

Characterization of plant diversity of pastures and volatile organic compound analysis in ewe's milk from a typical farm system in the Alta Murgia national Park (southern Italy): opportunities for a sustainable land use

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Abstract

In recent years, there has been an increasing interest of consumers in traditional dairy products whose origin from defined geographical areas is guaranteed. These products are appreciated for the distinctive sensorial characteristics, derived by the extensive grazing system on natural and artificial pastures and traditional cheese-making techniques. Moreover, a considerable public interest is also attributed to typical dairy products for their role in stimulating the economy of rural areas and in preserving environment and biodiversity. Here we present the results of a survey on the botanical composition of Alta Murgia pastures, together with the analysis of VOCs (volatile organic compounds) in milk from ewes reared under a typical farm system in the Alta Murgia National Park (Apulia region, southern Italy). Data from two less ordinary farm systems are also reported as external reference. The botanical composition of pastures was estimated by three modified Whittaker plots placed in the prevailing habitat types. Milk samples were analyzed for VOC compounds by head-space SPME/GC-MS. Consumer acceptance of cheese, made from each milk sample using

homogeneous traditional cheese-making techniques, was evaluated. The study provides interesting insights on the floral composition of Alta Murgia pastures and the first characterization of VOC profiles in ewe's raw milk from Alta Murgia.

Introduction

Due to major changes in rural production systems, traditional sheep farming in marginal areas such as Alta Murgia (Apulia region, southern Italy) has suffered a severe contraction during the last decades. As a consequence, a general tendency for abandonment of extensive sheep farming systems and intensification has been observed among the few remaining sheep farmers, with negative implications on the farmland environmental value. The recent establishment of the Alta Murgia National Park, together with the inclusion of the area into the Natura 2000 network (Habitat Directive 92/43/CEE) is giving rise to concerns by local farmers about constraints on livestock production. Efforts are being made in order to harmonize needs for economic profitability and environmental protection. Traditional sheep farming, characterized by the multifunctional nature of its production activities, would match well with the request for land use diversification and biodiversity conservation. In addition, pasture grazing is recognized as a key factor in preserving permanent grasslands from successional processes and conserving highly ecologically valuable semi-natural habitats (MacDonald *et al.*, 2000; Sarmiento, 2006). On the other side, qualification of local traditional dairy products may represent a key opportunity to improve socio-economic development in rural marginal areas such as Alta Murgia. Several studies have addressed the characterization of local and typical dairy products, some of them relating chemical and sensory properties of milk and cheese to botanical composition of natural pastures (Viallon *et al.*, 2000; Rubino and Claps, 2000; Chilliard *et al.*, 2001; Martin *et al.*, 2001; Bugaud *et al.*, 2002; Gorlier, 2002; Verdier-Metz *et al.*, 2002; Coulon *et al.*, 2004). The aim of this research was to provide a first characterization of the volatile organic compound (VOC) profiles in raw ewe's milk together with the results of a consumer acceptance test on cheese from a traditional farm system of the Alta Murgia National Park compared to different production systems in order to provide preliminary evidence about the link between chemical and sensory properties of products and the combined effect of natural and managerial factors.

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Materials and methods

Study area

The study was carried out in late spring (May and first half of June) in the Murge plateau, an internal area of the Apulia region (southeast of Italy). The top portion (above 350 m asl) of the north-western range (Alta Murgia) extends over 120.000 ha from the Bradanic valley toward the Adriatic Coast and was included in 2004 within the Alta Murgia National Park. The geological basement is made up of a thick layer of Jurassic-Cretaceous carbonate sedimentary rocks, covered on alternate tracts by thin and discontinuous Plio-Pleistocene deposits. The widespread presence of carbonate rocks allowed the formation of karst and slightly corrugated landscapes (Ricchetti *et al.*, 1988). According to the bioclimatic system of Rivas-Martinez (2004), the bioclimate of some meteorological stations representative of Alta Murgia was defined Mediterranean pluvioseasonal-oceanic, semicontinental, with mesomediterranean thermotypes and from dry to subhumid ombrotypes. Going up from the Adriatic coast, land use is characterized by olive groves, almond orchards and vineyards, on the most fertile soils, and mainly by (semi-)natural rocky grasslands in the remaining areas (Perrino *et al.*, 2006). Rocky grassland has been recently typified by the new association *Acino suaveolentis-Stipetum austroitalicae* (Forte *et al.*, 2005), assigned to the endemic alliance *Hippocrepido glaucae-Stipion austroitalicae* (Forte *et al.*, 2005) within the *Scorzonero villosae-Chrysopogonetalia grylli Horvatić et Horvat in Horvatić 1963 order and the Festuco-Brometea Br.-Bl. et Tx. ex Klika et Hadač 1944 class* (Forte *et al.*, 2005).

Farm selection

A farm considered representative of local traditional farming practices was chosen; within this farm (herein indicated as Farm 1), sheep were kept in a free ranging regime, grazing pasture all year round. Moderated hay supplementation was given only over the winter months. Pastures were composed of three main habitat types: seminatural rocky grassland (Hb 1); grassland with signs of ancient extensive land improvement (Hb 2) and set-aside (Hb 3). Set aside lands, previously cultivated with cereal and forage, were not ploughed and sown for four years and were used at the time of the study as free pasture for sheeps. Flock grazed daily both in grasslands and set-aside lands. Similar patchy landscapes are very common within the whole Alta Murgia area (Perrino *et al.*, 2006). Two other farms were also selected in the same area, since considered representative of different, not strictly traditional farm systems. In one farm (herein indicated as Farm 2), sheep were confined only at night and they grazed daily a mixed herbage of oat and ryegrass sown in November; regular supplementation with forages ensiled as round bales and concentrated feed was applied. In the second farm (herein indicated as Farm 3), sheep were exclusively indoor housed and fed a silage-free low-energy total mixed ration (TMR) mainly based on in-house produced wheat straw, hay, cereal grains and byproducts.

Pasture analysis

Plant composition of pastures was defined following the method described in Stohlgren *et al.* (1995). Three modified-Whittaker vegetation plots (Stohlgren *et al.*, 1995) were established in the main habitat types of Farm 1, one for each area. The modified-Whittaker plot was chosen because it samples a large area and allows detecting less abundant species (Stohlgren *et al.*, 1998). Each plot of 20×50 m (1000 mq) had nested in it: 1 subplot of 5×20 m (100 mq); 2 subplots of 2×5 m (10 mq) and 10 subplots of 0.5×2 m (1 mq). For the ten 1 mq subplots, all

plant species were identified and for each of them the cover percentage was visually estimated. For each of these species, only the mean cover value is reported in the species list of the entire plot. The other plant species recorded in the 10 mq and 100 mq subplots and in the remaining part of the plot (1000 mq) were added to the species list with the symbol x. In the numerical treatment of the data, a standard cover value of 0.1 was assigned to these species. The floristic analysis was based on the floras by Pignatti (1982), Fiori (1923/29) and Tutin *et al.* (1968/76); nomenclature was standardized using the checklist of the Italian Flora by Conti *et al.* (2005).

Milk volatile organic compounds analysis and cheese acceptance test

Concomitantly to pasture analysis, for each farm, milk samples were taken for chemical and SPME analysis that was carried out using 10 g of milk for each sample, hold 5 min in a thermostatic water bath at 80°C under constant stirring. Briefly, a 2-cm 50/30 m SPME fiber (Supelco Co., Bellefonte, PA, USA) was exposed to the headspace for volatile extraction and then desorbed in the GC-MS injection port. The volatile compounds in milk were identified by comparing mass spectra with those of Nist 107 and Nist 21 library. An additional 20 liters sample for each farm was concurrently collected and processed into cheese under uniform conditions and adopting traditional cheese-making techniques. After 3 months of ripening, cheese was administered to a random sample of individuals to test consumer acceptance by a blind taste test. The trial included also an artisanal eighth-months old ewe's milk cheese as an *external* reference; this sample was administered twice, in order to test for consumer opinion consistency.

Results and discussion

Botanical composition of pastures

Taxa (*species* and *subspecies*) recorded in the pastures were 180, belonging to 34 families (Table 1). In the habitat Hb1 number of species and families were 121 and 28, respectively; in Hb2, 134 and 31 whereas in set-aside lands (Hb3) 85 species belonging to 24 families were found. The highest cover values were estimated for *Stipa austroitalica* (30.8%), *Asphodelus ramosus* (15.1%) and *Scorzonera villosa* (11.1%) in Hb1; *A. ramosus* (28.7%), *Thymus spinulosus* (8.5%) and *S. villosa* (5.1%) in Hb2; *Dasyphyrum villosum* (34.0%), *Lolium perenne* (15.8%), *Trifolium scabrum* (11.7%), *Medicago disciformis* (10.4%) in Hb3. Regarding the botanical family composition, in grassland habitats (Hb1 and Hb2) more than 90% of total cover was given by 8 or 9 families. The most remarkable coverage (more than 10%) was recorded for *Poaceae*, *Asteraceae* and *Liliaceae*. In set-aside lands, 3 families gave more than 90% of total cover: *Fabaceae*, *Poaceae* and *Asteraceae*. Chorological spectra, weighted according to species cover (*data not shown*), highlighted a remarkable coverage of the endemic chorological type that accounted for 27.1% in the Hb1 and 10.3% in Hb2. On the contrary, in the set-aside lands this type accounted only for 3%. Grassland habitats (Hb1 and Hb2) harbour also several species with great relevance for conservation, such as *S. austroitalica*, defined as *priority specie* by the Habitat Directive (92/43/EEC) or *Iris pseudopumila*, rare and endemic species of few regions in the south of Italy.

Milk volatile organic compounds analysis

After pruning the unidentified peaks, a total of 66 volatile compounds were detected in milk by GC-MS (*data not shown*), 26 of them shared among the three farms. Free fatty acids (from C4 to C16, with

Table 1. Botanical families and species recorded in pastures and their mean cover percentage.

| Family and species | Mean cover (%) | | | Family and species | Mean cover (%) | | |
|--|----------------|-----|-----|---|----------------|-----|------|
| | Hb1 | Hb2 | Hb3 | | Hb1 | Hb2 | Hb3 |
| Apiaceae | | | | <i>Petrorhagia saxifraga</i> (L.) Link subsp. | | | |
| <i>Bupleurum baldense</i> Turra | 1.0 | 0.9 | 0.0 | <i>gasparrinii</i> (Guss.) Greuter & Burdet | 0.5 | x | 0.0 |
| <i>Cachrys libanotis</i> L. | 0.3 | 0.3 | 0 | <i>Silene italica</i> (L.) Pers. | 1.0 | x | 0.0 |
| <i>Cachrys pungens</i> Jan ex Guss. | x | 0.0 | x | <i>Silene nocturna</i> L. | 0.1 | 0.1 | 0.2 |
| <i>Daucus carota</i> L. | x | 0.1 | x | <i>Silene otites</i> (L.) Wibel | 0.1 | 0.0 | 0.0 |
| <i>Elaeostelinum asclepium</i> (L.) Bertol. | 2.9 | x | 0.0 | <i>Silene vulgaris</i> (Moench) Garcke subsp. <i>tenoreana</i> (Colla) Soldano & F. Conti | 0.0 | 0.1 | 0.0 |
| <i>Eryngium amethystinum</i> L. | 0.1 | 0.1 | x | Cistaceae | | | |
| <i>Eryngium campestre</i> L. | 2.8 | 0.7 | 0.6 | <i>Convolvulus cantabrica</i> L. | 0.8 | 0.5 | 0.3 |
| <i>Ferula communis</i> L. | 0.5 | 1.5 | 0.0 | <i>Convolvulus elegantissimus</i> Miller | 0.7 | 3.9 | 0.0 |
| <i>Scandix pecten-veneris</i> L. | 0.9 | 0.2 | 0.0 | <i>Cuscuta epithymum</i> (L.) L. | 0.4 | 0.1 | 0.0 |
| <i>Seseli tortuosum</i> L. | x | 0.0 | 0.0 | <i>Helianthemum salicifolium</i> (L.) Mill. | 1.7 | 1.2 | 0.3 |
| <i>Thapsia garganica</i> L. | 2.1 | 0.3 | 3.1 | Crassulaceae | | | |
| <i>Tordylium apulum</i> L. | x | 4.7 | 0.1 | <i>Sedum rupestre</i> L. | 0.2 | x | 0.0 |
| <i>Tordylium officinale</i> L. | x | 1.3 | x | Cyperaceae | | | |
| Asteraceae | | | | <i>Carex flacca</i> Schreber subsp. <i>serratula</i> (Biv.) Greuter | 0.3 | x | 0.0 |
| <i>Cardopatum corymbosum</i> (L.) Pers. | 0.1 | 0 | 0 | Dipsacaceae | | | |
| <i>Carduus micropterus</i> (Borbás) Teyber subsp. <i>perspinosus</i> (Fiori) Kazmi | 1.3 | 0.1 | x | <i>Sixalix atropurpurea</i> (L.) Greuter & Burdet | x | 3.1 | 0.2 |
| <i>Carduus pycnocephalus</i> L. subsp. <i>pycnocephalus</i> | 0 | x | 0.6 | Euphorbiaceae | | | |
| <i>Carlina corymbosa</i> L. | 0.1 | x | 0 | <i>Euphorbia exigua</i> L. | 0.5 | 0.6 | 0.1 |
| <i>Carlina lanata</i> L. | 0.3 | x | 0 | <i>Euphorbia falcata</i> L. | 0.0 | 0.1 | 0.1 |
| <i>Carthamus lanatus</i> L. subsp. <i>lanatus</i> | 0 | 1.1 | x | <i>Euphorbia nicaeensis</i> All. ssp. <i>japygica</i> (Ten.) Fiori | x | 1.0 | x |
| <i>Centaurea deusta</i> Ten. | 0 | x | 0.1 | Fabaceae | | | |
| <i>Centaurea solstitialis</i> L. | 0 | x | 0.1 | <i>Anthyllis vulneraria</i> L. | 0.1 | x | 0.1 |
| <i>Chondrilla juncea</i> L. | 0 | 0 | 0.3 | <i>Astragalus hamosus</i> L. | 0.6 | 0.3 | 0.5 |
| <i>Crepis corymbosa</i> Ten. | 0.1 | 2.4 | 3.4 | <i>Astragalus sesameus</i> L. | 0.3 | 0.0 | 0.0 |
| <i>Crepis neglecta</i> L. | 0 | 0.5 | 0 | <i>Coronilla scorpioides</i> (L.) W. D. J. Koch | 0.4 | 0.2 | 0.0 |
| <i>Crepis rubra</i> L. | 0.6 | 1.4 | 3.1 | <i>Hippocrepis biflora</i> Spreng | 0.3 | 0.7 | 0.1 |
| <i>Crepis vesicaria</i> L. | 0.2 | 0.2 | 0.4 | <i>Hippocrepis glauca</i> Ten. | x | 0.0 | 0.0 |
| <i>Crupina crupinastrum</i> (Moris) Vis. | 1.8 | 1.3 | 0 | <i>Lathyrus cicera</i> L. | 0.1 | 0.3 | 0.0 |
| <i>Filago eriocephala</i> Guss. | 0 | 0.1 | 0.1 | <i>Lathyrus ochrus</i> (L.) DC. | 0.0 | x | 0.0 |
| <i>Filago pyramidata</i> L. | 0.3 | 0 | 0 | <i>Medicago disciformis</i> DC. | 0.0 | 0.0 | 10.4 |
| <i>Hedypnois cretica</i> (L.) Dum. Cours. | 0.7 | 0.3 | x | <i>Medicago minima</i> (L.) L. | 0.2 | 0.3 | 0.2 |
| <i>Hypochaeris achyrophorus</i> L. | 1.2 | 0.3 | 0.8 | <i>Medicago orbicularis</i> (L.) Bartal. | 0.0 | 0.5 | 0.0 |
| <i>Lactuca serriola</i> L. | x | 0 | 0.2 | <i>Medicago rigidula</i> (L.) All. | 0.0 | 0.5 | 0.0 |
| <i>Onopordum illyricum</i> L. | 0 | 0.2 | 4.7 | <i>Medicago scutellata</i> (L.) Mill. | 0.0 | 0.1 | 0.0 |
| <i>Pallenis spinosa</i> (L.) Cass. subsp. <i>spinosa</i> | 0 | 0.2 | 0 | <i>Medicago turbinata</i> (L.) All. | 0.0 | 0.1 | 0.1 |
| <i>Picris hieracioides</i> L. | 0 | 0 | x | <i>Ononis reclinata</i> L. | 0.1 | 0.0 | 0.1 |
| <i>Reichardia picroides</i> (L.) Roth | x | 1.4 | 0.1 | <i>Scorpiurus muricatus</i> L. | 0.4 | 1.2 | 0.0 |
| <i>Rhagadiolus stellatus</i> (L.) Gaertn. | 0 | 0.2 | 0 | <i>Trifolium angustifolium</i> L. subsp. <i>angustifolium</i> | 3.0 | 1.4 | 0.1 |
| <i>Scolymus hispanicus</i> L. | 0 | 0.6 | 0 | <i>Trifolium campestre</i> Schreber | 0.7 | 1.0 | 0.5 |
| <i>Scolymus maculatus</i> L. | 0 | 0.3 | 0 | <i>Trifolium cherleri</i> L. | 0.0 | 0.0 | 1.2 |
| <i>Scorzonera villosa</i> Scop. subsp. <i>columnae</i> (Guss.) Nyman | 11.1 | 5.2 | 0.4 | <i>Trifolium mutabile</i> Port. | 0.1 | 0.3 | x |
| <i>Sonchus asper</i> (L.) Hill | 0 | 0 | 0.2 | <i>Trifolium scabrum</i> L. subsp. <i>scabrum</i> | 1.0 | 1.9 | 11.7 |
| <i>Sonchus tenerimus</i> L. | 0 | 0 | 0.1 | <i>Trifolium stellatum</i> L. | 0.1 | 1.0 | 39.0 |
| <i>Tragopogon porrifolius</i> L. | 0 | 0.2 | 0.1 | <i>Trifolium striatum</i> L. | 0.0 | 0.0 | 0.7 |
| <i>Urospermum dalechampii</i> (L.) F. W. Schmidt | 0.2 | x | 0 | <i>Trigonella gladiata</i> M. Bieb. | 0.0 | 0.1 | 0.0 |
| <i>Urospermum picroides</i> (L.) Scop. ex F. W. Schmidt | 0 | 0.1 | 0.2 | <i>Vicia onobrychioides</i> L. | x | 0.0 | 0.0 |
| <i>Xeranthemum inapertum</i> (L.) Mill. | 0.4 | x | x | <i>Vicia sativa</i> L. | 0.1 | 0.0 | 0.2 |
| Boraginaceae | | | | Gentianaceae | | | |
| <i>Echium asperrimum</i> Lam. | x | 0.2 | x | <i>Centaureum erythraea</i> Rafn | 0.0 | 0.1 | 0.0 |
| Brassicaceae | | | | Geraniaceae | | | |
| <i>Arabis hirsuta</i> (L.) Scop. | 0.1 | x | 0.0 | <i>Erodium ciconium</i> (L.) L'Hér. | 0.0 | 0.1 | x |
| <i>Biscutella didyma</i> L. | 0.0 | x | 0.0 | Guttiferae | | | |
| <i>Erysimum crassistylum</i> Presl | 0.1 | 0.1 | 0.1 | <i>Hypericum perforatum</i> L. | 0.0 | 0.0 | x |
| <i>Isatis tinctoria</i> L. | 0.0 | x | 0.0 | Iridaceae | | | |
| Caryophyllaceae | | | | <i>Hermodactylus tuberosus</i> (L.) Mill. | 0.0 | 0.1 | 0.0 |
| <i>Arenaria leptoclados</i> (Rchb.) Guss. | 0.0 | 0.0 | 0.9 | <i>Iris pseudopumila</i> Tineo | 0.2 | 0.0 | 0.0 |
| <i>Cerastium glomeratum</i> Thuill. | x | 0.0 | 0.0 | Labiatae | | | |
| <i>Cerastium pumilum</i> Curtis | 0.1 | 0.1 | 0.0 | <i>Ajuga chamaepitys</i> (L.) Schreb. | 0.0 | 0.0 | 0.4 |
| <i>Dianthus carthusianorum</i> L. | x | 0.0 | 0.0 | <i>Calamintha nepeta</i> (L.) Savi | 0.0 | x | 0.0 |
| <i>Dianthus garganicus</i> (Ten.) Brullo | x | 0.0 | 0.0 | To be continued on next page | | | |
| <i>Petrorhagia prolifera</i> (L.) P. W. Ball & Heywood | 0.4 | 0.2 | x | | | | |

the exception of the pentanoic acid) and hydrocarbons were the main groups of VOCs found in the milk samples. Free fatty acids (FFAs) are important components of cheese flavor, while hydrocarbons are secondary products of lipid auto-oxidation; they do not make a major contribution to the aroma, but may serve as precursors to other aroma compounds (Ortigosa *et al.*, 2001). Previous studies on ewe's milk VOC profile (Moio *et al.*, 1996) reported esters as being quantitatively the most important constituents of the volatile fraction of the ovine milk. Other authors indicated methyl ketones and hydrocarbons as the main groups of volatile compounds found in ewe's milk (Pereda *et al.*, 2008) and cattle milk (Mounchili *et al.*, 2005; Solano-Lopez *et al.*, 2006; Valero *et al.*, 2001). The total number of VOCs observed in milk from Farm 1 was 45,

49 in milk from Farm 2 and 36 in milk from Farm 3. The most represented compound in all the three samples was capric acid, followed by caprylic acid and lauric acid (Farm 1 and 2) or caprylic acid and the saturated hydrocarbon hexadecane (Farm 3). These results were discordant from those of Povolò *et al.* (2007) on grazing Sarda ewe's milk where none of the above mentioned compounds was detected by GC-MS. High levels of caprylic and capric acids were detected in the artisanal Serra da Estrela raw ewe's milk cheese (Tavaria *et al.*, 2004); moreover, high levels of caprylic, capric and lauric acids were detected in Maltese goat milk cheese by Chiofalo *et al.* (2004) who suggest that the remarkable presence of these three fatty acids may be correlated to integration of concentrate in the feeding regimen; this would explain

Continued from previous page.

| Family and species | Mean cover (%) | | |
|--|----------------|------|-----|
| | Hb1 | Hb2 | Hb3 |
| <i>Micromeria graeca</i> (L.) Benth. | x | 1.2 | 0.0 |
| <i>Phlomis herba-venti</i> L. | x | 0.4 | 0.1 |
| <i>Salvia argentea</i> L. | x | x | 0.1 |
| <i>Salvia verbenaca</i> L. | x | 0.1 | 0.0 |
| <i>Satureja montana</i> L. | 0.0 | 0.5 | 0.0 |
| <i>Sideritis romana</i> L. subsp. <i>romana</i> | 0.1 | x | 0.4 |
| <i>Stachys germanica</i> L. subsp. <i>sabvifolia</i> (Ten.) Gams | x | 0.0 | 0.1 |
| <i>Teucrium capitatum</i> L. subsp. <i>capitatum</i> | 0.1 | 0.0 | x |
| <i>Teucrium chamaedrys</i> L. | x | 0.2 | 0.0 |
| <i>Thymus spinulosus</i> Ten. | 4.1 | 8.5 | 0 |
| Liliaceae | | | |
| <i>Allium amethystinum</i> Tausch | x | 0.0 | 0.0 |
| <i>Allium sphaerocephalon</i> L. | 0.0 | x | 0.0 |
| <i>Allium tenuiflorum</i> Ten | x | 0.0 | 0.0 |
| <i>Asparagus acutifolius</i> L. | 0.1 | x | 0.0 |
| <i>Asphodeline lutea</i> (L.) Rchb. | 0.1 | x | 0.0 |
| <i>Asphodelus ramosus</i> L. subsp. <i>ramosus</i> | 15.1 | 28.7 | 0.5 |
| <i>Charybdis pancration</i> (Steinh.) Speta | 0.0 | x | 0.0 |
| Linaceae | | | |
| <i>Linum bienne</i> Miller | 0.1 | 0.0 | 0.0 |
| <i>Linum corymbulosum</i> Rchb. | 1.1 | 0.8 | 0.1 |
| <i>Linum strictum</i> L. | 1.0 | 2.1 | 0.4 |
| Malvaceae | | | |
| <i>Althea hirsuta</i> L. | x | 0.3 | 0.3 |
| <i>Malva sylvestris</i> L. | 0.0 | 0.0 | 1.4 |
| Orchidaceae | | | |
| <i>Orchis</i> sp. | 0.1 | 0.0 | 0.0 |
| <i>Serapias vomeracea</i> (Burm. f.) Briq. | x | 0.1 | 0.0 |
| Orobanchaceae | | | |
| <i>Orobanche schultzei</i> Mutel | 0.0 | x | 0.0 |
| Papaveraceae | | | |
| <i>Papaver hybridum</i> L. | 0.0 | 0.0 | x |
| <i>Papaver rhoeas</i> L. | 0.0 | 0.0 | 0.8 |
| Plantaginaceae | | | |
| <i>Plantago bellardi</i> All. | 0.1 | 0.1 | 0.0 |
| <i>Plantago lagopus</i> L. | 0 | 0.1 | 0.1 |
| <i>Plantago serraria</i> L. | x | x | 0.0 |
| Poaceae | | | |
| <i>Avena barbata</i> Pott ex Link | 1.7 | 1.3 | 0.0 |
| <i>Briza maxima</i> L. | 1.1 | 1.5 | 0.0 |
| <i>Bromus erectus</i> Huds. subsp. <i>erectus</i> | 4.7 | 0.0 | 0.0 |
| <i>Bromus hordeaceus</i> L. | 0.0 | 0.0 | x |
| <i>Bromus rubens</i> L. | 0 | 0.2 | 0.0 |
| <i>Bromus scoparius</i> L. | 0.1 | 1.0 | 0.0 |
| <i>Catapodium rigidum</i> (L.) C.E. Hubb. Ex Dony | 0.7 | 0.3 | 0.0 |
| <i>Cynosurus echinatus</i> L. | 0.0 | 1.2 | 0.0 |
| <i>Cynosurus effusus</i> Link | 1.0 | 0.3 | 0.0 |

| Family and species | Mean cover (%) | | |
|--|----------------|-----|------|
| | Hb1 | Hb2 | Hb3 |
| <i>Dactylis glomerata</i> L. subsp. <i>hispanica</i> (Roth) Nyman | 2.3 | 1.6 | 0.0 |
| <i>Dasypyrum villosum</i> (L.) P. Candargy, non Borbás | 0.9 | 4.1 | 34.0 |
| <i>Festuca circummediterranea</i> Patzke | 4.3 | 0.0 | 0.0 |
| <i>Gastridium ventricosum</i> (Gouan) Schinz & Thell. | 0.0 | 0.1 | 0.0 |
| <i>Hordeum bulbosum</i> L. | 0.0 | x | 0.0 |
| <i>Koeleria splendens</i> Presl | 4.8 | 0.0 | 0.0 |
| <i>Lagurus ovatus</i> L. | x | 1.0 | x |
| <i>Lolium perenne</i> L. | x | 2.1 | 15.8 |
| <i>Phleum hirsutum</i> Honck. subsp. <i>ambiguum</i> (Ten.) Tzvelev | x | 0.1 | 0.0 |
| <i>Poa bulbosa</i> L. | 0.9 | 0.5 | 0.0 |
| <i>Stipa austroitalica</i> Martinovský subsp. <i>austroitalica</i> | 30.8 | x | 0.0 |
| <i>Taeniatherum caput-medusae</i> (L.) Nevski | 0.1 | 0 | 0.0 |
| <i>Trachynia distachya</i> (L.) Link | 0.1 | 4.4 | 0.0 |
| <i>Triticum ovatum</i> (L.) Raspail | 2.5 | 3.1 | x |
| <i>Vulpia ciliata</i> Dumort. | 0.7 | 1.8 | 0.0 |
| Polygonaceae | | | |
| <i>Polygala monspeliaca</i> L. | 0.5 | 0.2 | 0.0 |
| <i>Rumex intermedium</i> DC. | x | 0.0 | x |
| <i>Rumex thyrsoides</i> Desf. | 0.0 | x | 0.0 |
| Primulaceae | | | |
| <i>Anagallis arvensis</i> L. | 0.1 | 0.3 | 0.0 |
| <i>Asterolinon linum-stellatum</i> (L.) Duby | 0.2 | 0.0 | 0.0 |
| Ranunculaceae | | | |
| <i>Nigella damascena</i> L. | 0.0 | x | x |
| Rhamnaceae | | | |
| <i>Rhamnus saxatilis</i> Jacq. subsp. <i>infectoria</i> (L.) P. Fourn. | x | 0.0 | 0.0 |
| Rosaceae | | | |
| <i>Potentilla detommassii</i> Ten. | 0.0 | 0.1 | 0.0 |
| <i>Prunus webbii</i> (Spach) Vierh. | 0.0 | 0.1 | 0.0 |
| <i>Pyrus spinosa</i> Forssk. | 1.5 | 2.0 | x |
| <i>Sanguisorba minor</i> Scop. subsp. <i>balearica</i> (Bourg. ex Nyman) Muñoz Garm. & C.Navarro | x | x | x |
| Rubiaceae | | | |
| <i>Asperula aristata</i> L. | 0.1 | 0.0 | 0.0 |
| <i>Galium cornudifolium</i> Vill. | 6.2 | 0.2 | 0.0 |
| <i>Sherardia arvensis</i> L. | 1.2 | 0.8 | 0.1 |
| Scrophulariaceae | | | |
| <i>Bartsia trixago</i> L. | 0.9 | 0.3 | 0.2 |
| <i>Verbascum macrurum</i> Ten. | 0.0 | 0.0 | 0.5 |
| <i>Verbascum niveum</i> Ten. | 0.0 | 0.0 | 0.5 |
| <i>Verbascum pulverulentum</i> Vill. | 0.0 | 0.0 | 1.1 |
| <i>Veronica arvensis</i> L. | 0.0 | x | 0.0 |
| Valerianaceae | | | |
| <i>Valerianella eriocarpa</i> Desv. | x | 0.1 | 0.0 |
| <i>Valerianella muricata</i> (Stev. ex M. Bieb.) J.W. Loudon | 0.0 | 0.2 | 0.0 |

the extremely higher occurrence of the saturated C8, C10 and C12 FFAs in the milk sample from the Farm 2, where animals were fed high-energy rations, compared to Farm 1 and 3.

Considering all the 66 VOCs identified in milk, factor analysis highlighted a clear differentiation among milk samples from the three farms (Figure 1) and in particular among the milk from Farm 1 and Farm 3 vs the milk from Farm 2. The first factor explained 67.6% of the total variance, while the second factor captured 32.4% of the total variance; therefore, the first two factors explained 100% of the total variance. The results of the factor analysis using data from the blind taste test are reported in Figure 2, while Table 2 presents in detail the average score (and standard deviation) assigned to cheese samples from each of the three considered farms, together with results obtained from the repeated administration of cheese from a different farm (taken as an external reference). These results are in agreement with those obtained from the SPME/GC-MS analysis, confirming a stronger differentiation among the milk from Farm 1 and Farm 3 vs the milk from Farm 2. Although these data suggest that grazing may have an influence both on milk VOC profile and cheese consumer acceptance, nonetheless, we cannot exclude the influence of unaccounted confounding factors such as different composition of diet supplementations, different farm genetic backgrounds, different hygienic quality of raw milk, etc.; the latter seems to have had a role since microbiological

contamination by gas producing organisms would explain the atypical cheese texture observed in the sample from Farm 2, which was characterized by a marked presence of small round eyes. This would also explain the results from the ranking of cheese samples made by consumers who widely preferred cheese from Farm 3 and 1 compared to cheese from Farm 2.

Results from the analysis of pastures showed the ecological relevance of grassland habitats on account of their high diversity and percentage of endemism. Moreover, grasslands showed also a high percentage of families with peculiar aromatic properties, such as *Labiatae* and *Apiaceae*, though some of them can be poorly attractive to grazing herds (e.g. *Apiaceae*). On the other hand, set aside lands showed a lower number of species, although families with high nutritional and productive value, such as *Fabaceae* and *Poaceae*, were well represented. These results are in agreement with data found by Tedone *et al.* (2008).

Results from milk VOC analysis highlighted free fatty acids (mainly capric, caprylic and lauric acids) and hydrocarbons as the main volatile compounds of ewe's milk in all three farms. Factor analysis of milk VOCs profiles, as well as factor analysis of data from the blind cheese taste test, allowed discriminating among farm samples. Although it was not possible in our study to separate the effects of grazing vs other management factors, these results suggest that the farm system can have a deep impact on differentiating dairy products. Considering the

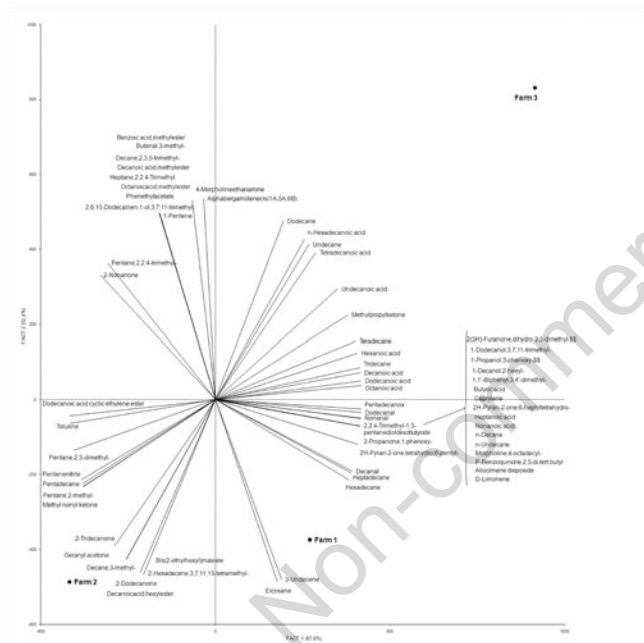


Figure 1. Scatterplot of factor scores for the first two principal components considering all the volatile organic compounds identified in milk.

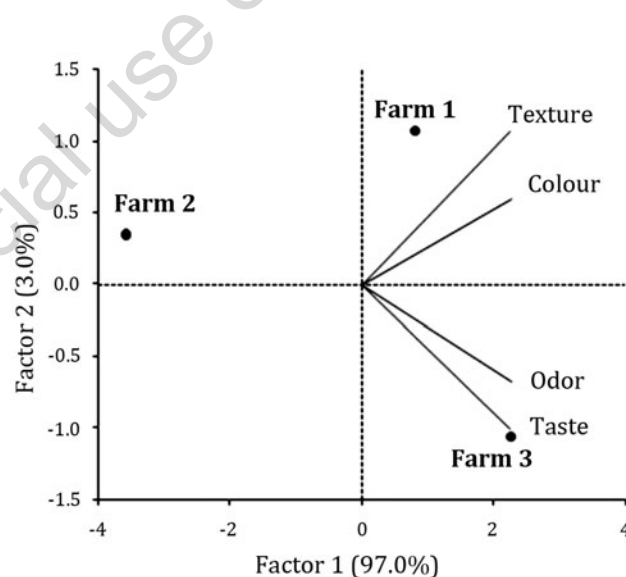


Figure 2. Scatterplot of factor scores for the first two principal components considering the consumer test parameters. Consumer preference assessed in a blind taste test on a 9-point hedonic scale.

Table 2. Average scores and standard deviation (SD) from the blind taste test for cheese samples from each of the three considered farms and from a different farm (taken as an external reference), administered twice.

| Sample origin | Colour | | Odour | | Taste | | Texture | |
|--|---------|------|---------|------|---------|------|---------|------|
| | Average | SD | Average | SD | Average | SD | Average | SD |
| Farm 1 | 7.13 | 1.13 | 6.70 | 1.42 | 6.56 | 1.68 | 6.72 | 1.48 |
| Farm 2 | 6.44 | 1.66 | 5.89 | 1.62 | 5.72 | 1.75 | 5.54 | 1.91 |
| Farm 3 | 7.19 | 1.36 | 7.09 | 1.36 | 7.07 | 1.60 | 6.69 | 1.61 |
| External reference (1 st serving) | 6.78 | 1.55 | 6.04 | 1.88 | 5.72 | 2.25 | 5.81 | 2.06 |
| External reference (2 nd serving) | 6.67 | 1.61 | 5.85 | 2.05 | 5.39 | 2.45 | 5.57 | 2.0 |

urgent needs for both an environmental and economic sustainability of the productive system within the Alta Murgia Park, preservation of traditional farming systems, mainly based on grazing practices, may be seen as an important strategy both for the conservation of grassland biodiversity (Forte and Vita, 1997; Perrino *et al.*, 2006, Terzi and Marvulli, 2006) and for providing local dairy products with a specific territorial footprint.

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