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Characterisation of Alpine highland pastures located at different altitudes: forage evaluation, chemical composition, *in vitro* digestibility, fatty acid and terpene contents

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Abstract

A survey was conducted over the summer of 2013 on two alpine pastures (P1 and P5), and three mountain pastures (P2, P3 and P4). The aim was to determine the botanical composition, pastoral value (PV), variation in chemical composition, gross energy, *in vitro* true digestibility (IVTD), *in vitro* indigestible neutral detergent fibre, fatty acid (FA) and terpene contents of pastures located at different altitudes. PV is highest in alpine pastures (25.7 and 26.9, for P1 and P5, respectively). Exploitation of pastures is intensive only for P1 and P2. Pastures differ in dry matter (DM), ash, crude protein, lipid, and gross energy content. Lignin content was significantly higher in P3 (125 g/kg DM), whilst in other pastures the variation in its average content (from 73 to 94 g/kg DM) was limited. All pastures had IVTD higher than 725 g/kg with the exception of P3 (659 g/kg). As far as FA content is concerned, in all pastures the most abundant were α-linolenic acid (from 495 to 583 g/kg of total FA) and linoleic acid (from 150 to 222 g/kg of total FA), while palmitic acid and oleic acid significantly differ among pastures. α-pinene, β-pinene and *p*-cymene were the most abundant terpenoids.

Keywords: Pasture; Lipid; Fibrous fractions; Crude protein; *In vitro* digestibility.

Introduction

Soil properties, altitude and climatic conditions influence pasture botanical composition which, in turn, influences palatability, nutritive value and digestibility for grazing ruminants (Collomb et al. 2002; Roukos et al. 2011), conferring unique organoleptic qualities to milk and its products (Viallon et al. 2000; Cornu et al. 2005; Tornambé et al. 2006; Bovolenta et al. 2009; Gorlier et al. 2012). Couvreur et al. (2006) found a linear relationship between the proportion of fresh grass in the cow diet and the rheological and nutritional properties of dairy products.

Several studies focus on the great biodiversity of pastures and on the high contents of terpenes and beneficial fatty acids (FAs) in dairy products derived from grass-fed ruminants, such as conjugated linoleic acid (CLA) and polyunsaturated fatty acid (PUFA) of the n-3 series (Parodi 2004; Dewhurst et al. 2006).

Boufaïed et al. (2003) studied the factors that can affect the FA concentrations of some forage crops and found that PUFA concentrations in forages could be increased by choosing species with higher FA concentrations, by harvesting the crop at an early stage of development and as fresh grass, and by increasing nitrogen fertilization.

Correlation between upland botanical species and chemical composition of dairy products has been considered in different studies, carried out in various European environments such as Alpine sites (Martin et al. 2005; Jayanegara et al. 2011; Gorlier et al. 2012) and Mediterranean sites (Skapetas et al. 2004; Mountousis et al. 2011; Roukos et al. 2011).

In light of this background, to provide valuable support for the natural environment and economic revitalization of local communities and, consequently, to help preserve a typical Italian Alpine food product, such as Plaisentif cheese, a correlation between the botanical composition and the chemical features of pastures has been investigated (Gai et al. 2014). Plaisentif cheese is a traditional bovine cheese, produced in the Chisone and Susa valleys, using raw milk of cows fed with meadow grass. Cheese factories and pastures are located at a minimum altitude of 1400 and 1700 m a.s.l., respectively. The peculiarity of this cheese is the fact that it is produced from pastures during the violet flowering period (from June to July).

The objective of this research was to quantify the variation in chemical composition, *in vitro* digestibility, FA and terpene contents of five Alpine highland pastures located at different altitudes in the Plaisentif cheese production area in the Piedmont Region (NW Italy).

Materials and methods

Pasture sites, sampling and phyto-pastoral analysis

Phyto-pastoral surveys in five pastures located at an altitude range of 1700-2030 m a.s.l. (*Table I*) were carried out. Three of these pastures are located in the Chisone valley and are identified with the following abbreviations: P1, P2, and P3 (Figure 1).

P1 is sited in Selleries, a hamlet in the municipality of Fenestrelle, at over 2000 m a.s.l.. The territory is classified as pasture and stone heap. There is significant rainfall throughout the year: about 946 mm of precipitation falls annually. According to Köppen & Geiger (1936), the climate is classified as temperate-humid with hot summer (Cfb). The average annual temperature in 2013 was 5.7 °C, while in the grazing period the average temperature was 12°C. The warmest month was July, with an average temperature of 15.3°C.

P2 is located in the Usseaux municipality, in the Pian dell'Alpe area, at an average altitude of 1900 m a.s.l.. This territory is classified as meadow and pasture. The Köppen-Geiger climate classification is Cfb. The average annual temperature is 6.8°C, while annual rainfall is 1026 mm. Values for the grazing period in 2013 were the same as indicated for P1.

P3 is sited in the Val Troncea Reserve, in the Pragelato municipality, at over 1900 m a.s.l.. This territory is classified as grazable brushwood, surrounded by larch and swiss pine forests. The Köppen-Geiger climate classification is Cfb. The average annual temperature in 2013 was 4.9°C, while in the grazing period it was 11.5°C. The annual rainfall was 647 mm, while in the grazing period it was 50.8 mm. The warmest month of the year was July, with an average temperature of 14.6 °C.

The other two pastures, P4 and P5, are located in the Susa valley (Figure 1).

P4 is sited in the Cesana municipality, near the San Sicario ski resort, at an average altitude of 1700 m a.s.l. The climate is temperate. According to Köppen & Geiger (1936), the climate is classified as Cfb. The average annual temperature in 2013 was 1.5 °C. Annual rainfall in 2013 was 713.6 mm. The driest month was September, with 16 mm. In the grazing period, rainfall was 315.6 mm, while most precipitation occurred in October, with 155.4 mm. The warmest month of the year was July, with an average temperature of 15.5 °C.

P5 is located the Granges des Alpes area, in the Sestriere municipality, at over 2060 m a.s.l.. The Piedmont Forestry map classifies this territory as pasture. The climate is cold and temperate. According to Köppen & Geiger (1936), the climate is classified as Dfc. There is significant rainfall throughout the year: the average annual rainfall is 1350 mm. The driest month is July, with 89 mm. The most precipitation falls in May, with an average of 128 mm. The 2013 average annual temperature was 3.2°C, while in the grazing period the average temperature was 9.8°C, with 547 mm of rain.

Lying on a metamorphic parent rock, the soils in P1, P2, and P3 belong to the order of Inceptisols, with a cambic subsurface horizon and no accumulation of clays or organic matter. By contrast, the soils in the pastures P4 and P5 belong to the order of Mollisols, and they feature a deep, high-organic-matter, nutrient-enriched surface horizon, shown as mollic epipedon on the Piedmont soil map (IPLA 2007). The grassland was not fertilized at the beginning of the growing season, and only received dairy cow manure from routine grazing during the late summer period.

Vegetation of investigated areas was surveyed using the linear analysis method proposed by Daget & Poissonet (1969) along 20 m transects laid out on representative and homogeneous meadows. A metric ribbon was used to trace these transects and an iron rod was inserted into the turf at 50 cm intervals (40 insertions along each transect). The plants in contact with the iron rod were recorded at each insertion.

Two samples (2 kg each) of herbage for chemical analysis were collected in the transect area of each pasture using the hand-plucking technique (Langlands 1974) for 1 h to simulate grazing. Each combined sample obtained from the grazing simulation was immediately refrigerated until arrival at the laboratory.

Vegetation species were identified with the Pignatti (1982) dichotomous key. Thereafter, the Species Frequency (SF) – the number of times a plant species is present in a given survey – and the Species Contribution (SC) – the ratio between the SF of a considered species and the summation of the SF of all present species – were computed. Finally, Pastoral Values (PV) were determined according to Daget & Poissonet (1972).

Chemical analysis

Each pooled sample of pasture was cuttered and mixed and then an aliquot of 200 g was used according to the AOAC method (1990) to determine dry matter content (#925.40) in duplicate. Another aliquot of 200 g was immediately refrigerated, freeze-dried and then brought to air temperature, ground in a Cyclotec mill (Tecator, Herndon, VA, USA) to pass through a 1 mm screen and stored for qualitative analyses.

Freeze-dried samples were analysed by methods of AOAC (1990) for N (#984.13), and ash (#923.03). Neutral detergent fibre (NDFom), acid detergent fibre (ADFom) and lignin were determined with the Ankom200 Fibre Analyser (Ankom Technology Corp., Macedon, NY, USA), following the Ankom Technology Method and corrected for residual ash. The NDF of herbage samples was analysed without sodium sulfite or α-amylase. The gross energy (GE) was determined using an adiabatic calorimeter bomb (IKA C7000, Staufen, Germany). Lipid content was quantified on freeze-dried samples according to Hara & Radin (1978). All determinations were performed in duplicate.

In vitro digestibility

In vitro digestibility was determined using the Ankom-Daisy procedure following the approach proposed by Robinson et al. (1999). Freeze-dried samples (0.25 ± 0.01 g) were double-weighed into F57 Ankom bags with a pore size of 25 µm, heat-sealed and then placed into an incubation jar. Each jar was a 5 l glass recipient with a plastic lid provided with a single-way valve which prevents the accumulation of fermentation gases, and was filled with 2 l buffered rumen fluid in anaerobic condition and placed into the Daisy^{II} Incubator (Ankom Technology Corp., Fairport, NY, USA). Rumen liquor was collected from rumen contents obtained at a slaughterhouse from cattle (two runs) from the same farm fed a fibre-rich diet (Spanghero et al. 2010). Heat (39°C) and agitation were maintained constant and uniform in the controlled chamber with continuous rotation. After 48 h incubation, the jars were emptied and the bags were gently rinsed and then washed with a neutral detergent solution at 100°C for 1 h and rinsed with distilled water into the Ankom200 Fibre Analyzer so as to remove bacterial cell walls and other endogenous products and incinerated to correct the residual NDF for the residual ash. Residual NDF was used to calculate indigestible NDF as portion of DM (iNDF) as follows:

$$iNDF = NDF\text{-}dNDF$$

$$dNDF = (NDF*NDFD/100)$$

$$NDFD=100-[W_3-(W_1*C_1)]*100/(W_2*NDF)$$

where W_1 is the filter bag weight, W_2 is the sample weight, W_3 is the final weight (filter bag+residue) after *in vitro* and sequential treatment with NDF solution, C_1 is a comparison of the blank filter bag weight after and before digestion treatment and NDF is the neutral detergent fiber content of the sample.

In vitro true digestibility (IVTD) was calculated using the following equation:

$$IVTD=1000-[W_3-(W_1*C_1)]*1000/(W_2*DM),$$

where: W_1 is the filter bag weight, W_2 is the sample weight, W_3 is the final weight (filter bag+residue) after in vitro and sequential treatment with NDF solution, C_1 is a comparison of the blank filter bag weight after and before digestion treatment and DM is the dry matter content of the samples.

Fatty acid analysis

FA analysis was performed on freeze-dried pasture (2 g) according to the method described by Revello Chion et al. (2010). The FA methyl esters in hexane were then injected into a gas chromatograph (GC 1000 DPC; Dani Instruments S.p.A., Cologno Monzese, Italy) equipped with a flame ionisation detector, a programmed temperature vaporizing injection port and a Supelcowax-10 fused silica column (60 m × 0.32 mm, 0.25 µm). The peak area was measured using a Dani DDS 1000 Data Station. Each peak was identified according to pure methyl ester standards (Supelco and Restek Corporation, Bellefonte, PA) and the data were expressed as relative values. The FA composition was expressed as g/kg of total FA.

Terpene analysis

Terpene analysis was carried out on freeze-dried pasture (200 mg) extracted without the use of solvents. Terpene analysis was performed according to the method described by De Noni & Battelli (2008) by means of dynamic headspace extraction (Dani Instruments S.p.A.), gas chromatography-mass spectrometry (Agilent Technology; 500 ml He_2 for 18 min at 65°C). Data were expressed as arbitrary units, as log10 of the peak area of the corresponding selected ion.

Statistical analysis

Univariate analyses. The variability in the chemical composition, FA, terpenes and nutritive value of the pastures were analysed for their statistical significance by means of one-way analysis of variance (ANOVA), using the Statistical Package for Social Science (SPSS 2002), to test the effect of location.

Multivariate analyses. The relationships between the locations were expressed in the form of average clusters, using a Cluster Hierarchical Analysis (CHA; StaBox V6.5, Grimmer Logiciels, Paris), according to Torri et al. (2013), applied directly to the assessment of the botanical features, considering the botanic family frequencies and the main forage groups (grasses, legumes, and forbs). The relationships between the locations and the chemical variables (chemical composition/nutritive value, fatty acids and terpenes) were primarily expressed by means of a distance matrix calculated through several Partial Least Square regressions (WinISI II V1.04, Infrasoft International, ISI State College, PA, USA). The R^2 coefficients, in calibration and cross-validation were both included in the distance matrix. Moreover, CHA dissimilarity elaborations were performed to build a dendrogram in order to establish the average cluster relationships. Finally, in order to correlate the botanic family frequencies of the pastures and each studied chemical parameter, some of the featured relationships were highlighted by using a CHA elaboration of similarities.

Results

Botanical composition of the pastures

A summary of the botanical composition in the five pastures is shown in *Table II*. A total of 64 species, belonging to 21 families, were identified in the total study area. In greater detail, P1 was dominated by two families, *Gramineae* and *Asteraceae* (45% and 22% of SC, respectively), while *Leguminosae* represented only 2.7% of the pasture; P2 was dominated by *Gramineae*, *Asteraceae* and *Leguminosae* (37%, 14% and 10% of SC, respectively); at site P3 *Gramineae*, *Cistaceae* and *Leguminosae* made up 33%, 18%, and 16% of SC, respectively; P4 was composed exclusively of *Gramineae* (60%), *Leguminosae* (31%) and *Asteraceae* (9%); P5 was dominated by *Gramineae*, *Asteraceae* and *Leguminosae* (47%, 22% and 12% of SC, respectively). In the total sampling, the most frequent plant species were: *Achillea millefolium*, *Bromus erectus*, *Carum carvi*, *Dactylis glomerata*, *Festuca rubra*, *Helianthemum nummularium*, *Lotus corniculatus*, *Phleum alpinum*, *Poa alpina*, *Poa trivialis*, and *Trisetum flavescens*.

As far as the botanic families are concerned (Figure 2), pasture P3 appeared to be the most heterogenous site; in fact, the maximum frequencies were recorded for *Apiaceae* and *Cistaceae*, while a reduction in frequency was shown for *Gramineae* (*Table III*). The other pastures appeared heterogenous in the following decreasing sequence: P4, P1, P2 and P5.

Pasture P4 was characterized by a dramatic reduction in forbs, in favour of legumes and grasses (*Table IV*), which can clearly be seen in Figure 3, while the other pastures were at the same level of similarity.

Chemical composition and in vitro digestibility

The chemical composition, gross energy and digestibility of the five pastures are presented in *Table V*. There were significant differences between the various pastures for all nutritional parameters and gross energy. Overall, the highest value of DM and GE was found in P1 and P4, with a DM content of 275.6 and 304.4 g/kg and a GE content of 18.1 and 18.2 MJ/kg DM, respectively. The P3 and P2 showed the lowest ash content ranging from 74.2 to 75.2 g/kg DM, respectively. As regards crude protein content, pastures P1 and P2 showed the highest values (127.5 and 118.4 g/kg DM, respectively), while the lowest values were recorded in pastures P5 and P4 (100.7 and 103.5 g/kg DM, respectively). There were no significant differences between the pastures with regard to NDF and ADF levels, whilst lignin differed. The NDF content of the pastures varied from a low of 588.0 g/kg for P4 to a high of 619.0 g/kg DM for P5. ADF content ranged from 338.6 to 370.0 g/kg. Lignin content was highest in P3 (125.5 g/kg DM), whilst in the other pastures there was limited variation in its average content (72.5–93.9 g/kg DM). All pastures had IVTD above 725 g/kg DM excluding P3 (658.8 g/kg DM), which resulted in higher iNDF values (341.2 g/kg DM).

Fatty acid profile

The pattern of FA in the pastures was characterised by a high proportion of α-linolenic acid (ALA, $C_{18:3\ n-3}$) and linoleic acid (LA, $C_{18:2\ n-6}$) and their sum makes up from 705 to 734 g/kg of total FA (*Table VI*). Palmitic acid (PA, $C_{16:0}$) and oleic acid (OA, $C_{18:1\ n-9}$) significantly differ ($P < 0.05$) between pastures, while there were no significant differences for other FAs.

Terpenes profile

The main terpenes found in all samples were as follows: α-pinene, camphene, β-pinene, δ 3-carene, limonene, *p*-cymene and allo-ocimene. Significant differences only occurred for δ 3-carene, with the highest value in P1 and the lowest value in P3 (*Table VII*). As a consequence, the highest levels of total terpenes (46.97 arbitrary units of log10) was recorded in P1, which is characterised by more forbs and fewer legumes, while the lowest levels of total terpenes were found in P3 and P5 (41.42 and 43.21 arbitrary units of log10, respectively), because these pastures were characterised by the highest amount of legumes.

Multivariate analyses

As far as the distance matrix analysis is concerned (*Table VIII*), considering all the chemical parameters, the terpene content resulted to be the best predictor (R^2cv=0.985) with all the locations being perfectly distinguished. The chemical composition/nutritional value parameters appeared to be intermediate (R^2cv=0.789), while the FA profile was less predictive (R^2cv=0.622). These features were graphically well represented by the CHA clusters reported in Figure 4.
As far as the CHA cluster of the botanic family frequencies and each chemical studied parameter are concerned (Figure 5), *Leguminosae* clearly appeared to be uncorrelated to the FA profile and terpene content. *Gramineae* were linked to crude protein, stearic acid (SA, C18:0) and camphene, respectively. The forb families were linked to several parameters. *Apiaceae*, *Cistaceae*, *Liliaceae*, *Brassicaceae*, *Euphorbiaceae* and *Primulaceae* were linked to lignin, iNDF, and limonene,

respectively. *Rosaceae* and *Valerianaceae* were linked to DM, ADFom, γ-linolenic acid (GLA, C18:3 n−6), and ALA, respectively. *Rubiaceae*, *Ranuncolaceae* and *Dipsaceae* were linked to lipids, NDFom, PA, OA, and LA, respectively. *Asteraceae* were linked to PA and five terpenes, respectively.

Discussion

Mountain and alpine pastures, such as those investigated in the current study, show very high species richness, as other authors have also observed (Kemp et al. 2003; Revello Chion et al. 2010; Jayanegara et al. 2011; Gorlier et al. 2012). This biodiversity has great importance for the sites' ecology, but also for cattle breeding, since animal products obtained in this context are generally characterised by high quality and organoleptic properties (Mountousis et al. 2011). At the same time, the presence of grazing animals improves botanical composition (Borer et al. 2014), provided carrying capacity is not exceeded.

The significant presence of *Asteraceae* and *Leguminosae* increases its attractiveness for cattle and influences the flavor of the cheese (Carpino et al. 2003). The predominance of these two families, besides *Gramineae*, has also been noticed in other studies (Skapetas et al. 2004; De Noni & Battelli 2008). In particular, *Gramineae* have a great resilience and an adaptive ability under grazing, and are more robust than *Leguminosae* and other dicotyledonous plants, with a high potential for regrowth (Skapetas et al. 2004).

PV is highest in alpine pastures (P1 and P5) due to the abundance of *Helictotrichon parlatorei*, *Achillea millefolium*, *Trifolium pratense*, *Phleum pratense*, *Onobrychis viciaefolia*, *Poa pratensis*, *Poa trivialis, Festuca pratensis, Festuca rubra,* and *Dactylis glomerata*, species with a significant Specific Index (Cavallero et al. 2002).

Within the chemical data, NDF with CP content indicate forage quality (Caccamo et al. 2010). Among species of alpine pastures, appreciable proportions of *Trifolium repens* were found in pasture P2 which had the highest CP and ash content. *Trifolium repens* is considered as a species with a high CP value, as demonstrated by Bovolenta et al. (2008), who found a CP value of 202 g/kg DM in a study carried out to investigate changes over time in chemical composition, digestibility and net energy for lactation contents of 12 species of alpine pastures during the vegetative season. The presence of similar NDF content in different pastures reflects the fact that the plants were at the same growth stage. NDF content has been ranked in the same order by other estimated values in the Alpine environment (Revello Chion et al. 2011). The higher level of lignin in pasture P3 resulted in the lowest forage digestibility (IVTD=659 g/kg) and highest indigestible proportion of NDF (341 g/kg). The results confirmed the study by Huhtanen et al. (2006), that identified fiber characteristics as being the most sensitive factor affecting forage nutritive value, since they can explain most of the variation in digestibility.

With regards to pasture FA content, significant variations among pastures were found for PA and OA (*Table VI*): indeed, the abundance of *Polygonum bistorta* in P2, P3 and P5 contributes to higher PA values, according to studies by Collomb et al. (2002), like so *Dactylis glomerata* (plentiful in P2, P4 and P5) and *Taraxacum officinale* (significant in P2 and P3), according to Wyss (2012).

The presence of *Festuca pratensis* (all pastures except P2), *Phleum pratense* (in pasture P5), and *Poa trivialis* (all pastures except P4) was positively correlated with the concentration of SA and PA (Collomb et al. 2002; Gorlier et al. 2012).

Cyperaceae in P2 and *Geraniaceae* in P2 and in P5 explain the higher OA values according to observations by Tejerina et al. (2011) and Gorlier et al. (2012).

In addition, the abundance of the *Asteracea* species (*Achillea millefolium*), in P2 and P5 led to a greater concentration of OA and PA (Nowak et al. 2010).

Taraxacum officinale (especially in P2), *Trifolium pratense* and *Galium verum* (especially in P5) led to high values of LA, according to observations by Collomb et al. (2002) and Wyss (2012).

PUFA contents may increase thanks to the presence of *Leontodon hispidus* and *Lotus corniculatus*, significant in P4, according to the observations by Collomb et al. (2002), and thanks to the interesting presence of *Festuca pratensis* in P1.

According to Bugaud et al. (2000), a decrease in the proportion of grasses and legumes compared to other botanical families (*Apiaceae* and *Geraniaceae*) causes a decrease in LA, and the appearance of rare minor FAs. High-altitude pastures show higher ALA content than fodder plains (Collomb et al. 1999).

The presence of GLA in all pastures is important from a nutritional point of view, because this FA has been shown to have important effects on several aspects of human and animal health (Fan & Chapkin 1998).

Gramineae and *Leguminosae* are characterised by lower contents of terpene components, while dicotyledonous species are more terpene-rich, as observed by various authors such as Collomb et al. (2002) and Battelli et al. (2004). In particular, some species of *Asteraceae*, *Lamiaceae* and *Apiaceae* can modify the terpenoid profile of milk (Mariaca et al. 1997; De Noni & Battelli 2008).

In P1 and P5, the significant presence of *Asteraceae* (22% of SC in both sites), and in particular species such as *Achillea millefolium* (8% and 10% of SC, respectively) entailed the higher presence of terpenes (specifically δ 3-carene and *p*-cymene, as demonstrated by Jaimand & Rezaee 2004) among the pastures studied.

Furthermore, in P5 a significant role is played by *Heracleum sphondylium*, a species of *Apiaceae* rich in α-pinene, camphene, β-pinene and *p*-cymene (Mariaca et al. 1997; Karuppusamy & Muthuraja 2011).

In P2, *Asteraceae* (14% of SC) and, in minimal part, *Apiaceae* (3%) contribute to the terpenic profile.

In P3, the terpenes derived principally from *Apiaceae* (10% of SC), expressly from *Carum carvi*, rich in limonene (Sedláková et al. 2003; Chemat et al. 2005), and in lower quantity from *Asteraceae* (7% of SC), specifically from *Achillea millefolium* and *Taraxacum officinale*.

Lamiaceae were present in all pastures, except in P4, though with a low SC (always under 3%). A significant contribution to the terpenic profile is given by *Acinos arvensis*, rich in allo-ocimene (Soulèles & Katsiotis 1988).

Belonging to other dicotyledonous families, but also significant, are the species *Geranium molle*, containing α-pinene (present in P1 and P2) and *Galium verum*, rich in *p*-cymene (P5) as analysed by Fedele et al. (2004). *Leucanthemum vulgare*, present in all pastures except in P4, also contains *p*-cymene. Although belonging to *Leguminosae*, *Trifolium repens* (present in all pastures, except in P5) is interesting, due to its abundance of camphene (Kitagawa et al. 1976).

Finally, P4 also presented good terpenic values, in spite of the scarceness of dicotyledonous species: in fact, *Dactylis glomerata* (Malecky & Broudiscou 2009) and *Festuca rubra* (Buchin et al. 1999) are rich in α-pinene and β-pinene. As in all pastures, the terpenic profile of P4 is influenced by the well-known *Achillea millefolium*.

Conclusion

The presence of some botanical species in highland pastures, such as *Achillea millefolium*, *Dactylis glomerata*, *Galium verum*, *Lotus cornicolatus*, *Taraxacum officinale* and *Trifolium repens*, boosts intake of molecules such as LA, ALA and GLA among FAs and α-pinene, β-pinene and *p*-cymene among terpenes, that could confer high nutritional properties to the dairy products.

Further studies are necessary to verify whether pasture botanical composition may enhance milk and Plaisentif cheese quality.

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1 Table I. Features of the pastures (P1, P2, P3, P4 and P5) in the 2013 grazing season.

Codes		P1	P2	P3	P4	P5
Animal units (n)		40	90	80	40	120
Grazing period		10/06–20/09	15/05–10/11	10/06–20/09	15/05–20/10	25/05–15/10
Sampling date		15/07/2013	15/07/2013	16/07/2013	18/07/2013	18/07/2013
Grazing area (ha)		60	100	100	100	390
Coordinates	Lat	45°2’50”	45°3’59”	44°57’29”	44°57’45”	44°57’20”
	Long	7°7'13”	7°1’57”	6°56’56”	6°48’55”	6°50’48”
Sampling altitude (m)		2023	1900	1930	1704	2070
Mean rainfall (mm)		58.5	58.5	50.8	63.1	109.4
Mean temperature (°C)		12.0	12.0	11.5	8.9	9.8
Pastoral value		25.7	22.0	23.6	23.1	26.9

Table II. Species frequencies of the pastures (P1, P2, P3, P4 and P5) before exploitation. Floristic nomenclature according to Pignatti (1982).

Family	Specie	P1	P2	P3	P4	P5
Amaranthaceae	*Chenopodium bonus-henricus*		10	1		
Apiaceae	*Anthriscus sylvestris*	2				
Apiaceae	*Carum carvi*	3	6	10		
Apiaceae	*Heracleum sphondylium*					1
Apiaceae	*Plantago media*			1		
Asteraceae	*Achillea millefolium*	6	15	1	2	11
Asteraceae	*Centaurea jacea*					1
Asteraceae	*Centaurea montana*	2				
Asteraceae	*Cirsium arvense*	2				
Asteraceae	*Leontodon autumnalis*		2			1
Asteraceae	*Leontodon hispidus*	1	2	2	5	2
Asteraceae	*Leucanthemum vulgare*	3	3	1		3
Asteraceae	*Solidago virgaurea*					4
Asteraceae	*Taraxacum officinale*	2	5	3		2
Brassicaceae	*Thlaspi perfoliatum*			2		
Caryophyllaceae	*Silene nutans*	5	8			1
Cistaceae	*Helianthemum nummularium*		5	19		
Cyperaceae	*Carex sempervirens*		1			
Dipsacaceae	*Knautia arvensis*					2
Dipsacaceae	*Scabiosa columbaria*					1
Euphorbiaceae	*Euphorbia cyparissias*			2		
Geraniaceae	*Geranium molle*	1	2			
Geraniaceae	*Geranium pratense*					1
Geraniaceae	*Geranium pyrenaicum*		7			
Gramineae	*Agropyron intermedium*				3	
Gramineae	*Agrostis alpina*	1	2			
Gramineae	*Agrostis tenuis*		9	1		
Gramineae	*Brachypodium caespitosum*					1
Gramineae	*Bromus erectus*		15	1		1
Gramineae	*Bromus hordeaceus*					4
Gramineae	*Dactylis glomerata*		18	2	13	13

Gramineae	*Festuca arundinacea*		4			
Gramineae	*Festuca ovina*		1	2		2
Gramineae	*Festuca pratensis*	5		1	1	2
Gramineae	*Festuca rubra*		2	6	5	5
Gramineae	*Helictotrichon parlatorei*	1	2			2
Gramineae	*Phleum alpinum*		1	3	14	
Gramineae	*Phleum pratense*					2
Gramineae	*Poa alpina*	1	2	1	13	
Gramineae	*Poa pratensis*	5	4		1	1
Gramineae	*Poa trivialis*	3	1	2		12
Gramineae	*Poa violacea*	14	2			
Gramineae	*Trisetum flavescens*	1	10	16		5
Lamiaceae	*Acinos arvensis*	2		1		1
Lamiaceae	*Salvia pratensis*		3	1		1
Leguminosae	*Hippocrepis comosa*		1			
Leguminosae	*Lathyrus pratensis*			3		
Leguminosae	*Lotus corniculatus*	1	2		25	4
Leguminosae	*Medicago lupulina*		1			
Leguminosae	*Onobrychis viciaefolia*		8	6		2
Leguminosae	*Trifolium pratense*		2	3		7
Leguminosae	*Trifolium repens*	1	6	5	1	
Liliaceae	*Allium vineale*		1	3		
Orobanchaceae	*Rhinanthus alectorolophus*		13			
Polygonaceae	*Polygonum bistorta*	1	6	4		3
Primulaceae	*Primula veris*			1		
Ranunculaceae	*Ranunculus acris*	1		1		2
Rosaceae	*Alchemilla vulgaris*		2	1		
Rosaceae	*Rubus spp.*	2				
Rubiaceae	*Galium mollugo*	4				
Rubiaceae	*Galium rotundifolium*		1			
Rubiaceae	*Galium verum*					7
Valerianaceae	*Valerianella locusta*	2				

Table III. Botanic family frequencies of the pastures (P1, P2, P3, P4 and P5).

Family	P1	P2	P3	P4	P5
Amaranthaceae	0	10	1	0	0
Apiaceae	5	6	11	0	1
Asteraceae	16	27	7	7	24
Brassicaceae	0	0	2	0	0
Caryophyllaceae	5	8	0	0	1
Cistaceae	0	5	19	0	0
Cyperaceae	0	1	0	0	0
Dipsacaceae	0	0	0	0	3
Euphorbiaceae	0	0	2	0	0
Geraniaceae	1	9	0	0	1
Gramineae	31	73	35	50	50
Lamiaceae	2	3	2	0	2
Leguminosae	2	20	17	26	13
Liliaceae	0	1	3	0	0
Orobanchaceae	0	13	0	0	0
Polygonaceae	1	6	4	0	3
Primulaceae	0	0	1	0	0
Ranunculaceae	1	0	1	0	2
Rosaceae	2	2	1	0	0
Rubiaceae	4	1	0	0	7
Valerianaceae	2	0	0	0	0

Table IV. Main forage groups (as mean percentages of the total number of species) of the pastures (P1, P2, P3, P4 and P5).

	P1	P2	P3	P4	P5	Mean
Grasses	44	37	33	60	47	44.2
Legumes	3	10	16	31	12	14.4
Forbs	53	53	51	9	41	41.4

Table V. Chemical composition (g/kg DM basis), gross energy (GE), *in vitro* true digestibility (IVTD) and *in vitro* indigestible neutral detergent fibre (iNDF) of the pastures (P1, P2, P3, P4 and P5).

	P1	P2	P3	P4	P5	SEM	*P* value
DM (g/kg)	275.6^{b}	232.1^{a}	270.3^{b}	304.4^{c}	256.4^{ab}	6.97	0.001
Ash	59.0^{ab}	75.2^{c}	74.2^{c}	55.9^{a}	62.3^{b}	1.97	0.001
Crude protein	118.4^{bc}	127.5^{c}	112.8^{abc}	103.5^{ab}	100.7^{a}	3.09	0.005
Lipid	24.7^{a}	26.2^{a}	26.6^{a}	25.8^{a}	31.4^{b}	0.83	0.044
NDFom	595.5	592.2	598.3	588.1	619.0	14.5	0.075
ADFom	338.6	361.1	370.0	356.6	347.7	21.6	0.487
Lignin	84.1^{a}	93.9^{ab}	125.5^{b}	72.5^{a}	77.7^{a}	14.7	0.011
GE (MJ/kg DM)	18.1^{b}	17.6^{a}	17.4^{a}	18.2^{b}	18.0^{b}	0.08	0.001
IVTD (g/kg DM)	725.2^{a}	750.4^{a}	658.8^{b}	741.2^{a}	728.5^{a}	23.2	0.007
iNDF (g/kg DM)	275.5^{a}	250.0^{a}	341.2^{b}	258.6^{a}	271.4^{a}	23.6	0.008

^{a,b,c} Means in the same row with unlike superscripts differ ($P < 0.05$).
NDFom: neutral detergent fibre; ADFom: acid detergent fibre.

Table VI. Fatty acid (FA) content (g/kg of total FAs) of the pastures (P1, P2, P3, P4 and P5).

	P1	P2	P3	P4	P5	SEM	*P* value
$C_{16:0}$	84.8[a]	101.6[b]	93.0[ab]	100.2[b]	94.5[b]	1.9	0.005
$C_{18:0}$	14.5	19.3	16.7	19.5	19.7	1.0	0.373
$C_{18:1n-9}$	26.7[a]	56.0[bc]	44.6[abc]	34.6[ab]	61.8[c]	4.4	0.022
$C_{18:2n-6}$	150.2	172.9	155.7	151.1	221.2	11.9	0.257
$C_{18:3n-6}$	34.9	19.9	16.1	31.2	19.7	2.6	0.074
$C_{18:3n-3}$	583.4	535.4	549.7	574.3	494.8	13.5	0.193
Unknown	105.3	94.7	124.2	89.0	88.2	4.0	0.069

[a,b,c] Means in the same row with unlike superscripts differ ($P < 0.05$).

Table VII. Terpene composition of the pastures (P1, P2, P3, P4 and P5).

	P1	P2	P3	P4	P5	SEM	*P* value
α-Pinene *	7.36	7.01	6.44	7.16	7.05	0.14	0.371
Camphene	6.31	6.13	5.95	5.98	5.66	0.12	0.634
β-Pinene	7.40	6.71	6.02	6.84	6.88	0.18	0.161
δ 3-Carene	5.59^{c}	5.32^{bc}	4.82^{a}	5.10^{ab}	5.21^{abc}	0.09	0.026
Limonene	6.08	5.99	6.63	5.86	5.17	0.23	0.442
p-Cymene	7.63	6.89	6.33	7.12	7.43	0.18	0.162
allo-Ocimene	6.60	5.96	5.23	6.44	5.81	0.20	0.165

* Data expressed as arbitrary units of $\log_{10}$ of the peak area of the corresponding selected ion.
^{a,b,c} Means in the same row with unlike superscripts differ ($P < 0.05$).

Table VIII. Distance matrix of the pastures (P1, P2, P3, P4 and P5) according to their chemical composition (10 parameters), fatty acids (7) and terpene (7) composition.

Chemical composition	P1	P2	P3	P4	P5	Mean R^2c/R^2cv
P1	0	0.993	0.908	0.861	0.996	*0.954*
P2	0.935	0	0.963	0.948	0.933	
P3	0.828	0.493	0	0.987	1.000	
P4	0.493	0.851	0.871	0	0.954	
P5	0.851	0.844	0.967	0.762	0	*0.789*
Fatty acids	P1	P2	P3	P4	P5	
P1	0	0.987	0.975	0.861	0.978	*0.809*
P2	0.819	0	0.444	0.643	0.402	
P3	0.952	0.718	0	0.976	0.924	
P4	0.718	0.761	0	0	0.895	
P5	0.761	0.363	0.952	0.648	0	*0.622*
Terpenes	P1	P2	P3	P4	P5	
P1	0	0.996	1.000	0.999	1.000	*0.999*
P2	0.933	0	0.999	0.999	1.000	
P3	0.999	0.999	0	0.999	1.000	
P4	0.999	0.998	0.996	0	1.000	
P5	0.998	0.935	0.996	1.000	0	*0.985*

Over diagonal: R^2calibration (R^2c); below diagonal: R^2cross-validation (R^2cv).

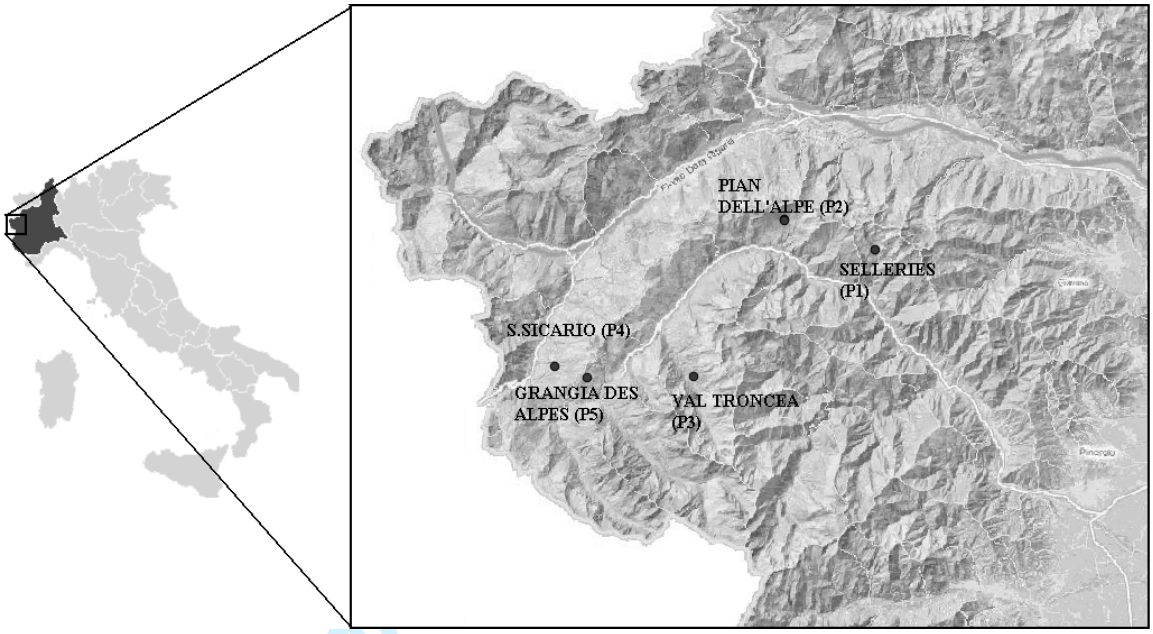

Figure 1. Geographical location of the pastures (P1, P2, P3, P4 and P5).

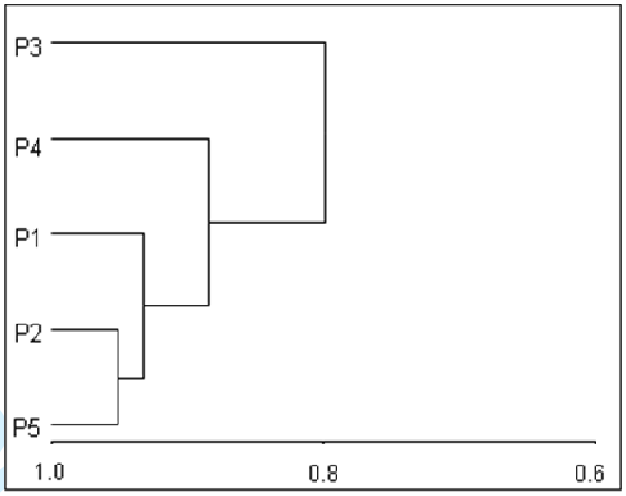

Figure 2. Cluster hierarchical analysis of the botanic family frequencies of the pastures (P1, P2, P3, P4 and P5), as reported in Table III.

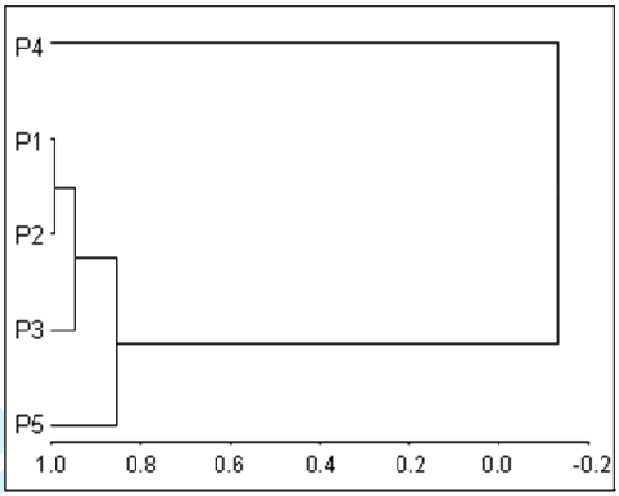

Figure 3. Cluster hierarchical analysis of the main forage groups of the pastures (P1, P2, P3, P4 and P5), as reported in Table IV.

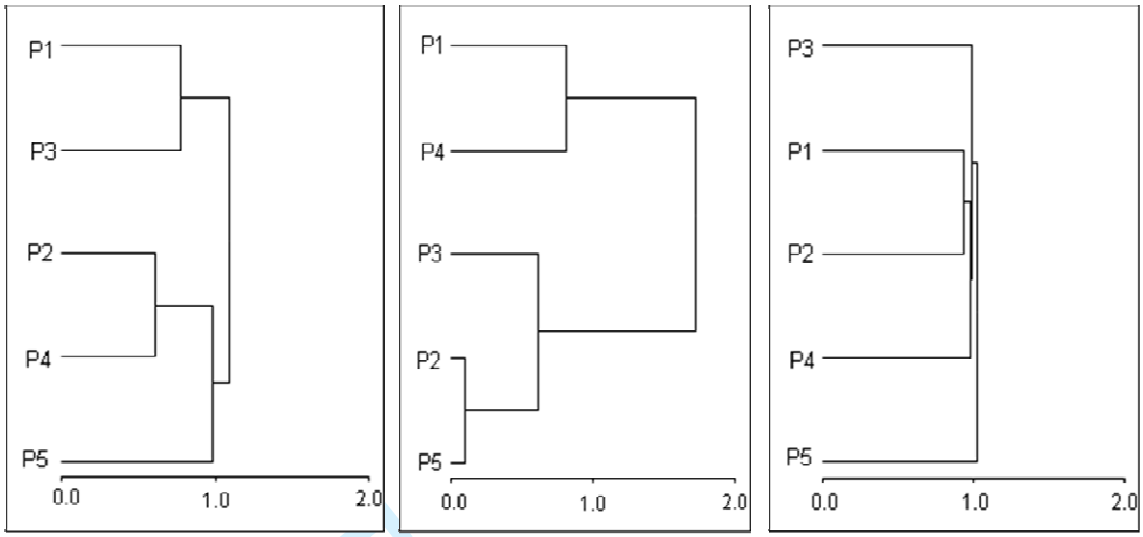

Figure 4. Cluster hierarchical analysis of the chemical composition (left), fatty acids (center) and terpenes content (right), on the basis of the distance matrix of the pastures (P1, P2, P3, P4 and P5) reported in Table VIII.

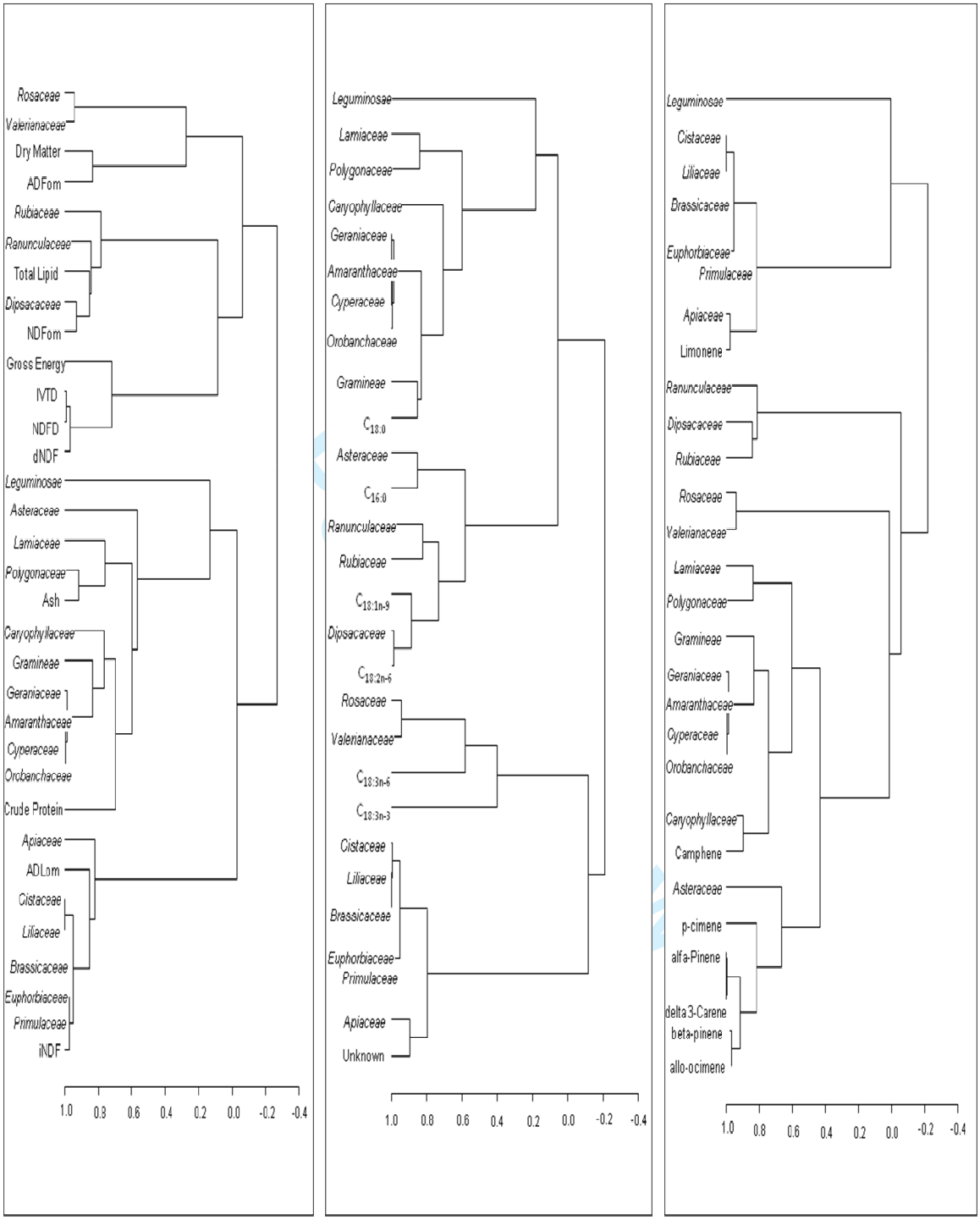

Figure 5. Cluster hierarchical analysis of the botanic family frequencies and the chemical composition (left), fatty acids (center) and terpenes content (right) of the pastures (P1, P2, P3, P4 and P5), respectively.