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Cheese ripening in nonconventional conditions: A multiparameter study applied to Protected Geographical Indication Canestrato di Moliterno cheese

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ABSTRACT

A multiparameter study was performed to evaluate the effect of *fondaco*, a traditional ripening cellar without any artificial temperature and relative humidity control, on the chemical, microbiological, and sensory characteristics of Protected Geographical Indication Canestrato di Moliterno cheese. Ripening in such a nonconventional environment was associated with lower counts of lactococci, lactobacilli, and total viable bacteria, and higher presence of enterococci, in comparison with ripening in a controlled maturation room. Moreover, fondaco cheese underwent accelerated maturation, as demonstrated by faster casein degradation, greater accumulation of free AA, and higher formation of volatile organic compounds. Secondary proteolysis, as assessed by liquid chromatography-mass spectrometry of free AA and low molecular weight peptides, did not show any qualitative difference among cheeses, but fondaco samples evidenced an advanced level of peptidolysis. On the other hand, significant qualitative differences were observed in the free fatty acid profiles and in the sensory characteristics. Principal component analysis showed a clear separation of the fondaco and control cheeses, indicating that ripening in the natural room conferred unique sensory features to the product.
Key words: fondaco cellar, proteolysis, liquid chromatography-mass spectrometry, peptide identification, sensory analysis

INTRODUCTION

The ripening environment plays a fundamental role on cheese quality because it regulates a series of physical-chemical, microbiological, and biochemical events that contribute to "build" the compositional and sensory profile of the product (van den Berg and Exterkate, 1993; Macedo et al., 1997; Pachlová et al., 2011). In ancient times, ripening took place in natural caves or cellars, in which temperature and relative humidity (**RH**) had limited variations over time. Today, environmental parameters are kept under strict control in artificial rooms that allow standardization of the ripening process, but caves and cellars have not been totally decommissioned. In most cases, they have been modified and equipped with control devices, as happens for some European blue (Fernández-Bodega et al., 2009) or semihard cheeses (Gobbetti et al., 1999; Dolci et al., 2009). Indeed, ripening in rooms without any artificial temperature and RH control has become rare; however, it is considered to highly contribute to cheese uniqueness (Bérard and Marchenay, 2006). Several papers have been published on this topic, mainly focusing on microbiological aspects (Torraca et al., 2015; Ozturkoglu Budak et al., 2016; Anelli et al., 2018, 2019).

Canestrato di Moliterno is an example of cheese ripened in an uncontrolled environment; it is manufactured in the Basilicata Region (southern Italy) from a mixture of ewe and goat milk and has received the Protected Geographical Indication (**PGI**) acknowledgment in 2010. Ripening takes place in ancient cellars called "*fondaci*," whose name derives from the Arabian word *funduq* meaning "warehouse" (European Union, 2010). Ancient fondaco rooms are present in the largest Italian seaside towns such as Geneva, Naples, and Venice, as well as in some mountain and rural towns, where they represented the basements of noble palaces and were used for both food storage and marketing. The structure of fondaco consists of several wide and communicating basement rooms, with walls at least 40-cm thick, 2 or more windows to allow moderate ventilation, and sloping floors to facilitate drainage (Pinarelli, 2006). Despite the fact that fondaco ripening is considered the basis of the PGI status of Canestrato di Moliterno (European Union, 2010), no investigation has explored its effect on cheese characteristics (Pirisi et al., 2011; Trani et al., 2016). As this traditional ripening is expensive and difficult to standardize, the producers wonder if it makes a real difference in the cheese quality compared with a rational process performed in a modern and

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strictly controlled ripening room. The purpose of the present investigation was to assess the differences in the quality characteristics of Canestrato di Moliterno hard cheeses ripened in fondaco or in an artificial room. As ripened cheese represents a very complex matrix, the study used a multiparameter approach based on the use of several analytical techniques, including gas and liquid MS, which were able to supply information on the evolution of the main macroconstituents and their metabolites.

MATERIALS AND METHODS

Cheese Samples

The investigation considered 8 wheels of Canestrato di Moliterno cheese (4 per type of ripening environment) from 2 different batches manufactured on 2 consecutive days at the beginning of May at a dairy farm located in the PGI geographical area (Gorgoglione, Basilicata, Italy). The cheeses were obtained from a mixture of sheep- and goat-farm milk (70/30) by following the official protocol of production. In brief, milk was thermized in the vat at 60°C × 15 min, cooled down at 38°C, and added with autochthonous starter culture (a lyophilized mix of lactococci and lactobacilli strains, developed and copyrighted by the PGI Consortium). After incubation for about 20 min, lamb rennet paste (0.3 g/L, 1:12,000 strength, Prodor) was added, and coagulation was obtained in about 30 min. The coagulum was cut to the size of 3 to 4 mm; the curd grains were scalded at 42°C under stirring and then were left to sediment to the vat bottom. After about 20 min, the whey was drained off and the compacted curd was cut in square blocks to be molded into plastic baskets. The cheeses, weighing about 3-kg each, were kept at 28 to 30°C for 12 h, salted in saturated brine for about 30 h, and then transferred into the artificial ripening room of the farm, set at 13 ± 2°C and 75 ± 5% RH. After 1 mo, 4 batches were transferred to the fondaco room [coded as **F** (A1, A2, B1, B2)], where they remained for 60 d, before coming back for continuing ripening in the artificial room, as indicated by the PGI official production protocol. The other 4 batches remained in the controlled room [coded as **C** (A1, A2, B1, B2)], at the farm until the end of ripening. All cheeses were taken for analyses after 6 mo of ripening.

Chemical, Microbiological, and Sensory Analyses

The following chemical analyses were performed: moisture by oven drying, pH by a pH meter equipped with a penetration probe (Hanna Instruments), NaCl by chloride analyzer (Sherwood Scientific Ltd.), fat (Soxhlet method), total nitrogen (Kjeldahl method), and water-soluble nitrogen (Kuchroo and Fox, 1982). For microbiological analyses, 10 g of cheese was diluted in 90 mL of 2% (wt/vol) sodium citrate solution and homogenized in a Waring blender (Waring Commercial). Serial dilutions were made in quarter-strength Ringer's solution and plated on specific medium (Oxoid) as follows: total mesophilic bacteria were counted on plate count agar incubated at 30°C for 48 h, lactobacilli were counted on de Man, Rogosa, and Sharpe agar at 37°C for 48 h under anaerobiosis, lactococci were counted in M17 agar at 37°C for 48 h, and enterococci were counted on Slanetz and Bartley medium at 45°C for 48 h. Sensory evaluation was performed by a panel of 9 trained assessors from the staff of the Section of Food Science and Technology at the Department of Soil, Plant and Food Sciences of the University of Bari (Bari, Italy). They were selected following international standards (ISO, 1993), and trained as reported in a previous paper (Trani et al., 2016). The panelists evaluated the samples by quantitative descriptive analysis as reported previously (Trani et al., 2016). The descriptors were quantified on a 6-point scale and were selected based on weight percentage (frequency of citations × perceived intensity). Only descriptors with a weight percentage greater than 30% were considered.

Proteolysis and Lipolysis

Primary proteolysis was evaluated by urea-PAGE using the protocol described by Andrews (1983). After running the gel, it was stained with blue silver as indicated by Candiano et al. (2004) and subjected to image analysis by using Quantity One software (version 4.6.3; BioRad). The main casein fractions were identified by comparison with a milk sample taken from the vat and with the data from the scientific literature. Secondary proteolysis was assessed by investigating the low-molecular-weight (**MW**) peptide and free AA profiles of the water-soluble extract ultrafiltered on a 10-kDa cutoff membrane (Amicon, Millipore Corp.). Peptides were studied by HPLC-MS using an Ultimate 3000 RS Dionex system (Thermo Fisher Scientific) composed by quaternary pump, autosampler, and column compartment. It was coupled with an LTQ Velos PRO Linear Ion Trap mass spectrometer by an electrospray interface (H-ESI; Thermo Fisher Scientific). The chromatographic conditions were as follows: gradient elution at 0.3 mL/min flow rate, from 10 to 50% solvent B (acetonitrile containing 0.1% formic acid) in A (water containing 0.1% formic acid) in 20 min. The column was a Kinetex C18 50 × 3.0 mm with a particle size of 2.6 μm (Phenomenex), controlled by thermostat at 35°C. All reagents were from Fluka and were LC-

MS grade. The electrospray interface temperature was 320°C for capillary and 280°C for probe, sheath gas flow was 30 psi, aux gas was 10 (arbitrary units), spray voltage was 3 kV in positive ion mode, and capillary temperature was 320°C. Data acquisition and analysis were performed using Trace Finder software v. 3.2 (Thermo Fisher). Each sample was acquired twice as follows: full scan mode with mass ranging from 50 to 1,800 amu and data dependent fragmentation with full scan from 520 to 2,000 Da, Zoom scan (the accurate mass measurement operational mode of the mass spectrometer), and tandem MS (MS^2) of the first 5 higher signals with minimum signal threshold of 500 counts. The MS^2 signals from full scan were obtained by using collision-induced dissociation, with normalized collision energy to 30 arbitrary units. The full scan was used to compare the chromatograms among samples, and the data-dependent scan was used for identification of peptides, their AA sequence, and mother protein throughout PEAK v.9 software (Bioinformatics Solutions Inc.). A tentative quantitation was made by comparing the area of peptide peaks with that of an eledoisin-related peptide standard (Lys-Phe-Ile-Gly-Leu-Met-NH_2, Sigma-Aldrich). A calibration curve was performed by serial dilution from a 10 mg/L stock solution. Analysis of free AA was performed using the EZ:faast LC/MS AA analysis kit (Phenomenex) as reported by Trani et al. (2016). Lipolysis was assessed by determination of free fatty acids (**FFA**) extracted, purified, and analyzed by GC as indicated in a previous paper (Trani et al., 2010).

Volatile Organic Compounds Analysis

Volatile organic compounds (**VOC**) were extracted at 37°C for 15 min, as reported in a previous paper (Faccia et al., 2018), after addition of 3-pentanone (81.3 ng) as internal standard for semiquantitation. A Triplus RSH autosampler was used, equipped with a divinylbenzene/carboxen/polydimethylsiloxane 50/30 mm solid-phase microextraction (SPME) fiber assembly (Supelco). The VOC were desorbed by exposing the fiber at 220°C for 2 min in the injection port of the gas chromatograph operating in splitless mode. The GC-MS analysis was performed using a Trace 1300 chromatograph equipped with a capillary column VF-WAX MS (60 m, 0.25 mm, 0.25 μm) coupled to a mass spectrometer ISQ Series 3.2 SP1 (Thermo Scientific). The operating conditions were as follows: oven temperatures of 50°C for 0.1 min; then, increase at 13°C/min to 180°C and at 18°C/min to 220°C, and held for 1.5 min. Source temperature was 250°C; ionization energy was 70 eV; scan range was 33 to 200 amu. Peak identification was done by means of Xcalibur V2.0 software (ThermoFisher Scientific) by matching with the reference mass spectra of the National Institute of Standards and Technology (NIST) library (https://www.nist.gov/nist-research-library); when available, pure standards were also used.

Statistical Analysis

The data were statistically processed by XLSTAT software (version 2020.1.3, Addinsoft). Discrete variables were described by their mode values and continuous variables by their means. For microbiological results, the means ± the standard deviation were calculated; the results of the chemical analyses were subjected to one-way ANOVA followed by Tukey's honestly significant difference test at a critical value for significance of $P < 0.05$; as for the sensory analysis, the nonparametric variables were compared by using the Kruskal Wallis test. Principal component analysis (**PCA**) was carried out for finding the correlations of the ripening environment with the microbiological counts and the most important low MW metabolites, whereas the Pearson correlation coefficient was computed to extrapolate the correlations among the sensory (taste and aroma attributes) and chemical variables.

RESULTS

Chemical, Microbiological, and Sensory Analyses

The mean gross composition (Table 1) and the microbiological profile of the 2 types of Canestrato di Moliterno were significantly different. fondaco cheese contained about 2% less moisture, was slightly more salted, and presented higher values of pH and water-soluble nitrogen than the control cheese. As for the fat and protein content, the significance of the differences depended on the calculation method as follows: on the dry basis, only fat was different (about 3% lower value in F samples). As to the microbiological profile, F samples were characterized by slightly higher counts of enterococci with respect to the C samples (8.8×10^8 vs. 2.6×10^8 cfu/g), and lower counts of total mesophilic (8.4×10^7 vs. 7.4×10^8 cfu/g), lactococci (7.6×10^7 vs. 4.8×10^8 cfu/g), and lactobacilli (5.6×10^7 vs. 4.6×10^8 cfu/g). Significant differences were also observed in the sensory characteristics, starting with the appearance of the cheese wheels (Supplemental Figure A, https://osf.io/n39yp/quickfiles). In fact, F samples presented slightly concave faces with darker color and an oily thicker rind. These differences suggested faster moisture loss and some fat sweating during ripening; these findings explained the differences found in the gross composition. The darker color of the rind could be also connected to an early Maillard reaction that can take

Table 1. Chemical composition of the cheeses ripened under different conditions (%, except pH; mean values ± SD)[1]

Item	C (on wb)	F (on wb)	C (on DM basis)	F (on DM basis)
pH	5.16±0.02[b]	5.27±0.02[a]		
Fat	34.31±0.51[a]	33.48±0.42[a]	51.73±0.77[a]	49.03±0.62[b]
Protein	24.84±0.35[b]	26.03±0.43[a]	37.45±0.53[a]	38.12±0.63[a]
NaCl	3.87±0.05[b]	4.21±0.09[a]	5.83±0.08[b]	6.17±0.13[a]
Moisture	33.67±1.05[a]	31.72±0.83[b]		
RI	13.87±0.22[b]	17.40±0.78[a]		

[a,b]Values in the same row for the same measure unit are statistically different at $P < 0.05$.
[1]RI = ripening index; C= controlled maturation room; F = fondaco; wb= wet basis.

place in long-ripened cheeses under particular conditions, such as low activity water value and availability of free carbonyl groups from lipid oxidation (Zamora and Hidalgo, 2011). As for flavor, 17 sensory attributes were developed based on quantitative descriptive analysis, the most of which significantly differed between the 2 types of samples (Table 2). All attributes were perceived as more intense in F samples, except for acid taste, whereas butter, toasted, and musty attributes were not perceived in C samples. The overall flavor of the cheese ripened in fondaco was described as "rough" and "typical," whereas that of the control cheese was defined as "mild" and "delicate" (results not shown).

Proteolysis

The urea-PAGE patterns (Figure 1) gave indications about primary proteolysis. The typical complex casein profile expected in a mixture of ovine and caprine milk was observed, with α_{S1}- and β-fractions resolved as multiple bands. Both fractions were hydrolyzed more rapidly in F samples, and α_{S1}-CN was almost totally degraded, as commonly reported in hard cheeses (Sousa et al., 2001). In addition to this, the band corresponding to its primary product of degradation, the α_{S1}-I fragment (24–199), was much less intense. Disappearance of this polypeptide is an indirect index of the level of secondary proteolysis because it tends to undergo further enzymatic hydrolysis as ripening proceeds. Detailed information about secondary proteolysis was derived from the determination of free AA and from the LC-MS study of the soluble nitrogen fraction. The free AA profiles (Figure 2) confirmed the presence of significant differences between the samples under study because the total concentration was higher in F samples (1,392.38 mg/kg vs. 643.85 mg/kg). The difference depended on all AA, except for Arg, and the increase with respect to the C samples varied from about 20 to more than 100%. The greatest increases were observed for Ser, Asn, Gly, Ala, Pro, Ile, Asp, and Glu. The cheese ripened in fondaco also contained higher level of some unconventional compounds that the EZ-fast method typically allows to quantify, such as α-aminobutyric and β-aminoisobutyric acids, citrulline, Gly-Pro dipeptide, and Orn. As for the LC-MS study, although the chromatograms presented very similar profiles, the total peptide peak area was significantly lower in F than in C samples (chromatograms not shown). The identification of peptides is reported in Tables 3 and 4, with details about the mother protein, AA sequence, and MW; the results of tentative quantification are also included. The calibration curve, along with the resulting plot slope, intercept, and correlation coefficient is reported as Supplemental Figure B (https://osf.io/n39yp/quickfiles). It is worth highlighting that, to our knowledge, the quantitation of peptides in the present experiment represents one of the first efforts in this area, as the studies available in the literature on peptide formation during cheese ripening are mostly qualitative. The chromatographic conditions applied and the instrumental features of the

Table 2. Sensory attributes (modal values) for Canestrato di Moliterno ripened under different conditions; C = controlled maturation room; F = fondaco; Sig = statistical significance

Item	C	F	Sig
Texture			
Soluble	3	4	*
Adhesive	3	3	
Crumbly	3	3	
Greasy	3	3	
Hard	2	3	*
Crystals	0	1	*
Odor			
Fermented	2	3	*
Sheep barn	1	2	*
Butter	0	2	*
Cheese rind	0	1	
Toasted	0	1	*
Musty	0	1	*
Taste			
Salty	3	4	*
Bitter	2	2	
Pungent	1	2	*
Acid	2	1	*
Umami	0	1	

*Indicates significant difference at $P < 0.05$.

mass detector allowed separation and identification of peptides with an upper limit of theoretical mass around 2,000 Da, whereas the lower limit was mainly represented by the background noise. In our conditions, the shortest sequence identified corresponded to a 741-Da MW octapeptide. Overall, 77 peptides were identified, of which 39 originated from β-CN, 33 from α_{S1}-CN, and 5 from α_{S2}-CN. All of them were present in both types of cheese, but the total concentration was about 18% higher in the control (708.60 ± 41.22 vs. 579.12 ± 63.54 mg/kg). However, when the values were normalized to the soluble nitrogen content, the difference appeared as much more relevant (about 62%). The MW distribution was as follows: 7 peptides had MW >2,000 Da, 21 were in the range of 1,500 to 2,000 Da, 44 were in the range of 1,000 to 1,500 Da, and 5 had MW <1,000 Da. Peptides with MW ranging from 1,000 to 2,000 Da were by far the most represented, accounting for 92.8% of total peptides in C samples and 96.6% in F samples. The first source for their formation was β-CN, and for the most part originated from AA regions 70 to 120 and 200 to 220. Higher presence of β-CN-derived peptides in hard cheese with respect to those deriving from other caseins was reported by Singh et al. (1995) in Cheddar and by Faccia et al. (2012) in ripened Cacioricotta, whereas Ferranti et al. (1997) reported α_{S1}-CN as the main source of low MW peptides in 21-mo-old Grana Padano. It must be underlined that the peptide profile of mature hard cheeses depends on a huge number of variables, including the ripening time, gross composition and weight of the cheese, type of rennet used, and salt concentration, and making a comparison among different types is very difficult.

Lipolysis and VOC Analysis

The quantification of FFA gave information about the status of the lipolysis process (Table 5). Different from free AA, the total FFA content was not significantly different between the 2 cheese types. Nevertheless, fondaco ripening resulted in formation of higher amounts of short-chain (C4–C8) and medium-chain (C10–C15) fatty acids, with an increase with respect to the control of about 40% and 21%, respectively. As for the single compounds, the most relevant differences regarded the concentration of butyric (C4), capric (C10), and linoleic (C18:2 *cis*-6) acids. Interestingly, 2 "uncommon" compounds, pentadecanoic (C15) and elaidic (C18:1 *trans*-9) acids, were detected only in F samples. The results of the VOC analysis are shown in Table 6. Sixty compounds were identified in the entire set of samples, 54 of which were present in C samples, and 57 in F samples. In both cheeses, the most important chemical groups, in order of abundance, were acids, ketones, alcohols, terpenoids, and esters. Acids and terpenoids were more abundant in C samples, whereas F samples contained higher amounts of ketones, alcohols, and esters. Acids were by far the most abundant chemical class in both cheeses, with butanoic and hexanoic acids representing about 79% of the total amount. Alcohols discriminated the samples quite well, as 2-heptanol, 1-hexanol, and 2-nonanol were higher in F samples, whereas 2-butanol and 2,3-butanediol were higher in C samples. In addition, 1-pentanol was only detected in F samples. Among esters and ketones, ethyl caproate, hexyl acetate, 2-nonanone, and 2-heptanone were the major characterizing compounds of F samples. Among the less abundant groups, alkanes and alkenes were more represented in C samples. Finally, some VOC were not in common between the 2 cheese groups; other than 1-pentanol, the C samples did not contain 2,2,4,6,6-pentamethylheptane, amyl acetate, heptyl acetate, and 5-hepten-2-1, 6 methyl, whereas the F samples did not evidence the presence of octane and 3-octene.

PCA and Pearson Correlation

The microbiological counts and the low MW metabolites (VOC, free amino acids, FFA) were included in a data set for multivariate statistical analysis (Figure 3). The 2 factors extracted from the PCA analysis

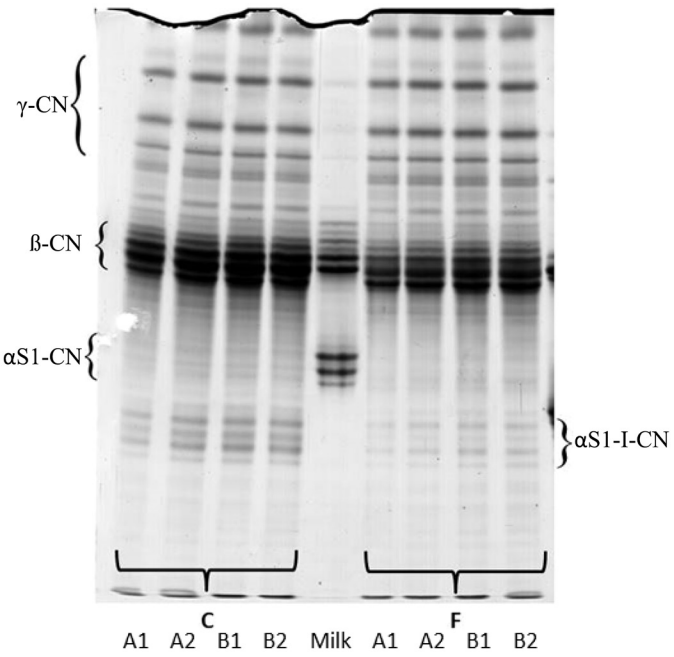

Figure 1. Urea-PAGE pattern of Canestrato di Moliterno cheeses ripened in controlled maturation room batches (C; A1, A2, B1, B2) and in fondaco batches (F; A1, A2, B1, B2).

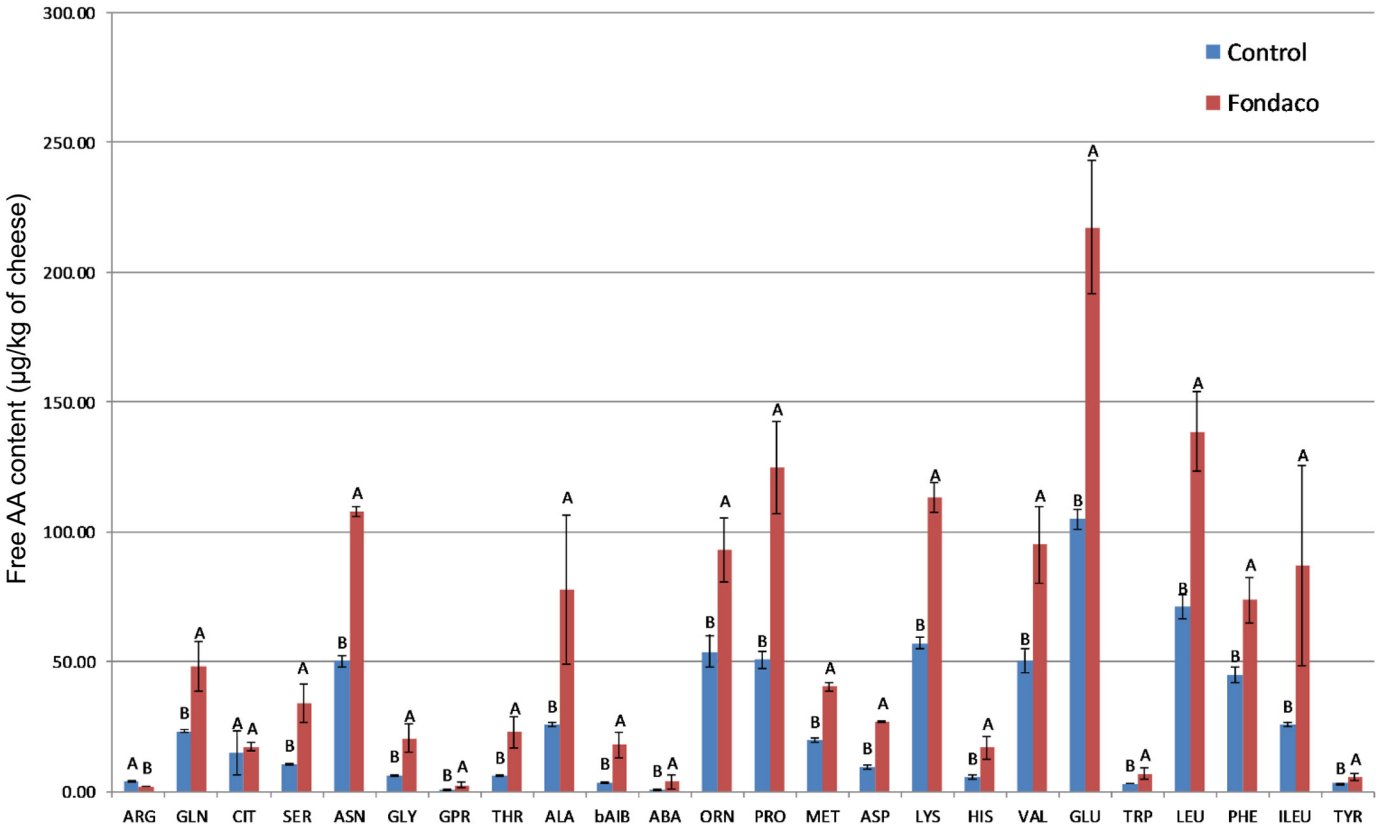

Figure 2. Free AA content of control and fondaco cheeses. Letters (A, B) indicate a significant difference in AA content between control and fondaco cheeses ($P < 0.05$; error bars indicate SE).

explained 99.4% of the variance, with a clear separation of F and C samples along principal component 1 (87.17% of variability explained). On the other hand, principal component 2 only explained 7.27% of variability, evidencing a slight separation of the 2 replicates of each type of cheese. The discrimination between the 2 cheeses was attributable to almost all parameters applied as follows: total and single FAA (except for Arg), single FFA (except for C18:1 *cis*-9), count of enterococci, and several classes of VOC (esters, ketones, alcohols, and lactones) characterized the cheese ripened in fondaco. Lactones, C15, C18, C6, and C18:1 *trans*-9 fatty acids showed a weak correlation. On the other hand, the counts of the other microbial groups, volatile alkanes, alkenes, aromatic compounds, terpenoids, and acids, were strongly correlated with the cheese ripened in the artificial room.

Figure 4 shows the Pearson correlation map including the sensory (only considering taste and aroma attributes) and the chemical data. In the figure, the variables that present strong correlation are in bright green or red (positive and negative correlations, respectively), whereas dull colors represent a less strong correlation based on the Pearson coefficient interval. For control cheese, all sensory descriptors resulted to have a strong positive correlation with VOC (average r = 0.866); the same was found for AA but with lower correlation coefficient (average r = 0.50). In the same way, FFA were positively correlated with all the sensory perceptions except for fatty acids from C10 to C18:1 *trans*-9. Also, for fondaco cheese, the sensory attributes had a strong positive correlation with VOC, but the correlation coefficient of AA was higher than in control (r = 0.86), except for "acid." Short-chain FFA (C6:0 and C8:0) were positively correlated with all the descriptors, in particular with the "pungent" descriptor that was indicated as much more intense by the panelists than in control cheese. Also, FFA with carbon chains >8 had positive correlations with the sensory descriptors except for C16:0, C18:1 *trans*-9, and C18:2 *cis*-9.

DISCUSSION

The present investigation allowed us to assess the presence of differences in Canestrato di Moliterno cheeses ripened in traditional-nonconventional (fondaco cellar) or artificial conditions (controlled ripening room), and to understand the role of the cellar in de-

Table 3. Peptides (mg/kg of cheese) detected in the water-soluble fraction of Canestrato di Moliterno cheeses ripened in controlled maturation room (C) and fondaco (F), with corresponding possible fragment identities and quantification (RT = retention time)

Fragment	Mother protein	Peptide sequence	$-\log_{10}$ *P*-value	Mass	Mass accuracy	*m*/*z*	z	RT	C, mg/kg		F, mg/kg	
									Mean	SD	Mean	SD
60–68*	α_{S2} goat	VRNANEEEY	20.16	1,122.4941	330.8	562.44	2	1.09	0.76	0.01	0.44	0.02
66–76*	α_{S1} goat/sheep	DQAMEDAKQMK	35.51	1,293.5693	−513.2	647.46	2	1.14	17.77	1.02	13.51	1.74
59–68*	α_{S2} goat	VVRNANEEEY	28.16	1,221.5625	111.2	611.86	2	1.27	0.70	0.03	0.29	0.01
62–68*	α_{S2} goat	NANEEEY	21.98	867.3246	119.5	868.44	1	1.38	1.03	0.06	0.51	0.03
56–67*	α_{S1} sheep	SKDIGSESIEDQ	40.22	1,306.5889	86.6	654.36	2	1.94	0.45	0.01	0.74	0.06
56–65*	α_{S1} sheep	SKDIGSESIE	28.44	1,063.5033	227.7	532.88	2	1.98	0.37	0.01	0.44	0.04
32–37*	α_{S1} goat/sheep	NENLLR	23.49	757.4082	52.5	758.46	1	2.49	0.48	0.02	0.31	0.01
58–67*	α_{S1} sheep	DIGSESIEDQ	33.77	1,091.4618	57.1	546.77	2	4.53	0.34	0.02	0.91	0.04
126–135	α_{S1} goat/sheep	IVPKSAEEQL	25.86	1,112.6077	61.2	557.35	2	4.96	0.64	0.03	0.59	0.13
192–202	β goat/sheep	AVPQRDMPIQA	29.5	1,224.6284	291.5	613.50	2	5.92	0.30	0.01	0.29	0.02
99–108*	α_{S1} goat/sheep	EDVPSERYLG	25.07	1,163.5458	274.7	582.94	2	6.05	0.34	0.01	0.57	0.07
181–190*	β goat/sheep	SQPKVLPVPQ	23.99	1,091.6339	541.9	547.12	2	6.17	1.28	0.09	0.71	0.08
125–135*	α_{S1} goat/sheep	EIVPKSAEEQL	24.8	1,241.6503	109.5	621.90	2	6.58	1.22	0.04	0.99	0.15
99–107*	α_{S1} goat/sheep	EDVPSERYL	23.22	1,106.5244	344.4	554.46	2	6.74	0.75	0.04	2.12	0.33
179–190*	β goat/sheep	SLSQPKVLPVPQ	30.09	1,291.75	216.6	647.02	2	7.59	0.69	0.03	0.43	0.05
100–109*	α_{S1} goat/sheep	DVPSERYLGY	27.05	1,197.5665	249.6	599.94	2	7.69	0.53	0.02	0.81	0.15
29–37*	α_{S1} sheep	EVLNENLLR	25.67	1,098.6033	90.8	550.36	2	7.8	0.56	0.03	0.41	0.03
120–129	α_{S1} goat/sheep	NVPQLEIVPK	36.99	1,135.66	392.2	569.06	2	8.22	1.36	0.10	2.58	0.44
99–112*	α_{S1} goat/sheep	EDVPSERYLGYLEQ	25.22	1,696.7944	47.6	849.44	2	9.44	0.24	0.00	0.29	0.01
100–112*	α_{S1} goat/sheep	DVPSERYLGYLEQ	30.89	1,567.7518	114.5	784.97	2	9.46	0.38	0.01	0.64	0.10
100–111*	α_{S1} goat/sheep	DVPSERYLGYLE	22.9	1,439.6932	314.1	721.08	2	9.69	0.36	0.02	0.46	0.04
30–37*	α_{S2} goat	NIFQEIYK	22.91	1,053.5494	46.3	527.81	2	9.71	0.33	0.01	0.29	0.01
40–48*	α_{S1} goat/sheep	VAPFPEVFR	22.86	1,060.5706	100.1	531.35	2	9.86	0.57	0.04	0.27	0.01
126–134*	β goat/sheep	FPKYPVEPF	21.67	1,122.575	154.9	562.38	2	9.87	0.72	0.02	0.89	0.08
207–219	β goat/sheep	QEPVLGPVRGPFP	30.22	1,391.7561	337.2	697.12	2	9.88	0.49	0.04	0.48	0.04
208–219*	β goat/sheep	EPVLGPVRGPFP	21.45	1,263.6975	322.8	633.06	2	9.89	0.43	0.05	0.59	0.07
100–110	α_{S1} goat/sheep	DVPSERYLGYL	23.58	1,310.6506	255.5	656.50	2	10.19	0.28	0.01	0.29	0.02
190–197	α_{S2} goat*	FAWPQYLK	25.13	1,051.5491	491	527.04	2	10.25	7.01	0.99	10.22	1.79
206–209*	β goat/sheep	YQEPVLGPVRGPFP	41.68	1,554.8193	100.4	778.50	2	10.38	0.65	0.05	0.55	0.04
104–113	α_{S1} goat/sheep	ERYLGYLEQL	31.88	1,282.6558	288.3	642.52	2	10.39	0.59	0.03	0.66	0.12
211–220*	β goat/sheep	LGPVRGPFPI	20.23	1,051.6178	106.3	526.87	2	10.4	6.98	0.98	10.04	2.69
30–38*	α_{S1} sheep	VLNENLLRF	28.55	1,116.6292	163.5	559.41	2	10.46	1.44	0.29	0.29	0.02
103–113	α_{S1} goat/sheep	SERYLGYLEQL	27.18	1,369.6877	222.5	686.00	2	10.5	0.53	0.02	0.62	0.14
39–48*	α_{S1} goat/sheep	VVAPFPEVFR	29.61	1,159.6389	91	580.88	2	10.57	0.69	0.06	0.31	0.02
210–220	β goat/sheep	VLGPVRGPFPI	34.03	1,150.6862	468.7	576.62	2	10.76	0.49	0.01	0.45	0.06
212–221*	β goat/sheep	GPVRGPFPIL	22.11	1,051.6178	138.5	526.89	2	10.78	6.93	0.98	10.07	2.67
29–38*	α_{S1} sheep	EVLNENLLRF	23.49	1,245.6718	380.3	624.08	2	10.96	0.37	0.00	0.28	0.01
101–113	α_{S1} goat/sheep	VPSERYLGYLEQL	25.35	1,565.809	145.6	784.03	2	11.15	0.35	0.01	0.35	0.03
124–134*	β goat/sheep	MPFPKYPVEPF	29.46	1,350.6682	382.9	676.60	2	11.16	0.34	0.01	0.29	0.01
207–220	β goat/sheep	QEPVLGPVRGPFPI	35.5	1,504.8401	254.6	753.62	2	11.36	0.97	0.11	0.81	0.10
41–47*	α_{S1} goat/sheep	APFPEVF	21.17	805.401	63.3	806.46	1	11.41	4.40	0.30	0.70	0.07
212–222	β goat/sheep	GPVRGPFPILV	26.14	1,150.6862	94.1	1,151.80	1	11.64	0.42	0.04	0.47	0.13
206–220*	β goat/sheep	YQEPVLGPVRGPFPI	38.91	1,667.9034	241	835.16	2	11.69	1.09	0.04	0.69	0.07
99–113*	α_{S1} goat/sheep	EDVPSERYLGYLEQL	36.79	1,809.8784	105.9	906.04	2	11.82	0.46	0.01	0.53	0.05
40–47*	α_{S1} goat/sheep	VAPFPEVF	20.14	904.4694	183	905.64	1	11.95	4.33	0.55	0.63	0.07
100–113	α_{S1} goat/sheep	DVPSERYLGYLEQL	27.1	1,680.8358	61.5	841.48	2	12.04	1.10	0.04	1.28	0.28
29–39	α_{S1} sheep	EVLNENLLRFV	33.3	1,344.7401	−204.3	673.24	2	12.19	0.25	0.00	0.24	0.01
210–221*	β goat/sheep	VLGPVRGPFPIL	29.71	1,263.7703	133.5	632.98	2	12.22	0.74	0.07	0.45	0.09
195–214	α_{S1} sheep	SDIPNPIGSENSGKITMPLW	24.89	2,155.062	20.2	1,078.56	2	12.26	0.24	0.00	0.23	0.00

Continued

Table 3 (Continued). Peptides (mg/kg of cheese) detected in the water-soluble fraction of Canestrato di Moliterno cheeses ripened in controlled maturation room (C) and fondaco (F), with corresponding possible fragment identities and quantification (RT = retention time)

Fragment	Mother protein	Peptide sequence	$-\log_{10}$ *P*-value	Mass	Mass accuracy	*m*/*z*	z	RT	C, mg/kg		F, mg/kg	
									Mean	SD	Mean	SD
99–108*	β goat/sheep	VPPFLQPEIM	24.06	1,169.6155	64.9	1,170.70	1	12.4	0.33	0.01	0.66	0.05
196–214	α_{S1} sheep	DIPNPIGSENSGKITMPLW	32.06	2,068.0298	−388.9	1,034.62	2	12.55	0.22	0.00	0.23	0.01
207–221*	β goat/sheep	QEPVLGPVRGPFPIL	30.79	1,617.9242	96.6	810.05	2	12.61	5.57	0.71	3.20	0.57
216–222	β goat/sheep	GPFPILV	21.05	741.4425	148.7	742.56	1	12.71	0.83	0.07	0.91	0.16
39–47*	α_{S1} goat/sheep	VVAPFPEVF	24.06	1,003.5378	47.8	1,004.59	1	12.72	2.60	0.44	0.56	0.07
208–221	β goat/sheep	EPVLGPVRGPFPIL	27.08	1,489.8656	108.3	746.02	2	12.78	5.60	1.09	5.94	1.15
210–220	β goat/sheep	VLGPVRGPFPILV	32.2	1,362.8386	105	682.50	2	12.89	2.02	0.12	1.80	0.45
108–116	α_{S1} goat/sheep	GYLEQLLRL	25.74	1,103.6339	391	553.04	2	12.94	0.30	0.02	0.29	0.02
206–221*	β goat/sheep	YQEPVLGPVRGPFPIL	42.71	1,780.9875	88.7	891.58	2	13	1.52	0.08	0.76	0.09
207–222*	β goat/sheep	QEPVLGPVRGPFPILV	39.6	1,716.9926	99.2	859.59	2	13.21	28.62	3.03	15.70	3.87
208–222	β goat/sheep	EPVLGPVRGPFPILV	37.1	1,588.9341	123.7	795.57	2	13.26	20.11	3.40	20.40	5.89
206–222*	β goat/sheep	YQEPVLGPVRGPFPILV	49.99	1,880.0559	121.5	941.15	2	13.5	3.36	0.24	1.66	0.25
77–92*	β goat/sheep	FTGPIPNSLPQNILPL	22.41	1,719.9559	110.2	861.08	2	13.64	26.92	2.89	14.64	3.66
97–108*	β sheep	VVVPPFLQPEIM	25.11	1,367.7522	37.2	684.91	2	13.9	0.43	0.01	0.98	0.15
205–222	β goat/sheep	LYQEPVLGPVRGPFPILV	44.99	1993.14	83.5	997.66	2	13.92	0.32	0.02	0.30	0.01
93–108*	β goat/sheep	TQTPVVVPPFLQPEIM	28.41	1,794.959	551.3	898.98	2	13.99	0.89	0.05	1.27	0.13
93–108	β goat/sheep	TQTPVVVPPFLQPEIM(+15.99)	28.97	1,810.9539	105.8	906.58	2	14.02	0.36	0.05	0.42	0.03
94–108	β goat/sheep	QTPVVVPPFLQPEIM	27.79	1,693.9113	19.7	847.98	2	14.1	0.28	0.02	0.31	0.01
95–108*	β goat/sheep	TPVVVPPFLQPEIM	30.5	1,565.8527	120.7	784.03	2	14.17	0.74	0.35	1.31	0.13
95–108	β goat/sheep	TPVVVPPFLQPEIM(+15.99)	30.87	1,581.8477	36.6	791.96	2	14.22	0.30	0.05	0.29	0.01
38–47	α_{S1} goat/sheep	FVVAPFPEVF	35.92	1,150.6062	26.2	576.33	2	14.36	0.24	0.01	0.21	0.00
75–92*	β goat/sheep	YPFTGPIPNSLPQNILPL	27.69	1980.072	506.1	991.54	2	14.53	0.36	0.04	0.52	0.03
204–222	β goat/sheep	LLYQEPVLGPVRGPFPILV	41.29	2,106.2241	76.5	1,054.20	2	14.58	0.23	0.00	0.23	0.00
197–205*	β goat/sheep	DMPIQAFLL	26.15	1,046.547	12.3	524.29	2	14.63	0.29	0.01	0.23	0.01
74–92	β goat/sheep	VYPFTGPIPNSLPQNILPL	36.66	2,079.1404	490.1	1,041.09	2	15.13	0.30	0.01	0.29	0.02
73–92	β sheep	LVYPFTGPIPNSLPQNILPL	31.34	2,192.2244	55.3	1,097.18	2	15.5	0.21	0.00	0.22	0.00
72–92*	β goat/sheep	SLVYPFTGPIPNSLPQNILPL	25.98	2,279.2566	435.6	1,141.13	2	15.61	0.22	0.01	0.26	0.01
203–222	β goat/sheep	FLLYQEPVLGPVRGPFPILV	22.67	2,253.2925	468.6	752.46	3	15.63	0.24	0.01	0.23	0.01

*Indicates significantly different concentration between C and F ($P < 0.05$).

Table 4. Number of peptides identified in the water-soluble fraction of Canestrato di Moliterno cheeses ripened in controlled maturation room (C) and fondaco (F), grouped by mother protein from which they are derived and by molecular weight (MW)

Item	Total number	Concentration (as mg/kg ± SD)		Concentration (as % water-soluble nitrogen ± SD)	
		C	F	C	F
Mother protein					
β-casein	39	398.92 ± 90.76	398.92 ± 90.76	1.42 ± 0.16[a]	0.88 ± 0.20[b]
α_{S1}-casein	33	179.00 ± 10.88[a]	133.24 ± 16.92[b]	0.52 ± 0.04[b]	0.30 ± 0.04[a]
α_{S2}-casein	5	39.32 ± 2.76	46.96 ± 11.28	0.11 ± 0.00	0.10 ± 0.01
Total	77	708.60 ± 41.22[a]	579.12 ± 63.54[b]	2.05 ± 0.13[a]	1.28 ± 0.14[b]
MW range					
>2,000 Da	7	6.64 ± 0.16	6.72 ± 0.20	0.02 ± 0.00	0.02 ± 0.00
1,500–2,000 Da	21	378.32 ± 44.8[a]	263.64 ± 62.92[b]	1.10 ± 0.12[a]	0.58 ± 0.12[b]
1,000–1,500 Da	44	279.32 ± 27.48	296.48 ± 53.08	0.81 ± 0.08	0.66 ± 0.12
<1,000 Da	5	44.32 ± 4.00[a]	12.28 ± 1.40[b]	0.13 ± 0.01[a]	0.03 ± 0.00[b]
Total	77	708.60 ± 41.22[a]	579.12 ± 63.54[b]	2.05 ± 0.13[a]	1.28 ± 0.14[b]

[a,b]Values of concentration in the same row, for each of the two measured units, bearing different letters, are different at $P < 0.05$.

termining the microbiological, chemical, and sensory characteristics. The higher values of the ripening index and pH supplied first evidence that fondaco cheese underwent faster maturation than control cheese. The results of the microbiological analyses supported this hypothesis because the prevalence of enterococci (an important representative of the nonstarter lactic acid bacteria group) over starter lactic acid bacteria (**LAB**) populations is a typical feature of an advanced ripening stage (Anastasiou et al., 2007; Colombo et al., 2009; Gobbetti et al., 2015). This is because enterococci are able to survive under the adverse conditions that form in cheese as ripening proceeds (i.e., low water activity, high salt concentration, lack of carbohydrates sources), whereas starter LAB tend to undergo to cell lysis (Crow et al., 1995; Serio et al., 2010). Despite their common presence in artisanal cheeses, enterococci have not yet received "generally recognized as safe" (GRAS) status. Further evidence of accelerated ripening in fondaco was derived from the proteolysis study. The more intense casein degradation and higher level of free AA clearly indicated that both primary and secondary proteolysis proceeded faster. In addition to this, the lower concentration of the peptides found by LC-MS analysis suggested that peptidolysis also reached a more advanced level in fondaco cheese. In fact, the ratio between the concentrations of total peptides and of total FAA was 0.42 in F and 1.10 in C samples. The value of such ratios derives from the balance between proteasic and peptidasic activity that, in turn, depends on the composition, abundance, and metabolic status of microbiota. As such, it is likely that higher peptidolysis was connected to faster lysis of starter LAB under the ripening conditions of fondaco because enterococci have low peptidasic activity (Sarantinopoulos et al., 2001).

As for lipolysis, the absence of significant differences in the total FFA amounts was not consistent, with accelerated ripening found in the proteolysis study. It is known that triglycerides degradation in hard cheeses is caused by indigenous and exogenous lipases, as well as from the lipolytic activity of microorganisms. The results suggested that the environmental conditions of the 2 ripening rooms did not significantly influence the lipase enzymes activity. As for the role of microorganisms, the weak lipolytic activity of starter LAB (Collins et al., 2003) and the limited difference in the counts of enterococci, which are much more lipolytic than LAB (Sarantinopoulos et al., 2001), might have accounted for the similar intensity of lipolysis. Nevertheless, the qualitative differences observed in the FFA patterns are worth deepening because they appear as potentially useful in discriminating the cheeses.

Table 5. Free fatty acid content (mg/g of fat, ±SD) of cheeses ripened in controlled maturation room (C) and fondaco (F)

Item[1]	C	% of total	F	% of total
C4:0	0.75±0.19[b]	3.78	1.09±0.14[a]	4.93
C6:0	0.19±0.23	0.96	0.39±0.01	1.76
C8:0	0.28±0.05	1.41	0.43±0.05	1.94
C10:0	0.93±0.05[b]	4.69	1.26±0.05[a]	5.70
C12:0	0.55±0.07	2.77	0.68±0.01	3.08
C14:0	1.10±0.10	5.54	1.41±0.06	6.38
C15:0	0.00±0.00[b]	—	0.14±0.20[a]	0.63
C16:0	6.31±0.90	31.79	7.08±0.16	32.02
C18:0	5.05±0.44	25.44	5.49±0.12	24.83
C18:1n9t	0.00±0.00[b]	—	0.47±0.66[a]	2.13
C18:1n9c	4.13±0.01	20.81	2.97±2.90	13.43
C18:2n6c	0.56±0.01[b]	2.82	0.69±0.01[a]	2.71
Total	19.85±1.15	100	22.11±3.68	100
SCFA	1.22±0.48[b]	6.15	1.91±0.10[a]	8.64
MCFA	2.57±0.24[b]	12.95	3.49±0.07[a]	15.78
LCFA	16.06±1.39	80.90	16.70±3.66	75.53

[a,b]Values bearing different superscripts in the same row are statistically different at $P < 0.05$.

[1]SCFA = short-chain fatty acid; MCFA = medium-chain fatty acid; LCFA = long-chain fatty acid.

Table 6. Volatile organic compounds (VOC) found in cheese samples[1]

VOC	Concentration, μg/kg of cheese				
	C	F	R	LRI	LRI ref
Acids					
Acetic acid	7.73[a]	7.30[b]	MS, LRI	1,438	1,480
Propionic acid	0.25[a]	0.22[a]	MS, LRI	1,524	1,554
Isobutyric acid	0.24[a]	0.14[a]	MS	1,552	
Butanoic acid	32.71[a]	29.30[b]	MS, LRI	1,610	1,630
Isovaleric acid	0.48[a]	0.27[a]	MS	1,653	
Hexanoic acid	26.22[a]	23.83[b]	MS	1,828	
Heptanoic acid	0.10[a]	0.10[a]	MS	1,932	
Octanoic acid	4.75[a]	4.56[a]	MS	2,036	
Nonanoic acid	0.09[a]	0.04[b]	MS	2,113	
Decanoic acid	1.72[a]	1.46[b]	MS	2,198	
Total	74.26[a]	67.20[b]			
Alcohols					
Ethanol	0.34[a]	0.55[a]	MS, LRI	906	932
2-Butanol	2.30[a]	0.85[b]	MS, LRI	998	975
2-Pentanol	0.75[a]	0.79[a]	MS, LRI	1,105	1,142
1-Butanol	0.36[a]	0.31[a]	MS, LRI	1,129	1,152
1-Pentanol	0.00[b]	0.06[a]	MS, LRI	1,230	1,256
2-Heptanol	0.68[b]	2.28[a]	MS, LRI	1,294	1,334
1-Hexanol	0.44[b]	0.79[a]	MS, LRI	1,329	1,354
2-Nonanol	0.18[b]	0.42[a]	MS, LRI	1,491	1,528
1-Octanol	0.16[a]	0.15[a]	MS, LRI	1,531	1,561
2,3-Butanediol	0.45[a]	0.15[b]	MS	1,561	
Total	5.65[b]	6.35[a]			
Ketones					
Acetone	0.04[a]	0.03[a]	MS, LRI	812	814
2-Heptanone	3.32[b]	4.36[a]	MS, LRI	1,167	1,185
2-Octanone	0.10[b]	0.17[a]	MS, LRI	1,265	1,251
Acetoin	0.16[a]	0.07[a]	MS, LRI	1,277	1,289
5-Hepten-2-one, 6 methyl	0.00[b]	0.03[a]	MS, LRI	1,316	1,340
2-Nonanone	7.02[b]	12.14[a]	MS, LRI	1,368	1,394
8-Nonen-2-one	0.17[a]	0.35[a]	MS	1,422	
2-Undecanone	0.26[a]	0.26[a]	MS, LRI	1,574	1,606
Total	11.07[b]	17.41[a]			
Terpenoids					
p-Menth-2-ene	2.15[a]	1.23[b]	MS	1,092	
α-Myrcene	0.06[a]	0.05[a]	MS, LRI	1,143	1,167
α-Phellandrene	0.41[a]	0.25[a]	MS, LRI	1,149	1,160
D-Limonene	2.62[a]	2.04[b]	MS, LRI	1,183	1,194
Total	5.24[a]	3.57[b]			
Esters					
Ethyl acetate	0.11[a]	0.06[a]	MS, LRI	845	893
Methyl lactate	0.04[a]	0.04[a]	MS	938	
Ethyl butyrate	0.35[a]	0.22[a]	MS, LRI	1,012	1,040
Butyl acetate	0.09[b]	0.35[a]	MS, LRI	1,051	1,077
sec-Butyl butyrate	0.13[a]	0.15[a]	MS, LRI	1,114	1,154
Butyl propionate	0.03[a]	0.04[a]	MS, LRI	1,122	1,120
Amyl acetate	0.00[b]	0.04[a]	MS, LRI	1,155	1,180
Butyl butyrate	0.08[b]	0.42[a]	MS, LRI	1,201	1,175
Ethyl caproate	0.76[b]	1.13[a]	MS, LRI	1,215	1,238
Hexyl acetate	0.23[b]	1.00[a]	MS, LRI	1,251	1,269
Heptyl acetate	0.00[b]	0.04[a]	MS, LRI	1,350	1,370
Butyl caproate	0.04[b]	0.25[a]	MS, LRI	1,389	1,420
Ethyl caprylate	0.12[a]	0.22[a]	MS, LRI	1,410	1,438
Total	1.97[b]	3.95[a]			
Alkanes					
Hexane	0.16[a]	0.06[a]	MS, LRI	600	600
Cyclopentane	0.04[a]	0.02[a]	MS	638	
Isooctane	0.05[a]	0.02[b]	MS	675	
Heptane	0.10[a]	0.04[a]	MS, LRI	700	700
Octane	0.03[a]	0.00[b]	MS, LRI	800	800
2,2,4,6,6-Pentamethylheptane	0.00[b]	0.02[a]	MS	939	
Decane	0.06[a]	0.03[b]	MS, LRI	975	1,000
Total	0.45[a]	0.20[b]			

Continued

Table 6 (Continued). Volatile organic compounds (VOC) found in cheese samples[1]

VOC	Concentration, µg/kg of cheese		R	LRI	LRI ref
	C	F			
Aromatic compounds					
Toluene	0.08[a]	0.04[b]	MS, LRI	1,022	1,040
Styrene	0.23[a]	0.20[a]	MS, LRI	1,241	1,261
Total	0.31[a]	0.24[b]			
Nitrogen containing compounds					
Methane, isocyano-	0.09[a]	0.06[b]	MS	986	
Total	0.09[a]	0.06[b]			
Sulfur compounds					
Dimethyl sulfone	0.08[a]	0.05[b]	MS	1,898	
Total	0.08[a]	0.05[b]			
Lactones					
Caprolactone	0.04[a]	0.06[a]	MS	1,693	
Total	0.04[a]	0.06[a]			
Aldehydes					
Valeraldehyde, 3-methyl	0.05[a]	0.04[a]	MS	1,063	
Total	0.05[a]	0.04[a]			
Alkene					
3-Octene	0.03[a]	0.00[b]	MS, LRI	824	846
Total	0.03[a]	0.00[b]			

[a,b]Values in the same row bearing different letters are different at $P < 0.05$.

[1]C = controlled maturation room; F = fondaco; R = identification method; LRI = linear retention index; LRI ref = values taken from Bianchi et al. (2007) and Natrella et al. (2020).

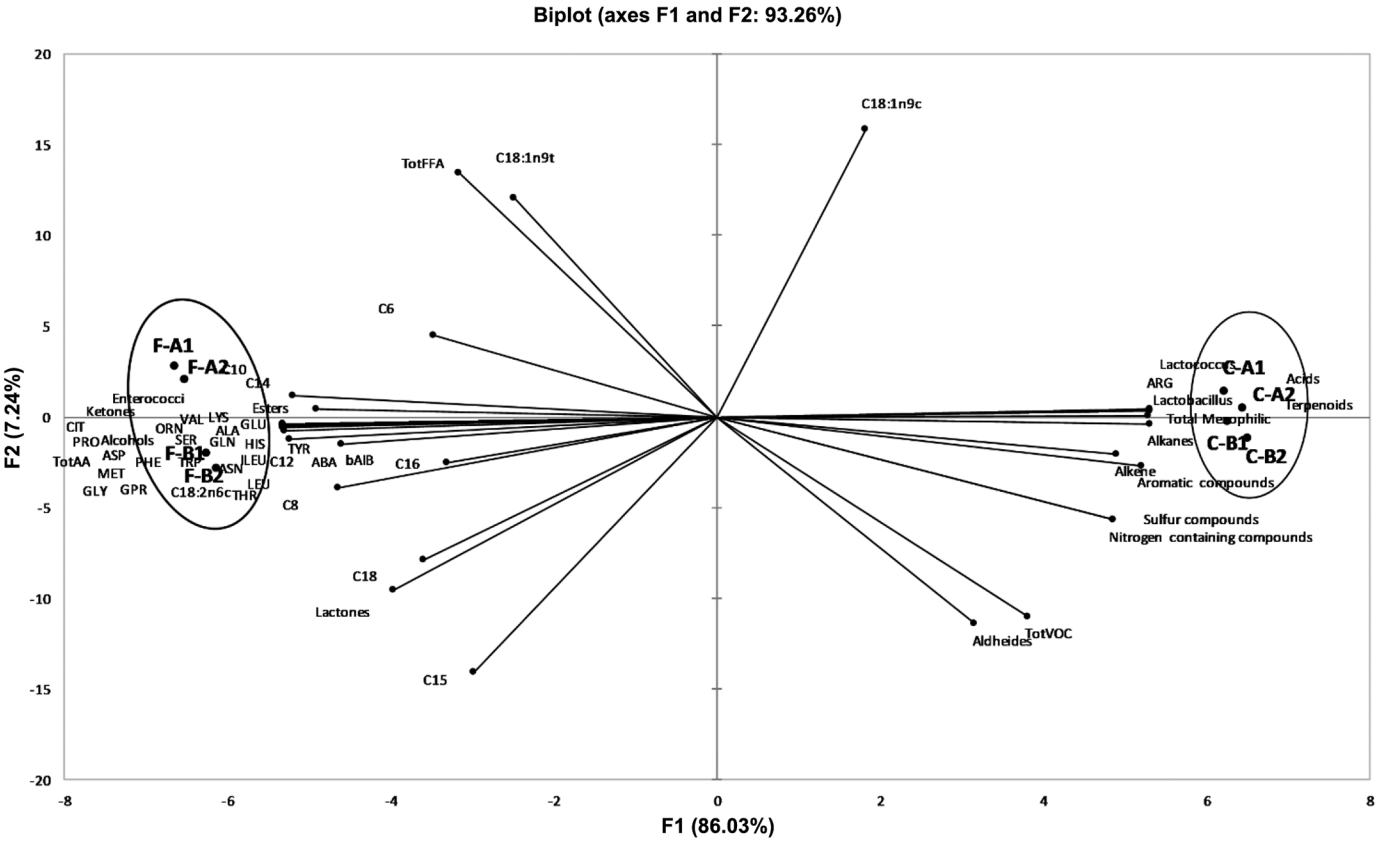

Figure 3. Principal component analysis of control (C; A1, A2, B1, B2) and fondaco (F; A1, A2, B1, B2) cheeses. F1 and F2 = principal components 1 and 2, respectively; TotFFA = total free fatty acids; TotAA = total free amino acids; TotVOC = total volatile organic compounds; C18:1n9t = elaidic acid; C18:1n9c = oleic acid; C18:2n6 = linoleic acid; C6 = hexanoic acid; C8 = octanoic acid; C12 = lauric acid; C14 = mirystic acid; C15 = pentadecanoic acid; C16 = palmitic acid; C18 = stearic acid.

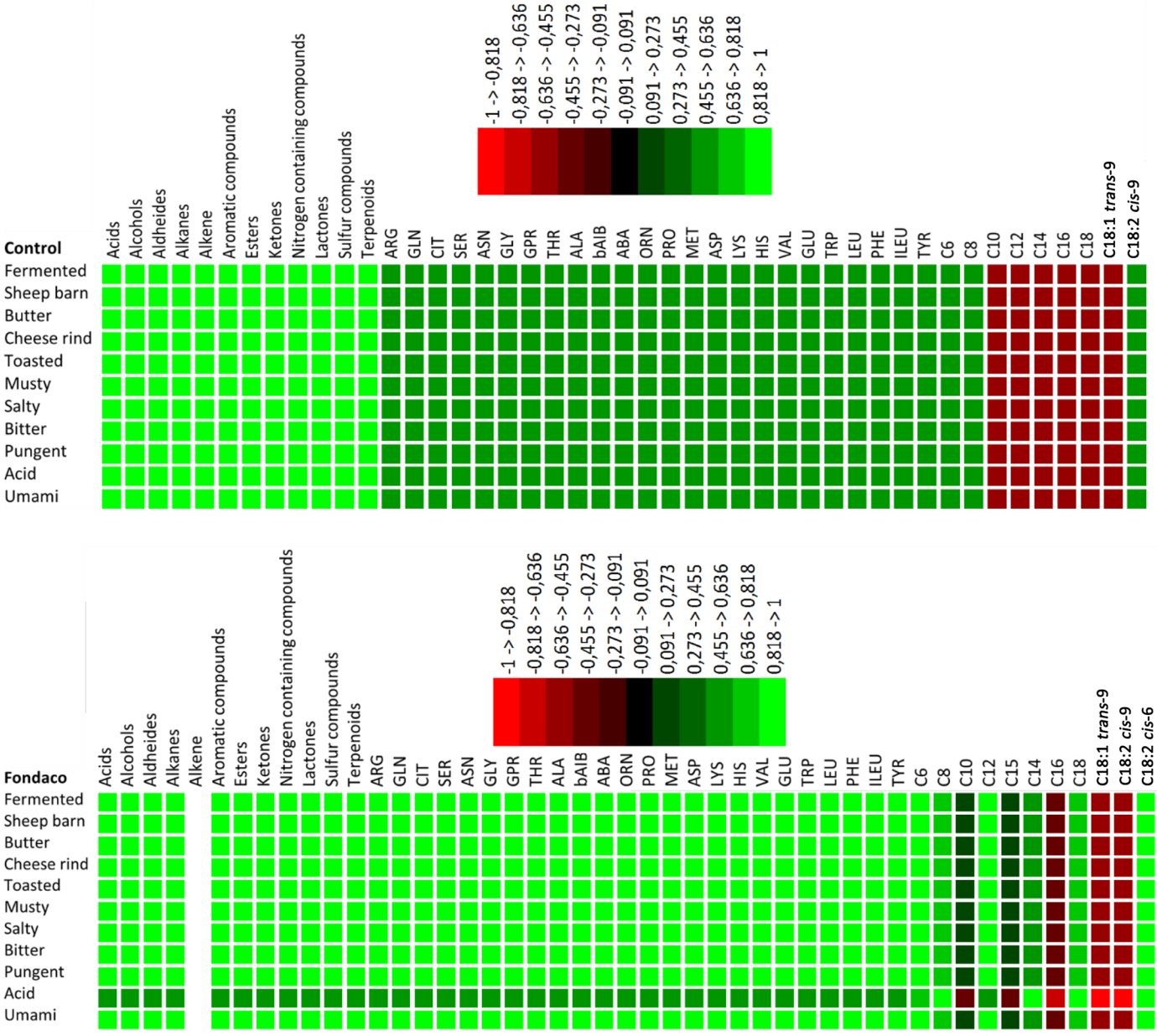

Figure 4. Pearson correlation map including the sensory (taste and aroma attributes) and the chemical data of the cheeses. Scale indicates r-values.

The different sensory characteristics of the 2 types of Canestrato di Moliterno demonstrated that fondaco ripening has a deep influence on the "perceivable quality" of the product. The "rough" and more intense flavor of the cheese ripened in fondaco matched well with the higher concentration of many compounds that are reported to exert strong effects on taste and aroma, such as butyric and caproic acids (Chávarri et al., 1999), ketones, alcohols, esters, and FAA (Collins et al., 2003). On the other hand, the abundance of acids in the VOC profile of the C samples matched well with the higher value of the acid perception reported by the panelists. Our findings agree with those reported in a recent study published by Bettera et al. (2020) on Nostrano Valtrompia Protected Designation of Origin cheese ripened under conventional and nonconventional conditions. In this work, the authors found differences in the characteristics of the cheeses and connected them to the seasonal fluctuations of the temperature in the uncontrolled ripening room. Our investigation did not contemplate constant monitoring of the temperature in fondaco, but the measurements done during the

periodical inspections of the cheeses (always performed in the morning) gave values ranging from a minimum of 14°C in June to a maximum of 17°C in July, versus the constant temperature of 13°C in the artificial room. Consequently, it is highly probable that the differences observed in the present study were also caused, or at least strongly influenced, by temperature fluctuations.

In conclusion, the results obtained demonstrated that ripening Canestrato di Moliterno in fondaco is responsible for the development of particular quality characteristics. Overall, the nonstandardized environmental conditions tended to accelerate maturation, with a related increase in the formation of low MW compounds responsible for flavor. However, this result must be considered within the context of the present experiment, in which the storage in fondaco took place in summer. In this period, the average temperature of the fondaco tends to be higher than that normally present in controlled rooms. As Canestrato di Moliterno is manufactured from January to May, it is likely that different results would be obtained when the cheese is transferred to fondaco in the cold season. In this case, we cannot exclude the possibility that slower maturation occurs, suggesting that the well-known seasonal variability of the cheese quality is caused not only by different quality of the milk but also by the ripening environment. The multiparameter approach allowed us to depict in detail the effect of ripening the cheese under different conditions and could be very useful to validate the authenticity of the product.

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