

## USE OF MONO- AND SESQUITERPENES FOR CHARACTERISATION OF MOUNTAIN CHEESES

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Received June 10, 2004, accepted October 15, 2004.

Delo je sprejeto 10. junija 2004, sprejeto 15. oktobra 2004.

### ABSTRACT

Terpenes are volatile compounds originating exclusively from plants that can be found in dairy products. Several studies have therefore been conducted to use the terpenes to trace the geographical origin or the nature of the feed supplied to animals. The vegetation of mountain pastures varies which results in different types of foraging. Our aim was to determine if the terpene profile can be used to distinguish the mountain pasture of origin. The mono- and the sesquiterpenes of ricotta cheese samples produced during the summer in three mountain farms were then determined with SPME-GC-MS. The ricotta cheeses were produced with cow and goat milk and every three days of production a sample was analysed. Obtained results showed that these compounds vary widely between samples due to the stage of plant development but discrimination among the farms was still possible. Hence mono- and sesquiterpenes can be used not only to distinguish between summer pasture cheeses and the ones produced during other periods but also as markers of each mountain pasture.

Key words: milk products / cheese / ricotta / characterisation / terpenes / sesquiterpenes / markers

## UPORABA MONO- IN SESKVITERPENOV ZA KARAKTERIZACIJO SIROV S PLANIN

### IZVLEČEK

Terpeni so hlapne snovi izključno rastlinskega izvora, ki jih lahko najdemo v mlečnih izdelkih. Opravljene so bile študije, v katerih so terpeni uporabljali za sledenje in ugotavljanje geografskega porekla krme, ki so jo zaužile živali. Razlike v vegetaciji na planinskih pašnikih se odražajo tudi v krmi. V tej raziskavi smo želeli proučiti možnost, če na osnovi profila terpenov lahko ugotovimo ali so se krave pasle na planinskem pašniku. Poleti smo s SPME-GC-MS ugotavljali mono- in seskviterpene v vzorcih sira "ricotta" s treh planinskih farm.. Vzorce sira "ricotta", ki so ga izdelovali iz kravjega in ovčjega mleka smo zbirali vsak tretji dan in opravili analize. Čeprav rezultati kažejo, da se vsebnost proučevanih snovi v vzorcih, zaradi razlik v razvojni fazi rastlin, zelo razlikuje, smo lahko ločevali vzorce z različnih farm. Mono- in seskviterpene lahko uporabimo za ločevanja med siri, ki so bili izdelani med pašno sezono in izven nje pa tudi kot markerje za posamezne planinske pašnike.

Ključne besede: mlečni izdelki / sir / ricotta / karakterizacija / terpeni / seskviterpeni / markerji

### INTRODUCTION

Animal feeding is a very important factor in cheese characterisation due to its action on bacteria and milk compounds such as fats, proteins, flavours and so on. Several studies have

highlighted the possibility to define a relation between cheese and its production area with the study of animal feed and above all with the study of terpene compounds (Dumont and Adda, 1978; Moio *et al.*, 1996; Mariaca *et al.*, 1997; Schehovic *et al.*, 1998; Viallon *et al.*, 1999; Bugaud *et al.*, 2000; Viallon *et al.*, 2000; Bugaud *et al.*, 2001a; Bugaud *et al.*, 2001b). These molecules are secondary metabolites of plants, well-known for their disinfectant (medicinal) and odorant (spice) properties. In the plant kingdom their qualitative and quantitative distribution is highly variable, but they are specie-specific thus many researchers have suggested that the analysis of such substances might improve the traceability of dairy products or meats originating from animals raised in specific geographical areas. Results have shown that the milk and the cheese from different production sites (lowland vs. highland) and seasons (winter vs. summer) can be distinguished (Jeangros *et al.*, 1997; Bosset *et al.*, 1999; Buchin *et al.*, 1999; Cornu *et al.*, 1999; Buchin *et al.*, 2002). In fact terpene compounds are more abundant in dicotyledones than in monocotyledons which are more abundant in alpine pastures. Terpenes are also more abundant in fresh grass than in hay. The aim of this work was to verify if mono- and sesquiterpens can also be used to distinguish the products from different highlands pastures and to define a traceability system for a typical mountain cheese.

## MATERIALS AND METHODS

Three mountain farms (Alpe Bancet, Alpe Gianna, Alpe Pra) were used in this study. The farms are located in Pellice Valley near Torino (Piedmont, North West Italy) at 1000–2500 m. A sample of ricotta cheese has been taken every four days for the period July 12/August 23 for Alpe Pra, for the period July 12/September 16 for Alpe Bancet and for the period July 14/September 16 for the Alpe Gianna. These differences of sampling are due to the different time of mountain grazing.

Each sample was vacuum packed in polyethylene bags and stored to  $-20\text{ }^{\circ}\text{C}$ . Samples of 2.5 g of ricotta cheese were taken and placed in a 10 mL glass vial (38 mm high and 22 mm in diameter) and sealed with 20 mm PTFE/Silicone septum caps (Supelco, Bellefonte, USA).

For the conditioning, the sample vial was placed in a  $53\text{ }^{\circ}\text{C}$  water bath for 10 min. The fiber used for the extraction was a DVB/Carboxen/PDMS 2 cm stable flex fiber (Supelco, Bellefonte, USA).

The volatile components were extracted using the static headspace method. During this step the fiber was exposed for 60 min in the headspace of the cheese with the vial maintained at  $53\text{ }^{\circ}\text{C}$  in a thermostatic bath. Every sample was analysed in triplicate.

The adsorbed molecules were desorbed by introducing the SPME fiber into the injector of a gaschromatograph (GC17A, Shimadzu, Tokyo, Japan) at  $270\text{ }^{\circ}\text{C}$  for 6 min in splitless mode. The volatile components were separated on a DB-WAX capillary column (30 m  $\times$  0.25 mm ID; film thickness 0.25  $\mu\text{m}$ ; J&W Scientific Inc., Folsom, CA, USA). The oven temperature program and the operating conditions were as follow: carrier gas helium at  $1\text{ mL min}^{-1}$ ; the column was maintained at  $35\text{ }^{\circ}\text{C}$  for 5 min, ramped at  $2\text{ }^{\circ}\text{C min}^{-1}$  to  $173\text{ }^{\circ}\text{C}$ , maintained at  $173\text{ }^{\circ}\text{C}$  for 1 min, ramped at  $15\text{ }^{\circ}\text{C min}^{-1}$  to 210 and finally maintained at  $210\text{ }^{\circ}\text{C}$  for 5 min. Mass spectra were recorded in the electron impact mode at an ionisation voltage of 70 eV in the 33–300 amu mass range. The ion source and the interface were maintained at  $220\text{ }^{\circ}\text{C}$ . Compounds identification was carried out with the mass spectra and retention times of standard compound, when available, or the NIST 12 and NIST62 mass spectral data base.

Statistical analysis was performed with Statistica ver. 6.0 (Statsoft Inc., Tulsa, OK, USA).

## RESULTS

The analysis of volatile compounds highlighted the presence of twenty-two monoterpenes (over all  $\alpha$ -pinene,  $\beta$ -pinene, camphene, *p*-cymene,  $\beta$ -myrcene and limonene) and sixteen sesquiterpenes such as  $\alpha$ -caryophyllene,  $\alpha$ -copaene and 9-epi-caryophyllene. Twenty-one monoterpenes were detected in the Alpe Bancet ricotta, 16 in the Alpe Gianna ricotta and 20 in the Alpe Pra ricotta. In these products also 13, 11 and 16 sesquiterpenes, respectively, were detected (Table 1).

Table 1. Monoterpenes (t) and sesquiterpenes (s) detected in the ricotta cheeses analysed for each mountain farm (n.i. – not identified)

Preglednica 1. monoterpeni (t) in seskviterpeni (s), odkriti v siru "ricotta" s posameznih gorskih farm (n.i. – ni identificirano)

		BANCET	GIANNA	PRA			BANCET	GIANNA	PRA
$\alpha$ -phellandrene	t	*	*	*	9-epi-caryophyllene	s	*	*	*
$\alpha$ -pinene	t	*	*	*	$\alpha$ -caryophyllene ( $\alpha$ -humulene)	s	*	*	*
$\alpha$ -terpineol	t	*	*	*	$\alpha$ -copaene	s	*	*	*
$\beta$ -myrcene	t	*		*	$\alpha$ -isocomene	s			*
bornyl acetate	t	*			$\beta$ -maaliene	s			*
$\beta$ -pinene	t	*	*	*	epi-cedrane	s	*	*	*
Camphene	t	*	*	*	isocaryophyllene	s		*	*
$\delta$ -3-carene	t	*	*	*	selinene	s	*	*	*
dihydro carveol acetate	t			*	sesquiterpene (n.i.)	s	*	*	*
D-verbenone	t	*		*	sesquiterpene (n.i.)	s	*		*
$\gamma$ -terpinene	t	*	*	*	sesquiterpene (n.i.)	s	*	*	*
Limonene	t	*	*	*	sesquiterpene (n.i.)	s	*	*	*
Linalool	t	*	*	*	sesquiterpene (n.i.)	s	*	*	*
Myrtenol	t	*	*	*	sesquiterpene (n.i.)	s	*		*
<i>p</i> -cymene	t	*	*	*	sesquiterpene (n.i.)	s			*
Sabinene	t	*	*	*	valencene	s	*	*	*
terpene (n.i.)	t	*	*	*					
terpene (n.i.)	t	*	*	*					
terpene (n.i.)	t	*		*					
terpene (n.i.)	t	*	*	*					
terpene (n.i.)	t	*	*	*					
terpene (n.i.)	t	*		*					

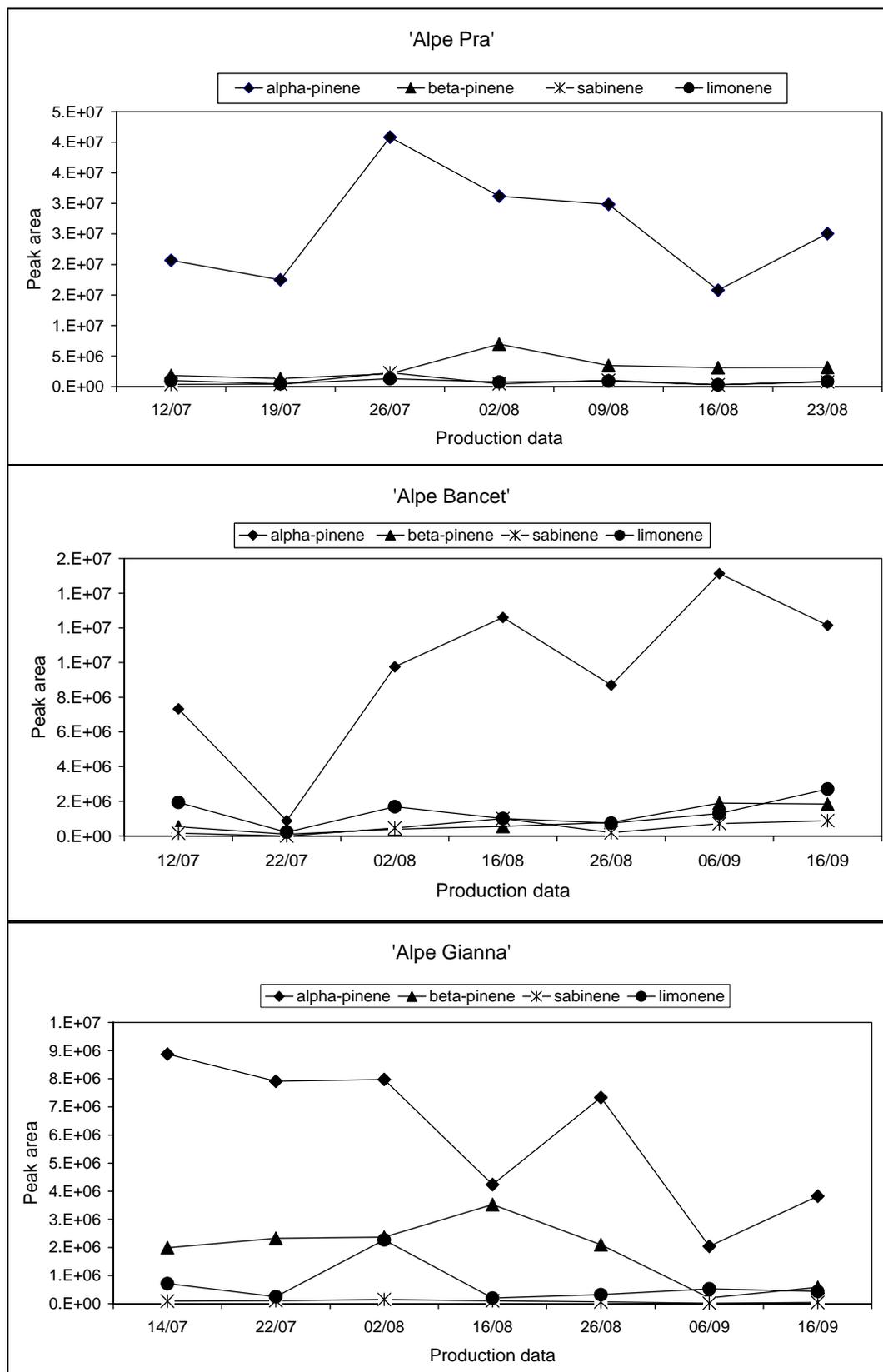


Figure 1. Trend of some terpenes (reported as area of base peak ion) for the three mountain farms ricotta cheeses during the grazing.

Slika 1. Trend gibanja posameznih terpenov v vzorcih sira "ricotta" s treh planinskih farm v pašni sezoni.

All these compounds showed a wide concentration variability due to the plant stage development and the pastured area during the mountain grazing. In Fig. 1 this effect is reported for some mono- and sesquiterpenes.

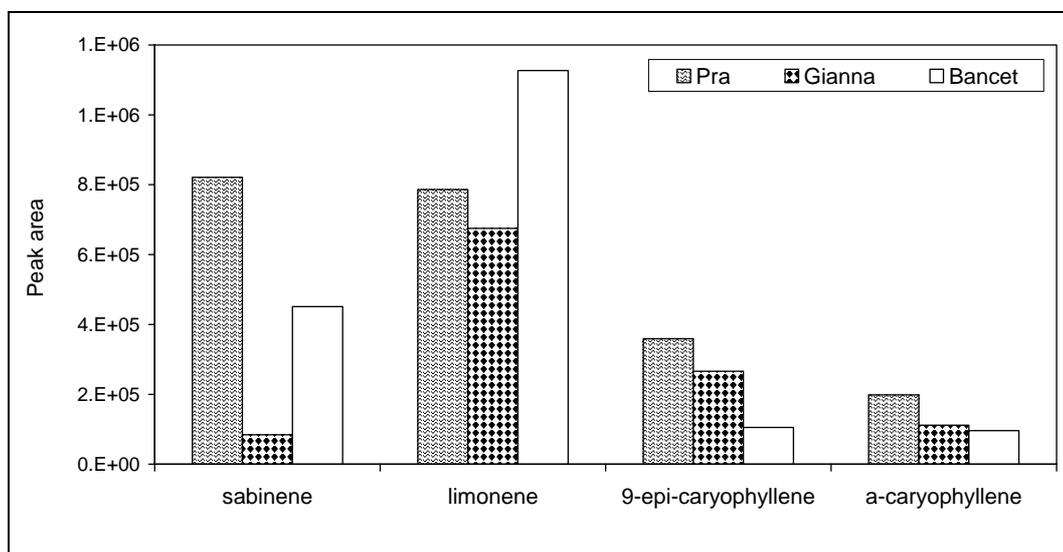


Figure 2. Area of base peak ion of two monoterpenes and two sesquiterpenes for the three mountain farms.

Slika 2. Površina vrhov dveh monoterpenov in dveh seskviterpenov na treh planinskih farmah.

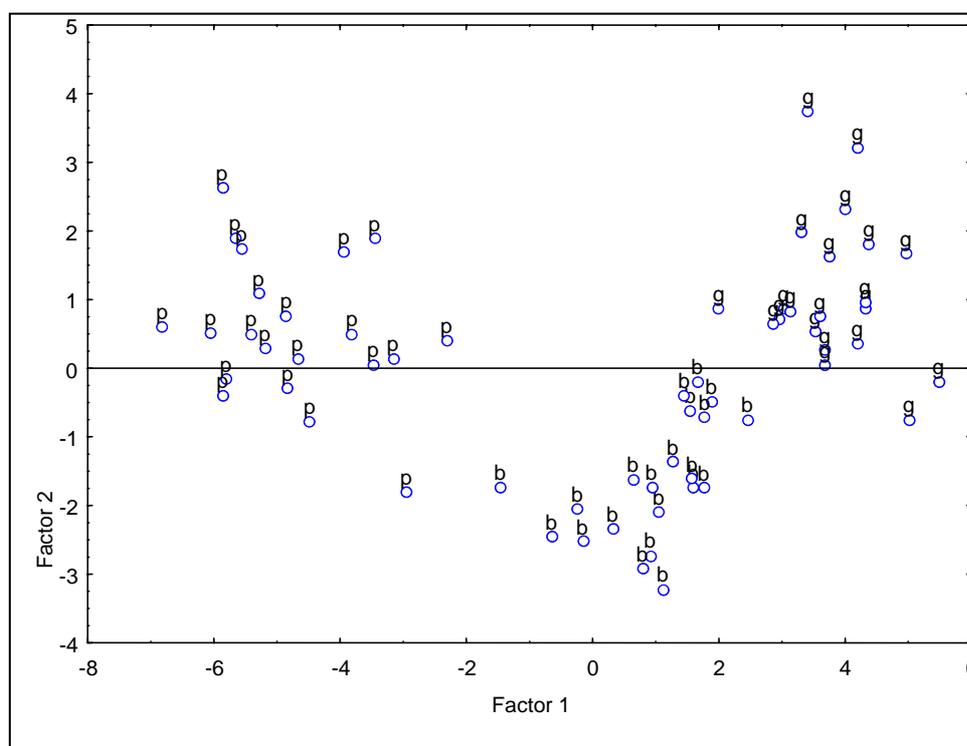


Figure 3. Score-plot with the ricotta cheese samples of the three mountain farms (g - Alpe Gianna; b - Alpe Bancet; p - Alpe Pra) calculated by terpene concentrations.

Slika 3. Porazdelitev vzorcev sira "ricotta" s treh planinskih farm (g - Alpe Gianna; b - Alpe Bancet; p - Alpe Pra) na osnovi koncentracije terpenov.

Although the concentrations of these compounds vary during the pasturing season, there are high differences between the three mountain farm ricotta cheeses (Fig. 2). Mono- and sesquiterpenes are particularly abundant in the Alpe Pra products and this is due to the mountain pastures with a small percentage of graminaceae and the presence of goat and ewe flocks. Concentration of mono- and sesquiterpenes in Alpe Bancet and Alpe Gianna is generally lower for the presence of cow herds and a high percentage of graminaceae in the pasture.

The differences between the three mountain farms can be highlighted with the Linear Discriminant Analysis calculated by areas of base peak ions for each detected terpenes (Fig. 3). The discrimination of Alpe Pra samples is excellent while for the other two farms some samples are misclassified. The refileing percentage is approximately 99% for Alpe Pra samples and 95% for the other farms. The differences between Alpe Bancet and Alpe Gianna are more evident at the end of the pasturing period (September) when, due to its northern exposure, there is still fresh grass in Alpe Gianna while the pasture is very reduced in Alpe Bancet.

## CONCLUSIONS

This study has confirmed the presence of mono- and sesquiterpenes in the mountain ricotta cheese in the mountain ricotta cheese much like those highlighted for other cheeses. These compounds can be used not only to distinguish between summer pasture products and the ones produced during other periods but also as markers of each mountain pasture. The study has also confirmed that SPME-GC-MS is a simple and effective technique for the study of terpene compounds.

## ACKNOWLEDGMENTS

Research supported by the Assessorato all'Agricoltura of Provincia di Torino, Italy.

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