TERPENES IN MILK AND TOMA CHEESE IN DIFFERENT FORAGE DIETS IN AN ALPINE VALLEY

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RIASSUNTO

Terpeni del latte e dei formaggi in diverse condizioni di alimentazione invernale ed estiva in una valle alpina.

Scopo del lavoro è stato quello di valutare l'effetto della tipologia di alimentazione delle bovine sulla variazione del contenuto in terpeni nel latte e nel formaggio prodotti in un ambiente alpino. La prova è stata condotta presso la Stazione Alpina Sperimentale di Sauze d'Oulx (TO) a 1.850 m s.l.m.. Il latte prodotto da una mandria di 40 vacche di razza valdostana con una produzione media annuale di circa 4.000 kg/latte è stato caseificato in diversi momenti dell'anno a Toma. In alcune prove di caseificazione sono stati valutati i profili terpenici dei latti e dei formaggi in funzione di due aspetti: l'effetto di razioni invernali a base di fieno o fieno-silo e l'effetto del momento di pascolamento nel periodo estivo di alpeggio. I terpeni sono stati valutati mediante P&T-GC/MS.

I terpeni presenti in maggior quantità sono risultati: α-pinene, camphene, β-pinene, δ 3-carene, β-mircene, β-ocimene, allo-cimene e D-limonene. Nei latti e nei formaggi prodotti nel periodo di pascolamento i terpeni sono risultati molto più elevati rispetto a quelli derivati da alimentazione a base di foraggi conservati. Per quanto riguarda il confronto tra l'alimentazione invernale a base di fieno e fieno-silo prodotti in loco o a base di fieno proveniente dalla pianura, si nota una riduzione dei principali terpeni presenti da cinque a tre. In conclusione l'utilizzo in razione di alte percentuali di foraggi prodotti in loco può preservare parzialmente il contenuto di terpeni nel latte e nei formaggi garantendo un più stretto legame con il territorio anche per le produzioni invernali.

INTRODUCTION

The vegetation of natural highland pastures is characterized by a very different botanical composition, particularly for dicotyledons, compared to that of the plains. A pasture rich in dicotyledons, mostly of natural highland pastures, is known to give cheese with flavor different from that produced from pasture rich in grass typical of plain. This different composition reflects different compounds that can be transferred to milk and cheese, especially terpenes, which are rich

in odours, and can be found in cheese flavour (Buchin et al., 2002). For this reason, cheeses from highlands are often highly appreciated. During winter, when conserved forages are fed, terpenes content in milk and cheeses may vary (Martin and Coulon, 1995). Furthermore, terpenes may be considered as biochemical indicators or markers to characterise cheeses originating from highlands (Mariaca et al., 1997).

The aim of this work was to evaluate the effect of different winter and summer diets for cows on the terpenoid composition of milk and cheese produced in an alpine environment.

MATERIALS AND METHODS

The experiment was carried out during the 2002-2003 period at the Experimental Alpine Station (1852 m a.s.l., Sauze d'Oulx, Italy) on 40 Valdostana cows producing an average of 4000 kg milk/year. This milk was used for the production of the traditional Alpine Toma cheese (diameter 300 mm, thickness around 10 mm, weighing ~ 6-8 kg, Photo 1). This cheese, made from raw whole milk, has a semi-cooked semi-soft curd. Cheese making was carried out in the experimental dairy plant equipped with two vats. Terpenoids were determined in milk and cheese in two cheese-making trials, based on winter and summer diets. The first trial was conducted during winter with two groups of cows offered hay or haylage from the same permanent meadow (located at 1800 m). The haylage was produced as wrapped big bales at a mean DM content of 50% obtained after 2 days of wilting, while the hay was harvested after 4 days of wilting. The date of cutting was 17 July with a medium-low forage quality. A cross-over design was used, with 10 days of adaptation followed by one experimental week for each of the two phases. On average, forages comprised 70% of total dry matter of the diet for each group. The second trial was carried out with grazing animals on a native Alpine pasture (from 1900 to 2200 m a.s.l., Photo 2) in two different moments of the morphological development of vegetation. The periods of grazing under consideration were early summer pasture (mid-June) and late summer (late-August). The animals grazed in one group only. The samplings of the two trials were drawn for 4 consecutive days for the winter trial, and one week (4 samplings) for the summer trial. Some milk samples were also collected during the period following winter trial, in which animals were feed with diets with hay come from lowland meadow (indicated as plain hay).

Cheeses, ripened for a period of about 70 days as per the usual procedure, were sampled and immediately frozen (–20°C). A total of 20 milk samples and 20 cheese samples were collected, 4 additive samples of milk come from plain hay were also collected. Milk samples were centrifuged for 10 min at 2100·g at 20°C, within 2-3 hours after sampling. Cream obtained was sealed and stored at –20°C in 10 ml glass vials until the second extraction of the fat phase. Cheese was finely grated and immediately submitted to fat extraction by means of a Sorvall RC-5B centrifuge. A sample of 20 g cream (at room temperature) or cheese was weighed in a 50 ml test tube (29x102 mm). In order to limit mechanical stress, the rate of centrifugation was increased by steps of 3000 rpm to a final gravity force of 27000·g, limiting the temperature to a maximum of 35°C. The complete cycle was performed in 1h. The liquid fat obtained was exactly weighed (1 g) in a 20 ml HS-vial and submitted to Purge and trap extraction by means of a Dani SPT37/50 apparatus. The parameters were: equilibrium, 65°C/20 min; extraction, 30 ml/min helium 15 min; adsorbent, 270 mg Tenax TA at 40°C; desorption at 280°C and injection for 3 min directly in the Agilent 6890N GC coupled with a Agilent 5973 Mass Selective Detector (E.I.). Chromatographic conditions: Column, 30 m, 0.25 mm (film 0.5 µm) HP-Innowax; oven temperature, 40°C (3 min) 12°C/min up to 230°C (5 min); carrier helium 7.13 psi. To increase the selectivity and sensitivity for the terpenes, the mass detector operated in SIM mode, the selected ions were 93 and 136. Results are expressed as percentages of the most abundant sums of the two masses at the specific retention time. An example of the chromatogram is shown in Figure 1.

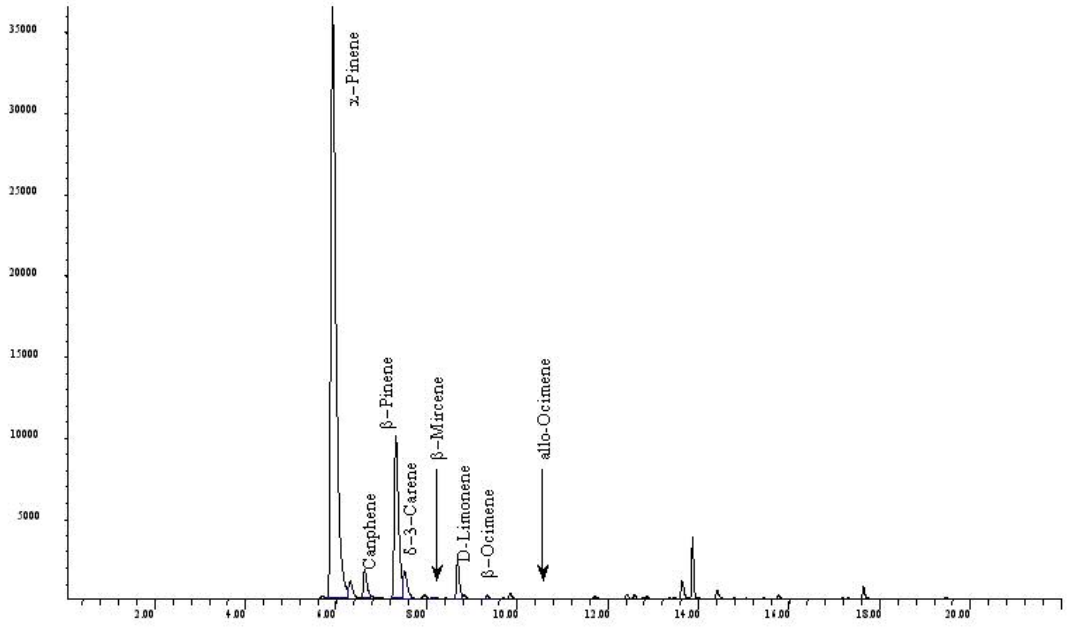

Figure 1. Total Ion Chromatogram resulting from masses 93 and 136 of a milk fat (early summer pasture) submitted to P&T extraction . Experimental conditions in the text.

RESULTS AND DISCUSSION

The botanical composition for the main botanical family of the permanent meadow, where hay and haylage were produced, and of the native alpine pasture grazed during summer are reported in Table 1. The Gramineae represents around 75 and 44% of the total contribution for meadow and pasture, respectively, while Leguminosae represent around 6% in meadow and 15.9% in pasture. In pasture the contribution of other families were relevant for the Compositae (10.2%) and Labiateae (15.9%). In these latter two families some species rich in terpenes were present, such as Achillea millefolium, Thymus spp., as reported by Viallon et al. (2000) and Cornu et al. (2001).

The main terpenes found in relevant amounts both in milk and cheeses were the following eight: α-pinene, camphene, β-pinene, δ-3-carene, β-myrcene, β-ocimene, allo-ocimene and D-limonene. All these terpenes were found in highland plants by Mariaca et al. (1997). The contents of monoterpenes extracted from milk fat during the summer trial showed the highest values for all the molecules considered (Table 2). Some molecules, such as β-myrcene, β-ocimene, camphene and δ-3-carene were undetected or at a very low level in winter diets in mountain hay, haylage and also plain hay. D-limonene content in all the studied winter diets made up around 1/3 of the amount

found in milk from pasture, while the amount of α- β-pinene ranged from 1.0 to 3.1% of the pasture samples. Among the winter diets, mountain hay showed higher values than haylage for all the terpenes, although these difference were not significant. The diet with plain hay showed similar values for D-limonene and δ-3-carene, while camphene and β-pinene were completely absent.

When cheeses are considered, pasture diets showed again the higher values of terpenes (Table 3). Wide differences in terpenes content were observed between cheeses produced from early summer pasture, characterized by plants in early stage of growth, and those produced in late summer, with grazed plants advanced in maturity. D-limonene, δ-3-carene, β-myrcene, β-ocimene were higher in early summer, while α-pinene, camphene, and β-pinene were higher in late summer cheeses. Allo-ocimene was present only in late summer cheeses.

Table 1. Botanical composition of permanent meadows and alpine pastures in the areas considered.

Plant family	Meadow	Pasture†
Poaceae	74.6	44.3
Leguminosae	6.1	15.9
Asteraceae	-	10.2
Lamiaceae	-	15.9
Others	19.4	13.7

†Main Species: **native permanent meadow** (93% of specific contribution): *Bromus inermis, Phleum pratense, Dactylis glomerata, Festuca rubra, Trifolium pratense, Lathyrus spp, Vicia cracca, Taraxacum officinalis, Carum carvi and Ranunculus spp.;* **pasture** (86.3% of specific contribution): *Avenula pubescens, Festuca gr. rubra, Festuca ovina, Trifolium badium, Onobrychis viciifolia, Achillea millefolium, Taraxacum officinale, Centaurea uniflora, Hieracium pilosella, Thymus serpillus, Salvia pratense*.

Table 2. Quantities of some monoterpenes extracted from milk fat during different diet periods. Values expressed as a percentage of the highest values of summer pasture.

*	D-limonene	α-pinene	camphene	β-pinene	δ-3-carene
Mountain Hay	27.1	3.4	1.0	2.7	1.3
Mountain Haylage	19.5	1.9	0.6	1.6	n.d.
Plain hay	23.6	0.9	n.d.	n.d.	1.0
Early summer pasture	84.9	92.2	90.9	88.1	87.5

* samples of late summer pasture were not analysed; n.d., not detected.

Table 3. Quantities of some monoterpenes extracted from cheese fat during different diet periods.

*	D-limonene	α-pinene	camphene	β-pinene	δ-3-carene	β-myrcene	β-ocimene
Mountain Hay	15.7	0.3	n.d.	0.3	0.2	n.d.	n.d.
Mountain Haylage	25.0	0.5	n.d.	0.4	1.2	n.d.	n.d.
Early summer pasture	85.5	27.6	11.6	22.2	48.0	79.2	89.4
Late summer pasture	74.3	79.0	77.6	88.2	26.4	13.9	6.8

* Plain hay milk was not cheese-made; n.d., not detected.

CONCLUSIONS

Terpens content of milk and cheeses produced in Alpine environment showed to be higher in summer diets based on grazing on native Alpine pasture. The winter diets based on high percentage of local forage (as hay or haylage) reduces the number of the main terpenes present from eight to five, while plain hay contains only three of the eight molecules. Therefore the use of locally produced forages could partially preserve the terpenes content. Thus terpene content could be investigated in evaluating cheese origin, also in winter production.

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