

## Determination of volatile compounds in cows' milk using headspace GC-MS

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**SUMMARY.** The composition of the volatile fraction of milk from cows was investigated in a survey of milk samples using a headspace sampling technique and gas chromatography coupled to mass spectrometry analysis (GC-MS). Milk samples were collected from 12 farms, selected for similar management, breed and level of production. Farms were also grouped according to the type of forage in the ration: (1) hay; (2) hay and maize silage; (3) hay, maize silage and grass silages. Forty-one compounds in milk were isolated and identified from GC-MS headspace analysis. Quantitatively, the most representative chemical class was ketones (eight compounds, 170  $\mu\text{g}/\text{kg}$ ), followed by aldehydes (nine compounds, 63  $\mu\text{g}/\text{kg}$ ), alcohols (eight compounds, 36  $\mu\text{g}/\text{kg}$ ), and lower amounts of hydrocarbons (six compounds), sulphur compounds (three compounds), esters (four compounds) and terpenes (three compounds). The novel headspace sampling technique, and the consequent reduction of sample pre-treatment, allowed the identification of low-molecular weight volatile compounds, and reduced the risk of producing artefacts during analysis.

Discriminant analysis was used to identify a classification criterion for milk samples, using type of forage in the ration as a grouping variable. Posterior probability error rate indicated that aldehydes provided one of the best discriminant criteria for grouping milks according to ration composition. When all 41 identified volatile compounds were included, discriminant analysis selected nine compounds (acetone, 2,3-butanedione, 2-butanone, ethanol, acetaldehyde, ethylacetate, ethylisovalerate, dimethylsulphone) that did not fail the tolerance test and which correctly classified 100% of the original cases.

**KEYWORDS:** Milk, cow, volatile compounds, GC-MS, discriminant analysis.

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The smell of raw milk is characteristic for each species (Moio *et al.* 1993*a*) and depends on the metabolism of the animal and interactions between the animal and its environment. Volatile compounds can be transferred to milk from inhaled air, from rumen gases or the digestive tract through the blood, and from there to the milk (Urbach, 1990, Desagé *et al.* 1996). Diet can influence the composition of volatile compounds in raw milk, either by interacting with rumen metabolism (Urbach, 1990) or by transferring odour-active molecules (Buchin *et al.* 1999). This latter route offers

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the possibility of deriving a fingerprint of cheeses produced in different areas or in different seasons (Verdier *et al.* 1995; Viallon *et al.* 1999). Volatile compounds can also arise from the activities of the indigenous microbiota in fresh milk (Moio, 1993a); such effects are strictly linked to the environment.

Another factor to be considered when assessing the volatile compounds of milk is the analytical method used. Although numerous methods for the determination of milk volatile compounds are known, the problem of accurate measurement remains, particularly for constituents of low molecular weight (Xanthopoulos *et al.* 1994). Several methods require the isolation of volatile compounds before separation by gas chromatography (GC) and, frequently, the procedure needs a pre-concentration step. To reduce potential artefacts, pre-treatments of sample must be carried out under mild conditions. Dynamic headspace techniques, involving the collection of the surrounding vapour phase, have the advantage of minimal manipulation of the sample, so avoiding contaminants or artefacts. Moreover, the fraction extracted should be closer to the genuine aroma perceived than that obtained by means of other extraction procedures (Fernández-García, 1996).

The aim of this work was to use a dynamic gas chromatography-mass spectrometry (GC-MS) headspace technique (Barcarolo *et al.* 1992) to analyse volatile compounds in milk. In addition, we investigated the potential of the volatile profile to discriminate milk samples according to type of forage eaten by cows, using a survey of raw milk samples collected from selected farms.

#### MATERIALS AND METHODS

##### *Experimental description*

During a fortnight in June, milks were sampled from 12 farms located in a 15-km<sup>2</sup> basin in the north-eastern part of Italy. Farms were selected from 83 candidates, for having similar breed (Italian Simmental), level of production, milking system and farm management. Type of forage in the ration was used as a grouping criterion, in order to have: (1) hay (H, four farms); (2) hay and maize silage (S, four farms); and (3) hay, maize silage and grass silage (G, four farms). Hays and silages were home grown, and hence were not standardized. Herds were composed of 60–70% pluriparous and 20–30% primiparous cows. Fresh cows were not present on any farm and about half of the animals were 4–16 weeks into lactation.

Milk samples from each farm were collected from two consecutive milkings (evening and morning), from all the cows (mean of 30 animals per farm). Evening samples were cooled to 4 °C, and pooled with the corresponding morning sample. Milk samples were collected from the farms within 3 h of the morning milking (7:00), deep-frozen within 1 h of collection and stored in glass bottles at –20 °C. Analyses were completed within 3 weeks. A further fresh milk sample for each farm was analysed for somatic cell count (Foss-o-Matic, mod. 180 Foss Electric, Hillerød, Denmark) and total bacterial count (Bactoscan, Foss Electric).

##### *Rations*

On all the farms, cows were offered a total mixed ration (TMR) *ad lib.* twice a day (at 7:00–9:00 and 16:00–18:00) for at least 2 months before the experiment began. On the day before milk collections, two TMR samples were taken from each farm and analysed for dry matter (DM), crude protein (N × 6.25), lipids (ether extract), neutral-detergent fibre (NDF, Van Soest *et al.* 1991) and non-structural carbohydrates (NSC, Nocek & Russel, 1988). NDF was determined after pre-treatment

Table 1. Dry matter intake, the composition of rations, and milk cell counts for the hay (H), maize silage (S) and grass silage (G) groups

(Values are means with SD for  $n = 4$ )

|  | Hay  |     | Maize silage |     | Grass silage |     |
|--|------|-----|--------------|-----|--------------|-----|
|  | Mean | SD  | Mean         | SD  | Mean         | SD  |
| DM intake, kg/d                        | 21.8 | 1.3 | 20.2         | 1.3 | 22.5         | 0.9 |
| Ingredient                             |      |     |              |     |              |     |
| Hay, lucerne, g/kg DM                  | 573  | 16  | 172          | 7   | 107          | 10  |
| Maize silage, g/kg DM                  | —    | —   | 591          | 20  | 507          | 18  |
| Grass silage, g/kg DM                  | —    | —   | —            | —   | 149          | 14  |
| Soybean meal, g/kg DM                  | 92   | 10  | 94           | 8   | 86           | 5   |
| Maize, g/kg DM                         | 298  | 15  | 143          | 18  | 137          | 18  |
| By-products†, g/kg DM                  | 37   | 4   | —            | —   | 14           | 2   |
| Total                                  | 1000 |     | 1000         |     | 1000         |     |
| Chemical analyses                      |      |     |              |     |              |     |
| Dry matter (DM), g/kg                  | 867  | 7   | 653          | 20  | 602          | 20  |
| Crude protein, g/kg DM                 | 147  | 6   | 152          | 6   | 166          | 3   |
| Ether extract, g/kg DM                 | 33   | 2   | 38           | 4   | 45           | 2   |
| Neutral detergent fibre, g/kg DM       | 380  | 15  | 355          | 19  | 322          | 10  |
| Non-structural carbohydrate, g/kg DM   | 369  | 16  | 385          | 18  | 395          | 17  |
| Ash, g/kg DM                           | 71   | 4   | 70           | 3   | 72           | 5   |
| Somatic cells count, $\times 10^3/l$   | 224  | 48  | 243          | 57  | 196          | 72  |
| Total bacterial count, $\times 10^3/l$ | 68   | 33  | 50           | 29  | 37           | 23  |

† Dehydrated sugar beet pulp and wheat bran.

with thermostable  $\alpha$ -amylase, without sodium sulphite and without correction for ash. The average ration formulations and chemical compositions for the three forage groups are shown in Table 1, together with milk analyses.

#### Headspace sampling

The headspace sampling technique was the one assessed by Barcarolo *et al.* (1992). A sample of milk (40 ml) was weighed into a 100-ml vial and mixed with 10 g NaCl. Internal standard (tetrahydrofuran, 17.8  $\mu\text{g}$ ) was added to the mixture. As pure standards were not available for all identified compounds, a response factor of 1 was set for each compound. Vials were sealed with an aluminium-rubber septum (Supelco Inc, Bellefonte, PA, USA) and conditioned at 80 °C for 15 min before the analysis. In this dynamic headspace technique, samples were purged by bubbling helium, and the stripping into a heated block (70 °C) was carried out for 150 s with helium at a rate of 8 ml/min. Volatile components were driven into a capillary tube that was inside a cryogenic trap (liquid nitrogen) maintained at  $-110$  °C, and connected in on-column mode to a capillary gas chromatograph (Carlo Erba GC 8000, Carlo Erba, 20090 Milan, Italy). The connection to the analytical column was not direct, as a Y press fit was inserted and connected to a vapour-exit valve. During the sampling step, helium was back-flushed through the analytical column with an outlet in the vapour-exit device, with the aim of avoiding any contamination of the analytical column.

#### Analysis by means of coupled GC-MS

At the end of sampling (purging) time, desorption of volatile components was achieved by heating the trap to 240 °C in 5 s and then by transferring volatiles to the capillary column in 20 s. The analytical column (low polarity, 7% phenyl-

Table 2. *Volatile compounds ( $\mu\text{g}/\text{kg}$ ) in raw milk samples for diets based on hay, maize silage and grass silage*(Values are means with SD for  $n = 4$ )

| Rt†                      | Volatile compound              | Hay    |        | Maize silage |       | Grass silage |        |
|--------------------------|--------------------------------|--------|--------|--------------|-------|--------------|--------|
|                          |                                | Mean   | SD     | Mean         | SD    | Mean         | SD     |
| <i>Ketones</i>           |                                |        |        |              |       |              |        |
| 6.91                     | Acetone                        | 203.52 | 64.16  | 106.60       | 39.33 | 162.66       | 90.25  |
| 11.22                    | 2,3-butanedione                | 2.70   | 4.91   | 0.30         | 0.20  | 0.21         | 0.17   |
| 11.67                    | 2-butanone                     | 5.38   | 3.25   | 15.82        | 13.80 | 19.07        | 4.24   |
| 16.45                    | 2-pentanone                    | 0.99   | 0.10   | 0.93         | 0.43  | 0.82         | 0.33   |
| 19.23                    | 2-hexanone                     | 0.20   | 0.17   | 0.44         | 0.35  | 0.53         | 0.17   |
| 24.29                    | 4-hydroxy-4-methyl-2-pentanone | 0.15   | 0.25   | 0.05         | 0.11  | 0.10         | 0.13   |
| 26.4                     | 2-heptanone                    | 0.16   | 0.12   | 0.14         | 0.12  | 0.17         | 0.05   |
| 30.57                    | 6-methyl-5-heptene-2-one       | 0.06   | 0.07   | 0.17         | 0.10  | 0.26         | 0.13   |
|                          | Total ketones                  | 213.14 | 65.23  | 124.44       | 50.73 | 183.80       | 94.38  |
| <i>Aldehydes</i>         |                                |        |        |              |       |              |        |
| 4.25                     | Acetaldehyde                   | 3.85   | 2.23   | 3.37         | 2.13  | 2.73         | 1.56   |
| 9.91                     | Isobutanal                     | 20.60  | 20.80  | 2.62         | 2.81  | 2.34         | 2.55   |
| 14.8                     | 3-methylbutanal                | 74.93  | 59.64  | 14.54        | 16.94 | 8.42         | 9.35   |
| 15.32                    | 2-methylbutanal                | 6.79   | 5.13   | 1.10         | 1.03  | 1.45         | 1.91   |
| 17.06                    | Pentanal                       | 0.26   | 0.23   | 0.38         | 0.33  | 3.21         | 3.31   |
| 17.29                    | 2,4-hexadienal                 | 0.04   | 0.08   | 0.07         | 0.06  | 0.14         | 0.15   |
| 22.2                     | Hexanal                        | 3.21   | 1.75   | 5.88         | 6.24  | 38.48        | 29.94  |
| 26.94                    | Heptanal                       | 0.38   | 0.15   | 0.48         | 0.33  | 1.17         | 1.30   |
| 35.3                     | Nonanal                        | 0.20   | 0.16   | 0.27         | 0.12  | 0.46         | 0.20   |
|                          | Total aldehydes                | 110.24 | 84.91  | 28.71        | 12.66 | 58.40        | 44.91  |
| <i>Alcohols</i>          |                                |        |        |              |       |              |        |
| 5.76                     | Ethanol                        | 34.40  | 39.85  | 18.16        | 20.09 | 1.87         | 1.12   |
| 13.36                    | Isobutanol                     | 0.55   | 0.89   | 0.08         | 0.12  | 0.05         | 0.03   |
| 15.13                    | n-butanol                      | 0.17   | 0.21   | 0.02         | 0.05  | 0.10         | 0.15   |
| 16.19                    | 1-penten-3-ol                  | 0.07   | 0.09   | 0.00         | 0.00  | 0.15         | 0.14   |
| 18.82                    | 3-methyl-1-butanol             | 14.69  | 16.56  | 4.48         | 3.35  | 1.55         | 0.59   |
| 20.44                    | 1-pentanol                     | 0.28   | 0.19   | 0.68         | 0.42  | 2.19         | 0.95   |
| 25.32                    | 1-hexanol                      | 0.22   | 0.16   | 0.55         | 0.53  | 0.88         | 0.24   |
| 26.71                    | 2-heptanol                     | 0.11   | 0.19   | 0.00         | 0.00  | 0.01         | 0.01   |
|                          | Total alcohols                 | 50.48  | 57.06  | 23.96        | 22.22 | 6.78         | 2.65   |
| <i>Hydrocarbons</i>      |                                |        |        |              |       |              |        |
| 4.88                     | 3-methylpentane                | 0.12   | 0.24   | 0.72         | 0.73  | 1.06         | 0.82   |
| 11.51                    | 1-hexene                       | 6.64   | 4.34   | 3.59         | 2.22  | 3.10         | 1.04   |
| 12.26                    | 3-methylfuran                  | 0.12   | 0.11   | 0.05         | 0.10  | 0.07         | 0.08   |
| 16.81                    | 1-heptene                      | 0.69   | 0.23   | 0.32         | 0.17  | 1.37         | 1.25   |
| 20.97                    | toluene                        | 1.94   | 0.67   | 1.45         | 0.65  | 1.14         | 0.30   |
| 21.79                    | 1-octene                       | 0.75   | 0.37   | 0.77         | 0.22  | 1.26         | 0.69   |
|                          | Total hydrocarbons             | 10.26  | 5.52   | 6.90         | 3.92  | 7.98         | 2.27   |
| <i>Sulphur compounds</i> |                                |        |        |              |       |              |        |
| 8.15                     | methylthiomethane              | 2.43   | 3.52   | 4.04         | 2.73  | 1.20         | 0.85   |
| 9.11                     | dimethylsulphone               | 0.05   | 0.10   | 0.11         | 0.16  | 0.13         | 0.14   |
| 19.86                    | methyldisulphide               | 0.05   | 0.10   | 0.14         | 0.18  | 0.15         | 0.19   |
|                          | Total sulphur compounds        | 2.53   | 3.71   | 4.28         | 2.90  | 1.47         | 0.93   |
| <i>Esters</i>            |                                |        |        |              |       |              |        |
| 8.53                     | Methylacetate                  | 0.12   | 0.10   | 0.18         | 0.12  | 0.16         | 0.08   |
| 12.62                    | Ethylacetate                   | 1.02   | 0.58   | 1.62         | 0.44  | 1.09         | 0.75   |
| 17.65                    | Ethylpropionate                | 0.11   | 0.16   | 0.07         | 0.06  | 0.00         | 0.00   |
| 24.63                    | Ethylisovalerate               | 0.00   | 0.00   | 0.05         | 0.09  | 0.00         | 0.00   |
|                          | Total esters                   | 1.25   | 0.66   | 1.91         | 0.46  | 1.24         | 0.83   |
| <i>Terpenes</i>          |                                |        |        |              |       |              |        |
| 29.08                    | $\alpha$ -pinene               | 0.13   | 0.05   | 0.07         | 0.06  | 0.05         | 0.02   |
| 32.9                     | Limonane                       | 0.38   | 0.25   | 0.29         | 0.16  | 0.28         | 0.04   |
| 34.03                    | p-cymene                       | 0.10   | 0.13   | 0.07         | 0.10  | 0.00         | 0.00   |
|                          | Total terpenes                 | 0.62   | 0.40   | 0.43         | 0.28  | 0.33         | 0.05   |
|                          | Total volatile compounds       | 388.51 | 203.93 | 190.61       | 42.01 | 260.00       | 125.00 |

† Rt, Retention time (min).

methylpolysiloxane) was a capillary fused-silica column 50 m × 0.32 mm I.D., coated with PS 264 (Mega, 20090 Milan, Italy) and of 3 μm film thickness.

The capillary gas chromatograph was coupled direct to a MD 800 mass spectrometer (Carlo Erba, 20090 Milan, Italy). GC conditions were: oven initial temperature, 40 °C; hold for 6 min; then programmed to 180 °C at a rate of 5 deg C/min; hold for 5 min; programmed to 200 °C at 7 deg C/min; hold for 3 min; and finally ramped to 240 °C at 10 deg C/min, with a 5-min final isotherm. Transfer line temperature was kept at 250 °C. The mass spectrometer scanned from  $m/z$  29 to  $m/z$  300 at a 0.5-s cycle time. The ion source was set at 200 °C and spectra were obtained by electron impact (70 eV). Compounds were tentatively identified by a study of the MS spectra and comparison with members of the NBS library.

#### Statistical analysis

Volatile compounds were considered individually, or grouped in classes according to the following chemical families: ketones, aldehydes, alcohols, hydrocarbons, sulphur compounds, esters and terpenes. Discriminant analysis (SPSS, 1997) was used to classify observations into the three groups of ration composition. The classification criterion was based on a within-group covariance matrix, and the posterior probability estimates of group membership for each class were calculated.

### RESULTS AND DISCUSSION

A total of 41 volatile compounds were detected and identified with headspace GC-MS analysis (Table 2). Within the chemical families found, aldehydes had the highest number of compounds (9), followed by alcohols (8), ketones (8), hydrocarbons (6), esters (4), sulphur compounds (3) and terpenes (3).

Ketones were the most abundant compounds in the milk samples, acetone being the main constituent. Previous research (Señorans *et al.* 1996; Nursten, 1997; Buchin *et al.* 1998) found acetone to be a component of fresh raw milk smell, but this did not appear in other studies (Moio *et al.* 1993a, 1996). Aldehydes were the second most abundant class of compounds, and they can derive from lipid (straight-chain aldehydes, as isobutanal, pentanal, esanal, hexenal, nonanal) or amino acid (branched-chain compounds, i.e. 2-methylbutanal, 3-methylbutanal) degradation (Moio *et al.* 1993a), whilst acetaldehyde is a by-product of the microbial activity of lactic flora (Adda, 1986). The third class of compounds was the alcohols, with ethanol and 3-methyl-1-butanol being the most abundant. Primary alcohols are probably formed by microbial reduction of the respective aldehydes and it is unlikely that they contribute to the odour of fresh milk, considering their relatively low concentrations (Moio *et al.* 1993a). Hydrocarbons, the fourth class of compound isolated from the volatile fraction, were rich in 1-hexene. The origin of aliphatic and aromatic hydrocarbons is not well understood. Referring to a semi-hard cheese, Buchin *et al.* (1998) discussed the presence of alkenes in relation to the type of feed, but the way in which the mode of preservation of the forage influences alkene contents is not known. Although only very small amounts of sulphur compounds, esters and terpenes were detected, this does not necessarily mean that their contribution to milk characteristics must be negligible. Low concentrations of dimethylsulphone have been associated with the characteristic odour of raw milk, and higher concentrations cause odour defects (Moio *et al.* 1996). Another sulphur compound, dimethylsulphide, has a very low threshold of olfactory perception (Verdier *et al.* 1995), and very small differences in concentration could change milk aroma. Terpenes in milk and in dairy

Table 3. *Discriminant analysis results for raw milk samples from cows eating diets based on hay, maize silage or grass silage, using volatile compounds as classification criteria*

| Discriminant variables | Variables in the model  | Milk samples in the correct group |   |   | Milk samples misplaced |   |   | % Correctly classified | Wilk's lambda for Test functions‡ |       |
|------------------------|---|-----------------------------------|---|---|------------------------|---|---|------------------------|-----------------------------------|-------|
|                        |   | H†                                | S | G | H                      | S | G |                        | 1-2                               | 2     |
| Ketones                | All   | 4                                 | 4 | 4 | —                      | — | — | 100.0                  | 0.035                             | 0.485 |
| Aldehydes              | All   | 4                                 | 4 | 4 | —                      | — | — | 100.0                  | 0.009                             | 0.180 |
| Alcohols               | Ethanol, isobutanol, n-butanol, 3-methyl-1-butanol, 1-hexanol, 2-heptanol   | 4                                 | 4 | 3 | —                      | 1 | — | 91.7                   | 0.011                             | 0.764 |
| Esters                 | All   | 2                                 | 3 | 4 | —                      | 1 | 2 | 75.0                   | 0.336                             | 0.746 |
| Hydrocarbons           | All   | 4                                 | 4 | 4 | —                      | — | — | 100.0                  | 0.022                             | 0.458 |
| Sulphur compounds      | All   | 3                                 | 1 | 4 | 2                      | 1 | 1 | 66.7                   | 0.562                             | 0.910 |
| Terpenes               | All   | 3                                 | 2 | 4 | 1                      | — | 2 | 75.0                   | 0.450                             | 0.811 |
| All compounds          | Acetone, 2,3-butanedione, 2-butanone, ethanol, acetaldehyde, ethylacetate, ethylisovalerate, dimethylsulphone, p-cymene | 4                                 | 4 | 4 | —                      | — | — | 100.0                  | 0.007                             | 0.396 |
| Sum of each class      | All   | 4                                 | 4 | 4 | —                      | — | — | 100.0                  | 0.008                             | 0.289 |

† H, hay based diet; S, maize silage based diet; G, grass silage based diet.

‡ Wilks' lambda is the ratio of the within-groups sum of squares to the total sum of squares and its values range from 0.0 to 1.0. Low values indicate strong group differences and values close to 1.0 no differences. Test of function labelled '1-2' tests the hypothesis that means of the two functions are equal in the three groups. Test labelled '2' is a successive test useful to identify whether or not the additional function reflects population differences or only random variation.

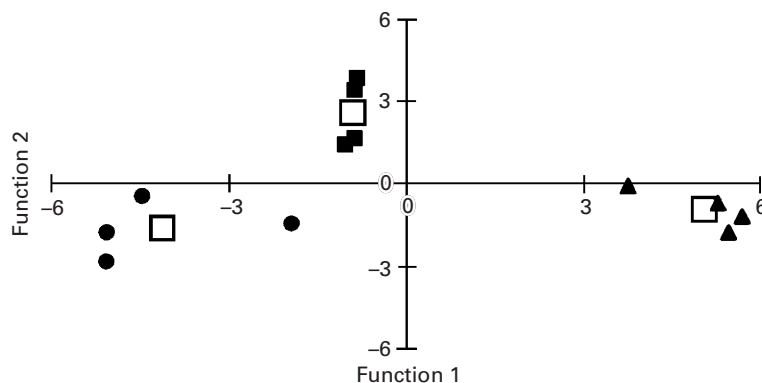


Fig. 1. Discriminant analysis: aldehydes. □, centroid; ■, H group; ●, S group; ▲, G group.

products (Verdier *et al.* 1995; Mariaca *et al.* 1997; Verdier-Metz *et al.* 1998; Buchin *et al.* 1999; Viallon *et al.* 1999) come from the plants eaten by the animals, particularly from dicotyledonous mixtures in highland pasture. According to these studies, their concentration and distribution can be used to discriminate between cheeses produced in lowlands and highlands. Esters can be formed within the mammary gland, from the esterification of short-chain alcohols and free fatty acids, but they can also indicate bacterial action (Moio *et al.* 1993*a,b*). Moreover, storage conditions of samples affect the relative concentration of different esters (Nursen, 1997).

The amount and distribution of volatile compounds in raw cow milk differed substantially from those described by Moio *et al.* (1993*a*), who reported that esters were the most represented constituents, followed by aldehydes and ketones, the latter being on average only 3% of total volatiles. The disagreement probably arises from the different methods of analysis. In the present investigation, no sample pre-treatment was used, whilst Moio *et al.* (1993*a*) used pre-concentration and distillation. According to Señorans *et al.* (1996) and Weidong *et al.* (1997), sample preparation could be a source of artefacts. Moreover, steam distillation under mild conditions, although considered a suitable method to improve detection of constituents present at very low concentration, could contribute to deviation from the natural distribution of the compounds in the volatile fraction.

To establish whether the measured volatile compounds could reflect the native composition of milk, a posterior classification of milk samples into the three groups differing for type of forage in the ration was accomplished using discriminant analysis. For this, all the identified compounds (41) or all the compounds within a class were used as independent variables in the discriminant analysis (Table 3). The multivariate test (Wilk's lambda for test functions) and posterior probability error rate indicated that the best discriminant criterion was based on all nine detected aldehydes (Fig. 1), which gave 100% of original grouped cases correctly classified. Comparable results were obtained by including all the 41 compounds in the discriminant analysis (Fig. 2), but in this case only 9 out of the 41 volatile compounds were used in the discriminant functions, the remaining 32 failing the tolerance test. Classification criteria included ketones (three compounds: acetone, 2,3-butanedione, 2-butanone), alcohols (one compound: ethanol), aldehydes (one compound: acet-aldehyde), esters (two compounds: ethylacetate, ethylisovalerate) and sulphur compounds (one compound: dimethylsulphone).



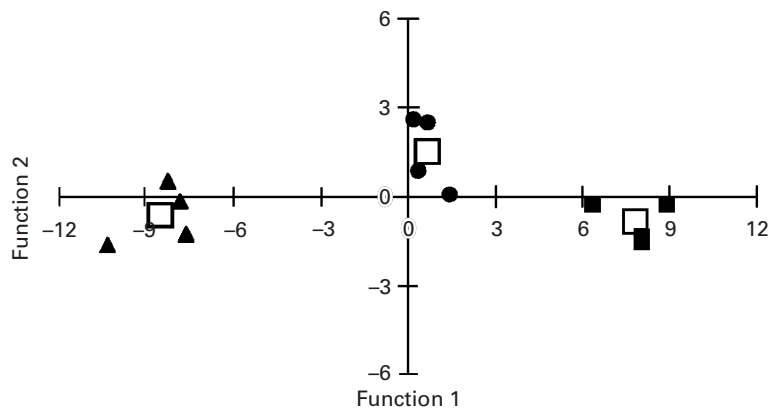


Fig. 2. Discriminant analysis: all compounds. □, centroid; ■, H group; ●, S group; ▲, G group.

The discriminant criteria of the classifications reported in Table 3 are not easy to understand. Ethanol can arise from silages, which can affect alcohol concentration in milk indirectly, via rumen metabolism, and directly by way of its presence in particular silage odours (Randby *et al.* 1999). Dimethylsulphone can also be influenced by diet composition, since it has been reported to be less concentrated in milk produced by ewes eating mixed-grain rations than in milk of ewes eating pasture (Urbach, 1990). On the other hand, diet composition did not affect ester concentrations in milk of sheep eating a mixed-grain ration or natural pasture (Moio *et al.* 1996). Terpenes and sesquiterpenes can arise in cheese from particular species of natural pastures (Viallon *et al.* 1999), and can provide a fingerprint of cheeses produced in different areas or in different seasons (Verdier *et al.* 1995), but were not included in the discriminant analysis.

Cross validations of discriminant functions were not performed, owing to the limited number of milk samples, and the correctness of classification was not verified. However, this preliminary study seems to indicate that ration compositions, forage quality in particular, can influence volatile compounds of raw milk.

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