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Determination of volatile fractions in raw milk and ripened cheese by means of GC-MS. Results of a survey performed in the marginal area between Italy and Slovenia

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ABSTRACT

The volatile fractions of milk and their evolution in ripened cheese were studied using purge and trap extraction and gas chromatography-mass spectrometry analysis (GC-MS). Eighteen samples of raw milk and cheese ripened for 70 days were collected in 2 consecutive years from dairy farms located in the border area between Italy and Slovenia. Twenty one volatile compounds not detected in the fresh milk used for cheese manufacturing, belonging mainly to the class of esters and alcohols, were found, while 8 compounds detected in milk were not found in cheese after 70 days of ripening. In cheese the majority of compounds increased significantly, even though the variations differed between the classes of volatile compounds. Concentration of alcohols, in particular ethanol, 1-propanol, 2-propanol, 3-methyl-1-butanol and 2-pentanol, increased considerably, while the content of ketones and terpenes was characterized by a significant, although small, rise that was largely related to a quantitative variation of only few compounds. Also esters, despite the high number of volatile compounds of new formation, showed a lower increase of concentration than that observed for the alcohols. Concentration of aldehydes, hydrocarbons and sulphur compounds remained unchanged, despite relevant variations involving the individual chemical compounds. Significant differences were also observed between the dairy product of the two countries, mainly for the concentration of volatile compounds belonging to the classes of alcohols, ketones and esters.

Key words: Dairy cow, Cheese, Volatile compounds, Alcohols, Esters.
RIASSUNTO
DETERMINAZIONE DELLE FRAZIONI VOLATILI NEL LATTE FRESCO E NEL FORMAGGIO STAGIONATO MEDIANTE L’UTILIZZO DELLA GASCHROMATOGRAFIA-SPETTROMETRIA DI MASSA (GC-MS).
RISULTATI DI UN’INDAGINE EFFETTUATA NELLA ZONA DI CONFINO TRA ITALIA E SLOVENIA
Le frazioni volatili del latte e la loro trasformazione nel formaggio stagionato sono state analizzate grazie all’utilizzo del sistema di estrazione “purge and trap” e all’analisi gas-cromatografica associata alla spettrometria di massa (GC-MS). Diciotto campioni di latte crudo e i corrispondenti campioni di formaggio stagionato per un periodo di 70 giorni sono stati raccolti per 2 anni consecutivi da alcuni allevamenti di bovine da latte situati nel territorio di confine tra Italia e Slovenia. Ventuno differenti composti volatili, non identificati nel latte crudo utilizzato per la produzione di formaggio e appartenenti principalmente alla classe degli esteri e degli alcoli, sono stati individuati nel prodotto trasformato e stagionato. Al contrario, 8 composti rilevati nel latte fresco non sono successivamente stati rinvenuti nei rispettivi formaggi stagionati. Nel formaggio la maggior parte dei composti ha subito un aumento significativo della concentrazione, ma la consistenza della variazione è cambiata in base alla classe di appartenenza. La concentrazione degli alcoli, in particolare dell’etanolo, dell’1-propanolo, del 2-propanolo, del 3-metil-1-butanol e del 2-pentanol, è stata caratterizzata da un aumento molto rilevante, mentre il contenuto in chetoni, composti solforati e terpeni ha subito un innalzamento significativo, ma non così sostanziale, e quasi sempre attribuibile alla variazione quantitativa di pochi composti. Anche gli esteri, nonostante l’elevato numero di composti di nuova formazione, sono stati caratterizzati da un aumento della concentrazione inferiore a quello rilevato nella classe degli alcoli. La concentrazione delle aldeidi, degli idrocarburi e dei composti solforati è rimasta sostanzialmente invariata, sebbene i singoli composti all’interno di ciascuna classe siano stati interessati in alcuni casi da rilevanti variazioni di tipo quantitativo. Differenze significative sono state riscontrate anche per le frazioni volatili dei prodotti caseari provenienti da Paesi diversi, in particolare per quanto riguarda la concentrazione di alcuni composti appartenenti alle classi degli alcoli, dei chetoni e degli esteri.

Parole chiave: Bovina da latte, Formaggio, Composti volatili, Alcoli, Esteri.

Introduction
The volatile composition of dairy products has been extensively investigated in milk of various species (Moio et al., 1993a, 1993b, 1996; Weidong et al., 1997) and in cheeses with different characteristics and origin, such as Cheddar (O’Riordan and Delahunty, 2003), Camembert (Kubicová and Grosch, 1998), Swiss Gruyère (Rychlik and Bosset, 2001), Cheddar (Bintsis and Robinson, 2004), Trentingrana (Biasioli et al., 2006) and Montasio (Toso and Stefanon, 2001). In some studies the generation and the modification of volatile compounds during cheese ripening (Dimos et al., 1996; Sunesen et al., 2002) has been investigated, but less information is available on the variation of volatile frac-
Volatiles fractions of dairy products from raw milk to ripened cheese with particular attention for those compounds that disappeared or showed an important increase or a de novo appearance in cheese compared with milk.

Material and methods

Experimental description

A survey of 9 dairy farms located in the border area between Italy and Slovenia were selected from the local Breeders Associations on the basis of diet, animal breed, farm management and level of milk production to represent local productive variability. The 6 Italian farms were located in Friuli Venezia Giulia Region within the provinces of Udine, Gorizia and Trieste, while the remaining 3 farms were located in the Slovenian Regions of Obalno-Kraska and Goriska. Samples of bulk milk and 70 days seasoned cheese were collected, in each farm in spring, during 2 consecutive years.

Milk was transformed in cheese directly at the farmhouse or in different cheese-factories. In all cases a batch of 1000 kg was used and milk was collected from two consecutive milkings and processed in the morning. Raw or pasteurised milk was warmed to 38/40°C, added with commercial graft to enhance the natural lactic flora and curdled for 45-60 min. After whey drainage fresh curd was placed in moulds and pressed. Cheeses were dry-salted or dipped in a saturated sodium chloride solution at 11°C for 24h. Ripening was performed in different seasoning rooms, in controlled humidity (78-85% relative humidity) and temperature (8-10°C), for 70 d until analysis.

Although cheese processing was performed using raw and pasteurised milk, various starter cultures and different salting methods, the final product, very common in the North East of Italy and in Slovenia, was in all cases a fatty cheese (fat>30%) characterized by a medium-hard and cooked paste and obtained exclusively from cow milk.

Feedings

Depending on farm management, feeds were administered as total mixed ration ad libitum or separated (maize and hay and/or lucerne). Animals were fed with different winter rations according with country and grazing pasture was not performed. In Slovenian farms (SL) grass silage and grass hay prevailed compared to maize silage and feed stuff whereas in Italian farms (IT) grass hay and feed stuff were the predominant component of the ration while grass silage was not used at all.

A week before bulk milk collection, individual feeds and TMR were sampled in each farm and analysed for dry matter (DM), crude protein (CP), lipids (ether extract, EE) and neutral-detergent fibre (NDF) (Goering and Van Soest, 1970). Starch was determined using the polarimetric method described by Martillotti et al. (1987).

The mean formulation and chemical composition of the rations of each group of farm are reported in Table 1.

Bulk milk and cheese sampling

One hour before curdling, a sample of bulk milk was collected from the cooled tank and 40 g were weighed in a 50 ml vial together with 10 g of NaCl. After adding tetrahydrofuran as internal standard, by means of a gas-chromatographic syringe (a volume of 0.02 μl equivalent to 17.8 μg) (Merck, Darmstadt, Germany), vials were sealed with an aluminium-rubber septum (Supelco Inc, Bellefonte, PA, USA) and frozen at -80°C until analysis. Another sample was collected in 50 ml sterile PE vial together with 100 μl of H2O2 as preservative and immediately delivered to the lab for chemical analysis. To evaluate concentration (%) of fat, protein,
lactose and dry matter, sample was submitted to IR spectrophotometry.

After an average of 70 (s.d. ±9.24) days of ripening, 1 cheese was collected from each farm or dairy. Cheese was immediately cut in pieces, removed from the rind and frozen. A sample was stored in sterile bag and frozen for chemical analysis. A further sample was frozen-grated and 10 g were weighed into a 50 ml glass vial. After addition of internal standard (17.8 μg of tetrahydrofuran) (Merck, Darmstadt, Germany), vials were sealed with an aluminium-rubber septum (Supelco Inc, Bellefonte, PA, USA) and frozen at -80°C before analysis. A sample of about 500 g was collected in a PE sterile bag and immediately analyzed to evaluate fat, protein, carbohydrates and dry matter percentage in according to AOAC Official Methods (1995).

The average chemical composition of bulk milk and cheese and the significant differences between Italian and Slovenian dairy products are reported in Table 2.

### Table 1. Dry matter (DM) intake and composition of rations of Italian (IT) and Slovenian (SL) dairy farms.

|                     | IT       | SL       |
|---------------------|----------|----------|
| DM intake: (kg/d)   | Mean     | SD       | Mean     | SD       |
| Hay, lucerne g/kg DM| 501.50   | 129.10   | 99.70    | 87.90    |
| Maize silage        | 121.00   | 148.00   | 38.10    | 66.00    |
| Grass silage        |          |          | 582.50   | 106.00   |
| Feed stuff          | 377.50   | 72.40    | 297.70   | 98.10    |
| Total               | 1000.00  |          | 1000.00  |          |

**Chemical analysis:**

|                  | IT   | SL   |
|------------------|------|------|
| Dry matter g/kg  | 792.60| 792.80 |
| Crude protein g/kg DM | 131.40| 139.60 |
| Ether extract    | 26.90| 25.60 |
| NDF              | 405.30| 457.20 |
| Starch           | 209.20| 133.70 |
| Ash              | 69.10| 75.90 |

**SD:** Standard Deviation.

Headspace sampling and analysis by means of coupled GC-MS

Milk and cheese samples were analysed with the head space sampling technique (Barcarolo and Casson, 1997). Vials were conditioned at 70°C for 15 min before analysis, then stripping was carried out for 120 sec. Stripping was realized with helium, at a rate of 10 ml/min. The analytical column used was a capillary fused-silica column 50 mX0.32 mm I.D., coated with PS 264 (Mega, Milan, Italy), 3 μm film thickness. The capillary GC system (Carlo Erba GC 8000) was coupled directly to a MD 800 mass spectrometer (Carlo Erba GC 8000). GC condi-
Concentrations were the following: initial temperature of oven of 40°C, 6 min holding, then programmed to 180°C at a rate of 5°C/min, then 5 min at 180°C, then at 7°C/min to 200°C held 10 min. Transfer line temperature was kept at 250°C. A mass spectrometer scanned from m/z 29 to m/z 300 at 0.5 s cycle time. The ion source was set at 180°C and spectrum was obtained by electron impact (70 eV). Identification of compounds was carried out by comparison of retention times and mass spectra of known standards, members of the NBS library. The relative abundance of each volatile compound was obtained as the ratio of its peak to the area of tetrahydrofuran peak. (1000×peak area/internal standard peak area). In the attempt to compare the concentration of the same volatile compounds into two matrices with different moisture content, bulk milk and ripened cheese, the concentration of aroma constituents were related to DM percentage of the respective dairy product and expressed in µg/100g DM. For this, the concentration of each volatile compound (expressed in µg/kg) was divided for the DM content of the matrix (expressed in g/100g DM) and than multiplied for 10.

**Statistical analysis**

Volatile compounds were considered individually and grouped in classes according to the following chemical families: ketones, aldehydes, alcohols, hydrocarbons, sulphur compounds, esters and terpenes. The significance of volatile compound concentrations from milk to cheese was evaluated with the univariate ANOVA model considering type of dairy product as fix factor (SPSS, 1997).

**Results and discussion**

**Volatile fractions of milk**

The headspace sampling technique allowed the detection, in bulk milk samples, of 40 volatile compounds, which were grouped according to chemical classes in alcohols, aldehydes, ketones, esters, hydrocarbons,

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**Table 2. Chemical composition of bulk milk and cheese produced in Italian and Slovenian farms.**

| IT         | SL         | P  |
|------------|------------|----|
| **Bulk milk:** |           |    |
| Fat %      | 3.85       | 3.39 | ns |
| Protein    | 3.47       | 3.43 | 0.16 | ns |
| Lactose    | 4.89       | 5.01 | 0.14 | *  |
| Dry matter | 9.11       | 9.18 | 0.17 | ns |
| **Cheese:** |           |    |
| Fat %      | 33.21      | 35.17 | 1.75 | *  |
| Protein    | 25.63      | 25.91 | 0.76 | ns |
| Carbohydrates | 1.77      | 1.72 | 0.93 | ns |
| Dry matter | 65.02      | 67.01 | 1.33 | ***|

SD: Standard Deviation.

* **,** *** = significant at P<0.05, P<0.01 and P<0.001, respectively; ns=not significant.
sulphur compounds and terpenes. Table 3 summarizes the mean concentration of each volatile compound detected in milk and cheese produced respectively in Italy and Slovenia and the significance of the differences from fresh milk to 70 d ripened cheese.

As reported in previous studies on volatile fractions of milk (Nursten, 1997; Buchin et al., 1998; Toso et al., 2002), ketones resulted the most abundant class of compounds (237.74 µg/100g DM) with acetone (209.26 µg/100g DM) and 2-butanone (25.32 µg/100 g DM) as main constituents. These compounds were frequently detected in raw milk (Badings and Neeter, 1980; Badings et al., 1985) and the presence of acetone, likely related to the metabolism of cows during lactation, was found to be highly and negatively correlated with stale flavour in UHT milk (Jeon et al., 1978).

Esters (120.30 µg/100g DM), especially ethylacetate (112.19 µg/100g DM), propylpropionate (3.33 µg/100g DM) and propylacetate (2.68 µg/100g DM), were the second more representative group of volatile compounds. In fresh milk these compounds generally represent less than 1% of the volatile fraction (Stefanon et al., 2002) and this low presence is likely due to the esterase activity of the mammal gland (Moio et al., 1993a). The high percentage of esters observed in Italian and Slovenian bulk milk can be related to the cold storage before sampling. During this phase, as reported by Nursten (1997), alcohols are partially esterified with volatile acids and converted in propyl- and ethyl-esters from the enzymatic activity of lactic bacteria (Moio et al., 1993a).

The concentration of alcohols in milk (23.15 µg/100g DM) was low and the more abundant compounds were 1-propanol (10.86 µg/100g DM), ethanol (6.45 µg/100g DM) and methanol (4.24 µg/100g DM). The presence of these alcohols could be associated with a direct transfer from feeds, especially silages and corn silages (Stefanon et al., 2002; Stefanon and Procida, 2003).

Aldehydes (13.91 µg/100g DM), sulphur compounds (6.33 µg/100g DM), hydrocarbons (1,56 µg/100g DM), and terpenes (0,39 µg/100g DM) were detected in small concentrations, although they can largely contribute to milk characteristics, due to their low perception threshold. Acetaldehyde (1.82 µg/100g DM) is a by-product of lactic microflora activity (Adda, 1986) and represents the main aroma component of fermented milk (Marshall, 1984). Dimethylsulfone (0.75 µg/100g DM) has been associated with the odour of raw milk already at low concentrations and with odour defects at higher concentrations (Moio et al., 1996). Aromatic and aliphatic hydrocarbons were detected in low concentration and, in view of the fact that they are considered to have a high perception threshold (Moio et al., 1993a), probably did not play a remarkable role in bulk milk aroma.

The comparison between milk produced in Italy and Slovenia have not shown significant differences in the concentration of volatile fractions, with the exception of few compounds such as 2-butanone, an aliphatic methyl ketone originating from the oxidation of free fatty acids by microbial metabolism (Barbieri et al., 1994; Urbach 1997), that resulted to be more abundant in the Italian milk.

**Volatile fractions of cheese**

The volatile fractions changed considerably on the quantitative aspect from raw milk to ripened cheese. As shown in Table 3, 21 aroma compounds not detected in raw milk were found in cheese, while 8 compounds detected in raw milk were not found in cheese after 70 days of ripening. The new aroma constituents belong mainly to the...
groups of alcohols, which are formed during cheese maturation as a consequence of the low redox potential of substrate, and esters, which are related to the presence of alcohols (Fernández-García et al., 2004a).

Alcohols (2252.31 µg/100g DM) were the most representative class of compounds detected using headspace sampling GC-MS. In ripened cheese, compared to bulk milk, this predominance was mainly due to the great significant increase of ethanol, 1-propanol, 2-propanol, 3-methyl-1-butanol and 2-pentanol. These compounds generally originate from the reduction of aldehydes and methyl-ketones (Moio et al., 1993b; Engels et al., 1997), but the considerable increase in ethanol content could also be related to lactose fermentation and amino acid metabolism (Molimard and Spinnler, 1996). n-butanol and 4 secondary alcohols (isobutanol, 2-propanol, 2-hexanol, and 2-heptanol) were considered as compounds of new formation in ripened cheese because they were not detected in raw milk. Secondary alcohols are formed as a consequence of the presence of moulds (Buchin et al., 1998) by enzymatic reduction of the corresponding methyl ketones which themselves derived from fatty acids by β-oxidation or from β-ketoacids (Molimard and Spinnler, 1996).

The remaining classes of volatile compounds increased in cheese to a lesser extent when compared with alcohols. The concentration of ketones (396.54 µg/100g DM) increased, but 4-hydroxy-4-methyl-2-pentanone completely disappeared and acetone, the main constituent of milk, resulted significantly reduced. Keen et al. (1974) reported that enzymes from bacteria present in raw milk reduce 2,3-butanedione and acetone to 2-butanol and 2-butanone, the latter a compound characterized by a significant increase and found at high levels in cheeses considered in this study as well as in other raw milk cheese (Izco and Torre, 2000; Fernández-García et al., 2004b). On the contrary, aliphatic ketones (2-butanone, 2-pentanone, 2,3-butanedione and 2-heptanone) were characterized by a pronounced growth. Stefanon and Procida (2004) reported that aliphatic and branched ketones increased in cheese during ripening and that their concentration likely depends on indigenous microflora. The appearance of new compounds was not observed within this volatile class.

Esters (157.16 µg/100g DM) underwent numerous variations from a qualitative viewpoint, with a significant increase in the global concentration associated with a de novo formation of 12 compounds (methylformate, ethylformate, 2-butylacetate, amylformate, ethylbutyrate, butylacetate, isopropylbutyrate, butylpropionate, isoamylacetate, propylbutyrate, isobutylbutyrate and ethylhexanoate). Components with the higher increase were ethyl- and propyl-esters and this was probably due to the abundant presence of ethanol, 1-propanol and 2-propanol. As reported by Bosset and Liardon (1984), reactions of esterification occur between short to medium-chain fatty acids contained in cheese, and primary and secondary alcohols result from lactose and amino acid metabolism.

Aldehydes (20.86 µg/100g DM) had a lower and substantially unchanged concentration in comparison with the previous volatile groups. Three compounds, butanal, pentanal and 2,4-hexadienal, observed in milk, were not detected in the ripened cheese, while 2-propenal and octanal were newly formed, likely from β-oxidation of unsaturated fatty acids (Collomb and Spahni, 1996). Sulphur compounds (9.80 µg/100g DM) were detected in milk and in cheese. Methyldisulfide underwent a relevant increase from milk to cheese and methanethiol and dimethyltrisulfide newly appeared. These variations are commonly related to methionine degradation in cheese (Yvon and Rijnen, 2001).
Table 3. Mean content of volatile compounds detected in Italian (IT) and Slovenian (SL) milk and 70 d ripened cheese: values related to dry matter content (mg/100g DM).

| Compound          | IT       | SL       | IT       | SL       | Product | Country | Product x Country |
|-------------------|----------|----------|----------|----------|---------|---------|------------------|
|                   | mean SD  | mean SD  | mean SD  | mean SD  | P value | P value | P value          |
| **Alcohols:**     |          |          |          |          |         |         |                  |
| Methanol          | 4.63     | 6.42     | 3.46     | 3.28     | 14.07   | 23.43   | 5.35  8.46 ns.  |
| Ethanol           | 6.71     | 9.92     | 5.94     | 5.80     | 1666.27 | 823.94  | 1478.71 1674.99 *** ns  |
| 2-Propanol        | nd       | nd / nd  | nd       | 87.49    | 60.07   | 201.68  | 138.96 *** ns  |
| 1-Propanol        | 9.75     | 6.48     | 13.10    | 5.41     | 181.78  | 126.92  | 387.66 215.89 *** ns  |
| Isobutanol        | nd       | nd / nd  | nd       | 21.89    | 21.14   | 13.87   | 9.06 *** ns  |
| n-Butanol         | nd       | nd / nd  | nd       | 85.44    | 109.03  | 83.53   | 83.91 ** ns  |
| 1-Penten-3-ol     | 0.30     | 0.43     | 0.43     | 0.56     | nd / nd | nd / nd | ** ns  ns  |
| 2-Pentanol        | 0.42     | 0.57     | 0.50     | 0.65     | 34.93   | 27.71   | 108.56 77.77 *** ** ** |
| 3-Methyl-1-butanol| 0.20     | 0.12     | 0.27     | 0.14     | 86.66   | 76.88   | 70.89   80.38 *** ns |
| 2-Methyl-1-butanol| 0.08     | 0.07     | 0.10     | 0.05     | 10.45   | 7.77    | 17.23   17.76 *** ns |
| 1-Pentanol        | 0.21     | 0.50     | 0.52     | 0.71     | nd / nd | nd / nd | ** ns  ns  |
| 2-Hexanol         | nd       | nd / nd  | nd       | 0.10     | 0.13    | 0.10    | 0.22 * ns  |
| 1-Hexanol         | 0.14     | 0.11     | 0.26     | 0.24     | 1.33    | 1.29    | 1.82    2.41 ** ns  |
| 2-Heptanol        | nd       | nd / nd  | nd       | 1.48     | 1.07    | 3.20    | 1.13 *** ** ** |
| Total alcohols    | 22.43    | 19.35    | 24.56    | 11.13    | 2192.11 | 868.76  | 2372.71 1984.02 *** ns |
| **Aldehydes:**    |          |          |          |          |         |         |                  |
| Acetaldehyde      | 2.01     | 2.89     | 1.44     | 1.47     | 1.77    | 1.91    | 4.02    5.29 ns  |
| 2-Propanal        | nd       | nd / nd  | nd       | 0.26     | 0.90    | 0.73    | 1.56 ns  ns  |
| Butanal           | 0.02     | 0.05     | 0.05     | 0.07     | nd / nd | nd / nd | * ns  ns  |
| 3-Methylbutanal   | 0.42     | 0.64     | 0.07     | 0.08     | 13.39   | 31.54   | 14.35   21.59 ns  |
| Pentanal          | 1.06     | 1.69     | 1.33     | 0.84     | nd / nd | nd / nd | ** ns  ns  |
| 2,4-Hexadienal    | 0.77     | 1.55     | 0.60     | 0.53     | nd / nd | nd / nd | * ns  ns  |

Continued >>
|   |   |   |   |   |   |   |   |   |   |   |   |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 21.96 | hexanal | 10.80 | 18.44 | 4.26 | 5.04 | 3.09 | 3.79 | 1.29 | 0.63 | ns | ns | ns |
| 26.75 | heptanal | 0.75 | 0.75 | 0.82 | 0.87 | 0.36 | 0.44 | 0.41 | 0.58 | ns | ns | ns |
| 36.06 | nonanal | 0.49 | 0.32 | 0.53 | 0.35 | 1.19 | 0.67 | 0.92 | 0.27 | ** | ns | ns |
| 31.09 | octanal | nd | / | nd | / | 0.25 | 0.27 | 0.26 | 0.30 | ** | ns | ns |
| **Total aldehydes** | 16.32 | 22.49 | 9.09 | 6.57 | 20.30 | 34.47 | 21.98 | 21.35 | ns | ns | ns |

**Ketones:**

|   |   |   |   |   |   |   |   |   |   |   |   |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 6.46 | acetone | 191.82 | 63.70 | 244.13 | 123.34 | 25.45 | 30.56 | 61.76 | 25.41 | *** | ns | ns |
| 10.82 | 2,3-butanedione | 0.13 | 0.19 | 0.23 | 0.23 | 10.22 | 11.30 | 71.57 | 67.96 | *** | ** | ** |
| 11.30 | 2-butanone | 29.64 | 13.11 | 16.68 | 10.33 | 224.64 | 180.46 | 391.58 | 377.43 | *** | ns | ns |
| 16.15 | 2-pentanone | 0.71 | 0.38 | 0.70 | 0.30 | 24.32 | 35.26 | 82.01 | 46.95 | *** | ** | ** |
| 16.49 | 3-methyl-2-pentanone | 0.89 | 1.67 | 0.91 | 1.05 | 0.27 | 0.38 | 1.27 | 2.09 | ns | ns | ns |
| 19.81 | 2-hexanone | 0.46 | 0.31 | 0.25 | 0.20 | 0.16 | 0.22 | 0.48 | 0.84 | ns | ns | ns |
| 29.41 | 4-hydroxy-4-methyl-2-pentanone | 0.58 | 0.84 | 0.66 | 0.45 | nd | / | nd | / | ** | ns | ns |
| 27.06 | 2-heptanone | 0.17 | 0.16 | 0.09 | 0.09 | 2.14 | 1.35 | 4.12 | 2.30 | *** | * | * |
| 31.22 | 6-methyl-5-hepten-2-one | 0.14 | 0.19 | 0.46 | 0.65 | 0.87 | 0.45 | 0.65 | 0.34 | ** | ns | ns |
| **Total ketones** | 224.55 | 68.49 | 264.12 | 121.99 | 288.09 | 233.32 | 613.46 | 429.95 | * | * | ns |

**Esters:**

|   |   |   |   |   |   |   |   |   |   |   |   |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 4.64 | methylformate | nd | / | nd | / | 0.11 | 0.12 | 0.14 | 0.27 | * | ns | ns |
| 7.50 | ethylformate | nd | / | nd | / | 0.95 | 0.28 | 1.27 | 0.92 | *** | ns | ns |
| 8.06 | methylacetate | 0.27 | 0.19 | 0.22 | 0.20 | 0.49 | 0.51 | 0.60 | 0.37 | * | ns | ns |
| 12.25 | ethylacetate | 111.12 | 15.87 | 114.33 | 23.31 | 62.25 | 9.93 | 58.30 | 10.52 | *** | ns | ns |
| 12.68 | propylformate | 1.45 | 0.76 | 2.30 | 3.29 | 36.22 | 19.05 | 48.63 | 36.91 | *** | ns | ns |
| 17.38 | ethylpropionate | 0.12 | 0.05 | 0.07 | 0.04 | 1.01 | 0.63 | 19.12 | 22.24 | ** | ** | ** |
| 17.53 | propylacetate | 2.68 | 0.61 | 2.67 | 1.27 | 3.35 | 3.06 | 9.19 | 6.35 | ** | * | * |
| 18.83 | methylbutirate | 0.01 | 0.02 | 0.03 | 0.04 | 0.18 | 0.45 | 0.06 | 0.07 | ns | ns | ns |
| 19.85 | 2-butylacetate | nd | / | nd | / | 0.72 | 0.62 | 1.47 | 1.82 | ** | ns | ns |
| 20.20 | amyifomate | 0.90 | 0.96 | 0.93 | 1.28 | 0.45 | 0.81 | 0.46 | 0.99 | ** | ns | ns |
| 21.87 | ethylbutyrate | nd | / | nd | / | 23.11 | 22.94 | 50.30 | 79.62 | ** | ns | ns |

*Continued >>*
**Table 3**  >> Continuation

|        | Propylpropionate | Butylacetate | Isopropylbutyrate | Butylpropionate | Isoamylacetate | Propylbutyrate | Isobutyrlbutyrate | Ethylhexanoate | Total Ester        |
|--------|------------------|--------------|-------------------|-----------------|----------------|----------------|-------------------|----------------|-------------------|
|        | 22.29            | 3.56         | 1.22              | 2.86            | 1.45           | 0.46           | 2.21              | 0.73           | 119.21            |
|        | 22.51            | nd           | nd                | 1.37            | 1.70           | 1.57           | 0.90              | nd             | 17.81             |
|        | 24.57            | nd           | nd                | 0.04            | 0.07           | 0.01           | 0.04              | nd             | 122.49            |
|        | 24.98            | nd           | nd                | 0.09            | 0.27           | 0.27           | 0.41              | nd             | 27.91             |
|        | 25.45            | nd           | nd                | 0.48            | 0.66           | 0.96           | 1.39              | nd             | 136.09            |
|        | 27.14            | nd           | nd                | 0.17            | 0.50           | 0.40           | 0.81              | nd             | 43.00             |
|        | 28.93            | nd           | nd                | 0.08            | 0.15           | 0.17           | 0.20              | nd             | 199.30            |
|        | 30.67            | nd           | nd                | 3.03            | 1.91           | 3.66           | 3.23              | nd             | 119.30            |
|        | ** Total Esters  | 119.21       | 17.81             | 122.49          | 27.91          | 136.09         | 43.00             | 199.30         | ** 199.30         |

**Hydrocarbons:**

|        | 1-hexene         | 0.13         | 0.28              | 0.07            | 0.09           | nd             | nd                | nd             | ns                |
|        | Toluene          | 0.97         | 0.20              | 2.17            | 1.56           | 0.97           | 0.20              | 1.53           | 0.82              |
|        | 1-octene         | 0.11         | 0.33              | 0.04            | 0.07           | nd             | nd                | nd             | ns                |
|        | ** Total Hydrocarbons** | 1.20       | 0.55              | 2.28            | 1.54           | 0.97           | 0.20              | 1.53           | 0.82              |

**Sulphur compounds:**

|        | Methanethiol     | nd           | nd                | nd              | 0.22           | 0.39           | 0.10              | 0.15           | ns                |
|        | Methylthiomethane| 7.59         | 8.73              | 1.37            | 0.92           | 1.35           | 1.54              | 1.11           | 0.75              |
|        | Dimethylsulfone  | 0.78         | 0.43              | 0.70            | 0.65           | 3.58           | 3.22              | 1.78           | 1.88              |
|        | Methyl disulfide | 0.09         | 0.26              | 0.02            | 0.03           | 3.63           | 3.70              | 4.96           | 3.70              |
|        | Dimethyltrisulfide| nd           | nd                | nd              | 1.19           | 1.33           | 2.04              | 1.66           | ns                |
|        | ** Total Sulphur Compounds** | 8.45       | 8.48              | 2.09            | 0.66           | 9.75           | 5.87              | 9.90           | 5.15              |

**Terpenes:**

|        | Alpha-pinene     | 0.34         | 0.21              | 0.48            | 0.19           | 0.57           | 0.46              | 1.32           | 1.00              |
|        | Limonene         | nd           | nd                | nd              | 0.49           | 0.62           | 0.96              | 1.33           | ns                |
|        | ** Total Terpenes** | 0.34       | 0.21              | 0.48            | 0.19           | 1.07           | 1.07              | 2.28           | 2.28              |

**Total Volatile Compounds**

|        | 392.51           | 74.81        | 425.13            | 121.75          | 2648.38        | 840.66         | 3221.16           | 2458.62        | ns                |

**SD=standard deviation; nd=not detected; ns=not significant.**

*,**,**=significant at P<0.05, P<0.01 and P<0.001, respectively.
Terpenes (1.47 µg/100g DM) are considered to originate mainly from feed components such as hays and pastures (Viallon et al., 1999; Tornanbé et al., 2006) and, as a consequence, they should remain at the same concentration in milk and cheese. However, in milk, limonene was not detected and α-pinene had a significantly lower concentration, but this could be related to a major dilution of the compounds in milk rather than in cheese.

As observed also by Stefanon and Procida (2004) in Montasio cheese, hydrocarbons were detected in very small concentrations (1.16 µg/100g DM), but they are not generally considered important contributors to food flavours.

Considering volatile fractions of cheese produced in Italy and Slovenia, some important differences were found especially in the classes of alcohols, ketones and esters (Table 3).

Among alcohols the concentration of 4 compounds, 1-propanol, 2-propanol, 2-pentanol and 2-heptanol, was significantly higher in cheese coming from Slovenia. This could be due to the different treatment used for milk before curdling. Fernandez et al. (2002) observed a significant prevalence of n-alkanols in raw milk cheese compared with pasteurized milk cheese, and while in Italy both raw - but mainly pasteurised - milk was used for cheese making, depending on the farm and cheese factory, in Slovenia exclusively raw milk was employed.

Also, the class of ketones, in particular acetone, 2,3-butanedione, 2-pentanone and 2-heptanone, was characterized by a significant prevalence in Slovenian cheese. Since methyl ketones derive from oxidation of free fatty acids by microbial metabolism, and branched compounds can originate from amino acid degradation (Barbieri et al., 1994; Urbach, 1997), the different presence of these compounds in Italian and Slovenian cheese could depend upon the activity of different indigenous microflora. Nevertheless, higher content of the mentioned compounds is sometimes related to a diet rich in hay and grass silage (Stefanon and Procida, 2004) like that used in Slovenia.

In the class of esters only 3 compounds - ethylpropionate, propylacetate and propylpropionate - significantly differed in cheeses produced in Italy and Slovenia. Since esters in dairy products are mainly formed for enzymatic or chemical reaction of free fatty acids with primary alcohols (Stefanon and Procida, 2004), the higher content of propyl esters observed in Slovenian cheese could be a direct consequence of the highest value of 1- and 2-propanol found for these samples.

Conclusions

The volatile fractions of 70 d ripened cheese sampled in Italian and Slovenian farms, detected by means of coupled GC-MS, were quantitatively and qualitatively different from those of the corresponding raw milk. The number of volatile compounds varied from 40 to 55, respectively, in milk and cheese. Eight compounds detected in the raw milk were not found in cheese after 70 days of ripening while 21 aroma compounds, not detected in milk were found in cheese. The statistical analysis made it possible to identify significant variations from fresh milk to ripened cheese. In particular, the class of alcohols was the only affected by a high increase in concentration, while esters and ketones were characterized by a low rise and the remaining groups, such as aldehydes, hydrocarbons, sulphur compounds and terpenes, were characterized by substantial stability or by variations as respects a few individual chemical compounds. Milk and cheese samples were collected for 2 consecutive years from different farms and cheese factories and cheese was obtained...
with different starter cultures and ripened in different seasoning rooms; therefore, the significant increases in alcohols, esters and ketones could indicate that their formation is mainly attributable to the ripening process. However, as reported in other studies about cheese flavour formation (Sousa et al. 2001; Curioni and Bosset, 2002; Collins et al., 2003), numerous chemical and enzymatic reactions take place during ripening and, consequently, it is not easy to define or predict the qualitative and quantitative variations of the volatile classes, not even when considering a product with standard characteristics. This aspect is confirmed by the results obtained comparing milk and cheese produced in different countries. The comparison indicated that many factors, such as animal feeds, composition of pasture, milk fat composition or animal breed, are involved in the composition of volatile fractions in milk and cheese.

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