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Volatile compounds in herbage intake by goats in two different grazing seasons

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SUMMARY – Variations in volatile organic compound (VOC) composition of herbage grazed by goats, in two seasons, were investigated. Fifteen non-supplemented lactating goats grazed for 8 hours/day an area of 1.2 ha, from March to middle spring and from middle spring to early June. Artificial diets were formed on the basis of grazing behaviours estimated on five areas of 2x2 m, randomly distributed in the pasture. The contribution of each species to the diet was estimated by the ratio between the number of plants grazed for single species, and the number of plants, for the same species, present in the delimited area before grazing. VOC composition was analysed by HRGC-MS. Evaluation of VOC content in herbage showed maximum concentrations in spring, when the grazed herbage was composed especially by dicotyledons and by apical leaves, flower bud and flowers. Alcohols and ketones were the most abundant compounds both in winter and in spring. In winter, the monoterpenes were higher than sesquiterpenes, in spring, however, the situation inverted. Herbage grazed in winter and spring showed the same alcohol, ketone and monoterpene profile, whereas the sesquiterpene profile exhibited substantial differences. In winter only five sesquiterpenes were found in grazed herbage, while in spring 21. These results showed that season and animal behaviour were the dominant factors that influenced the richness in secondary metabolites in grazed herbage.

Keywords: Goat, grazed herbage, alcohols, ketones, terpenes.

RESUME – *"Composés volatils de l'herbe ingérée par des chèvres au pâturage au cours de deux saisons". L'objectif de cet essai est d'étudier les variations des composés volatils dans l'herbe ingérée par des chèvres au pâturage au cours de deux saisons. Quinze chèvres sans complémentation ont pâturé chaque jour pendant des heures sur une prairie naturelle d'environ 1,2 ha à partir du mois de mars jusqu'à mi-avril et de mi-avril jusqu'au début juin. La composition botanique de l'herbe ingérée a été estimée sur la base du comportement alimentaire sur cinq parties de 2x2 m choisies au hasard dans le pâturage. L'incidence de chaque espèce dans le régime a été déterminée par le rapport entre les plantes pâturées de chaque espèce et le nombre de plantes de la même espèce présentes dans les parties avant pâturage. La composition en composés volatils (VOC) a été analysée par HRGC-MS. L'évolution en VOC dans l'herbe ingérée a montré des concentrations maximales au printemps, quand l'herbe ingérée est composée particulièrement par des dicotylédones et par les feuilles apicales, le bouton à fleur et les fleurs. Les alcools et les cétones étaient les composés les plus abondants en hiver et au printemps. En hiver, les monoterpènes étaient plus abondants que les sesquiterpénes. La situation inverse a été constatée au printemps. L'herbe pâturée, en hiver et au printemps, a montré le même profil pour les alcools, les cétones et les monoterpènes. Le profil des sesquiterpènes a varié considérablement. En hiver seulement cinq sesquiterpènes ont été trouvés dans l'herbe pâturée, alors qu'au printemps il y en avait 21. Ces résultats montrent que la saison et le comportement alimentaire de l'animal étaient les principaux facteurs qui affectent la richesse en métabolites secondaires de l'herbe pâturée.*

Mots-clés : *Chèvre, herbe pâturée, alcools, cétones, terpènes.*

Introduction

The diet of grazing animals is very different from season to season, according to the variability of pasture botanical composition. This botanical diversification could be a source of enrichment, or impoverishment, in secondary metabolites of milk and therefore of cheese, considering that these compounds change in quantity and type from plant to plant. Some plants, such as *Festuca pratensis* and *Dactylis glomerata,* contain few terpenoids, others (*Mentha longifolia*, *Heracleum sphondylium*), instead, contain many of them (Mariaca *et al.,* 1997). However, several factors may affect the distribution and abundance of secondary metabolites, such as seasonal climatic changes, the kind of aerial plant part and natural enemies (Geervliet *et al.,* 1997). Some terpenoids, such as linalool and geraniol decrease as temperature increases; on the contrary, others, such as citronellol (Rajeswara Rao *et al.,* 1996) increase. The quantity and the composition of essential oils from plants are closely related to the plant's developmental stage. In peppermint (*Menthax piperita*), Rohloff (1999) found that β-pinene 1,8-cineole and sabinene decreased concentrations from apical to basal leaves, while limonene and p-cymene increased. In yarrow (*Achillea millefolium*), Rohloff *et al.* (2000) observed that during the vegetative stage sabinene and 1,8-cineole were abundant, camphor and borneol during early bloom, whereas during full bloom alfa and beta pinene and alfa thujone were more abundant. According to these results it seems that volatile compounds increase as the plant develops. On the basis of this information, it seems that winter and spring diets can be greatly diversified one from another, because they change considerably the botanical composition of pasture and aerial parts selected by goats.

This hypothesis seems to be supported by the results obtained in milk or cheese. Fedele (2001a) observed that in winter, when graminaceous plants (grasses) were dominant in the pasture, milk was particularly rich in hydrocarbons and alcohols; in spring, when the different vegetable categories are balanced, alcohols and ketones predominated. Scehovic (1991) reported that cheese produced from a pasture rich in dicotyledons had a different flavour than that produced from pasture rich in Gramineae. Since the botanical composition of native pasture changes from a dominance of monocotyledons (grasses) in winter to a dominance of dicotyledons (legumes and forbs) in spring, it seems that this variability modified goat's selectivity, also modifying secondary metabolites content in the diet. The objective of this study was, exactly, to evaluate the effect of botanical composition on the content and profile of secondary metabolites of herbage grazed by goats.

Materials and methods

Pasture and grazing trials

A native herbaceous pasture in a Basilicata valley (Southern Italy) at 360 m a.s.l. was used for this experiment. The botanical composition changed considerably from season to season, according to climatic conditions, cold in winter (from -6 to 8°C) and temperate in spring (from 16 to 23°C). Grasses, and particularly *Lolium perenne, Dactylis glomerata* and *Bromus* spp*.,* developed in winter, while legumes (*Medicago polimorpha*, *Trifolium repens*, *Vicia* spp.) and certain forbs (*Ranunculus bulbosus*, *Asperula odorosa*, *Daucus carota*, *Geranium molle*, etc.) during spring. Fifteen non-supplemented lactating goats grazed for 8 hours/day an area of 1.2 ha from March to May in two experimental periods: winter (from March to middle April) and spring (from middle April to early June), on five areas of 2x2 m, randomly distributed in the pasture. The contribution of each species to the diet was estimated by measuring the ratio between the number of plants grazed of a single species and the number of plants of the same species present in the delimited area before grazing. On the basis of this information an "artificial diet" was formulated. For each species, plant samples corresponding to those really browsed by goats were cut from an un-grazed area and mixed in the same estimated proportions, and immediately stored in liquid nitrogen. This diet, so formed, was used for chemical analyses.

Herbage intake was estimated by the difference between herbage mass, measured on an un-grazed area, and post-grazing herbage mass measured in experimental grazed areas, on five 2x2 m sampling units.

Volatile organic compounds (VOC) determination

The VOC content in the diet sample was determined by a modified headspace technique. A gram of diet was flushed with pure helium and VOC released were collected into adsorption traps, filled with graphitic sorbents until a quantitative extraction was achieved. VOC were analysed by HRGC-MS after thermal desorption of traps performed at 250°C. The separation was carried out on a capillary column (50 m x 0.32 mm ID) internally coated with a non-polar silicon phase. VOC were identified on the basis of their mass spectra. Selected ions were used to quantify overlapping peaks or those present at trace levels.

Results and discussion

Botanical composition of herbage intake

The higher availability of grasses in the pasture (88-85%) during winter prevented goats from having the possibility to select many species. For this reason, their diet (Table 1) was made up of 90% of grasses, especially *Lolium perenne* (60%) and *Dactylis glomerata* (25%).

Table 1. Botanical composition of herbage intake by goats (%) during winter and spring (species selected over 5%)

Species selected	Winter (from March to middle spring)	Spring (from middle spring to May)
Lolium perenne	60	10
Dactylis glomerata	25	8
Phleum pratense	5	5.5
Poa pratensis		6
Medicago polimorfa		13
Medicago lupolina		8
Asperula odorosa		12
Rumex sp.		6.5
Geranium molle		8
Total grasses	92	39.5
Total legumes	1	24
Total forbs	7	36.5
Specie selected	8	28
Intake (g/day DM)	520	680

During spring, with increasing temperature, the botanical composition of pasture significantly changed. The number of species increased from 12 in winter to 37 in spring. Goats adapted their behaviour selecting other 20 species (28 vs 8), modifying the proportion between the different botanical families: grasses decreased from 92% to 39.5%, legumes increased from 1 to 24% and forbs from 7 to 36.5%.

The VOC content of herbage intake

Seasonal evolution of volatile compound content in herbage selected by goats is reported in Fig. 1. The most important constituents of winter and spring herbage were alcohols. These compounds and the sesquiterpenes increased (16,280 vs 93,190 ng/g DM and 209 vs 3,332 ng/g DM respectively); in a different way, ketones and monoterpenes showed smaller variability (5,856 vs 6,246 ng/g DM and 1,390 vs 2,057 ng/g DM respectively).

These differences, in any case, could be cancelled or increased, depending on the level of herbage intake. In our case, since the intake was higher in spring (680 vs. 520 g /day DM), the differences between the two seasons increased considerably (Fig. 2). During winter, goats ate fewer VOC than in spring (75 times for alcohols, 21 times for sesquiterpenes, 2.0 times for monoterpenes and 1.5 times for ketones).

The ratio of terpenes and other secondary metabolites was similar (7/93) in the two seasons, while the ratio between mono- and sesquiterpenes changed from 88/12 to 38/62 for winter and spring, respectively. No esters were found in herbage grazed in winter and spring.

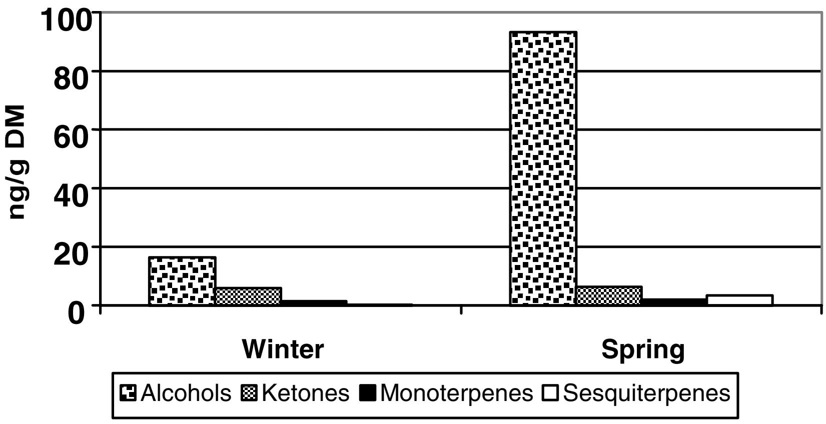

Fig. 1. Diet's VOC content in winter and spring.

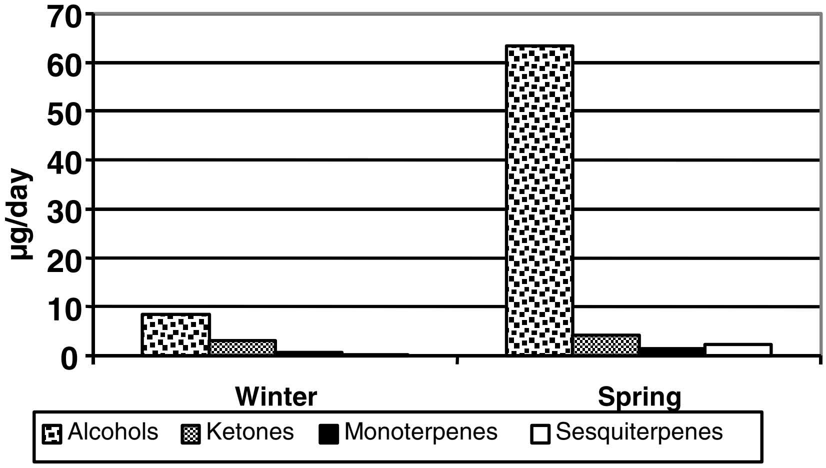

Fig. 2. VOC intake by goats in winter and spring.

The higher content of secondary metabolites of spring grazed herbage may be due to the contemporaneous action of two factors: higher presence of dicotyledons and the goat's preferential selection of apical leaves, flower buds and flowers. These selected parts and the dicotyledons are particularly rich in secondary metabolites (Schantz and Ek, 1971; Mariaca *et al.*, 1997; Rohloff, 1999; Rohloff *et al.*, 2000). Such high presence of alcohols and ketones in grazed herbage (over 90%) suggests that the aroma of milk and cheese can be influenced by these metabolites, since these many alcohols and ketones are used by the fragrance industry to prepare perfume compositions with characteristic notes (fruit, green odour, mushroom, etc.). Until now, only the effect of mono- and sesquiterpenes on milk or cheese aroma has been investigated (Dumont *et al.*, 1981; Scehovic, 1991; Bosset *et al.*, 1994). The season also modified these metabolites, especially their ratio. The ratio between mono- and sesquiterpenes changed from 88/12 in winter to 38/62 in spring.

The ratio seemed to be influenced by the type of plant. In some plants, such as *Meum, Achillea*, *Tymus*, etc., the mono- and sesquiterpenes ratio was equivalent; in others, such as *Pimpinella, Taraxacum, Plantago*, etc., sesquiterpenes were predominant (Cornu *et al.*, 2001). The ratio between these metabolites in milk reflected that of herbage, as found by Claps *et al.* (2003), who reported a mono/sesquiterpenes ratio of 44/56 in winter and of 18/82 in spring in goat milk.

Alcohol and ketone profiles

Winter and spring herbages showed the same alcohol and ketone profiles, but different molecule concentrations (Fig. 3). Winter herbage was richer in 2-propanol (72.3% vs 62.6%), 1-propanol and 2-butanol alcohols, while spring herbage in ethanol (30.3% vs 16.5%) and 1- and 2-penthanol. The concentration of ketones changed from winter to spring. 2-nonanone (4.5% vs 0.4%) and 2,3 butanedione (3.0% vs 1.2%) were higher in winter herbage than in spring, whereas 2-propanone was higher in spring than in winter (90.2% vs 81.3%).

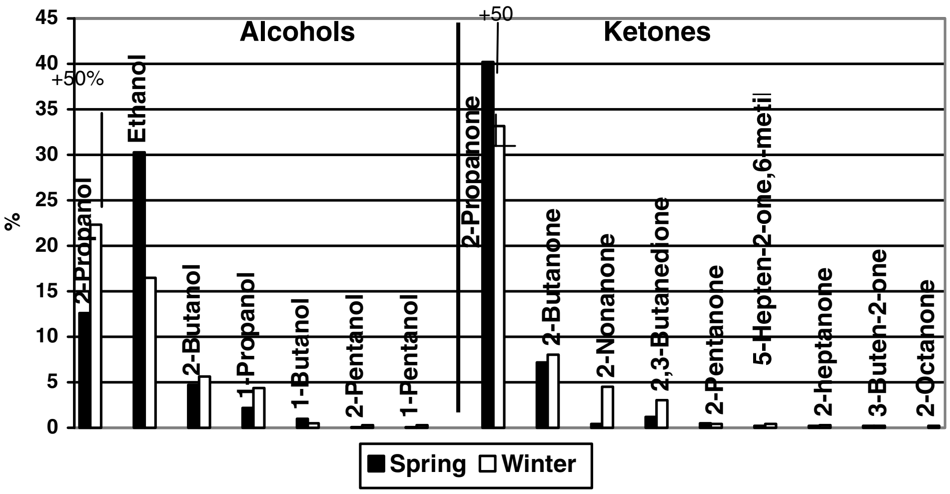

Fig. 3. Profile of alcohols and ketones in herbage selected by goats.

Since the only difference between the two seasons was observed in the concentration of each molecule, these secondary metabolites were not supposed to affect milk or cheese characteristics. Almost all these molecules are contained also in cheese. Bosset and Liardon (1994) reported that ethanol, 2-butanol and 1-butanol were found in high quantities in the Swiss Gruyère cheese, but no 2-propanol was found.

Mono and sesquiterpene profiles

Figure 4 shows mono and sesquiterpene profiles in winter and spring herbage. Twenty monoterpenes were identified in winter herbage and nineteen in the spring one (Fig. 4), but some molecules found in a season were absent in the other. Δ-3-Carene, α-terpinene and cis-β-ocimene were identified only in winter herbage, cis and trans-linalol oxide only in spring herbage. Winter herbage was characterized by higher contents of p-cymene (29.9% vs 21.5%), β-phellandrene (15.5% vs 9.9%) and α-pinene (15.4 vs 9.5%), and spring herbage by higher contents of linalool (30.5% vs 5%) and α+y terpineol (5.2% vs 2.0%).

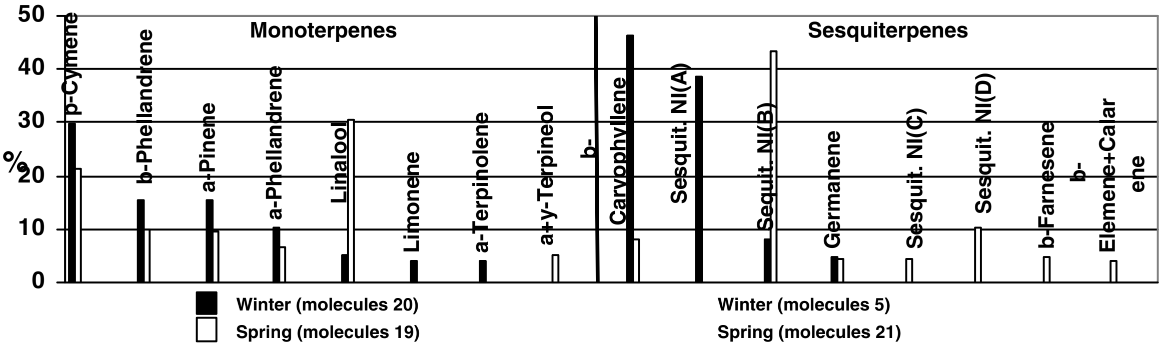

Fig. 4. Profile of mono and sesquiterpenes in herbage selected by goats (values over 4%).

The biggest difference between the two seasons was observed in the sesquiterpene profile (Fig. 4). In winter herbage only five molecules were found, in spring herbage twenty-one. In the first season, β-caryophyllene and two unidentified sesquiterpenes (A and B) accounted for 92.2 of total sesquiterpenes (46.4%, 38.5% and 8.7%, respectively). In spring β-caryophyllene decreased from 46.4% to 8% and the unidentified sesquiterpenes (B) increased from 8.7% to 43.5%. In this season another unidentified sesquiterpenes (D) were accounted for 10.3%, and β-farnesene 4.5% and β-elemene+calarene 4.2%, absent in winter herbage, were identified.

A large number of these molecules was found also in milk and cheese (Fedele, 2001b; Bosset *et al*., 1994; Coulon *et al*., 2000), but their specific contribution to the sensorial characteristics is not yet well known.

The importance of monoterpenes decreased from winter to spring, and that of sesquiterpenes increased. The last ones were, probably, the most important for the aroma of spring cheese.

Conclusions

Season and animal behaviour were the dominant factors that influenced richness in secondary metabolites of herbage selected by animals. In winter the lower availability of botanical families and plant species in the pasture explained the low presence of secondary metabolites in grazed herbage. The relatively high abundance of alcohols and ketones in winter, still higher in spring, suggested that these compounds could have a role on determining milk and cheese aroma. The high abundance of p-cymene monoterpenes and β-caryophyllene and unidentified sesquiterpenes (A) in winter, linalool and unidentified sesquiterpenes (B) in spring suggested that these terpenes could be used as markers of winter and spring native pastures. The abundance of sesquiterpene compounds in spring could explain why shepherds assert that the cheeses produced in spring and summer have a more pronounced flavour and taste.

References

Bosset, J.O., Bütikofer,U., Gauch, R. and Sieber, R. (1994). Caractérisation de fromages d'alpages subalpins suisses: Mise en évidence par Purge and Trap des arômes volatils de ces fromages [Occurrence of terpenes and hydrocarbons in Swiss Gruyère and Etivaz Highland cheese using dynamic headspace GC-MS analysis of their volatile compounds]. *Schweiz. Milchwirtssch. Forsch*., 23: 37-41.

Bosset, J.O. and Liardon, R. (1994). *The Aroma Composition of Swiss Gruyére Cheese: II. The Neutral Volatile Components*. Information-FAM, Mars 1994/280 W, 6-9.

Claps, S., Rubino, R., Fedele, V., Morone, G. and Di Trana, A. (in press). Effect of concentrate supplementation on milk production, chemical features and milk volatile compounds in grazing goats. In: *Sustainable Grazing, Nutritional Utilization and Quality of Sheep and Goat Products*, Proceedings of the 1st Joint Seminar of the FAO-CIHEAM Sheep and Goat Nutrition and Mountain and Mediterranean Pasture Networks, Granada (Spain), 2-4 October 2003 (this issue).

Cornu, A., Carnat, A.P., Martin, B., Coulon, J.B., Lamaison, J.L. and Berdagué, J.L. (2001). Solid-Phase Microextraction of Volatile Components from natural grassland plants. *J. Agric. Food Chem.,* 49: 203-209.

Coulon, J.B., Martin, B., Verdier-Metz, I., Buchin, S. and Viallon, C. (2000). Etude du lien entre terroir et produit dans le cas de fromages AOC : Influence de la composition floristique des fourrages sur les caractéristiques chimiques et sensorielles des fromages affinés. *7èmes Recontres Recherches Ruminant*, Paris, 6-7 December 2000, pp. 304-307.

Fedele, V. (2001a). Grazing for different quality of cheese. *Caseus International*, 2: 36-40.

Fedele, V.(2001b). Feeding and product quality. In: *Nutrition and Feeding Strategies of Sheep and Goats under Harsh Climates. 9th Seminar of the FAO-CIHEAM Sub-Nework on Sheep and Goat Nutrition*, Hammamet (Tunisia), 8-10 November 2001.

Dumont, J.P., Adda, J. and Rousseaux, O. (1981). Exemple de variation de l'arôme à l'intérieur d'un même type de fromage: Le Comté. *Lebensm-Swiss Technol*., 13: 198-202.

Geervliet, J.B.F., Posthumus, M.A., Vet, L.E.M. and Dicke, M. (1997). Comparative analysis of Headspace volatiles from different caterpillar-infested or uninfested food plats of Pieris species. *Journal of Chemical Ecology*, Vol.23, No. 12: 2935-2954

Mariaca, R., Berger, T., Gauch, R., Imhof, M., Jeangros, B. and 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. *J. Agric.Chem.*, 45(11): 4423-4434.
Rajeswara Rao, B.R., Kaul, P.N., Mallavarapu, G.R. and Ramesh, S.(1996). Effect of seasonal climatic changes on biomass yield and terpenoid composition of Rose-scented geranium. *Biochemical systematics Ecology*, Vol. 24, No. 7/8, pp. 627-635.
Rohloff, J. (1999). Monoterpenes composition of essential oil from peppermint (*Mentha* x *piperita* L.) with regard to leaf position using Solid-Phase Microextraction on Gas Chromatografy/Mass Spectrometry Analysis. *J. Agric. Food Chem.*, 47: 3782-3786.
Rohloff, J., Skagen, E.B., Stehen, A.H. and Iversen, T.H. (2000). Production of yarrow (*Achillea millefolium* L.) in Norway: Essential oil content and quality. *J. Agric. Food Chem.*, 48: 6205-6209.
Schantz, V.M. and Ek, B.S. (1971). Ueber die Bildun von ätherischem oel in Kümmel, *Carum carvi* L. (About the formation of essential oil in caraway, *Carum carvi* L.). *Sci. Pharm.*, 39: 82-101.
Scehovic, J. (1991). Considérations sur la composition chimique dans l'évolution de la qualité des fourages des prairies naturelles. *Rev. Suisse Agric.*, 23: 305-310.