among VOC data. We included 10 cheese samples and 5 chemical classes of VOC, selected as input variables, in a correlation matrix. Three PC cumulatively explained 86.1% of the total variability in the volatile profile of the analyzed samples. Based on scree plots (eigenvalue > 1), the first 2 PC, explaining 72.3% of the overall variance (Figure 3), were adopted. The loading vectors in the PC1 vs. PC2 cartesian diagram ran in all the 4 quadrants of the plot (Figure 3a). The opposite curves suggested that the corresponding variables were negatively correlated. An exception was represented by alcohols and aldehydes with high positive loadings (>0.5) lying close to each other, in this way being positively correlated. On the opposite side, acids prevailed for negative loading. In relation to PC2, esters and ketones showed the major absolute loadings $> |0.6|$. The distance among cheese samples in the score plot was proportional to their relationship. We observed a scattered distribution of cheese samples in the score plot (Figure 3b). Principal component 1 distinguished samples C and D for their high levels of alcohols and aldehydes and sample A for large amounts of esters. Principal component 2 differentiated sample G for acid content and sample H for the high level of ketones. No clear distinction was possible between cheese samples A to H versus I and L. In summary, multivariate analysis showed a large variability of the VOC profile in Strachitunt samples.

CONCLUSIONS

Physico-chemical properties and the proteolytic and volatile profiles of Italian PDO Strachitunt differed from those typical for the other blue-veined cheeses. The analytical profile of Strachitunt differed for the acid pH of the paste, the reduced hydrolysis of CN fractions, and the abundance of esters and alcohols among VOC. The variable extent of biochemical phenomena occurring during ripening of blue-veined cheeses and resulting from the heterogeneity in the distribution of blue and white zones of the matrix was also inferred from the large variability of results in Strachitunt. In particular, the scarce standardization was attributed to the erratic adventitious contamination by mold spores of the cheese milk, the dual-curd method of cheesemaking, and the large time variability between the piercing time and the end of ripening. Our results represent an added value and a contribution for an extended comprehension of this variety of cheeses.

REFERENCES

Andrews, A. T. 1983. Proteinases in normal bovine milk and their action on caseins. J. Dairy Res. 50:45–55.

Belotti, E., M. Brasca, and R. Lodi. 2003. Un formaggio tradizionale delle valli bergamasche: lo strachitunt. Sci. Tecn. Latt. Cas. 54:391–416.

Bertolino, M., P. Dolci, M. Giordano, L. Rolle, and G. Zeppa. 2011. Evolution of chemico-physical characteristics during manufacture

and ripening of Castelmagno PDO cheese in wintertime. Food Chem. 129:1001–1011.
Cantor, M. D., T. Van den Temple, T. K. Hansen, and Y. Ardö. 2004. Blue cheese. Pages 175–178 in Cheese: Chemistry, Physics and Microbiology. Vol. 1. P. F. Fox, P. L. H. McSweeney, T. M. Cogan, and T. P. Guinee, ed. Elsevier, London, UK.
Commission Regulation (EC). 1996. No 1107/96 of 12 June on the registration of geographical indications and designations of origin under the procedure laid down in Article 17 of Council Regulation (EEC) No 2081/92. Off. J. Eur. Union L148:1–10.
Cornelli, U., G. Bondiolotti, G. Battelli, G. Zanoni, A. Finco, and M. Recchia. 2015. Activity of 30 different cheeses on cholesterol plasma levels and OBRI (Oxidative Balance Risk Index) in a rat model. Int. J. Food Sci. Nutr. 66:383–390.
Corrëa Lelles Nogueira, M. C. L., G. Lubachevsky, and S. A. Rankin. 2005. A study of the volatile composition of Minas cheese. Lebensm. Wiss. Technol. 38:555–563.
Diezhandino, I., D. Fernández, L. González, P. L. H. McSweeney, and J. M. Fresno. 2015. Microbiological, physico-chemical and proteolytic changes in a Spanish blue cheese during ripening (Valdeón cheese). Food Chem. 168:134–141.
Engels, W. J. M., R. Dekker, C. de Jong, R. Neeter, and S. A. Visser. 1997. A comparative study of volatile compounds in the water-soluble fraction of various types of ripened cheese. Int. Dairy J. 7:255–263.
European Commission. 2014. Commission Implementing Regulation (EU) No 244/2014 of 7 March entering a name in the register of protected designations of origin and protected geographical indications (Strachitunt PDO). Off. J. Eur. Union L 74:31–32.
European Commission. 2016. Publication of an application pursuant to Article 50(2)(a) of Regulation (EU) No 1151/2012 of the European Parliament and of the Council on quality schemes for agricultural products and foodstuffs. Off. J. Eur. Commun. C290:5–8.
Fernández-Salguero, J. 2004. Internal mould-ripened cheeses: Characteristics, composition and proteolysis of the main European blue varieties. Ital. J. Food Sci. 16:437–445.
Fox, P. F., T. P. Guinee, T. M. Cogan, and P. L. H. McSweeney, ed. 2000. Fundamentals of Cheese Science. Aspen Publishers Inc., Gaithersburg, MD.
Fox, P. F., P. L. H. McSweeney, T. M. Cogan, and T. P. Guinee, ed. 2004. Cheese Chemistry, Physics and Microbiology. Chapman and Hall, London, UK.
Gallois, A., and D. Langlois. 1990. New results in the volatile odorous compounds of French cheeses. Lait 70:89–106.
Gkatzionis, K., R. S. T. Linforth, and C. E. R. Dodd. 2009. Volatile profile of Stilton cheeses: Differences between zones within a cheese and dairies. Food Chem. 113:506–512.
Gobbetti, M., R. Burzigotti, E. Smacchi, A. Corsetti, and M. De Angelis. 1997. Microbiology and biochemistry of Gorgonzola cheese during ripening. Int. Dairy J. 7:519–529.
Hayaloglu, A. A., E. Y. Brechany, K. C. Deegan, and P. L. H. McSweeney. 2008. Characterization of the chemistry, biochemistry and volatile profile of Kuflu cheese, a mould-ripened variety. LWT-Food Sci. Technol. 41:1323–1334.
ISO (International Organization for Standardization). 2004a. Cheese and processed cheese—Determination of the total solids content (Reference method). ISO 5534. ISO, Geneva, Switzerland.
ISO (International Organization for Standardization). 2004b. Cheese and processed cheese—Determination of fat content—Gravimetric method (Reference method). ISO 1735. ISO, Geneva, Switzerland.
ISO (International Organization for Standardization). 2008. Processed cheese products—Determination of nitrogen content and crude protein calculation—Kjeldahl method. ISO 17837. ISO, Geneva, Switzerland.
ISO (International Organization for Standardization). 2011. Cheese and processed cheese—Determination of the nitrogenous fractions. ISO 27871. ISO, Geneva, Switzerland.
ISO (International Organization for Standardization). 2012. Determination of water activity. ISO/TC 34 Foodstuffs. ISO, Geneva, Switzerland.
Lawlor, J. B., C. M. Delahunty, J. Sheehan, and M. G. Wilkinson. 2003. 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.
Moio, L., P. Piombino, and F. Addeo. 2000. Odour-impact compounds of Gorgonzola cheese. J. Dairy Res. 67:273–285.
Preedy, V. R., R. R. Watson, and V. B. Patel, ed. 2013. Handbook of Cheese in Health: Production, Nutrition and Medical Sciences. Academic Publishers, Wageningen, the Netherlands.
Prieto, B., I. Franco, J. M. Fresno, A. Bernardo, and J. Carballo. 2000. Picon Bejes-Tresviso blue cheese: An overall biochemical survey throughout the ripening process. Int. Dairy J. 10:159–167.
Sousa, M. J., Y. Ardö, and P. L. H. McSweeney. 2001. Advances in the study of proteolysis during cheese ripening. Int. Dairy J. 11:327–345.
Veloso, A. C. A., N. Teixeira, A. M. Peres, A. Mendonça, and I. M. P. L. V. O. Ferreira. 2004. Evaluation of cheese authenticity and proteolysis by HPLC and urea–polyacrylamide gel electrophoresis. Food Chem. 87:289–295.
Voigt, D. D., F. Chevalier, M. C. Qian, and A. L. Kelly. 2010. Effect of high-pressure treatment on microbiology, proteolysis, lipolysis and levels of flavour compounds in mature blue-veined cheese. Innov. Food Sci. Emerg. Technol. 11:68–77.
Williams, A. G., and S. E. Withers. 2010. Microbiological characterisation of artisanal farmhouse cheeses manufactured in Scotland. Int. J. Dairy Technol. 63:356–369.
Wolf, I. V., M. C. Perotti, and C. A. Zalazar. 2011. Composition and volatile profiles of commercial Argentinean blue cheeses. J. Sci. Food Agric. 91:385–393.
Zarmpoutis, I. V., P. L. H. McSweeney, and P. F. Fox. 1997. Proteolysis in blue-veined cheeses: An intervarietal study. Ir. J. Agric. Food Res. 36:219–229.