Integrating forage-system and analytical approaches for the certification of origin and the traceability of mountain cheese

G. Lombardi[1] and G. Zeppa[2]
[1] *University of Turin - Dept. AGROSELVITER, Grazing-land Management Unit, via L. da Vinci 44, 10095 Grugliasco (TO) - Italy Email: giampiero.lombardi@unito.it*
[2] *University of Turin - DIVAPRA, Food Technology Sector, via L. da Vinci 44, 10095 Grugliasco (TO) - Italy*

Keywords: artificial neural networks, GC-MS, vegetation composition, pastoral cadastre, tepenes

Introduction Since some years, consumers have showed in Italy an increasing interest towards typical products, in particular towards wine and cheese, especially when they are perceived as exclusive/niche products. Among typical products, some mountain cheeses have niche product features: small quantities, short production period (they are not available for the whole year), well geographically delimited area of origin, small number of producers, and they are generally produced in mountains or marginal areas. Gourmets' interest towards such products has mainly two effects: i) the development of rural tourism, generally directed towards the exploration of the *terroir* where cheeses are produced (to satisfy tourists sense of naturalness); ii) the increase of cheese value added, resulting from final price growth because of a reduced supply, compared with a large demand from the market. Consequently, producers and consumers need to be guarantee about the origin and the characteristics of such "high value added" cheeses, and to be protected from an illicit use of trademarks, through means by which verifying the link between the products and their *terroir*. To verify this link at least two different approaches are available: i) a forage system - territorial approach by, for instance, "pastoral cadastre" (Cavallero *et al.*, 2001), which is based on the computation of peak cheese yields from each summer pasture, or from each grazing sector of the pasture, that producers should not exceed; ii) an analytical approach by, for instance, site-specific natural isotope fractionation-nuclear magnetic resonance (SNIF-NMR), or gas chromatography-mass spectrometry analyses (GC-MS) that may allow a unique identification of the product through marker isotopes/molecules inside cheese (Zeppa et al., 2005). The former approach requires specific skills to draw a detailed map of vegetation (that is the basis of cadastre) and the control of effective dairy livestocks and milk yields, but it has little or no cost for laboratory analyses. With the latter the link has more objective basis, but laboratory analyses cannot be performed at large scale as they generally are expensive, destructive and time consuming.

To overcome the limits of the two approaches, a food chain approach combining "pastoral cadastre" and GC-MS analyses is proposed in this paper. It was applied for the certification of the origin and the traceability of *Saras del Fén* ricotta cheese. The *Saras del Fén* is produced during summer in a small valley near Turin (Italy), using a mixture of cow and/or sheep and/or goat whey added to cow and/or sheep and/or goat milk (10% on average). The whey is heated gradually at 60–70 °C. At this temperature the whey is added to the mixture of milks and then heated to 80 °C. Once this temperature is reached, the whey is coagulated with citric acid or magnesium sulphate. When the curd has formed, the whey–curd mixture is heated to 90–95 °C, then the curd is finally removed, salted and placed in linen cloths. These bags are hung for 24–48 h forming a characteristic half-sphere shape. At the end of this time, the ricotta cheese is removed from the linen cloths, if necessary salted dry, and ripened for at least 21 days at 8–12 °C in curing rooms.

Material and methods The forage system of 4 mountain farms leading animals to 3 summer pastures in Val Pellice (NW Italian Alps) was studied, starting from botanical composition of the native pastures exploited during cheese production period. Their pastoral value and their potential in supplying compounds that might be used as markers were determined. The botanical composition was surveyed according to the following protocol: i) delimitation of the grazing sectors of each summer pasture; ii) delimitation, inside each sector, of the borders of areas with homogenous vegetation (*facies*); iii) phyto-pastoral survey according to Daget & Poissonnet (1969) methodology, inside each homogeneous area; iv) survey of *facies* ecologic characters (morphology, altitude, aspect, slope, soil properties, etc.). Altogether 211 surveys were carried out on the 3 summer pastures.

The vegetation data were analysed by computing single species percent contribution to vegetation composition (CS) of each transect. Then, surveys were classified by cluster analysis, using Pearson correlation as similarity coefficient and average linkage as agglomeration method. Each cluster partition identified a unique vegetation type at a first level, which could be split into sub-types, at a lower level. The goodness of classification was checked with a bootstrap validation procedure (Whishart, 1999) performed starting from the original data set. The vegetation composition was then used to compute the forage values of the grassland with the pastoral value method (Daget & Poissonet, 1972) which, by further analysis, returned the carrying capacity of the grazing-land.

The pastoral management of the three mountain farms was surveyed by interviews to shepherds and direct observation. Data about grazing species, grazing management (use of fences, movement of herds, distribution of

water and mineral dispensers, time spent inside each grazing sector and indoor), milking (hand or machine milking, frequency and yields), and animal waste management were collected.
Joining vegetation and management data, "cadastral units" - which are the basic units of pastoral cadastre - were defined. They are grazing areas characterized by contiguousness, homogeneity of herbaceous cover, vegetation type and carrying capacity, which a given species or category of domestic animals can profitably exploit supplying a specific animal production, whose maximum may be computed on the basis of average milk production and yield of cheese-making. Vegetation and ecologic characters, carrying capacity, milk and cheese yields, and suggestions for optimum grazing management were reported for each cadastral unit.
For the individuation of markers, the 4 farms plus 5 other mountain farms (F1-F5), add for better control of variability, were considered. During summer approximately every 7 days a sample of ricotta cheese was taken from each farm. 132 ricotta cheese samples were then analysed to define mono- and sesquiterpene composition (3 replicates for each sample). After headspace solid-phase microextraction (HS-SPME), gas chromatography–mass spectrometry analyses (GC-MS) were performed. Compound identification was carried out with the mass spectra and retention times of standard compounds, NIST12, NIST62 (National Institute of Standards and Technology, USA) and Adams mass spectral data bases, and the retention indexes were calculated. Further details on analytical methods used are reported in Zeppa *et al.* (2005).
To define a traceability of cheese and possibly identify producers, artificial neural networks (ANNs) with the terpene compounds as input neurons and the farms as output neurons were used. ANNs were chosen because they are able to learn intelligently through an automatic process and define a model that can be used for the classification of unknown samples and the identification of the cheese-maker. ANNs were generated with NeuroShell 2 (Ward System Groups Inc., USA). The network architecture used was a three-layer, fully interconnected, feed-forward type. The default 3 layers network with 21 nodes in the input, 22 in the hidden and 9 in the output layer was used for monoterpenes. A network with 16 nodes in the input, 22 in the hidden and 9 in the output layer was used for sesquiterpenes. To avoid network overtraining, an implemented procedure of NeuroShell 2 was used. It creates an entirely separate set of data, called test-set, and uses it to evaluate how well the network is predicting. The network learning was carried out with a limit of 200000 events after the test-set minimum mean value of re-classification error was reached. ANNs were trained using a training set with 70% of the samples, and validated using remaining 30%.

Results With regard to vegetation, 14 vegetation types and 33 sub-types were identified by cluster analysis in the 3 summer pastures studied (**Table 1**). The presence of only two (FnAt, PfCsAr) out of the 33 sub-types in all the summer pastures indicated that a wide diversity of types occurred among the 3 summer pastures, not only within the same pasture. The vegetation of 53% of the surface exploited by animals was of anthropic origin, resulting from grazing management that was unchanged for a long time. The remaining portion was represented by high altitude types, influenced more by climate, morphology and soil water conditions than by management. While the first types are mainly dominated by grasses, high altitude types are rich in dycotiledons that may influence cheese sensorial properties and supply marker terpenes (Mariaca *et al.*, 1997; Bugaud *et al.*, 2001).
The diversity of vegetation resulted first of all in a diversity of forage values, which ranged from 0.5 to 31.6, and of carrying capacity, consequently. By settling the exploitation in the frame of a "pastoral cadastre", whose organization takes into account not only vegetation, but also the needs of grazing management and the morphology of the land, dairy productions might take advantage of such a diversity. With the goal of maximizing cheese diversity and controlling the production at the same time, a pastoral cadastre was drawn for the 3 summer pastures (in the first part of **Table 2** summary data for all the pastures are reported, while a detailed example for one of them is reported in the second part). On the basis of carrying capacity, mean lactation curve of bred races, milk harvest, and surveyed mean yield of cheese-making, *Saras del Fèn* potential yields (which is the main achievement of the pastoral cadastre in the view of the cheese labelling and certification of origin) were determined. The comparison between ricotta productive potential and current production highlighted that potential was on average widely unexploited (44% on average), as a consequence of reduced stocking-rates (30000 grazing days exploited overall of which only 53% by dairy animals) in comparison with carrying capacity (37500 grazing days), and also of a generalised inappropriate grazing management.
Ricotta cheese samples were analysed to verify the hypothesis that vegetation diversity among summer pastures results in a different terpene composition among cheeses, allowing traceability and affecting cheese sensorial properties (whose evaluation was not the objective of this study). By GC-MS analyses 22 mono- and 16 sesquiterpenes, all present in the farms for which pastoral cadastre was drawn, were detected (**Table 3**). Their number per farm ranged from 16 to 21 for monoterpenes, and from 5 to 16 for sesquiterpenes. The most widespread monoterpenes were α-pinene, camphene, β-pinene, δ-3-carene, and limonene. For the sesquiterpenes the most important were α-copaene, selinan, 9-epi-caryophyllene, α-caryophyllene, isocaryophyllene. All mono-sesquiterpenes found were previously reported in some alpine species (Mariaca *et al.*, 1997; Cornu *et al.*, 2001).

Table 1 Vegetation types of the 3 summer pastures studied

Vegetation type	sub-type	summer pasture[1]			dominant species	VP
		Bn	Gn	Pr		
	code	ha	ha	ha	(CS, percent specific contribution in brackets)	
Agrostis rupestris	Ar	16.7	-	-	A. rupestris (22), Potentilla grandiflora (8)	15.1
	PfCsAr	11.9	1.0	20.5	Potentilla grandiflora (15), Veronica allionii (8), Geum montanum (8)	11.4
Carex curvula	CcPy	-	12.4	-	C. curvula (26), Polygonum viviparum (15)	4.1
	CcTa	-	1.0	-	C. curvula (38), T. alpinum (22)	17.5
	VuCc	-	7.0	-	V. uliginosum (49), C. curvula (24)	.5
Carex sempervirens	CsFoAf	-	-	30.0	C. sempervirens (15), F. gr. ovina (15), Avenella flexuosa (14)	14.3
	CsFv	-	9.5	38.1	C. sempervirens (19), F. gr. violacea (13)	15.6
	CsPi	-	-	1.0	C. sempervirens (21), Plantago serpentina (14)	17.5
Festuca gr rubra	FnAt	7.9	23.2	71.4	F. gr. rubra (18), A. tenuis (13)	26.6
	FnCs	22.5	-	-	F. gr. rubra (12), C. sempervirens (8), Ranunculus gr. montanus (7)	21.6
	PaFn	6.6	-	9.0	Poa alpina (24), F. gr. rubra (12)	31.6
Festuca gr ovina	FoAr	0.4	-	-	F. gr. ovina (16), A. rupestris (9), V. uliginosum (8)	12.5
	FoHnCs	0.5	-	2.8	F. gr. ovina (19), Helianthemum nummularium (17), C. sempervirens (13)	18.0
	FoOm	-	-	0.4	F. gr. ovina (20), Onobrychis montana (13)	23.5
	FoPf	-	-	1.8	F. gr. ovina (11), Potentilla grandiflora (10), Anthoxanthum alpinum (9)	24.0
Festuca gr violacea	FvGm	58.2	-	-	F. gr. violacea (14), Geum montanum (12), Potentilla grandiflora (8)	15.6
	FvHs	-	-	7.8	F. gr. violacea (25), Helictotrichon sedenense (24)	14.6
	FvLa	-	5.4	15.8	F. gr. violacea (22), L. alpinus (16), Polygonum viviparum (9)	17.1
	FvTs	-	-	14.7	F. gr. violacea (17), Thymus gr. Serpyllum (11), Veronica allionii (10)	17.2
	AaPfFv	-	15.7	-	A. alpina (15), Potentilla grandiflora (13), F. gr. violacea (12)	19.6
	TsFvHs	-	-	7.2	Thymus gr. serpyllum (9), Alch. gr. alpina (9), Euphorbia cyparissias (9), F. gr. violacea (7)	4.8
Juniperus nana	JnHnCg	4.0	-	-	Juniperus nana (28), Helianthemum nummularium (15), C. ferruginea (10)	18.3
Nardus stricta	Ns	16.4	-	11.1	N. stricta (22), T. alpinum (5)	18.1
	FvNsVu	5.0	-	21.7	F. gr. violacea (11), V. uliginosum (9), N. stricta (7)	19.1
Lotus alpinus	PyLa	-	5.7	-	Polygonum viviparum (12), L. alpinus (11), Avenella flexuosa (9)	13.8
Salix herbacea and /or	ShArAp	12.3	-	0.4	Sal. herbacea (17), A. rupestris (8), Alch. pentaphyllea (7)	12.2
Alchemilla pentaphyllea	ApPg	17.2	-	9.0	Alch. pentaphyllea (28), Plantago alpina (10)	16.7
Sesleria varia	Sv	-	5.5	-	S. varia (31), Globularia cordifolia (10)	11.8
	SvSr	-	-	7.8	S. varia (28), Sal. reticulata (26), Hedysarum hedysaroides (11)	9.9
Trifolium alpinum	Ta	-	36.2	-	T. alpinum (44), Alch. gr. alpina (16)	25.1
	LmTa	-	32.4	-	Ligusticum mutellina (22), T. alpinum (13)	20.5
Vaccinium myrtillus	VmPv	-	-	2.3	V. myrtillus (22), Poa violacea (18), Rhododendron ferrugineum (13)	8.4
	FnAfVm	-	76.1	5.9	F. gr. rubra (12), V. myrtillus (9), Avenella flexuosa (9)	19.0

[1] in the 3 columns the total surface of each sub-type, when present, is reported. Summer pastures codes: Bn = Alpe Bancet, exploited by farm B; Gn = Alpe Gianna, farm G; Pr = Partia d'Amount, farms P and R.

Table 2 Summary data of summer pastures pastoral cadastre and example of by unit data for Alpe Gianna

Summer pasture	farm	cadastral unit	surface total	surface exploitable	altitude min-max	vegetation type primary	vegetation type second.	VP	carrying capacity		grazing animals[1]	*Saras del Fén* C[2]	*Saras del Fén* S+G[2]
		n°	ha	%	m				d ha^{-1}	d total		kg	kg
Bn	B	9 tot	251	71	1850-2780			17	39	6927		2303	434
Gn	G	8 tot	391	66	1700-2670			19	57	13081		7761	1924
Pr	P-R	11 tot	375	74	1710-2670			19	63	17476		6419	1429
Details for Gn summer pasture and G farm													
		1	93.2	64	1750-2100	FnAfVm	-	19	75	4461	C,S,G	3022	649
		2	21.8	79	1700-1980	FnAt	-	27	130	2243	C,S,G	1444	416
		3	12.7	98	1950-2070	FnAt	Ta	25	98	1221	C,S,G	826	210
		4	44.1	62	2050-2300	Ta	-	24	56	1530	C,S,G	1046	210
		5	75.4	47	2050-2650	LmTa	PyLa	19	37	1312	C,S,G	750	155
		6	28.9	68	2050-2400	FnAfVm	FnAt	19	49	968	C,S,G	673	161
		7	72.8	35	2300-2600	CcTa	CsFv	12	24	623	S,G	-	66
		8	42.1	72	2100-2670	AaPfFv	CcPy	13	24	723	S,G	-	57

[1] C = dairy cattle; S = sheep; G = goats.

[2] potential ricotta cheese yields in the case of dairy cattle (C) and sheep (70% of flock) + goat (30%) grazing (S+G).

Some terpenes were less diffused, and some others were constantly found only in the cheese of one farm, which is a good assumption for traceability. In general terpene composition was complex, so that the use of ANNs to characterize farm cheeses was justified, besides the possibility of classification of further unknown samples. Mono- and sesquiterpenes were used as input neurons in two separate networks with farms as output. The mean reclassification values of ANNs were 99% and 93% for mono- and sesquiterpenes respectively, reaching 100% in 6/9 farms for mono- and 3/9 farms for sesquiterpenes.

Table 3 Mono- and sesquiterpenes detected in ricotta samples of each mountain farm (B,G,P,R + F1-F5).

Monoterpenes / farm:	B	G	P	R	F1	F2	F3	F4	F5	Sesquiterpenes / farm:	B	G	P	R	F1	F2	F3	F4	F5
Monoterpene (ni 1)	*	*	*				*	*	*	α-Copaene	*	*	*	*			*	*	*
α-Pinene	*	*	*	*	*	*	*	*	*	β-Maaliene			*						
Monoterpene (ni 2)	*	*	*	*			*	*	*	Isocomene			*						
Monoterpene (ni 3)			*						*	Sesquiterpene (ni 1)			*						
Camphene	*	*	*	*	*	*	*	*	*	Selinan	*	*	*					*	*
β-Pinene	*	*	*	*	*	*	*	*	*	9-epi-Caryophyllene	*	*	*	*	*	*	*	*	*
Sabinene	*	*	*	*	*		*	*	*	Sesquiterpene (ni 2)	*	*	*				*	*	*
δ-3-Carene	*	*	*	*	*			*	*	Sesquiterpene (ni 3)	*		*					*	*
Monoterpene (ni 4)	*		*	*	*			*	*	α-Caryophyllene	*	*	*	*	*		*	*	*
β-Myrcene	*		*	*			*	*	*	epi-Cedrane	*	*	*	*		*	*	*	*
Monoterpene (ni 5)	*	*	*	*		*	*	*	*	Sesquiterpene (ni 4)	*	*	*	*				*	
Limonene	*	*	*	*	*	*	*	*	*	Sesquiterpene (ni 5)	*		*					*	*
β-Phellandrene	*	*	*	*			*	*	*	Isocaryophyllene	*	*	*			*			*
γ-Terpinene	*	*	*	*	*		*	*	*	Sesquiterpene (ni 6)	*	*	*						
p-Cymene	*	*	*	*	*	*	*	*	*	Sesquiterpene (ni 7)	*	*	*				*	*	*
Monoterpene (ni 6)	*	*	*	*	*	*	*	*	*	Valencene	*	*	*					*	*
Monoterpene (ni 7)			*																
Linalool	*	*	*	*	*	*		*	*										
Bornyl acetate	*								*										
Verbenone	*		*		*		*		*										
α-Terpineol	*	*	*		*			*	*										
Myrtenol	*	*	*	*				*	*										

Conclusions

With the pastoral cadastre the maximum quantity of cheese for the *terroir* of *Saras del Fén* was defined for each cadastral unit. Two goals were achieved at the same time: the control of produced quantities and a characterisation of productions by linking cheese production to a dominant vegetation type, which itself may result in cheese valorisation. Furthermore, reorganization of grazing, which is a requirement for cheese production diversification, was offered by cadastre (resulting in the possibility to increase the quantity of labelled product). Nevertheless, the cadastre returned cheese yields referred to standard conditions of herd productivity and carrying capacity, so that at least two controls of productivity during grazing season to adjust lactation curve and a constant control of dairy heads will be required at the operating stage.

By GC-MS analyses and subsequent ANNs data analysis, the objective of obtaining a traceability of cheese and of identifying the producer was achieved. Furthermore the possibility of using mono- and sesquiterpenes not only to distinguish between summer pasture cheeses and the ones produced during other periods (which has already been reported by several authors), but also as markers for each mountain pasture or farm was showed.

The two approaches together supply all the data to draw a complete product label, with information about the whole food chain and an "identity card" of the product, so that a cross control system to protect producers and consumers from fakes coming either from inside or outside cheese territory of origin may be established.

Finally, even if the investigation about the link between vegetation and terpene cheese composition still is at its beginning, the variability of botanic composition of the grazing-land seemed to result in important differences of terpenes composition among summer pastures and/or farms.

References

Cavallero A., Lombardi G., Tagliatori C., Martinasso B., 2001. Proposta di Catasto Pastorale delle Valli Ossolane. Regione Piemonte, 151 pp.

Cornu A., Carnat A.P., 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.

Daget Ph., Poissonet J., 1969. Analyse phytologique des prairies. Application agronomiques. CNRS - CEPE Montpellier, document n.48.

Daget Ph., Poissonet J., 1972. Un procède d'estimation de la valeur pastorale des pâturages. Fourrages 49:31-40.

Mariaca R.G., Berger T.F.H., Gauch R., Imhof M.I., Jeangros B., Bosset J.O., 1997. Occurrence of volatile mono- and sesquiterpenoids in highland and lowland plant species as possible precursors for flavor compounds in milk and dairy products. Journal of Agricultural Food Chemistry, 45:4423-4434.

Wishart D., 1999. Clustan graphics primer: a guide to cluster analysis. Clustan Limited, 60 pp.

Zeppa G., Giordano M., Bertolino M., Gerbi V., 2005. Application of artificial neural network on mono- and sesquiterpenes compounds determined by headspace solid-phase microextraction–gas chromatography–mass spectrometry for the Piedmont ricotta cheese traceability. Journal of Chromatography A, 1071(1-2):247-253.