

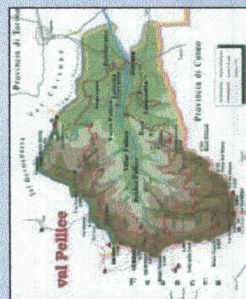


characterisation of terpenes and sesquiterpenes in Piedmont ricotta cheese

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Animal feeding is a very important factor in cheese characterisation due to its action on milk compounds such as fats, proteins, flavours, bacteria and so on. Several studies have then 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. These molecules are present in many plants but above all in dicotyledons and their content is related to botanical species (dicotyledons and particularly Apiaceae and Umbelliferae are richer than monocotyledons such as Poaceae) and to the plant's phenological phase. The transfer of these compounds to the milk can change its composition and particularly its microbiological and aroma characteristics. Several studies were then conducted to use the terpenes to trace the geographical origin or the nature of the feed supplied to animals. Results showed that milk from different production sites (lowland vs. highland) and seasons (winter vs. summer) can be distinguished but there is no research devoted to differentiate the production site located in the same area. As the obtained products from highland pastures in the same valley are very different, differentiation using terpenes and sesquiterpenes of these products could be possible. The aim of this work was to use terpene and sesquiterpene composition obtained by HS-SPME-GC-MS to define a traceability of cheese and the identification of producer.



FARM NAME	IDENTIFICATION CODE	ALTITUDE (m)	PASTURE SURFACE (ha)	HERB COMPOSITION
Alpe Bionda	G	1660-2000	24	20 goats
Alpe Pini Verdi	C	1780-2000	227	10 cows
Alpe Pini Verdi	CT	1780-2000	227	10 cows
Alpe Bionda	G	1660-2000	24	10 goats
Alpe Bionda	P	1660-2000	24	10 goats
Alpe Bionda	R	1660-2000	24	10 goats
Alpe Bionda	T	1660-2000	24	10 goats
Alpe Bionda	M	1660-2000	24	10 goats

MATERIALS AND METHODS

The study was conducted with a ricotta cheese named 'Saras del Fien' produced only during the summer in the alpine farms located in a small alpine valley of Piedmont (North West Italy) and ripened at least 21 days.

- The products of nine mountain farms were analysed.
- During the mountain grazing approximately every 7 days a sample of ricotta cheese was taken from each farm, put into vacuum-packed polyethylene bags and stored at -20°C.

INSTRUMENTATION AND GASCHROMATOGRAPHIC/MASS SPECTROMETRY CONDITIONS

- Analysis by SPME with a DVB/Carboxen/PDMS stable fibre (Supelco)
- Aroma extraction: 2.5 g of ricotta; conditioning for 10 min at 53 °C; extraction for 1 h at 53 °C; desorption for 6 min at 270 °C in splitless
- Analysis by GC-MS (column DB-WAX fused silica column, 30 m x 0.25 mm i.d., 0.25 µm film thickness)
- Carrier gas He at 1 ml/min
- Oven temperature: 35°C for 5 min, increased to 173 °C at a rate 2°C/min for an additional 1 min then increased to 210°C at a rate of 15°C/min and an additional 5 min
- Mass spectra recorded in TIC mode; ionisation voltage of 70 eV; 33-300 amu mass range; ion source and interface at 220°C
- Compound identification with the mass spectra and retention times of standard compounds, when available, or/and the NIST 12, NIST62 and Adams mass spectral data base

CHEMOMETRIC PROCESSING

- Artificial Neural Network (ANN) analysis with a three-layer, fully interconnected, feed-forward type
- 21 nodes in the input, 22 in the hidden and 9 in the output layer were used for the terpenes
- 16 nodes in the input, 22 in the hidden and 9 in the output layer were used for the sesquiterpenes
- Learning rate of 0.1 and momentum of 0.1
- For the input slab the linear activation function was used while the logistic functions were used for hidden and output layer slabs
- Inputs of the data sets were normalised in the 0-1 range before use in training and testing of the ANN
- The network learning was carried out with a limit of 200,000 events after the test set's minimum mean value of re-classification error was reached
- Samples randomly subdivided into a training set (70%) and a validation set (30%)
- The ANN construction process, from the two data set extractions through learning, was repeated five times.

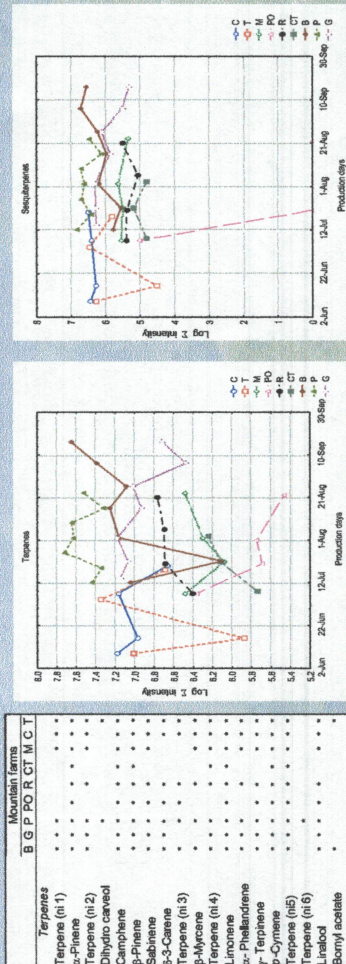
RESULTS

Over all 22 terpenes and 16 sesquiterpenes were detected. Depending on mountain farm and grazing period the total number of terpenes and sesquiterpenes varied from 27 to 37.

The more widespread terpenes are α -Pinene, Camphene, β -Pinene, δ -3-Carene, Limonene already reported in some alpine grassland herbs. For the sesquiterpenes the most important are α -Copaene, Selinon, 9-epi-Caryophyllene and Isocaryophyllene.

All these compounds showed a wide concentration variability due above all to the different composition of herds, but in each farm the herd changed pasture during the mountain grazing, according to the vegetative phase of herbs thus the differences for type and concentration of terpene and sesquiterpene compounds are given also to the different facies in the pasture and the vegetative phase of heated herbs.

Despite this concentration variability during the pasture, there are high differences between the nine mountain farm ricotta cheeses. These differences can be highlighted with the Artificial Neural Networks where the mean reclassification value is 99% with terpenes and 93% with sesquiterpenes.



Terpene and sesquiterpene evolution for each farm during the mountain grazing. For each sample the reported values are the sum of intensity of base peaks evaluated in TIC of all terpenes and sesquiterpenes detected.

Mountain farm	Sesquiterpenes	Terpenes
B	97	97
C	100	100
CT	95	100
G	95	100
M	100	100
P	99	100
PO	83	98
R	100	96
T	66	100

Mean reclassification value (%) for each mountain farm calculated with five cycles of learning using only the sesquiterpene or the terpene compounds

CONCLUSIONS

Terpenes and sesquiterpenes can be used not only to distinguish between summer pasture cheeses and the ones produced during other periods as reported by more Authors but can be used also as markers for each mountain pasture. Very important for this approach could be the ANNs since they constitute an efficient tool for characterising and discriminating food products. Moreover the ANNs could be used for the classification of samples not taken into account during learning for the control of commercialised products. But for a good interpretation of differences between mountain farms it is necessary to know exactly the botanical composition of the highlands and above all the terpene and sesquiterpene composition of each facies plant and their evolution according to phenological phase.

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