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Terpenes and fatty acid profiles of milk fat and "Bitto" cheese as affected by transhumance of cows on different mountain pastures

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Abstract

The evolution of fatty acid (FA) and terpenoid profiles was studied in milk ($n = 20$) and "Bitto" ($n = 3$), a protected designation of origin cheese produced in a restricted Italian alpine area. Milk came from 25 Italian Brown cows successively grazing pastures at 1400, 2100 and 2200 m during transhumance in June–September 2006. The fat matter was analyzed for FAs and terpenes by means of gas chromatography and purge & trap/gas chromatography–mass spectrometry, respectively. FA composition of milk fat varied significantly ($p < 0.0001$) in relation to contents of conjugated linoleic acid (CLA), stearic, linoleic and *trans*-vaccenic acids. Similar monoterpene profiles characterized milk fat from cows grazing the different pastures and the highest amount of terpenes was measured in milk coming from cows grazing at 1400 m. High levels of δ3-carene in milk fat were likely related to the important presence of *Ligusticum mutellina* in the pasture. Only negligible amounts of sesquiterpenes were detected in milk fat whereas they were the most abundant class in fodder. Both FA and terpene profiles of ripened (70 days) cheeses resembled those of the original milks. Overall, results confirm the influence of the botanical composition of mountain pastures both in enhancing the ruminal synthesis of CLA and in modifying the FA and terpenoid profiles of milk and "Bitto" cheese. Nevertheless, neither the FA nor the terpenoid profiles revealed here can be considered as "unique" to "Bitto" cheese and, for this reason, they can hardly be assumed to be biomarkers for defining a specific relationship among grazing area, milk and "Bitto" cheese. They better represent the chemical fingerprint of the cow feeding, adopted in mountain areas.

Keywords: "Bitto" cheese; Cow feeding; Terpenes; Fatty acids; Gas chromatography–mass spectrometry

1. Introduction

There are many conditions needed to satisfy the growing demand for high quality cheese made from raw whole milk. These conditions are still adopted in mountain regions of northern Italy where several protected designation of origin (PDO) cheeses are produced. Among them, "Bitto" cheese is a traditionally made semi-cooked, semi-hard cheese, cylindrically shaped (30–50 cm × 10–12 cm) with concave sides and sharp edges of about 10–25 kg. The paste has a compact structure with sparse partridge-eye holes, the color varies from white to straw yellow according to the degree of ripening which can last from 70 days up to several years. In 1996, it was awarded the certificate of PDO by the European Community (Commission Regulation, 1996). "Bitto" cheese is made from cow milk of Italian Brown breed and the addition of not more than 10% goat milk is allowable. According to its PDO rules, it is produced according to traditional cheesemaking practices in a well-defined Italian alpine area only between June 1st and September 30th. In this interval, herds move from intermediate to the highest elevations, following the richest pastures, and then move down to the former ground where new growth has sprouted.

The natural environmental conditions of the production area are more or less favourable for the growth of certain types of herbs and grass which constitute the grazing

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pastures. It follows that some of the characteristics of matured cheese depend upon the original features of the milk and hence on the conditions, mainly feeding, under which the milk has been produced. Several authors have established a relationship between fat composition of milk and grazing of cows on mountain pastures (Berard et al., 2007; Bugaud, Buchin, Coulon, & Hauwuy, 2001a; Bugaud, Buchin, Coulon, Hauwuy, & Dupont, 2001b; Collomb, Butikofer, Sieber, Jeangros, & Bosset, 2002; Favaro, Magno, Boaretto, Bailoni, & Mantovani, 2005; Fernandez et al., 2003; Mariaca et al., 1997; Stockdale et al., 2003; Tornambè et al., 2006; Viallon et al., 2000). In this regard, attention has been paid to the level of polyunsaturated fatty acids (PUFA), especially conjugated linoleic acid (CLA), in milk fat. CLA naturally occurs in milk and it is believed to beneficially modulate several important physiological functions (Pariza, Park, & Cook, 2001; Tanaka, 2005). Although most of the CLA of milk fat are synthesized in the mammary gland (Griinari et al., 2000), part of it represents an intermediate of ruminal biohydrogenation of linoleic acid (Tanaka, 2005). For this reason, pasture feeding yielding higher levels of PUFA causes higher CLA content of milk fat compared to feeding conserved forage (Jahreis, Fritsche, & Steinhart, 1997).

Terpenes are a group of lipophilic aliphatic compounds originating from the secondary metabolism of plants, usually as semiochemicals in defence against insects (Paré & Tumlinson, 1999). According to their general formula, mono and sesquiterpenes are made up of 2 or 3 isoprenoid units, respectively. Terpenes are abundant in certain plant families, especially dicotyledons such as *Apiaceae* and *Asteraceae*, but not in *Fabaceae*, while they are scant in monocotyledons such as *Poaceae*. In addition, production of terpenes is regulated by environmental factors; thus different qualitative and quantitative terpene profiles can be expected for pastures of lowlands or highlands.

Accordingly, either fatty acid (FA) composition or terpene profiles have been proposed as biomarkers of the chain plant–animal–milk–cheese for tracing mountain cheeses, including some Italian PDO mountain cheeses (Berard et al., 2007; Favaro et al., 2005). To this end, dynamic head-space extraction, purge and trap and head-space solid-phase microextraction coupled to gas chromatography–mass spectrometry (GC–MS) proved to be suitable analytical tools for analyzing fatty and terpene fractions of milk and cheese (Berard et al., 2007; Cornu et al., 2001; Favaro et al., 2005; Mallia, Fernandez-Garcia, & Bosset, 2005; Viallon et al., 1999).

To our knowledge, no studies have been carried out on FA and terpenoid profiles of milk and "Bitto" cheese as affected by changing of grazing pasture during the complete period of transhumance of the cows. This work has been undertaken to find a relationship among these chemical features and seasonal grazing of cows on different mountain pastures with the final goal being to ascertain the significance of FA and terpenoid profiles as biomarkers of PDO "Bitto" cheese.

2. Materials and methods

2.1. Experimental design

Twenty five multiparous Italian Brown cows, yielding 10–13 kg/d, were concerned in this work. The animals were approximately at the same stage of lactation during the experimentation from June to September 2006 (Table 1). During the entire experimentation, cows received all their daily feed as pasture, as provided by the PDO specifications in force in 2006, which did not allow supplementation with concentrate (Commission Regulation, 1996). The feeding was controlled to ensure sufficient energy supply. Four experimental periods were identified (P1–P4), during which cows moved to three (L1, L2 and L3) different locations at 1400, 2100 and 2200 m altitudes (Table 1). These locations are within the alpine region of Italy where "Bitto" cheese can be manufactured according to the PDO specifications.

During the experimental periods, milk samples were taken on different days (Table 1). Four additional samples were collected in May ($n = 2$) and October ($n = 2$), i.e. 30 days before and after cow transhumance, when herds were housed indoors at 1200 m altitude and they were fed forage coming from the neighbourhood of the cowshed. In May and October, cows were also fed with 3 kg/d of commercial concentrate based on corn, wheat, barley and soybean.

The experimental design turned out to be unbalanced due to the need to respect the normal mountain grazing practice; thus it reflects the normal calendar of herd transhumance in that alpine area.

2.2. Pastures and animal diets

The mean botanical composition of the pastures of each location was the average of surveys on several 10 m^2 parcels. Relative cover of a species was calculated as the ratio of the recorded presence for a given species to the sum of presences of all species and expressed as a percentage (Table 2).

From 1st to 28th June 2006 (P1), cows were farmed in L1 at 1400 m altitude. Herds grazed pastures whose botanical composition (3 surveys) included *Festuca rubra* (13%), *Dactylis glomerata* (12%), *Poa* (8%), *Avenula vescicolor* (7%), *Silene vulgaris* (7%), *Trifolium pratense* (5%), *Salvia pratensis* (5%) and *Heracleum sphondylium* (4%). *Poaceae* represented 43% of the herbaceous families, followed by *Asteraceae* (15%), *Fabaceae* (10%), *Labiateae* (7%), *Caryophyllaceae* (7%), *Apiaceae* (6%) and *Campanulaceae* (4%).

From 29th June to 16th July 2006 (P2), cows grazed in L2 at 2100 m altitude. Six surveys for botanical composition mainly showed the presence of *Poa alpina* (16.7%). *Phleum alpinum*, *F. rubra* and *Potentilla aurea* were present at 10% coverage each. *Leodonton helveticus* (5%), *Ligusticum mutellina* (2.5%), *A. versicolor* (4%) and *Alchemilla vulgaris* (5%) were particularly present in small areas of this location. Overall, 3 main plant families were present: *Poaceae* (63%), *Rosaceae* (15%) and *Asteraceae* (11%).

Table 1
Experimental design adopted in this work

Period (date)	P_1 (1st–28th June)	P_2 (29th June–16th July)	P_3 (17th July–10th September)	P_4 (11th–30th September)
Location (Altitude, m asl)	L_1 (1400)	L_2 (2100)	L_3 (2200)	L_1 (1400)
Milk sampling day, D (milk samples, *n*)	D_5, D_6, D_{15}, D_{16}, D_{26}, D_{27} ($n = 6$)	D_5, D_6, D_{13}, D_{14} ($n = 4$)	D_8, D_9, D_{22}, D_{23}, D_{36}, D_{37}, D_{48}, D_{49} ($n = 8$)	D_8, D_9 ($n = 2$)
Cheesemaking day, D (cheese samples, *n*)	D_{15} ($n = 1$)	D_{13} ($n = 1$)	D_{22} ($n = 1$)	

Table 2
Main botanical families (as mean percentage of total number of species) present in the pasture of the different grazing sites

	Period (botanical surveys, *n*)			
	P1 ($n = 3$)	P2 ($n = 6$)	P3 ($n = 3$)	P4 ($n = 2$)
Equisetaceae				4
Monocotyledons				
Poaceae	43	63	45	38
Cyperaceae	1		14	
Dicotyledons				
Asteraceae	15	11	5	10
Fabaceae	10	3	4	11
Ranunculaceae		1	2	9
Rosaceae		15	3	10
Apiaceae	6	3	5	4
Geraniaceae				8
Lamiaceae	7			1
Plantaginaceae	3			
Caryophyllaceae	7			
Polygonaceae			3	5
Campanulaceae	4	1	2	
Ericaceae			17	
Rubiaceae	2	1		
Other families	2	2		5

For period definition, see Table 1.

On 17th July 2006, herds were moved to L3 (2200 m altitude) where they rested until 10th September (P3). *Poaceae* (45%) was the dominant family present in the pasture (3 surveys). Other families found in the pasture were *Ericaceae* (17%), *Cyperaceae* (14%), *Asteraceae* (5%), *Apiaceae* (5%) and *Fabaceae* (4%). Among *Apiaceae*, *L. mutellina* was the only represented species. A large coverage of the poorly palatable plant *Loiseleuria procumbens* (*Ericaceae*) was also observed.

After L3, cows came back to location L1 until the end of September, 2006 (P4). Botanical composition (2 surveys) showed the presence of *Poaceae* (38%) and similar proportions of *Rosaceae* (10%), *Fabaceae* (11%), *Asteraceae* (10%), *Ranuncolaceae* (9%) and *Geraniaceae* (8%).

2.3. Cheese manufacture

The cheeses ($n = 3$) were manufactured from milk produced by cows fed on different pastures (Table 1). Cheesemaking was carried out using the milk produced at least 13 days after cows moved to a new location. The cheesemaking was carried out immediately after milking and according to the procedure provided for "Bitto" cheese. In particular, about 300 kg of milk was processed without any standardisation. Once transferred in the copper vat, milk was added with calf rennet powder (1:125,000). Clotting time was about 40 min and after a firming period of 10 min, the curd was cut into grains of ca. 3 mm, left standing for 5 min, stirred into the whey and cooked by indirect heating at 43 and 50 °C over a period of 40 min. Then, the curd was stirred into the whey for 20 min, left to settle down and allowed to rest under whey for 20 min. The whey was drained off and the curd was removed with a cloth, placed into moulds and pressed for 24 h. The cheese was then removed from the moulds, left at 10–15 °C for 12 h and manually salted on the surface. All cheeses ripened (70 days) in the same cellar.

2.4. Sampling

2.4.1. Pasture sampling

Samples of different pastures were obtained by mixing cut-grass from the sites where botanical surveys were made. Grass was cut the day before the milks were sampled and it was vacuum-packed in polyethylene bags and stored at −18 °C. After freeze-drying, samples were ground before analysis.

2.4.2. Milk sampling

Each milk sample was a mixture of milk coming from all 25 cows. After sampling, milk was immediately frozen in a glass bottle. Before analysis, milk samples were thawed at 20 °C and the cream was separated by spinning the milk for 15 min at 5000*g* at 4 °C. Creams were further used for fat extraction.

2.4.3. Cheese sampling

Samples of 70-days ripened "Bitto" cheese were derived from cheesemaking of milk sampled on D15, D13 and D22 for P1, P2 and P3, respectively (Table 1). Cheese samples were vacuum-packed in polyethylene bags and stored at −18 °C. Immediately before analysis, they were defrosted at 20 °C. For analysis, 500 g of cheese was ground after rind removal (10 mm).

2.5. Reagents

α-Pinene, β-pinene, camphene, β-mircene, α-phellandrene, δ3-carene, α-terpinene, γ-terpinene, *p*-cymene,

limonene, terpineol, linalool, β-ocymene, β-caryophyllene, and α-humulene were purchased from Aldrich/Fluka (Milan, Italy) and used as purchased.

Standard fatty acid methyl esters (FAMEs) mix, Supelco 37 Component (47885-U), was from Supelco (Supelco Park, Bellafonte, PA).

All other reagents were of analytical grade.

2.6. Fat extraction

2.6.1. Milk

Preliminary centrifugation of the whole raw milk (300 ml) was conducted as mentioned above. Forty grams of the obtained cream was transferred into a 50 ml polycarbonate tube (29 × 102 mm), then submitted for 90 min to a second centrifugation at 35 °C at 27,000*g* in a Superspeed Centrifuge RC-5B (Sorvall, Newtown, CT). The clear supernatant was transferred in a screw-cap bottle and immediately stored at −20 °C.

2.6.2. Cheese

Forty grams of ground cheese was transferred into a 50 ml polycarbonate tube (29 × 102 mm) and directly submitted to centrifugation at 35 °C at 27,000*g*. The clear supernatant was transferred in a screw-cap bottle and immediately stored at −20 °C.

2.7. Fatty acid analysis

As the choice of methylation reagent is critical in the analysis of polyunsaturated FAs as methyl esters, the derivatization method, involving 2M sodium methoxide in methanol, was used. Analysis of FAMEs by means of GC was carried out according to Christopherson and Glass (1969). FAs were identified by comparing their retention times with those of the reference standard mix of FAMEs.

Data were expressed as percentage of FAMEs.

2.8. Terpenes extraction, separation, identification and quantitation

Two hundred milligrams of ground freeze-dried pasture or 2 ml of melted fat was put into a head-space crimp-top glass bottle (20 ml, Agilent, Santa Clara, CA) sealed with a PTFE-silicone septum and introduced in the purge and trap bath at 65 °C (PT 37.50 Dani Instrument, Cologno Monzese, I) allowing it to equilibrate for 10 min. Volatile compounds were purged with 500 ml of helium (high grade purity) during 18 min, trapped on the Tenax-TA trap (270 mg) at 40 °C, desorbed at 280 °C for 3 min and directly introduced to the GC column. The analytes were separated and identified by GC–MS using an Agilent Technologies 6890N/5973N gas chromatograph–electron impact (70 eV) mass spectrometer equipped with a 30 m × 0.25 mm × 0.50 μm crosslinked polyethylene glycol column (HP-Innowax 19091N Agilent Technologies). Separating conditions were as follows: carrier gas, helium (1.0 ml min^{-1}); oven programme, 50 °C (3 min) (8 °C min^{-1}) to 162 °C, (17 °C min^{-1}) to 230 °C (4 min).

Three extractions were performed for each sample. To check the presence of carry-over effects, blank extractions were conducted regularly.

Identification of terpenoids was done by comparing retention times with those of the pure standards. The identification was also performed by comparing the mass spectra with those of the pure standards or those stored in the National Institute of Standards and Technology (NIST), US Government library.

Mono and sesquiterpenes were detected in the Selected Ion Mode by monitoring their characteristic ions at *m*/*z* 68, 93 and 161. Quantitation was performed by integrating the Q_{93} and the Q_{161} ion peaks for mono and sesquiterpenes, respectively, using the MS-Chemstation software (Agilent Technologies). Limonene was quantitated by its Q_{68} peak. The results were expressed in arbitrary units.

2.9. Statistical analyses

ANOVA was carried out using the Stat-View software, version 5.1 (SAS Institute, Inc., Cary, NC).

3. Results and discussion

3.1. Botanical composition of grazing pastures

The grazing sites here considered are located within an area of approximately 10 km^2 in Valtellina, an alpine valley in Northern Italy. Due to the presence of extensive pastures, cows have been moved from L1 to L4, as reported in Table 1. The experimentation was conducted under real conditions of milk and "Bitto" cheese production to cover the natural botanical diversity of pastures that cows normally graze during transhumance in spring-summer. Herds were allowed to graze the studied pastures which presented *Poaceae* as the dominant (38–63%) plant family (Table 2). The highest proportion of *Poaceae* characterized the pasture of L2 and it was related to manure, due to frequent staying of cows in the flat zones of this location. Plants belonging to this family (and to *Fabaceae*) are reported to contain low amounts of terpenes and, for this reason, they were not expected to appreciably enrich milk fat in these volatile compounds (Bugaud et al., 2001a; Collomb et al., 2002). Proportion and type of dicotyledonous families varied according to the site, and for the location L1 according to the considered period (i.e. P1 or P4). On the whole, more than 30% of dicotyledons coverage was observed in each of the pastures, *Asteraceae*, *Cyperaceae*, *Rosaceae* and *Apiaceae* being the families most widely represented. Dicotyledonous species are more terpene-rich than are *Poaceae* and, moreover, they could be responsible for the occurrence of the most abundant FAs in milk fat (Collomb et al., 2002). In particular, *Asteraceae* and *Rosaceae* positively correlate with CLA and *trans*-vaccenic (TVA) contents (Collomb et al., 2002). *Asteraceae* were

present at 5–15% coverage in all the studied pastures whereas *Rosaceae* were found in L1 (during P4) and in L2. Among monocotyledons, *Cyperaceae* were present only in L3. Similar proportion (3–6%) of *Apiaceae* characterized the pastures, even if different plants predominated according to either the location or the period considered. This family is not reported to correlate with FA composition of milk fat (Collomb et al., 2002). On the other hand, *Apiaceae* include several monoterpene-rich plants whose presence in the pasture can modify the terpenoid profile of milk, as demonstrated for *L. mutellina*. This species predominated in the pasture of L3 and it has been related to the presence of δ3-carene in Ossolano cheese manufactured in the mountain areas of Piedmont (I) (Zeppa, Gerbi, & Tallone, 2002).

The botanical composition of the studied pastures was influenced by both the geomorphological characteristics of the locations considered here (soil composition, slope, exposure to the sun) and the climate conditions in the period June–September 2006. All these factors can determine the important variation of botanical composition of the same pasture from one year to the next. From this point of view, what is recorded in this work is a picture, among others, of the botanical composition of pastures within the alpine area in which "Bitto" cheese can be manufactured.

Table 3
Mean fatty acid composition of milk fat by period/location

	P_1/L_1		P_2/L_2		P_3/L_3		P_4/L_1		*P*
	x	σ	*x*	σ	*x*	σ	*x*	σ	
$MeC_{4:0}$	3.63	0.134	3.63	0.082	3.86	0.194	3.78	0.006	0.0408
$MeC_{6:0}$	2.06	0.198	1.90	0.122	2.15	0.113	2.08	0.029	0.0843
$MeC_{8:0}$	1.05	0.124	0.95	0.093	1.15	0.083	1.09	0.024	0.0328
$MeC_{9:0}$	0.01	0.003	0.01	0.002	0.02	0.004	0.02	0.001	0.0032
$MeC_{10:0}$	2.05	0.328	1.87	0.245	2.36	0.210	2.22	0.065	0.0291
$MeC_{10:1}$	0.22	0.033	0.21	0.016	0.29	0.020	0.29	0.011	<.0001
$MeC_{11:0}$	0.02	0.006	0.02	0.003	0.03	0.007	0.03	0.004	0.0078
$MeC_{12:0}$	2.23	0.361	2.09	0.243	2.77	0.215	2.60	0.075	0.002
$MeC_{12:1}$	0.05	0.009	0.05	0.004	0.07	0.006	0.07	0.001	<.0001
$MeC_{i13:0}$	0.09	0.013	0.10	0.007	0.14	0.011	0.14	0.001	<.0001
$MeC_{a13:0}$	0.02	0.002	0.02	0.001	0.03	0.002	0.03	0.004	<.0001
$MeC_{13:0}$	0.06	0.011	0.06	0.005	0.08	0.006	0.09	0.002	<.0001
$MeC_{i14:0}$	0.15	0.024	0.15	0.005	0.21	0.018	0.22	0.004	<.0001
$MeC_{14:0}$	8.85	1.282	8.10	0.623	10.2	0.263	10.2	0.320	0.0021
$MeC_{14:1}$	0.67	0.090	0.64	0.048	0.91	0.035	0.99	0.039	<.0001
$MeC_{i15:0}$	0.30	0.038	0.35	0.015	0.42	0.036	0.42	0.001	<.0001
$MeC_{a15:0}$	0.56	0.091	0.62	0.017	0.85	0.060	0.80	0.023	<.0001
$MeC_{15:0}$	1.01	0.132	1.03	0.031	1.27	0.065	1.32	0.047	<.0001
$MeC_{15:1}$	0.06	0.014	0.05	0.002	0.06	0.004	0.09	0.003	0.0012
$\Sigma MeC_{9+11+13+15\ linear}$	*1.09*	*0.150*	*1.12*	*0.037*	*1.40*	*0.077*	*1.45*	*0.049*	*<.0001*
$MeC_{i16:0}$	0.27	0.018	0.28	0.004	0.33	0.015	0.33	0.022	<.0001
$MeC_{16:0}$	26.8	1.636	23.9	0.494	24.1	0.719	26.4	0.205	0.0004
$MeC_{16:1}$	1.69	0.159	1.70	0.071	1.45	0.039	1.52	0.042	0.0009
$\Sigma MeC_{13+14+15+16\ branched}$	*1.38*	*0.181*	*1.52*	*0.042*	*1.98*	*0.103*	*1.93*	*0.043*	*<.0001*
$MeC_{i17:0}$	0.51	0.022	0.54	0.002	0.59	0.011	0.54	0.004	<.0001
$MeC_{a17:0}$	0.54	0.027	0.55	0.009	0.58	0.023	0.51	0.010	0.0043
$MeC_{17:0}$	0.81	0.080	0.78	0.038	0.73	0.022	0.70	0.020	0.0314
$MeC_{17:1}$	0.46	0.076	0.43	0.034	0.36	0.013	0.34	0.016	0.0032
$MeC_{i18:0}$	0.08	0.015	0.07	0.008	0.06	0.003	0.05	0.006	0.0002
$MeC_{18:0}$	13.1	0.646	14.5	0.472	14.2	0.156	12.1	0.023	<.0001
$MeC_{18:1c9}$	24.5	3.781	26.1	1.426	21.7	0.684	20.6	0.588	0.0135
$MeC_{18:1t11}$	2.88	0.300	3.76	0.126	3.79	0.326	3.82	0.143	<.0001
$MeC_{18:1\text{-}A}$	0.56	0.113	0.50	0.026	0.48	0.058	0.59	0.059	0.199
$MeC_{18:1\text{-}B}$ *t*15 or *c*15 or *t*16	0.25	0.068	0.27	0.030	0.25	0.019	0.33	0.060	0.1593
$MeC_{18:1\text{-}C}$	0.28	0.053	0.30	0.019	0.31	0.021	0.36	0.033	0.0567
$MeC_{18:2}$	1.75	0.142	1.58	0.072	1.27	0.114	1.67	0.030	<.0001
$MeC_{18:2a}$	0.45	0.096	0.52	0.031	0.44	0.035	0.79	0.043	<.0001
$MeC_{18:3}$	0.77	0.040	0.70	0.046	0.60	0.068	0.82	0.059	<.0001
$MeC_{18:2conj}$	0.91	0.063	1.25	0.070	1.40	0.125	1.62	0.043	<.0001
$MeC_{20:0}$	0.20	0.019	0.22	0.007	0.27	0.015	0.27	0.011	<.0001
$MeC_{20:1}$	0.20	0.017	0.23	0.012	0.27	0.007	0.28	0.004	<.0001

Significant values of analyses of variance (*P*) are also indicated.
Data expressed as a percentage of total percentage of FAMEs; *x*: mean value; and σ: standard deviation.
For period and location definitions, see Table 1.

3.2. Fatty acid profile

The analysis of variance (ANOVA), for periods P1–P4 of single FAs, showed that the most susceptible FAs were the branched *i*- and *ai*- C13, C14, C15 and C16 (Table 3). Regarding the C18 family, the most susceptible were CLA, TVA, stearic and linoleic acids. All the mentioned FAs showed a $p < 0.0001$. The poorest association was encountered for C4:0–C10:0 and some isomers of C18:1 (not TVA). The level of total CLA in milk fat varied from 0.81% to 1.65% (total FAMEs basis) (Fig. 1a). These values are lower than those (2.23% ± 0.42) reported for Ossolano, an Italian mountain cheese, produced in summer (Zeppa, Giordano, Gerbi, & Arlorio, 2003). The effect of the altitude of the pasture on CLA level of milk fat has been well investigated and it was correlated with the specific botanical composition of pastures (Bugaud et al., 2001a; Ledoux et al., 2005). Indeed, the negative effect exerted by either lowland pastures or silage feeding on CLA content of fat milk has been pointed out (Jahreis et al., 1997) and, under such feeding conditions, CLA values lower than 0.6% can be expected (Dhiman, Anand, Satter, & Pariza, 1999; Kelly, Kolver, Baumann, Van Amburgh, & Muller, 1998). During the experimentation, CLA level in milk fat was in the range 0.81–1.06% during P1 and then it rose to 1.20–1.32% in P2 (Fig. 1a). Further increase, up to 1.6%, was observed when cows moved to the highest grazing site (L3) where a remarkable proportion of *Cyperaceae* was present in the pasture (Table 2). As reported above, this plant family positively influences the level of CLA in milk fat (Collomb et al., 2002). The two milk samples collected during P4 showed the highest CLA levels (1.58% and 1.65%) which almost doubled those found in milk fat when cows grazed in the same location (L1) during P1. This feature could be explained by the change of botanical composition of pasture occurring from June to September (Table 2). Indeed, contrary to period P1, the presence of *Rosaceae* was recorded and this could have contributed to increase the CLA content of the milk fat (Collomb et al., 2002). As expected, the lowest CLA levels (0.60–0.64%) characterized fat from milk samples collected either before or after herd transhumance when cows were fed forage indoors at 1200 m and feeding was supplemented with commercial concentrate (Fig. 1a).

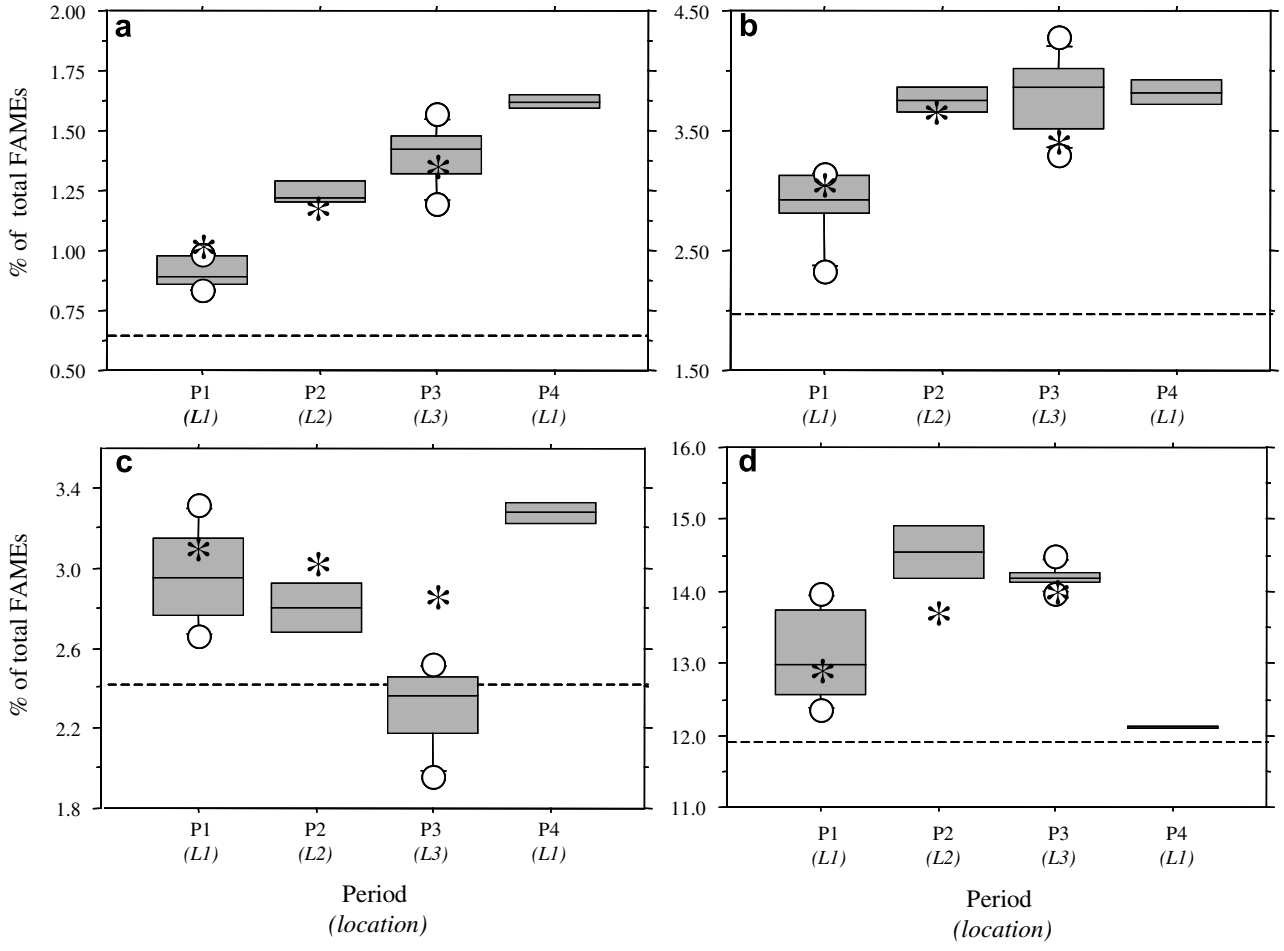

Fig. 1. Box–whisker plots of contents of the CLA (a), TVA (b), sum of linoleic and linolenic acids (c) and stearic acid (d) of milk fat by period/location. Asterisks refer to "Bitto" cheese data. A mean value relative to milk samples collected before and after cow transhumance is indicated for comparison (dotted line). Data expressed as a percentage of total percentage of FAMEs. For period and location definitions, see Table 1.

CLA level did not change in milk sampled 2–4 days after the cows moved to a new grazing location (not shown). This accounts for the delay (about 7 days) in stabilisation of biohydrogenation phenomena occurring in rumen and leading to CLA synthesis (Ryhanen et al., 2005). Mean levels of CLA recovered in milk samples collected during the same period differed slightly (mean *cv*: 6.0%) from each other (Table 3). Taking into account the length (<30–40 days) of the studied periods, this feature supports the hypothesis that change of plant phenological stage did not strongly affect the CLA level. Overall, data confirm that the alpine pastures positively influence CLA level in milk fat, likely increasing the availability of CLA precursors in fodder and then in rumen. In this regard, the presence of particular dicotyledonous families (*Asteraceae*, *Apiaceae* and *Rosaceae*) seems relevant for increasing the level of PUFA present in the fodder (Collomb et al., 2002). The role exerted by biohydrogenation phenomena in increasing CLA level was supported by the observed variation in the levels of TVA and stearic acid in milk fat (Fig. 1b and d). From P1 to P3, levels of stearic acid and TVA increased from 12.3% to 14.8% and from 2.3% to 4.3%, respectively. In period P4, the mean level of TVA was 3.8% whereas that of stearic acid was lower than that recovered in P1, despite the CLA value being two times higher. Moreover, the sum of linoleic and α-linolenic was higher (3.3% on average) in P4 than in P1 (2.3–3.3%) (Fig. 1c). The same applied for TVA level (3.7–3.9% vs. 2.4–3.1%) (Fig. 1b). Either incomplete biohydrogenation or different rate of intermediates hydrogenation could be hypothesized. Indeed, different authors have found that, in feeding sheep diets containing PUFA, the conversion rates of linoleic and α-linolenic acid to TVA were more rapid than that of TVA to stearic acid (An, Kang, Izumi, Kobayashi, & Tanaka, 2003; Izumi, An, Kobayashi, & Tanaka, 2002). Taking into account all the experimental periods, levels of linoleic and α-linolenic acids (1.9–3.3%) (Fig. 1c) were lower than those (6.5–9.0%) reported by Bugaud et al. (2001a) for milk from cows grazing mountain pastures. By contrast, they were similar (2.6–4.2%) to those reported by the same author for cows fed either silage or lowlands hay. Anyway, Bugaud et al. (2001a) found levels of these PUFA to be influenced by a lower biohydrogenation activity in the rumen rather than by a particular botanical composition of pasture.

It was not possible to identify a particular effect of grazing pastures on the level of saturated FAs C4:0–C14:0. Sum of them ranged from 17% to 25% and, in this regard, the percentage of *Poaceae* coverage seemed not to affect their concentration in milk fat (Tables 2 and 3). Lieber, Kreuzer, Nigg, Wettstein, and Scheeder (2005) observed a decrease of saturated FAs in milk fat from cows grazing at 2000 m altitude with respect to milk fat coming from cows grazing at 400 m. In the latter case, 50% reduction of levels of C10:0 and C12:0 was observed. According to Collomb et al. (2002), the presence of *Poaceae* positively correlates with saturated FAs (C4:0–C22:0) content of milk whereas the presence of *Asteraceae* and *Rosaceae* does not. In this work, this effect was not identified and levels of C4:0–C14:0 did not vary according to percentage of these plant families in the pasture. Levels of palmitic acid in milk fat significantly ($p < 0.001$) differed according to periods and it decreased from L2 to L3 (Table 3). Less significant ($p < 0.0135$) difference was observed for oleic acid (Table 3). According to Collomb et al. (2002), occurrence of MUFA is poorly dependent on the botanical composition of pasture since their production follows regulation mechanisms different from those of other FAs.

As far as FA composition of "Bitto" cheese is concerned, the highest CLA level (1.27%) characterized cheese manufactured in P3 (Table 4). It must be noted that FA composition of cheese slightly differed from that of the

Table 4
Fatty acid composition of "Bitto" cheese fat by period/location

	P_1/L_1	P_2/L_2	P_3/L_3
$MeC_{4:0}$	3.67	3.67	3.12
$MeC_{6:0}$	2.05	1.80	1.90
$MeC_{8:0}$	1.05	0.89	0.94
$MeC_{9:0}$	0.01	0.01	0.01
$MeC_{10:0}$	2.08	1.82	1.80
$MeC_{10:1}$	0.23	0.21	0.21
$MeC_{11:0}$	0.02	0.02	0.02
$MeC_{12:0}$	2.31	2.20	2.00
$MeC_{12:1}$	0.05	0.05	0.05
$MeC_{i13:0}$	0.10	0.10	0.10
$MeC_{a13:0}$	0.02	0.02	0.02
$MeC_{13:0}$	0.07	0.06	0.06
$MeC_{i14:0}$	0.16	0.17	0.16
$MeC_{14:0}$	9.20	8.57	7.79
$MeC_{14:1}$	0.72	0.68	0.62
$MeC_{i15:0}$	0.33	0.34	0.33
$MeC_{a15:0}$	0.62	0.62	0.58
$MeC_{15:0}$	1.14	1.05	0.98
$MeC_{15:1}$	0.07	0.05	0.05
$\Sigma MeC_{9+11+13+15\ linear}$	*1.25*	*1.14*	*1.07*
$MeC_{i16:0}$	0.29	0.28	0.26
$MeC_{16:0}$	27.0	24.9	23.9
$MeC_{16:1}$	1.66	1.75	1.75
$\Sigma MeC_{13+14+15+16\ branched}$	*1.51*	*1.53*	*1.44*
$MeC_{i17:0}$	0.52	0.53	0.53
$MeC_{a17:0}$	0.56	0.54	0.54
$MeC_{17:0}$	0.83	0.79	0.80
$MeC_{17:1}$	0.45	0.47	0.47
$MeC_{i18:0}$	0.08	0.08	0.07
$MeC_{18:0}$	12.9	13.7	14.1
$MeC_{18:1c9}$	23.0	25.6	27.4
$MeC_{18:1t11}$	3.12	3.60	3.42
$MeC_{18:1\text{-}A}$	0.60	0.43	0.60
$MeC_{18:1\text{-}B}$ *t*15 or *c*15 or *t*16	0.23	0.21	0.28
$MeC_{18:1\text{-}C}$	0.28	0.25	0.31
$MeC_{18:2}$	1.84	1.66	1.81
$MeC_{18:2a}$	0.54	0.52	0.57
$MeC_{18:3}$	0.78	0.84	0.84
$MeC_{18:2conj}$	1.00	1.20	1.27
$MeC_{20:0}$	0.20	0.22	0.22
$MeC_{20:1}$	0.22	0.24	0.25

Data expressed as a percentage of total percentage of FAMEs.
For period and location definitions, see Table 1.

corresponding milk, likely due to the partial hydrolysis of triglycerides following lipase activity. Despite this, variations of CLA, TVA linoleic, linolenic and stearic acids were quite similar to those of milk (Fig. 1a–d). The levels of CLA found in this work were lower than those found in Ossolano cheese (2.95 ± 0.42%) produced in summer using milk from cows fed exclusively green forage in mountain pastures located between 1500 and 2300 m (Zeppa et al., 2003). The same applied for levels of linolenic and linolenic acids which were about 25% lower.

3.3. Terpenoid profile

As is well known, palatability of plants depends on their botanical and phenological characteristics. Since it was not possible to ascertain which single plants cows preferentially ingested, the terpene composition of fodder samples representative of the whole pasture of each location was considered. The monoterpene profiles of pastures narrowly resembled those of milk samples (Fig. 2). On the whole, the highest content of monoterpenes was revealed in the pasture sampled during P3. Obviously, a quantitative relationship between pastures and milk was not established since fodder samples were directly submitted to terpene extraction whereas only the fat phase of milk was considered for the same purpose. Sesquiterpenes profiles of pastures and milk fat were not comparable, as the presence of long-chain terpenes was negligible in the latter (not shown). The terpenoid profile of milk samples mainly showed the presence of the monoterpenes: α-pinene, β-pinene, β-mircene, sabinene, camphene, δ3-carene and limonene (Table 5). Nevertheless, a great variability of the monoterpene content was observed, even in milks sampled in the same period, as demonstrated by values of standard deviation which largely exceeded the analytical error (±8%) of GC–MS analysis (Table 5). Overall, the qualitative profile of monoterpenes did not vary according to the periods considered here and, with the exception of δ3-carene and limonene, the mean level of terpenes generally decreased from P1 to P4 (Fig. 3). On the whole, the highest content of monoterpenes was revealed in milk from cows grazing in L1 during P1 (Table 5). In this location, *H. sphondylium* constituted the predominant species among *Apiaceae*. This plant has high levels of several monoterpenoids, including α-pinene, β-pinene, β-mircene and camphene (Mariaca et al., 1997). According to Bugaud et al. (2001a), leguminous dicotyledons, especially *Apiaceae*, are the herbaceous family mostly related to increase of terpenoid level in milk from cows grazing mountain pastures. The pasture of location L1 during P4 showed significant presence of nine plant families, four of them (*Ranunculaceae*, *Rosaceae*, *Geraniaceae* and *Polygonaceae*) not present in P1. Despite this, milk from cows grazing on this pasture showed a lower monoterpenes content (Table 5). During P2 and P3, *L. mutellina* was the main Apiaceous plant present in the pasture with 2.5% and 5.0% coverages, respectively. Accordingly, milk samples collected in P2 and P3

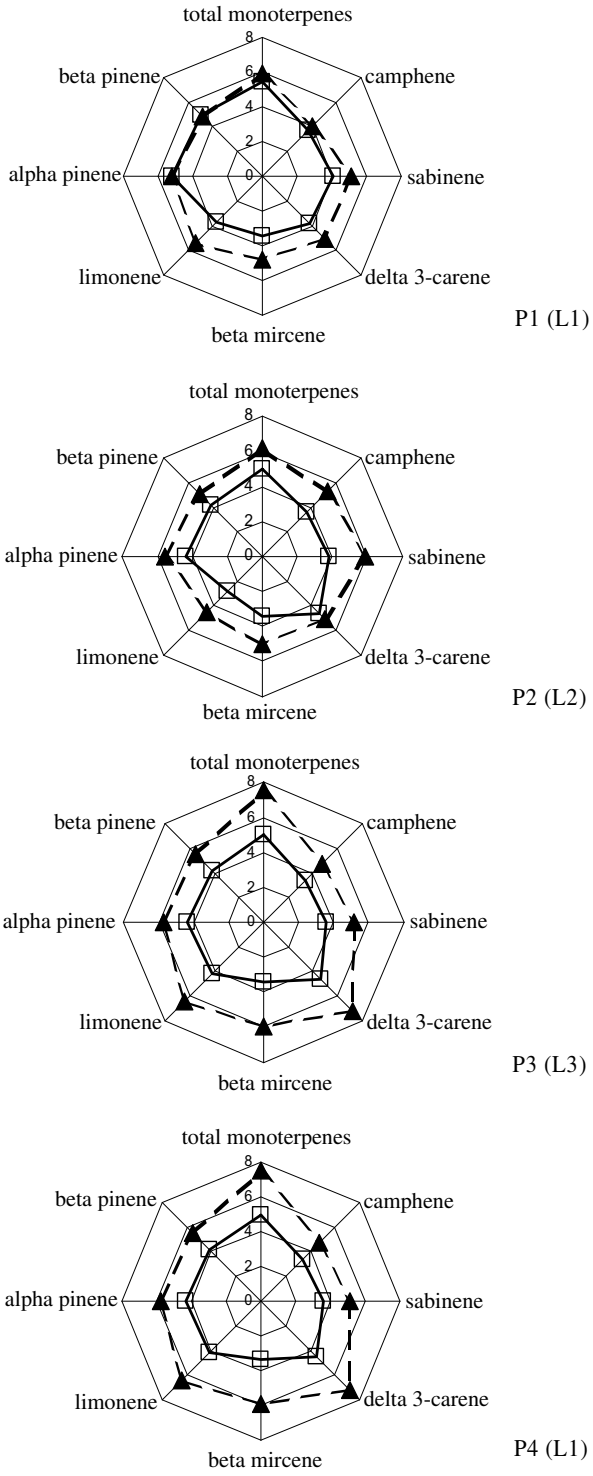

Fig. 2. Radar plots showing the monoterpene profiles of pastures (--▲--) and milk fat (–□–) according to period (P)/location (L). Abundance of terpenes is reported as the logarithmic relative peak intensities of Q_{ion}.

presented the highest levels of δ3-carene, which represents one of the major monoterpenoids present in *L. mutellina* (Mariaca et al., 1997). The same finding was reported for "Bettelmat", an Ossolano-type cheese produced in a very small Italian alpine area where *L. mutellina* is largely present in the pasture (Zeppa et al., 2002). Despite this plant

Table 5
Mean terpene compositions of milk fats by period/location

	P_1/L_1		P_2/L_2		P_3/L_3		P_4/L_1	
	x	σ	x	σ	x	σ	x	σ
α-Pinene	169,844	156,584	23,810	15,852	22,262	22,458	7761	4808
Camphene	5889	5225	3477	2083	2431	3093	1294	788
β-Pinene	89,563	101,134	12,738	8179	13,971	6744	6119	4014
Sabinene	11,133	17,044	6448	6198	3903	2864	1394	1009
δ-3-Carene	7225	8989	40,515	30,690	38,597	50,842	104	27
β-Mircene	2660	3651	2663	2408	2417	3130	174	70
Limonene	5409	5470	762	408	13,722	2302	366	235
Total terpenes	372,107	286,250	170,797	62,246	177,687	61,220	97,595	11,092

Data expressed as arbitrary units of the peak area of Q_{ion}; x: mean value; and σ: standard deviation.
For period and location definitions, see Table 1.

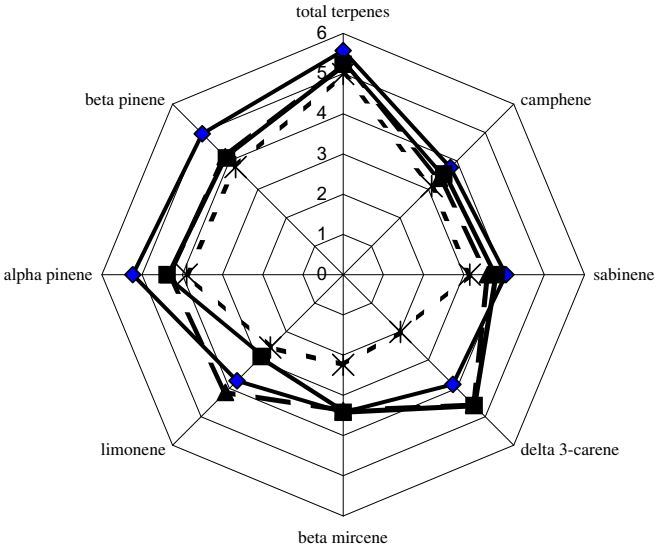

Fig. 3. Radar plots showing the mean terpenoid profiles of milk samples collected in P1 (–◆–), P2 (–■–), P3 (--▲--) and P4 (--✳--). Abundance of terpenes is reported as the logarithmic relative peak intensities of Q_{ion}. (Total terpenes refers to the sum of mono and sesquiterpenes.)

containing many other terpenes, as does *H. sphondylium*, amounts of α-pinene, β-pinene, sabinene and camphene did not increase in milk fat sampled during P2 and P3. The highest content of limonene was observed in milks sampled in P3, despite this compound being commonly present in many plants.

The monoterpene patterns of ripened "Bitto" samples mostly resembled that of the original milk with regard to type and proportion of terpenoid compounds (Fig. 4), thus confirming that the ripening has almost no effect on the terpene levels in cheese (Dumont & Adda, 1978). Camphene, α- and β-pinenes were also the main terpenes found in "Fontina Valle d'Aosta", a PDO cheese produced in the Italian alpine region (Berard et al., 2007). β-Caryophyllene, α-pinene and β-myrcene were the most abundant terpenes found in "Ossolano" cheese whereas β-pinene and limonene were detected at trace levels (Zeppa et al., 2002). Only negligible amounts of β-ocymene and β-caryophyllene were found in fat from milk and "Bitto" cheese. The same finding was reported by Viallon et al. (1999) for Saint Nectaire-type cheese. Nevertheless, some sesquiterpenes have been

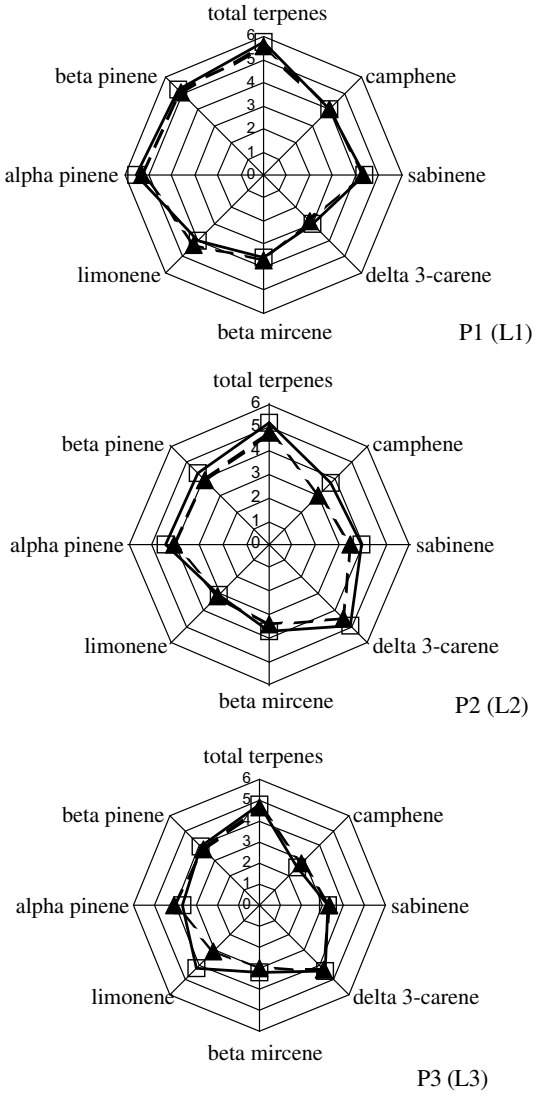

Fig. 4. Radar plots showing the terpene profiles of milk (--▲--) and derived "Bitto" (–□–) cheese according to period (P)/location (L). Abundance of terpenes is reported as the logarithmic relative peak intensities of Q_{ion}. (Total terpenes refers to the sum of mono and sesquiterpenes.)

reported as possible biomarkers of mountain cheese (Favaro et al., 2005; Mariaca et al., 1997).

The revealed terpenoid profiles of milk and "Bitto" cheese were partially determined by the characteristics of pastures which, however, were in quick evolution (plant type and development), depending on several environmental factors. Consequently, these characteristics are not easily predictable and they can be considered as typical of the studied locations only. As reported by Tornambè et al. (2006), this can impair the reliability of the terpenoid profile as a chemical tracer of the origin of cheese and, in this work, of "Bitto". Moreover, with the exception of δ3-carene, the terpenes recovered in milk and "Bitto" cheese are largely present in many plants from alpine pastures and thus in milk produced by cows grazing these pastures (Mariaca et al., 1997).

More monoterpenes and higher amounts of sesquiterpenes were extracted from pasture samples (data not shown). This fact is obviously related to the different contribution of terpenes to the total amount of volatiles extracted from pasture or milk and cheese fat. The strong interaction of terpenes with the lipophilic matrix of milk and cheese has to be considered as well (Viallon et al., 2000). In general, a weak relationship between terpene profile of fodder and milk/cheese was observed. This discrepancy can be related to the biochemical phenomena occurring in the rumen which are responsible for both degradation and hydrogenation of terpenes coming from feed (Hylemon & Harder, 1999; Schlictehtherle Cerny, Imhof, Fernandez Garcia, & Bosset, 2004; Viallon et al., 2000). Moreover, the great heterogeneity of plants could impair the analytical significance of the sampled pasture which may not be truly representative of what the cows effectively grazed.

4. Conclusions

The present paper has demonstrated that the FAs and terpenoid profiles of milk used for manufacturing "Bitto" cheese partially changed as the botanical characteristics of mountain pastures changed. Results confirmed the importance of the botanical composition of pastures in promoting CLA biosynthesis and in enriching the terpene profiles of milk and "Bitto" cheese. These chemical changes can be considered as "native" for the milk and the derived cheese produced in the alpine areas. Nevertheless, neither the FA nor the terpene profiles revealed in this study can be assumed as unique for "Bitto" cheese. Consequently, they cannot be considered reliable analytical tools for tracing "Bitto" cheese or milk originating from the area granted the "Bitto" PDO label. More likely, they seem valuable as a chemical fingerprint for characterizing the cow feeding adopted in mountain areas.

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References

An, J. K., Kang, C. W., Izumi, Y., Kobayashi, Y., & Tanaka, K. (2003). Effects of dietary fat sources on occurrences of conjugated linoleic acid and *trans*-fatty acids in rumen contents. *Asian–Australian Journal of Animal Science, 16*, 222–226.

Berard, J., Bianchi, F., Careri, M., Chatel, A., Mangia, A., & Musci, M. (2007). Characterization of the volatile fraction and of free fatty acids of "Fontina Valle d'Aosta", a protected designation of origin Italian cheese. *Food Chemistry, 105*, 293–300.

Bugaud, C., Buchin, S., Coulon, J. B., & Hauwuy, A. (2001a). Relationship between flavour and chemical composition of abundance cheese derived from different types of pastures. *Lait, 81*, 757–773.

Bugaud, C., Buchin, S., Coulon, J. B., Hauwuy, A., & Dupont, D. (2001b). Influence of the nature of alpine pastures on plasmin activity, fatty acid and volatile compound composition of milk. *Lait, 81*, 401–414.

Christopherson, S. W., & Glass, R. L. (1969). Preparation of milk fat methyl esters by alcoholysis in an essentially non-alcoholic solution. *Journal of Dairy Science, 52*, 1289–1290.

Collomb, M., Butikofer, U., Sieber, R., Jeangros, B., & Bosset, J. O. (2002). Correlation between fatty acids in cows milk fat produced in the lowlands, mountains and highlands of Switzerland and botanical composition of the fodder. *International Dairy Journal, 12*, 661–666.

Commission Regulation (EC). (1996). No. 1107/96 of 12 June 1996, Published in the Official Journal of the European Union, L148 of 21 June 1996.

Cornu, A., Carnat, A., Martin, B., Coulon, J. B., Lamaison, J. L., & Berdagué, J. L. (2001). Solid-phase microextraction of volatile components from natural grassland plants. *Journal of Agricultural Food Chemistry, 49*, 203–209.

Dhiman, T. R., Anand, G. R., Satter, L. D., & Pariza, M. W. (1999). Conjugated linoleic acid content of milk from cows fed different diets. *Journal of Dairy Science, 82*, 2146–2156.

Dumont, J. P., & Adda, J. (1978). Occurrence of sesquiterpenes in mountain cheese volatiles. *Journal of Agricultural and Food Chemistry, 26*, 364–367.

Favaro, G., Magno, F., Boaretto, A., Bailoni, L., & Mantovani, R. (2005). Traceability of Asiago Mountain Cheese: a rapid, low-cost analytical procedure for its identification based on solid-phase microextraction. *Journal of Dairy Science, 88*, 3426–3434.

Fernandez, C., Astier, C., Rock, E., Baptiste, J., Coulon, J. B., & Berdague, J. L. (2003). Characterization of milk by analysis of its terpene fractions. *International Journal of Food Science and Technology, 38*, 445–451.

Griinari, J. M., Corl, B. A., Lacy, S. H., Chouinard, P. Y., Nurmela, K. V. V., & Baumann, D. E. (2000). Conjugated linoleic acid is synthesized endogenously in lacting dairy cows by delta 9-desaturase. *Journal of Nutrition, 130*, 2285–2291.

Hylemon, P. B., & Harder, J. (1999). Biotransformation of monoterpenes, bile acids, and other isoprenoids in anaerobic ecosystems. *FEMS Microbiology Reviews, 22*, 475–488.

Izumi, Y., An, J. K., Kobayashi, Y., & Tanaka, K. (2002). Effects of fresh grass feeding on the formation of conjugated linoleic acid (CLA) and vaccenic acid (*trans*-11C18:1) in the rumen. *Proceedings of Japanese Society for Rumen Metabolism and Physiology, 15*, 43–46.

Jahreis, G., Fritsche, J., & Steinhart, H. (1997). Conjugated linoleic acid in milk fat: high variation depending on production system. *Nutrition Research, 17*, 1479–1484.

Kelly, M. L., Kolver, E. S., Baumann, D. E., Van Amburgh, M. E., & Muller, L. D. (1998). Effect of intake of pasture on concentrations of

conjugated linoleic acid in milk lactating cows. *Journal of Dairy Science, 81*, 1630–1636.

Ledoux, M., Chardigny, J. M., Darbois, M., Soustre, Y., Sébédio, J. L., & Laloux, L. (2005). Fatty acids composition of French butters, with special emphasis on conjugated linoleic acid (CLA) isomers. *Journal of Food Composition and Analysis, 18*, 409–425.

Lieber, F., Kreuzer, M., Nigg, D., Wettstein, H. R., & Scheeder, M. R. L. (2005). A study on the causes for the elevated $n-3$ fatty acids in cows' milk of alpine origin. *Lipids, 40*, 191–202.

Mallia, S., Fernandez-Garcia, E., & Bosset, O. J. (2005). Comparison of purge and trap and solid phase microextraction techniques for studying the volatile aroma compounds of three European PDO hard cheeses. *International Dairy Journal, 15*, 741–758.

Mariaca, R. G., Berger, F. H., Gauch, R., Imhof, M. I., Jaegros, B., & Bosset, J. O. (1997). Occurrence of volatile mono- and sesquiterpenoids in highland and lowland plant species as possible precursors for flavour compounds in milk and dairy products. *Journal of Agricultural and Food Chemistry, 45*, 4423–4424.

Paré, P. W., & Tumlinson, J. H. (1999). Plant volatiles as a defence against insects herbivores. *Plant Physiology, 121*, 325–331.

Pariza, M. W., Park, Y., & Cook, M. E. (2001). The biologically active isomers of conjugated linoleic acid. *Progress in Lipid Research, 40*, 283–298.

Ryhanen, E. L., Tallaavara, K., Griinari, J. M., Jaakkola, S., Mantere-Alhonen, S., & Shingfield, K. J. (2005). Production of conjugated linoleic acids enriched milk and dairy products from cows receiving grass silage supplemented with a cereal based concentrate containing rapeseed oil. *International Dairy Journal, 15*, 207–217.

Schlictehtherle Cerny, H., Imhof, M., Fernandez Garcia, E., & Bosset, J. O. (2004). Changes in terpene composition form pasture to cheese. *Mitteilungen Lebensmittel Hygiene, 95*, 681–688.

Stockdale, C. R., Walker, G. P., Wales, W. J., Dalley, D. E., Birkett, A., Shen, Z., et al. (2003). Influence of pasture and concentrates in the diet of grazing dairy cows on the fatty acid composition of milk. *Journal of Dairy Research, 70*, 267–276.

Tanaka, K. (2005). Occurrence of conjugated linoleic acid in ruminant products and its physiological functions. *Animal Science Journal, 76*, 291–303.

Tornambè, G., Cornu, P., Pradel, N., Kondjoyan, A. P., Carnat, M., Petit, M., et al. (2006). Changes in terpene content in milk from pasture-fed cows. *Journal of Dairy Science, 89*, 2309–2319.

Viallon, C., Martin, B., Verdier-Metz, I., Pradel, P., Garel, J. P., Coulon, J. B., et al. (2000). Transfer of monoterpenes and sesquiterpenes from forages into milk fat. *Lait, 80*, 635–641.

Viallon, C., Verdier-Metz, I., Denoyer, C., Pradel, P., Coulon, J. B., & Berdagué, J. L. (1999). Desorbed terpenes and sesquiterpenes from forages and cheeses. *Journal of Dairy Research, 66*, 319–326.

Zeppa, G., Gerbi, V., & Tallone, G. (2002). Aspetti tecnologici, analitici e sensoriali. In *Reione Piemonte-Assessorato Agricoltura, Il formaggio Ossolano* (pp. 215–244). Torino: Regione Piemonte.

Zeppa, G., Giordano, M., Gerbi, V., & Arlorio, M. (2003). Fatty acid composition of Piedmont "Ossolano" Cheese. *Lait, 83*, 167–173.