Food Chemistry 129 (2011) 1001–1011

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Evolution of chemico-physical characteristics during manufacture and ripening of Castelmagno PDO cheese in wintertime

Marta Bertolino *, Paola Dolci, Manuela Giordano, Luca Rolle, Giuseppe Zeppa

Dipartimento di Valorizzazione e Protezione delle Risorse Agroforestali, Settore di Microbiologia Agraria e Tecnologie Alimentari, Facoltà di Agraria, Università degli Studi di Torino, Via Leonardo da Vinci 44, Grugliasco, TO, Italy

ARTICLE INFO

Article history:
Received 8 October 2010
Received in revised form 25 March 2011
Accepted 11 May 2011
Available online 15 May 2011

Keywords:
Castelmagno PDO cheese
Chemistry
Proteolysis
Volatile compounds
Texture
Evolution
Ripening

ABSTRACT

Biochemical, volatile and textural profiles during manufacture and ripening were determined in samples of Castelmagno PDO cheese obtained from three different batches in the main artisan cheese plant of Castelmagno PDO production area. At the end of manufacture, samples were characterised by a pH of 6.57% and 52.4% moisture content. The HPLC analysis of organic acids and sugars showed the exhaustion of lactose content, while Urea-PAGE indicated extensive primary proteolysis of both β-casein and α_{s1}-casein. During ripening, cheeses were characterised by high degradation of β-casein and α_{s1}-casein, due to bacterial action. RP-HPLC profiles showed a high production of peptides eluted between 20 and 30 min. In total, 92 volatile compounds were identified in cheese headspace. Texture profiles showed an increase in hardness, gumminess, chewiness and adhesiveness values, as well as a decrease in cohesiveness during ripening.

1. Introduction

Castelmagno PDO cheese is one of the most important Italian hard cheeses and was given the Protected Denomination of Origin label (PDO) in 1996. It takes its name from the homonymous small town in Piedmont (Northwest Italy) where it was originally produced. Currently, the production area is limited to three municipalities (Castelmagno, Pradleves and Monterosso Grana) in the Province of Cuneo in Piedmont. The cheese is produced by six manufacturers (2 industrial and 4 artisanal dairy plants). It is usually made from raw cow milk obtained from two consecutive milkings. The evening milk may be partially skimmed after overnight creaming at 15 °C in shallow and large diameter tanks. Such semi-skimmed milk is mixed at a 1:1 ratio with the whole milk collected during the successive morning milking. A small percentage of ewe or goat's milk may be added to cow's milk, although such practice is not currently in use. Production technology does not allow the use of starter cultures, so acidification is due to indigenous lactic acid bacteria and milk is coagulated with liquid calf rennet at 32–38 °C. The curd is transferred to molds and harvested for at least 18 h for complete whey elimination. Then the curd is left at 10 °C for a period of 2–4 days under the whey obtained from previous cheesemaking. The curd is then milled, dry-salted and strongly pressed. Finally, the cheese is placed in natural caves where ripening takes place at 10–12 °C and 85–90% humidity for at least 60 days. The cheese has a cylindrical shape, measuring 12–20 cm high and 15–25 cm in diameter, and weighing 2–7 kg. *Penicillium* spp., from the environment, occasionally colonises the interior part of the cheese during the final phase of ripening. Due to the presence of this colonisation, the Castelmagno PDO cheese is usually considered a hard blue cheese variety (Gobbetti, 2004; Gobbetti & Di Cagno, 2002; Ottogalli, 2001) but nowadays the cheese is marketed before the appearance of mould.

Although a little is known about the microbiology of Castelmagno PDO cheese (Dolci et al., 2008; Dolci, Alessandria, Rantsiou, Bertolino, & Cocolin, 2010), there are no studies on the technology, gross composition, glycolysis, proteolysis, lipolysis, volatile and textural profiles of this cheese. Therefore, the aim of this research was to determine the biochemical, volatile and textural profiles of Castelmagno PDO cheese. Since its production process consists of 4–5 days, it influences the biochemical pathways that determine the final characteristics of the cheese. As a consequence, it was also necessary to analyse the samples during Castelmagno PDO cheese manufacture and not only during ripening.

2. Materials and methods

2.1. Materials

Samples were taken from three batches of Castelmagno PDO cheese produced in the main artisanal dairy plant in the town of

* Corresponding author. Tel.: +39 0116708705; fax: +39 0116708549.
E-mail address: marta.bertolino@unito.it (M. Bertolino).

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doi:10.1016/j.foodchem.2011.05.060

Castelmagno (Piedmont, Italy) during the wintertime. The three batches were produced on different consecutive days (A, B, C) by using milk from the same farm. The cow milk used in cheese production had a pH of 6.59 ± 0.01, and contained 4.51 ± 0.17% lactose, 3.45 ± 0.07% protein and 3.40 ± 0.32% fat. For each batch, the milk (A1, B1, C1), the curd after the cut (A2, B2, C2), the curd after 24 h (A3, B3, C3), the curd after 3 days under the whey (A4, B4, C4), and the cheese after 3 (A5, B5, C5), 30 (A6, B6, C6), 60 (A7, B7, C7), 90 (A8, B8, C8) and 150 (A9, B9, C9) days of ripening were sampled. Samples were transferred to the laboratory under refrigerated conditions and milk was immediately analysed for pH and gross composition. Cheesemaking samples (A2, B2, C2, A3, B3, C3, A4, B4 and C4) were analysed immediately for pH and an aliquot was also frozen and subsequently used for compositional, glycolysis, proteolysis and volatile analysis. Cheese ripening samples (from A3 to C9) were immediately analysed for pH and texture profile and an aliquot was also frozen and subsequently used for compositional, glycolysis, proteolysis and volatile analysis.

2.2. Methods

2.2.1. Compositional analysis

Milk samples were analysed for lactose, protein and fat content by using a Milko Scan™ FT 120 (Foss, Padova, Italy). Cheese samples during manufacturing and ripening were analysed for: moisture by the oven drying method at 102 °C (IDF, 1982), salt by titration with $AgNO_3$ (IDF, 1988), total protein and pH 4.6-soluble nitrogen by Kjeldhal method (IDF, 1993), and fat by the FIL-IDF Standard 5A method (1969). The pH was determined with a Portamess 913 pHmeter (Knick, Berlin, Germany), placing the penetration electrode in contact with the sample mass. All analyses were performed in triplicate.

2.2.2. Assessment of proteolysis

The pH 4.6-insoluble and -soluble extracts were prepared according to the method of Kuchroo and Fox (1982), which was slightly modified, as outlined by Hayaloglu, Guven, Fox, Hannon and McSweeney (2004). Urea-polyacrylamide gel electrophoresis (Urea-PAGE) was performed on the insoluble fraction, using a Protean II xi vertical slab-gel unit (Bio-Rad Laboratories Ltd., Watford, UK) according to the method of Shalabi and Fox (1987). The gels were stained directly with Coomassie Brilliant Blu G-250, using the method of Blakesley and Boezi (1977) and destained using distilled water. After destaining, gel slabs were digitised by a scanner (Epson Perfection 1650, Seiko Epson Corporation, Nagano, Japan). Scans of the electrophoretograms were used to quantify bands, using densitometric software (Image Master TotalLab 1D Gel analysis v 1.11 software, Nonlinear Dynamics Ltd., Newcastle-upon-Tyne, UK). Similar bands were recognised visually, as described by McSweeney, Poochet, Fox and Healy (2004) and peak volumes of corresponding bands were quantitatively determined.

Peptides of the pH 4.6-soluble fraction of cheeses were determined by RP-HPLC, using the method described by Hayaloglu, Guven, Fox, Hannon, and McSweeney (2004), utilising an HPLC system (Thermo Electron Corporation, Waltham, MA, USA) equipped with an isocratic pump (P1000), and a multiple autosampler (AS3000) fitted with a 20 μl loop and a UV detector (UV100) set at 214 nm.

Individual free amino acids (FAA) of the pH 4.6-soluble fractions of cheeses were prepared and analysed according to the method of Bertolino, Zeppa, Gerbi, and McSweeney (2008).

2.2.3. Assessment of organic acid, sugars, diacetyl and acetoin

Organic acids (citric, orotic, pyruvic, lactic, oxalic, hippuric, isobutyric, valeric and isovaleric), sugars (lactose, glucose and galactose), diacetyl and acetoin were determined by high performance liquid chromatography according to the method of Zeppa and Rolle (2008). Five grams of sample were added to 25 ml of 0.013 N H_2SO_4 (mobile phase) and homogenised for 10 min with a Stomacher blender (PBI, Milano, Italy). The extract was subsequently centrifuged for 5 min at 2500*g* and the supernatant was filtered through a PTFE 0.20 μm disposable syringe membrane filter (Sartorius AG, Göttingen, Germany). The HPLC system (Thermo Electron Corporation, Waltham, MA, USA) was equipped with an isocratic pump (P1000), a multiple autosampler (AS3000) fitted with a 20 μl loop, a UV detector (UV100) set at 210 and 290 nm and a refractive index detector (RI-150). The analyses were performed isocratically, at 0.8 ml/min and 65 °C, with a 300 × 7.8 mm i.d. cation exchange column (Aminex HPX-87H) equipped with a Cation H^+ Microguard cartridge (Bio-Rad Laboratories, Hercules, CA, USA). Three replicates for each sample were analysed. The data treatments were carried out using the ChromQuestTM chromatography data system (ThermoQuest, Inc., San Jose, CA, USA). Analytical grade reagents were used as standards (Sigma–Aldrich Corporation, Milan, Italy).

2.2.4. Volatile compounds analysis

Grated homogenised sample (5 g) was placed in a 40 ml vial fitted with a PTFE silicone septum (Supelco, Bellefonte, PA, USA), through which the SPME syringe needle, fitted with a Stable Flex 2 cm–50/30 μm divinylbenzene-carboxen-polydimethylsiloxane (DVB-CAR-PDMS) fibre (Supelco, Bellefonte, PA, USA), was introduced. The internal standard was methyl nonaoate (Sigma–Aldrich) at a final concentration of 80.4 μg/kg in the sample (Katechaki, Panas, Rapti, Kandilogiannalis, & Koutinas, 2008). The vial was placed in a heat/stir plate at 80 °C for 35 min for the absorption phase. After exposure in the headspace (HS), the fibre with the analytes was retracted and transferred to the injector, which was operated in the splitless mode at a temperature of 280 °C for 4 min. Compound identification was achieved with a Shimadzu GC-17A gas chromatograph (GC) coupled with a Shimadzu QP5000 quadrupole mass spectrometer (Shimadzu Corporation, Kyoto, Japan). The GC was equipped with a DB-wax column (30 m, 0.25 mm i.d., and 0.25 μm film thickness) and a split/splitless injector. The carrier gas was ultrahigh purity (99.999%) helium with a flow rate of 1 ml/min. The following column temperature programming sequence was used: an initial temperature of 35 °C for 3 min, increased to 110 °C at a rate of 5 °C/min, increased to 240 °C at a rate of 10 °C/min and a final extension at 240 °C for 10 min. Mass spectra were recovered in the electron impact mode at an ionisation voltage of 70 eV. The ion source and the interface were maintained at 250 °C. Identification was achieved by comparison to standard compounds where available, and/or using the NIST 12 and the NIST 62 data base (National Institute of Standards and Technology, Gaithersburg MD, USA).

2.2.5. Texture analysis

For texture analysis, samples were cut with a thin blade into 20 mm squared cubes and immediately analysed. The TPA test was carried out using a Universal Testing Machines (UTM) TA-XT2i Texture Analyzer® (Stable Micro System, UK) equipped with a 25 kg loadcell and HDP/90 platform. Samples were compacted in height to 30% of the original using a crosshead speed of 0.8 mm/s and a P-35 DIA cylinder stainless flat probe (Blazquez et al., 2006; Kapoor, Metzger, Biswas, & Muthukummarappan, 2006). Each sample was subjected to a two-cycle compression with 5 s between cycles (Drake, Gerard, Truong, & Daubert, 1999). For the acquisition of the force–time curve, a Texture Export Exceed software rel. 2.54 (Stable Micro Systems, Godalming, UK) was used. According to Gunesakaran and Mehemet Ak (2003), the following parameters were measured from the force–time curves (Fig. 1): hardness (N, as F_1 maximum force), cohesiveness (adimensional, as $(A_2)/(A_1 + A_{1W})$), adhesiveness (mJ, as A_3), gumminess (N, as hardness × cohesiveness), springiness (mm, as d_2), chewiness (mJ, as gumminess × springiness) and resilience (adimensional, as (A_{1w}/A_1)). For each batch and point of ripening, five analyses were performed.

2.2.6. Statistical analysis

The distribution and the differences in the compositional parameters, organic acids, sugars, diacetyl, acetoin, free amino acids, and textural parameters of Castelmagno PDO samples were analysed using Brown–Forsythe test of homogeneity of variance, ANOVA and the Duncan mean comparison test, respectively, to underline the normal distribution of the data and differences during the manufacture and ripening of cheeses. Calculation was performed by Statistica 7.0 Software (Statsoft, Tulsa, USA).

3. Results and discussion

3.1. Compositional analysis

The averages of pH, moisture, salt, fat, protein and pH 4.6-soluble nitrogen contents of Castelmagno PDO samples during manufacturing and after 3, 30, 60, 90, 150 days of ripening are shown in Table 1. The pH of cheeses was between 6.57 and 4.71 during manufacturing and between 4.71 and 5.02 during ripening, due to the microbial ecosystem evolution, as reported by Dolci et al. (2010). The pH average value during Castelmagno PDO market life (after 60 days of ripening), was 4.94 lower than that reported by Gobbetti and Di Cagno (2002). According to moisture data after 60 days of ripening (period after which the cheese can be sold), Castelmagno PDO cheese can be categorised as a hard cheese with an average value of 35.5% (McSweeney, Ottogalli & Fox, 2004). After 3 days under the whey, the curd is ground, pressed and formed with a high loss of whey. As a consequence, samples at 3 days of ripening showed a large decrease in moisture content.

Low salt levels found during Castelmagno PDO manufacturing (0.75%) were due to the fact that it is a dry-salted cheese and salt is added during the curd grinding at the end of cheesemaking. During ripening, salt levels increased to an average value of 2.46%, which was in line with data reported by Delforno (1960).

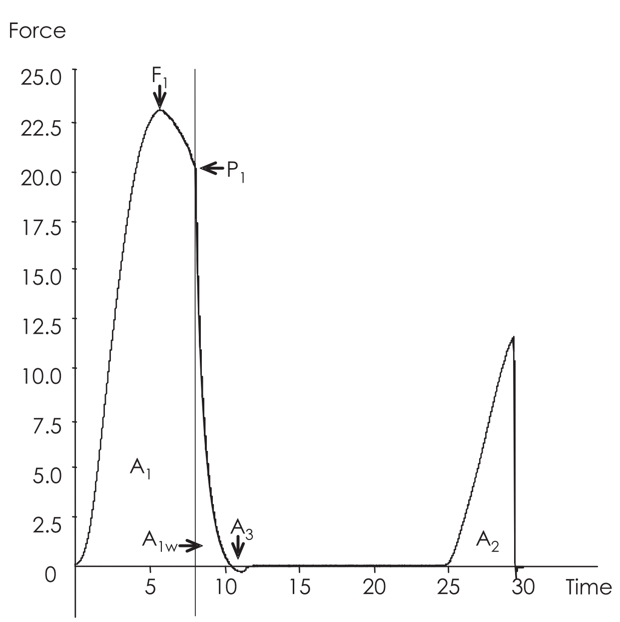

Fig. 1. Example of TPA profile of Castelmagno PDO cheese (N, mJ or non-dimensional).

Fat content increased from an average value of 23.2% during the manufacture period to an average of 31.1% after 60 days. The second one was lower than that reported by Gobbetti (2004) and Merlo (2001) but within the range reported by Delforno (1960).

Protein content increased from an average value of 19.3% during manufacture to an average of 26.6% after 60 days, whilst the pH 4.6-soluble nitrogen rose from 7.13% to 13.7% as a consequence of the decrease of moisture. Protein content during market life was higher than that reported by Gobbetti (2004).

3.2. Assessment of proteolysis

The data for pH 4.6-SN level in Castelmagno PDO samples are shown in Table 1. During cheese manufacturing, pH 4.6-SN decreased as a consequence of its use as a nutritional requirement by LAB (Monnet, Condon, Cogan & Gripon, 1996) and due to its diffusion in whey as a consequence of the attainment of equilibrium of soluble constituents into two solutions, as reported for the cheese ripening in brine (Abd El-Salam and Alichanidis, 2004). Contrarily, pH 4.6-SN increased during the ripening period, due to the breakdown of casein into peptides and amino acids by the action of chymosin, plasmin, and bacteria.

Urea-PAGE electrophoretograms of the pH 4.6-insoluble fractions of Castelmagno PDO cheese (batch A) during manufacture and ripening are shown in Fig. 2. Bands in the electrophoretograms and the densitometric analysis (data not reported) showed that the degradation of β- and α_{s1}-caseins occurs early, during cheese manufacturing, due to the rennet and plasmin activity effects. However it can be seen that neither β- nor α_{s1}-casein were totally degraded at the end of ripening but α_{s1}-casein hydrolysis rate was greater than that of β-casein during all stages of ripening. As a consequence of α_{s1}-casein hydrolysis, in all electrophoretogram samples, the band corresponding to α_{s1}-I-casein (α_{s1}–CN f24–199), which is the first product of rennet action on α_{s1}-casein, was present; from the third day of ripening, other bands, corresponding to other peptides, appeared (marked as z α_{s1}-CN) these are characterised by faster mobilities than α_{s1}-I-casein (α_{s1}-CN f24–199) which are degradation products of α_{s1}-casein due to rennet and indigenous milk proteinases' action. From the thirtieth day of ripening, cheeses showed a band corresponding to the peptide α_{s1}-I-casein (α_{s1}-CN f102–199). Among γ-caseins (the polypeptides produced by the action of plasmin on β-caseins), the γ_2-casein [β-casein (f106–209)] was present at the highest concentration, followed by γ_3- [β-casein (f108–209)] and γ_{1-} [β-casein (f29–209)] caseins. The RP-HPLC profiles of the pH 4.6-soluble fractions of Castelmagno PDO cheese (batch A) are shown in Fig. 3. To compare the chromatographic data obtained by RP-HPLCs, visual identifications of similar peaks were evaluated. Common peaks were evident in the region 5–8 min in the chromatograms of all, with an increase in concentration during ripening. Similar peptides eluted with retention times of 11–18 and 24–29 min were observed in samples 24 h after manufacturing until the 150th day of ripening, with increased concentration during ripening. All of these regions were composed principally of amino acids and hydrophilic peptides (Gonzales del Llano, Polo, & Ramos, 1995; Pavia, Trujillo, Guamis, & Ferragut, 2000). However, qualitative and quantitative differences were observed in the region included between 30 and 50 min, considered to be composed mainly of hydrophobic peptides (Gonzalez del Llano et al., 1995; Pavia et al., 2000). Cheese proteolysis was also monitored by determining the levels of individual free amino acids (FAAs). These data are listed in Table 2. Overall, the total concentration of FAAs increased considerably from the end of manufacture (14.8 ± 4.89 mg/g of cheese) to the end of ripening 98.6 ± 14.1 mg/g of cheese. Glutamic acid, valine, leucine, phenylalanine and lysine were the FAAs characterised by the highest concentration during cheese manufacturing whilst the most common

Table 1
Mean values ± standard deviation for the gross composition of Castelmagno PDO cheese during its production and its ripening and result of variance analysis.

	Manufacture of cheese			Days of ripening					
	Cut of the curd	After 24 h	Curd after 3 days under whey	3	30	60	90	150	Statistical significance
pH	6.57 ± 0.01[a]	5.06 ± 0.02[a]	4.71 ± 0.07[a]	4.74 ± 0.02[b]	4.71 ± 0.08[b]	4.80 ± 0.11[c]	5.02 ± 0.10[c]	4.99 ± 0.05[c]	***
Moisture (% w/w):	54.6 ± 1.35[a]	53.4 ± 1.34[b]	52.4 ± 0.34[b]	45.5 ± 0.65[c]	40.1 ± 0.99[d]	37.7 ± 0.79[e]	35.2 ± 0.31[f]	33.5 ± 0.82[g]	***
NaCl (% w/w):	0.08 ± 0.01[a]	0.07 ± 0.03[a]	0.08 ± 0.03[a]	2.14 ± 0.24[b]	2.50 ± 0.40[b,c,d]	2.75 ± 0.54[c,d]	2.24 ± 0.35[b,c]	2.69 ± 0.14[c,d]	***
Fat (% w/w):	22.5 ± 0.44[a]	23.9 ± 0.21[b]	23.3 ± 0.46[b]	25.8 ± 0,76[c]	29.0 ± 1.03[d]	29.9 ± 1.09[d]	31.4 ± 0.57[e]	31.9 ± 0.79[e]	***
Protein (% w/w):	18.4 ± 0.07[a]	19.5 ± 0.20[b]	20.0 ± 0.08[b]	20.9 ± 0.02[c]	23.3 ± 0.02[d]	26.0 ± 0.08[e]	27.1 ± 0.09[e,f]	26.7 ± 0.01[e,f]	***
pH 4.6-solubleN (% total N)	8.09 ± 0.15[a]	7.72 ± 0.90[a]	5.59 ± 0.19[b]	5.53 ± 0.30[b]	7.85 ± 0.22[c]	9.72 ± 0.10[d]	12.4 ± 0.24[e]	15.0 ± 0.28[f]	***

Mean data for the three batches of Castelmagno PDO cheeses analysed in triplicate.
a, b, c, d, e, f: Different letters in the same row indicate significant statistical differences (Duncan Test, $p < 0.05$).
ns = not significant.
*$P < 0.05$.
**$P < 0.01$.
*** $P < 0.001$.

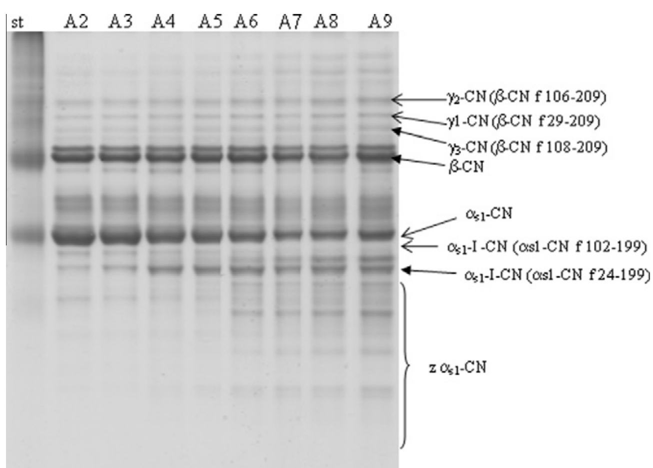

Fig. 2. Urea-polyacrylamide gel electrophoretrograms of Castelmagno PDO cheese (batch A) during its production (A2 = cut of curd; A3 = after 24 h; A4 = curd after 3 days under whey) and its ripening (A5 = 3 days of ripening; A6 = 30 days of ripening; A7 = 60 days of ripening; A8 = 90 days of ripening; A9 = 150 days of ripening). Lane st = Na-caseinate.

FAAs during ripening were aspartic acid, glutamic acid, valine, leucine and phenialanine. Most of these amino acids were previously found to be present at high concentration in several hard or extra-hard Italian cheese varieties (Albenzio et al., 2001; Gobbetti, 2004; Resmini, Pellegrino, Hogenboom, & Bertuccioli, 1988).

3.3. Assessment of organic acids, sugars, diacetyl and acetoin composition

Organic acids, sugars, diacetyl and acetoin concentrations of Castelmagno PDO samples are listed in Table 3.

Lactose metabolism was totally complete at the end of manufacture but already, after just 24 h, most of the lactose had been converted into lactate by the growth of starter bacteria or by its loss into whey, as reported by McSweeney (2004). Glucose and galactose were also present at very low concentrations (0.01 ± 0.01 mg/g of cheese and 0.11 ± 0.02 mg/g of cheese, respectively) and they were already absent in curd after 3 days under the whey samples and 3-day-old cheeses, respectively, due to their use by lactic acid bacteria (LAB) and non-starter lactic acid bacteria (NSLAB) as substrates of growth (Michel & Martley, 2001). During ripening, lactic acid was the main organic acid in all samples,

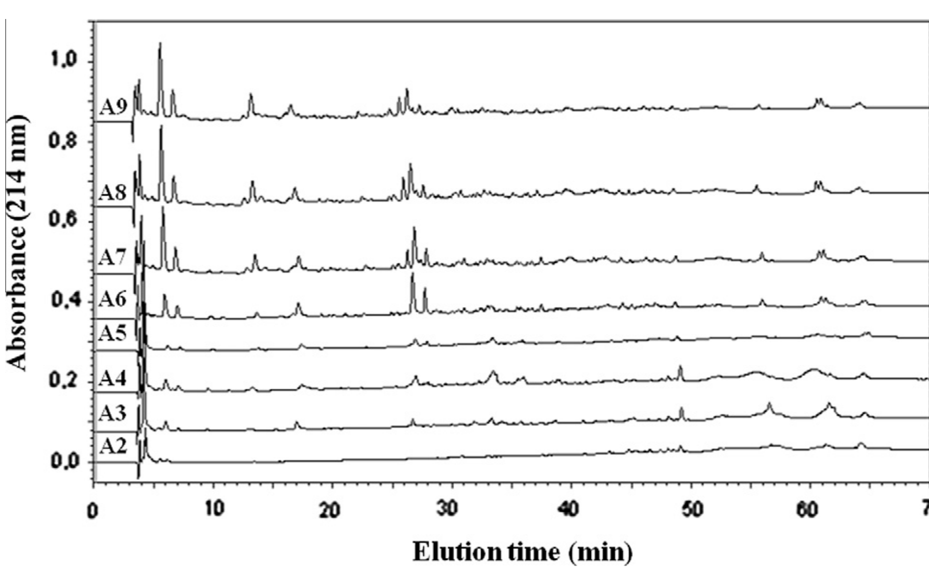

Fig. 3. RP-HPLC chromatograms of the pH 4.6-soluble fraction of Castemagno PDO cheese (batch A) during the manufacture (A2 = cut of curd; A3 = after 24 h; A4 = curd after 3 days under whey) and its ripening (A5 = 3 days of ripening; A6 = 30 days of ripening; A7 = 60 days of ripening; A8 = 90 days of ripening; A9 = 150 days of ripening).

Table 2
Mean values ± standard deviation of free amino acids composition of Castelmagno PDO cheese (mg/g) during its manufacture and ripening.

	Manufacture of cheese			Days of ripening					
	Cut of the curd	After 24 h	Curd after 3 days under whey	3	30	60	90	150	Statistical significance
Aspartic acid	0.07 ± 0.06[a]	0.29 ± 0.05[a]	0.65 ± 0.19[a]	0.74 ± 0.03[a]	2.05 ± 0.55[b]	4.45 ± 0.25[c]	5.24 ± 0.47[c]	7.98 ± 0.30[d]	***
Threonine	0.02 ± 0.01[a]	0.15 ± 0.03[a]	0.44 ± 0.12[a,b]	0.55 ± 0.02[a,b]	0.85 ± 0.20[b]	1.70 ± 0.29[c]	1.90 ± 0.43[c]	2.67 ± 0.22[d]	***
Serine	0.02 ± 0.01	0.14 ± 0.05	0.38 ± 0.19	0.45 ± 0.18	0.58 ± 0.22	1.00 ± 0.56	1.19 ± 0.51	1.65 ± 0.72	ns
Glutamic acid	0.45 ± 0.26[a]	1.55 ± 0.14[a,b]	2.69 ± 1.10[a,b]	3.80 ± 0.09[a,b]	4.02 ± 1.14[a,b,c]	7.94 ± 1.63[b,c]	6.74 ± 1.16[c,d]	11.4 ± 1.96[d]	***
Glycine	0.04 ± 0.03[a]	0.04 ± 0.02[a]	0.12 ± 0.03[a,b]	0.32 ± 0.02[a,b]	0.57 ± 0.11[b]	1.29 ± 0.24[c]	1.58 ± 0.01[c]	2.66 ± 0.51[d]	***
Alanine	0.03 ± 0.01[a]	0.22 ± 0.01[a]	0.87 ± 0.18[a]	1.20 ± 0.02[a]	1.56 ± 0.24[a,b]	2.74 ± 0.15[b]	3.01 ± 0.09[b]	5.66 ± 1.10[c]	***
Cysteine	0.04 ± 0.02[a]	0.06 ± 0.02[a]	0.14 ± 0.07[a]	0.32 ± 0.03[a,b]	0.44 ± 0.01[a,b]	0.63 ± 0.04[b,c]	0.91 ± 0.08[c,d]	1.29 ± 0.66[d]	***
Valine	0.16 ± 0.05[a]	0.50 ± 0.04[a]	1.02 ± 0.28[a,b]	1.76 ± 0.09[a,b]	2.54 ± 0.09[b]	4.83 ± 0.05[c]	6.28 ± 0.04[c]	9.78 ± 2.54[d]	***
Methionine	0.02 ± 0.02[a]	0.13 ± 0.02[a]	0.27 ± 0.09[a]	0.80 ± 0.06[a]	1.43 ± 0.22[b]	2.91 ± 0.53[c]	3.47 ± 0.21[d]	4.89 ± 0.20[e]	***
Isoleucine	0.01 ± 0.02[a]	0.19 ± 0.06[a]	0.40 ± 0.14[a]	0.70 ± 0.04[a]	1.00 ± 0.05[a,b]	2.28 ± 0.30[b,c]	3.37 ± 0.07[c]	5.46 ± 1.45[d]	***
Leucine	0.12 ± 0.08[a]	0.70 ± 0.12[a,b]	2.09 ± 0.54[a,b]	4.61 ± 2.81[b]	7.50 ± 1.36[c]	13.6 ± 1.81[d]	16.0 ± 1.11[e]	20.2 ± 0.33[f]	***
Tyrosine	0.10 ± 0.06[a]	0.42 ± 0.12[a,b]	0.65 ± 0.27[b]	0.58 ± 0.05[a,b]	0.80 ± 0.28[b]	1.33 ± 0.09[c]	1.55 ± 0.10[c]	1.50 ± 0.07[c]	***
Phenylalanine	0.10 ± 0.07[a]	0.71 ± 0.14[a,b]	1.81 ± 0.44[b]	3.35 ± 1.71[b]	4.82 ± 0.42[c]	7.76 ± 0.31[d]	9.26 ± 0.35[e]	11.9 ± 1.84[f]	***
Histidine	0.14 ± 0.07[a]	0.60 ± 0.13[a,b]	1.00 ± 0.25[b]	1.82 ± 0.09[b]	2.44 ± 0.08[c]	3.31 ± 0.05[d]	3.57 ± 0.23[d]	4.03 ± 0.83[d]	***
Lysine	0.13 ± 0.05	0.71 ± 0.38	1.15 ± 0.65	1.66 ± 0.56	2.46 ± 0.41	2.49 ± 0.80	4.51 ± 0.03	3.98 ± 0.25	ns
Arginine	0.01 ± 0.01	ND	0.07 ± 0.09	0.02 ± 0.01	0.05 ± 0.11	ND	ND	ND	ns
Proline	0.11 ± 0.14[a]	0.64 ± 0.18[a]	1.03 ± 0.26[a]	1.03 ± 0.28[a]	1.05 ± 0.23[a]	1.19 ± 1.26[a]	1.41 ± 0.01[a]	3.57 ± 1.15[b]	***
Total free amino acids	1.57 ± 0.97	7.05 ± 1.51	14.8 ± 4.89	23.7 ± 6.09	34.2 ± 5.72	59.4 ± 8.36	70.0 ± 4.90	98.6 ± 14.1	

Mean data for the three batches of Castelmagno PDO cheeses analysed in triplicate.
a, b, c, d, e, f: Different letters in the same row indicate significant statistical differences (Duncan Test, $p < 0.05$).
ND: not detected.
ns = not significant.
*$P < 0.05$.
**$P < 0.01$.
*** $P < 0.001$.

representing approximately 95% of the total organic acid content in 3 day-old cheeses and 78% of total organic acid content in 150 day-old cheeses. The mean lactic acid concentration during Castelmagno market life was similar to that observed for Cheddar and Colby cheeses (Mullin & Emmons, 1997) but higher than that already reported for Castelmagno PDO cheese (Dolci et al., 2008; Zeppa & Rolle, 2008). Citric acid was present with the highest concentration (1.32 ± 0.11 mg/g of cheese) in 24 h-old cheeses; it then decreased to 0.03 ± 0.02 mg/g of cheese in 3 day-old cheeses, due to its metabolisation by Cit^+ strains of LAB or NSLABS into acetate, acetoin and diacetyl (McSweeney & Fox, 2004). In particular, all Castelmagno PDO samples demonstrated a higher concentration of diacetyl than acetoin, which can also be derived from the metabolism of pyruvate by NSLAB. The acetic acid concentration increased during manufacture to a final level of 0.81 ± 0.08 mg/g of cheese in samples at 3 days under the whey; it then decreased during the ripening phase. In 150 day-old cheeses, it was found at a concentration of 0.50 ± 0.03 mg/g of cheese. Acetate is produced from lactose, lactic acid or citric acid metabolisms or from the catabolism of amino acids. Many authors have reported that its concentration in different PDO cheeses, such as Cheddar, Camembert, Beaufourt, Canestrato Pugliese, Murazzano, Raschera, Robiola di Roccaverano and Toma Piemontese, ranged from 0.18 to 1.89 mg/g of cheese. (Bouzas, Kantt, Bodyfelt, & Torres, 1991; Faccia, Gambacorta, Lamacchia, & Luccia, 2004; Mullin & Emmons, 1997; Zeppa & Rolle, 2008). The propionic acid concentration increased from manufacture to the end of ripening where it was found to be 1.43 ± 0.14 mg/g of cheese, representing 7.3% of total organic acid content. The propionic acid is produced from lactic acid metabolism by *Propionobacterium* spp., as reported by McSweeney (2004) or from the lypolytic activities of starter and secondary microflora, as reported by Collins, McSweeney, and Wilkinson (2004). Iso-butyric acid was detected only in samples after 90 days of ripening with a mean concentration of 0.68 ± 0.18 mg/g of cheese. Iso-valeric acid concentration increased during Castelmagno PDO production and in the 150 day-old cheeses it was detected at a concentration of 0.69 ± 0.28 mg/g of cheese, representing 3.5% of total organic acid content.

3.4. Volatile compound analysis

Volatile compounds identified in Castelmagno PDO samples by HS-SPME-GC/MS during manufacturing and ripening are shown in Table 4. In total, 92 compounds were detected: 15 acids, 28 esters, 13 ketones, 12 aldehydes, 13 alcohols, 3 lactones, 3 hydrocarbons and 6 compounds which could not be classified in these chemical groups. Acids constituted the main chemical class during manufacturing (mean concentration of 88.6% w/w of total volatile compounds) and during ripening (77.9% w/w of total volatile compounds concentration). Acids can originate from three biochemical pathways: lipolysis, proteolysis and glycolysis (Curioni & Bosset, 2002). During manufacturing, the most abundant acids were acetic, decanoic, dodecanoic, hexanoic and octanoic acids. Acetic acid increased in concentration from the beginning to the end of manufacture and could have a microbial origin as a product of lactose fermentation, due to the growth of lactic and propionic bacteria (McSweeney & Fox, 2004), which are abundant in this cheese, as shown by microbiological data (Dolci et al., 2010). The other acids were derived from the action of esterases and lipases present in raw milk used for Castelmagno PDO cheesemaking. During cheese ripening, the highest acid concentrations were found for acetic, butyric, decanoic, dodecanoic, hexanoic and octanoic acids.

Esters are important common constituents of the volatile fraction of cheese. Different esters have been reported, such as methyl, ethyl, propyl and butyl esters, as a reaction of free fatty acid with ethanol, methanol, propanol and butanol in different cheese varieties (Liu, Holland, & Crow, 2004). Ester formation is correlated with the growth of lactic acid bacteria and *Micrococcaceae* (Gripon, Monnet, Lambert, & Desmazeaud 1991). In Castelmagno PDO samples, ester concentration represented 1.41% of the total volatile compounds concentration during manufacturing and 5.64% during cheese ripening. Ethyl esters were the predominant esters in

Table 3
Mean values ± standard deviation of organic acids, sugars, diacetyl and acetoin concentrations of Castelmagno PDO (mg/g) cheese during its manufacture and ripening.

	Manufacture of cheese			Days of ripening					
	Cut of the curd	After 24 h	Curd after 3 days under whey	3	30	60	90	150	Statistical significante
Lactose	36.7 ± 2.06^{a}	6.13 ± 1.71^{b}	0.72 ± 0.49^{c}	ND	ND	ND	ND	ND	***
Glucose	0.01 ± 0.01^{a}	0.01 ± 0.01^{b}	ND	ND	ND	ND	ND	ND	***
Galactose	0.10 ± 0.01^{a}	0.11 ± 0.02^{b}	0.12 ± 0.03^{b}	0.03 ± 0.01^{c}	ND	ND	ND	ND	***
Lactic acid	0.90 ± 0.90^{a}	20.3 ± 2.68^{b,c}	31.1 ± 3.54^{d}	30.6 ± 2.58^{d}	25.0 ± 2.41^{b}	19.1 ± 0.90^{c}	17.7 ± 2.07^{c}	15.3 ± 1.45^{c}	***
Diacetyl	0.04 ± 0.01^{a}	0.20 ± 0.13^{a}	0.66 ± 0.10^{a,b,c}	0.52 ± 0.08^{a,b}	0.74 ± 0.25^{a,b,c}	1.17 ± 0.39^{b,c,d}	1.39 ± 0.35^{c,d}	1.59 ± 0.20^{d}	***
Acetoin	ND	ND	0.02 ± 0.01^{a}	0.02 ± 0.01^{a}	0.02 ± 0.00^{a}	0.04 ± 0.00^{b}	0.06 ± 0.01^{c}	0.06 ± 0.02^{d}	***
Citric acid	1.24 ± 0.04^{a}	1.32 ± 0.11^{b}	0.30 ± 0.17^{c}	0.03 ± 0.02^{d}	0.03 ± 0.01^{d}	0.04 ± 0.02^{d}	0.07 ± 0.03^{d}	0.09 ± 0.02^{d}	***
Pyruvic acid	0.04 ± 0.02^{a}	0.12 ± 0.06^{a}	0.43 ± 0.17^{b}	0.41 ± 0,11^{b}	0.12 ± 0.04^{a}	0.04 ± 0.03^{a}	0.03 ± 0.02^{a}	0.02 ± 0.01^{a}	***
Formic acid	0.03 ± 0.03	0.04 ± 0.04	0.02 ± 0.01	0.03 ± 0.01	0.02 ± 0.00	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	ns
Acetic acid	ND	0.13 ± 0.05^{a}	0.81 ± 0.08^{b}	0.77 ± 0.06^{c}	0.64 ± 0.02^{d}	0.57 ± 0.05^{d,e}	0.51 ± 0.03^{e}	0.50 ± 0.03^{e}	***
Propionic acid	ND	0.08 ± 0.02^{a}	0.08 ± 0.03^{a}	0.09 ± 0.03^{a}	0.54 ± 0.06^{b}	0.83 ± 0.08^{c}	1.23 ± 0.13^{d}	1.43 ± 0.14^{e}	***
Oxalic acid	0.42 ± 0.05^{a}	0.56 ± 0.07^{b}	0.18 ± 0.09^{c}	0.03 ± 0.01^{d}	ND	ND	0.01 ± 0.01^{d}	0.01 ± 0.01^{d}	***
Orotic acid	0.04 ± 0.02^{a}	0.02 ± 0.01^{b}	0.01 ± 0.01^{c}	0.01 ± 0.01^{c,d}	ND	ND	0.01 ± 0.01^{c,d}	0.01 ± 0.01^{c,d}	***
Iso-butyric acid	ND	ND	ND	ND	ND	ND	0.19 ± 0.28^{a}	1.17 ± 0.09^{b}	***
Butyric acid	0.06 ± 0.05^{b}	0.01 ± 0.01^{a}	0.15 ± 0.02^{c,d}	0.14 ± 0.03^{c,d}	0.11 ± 0.02^{b,c}	0.15 ± 0.01^{c,d}	0.19 ± 0.04^{d}	0.19 ± 0.04^{d}	***
Iso-valeric acid	ND	0.01 ± 0.01^{a}	0.01 ± 0.01^{a}	0.11 ± 0.06^{a}	0.43 ± 0.09^{b}	0.44 ± 0.08^{b}	0.48 ± 0.06^{b}	0.69 ± 0.28^{b}	***
n-Valeric acid	ND	0.01 ± 0.00	0.01 ± 0.00	ND	ND	ND	0.01 ± 0.02	0.03 ± 0.02	ns
Hippuric acid	0.01 ± 0.01	ND	ND	ND	ND	ND	ND	ND	***
Uric acid	0.02 ± 0.00^{a}	0.02 ± 0.00^{a}	0.03 ± 0.00^{b}	ND	ND	ND	0.04 ± 0.01^{c}	0.05 ± 0.01^{d}	***

Mean data for the three batches of Castelmagno PDO cheeses analysed in triplicate.
a, b, c, d, e: Different letters in the same row indicate significant statistical differences (Duncan test, $p < 0.05$).
ND: not detected.
ns = not significant.
* $P < 0.05$.
** $P < 0.01$.
*** $P < 0.00$.

analysed samples due to the high concentration of ethanol arising from lactose fermentation or amino acid catabolism. Among esters during Castelmagno PDO manufacturing, ethyl hexanoate, ethyl octanoate and ethyl decanoate concentrations represented 72.3% w/w of total ester concentration and ethyl butanoate and ethyl acetate represented 8% w/w of total ester concentration. The concentration of all esters identified in Castelmagno PDO samples increased during ripening and ethyl hexanoate, octanoate and decanoate represented 74% w/w of total ester concentration during this time. The increase of these esters could be associated with the decrease in corresponding acids. Ethyl hexanoate was also identified as the most abundant ester in other PDO cheeses, such as Grana Padano (Moio & Addeo, 1998), Parmigiano Reggiano (Bellesia et al., 2003) and Pecorino Romano (Di Cagno et al., 2003).

Ketones were the second most abundant compounds isolated in Castelmagno PDO samples, with a mean percentage of 2.04% (w/w of total volatile compounds concentration) during manufacturing and 8.25% (w/w of total volatile compounds concentration) during ripening. They are formed by enzymatic oxidation of free fatty acids to β-ketoacids and their consequent decarboxylation to ketones. They are very important compounds for dairy products because they have very particular odours and low perception thresholds (McSweeney, 2004; McSweeney & Sousa, 2000). In total, 12 ketones were identified in Castelmagno PDO samples; 2-butanone, 2-pentanone and 2-heptanone were the most abundant. Acetoin originates from citrate metabolism as a reduction of diacetyl by the action of lactic acid bacteria (McSweeney & Fox, 2004). The highest concentration of acetoin was detected during cheesemaking at the cut of the curd (44.4 ± 75.7 μg/kg of cheese); its concentration then decreased until the 90th day of ripening. In 150 day-old cheeses, its concentration was 3.25 ± 2.59 μg/kg of cheese and this decrease could be due to its reduction to butanone, as reported by Urbach (1993).

Aldehydes were present with the highest concentration (4.50% w/w of total volatile compounds concentration) at the end of manufacture of Castelmagno PDO cheeses. This concentration decreased to a mean value of 0.18% w/w of total volatile compounds concentration during ripening because they were rapidly converted to the corresponding alcohols or acids (Lemieux & Simard, 1992). During manufacturing, hexanal, heptanal and 2-nonenal were the aldehydes with the highest concentrations and represented 37%, 15% and 30% (w/w), respectively, of the total aldehydes concentration of the curd after 3 days under whey. During ripening, the aldehydes with the highest concentration were acetaldehyde, *trans* 2-hexenal and hexanal with concentrations that represented 45%, 17% and 13% w/w of total aldehydes concentration. Acetaldehyde, which represented nearly half the concentration of total aldehydes during ripening, could derive from the breakdown of threonine, from the lactose metabolism, or by the oxidation of ethanol (McSweeney & Sousa, 2000).

Alcohols were abundant during Castelmagno PDO manufacturing with a mean percentage of 3.56% (w/w of total volatiles detected), whilst these levels increased during ripening to a mean percentage of 6.18% (w/w of total volatile compounds concentration). Ethanol was the most abundant. It is a product of lactose fermentation or amino acid catabolism and it is the alcohol that contributes to the formation of ethyl esters. Primary alcohols are produced by the reduction of aldehydes derived by the catabolism of the amino acids (Moio & Addeo, 1998) and were present, during manufacture, at a final concentration of 7.11 ± 1.31 μg/kg of cheese

Table 4
Mean values ± standard deviation of volatile compound concentrations of Castelmagno PDO (δg/kg) cheese during its manufacture and ripening.

Compounds	Manufacture of cheese				Days of ripening				
	LRI[a]	Cut of the curd	After 24 h	Curd after 3 days under whey	3	30	60	90	150
Acids									
Acetic acid	1438	29.0 ± 38.8	17.9 ± 8.61	125 ± 64.7	136 ± 13.3	249 ± 92.5	136 ± 71.4	103 ± 24.1	142 ± 60.1
Propanoic acid	1528	ND	ND	ND	ND	4.85 ± 4.93	12.7 ± 14.3	11.7 ± 4.95	21.8 ± 7.87
Isobutyric acid	1560	0.18 ± 0.32	ND	ND	0.48 ± 0.05	3.43 ± 0.58	2.70 ± 2.73	1.17 ± 0.60	6.76 ± 4.44
Butyric acid	1620	141 ± 83.0	8.02 ± 2.57	178 ± 89.8	167 ± 8.63	397 ± 116	235 ± 120	163 ± 50.1	279 ± 166
Isovaleric acid	1665	1.43 ± 2.48	0.60 ± 0.17	1.90 ± 0.73	1.18 ± 020	16.3 ± 3.82	10.3 ± 9.50	4.90 ± 1.79	59.4 ± 46.8
Valeric acid	1736	2.11 ± 1.46	0.26 ± 0.04	0.75 ± 0.33	0.52 ± 0.05	2.97 ± 0.61	1.69 ± 0.51	1.49 ± 0.65	3.62 ± 2.30
Hexanoic acid	1842	329 ± 188	310 ± 7.13	780 ± 343	542 ± 177	1342 ± 678	1002 ± 358	733 ± 252	1094 ± 699
Heptanoic acid	1950	10.2 ± 6.34	2.59 ± 0.69	4.25 ± 2.35	1.58 ± 0.59	11.2 ± 5.38	9.36 ± 5.46	6.93 ± 4.68	15.3 ± 12.9
Octanoic acid	2056	523 ± 249	516 ± 16.3	156 ± 80.8	103 ± 38.6	637 ± 199	509 ± 285	381 ± 164	721 ± 518
Nonanoic acid	2163	19.0 ± 11.2	4.03 ± 0.94	9.13 ± 2.73	1.47 ± 2.31	11.7 ± 3.41	10.0 ± 4.77	9.47 ± 6.06	22.4 ± 18.4
Decanoic acid	2268	594 ± 336	280 ± 26.2	260 ± 114	128 ± 59.2	693 ± 204	570 ± 329	449 ± 197	842 ± 554
Undecanoic acid	2378	14.7 ± 13.4	0.43 ± 0.46	ND	ND	1.94 ± 3.36	ND	7.61 ± 5.79	15.2 ± 19.9
Benzoic acid	–	3.44 ± 2.53	1.30 ± 0.41	5.41 ± 2.05	3.95 ± 0.63	8.56 ± 1.21	4.78 ± 3.03	3.88 ± 1.99	9.61 ± 15.60
Dodecanoic acid	–	246 ± 155	20.8 ± 8.41	54.2 ± 26.8	18.9 ± 16.3	188 ± 44.8	164 ± 107	148 ± 84.0	291 ± 218
Tetradecanoic acid	–	1.72 ± 2.99	ND	ND	ND	ND	ND	5.71 ± 6.30	1.65 ± 2.85
Total		1915 ± 1090	1162 ± 71.9	1575 ± 728	1104 ± 316	3567 ± 1357	2668 ± 1311	2029 ± 803	3524 ± 2346
Percentage (%)[b]		93.4	87.0	85.5	88.1	75.9	70.7	76.2	78.7
Esters									
Ethyl acetate	880	0.13 ± 0.14	0.30 ± 0.07	1.12 ± 0.59	0.60 ± 0.18	2.05 ± 1.06	1.88 ± 1.81	0.97 ± 0.21	1.42 ± 0.04
Methyl butanoate	978	0.03 ± 0.05	0.22 ± 0.20	0.96 ± 0.55	ND	ND	1.14 ± 1.25	0.30 ± 0.53	1.03 ± 1.04
Ethyl butanoate	1030	0.72 ± 0.21	1.34 ± 1.72	2.27 ± 0.66	1.09 ± 0.30	6.89 ± 4.26	11.5 ± 15.8	5.92 ± 1.37	7.76 ± 3.89
Butyl butanoate	1120	ND	ND	ND	ND	ND	ND	0.22 ± 0.20	0.35 ± 0.60
Butyl 3-methyl-butanoate	1257	ND	ND	ND	0.07 ± 0.12	1.95 ± 0.50	1.90 ± 2.10	1.09 ± 0.31	1.61 ± 0.68
Ethyl hexanoate	1226	0.95 ± 0.87	3.09 ± 4.11	22.9 ± 13.2	16.1 ± 1.51	120 ± 5.30	111 ± 10.9	75.5 ± 1.49	89.1 ± 44.1
n-Hexyl acetate	1265	ND	ND	ND	ND	ND	0.25 ± 0.29	0.26 ± 0.17	0.52 ± 0.13
Propyl hexanoate	1312	ND	ND	ND	ND	8.09 ± 8.15	12.2 ± 13.8	15.1 ± 8.63	14.9 ± 5.03
Ethyl heptanoate	1325	ND	0.22 ± 0.38	ND	ND	0.40 ± 0.68	0.90 ± 1.56	0.25 ± 0.22	0.78 ± 0.87
Ethyl octanoate	1336	2.96 ± 2.04	4.47 ± 6.60	1.87 ± 0.70	1.25 ± 0.65	37.0 ± 45.4	44.7 ± 69.4	22.5 ± 9.58	18.0 ± 6.98
Allyl caproate	1364	ND	ND	ND	ND	0.07 ± 0.13	0.05 ± 0.08	0.23 ± 0.05	0.49 ± 0.32
n-Heptyl acetate	1366	ND	ND	ND	ND	ND	1.09 ± 1.88	0.25 ± 0.23	0.32 ± 0.28
Methyl octanoate	1380	0.33 ± 0.57	1.46 ± 2.53	ND	ND	0.81 ± 1.41	3.04 ± 5.27	1.15 ± 2.00	1.48 ± 1.54
n-Hexyl butanoate	1408	ND	ND	ND	ND	0.43 ± 0.52	0.70 ± 0.56	0.69 ± 0.14	0.93 ± 0.30
Propyl octanoate	1513	ND	ND	ND	ND	3.20 ± 4.43	2.67 ± 3.83	3.07 ± 2.13	2.51 ± 1.18
Ethyl nonanoate	1529	0.19 ± 0.19	ND	ND	ND	0.99 ± 1.71	ND	0.08 ± 3.48	ND
Methyl decanoate	1588	2.83 ± 1.52	2.10 ± 3.38	2.91 ± 1.48	0.66 ± 0.14	12.0 ± 8.68	11.7 ± 9.08	10.5 ± 4.85	17.9 ± 15.4
n-Hexyl hexanoate	1605	ND	ND	ND	ND	2.45 ± 1.32	1.89 ± 0.74	1.72 ± 0.59	2.83 ± 1.63
Ethyl decanoate	1636	6.71 ± 7.03	5.84 ± 10.12	3.09 ± 2.69	2.26 ± 1.33	56.2 ± 63.1	52.2 ± 80.4	46.1 ± 30.4	65.9 ± 72.3
Ethyl 9-decenoate	1687	0.82 ± 0.43	0.53 ± 0.91	ND	ND	5.88 ± 6.49	5.86 ± 8.68	5.47 ± 3.46	7.79 ± 9.55
Methyl undecanoate	1694	ND	ND	ND	ND	0.06 ± 0.10	ND	0.03 ± 0.05	0.23 ± 0.41
Propyl decanoate	1724	ND	ND	ND	ND	5.79 ± 5.08	3.51 ± 4.76	3.38 ± 2.21	3.61 ± 1.85
Ethyl undecanoate	1741	ND	0.07 ± 0.12	ND	ND	0.55 ± 0.95	ND	0.45 ± 0.78	ND
Methyl dodecanoate	1799	0.67 ± 1.16	0.42 ± 0.73	ND	ND	ND	ND	1.09 ± 1.88	ND
2-Phenyl ethyl acetate	1808	ND	ND	ND	0.05 ± 0.08	9.18 ± 6.55	2.78 ± 4.82	3.54 ± 1.15	2.44 ± 2.11
Propyl dodecanoate	1927	ND	ND	ND	ND	0.57 ± 0.52	0.60 ± 1.04	0.74 ± 0.19	0.48 ± 0.51
Ethyl tetradecanoate	2052	ND	0.17 ± 0.29	ND	ND	5.37 ± 1.62	0.10 ± 0.18	5.62 ± 4.00	1.06 ± 1.04
Methyl tetradecanoate	2008	ND	0.08 ± 0.14	ND	ND	ND	ND	0.40 ± 0.44	0.33 ± 0.35
Total		16.3 ± 14.2	20.3 ± 31.3	35.1 ± 19.9	22.1 ± 4.31	280 ± 168	272 ± 238	209 ± 80.7	244 ± 172
Percentage (%)		0.80	1.52	1.91	1.76	5.96	7.20	7.83	5.44
Ketones									
Acetone	810	0.81 ± 0.38	0.32 ± 0.14	0.40 ± 0.10	0.73 ± 0.32	6.81 ± 6.85	4.72 ± 5.04	1.28 ± 1.14	2.42 ± 1.04
2-Butanone	896	0.17 ± 0.17	0.24 ± 0.21	0.55 ± 0.29	0.53 ± 0.37	32.0 ± 26.6	73.6 ± 85.3	58.2 ± 10.7	46.9 ± 26.0
2-Pentanone	965	0.22 ± 0.24	ND	2.75 ± 3.15	0.94 ± 1.33	82.1 ± 9.89	54.7 ± 76.1	14.0 ± 14.3	21.3 ± 15.9

(*continued on next page*)

Table 4 (*continued*)

Compounds	Manufacture of cheese				Days of ripening				
	LRI[a]	Cut of the curd	After 24 h	Curd after 3 days under whey	3	30	60	90	150
Diacetyl	970	3.97 ± 6.56	ND	ND	2.75 ± 1.62	0.67 ± 1.16	ND	ND	ND
2-Hexanone	1069	ND	ND	ND	ND	0.43 ± 0.74	1.74 ± 2.75	0.28 ± 0.49	0.81 ± 0.18
2-Heptanone	1171	0.68 ± 1.18	ND	ND	1.60 ± 0.80	25.0 ± 14.4	77.6 ± 13.9	13.1 ± 5.19	41.4 ± 23.8
Acetoin	1270	44.4 ± 75.7	10.8 ± 6.82	27.8 ± 23.6	27.0 ± 15.5	11.1 ± 6.17	3.86 ± 2.40	1.84 ± 1.05	3.25 ± 2.59
2-Octanone	1274	ND	ND	ND	ND	0.70 ± 063	1.85 ± 2.64	0.38 ± 0.12	1.16 ± 0.70
2-Nonanone	1379	2.27 ± 1.21	0.10 ± 0.18	0.99 ± 0.13	1.03 ± 0.20	34.2 ± 25.7	65.4 ± 82.8	24.4 ± 10.6	57.7 ± 26.3
8-Nonen-2-one	1432	0.11 ± 0.19	ND	ND	ND	5.99 ± 5.93	11.0 ± 14.4	3.18 ± 1.16	10.1 ± 4.88
3,5-Octadien-2-one	1506	1.88 ± 1.09	3.66 ± 1.65	10.5 ± 4.43	ND	ND	ND	ND	ND
Benzyl methyl ketone	1717	ND	ND	ND	ND	0.23 ± 0.40	ND	ND	0.42 ± 0.46
2-Tridecanone	1809	ND	ND	ND	ND	257 ± 44.5	0.76 ± 1.14	ND	2.09 ± 2.97
Total		54.5 ± 86.7	15.1 ± 9.00	42.9 ± 31.7	34.6 ± 20.2	456 ± 143	525 ± 342	209 ± 70.0	316 ± 146
Percentage (%)		2.66	1.13	2.33	2.76	9.70	13.92	7.84	7.06
Aldehydes									
Acetaldehyde	–	ND	0.35 ± 0.25	1.61 ± 1.79	2.79 ± 1.61	4.24 ± 1.79	2.62 ± 0.36	0.20 ± 0.17	1.11 ± 1.36
3-Methyl butanal	911	0.54 ± 0.87	0.25 ± 0.24	1.68 ± 0.95	0.56 ± 0.15	1.20 ± 0.87	0.21 ± 0.24	0.14 ± 0.12	0.70 ± 0.61
Hexanal	1071	1.31 ± 0.60	26.4 ± 10.5	30.2 ± 26.5	1.51 ± 0.17	0.98 ± 0.85	0.06 ± 0.10	0.27 ± 0.47	0.42 ± 0.72
Heptanal	1171	ND	4.81 ± 4.66	12.4 ± 9.10	ND	ND	ND	ND	ND
trans-2-Hexenal	1203	ND	2.42 ± 1.20	2.80 ± 2.51	ND	4.11 ± 7.12	ND	ND	ND
Octanal	1272	ND	4.13 ± 3.09	ND	ND	ND	ND	ND	ND
2-Heptenal	1307	ND	1.96 ± 1.78	2.90 ± 2.19	ND	ND	ND	ND	ND
Nonanal	1383	ND	6.57 ± 2.97	ND	0.15 ± 0.27	ND	ND	ND	ND
2-Octenal	1412	ND	4.56 ± 2.37	5.03 ± 4.77	ND	ND	ND	ND	ND
Benzaldehyde	1504	0.37 ± 0.35	0.80 ± 0.27	1.39 ± 1.72	ND	ND	ND	ND	0.59 ± 1.03
2-Nonenal	1524	1.06 ± 0.96	19.0 ± 11.4	24.9 ± 22.9	0.62 ± 0.15	ND	0.04 ± 0.07	0.08 ± 0.14	0.09 ± 0.15
Decanal	1767	ND	ND	ND	ND	0.88 ± 1.53	ND	0.12 ± 0.21	0.61 ± 0.15
Total		3.28 ± 2.78	71.3 ± 38.8	83.0 ± 72.5	5.64 ± 2.34	11.4 ± 12.2	3.00 ± 0.91	0.81 ± 1.11	3.52 ± 4.02
Percentage (%)		0.16	5.34	4.50	0.45	0.24	0.08	0.03	0.08
Alcohols									
Ethanol	930	8.19 ± 8.15	43.0 ± 42.9	57.2 ± 39.9	27.8 ± 13.1	30.2 ± 7.50	62.4 ± 59.5	46.0 ± 39.6	157 ± 107
2-Butanol	1025	ND	ND	ND	ND	9.84 ± 6.95	36.0 ± 46.0	50.3 ± 12.3	40.1 ± 6.98
Isobutanol	1097	0.14 ± 0.24	ND	ND	1.44 ± 0.43	2.08 ± 1.61	1.26 ± 0.81	0.80 ± 0.49	0.96 ± 0.99
2-Pentanol	1123	ND	ND	ND	0.11 ± 0.19	33.6 ± 9.21	41.0 ± 47.2	14.6 ± 10.4	16.1 ± 11.6
1-Butanol	1144	ND	ND	ND	0.53 ± 0.09	1.92 ± 0.93	2.29 ± 1.95	3.59 ± 0.83	3.61 ± 1.40
Isopentanol	1206	6.94 ± 12.03	2.38 ± 2.06	14.8 ± 6.68	10.6 ± 2.22	47.4 ± 24.7	21.0 ± 19.3	13.4 ± 4.88	17.7 ± 8.69
2-Heptanol	1320	ND	ND	ND	ND	32.84 ± 32.10	36.6 ± 44.3	15.6 ± 7.88	22.6 ± 4.29
1-Hexanol	1351	0.36 ± 0.34	1.28 ± 0.55	3.30 ± 1.31	1.80 ± 0.58	6.19 ± 3.99	6.07 ± 2.05	8.56 ± 3.34	8.47 ± 2.76
2-Nonanol	1519	ND	ND	ND	ND	45.7 ± 70.4	23.8 ± 22.6	16.0 ± 2.02	28.6 ± 7.87
2,3-butanediol d,l	1535	6.65 ± 11.52	ND	ND	1.70 ± 1.05	9.52 ± 3.83	7.55 ± 7.81	4.04 ± 1.81	5.08 ± 0.91
1-Octanol	1555	ND	2.17 ± 1.99	ND	0.07 ± 0.12	ND	0.19 ± 0.19	0.34 ± 0.12	0.78 ± 0.33
2,3-butanediol meso	1573	13.6 ± 23.6	5.51 ± 3.92	14.3 ± 5.18	19.4 ± 1.60	40.5 ± 20.4	15.1 ± 6.43	4.95 ± 4.31	5.69 ± 3.53
Benzyl alcohol	1883	ND	ND	ND	ND	1.43 ± 1.60	0.53 ± 0.92	ND	ND
Total		35.9 ± 55.9	54.3 ± 51.4	89.6 ± 53.1	63.6 ± 19.4	261 ± 183	254 ± 259	178 ± 90.0	307 ± 156
Percentage (%)		1.75	4.07	4.86	5.06	5.56	6.72	6.69	6.85
Lactones									
δ-Octalactone	1609	2.63 ± 0.99	0.86 ± 0.30	3.90 ± 2.26	1.81 ± 0.51	3.73 ± 1.67	2.68 ± 1.24	1.90 ± 0.69	2.88 ± 1.50
δ-Decalactone	2157	8.81 ± 4.35	6.65 ± 3.29	3.61 ± 6.26	12.6 ± 11.1	80.1 ± 48.9	23.1 ± 6.18	16.6 ± 0.52	41.9 ± 42.8
δ-Dodecalactone	–	8.26 ± 6.37	3.82 ± 4.67	4.23 ± 1.62	1.51 ± 1.84	15.9 ± 7.84	9.77 ± 5.82	8.20 ± 3.74	13.8 ± 12.2
Total		19.7 ± 11.7	11.3 ± 8.26	11.7 ± 10.1	15.9 ± 13.5	99.8 ± 58.4	35.5 ± 13.3	26.7 ± 4.95	58.6 ± 56.5
Percentage (%)		0.96	0.85	0.64	1.27	2.12	0.94	1.00	1.31
Hydrocarbons									
Decane	1000	0.62 ± 0.22	0.45 ± 0.04	1.89 ± 0.77	1.45 ± 0.10	2.21 ± 1.04	1.35 ± 0.73	1.50 ± 1.20	0.24 ± 0.28
Undecane	1100	ND	ND	ND	2.26 ± 1.32	0.86 ± 0.89	ND	ND	0.08 ± 0.14
Dodecane	1200	ND	ND	ND	0.36 ± 0.37	2.87 ± 3.23	2.77 ± 2.16	1.83 ± 0.89	0.79 ± 0.95

Total		0.62 ± 0.22	0.45 ± 0.04	1.89 ± 0.77	4.07 ± 1.78	6.04 ± 5.33	4.12 ± 2.89	3.33 ± 2.09	1.11 ± 1.36
Percentage (%)		0.03	0.03	0.10	0.32	0.13	0.11	0.12	0.02
Other compounds									
Phenolic compounds									
1,3-Dimethoxybenzene	1740	ND	ND	ND	ND	0.80 ± 1.38	ND	0.32 ± 0.55	1.12 ± 1.50
Phenol	2000	ND	ND	ND	0.26 ± 0.02	0.21 ± 0.20	ND	ND	8.86 ± 9.74
Terpenes									
Limonene	1181	ND	ND	ND	ND	0.10 ± 0.17	ND	ND	0.70 ± 1.22
N-compounds									
N,N-Dibutyl formamide	1773	0.18 ± 0.30	ND	ND	ND	ND	ND	ND	ND
Aromatic alcohol									
Phenyl ethyl alcohol	1907	5.84 ± 9.56	0.98 ± 0.13	3.35 ± 1.61	2.51 ± 0.73	17.8 ± 6.63	12.5 ± 13.4	7.44 ± 2.36	10.2 ± 4.14
S-compounds									
Dimethyl disulphide		ND	ND	ND	ND	ND	ND	ND	2.21 ± 2.87
Total		6.01 ± 9.87	0.98 ± 0.13	3.35 ± 1.61	2.78 ± 0.75	18.9 ± 8.38	12.5 ± 13.4	7.76 ± 2.91	23.1 ± 19.5
Percentage (%)		0.29	0.07	0.18	0.22	0.40	0.33	0.29	0.52

Mean data for the three batches of Castelmagno PDO cheese analysed in triplicate.
ND: not detected.
[a] LRI: linear retention index.
[b] Percentage (%): percentage of volatile compounds of each chemical group in each step of manufacture and ripening analysed.

and a final concentration at the end of ripening of 56.9 ± 15.7 μg/kg of cheese. Instead, secondary alcohols, formed by enzymatic reduction from the corresponding methyl ketones, which are produced from fatty acids (Collins et al., 2004), were not detected during manufacture but only during ripening. The branched-chain alcohols detected (2-methyl-1-butanol and 3-methyl-1-butanol), were derived from the reduction of aldehydes produced from the catabolism of isoleucine and leucine, respectively (Yvon & Rijnen, 2001), and were present at final concentrations of 14.8 ± 6.68 and 18.6 ± 9.68 μg/kg of cheese at the end of manufacture and ripening, respectively.

Among the lactones, three δ-lactones (δ-octalactone, δ-decalactone and δ-dodecalactone) were detected. Lactones are cyclic compounds formed by the intramolecular esterification of hydroxyl fatty acids through the loss of water (Molimard & Spinnler, 1996). Lactones were present with a mean percentage of 0.82% w/w of total volatile compounds concentration during manufacture of cheese and a mean percentage of 1.33% w/w of total volatile compounds concentration during ripening. Lactones represented only a very small portion (c.a. 0.1% of total volatile compounds concentration) in other cow's milk PDO cheeses, such as Grana Padano (Moio & Addeo, 1998) but a higher portion in other ewes' milk PDO cheeses, e.g. Canestrato Pugliese (ca 7.8% w/w of total volatile compounds concentration) or Fiore Sardo (ca. 8.1% of total volatile compounds concentration) or Pecorino Romano PDO (ca. 9.2% w/w of total volatile compounds concentration) (Di Cagno et al., 2003).

3.5. Textural analysis

The mean values obtained for texture parameters of TPA, obtained during ripening, are shown in Table 5.

Texture profile analysis presented changes for all variables during ripening, in particular in the first 30 days and between 90 and 150 days. In general, hardness, adhesiveness gumminess and chewiness values increased up to 3 days, while the opposite behaviour was noted for cohesiveness and resilience parameters.

Hardness, the force necessary to attain a given deformation (Szczesniak, 2002) increased particularly at the beginning of ripening (first 30 days), was maintained for up to 90 days of ripening and increased significantly in the last 60 days (about 18 N, from 25.88 ± 4.13 to 43.15 ± 5.50). Similar behaviours were also found in gumminess and chewiness properties (respectively defined as the force and energy required to masticate cheese into a uniform state before swallowing). Also adhesiveness, (the work necessary to overcome the attractive forces between the cheese and the contact surfaces of the universal testing machine probe) (Tunick, 2000), showed a large increase in the last two months of ripening (0.45 ± 0.15 mJ). On the other hand, small differences in springiness (measure of the distance recovered by the cheese sample during the time between the end of the first bite and start of second bite), were registered throughout the entire ripening period. Instead, the parameters of cohesiveness (measure of the strength of the internal bonds of the protein mycelium) (Tunick, 2000), and resilience, (a dimensionless parameter which represents the ability of the cheese to regain its original position after the first compression) (Chevanan, Muthukumarappan, Upreti, & Metzger, 2006), were characterised by a strong decrease in the values between 3 and 30 days, respectively −0.07 ± 0.01 and −1.51 ± 0.63. The values of these parameters also showed a decrease between 90 and 150 days.

Therefore, three distinct phases in texture development took place during Castelmagno PDO cheese ripening: the first between 3 and 30 days, the second, from 30 to 90 days and the third, from 90 to 150 days.

Table 5
Mean value ± standard deviation of TPA parameters of Castelmagno PDO cheese during its ripening.

	Days of Ripening					
	3	30	60	90	150	Statistical significance
Hardness (N)	12.44 ± 2.79[a]	23.21 ± 4.46[b]	24.07 ± 3.36[b]	25.88 ± 4.13[b]	43.15 ± 5.50[c]	***
Cohesiveness (–)	0.28 ± 0.06[a]	0.21 ± 0.05[b]	0.25 ± 0.04[a]	0.20 ± 0.06[b]	0.17 ± 0.03[b]	***
Adhesiveness (mJ)	−0.04 ± 0.04[a]	−0.21 ± 0.13[b]	−0.13 ± 0.08[a,b]	−0.12 ± 0.05[a,b]	−0.57 ± 0.20[c]	***
Gumminess (N)	3.51 ± 1.34[a]	4.91 ± 1.75[a,b]	6.09 ± 1.41[b,c]	5.24 ± 1.70[b]	7.51 ± 1.77[c]	***
Springiness (mm)	3.27 ± 0.23[a]	3.31 ± 0.23[a]	3.58 ± 0.18[b]	3.57 ± 0.24[b]	3.18 ± 0.42[a]	***
Chewiness (mJ)	11.53 ± 4.40[a]	16.41 ± 6.34[a,b]	21.91 ± 5.29[b,c]	18.92 ± 6.47[b,c]	24.00 ± 7.69[c]	***
Resilience (–)	3.15 ± 2.26[a]	1.64 ± 1.63[b]	1.63 ± 1.15[b]	1.41 ± 1.30[b,c]	0.79 ± 0.31[c]	***

Mean data for the three batches of Castelmagno PDO cheese analysed in quintuplicate.
a, b, c: Different letters in the same row indicate significant statistical differences (Duncan test, $p < 0.05$).
ns = not significant.
* $P < 0.05$.
** $P < 0.01$.
*** $P < 0.001$.

4. Conclusions

During the manufacture of Castelmagno PDO cheese, it was possible to detect the conclusion of lactose metabolism with the total conversion of lactose into lactate and the commencement of the primary proteolyses. The volatile profile was characterised by a high level of acids, in particular of hexanoic, octanoic and decanoic acids, which are the primary products of lipolysis metabolism.

During ripening of Castelmagno PDO cheeses, it is possible to observe high degradation of α_{s1}-casein with an increase of all its degradation products, and evolution of the hydrophilic peptides associated also with the highest concentrations of glutamic acid, valine, leucine phenylalanine and lysine. The volatile profiles of Castelmagno PDO cheese during ripening are characterised by a decrease in acid compounds and an increase in ketones and alcohols as a consequence of free fatty acid metabolism. Texture profiles show increases of hardness, gumminess, chewiness and adhesiveness properties, and a diminution of cohesiveness.

However, to fully characterise the Castelmagno PDO cheese it will also be necessary to analyse samples of Castelmagno PDO during the summer period, when the producers transfer the herd to grassland where cows eat fresh forage that both directly and indirectly influences the organic acid and volatile profile of the obtained products. At the same time, the producers change the caves, an act that can influence microbiological effects and, as a consequence, the biochemical pathways of Castelmagno PDO cheese during ripening.

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