Seasonal evolution of volatile compounds content and aromatic profile in milk and cheese from grazing goat

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Abstract

The objective of this study was to characterise the sensory profile of goat milk and cheese evaluated in three different seasons on the basis of alcohols, ketones, esters and terpenes content and profile. For this purpose, a trained panel of eight people was used, which sniffed the pure compounds corresponding to those found in all the products.

One group of 15 lactating goats grazed a local pasture from March to July. Daily grazing time was approximately 8 h/day. During winter, spring and summer, herbage intake and the contribution of each species to the grazing diet were estimated by difference of herbage mass weight. Volatile organic compounds (VOC) content in herbage diet, milk and cheeses was determined by a multiple dynamic headspace extraction and GC–MS. The “fragrance” profile was described using the single pure compounds found in milk and cheese. The results showed that from winter to spring especially ketones content increased in milk, while no variation was observed for the other compounds. Summer milk was characterised mostly by terpenes.

Also, the “fragrance” profile changed with grazing season. Winter milk was characterised by wood pulp-hay, green-herbaceous and blue-cheese notes, while in the spring by wood pulp-hay, blue-cheese, hot milk and musty. In the summer, the profile totally changed. In this season resinous, citrus, mint and fruity were the dominant notes.

In comparison to herbage and milk, cheeses were less rich in volatile compounds. Not significant variation was observed in cheeses from a season to another, but their sensory profile was dominated by sweet odour.

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1. Introduction

In fresh products, such as fruit, vegetable and milk, flavour components are very abundant. The odours can be elicited by numerous classes of compounds, such as terpenes, alcohols, ketones, etc., but not all these compounds have significant effect on the overall odours. Recently, only the terpenes were emphasised for their capability to characterise flavour and aroma of milk.

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and dairy products (Mariaca et al., 1997; Buchin et al., 1999; Coulon et al., 2000; Viallon et al., 2000). These volatile compounds occur in greater amounts in milk and dairy products when the animals are fed with fresh herbage, especially with dicotyledons (Sehovic, 1991; Mariaca et al., 1997; Cornu et al., 2001). In a previous study, Fedele et al. (2000) observed that at the beginning of May milk from grazing goats was characterised by high content of aliphatic hydrocarbons, while at the end of May, alcohols and ketones were dominant. This variation could be explained by the change of pasture botanical composition, considering that distribution and abundance of volatile compounds depend on plant species (Mariaca et al., 1997). However, several other factors may affect the abundance of volatile compounds, such as development stage of the plant and the kind of aerial plant part (Roholoff, 1999), and seasonal climatic changes (Rajeswara Rao et al., 1996).

The diet of all grazing animals, and especially of goats, is almost different from one season to another, because they change the plants and the aerial parts selected, according to the plant communities available in the pasture, the development stage and the climatic condition (Fedele et al., 1993). This diet diversification could influence the volatile compounds content in milk and cheese, and especially the presence and abundance of molecules that have a significant effect on the flavour and aroma.

The objective of this study was to evaluate the effect of grazing season on the fragrance profile in milk and cheese.

2. Materials and methods

2.1. Pasture and grazing trials

A native herbaceous pasture in a Basilicata valley (Italy) at 380 m s.l. was used for this experiment. It was carried out during three periods, corresponding to three seasons: winter (from 4 to 24 March), spring (from 8 to 26 May) and summer (from 24 June to 14 July). An area of 1.2 ha was divided into two equal paddocks with similar herbage availability and botanical composition. From March to July, one group of 15 lactating Siriana goats alternately grazed for 8 h/day these two paddocks. The length of the grazing period in each paddock was variable in relation to the season and the herbage availability (meanly 10 days in winter and summer, and 3 weeks in spring).

In each period, one milk sample was collected from each goat, at regular intervals of 7 days, for a total of three samples/head. The milk samples were mixed together into three cumulative samples per season and a quote was immediately submitted to volatile compounds extraction. Caciotta cheeses were made from the residual milk and stored in liquid nitrogen at 1 and 20 days of age. The day after milk sampling, herbage intake was estimated by difference between the weight of herbage mass cut on five 2 m × 2 m un-grazed squares, randomly distributed in each paddock, and post-grazing herbage mass cut in five 2 m × 2 m squares in the experimental area. The contribution of each species to the grazing diet was estimated on the same five 2 m × 2 m post-grazing squares. It was calculated for each species by ratio between the number of plants grazed for single species and the number of plants, for the same species, present in each area before grazing. On the basis of this information an “artificial diet” was formed. For each species, plant samples corresponding to those really browsed by goats (both for species and part of plant) were cut from an un-grazed area and mixed in the same estimated proportions and immediately stored in liquid nitrogen.

2.2. Cheese-making

The raw whole milk was filtered and heated in a stainless steel vat to 36 °C. Liquid calf rennet was added in the amount of 35 ml/100 l of milk. After 20–25 min, at the end of coagulation, the curd was cut with a knife in blocks 10 cm distant, and then perpendicularly at the same distance; after 5 min of rest, the curd was broken into walnut sized pieces. The curd was placed into plastic cylindrical moulds of 113 mm × 80 mm.

2.3. Volatile organic compounds (VOC) determination

VOC content in herbage diet, milk and cheeses, was determined by a multiple dynamic headspace extraction and GC-MS (Fedele et al., 2000; Ciccioli et al., 2004). The samples were transferred in a glass container, flushed with pure helium at rate of 200 ml/m. The outlet of the extraction system was connected to a sampling train comprised of two adsorption traps
set in series. The first trap was filled in with equal volume (1 ml) of tenax TA (Aldrich Chemical Co., Milwaukuee, USA), and Carbograph 5, supplied by Lara S.r.l., Rome, Italy. The second trap was filled in with the same volumes of packing but the adsorbent used were Carbograph 1 and Carbograph 5, also supplied by Lara S.r.l. Volatile compounds were analysed by GC–MS after thermal desorption of traps performed at 250 °C. The separation was carried on a capillary column (50 m × 0.32 mm i.d.) internally coated with a non-polar silicon phase. VOCs were identified on the basis of their mass spectra. Selected ions were used to quantify overlapping peaks or those present at trace levels.

To avoid the dilution effect due to the different water content in herbage, milk and cheeses, the data reported were calculated on 100 g of dry matter.

3. Aromatic characterisation of milk and cheese

In this trial, milk and cheese were not evaluated by conventional sensory analysis, but the compounds detected by GC–MS in milk and cheese were used for defining the fragrant profile of these products. Single pure compounds (Sigma–Aldrich, Milan, Italy) corresponding to those identified in milk and cheese were used. Eight trained panellists sniffed five pure compounds per session, contained in small vials. They were asked to give a description of the odour perceived. In order to reduce the number of descriptors, it was established to form some classes of odours in which to include the descriptors of similar meaning (Table 1).

The value of the assessment of each class was the sum of the values for each descriptor measured by GC–MS.

<table>
<thead>
<tr>
<th>Classes of odours used to describe sensory profile of milk and cheese</th>
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<tbody>
<tr>
<td>Classes of odours</td>
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<tr>
<td>Citrus</td>
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<tr>
<td>Fruity</td>
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<tr>
<td>Green/herbaceous</td>
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<tr>
<td>Woody/hay</td>
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<tr>
<td>Solvent/ethereal</td>
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<tr>
<td>Resinous</td>
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<tr>
<td>Musty</td>
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The values of the classes of odours and of the single descriptors (mint, soapy, hot milk, etc.) were used for the statistical analyses.

4. Statistical methods

The data of VOC content in herbage, milk and cheese were processed by analysis of variance by including the season period in the model. The repeated measure ANOVA model was used for the sensory data. Analyses were done using SAS procedures (SAS 1989).

5. Results

5.1. Diet botanical composition and herbage intake

Winter pasture was dominated by grasses (85–88%), consequently goats adapted their grazing behaviour selecting above all these species and, among them, *Lolium perenne* and *Dactylis glomerata*. These two species contributed to the diet in a high percentage (60 and 25%, respectively). In spring, with the increase of the temperature, also legumes and forbs developed in the pasture. Goats adapted their grazing behaviour selecting 20 species more than in winter (8 versus 28), modifying the proportion among the different botanical categories in the diet: grasses decreased from 92 to 39.5%, legumes increased from 1 to 24% and forbs from 7 to 36.5%. In the summer pasture, grasses and legumes decreased and forbs increased, so goats selected mostly forbs (71%). According to pasture herbage availability (1200 ± 210 kg/ha DM in winter, 2500 ± 310 kg/ha DM in spring and 700 ± 130 kg/ha DM in summer), also the intake level changed: it was higher in spring, 680 g/day DM versus 520 g/day DM in winter and 490 g/day DM in summer.

5.2. Seasonal evolution of volatile compounds

Fig. 1 shows that no esters were found in herbage samples in all three seasons. Alcohols increased significantly ($P < 0.05$) from winter (8.5 mg/100 g DM) to spring (33.3 mg/100 g DM). Ketones content was not significantly different from a season to another (2.9 mg/100 g DM versus 4.2 mg/100 g DM), while
terpenes increased significantly ($P < 0.05$) from winter to summer (0.832 mg/100 g DM versus 7.2 mg/100 g DM).

In the winter milk, alcohols and ketones were dominant (2.7 and 10.1 mg/100 g DM, respectively), but while the first category decreased significantly in spring ($P < 0.05$), the second one increased ($P < 0.05$). From the first two seasons to the summer, only the terpenes increased at significant level ($P < 0.05$) and reached the highest value (31.2 mg/100 g DM).

Summer cheese samples were not analysed because they showed clear defect due to probable

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**Fig. 1.** Seasonal evolution of volatile compounds in herbage, milk and cheese.

**Fig. 2.** Aromatic sensory profile of milk from winter (W), spring (Sp) and summer (Su).
contamination. No significant variations from a season to another were observed for both cheeses (1 and 20 days-aged). Passing from herbage to milk to cheese, each class of volatile compound showed a different trend: alcohols decreased from herbage to milk (65% versus 7%) and then increased up to reach 48% in the cheese 20 days-aged. Ketones compounds, on the contrary, increased from herbage to milk (12% versus 58%) and then decreased in both cheeses at very low level.

5.3. Fragrance profile

The sensory profiles of milk (Fig. 2), cheese 1 day-aged (Fig. 3) and cheese 20 days-aged (Fig. 4) are reported.

Milk from winter, spring and summer showed different sensory profiles (Fig. 2). Winter and spring milk showed higher wood pulp-hay odour attributes ($P < 0.05$) than summer milk. The profile of winter milk was also characterised by musty and hot milk odours. Spring milk was significantly different from the others ($P < 0.05$) for soapy, blue-cheese and hot milk odours. In summer milk, the profile totally changed. Milk from this season was significantly ($P < 0.05$) characterised by resinous, citrus, mint, green-herbaceous and fruity odours.

Grazing season affected also cheese’s sensory profile. The profile of winter cheese 1 day-aged (Fig. 3) was significantly ($P < 0.05$) characterised by sweet, green-herbaceous, solvent-etheral and citrus odours, while spring cheese by blue-cheese, medicinal and soapy. The profile of winter cheese 20 days-aged (Fig. 4) was similar to the corresponding cheese 1 day-aged, but in this profile medicinal odours reached the same importance of sweet odour. The sensory profile of spring cheese 20 days-aged was very poor but significantly ($P < 0.05$) characterised by the same odours of the corresponding winter cheese. Its profile was dominated by sweet odour.

6. Discussion

The differences in content and profile of fragrance compounds in herbage, milk and cheese seemed clearly associated to the pasture’s and diet’s botanical seasonal variation. From winter to spring and summer, the decrease of grasses and the increase of other botanical classes in the goat’s diet caused the large increase of alcohols. In the corresponding milk samples, this class of compounds has not reached the same level of dominance than in the grazed herbage, it was rather replaced in this role by ketones. The decrease of alcohol content from herbage to milk can be explained considering that secondary alcohols were reduced in methyl ketones by reductase activity.
Concerning terpenes content, on the light of the actual knowledge it is not simple to explain why all sesquiterpenes decreased, from winter to spring, from herbage to milk, and on the other side, why some monoterpenes (e.g. camphene, myrcene and limonene) decreased and some others (e.g. $\alpha$ and $\beta$ pinene) increased. One hypothesis is that higher herbage intake in spring (30–40% more than in the other seasons) modified the digestive and metabolic processes, causing deviation in terpenes absorption.

In summer, when goats grazed mostly forbs, terpenes increased in grasses and in milk, becoming dominant. This phenomenon showed that in summer no limitation opposed to the terpenes transferring from herbage to milk. Probably, the lower intake and lower herbage fermentescibility have created better condition in the ruminal environment, improving the bio-availability for the absorption. Cheeses were poorer in volatile compounds than the corresponding milk, probably due to the volatility of these classes of compounds. It is very likely that during the milk heating a quote of volatile compounds evaporated. However, other factors such as enzymatic and microbial activities, the interaction between aromatic molecules and the matrix, and others still unknown, could have affected the content and the extraction capability (Molinard and Spinnler, 1996).

The odorous profile of winter and spring milk and cheeses was due especially to ketones, alcohols and esters. Octane-2-one, nonan-2-one ketones and hexanoic acid methyl ester were the responsible for fruity notes, the first two compounds and 1-propanol alcohol were responsible for musty notes; 1 and 2-exanol alcohols were responsible for resinous notes, while octanoic acid methyl ester and limonene terpenoid were responsible for citrus notes. In summer, the odorous profile was characterised especially by terpenes. The green, citrus, mint and resinous notes were due above all to $\alpha$-phellandrene, limonene and linalool, $\beta$-phellandrene, terpinolene and terpineol, respectively. On the basis of these results, seemed that the presence of the terpenes in the milk and cheese depended on the forbs and especially to the Geranium molle, Asperula odorosa, Cichorium intybus and Galium verum, very grazed in the summer season.

7. Conclusion

The numerous compounds and the mechanism involved in milk and cheese odours are not well known.
The transferred compounds continually changed in quantity and type with the progressing of seasons and for this reason also the fragrance profile of products changed. Perhaps, up to now the attention was devoted only or mostly to the terpenes, neglecting the other classes of compounds. This study showed that in winter and spring the terpenes’ was not the most important class in the note of milk. These compounds reached important quantity only in summer.

This type of approach to the study of the fragrance profile was useful simply to appraise what and how many molecules could potentially contribute to determine the organoleptic quality perceived by the consumers. In fact, the odours or flavours perceived tasting dairy products are not due to the simple sum of molecules but to the interaction among all compounds (Forss, 1979; Monso et al., 1996).

References


