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Characterization of the volatile fraction and of free fatty acids of "Fontina Valle d'Aosta", a protected designation of origin Italian cheese

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Abstract

The characterization of the volatile fraction of 24 samples of "Fontina Valle d'Aosta", a protected designation of origin (PDO) semi-hard Italian cheese, was performed using the dynamic headspace extraction technique coupled with gas chromatography–mass spectrometry. Seventy-four volatile compounds, belonging to several chemical classes, mainly alcohols, sulfur and carboxylic compounds, were identified, allowing characterization of the distinct flavour of "Fontina Valle d'Aosta PDO" cheese. The characterization of the cheese was done also in terms of free fatty acids and the mechanism of formation of some classes of compounds is considered. Determinations of fat, N-Kjeldhal and dry matter were also carried out in order to obtain a more complete description of the properties of this PDO Italian cheese.

Keywords: Fontina cheese; Volatile compounds; Free-fatty acids; Dynamic headspace technique; Gas chromatography–mass spectrometry

1. Introduction

The acquisition of the protected designation of origin (PDO) denomination, describing a foodstuff produced and processed in a well defined geographical area using recognized know-how, poses a fascinating challenge to the producers of the European Union. In fact, nowadays consumer attention is increasingly focussed on certified quality products.

About 30 Italian cheeses have obtained the PDO denomination (web site: http://www.politicheagricole.it) and among them is the "Fontina Valle d'Aosta" cheese. It is a typical semi-hard cheese, produced in the mountains of Valle d'Aosta, a small region in the north-west of Italy, at 2000 m height, following well-established traditional rules. It is produced from raw milk obtained exclusively from native breedes, fed only with forage grown in the same valley (Fontina Valle d'Aosta PDO Production rules, 1955), using bovine rennet as coagulant and adding 3 different starter cultures selected by the Institute Agricole Regional of Aosta. "Fontina PDO Valle d'Aosta" cheeses are cylindrical in shape and weigh 8–12 kg each and are usually commercialized with 5–6 months ripening. This product obtained the protected designation of origin in 1996 (Commission Regulation, 1996), accordingly to the Council Regulation (EC) No 2081/92 of 14 July 1992 concerning the protection of geographical indications and designations of origin for agricultural products and foodstuffs.

Owing both to the low number of producers and to the small area of production, "Fontina Valle d'Aosta PDO" manufacture is rather limited, so many "Fontina-like"

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cheeses, industrial imitations of the PDO product and produced using pasteurised milk, are commercialized, being competitive by their lower prices.

In this context, the characterization of the aromatic profile of "Fontina Valle d'Aosta PDO" cheese represents an important tool for quality evaluation purposes. In fact, the acceptability of cheese by consumers depends on its sensory qualities, flavour being a distinctive feature of the product. In food analysis, the importance of the aromatic profile has already been emphasised by different studies; thus volatile compounds are the chemical fingerprint of the analyzed products (Barbieri et al., 1994; Barron et al., 2005; Bianchi, Careri, & Musci, 2005).

In cheese manufacturing, aroma is influenced by different factors; among them, milk origin and treatment (raw and pasteurised milk) are important parameters since products of different qualities can be obtained in different situations (Awad, 2006; Buchin et al., 1998; Fernandez-Garcia, Carbonell, & Nunez, 2002). In particular, when milk pasteurisation is performed, cheeses characterized by a more homogeneous quality than those obtained from raw milk are produced.

However, raw milk products represent a significant proportion of the ripened cheeses produced in Europe, particularly in Italy, France and Switzerland (Grappin & Beuvier, 1997). In these products, native enzymes and wild microbiota can act, both beneficially and negatively, thus providing the "typical" cheese aroma (Beuvier et al., 1997; Buchin et al., 1998).

Different studies have been performed on European raw milk cheeses in which volatile compounds have been analyzed mainly by using "solvent-free" techniques, such as dynamic headspace (DHS), purge-and-trap (P&T) and solid phase microextraction (SPME) coupled to gas chromatography–mass spectrometry (GC–MS) analysis (Carbonell, Nunez, & Fernandez-Garcia, 2002; Fernandez-Garcia, Carbonell & Nunez, 2002; Mallia, Fernandez-Garcia, & Bosset, 2005).

Taking into account that, up until now, only one study, using analytical parameters such as alkaline phosphatase activity and isotope ratios has been performed in order to characterize the "Fontina Valle d'Aosta PDO" (Pillonel, Butikofer, Rossmann, Tabacchi, & Bosset, 2004), and that no other studies have been performed on the volatile compounds of the aromatic profile of this typical product, the aim of this work was the characterization of the aroma of this PDO cheese by means of the DHS-GC–MS technique. Hypotheses on the mechanism of formation of major classes of compounds are also provided.

The obtained results will be used as starting-point for further studies of the influence of seasonality, as well as of microbiological effects (use of different starter cultures), on the volatile fraction of this typical Italian cheese.

In addition, other analyses, involving the evaluation of dry matter, fat, N-Kjeldahl and free fatty acids (FFA), were carried out in order to obtain a more complete characterization of this typical product.

2. Materials and methods

2.1. Reagents

2-Propanol, boric acid, propionic acid (C3), hexanoic acid (C6) (all >99.5% purity), hexane, nonane, decane, undecane, dodecane, tridecane, tetradecane, pentadecane, hexadecane, heptadecane, β-pinene, acetic acid (C2), butyric acid (C4), heptanoic acid (C7), tetradecanoic acid (C14), hexadecanoic acid (C16) and oleic acid (C18:1) (all >99% purity), octane, α-pinene and dodecanoic acid (C12) (all >98% purity), camphene, α-terpinene, formic acid and octadecanoic acid (C18) (all >95% purity), octanoic acid (C8) and decanoic acid (C10) (>96% purity), were from Sigma–Aldrich (Milan, Italy). Diethyl ether (99.8% purity) and ammonium hydroxide solution were from Fluka (Buchs SG, Germany). Phosphosulfuric acid, sulfuric acid 96%, sodium hydroxide and sodium sulfate were from Carlo Erba (Milan, Italy). Hydrochloric acid (36–38%) was from J. T. Baker (Deventer, The Netherlands).

2.2. Cheese samples

Twenty-four "Fontina Valle d'Aosta PDO" samples, produced during summer 2004, were provided by the Institut Agricole Regional of Aosta after 5 months of ripening. The quality assessment of the analyzed samples was performed by the "Cooperativa Produttori Latte Fontina" and the samples were defined as 'First Quality' products. The cheese samples were part of the normal production of 'Fontina PDO Valle d'Aosta', located within an area of 35,000 ha in the Valle d'Aosta region, from bovines at the end of their lactation stage. Vats of 600–800 l were used, adding 3 stocks of *Streptococcus thermophilus* (M16PTZA4'96, MTH17CL3'96, MT17BA7'96), as starters. These stocks were isolated by the Institute Agricole Regional of Aosta from 'Fontina Valle d'Aosta PDO' cheeses produced in the alpine pasture (Andrighetto, Borney, Barmaz, Stefanon, & Lombardi, 2002). Bovine rennet was used to coagulate milk in 30–45 min at 36 °C. Bovine rennet (1.5 g) were used for 100 l of milk. Curd was cut to maize grain size and gradually heated to 48 °C. Starting from 36 °C, 30 min were required to reach the temperature of 42 °C and a further 15 min to pass from 42 °C to 48 °C. When this temperature was reached, heating was immediately switched off and the cheeses were pressed for 12 h and manually salted. 'Fontina PDO Valle d'Aosta' cheeses were ripened at 10–12 °C on deal tables and >85% RH during 5 months. During the first ripening month, the samples were daily turned upside down and manually salted on alternate days. More precisely, a dry-salting procedure was alternated with a brushing step performed by using a saturated salty-solution. Afterwards, only the brushing step with the same saturated salty-solution was carried out for a time of 50 days.

Before analysis, all the cheese samples were cut into sectors, the rind was removed and all the samples were frozen

under liquid nitrogen, ground in a domestic blender and stored at −20 °C in screw-cap glass vials prior to analysis.

2.3. Dynamic headspace

Ten grams of finely ground cheese were placed in a 200 ml Erlenmeyer flask at a temperature of 40 °C and submitted to the dynamic headspace extraction for 30 min using purified nitrogen (60 ml min^{-1}). The extracted volatiles were concentrated on a Tenax TA® trap (Chrompack, Middelburg, The Netherlands) filled with 90 mg, 20–35 mesh, of the adsorbent material. The adsorbent trap was then back-flushed with the purified gas for 5 min to remove trapped moisture. Volatiles were automatically thermally desorbed and transferred to the GC column by using a TCT thermal desorption cold trap (TD800, Fisons Instruments, Milan, Italy). Desorption was performed at 280 °C for 10 min under a helium flow (10 ml min^{-1}): the volatile compounds were cryofocussed in a glass-lined tube at −120 °C with liquid nitrogen and then injected into the GC capillary column by heating the cold trap to 240 °C.

Three independent DHS extractions were performed for each sample.

In order to verify possible environmental contamination, blank analyses were carried out using an empty 200 ml Erlenmeyer flask, following the same procedure as for the sample.

To assess the presence of carry-over effects, the adsorbent trap was also desorbed before and after each entire sampling procedure.

2.4. GC–MS analysis

A TRACE GC 2000 gas chromatograph (Thermo Electron Corporation, Walthan, MA, USA) equipped with a Finningan TRACE MS mass spectrometer (Thermo Electron Corporation) was used for GC–MS analysis. Helium was used as the carrier gas at a flow rate of 1 ml min^{-1}. Chromatographic separation was performed on a 30 m × 0.25 mm, d_f 0.25 μm Supelcowax-10™ capillary column (Supelco, Palo Alto, CA, USA). The following GC oven temperature programme was applied: 35 °C for 8 min, 6 °C min^{-1} to 60 °C, 4 °C min^{-1} to 160 °C, 20 °C min^{-1} to 200 °C, 200 °C held for 1 min. Transfer line and source were maintained at 250 °C and 230 °C, respectively.

Electron impact (EI) mass spectra were recorded at 70 eV ionization energy, scanning the mass spectrometer from 35 to 350 amu (scan time, 0.5 s).

Signal acquisition and data processing were performed using the Excalibur V 1.2 (Thermo Electron Corporation). The identification of the volatile compounds was performed by comparing the obtained mass spectra with those stored in the National Institute of Standards and Technology (NIST) US Government library. Finally, Kovàts retention indices (KI) were calculated for the GC peaks by interpolation of the retention times of the volatile compounds with those of normal alkanes (C8–C17) analyzed under the same chromatographic conditions. Calculated KI were compared with those stored in a home-made database (Bianchi, manuscript in preparation) obtained for the same kind of stationary phase (polyethylenglycole) by injection of pure standards.

To evaluate semiquantitative differences in the aromatic profile of different samples, GC peak areas were calculated as total ion current for the identified compounds.

2.5. Fat, N-Kjeldahl, dry matter

Determinations of fat content, dry matter and nitrogen fraction were carried out, following the procedure reported in the Gazzetta Ufficiale della Repubblica Italiana (Gazzetta Ufficiale della Repubblica Italiana n.229 del 2 ottobre, 1986). The fat content was determined following the Schmidt–Bondyzynsky–Ratzlaff procedure, except for the hydrolysis step, as described in Section 3.

2.6. Free-fatty acids

Free-fatty acids were determined following a procedure reported elsewhere (de Jong & Badings, 1990).The extraction procedure was replicated 3 times.

For each acid, a suitable regression curve was calculated in the following ranges:

C2; 200–400 mg/kg.
C3, C4, C14, C18; 50–150 mg/kg.
C6, C8, C10, C12; 25–75 mg/kg.
C16, C18:1; 100–200 mg/kg.

C7 was used as internal standard at the concentration of 100 mg/kg, whereas C15 was used as standard of extraction.

Three concentration levels were analyzed, performing three measurements at each level. Statistical analyses (Bartlett, lack-of-fit and Mandel test) were performed to check the goodness of fit and linearity (Draper & Smith, 1981).

The significance of the intercept (significance level 5%) was established by running a *t*-test. Intra-day repeatability and between-day precision, estimated over five days (Box, Hunter, & Hunter, 1978) were calculated in terms of relative standard deviations (RSD) on two concentration levels, performing three replicates at each level.

Trueness was evaluated in terms of the presence of matrix effect and of recovery rate (RR%) (EURACHEM Guide, 1998) at two concentration levels (near the lowest and highest levels of the regression curves calculated for each analyte) by using the following expression:

$$RR\% = \frac{\overline{c_{obs}}}{c_{spike}} \times 100$$

where $\overline{c_{obs}}$ is the mean concentration of the fortified sample, and c_{spike} is the spiked concentration. All the measurements were triplicated.

The presence of matrix effect was evaluated by comparing the slopes of the regression models obtained by using the external standard and the standard addition method, respectively.

3. Results and discussion

3.1. DHS-GC–MS analysis

The volatile fraction of "Fontina Valle d'Aosta PDO" cheese was characterized by considering a representative number of the Fontina cheese samples.

The DHS-GC–MS analysis allowed us to identify 74 volatile compounds (Table 1). Among them, a total of 14 alcohols, 11 esters, 10 ketones, 9 aldehydes, 7 terpenes, 6 aromatic hydrocarbons, 4 hydrocarbons, 4 sulfur compounds, 3 organic acids, 3 furans and 3 halogen compounds were found.

The good repeatability of the utilised procedure was proved by a coefficient of variation of the method lower than 10% ($n = 3$). As a consequence, the observed variability of data on the 24 analyzed samples, as reported in Table 1, allowed characterization of the "Fontina Valle d'Aosta PDO" cheese summer production.

The observed variability is in accordance with other studies on cheese characterization (Fernandez-Garcia, Carbonell & Nunez, 2002; Mallia et al., 2005).

The headspace fraction of "Fontina Valle d'Aosta PDO" cheese contained both linear and branched chain aldehydes. Among them, hexanal and 3-methylbutanal were the most abundant compounds. Aliphatic aldehydes are products of autoxidation of unsaturated fatty acids: autoxidation proceeds via hydroperoxides which undergo further degradation to hydrocarbons, alcohols and carbonyl compounds (Barbieri et al., 1994; de Man, 1990). Aldehydes may also derive from amino acids during ripening (Strecker degradation): at the beginning of curing (pH 5.4 ± 0.2), amino acids are decarboxylated to amines and then oxidation reactions take place at higher pH (pH 5.9 ± 0.1) (Belitz & Grosch, 1987). Branched chain aldehydes are normally found in cheese and in some varieties they are considered to be key flavour compounds (Bosset & Gauch, 1993).

The major ketone was 2-butanone, but considerable levels were also found of 2-pentanone, 2-heptanone, acetone and the diketones 2,3-butandione (diacetyl) and 2,3-pentandione. Noteworthy is the absence of 3-hydroxy-2-butanone (acetoin). 2-Pentanone and 2-heptanone have been already identified as the prevailing ketones in the volatile fraction of Parmigiano (Barbieri et al., 1994), whereas acetoin has been identified at higher concentrations in pasteurised milk cheeses than in raw milk cheeses (Fernandez-Garcia, Carbonell & Nunez, 2002; Fernandez-Garcia, Serrano, & Nunez, 2002). Methyl ketones are considered to derive from free fatty acids enzymatically oxidised to β-ketoacids. Consequently, these products are decarboxylated to alkan-2-ones with the loss of one carbon atom (McSweeney & Sousa, 2000). Diacetyl, is metabolized by adventitious bacterial activity to 2-butanone and 2-butanol, which were found in the "Fontina Valle d'Aosta PDO" aromatic fraction, thus confirming the prevalence of the non-starter bacterial activity in these products (Mallia et al., 2005).

Regarding alcohols, the evaluation of the "Fontina Valle d'Aosta PDO" GC profile indicated that 2-butanol was the most abundant compound, followed by 3-methyl-1-butanol, 1-propanol and ethanol. Similar results have been observed also in the case of the aromatic fraction of other raw milk cheeses, in which all these components were found in high amounts (Carbonell et al., 2002; Fernandez-Garcia, Carbonell & Nunez, 2002; Fernandez-Garcia, Serrano & Nunez, 2002). The large amount of 2-butanol could be formed by reduction of 2,3-butandione to 2,3-butandiol by the starter bacteria and subsequent reduction to 2-butanol due to the high activity of non-starter lactic acid bacteria during ripening (Urbach, 1993). Generally, primary alcohols originate from the corresponding aldehydes produced from fatty acids and from amino acid metabolism (Barbieri et al., 1994). Among these, ethanol may be formed by lactose metabolism or by reduction of acetaldehyde. Secondary alcohols, are obtained by the enzymatic reduction of methyl ketones (Molimard & Spinnler, 1996).

The most abundant ester was ethyl acetate, followed by ethyl butanoate, ethyl propanoate and propyl acetate. It is notable that, also in Manchego cheese, ethyl esters have been reported at higher levels in raw milk cheese than in pasteurised milk cheeses (Fernandez-Garcia, Carbonell & Nunez, 2002). These compounds are produced by enzymatic or chemical reactions of fatty acids with primary alcohols, so alcohol concentration is a limiting factor in ester production.

Among aromatic hydrocarbons, toluene was the most abundant compound, in accordance with results for volatiles of other cheese products (Carbonell et al., 2002; Fernandez-Garcia, Carbonell & Nunez, 2002; Fernandez-Garcia, Serrano & Nunez, 2002).

Among terpenes, α-pinene, β-pinene and camphene were the most abundant compounds. As already reported in other studies, their presence can be related to the fodder given to the cows (Carbonell et al., 2002; Fernandez-Garcia, Serrano & Nunez, 2002).

Among hydrocarbons, 4 compounds were identified, octane and 2-octene being characterized by the highest responses. High levels of octane have previously been found in other raw milk cheeses, e.g. Spanish Manchego cheese (Fernandez-Garcia, Carbonell & Nunez, 2002). Hydrocarbons can have a feed origin (Carbonell et al., 2002; Ziino, Condurso, Romeo, Giuffrida, & Verzera, 2005), but they can also be produced during ripening as a result of lipid autoxidation (Barbieri et al., 1994). Halogen compounds, have been already reported in other cheeses and ascribed to probable external contamination (Fleming-Jones & Smith, 2003). Finally, dimethyldisulfide was the most abundant sulfur compound found in the "Fontina Valle d'Aosta PDO" volatile fraction. The origin of many

Table 1
Volatile compounds identified in the Fontina PDO samples ($n = 24$)

	ID[a]	KI_{calc}	KI_{tab}[b]	Occurrence[c]	Chromatographic response (peak area) mean ± Std. Dev[d] × 10^3
Linear aldehydes					
Butanal	MS; KI	880	878	16	4300 ± 4600
Pentanal	MS; KI	985	977	16	3600 ± 3800
Hexanal	MS; KI	1082	1080	24	34,000 ± 54,000
Heptanal	MS; KI	1191	1186	14	1300 ± 1500
Octanal	MS; KI	1290	1286	10	1600 ± 1800
Nonanal	MS; KI	1398	1396	9	1100 ± 1200
Branched chain aldehydes					
2-Methylpropanal	MS; KI	812	814	23	690 ± 540
2-Methylbutanal	MS; KI	916	914	23	4200 ± 3200
3-Methylbutanal	MS; KI	920	917	24	18,000 ± 16,000
Ketones					
Acetone	MS; KI	819	814	24	7300 ± 6600
2-Butanone	MS; KI	907	901	24	330,000 ± 280,000
3-Buten-2-one	MS; KI	946	953	14	1500 ± 1700
2-Pentanone	MS; KI	979	980	10	20,000 ± 23,000
4-Methyl-2-pentanone	MS; KI	999	1008	12	1900 ± 1400
(?)-Penten-(?)-one	MS	1035		4	2300 ± 1100
2-Heptanone	MS; KI	1188	1185	12	7300 ± 1000
2-Nonanone	MS; KI	1392	1394	10	800 ± 1000
Diketones					
2,3-Butandione	MS; KI	988	986	23	7600 ± 7500
2,3-Pentandione	MS; KI	1069	1071	14	8000 ± 10,000
Primary alcohols					
Ethanol	MS; KI	937	932	24	25,000 ± 28,000
1-Propanol	MS; KI	1051	1052	24	30,000 ± 31,000
1-Butanol	MS; KI	1153	1152	19	14,000 ± 19,000
1-Pentanol	MS; KI	1261	1256	21	2000 ± 3000
1-Hexanol	MS; KI	1359	1354	13	1100 ± 1200
Secondary and tertiary alcohols					
2-Propanol	MS; KI	970	975	10	5700 ± 5600
2-Butanol	MS; KI	1039	1035	24	600,000 ± 350,000
3-Pentanol	MS; KI	1113	1112	5	1100 ± 1000
1-Penten-3-ol	MS; KI	1177	1176	10	1500 ± 1400
3-Hexanol	MS; KI	1194	1207	5	1800 ± 1800
2-Heptanol	MS; KI	1326	1334	17	900 ± 1200
Branched chain alcohols					
2-Methyl-1-propanol	MS; KI	1107	1097	23	7000 ± 8000
3-Methyl-1-butanol	MS; KI	1222	1215	24	50,000 ± 80,000
2-Ethyl hexanol	MS; KI	1492	1492	4	540 ± 530
Ethyl esters					
Ethyl acetate	MS; KI	891	893	24	61,000 ± 51,000
Ethyl propanoate	MS; KI	959	957	24	14,000 ± 17,000
Ethyl isobutanoate	MS; KI	969	960	21	1700 ± 1700
Ethyl butanoate	MS; KI	1046	1040	22	17,000 ± 20,000
Ethyl isocaproate	MS	1197		6	3000 ± 3800
Ethyl hexanoate	MS; KI	1242	1238	23	2400 ± 3200
Ethyl heptanoate	MS; KI	1327	1331	5	1300 ± 1300
Ethyl octanoate	MS; KI	1439	1438	10	550 ± 470
Other esters					
Propyl acetate	MS; KI	977	976	11	11,000 ± 14,000
Butyl acetate	MS; KI	1085	1077	10	2200 ± 1500
propyl butanoate	MS; KI	1133	1123	11	3200 ± 1900
Aromatic hydrocarbons					
Benzene	MS; KI	938	936	24	3100 ± 1500
Toluene	MS; KI	1043	1040	24	76,000 ± 59,000
Ethylbenzene	MS; KI	1121	1125	11	3100 ± 3700
p-Xylene	MS; KI	1129	1127	10	310 ± 250

(*continued on next page*)

Table 1 (*continued*)

	ID[a]	KI_{calc}	KI_{tab}[b]	Occurrence[c]	Chromatographic response (peak area) mean ± Std. Dev[d] × 10^3
m-Xylene	MS; KI	1136	1132	10	270 ± 260
o-Xylene	MS; KI	1175	1182	9	670 ± 720
Terpenes					
α-Pinene	MS; KI	1017	1010	24	6400 ± 6700
Terpene (not identfied)	MS	1027		10	1000 ± 570
Camphene	MS; KI	1060	1053	14	3500 ± 4700
β-Pinene	MS; KI	1102	1095	20	4500 ± 3500
Limonene	MS; KI	1193	1194	10	1000 ± 700
p-Cymene	MS; KI	1270	1266	4	1300 ± 890
α-Terpinolene	MS; KI	1280	1276	10	1100 ± 1000
Aliphatic hydrocarbons					
Hydrocarbon (not identfied)	MS	<800	<800	19	1900 ± 1600
Hydrocarbon (not identfied)	MS	<800	<800	10	2000 ± 2200
Octane	MS; KI	800	800	22	5500 ± 5500
2-Octene	MS; KI	843	846	23	5500 ± 4800
Sulfur compounds					
S-methyl-thioacetate	MS	1056		20	3900 ± 4700
Dimethyl disulfide	MS; KI	1080	1075	24	130,000 ± 140,000
S-methyl thiopropionate	MS	1131		8	3600 ± 4300
Dimethyl trisulfide	MS; KI	1381	1383	9	520 ± 670
Organic acids					
Acetic acid	MS	1480		5	240 ± 130
Propanoic acid	MS	1554		4	190 ± 150
Butanoic acid	MS	1630		4	100 ± 80
Furans					
2-Methyl furan	MS; KI	872	876	16	1900 ± 1700
2-Ethyl furan	MS; KI	950	945	21	700 ± 570
2-Pentyl furan	MS; KI	1236	1240	18	500 ± 290
Halogen compounds					
Halogen compound (not identfied)	MS	<800	<800	6	500 ± 100
Dichloromethane	MS; KI	933	927	14	600 ± 500
Chloroform	MS; KI	1020	1018	16	2400 ± 2000

[a] ID: MS = identification by comparison with NIST mass spectrum, KI = identification by comparison with Kovats indices.
[b] KI: identification by comparison with KI home-made database.
[c] Number of samples (out of 24) in which the component was detected.
[d] Referred to all the measurements performed on the 24 samples.

sulfur compounds in cheese is usually associated with the production of methanethiol by bacterial cultures used in the cheese-making process. It is known that the bacterial metabolism of this compound leads to the generation of a range of compounds, e.g. dimethyldisulfide, dimethyltrisulfide and thioesters, such as *S*-methylthioacetate (Cuer, Dauphin, Kergomard, Dumont, & Adda, 1979; Molimard & Spinnler, 1996). In particular, the high amount of dimethyldisulfide found in the Fontina cheese samples could be explained by taking into account that micro-organisms such as *B. linens* were found in the analyzed product. Since these micro-organisms have already been identified as key agents of sulfur compound production in cheese (Karahadian, Josephon, & Lindsay, 1985; Molimard & Spinnler, 1996), the presence of dimethyldisulfide could be related to the presence of *B. linens*. Further studies will be carried out in order to evaluate the production of this sulfur compound during ripening.

As a general comment, the obtained results were in agreement with literature data. In fact, high concentrations of compounds such as diacetyl, ethyl acetate and 2-butanol, as well as the absence of volatiles such as acetoin, have been already reported in previous studies on the characterization of different raw-milk cheeses.

These results demonstrate that characterization of the aromatic profile of "Fontina Valle d'Aosta PDO" cheese could represent a powerful tool to protect this typical dairy product.

3.2. Fat, N-Kjeldahl, dry matter

For fat determination, the official method of analysis (Gazzetta Ufficiale della Repubblica Italiana n.229 del 2 ottobre, 1986) was simplified by omitting the hydrolysis step. The analysis of the same sample ($n = 3$) performed, with and without the hydrolysis step, did not produce significantly different results (25.6 ± 1.8% vs 25.63 ± 0.09% obtained with the modified method). Instead, the procedure proved to be more simple and precise, thus resulting in a method improvement.

As requested by the rules of production (Fontina Valle d'Aosta PDO Production rules, 1955), all "Fontina Valle

d'Aosta PDO" samples submitted to analysis had a fat content (corrected for the dry matter) above 45% with a mean value of 48 ± 3%.

Finally, a mean content of 24.9 ± 0.8% was found for the nitrogen fraction by using the Kjeldhal technique, whereas a mean value of 61 ± 2% was obtained for the dry matter determination.

3.3. Free-fatty acids

"Fontina Valle d'Aosta PDO" cheese samples were subsequently also characterized in terms of free fatty acids, since it is well known that these compounds directly contribute to the cheese aroma, as also do some volatile components derived from their catabolism during proteolysis.

Since the dynamic headspace method used for volatile compound sampling proved to be inadequate for the extraction of free fatty acids, these compounds were determined by using the procedure previously reported by de Jong and Badings (1990). In a preliminary study, we tested method reliability by assessing trueness (matrix effect and recovery) and precision since, in that procedure, some validation parameters were missing.

Matrix effect was evaluated by applying the standard addition method. The absence of proportional systematic errors was verified, since no significant difference was observed between the slopes of the regression models calculated using the external standard and the standard addition method ($t_{calc} < t_{tab(\alpha=0.05,14)}$), so it can be concluded that the free fatty acids can be determined using the external standard approach. Good results were obtained, both in terms of intra-day repeatability and between-day precision at the two concentrations tested. In the case of intra-day repeatability, RSD percentages below 3% at the lowest concentration and below 2% at the highest one were calculated. Between-day precision was evaluated, verifying homoscedasticity and performing ANOVA on data acquired over five days for each concentration. At 95% confidence level, ANOVA showed that mean values were not significantly different ($p > 0.05$), attesting to a good between-day precision of the method. RSD percentages below 5% at both concentrations were calculated.

Table 2
Free fatty acid contents

Free fatty acids	Fontina PDO (mg/kg)[a]
C2	59 ± 19
C3	70 ± 67
C4	46 ± 37
C6	19 ± 16
C8	17 ± 12
C10	24 ± 14
C12	36 ± 17
C14	92 ± 44
C16	220 ± 90
C18	100 ± 31
C18:1	270 ± 130

[a] Mean value calculated on 24 samples (3 replicated measurements for each sample).

Extraction recoveries in the 81.5 ± 0.5% ($n = 3$) and 103.5 ± 0.8% ($n = 3$) range for both the levels were calculated. These findings showed the good efficiency of the extraction technique in terms of extraction recovery, as well as repeatability.

Finally, as shown in Table 2, free-fatty acids were determined in the 24 "Fontina Valle d'Aosta PDO" samples. As already observed in other studies (Barbieri et al., 1994), the highest concentrations were found for longer chain C_{14}–$C_{18:1}$ FFAs. In the biogenesis of these compounds, it is known that linear FFAs containing four or more carbon atoms are generally produced by lipolysis of milk fat, whereas acetic acid derives from different processes, including the oxidation of lactose by lactic acid bacteria and the catabolism of alanine and serine by lactic acid bacteria (Ur-Rehman et al., 2000).

4. Conclusions

This work provides the first characterization of the volatile composition of "Fontina Valle d'Aosta PDO". The DHS-GC–MS technique used in this study proved to be useful for the characterization of the aromatic profile of this cheese, showing the important role that volatiles play in assessing and preserving the quality of typical products. 2-Butanol, dimethyldisulfide and 2-butanone were the most abundant compounds found in the volatile fraction of "Fontina Valle d'Aosta PDO". Sensory studies, as well as the evaluation of the effects of ripening conditions and microbiological activity on the cheese-making process are in progress to determine the role that each volatile compound plays in the flavour of "Fontina Valle d'Aosta PDO" cheese. In addition, determination of nitrogen fraction, fat, dry matter and of free fatty acids allows us to obtain a more complete characterization of this typical cheese.

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