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A STUDY ON ORGANIC ACID, SUGAR AND KETONE CONTENTS IN TYPICAL PIEDMONT CHEESES

INDAGINE SUL CONTENUTO IN ACIDI ORGANICI, ZUCCHERI E CHETONI
DI FORMAGGI TIPICI PIEMONTESEI

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ABSTRACT

The dairy industry is a very important sector for the Piedmont (Italy) economy with ten Protected Denomination of Origin (PDO) cheeses and fifty-five classified as "Traditional". Among these "Traditional" cheeses, some are very interesting because of the quantity produced, cheese-making procedures used and sensory characteristics. Therefore, these cheeses might qualify for the PDO designation, but chemical information is lacking and only their gross composition is usually known.

RIASSUNTO

Il settore lattiero caseario costituisce per il Piemonte uno dei principali comparti produttivi e dei 33 formaggi DOP italiani ben dieci interessano il Piemonte e 55 sono quelli tradizionali. Fra questi ultimi alcuni per quantità prodotte o per caratteristiche tecnologiche, compositive e sensoriali risultano di estremo interesse e quindi potenzialmente soggetti alla attribuzione di una futura DOP, ma le conoscenze sulla loro caratteristiche compositive si limitano in genere esclusivamente ai macrocom-

- Key words: acetoin, cheese, diacetyl, organic acid, Piedmont cheeses, sugar -

The aim of this study was to characterise some Piedmont cheeses by studying important compounds responsible for their flavour. Sugars (lactose, glucose and galactose), organic acids (citric, orotic, pyruvic, lactic, oxalic, hippuric, formic, acetic, propionic, butyric, isobutyric, valeric and isovaleric) and ketones (diacetyl and acetoin) were quantitatively determined in 460 samples from 17 cheese varieties using a simple isocratic HPLC method. Lactose concentrations varied from 0 to 73.9 mg/g of cheese and were inversely correlated to ripening time. Glucose concentrations ranged from 0 to 4.3 mg/g of cheese while galactose showed a maximum value of 9.4 mg/g of cheese. The range of diacetyl and acetoin were from 0 to 4.3 mg/g and from 0 to 0.2 mg/g of cheese, respectively. Among the organic acids, lactic acid was the most abundant (0.3 to 39 mg/g). Citric acid was also very important with a maximum value of 4 mg/g. High variability characterised the carboxylic acids due to differences in raw material, cheese-making technique and use of a starter. For all compounds high variability was observed due to home-made production, but nevertheless it was possible to obtain a good characterisation of all these cheeses.

In questo lavoro sono riportati i risultati di uno studio di caratterizzazione compositiva volto a valutare in 460 campioni di 17 formaggi piemontesi il contenuto di alcuni componenti minori quali zuccheri (lattosio, glucosio, galattosio), acidi organici (citrico, orotico, piruvico, lattico, ossalico, ippurico, formico, acetico, propionico, butirrico, isobutirrico, valeric ed isovaleric) e chetoni (diacetile ed acetoino) separati mediante HPLC. Il contenuto in lattosio è risultato compreso fra 0 e 73,9 mg/g di formaggio ed inversamente correlato al tempo di stagionatura. La concentrazione di glucosio è risultata compresa fra 0 e 4,3 mg/g per il galattosio mentre il valore massimo è risultato di 9,4 mg/g. La concentrazione di diacetile e di acetoino sono risultate comprese fra 0 e 4,3 mg/g e fra 0 e 0,2 mg/g rispettivamente. Fra gli acidi l'acido lattico risulta il più abbondante (0,3-39 mg/g). Molto importante altresì l'acido citrico con un massimo di 4 mg/g. Molto elevata per tutti i prodotti la variabilità in relazione alla artigianalità delle produzioni benché una loro discriminazione sia risultata comunque possibile.

INTRODUCTION

In the Piedmont region (northwestern Italy) with its vast area of land available for pasture, dairy farming and cheese-making have become highly developed and are of great economic and cultural importance.

Of the thirty-three Italian cheeses with the Protected Denomination of Origin (PDO) according to European Regulation 510/06 (European Union, 2006), ten are produced in the Piedmont region

and elsewhere, while seven (Bra duro, Bra tenero, Castelmagno, Robiola di Roccaverano, Murazzano, Toma Piemontese and Raschera) are produced exclusively in the Piedmont.

In addition to these PDO cheeses, about 55 cheeses, defined as "Typical" according to Italian law 350/99 (Decreto Presidente Repubblica, 1999), are also produced in Piedmont. While they are usually only available at local markets, there might be a way for farm cheesemakers to make a higher profit if they

are well-defined and their characteristics are better known. Unfortunately, information about the chemical characteristics of these cheeses is very scarce and only pertains to their gross composition (COISSON *et al.*, 2000; PATTONO *et al.*, 2001; ZEPPA *et al.*, 2003a, b; 2005).

Among the chemical, sensory and textural parameters, flavour profile is very important for cheese characterisation because it is strongly variety- or type-specific. This profile is influenced by many compounds, including organic acids, sulphur compounds, lactones or ketones, formed as a result of hydrolysis of milk fat during lipolysis, bacterial growth, normal ruminant metabolic processes and the addition of acidulants during cheesemaking (ADDA *et al.*, 1982; Fox *et al.*, 2004). Hence, quantitative determination of these compounds is especially important when studying flavour and nutritional qualities; they serve as indicators of microbial metabolism and as a classification index for cheese varieties (ADDA *et al.*, 1982; URBACH, 1993; FOX *et al.*, 2004).

The study focused on the following cheeses: Bra duro PDO, Bra tenero PDO, Castelmagno PDO, Cevrin di Coazze, Maccagno, Murazzano PDO, Murianengo, Plaisentif, Raschera PDO, Robiola d'Alba, Robiola di Roccaverano PDO, Saras del Fen, Toma del lait brusc, Tomino di Melle, Toma Piemontese PDO, Tomino di Talucco and Tuma 'd Trausela.

The Bra duro PDO is an uncooked, hard-cheese made with raw or pasteurized cow milk and ripened for almost 6 months. Starters are only used in the cheese made with pasteurized milk. The cheese is brine-salted; its cylindrical shape has a diameter of 30-40 cm and a height of 7-9 cm.

The Bra tenero PDO, Raschera PDO and Toma Piemontese PDO are uncooked semi-hard cheeses, made from raw or pasteurized cow milk and ripened for at least 2 months. Also for these cheeses, starters are only used in the cheeses

made with pasteurized milk. Cheeses are brine- or dry salted. Toma Piemontese PDO and Bra tenero PDO are cylindrical (diameter of 30-40 cm and height of 7-15 cm) while Raschera PDO is square (side of 40 cm and height of 12-15 cm).

The Castelmagno PDO is a semi-fat, pressed, hard-paste, mould-inoculated cheese made from raw cow milk and a small quantity of ewe and/or goat milk and is ripened for 2-5 months. The production regulations prohibit the use of starters. The uniqueness of this cheese is that the curd is immersed in the whey for at least 48 hours. Its subsequent mincing in a special grinder and moulding imparts the characteristic very hard, granular and "chalky" consistency of this cheese. The cylindrical shape has a diameter of 15-25 cm and a height of 20-25 cm.

The Cevrin di Coazze cheese is a typical cheese that is produced by only five farmers. A mix of raw goat and cow milks is coagulated with liquid calf rennet at 40°-45°C. Starters are not used and the cheese is dry-salted. The cylindrical shape has a diameter of 15-18 cm and an height of 4-5 cm.

The Maccagno cheese is produced with raw cow milk coagulated with calf rennet at 35°-37°C. Starters are not used and the cheese is dry-salted. The cylindrical shape has a diameter of 18-20 cm and a height of 6-8 cm.

Murazzano PDO is a soft-cheese made from raw sheep milk or a mix of raw cow and sheep milk coagulated with liquid rennet and ripened for up to 10 days. The cylindrical shape has a diameter of 10-15 cm and a height of 3-4 cm.

Murianengo is a blue cheese produced in a small alpine area of Piedmont, near France, with raw cow milk coagulated with liquid calf rennet and ripened for almost 6 months. The milk is left to sour at room temperature for 12 hours then skimmed and added to the same quantity of fresh raw milk. Starters are not used and the cheese is dry-salted.

The cylindrical shape has a diameter of 20-25 cm, a height of 8-20 cm and weighs 8-10 kg.

Plaisentif is an ancient cheese produced with partially skimmed raw cow milk in a small mountain area in the Turin Province. The cylindrical shape has a diameter of 15-18 cm and a height of 4-5 cm. According to the regulations, it is only produced in June when the *Viola calcarata* L. flowers are present in the pastures. The milk is coagulated with liquid calf rennet at 30°-32°C. Starters are not used. The cheese is dry-salted.

The Robiola d'Alba and Robiola di Roccaverano PDO cheeses are similar to the Murazzano PDO cheese but are produced with pasteurized cow or sheep milk inoculated with starters and coagulated without rennet. All these cheeses are ripened for up to 3 days.

The Saras del Fen is a ripened ricotta cheese produced with a whey mix obtained from goat, sheep and cow milks. The whey is coagulated with citric acid and the product is ripened for at least 20 days. The cheese is spherical with a diameter of 12-15 cm and weight 500-800 g.

The Toma del lait brusc cheese is produced with naturally acidified cow milk. It is similar to Castelmagno PDO cheese but is only produced in some alpine areas. The cheese is very hard, granular and "chalky". The cylindrical shape has a diameter of 15-25 cm and a height of 20-25 cm.

Tomino di Melle is a fresh cheese (about 5 days of ripening) produced with fresh raw cow milk coagulated with liquid calf rennet at 30°-32°C. Starters are not used and the cheese is dry-salted. The cylindrical shape has a diameter of 10-15 cm and a height of 2-2.5 cm.

Tomino di Talucco is a fresh cheese produced with a mix of raw goat and cow milks. The cylindrical shape has a diameter of 10-12 cm and a height of 2-2.5 cm.

Tuma 'd Trausela is produced by only

four farmers. It is a very fresh cheese without a crust. The milk is coagulated with calf rennet; the curd, without cutting, is drained for 8-10 hours before being sold.

The purpose of this study was to quantify the following flavour compounds: sugars, ketones and organic acids in these seventeen important Piedmont cheeses.

MATERIALS AND METHODS

Samples

Four-hundred and sixty samples from seventeen cheese varieties were collected and analyzed (Table 1). Samples were collected in 2005 from the cheese-makers of practically all of the cheese varieties. Due to the many cheese-makers who produce the Toma Piemontese PDO cheese only about 60% of the producers participated.

One or two cheeses from different batches were furnished directly by the producers. All samples were collected from a whole cheese ripened according to the specific cheese-making regulations (Table 1) and stored at -18°C after arrival in the laboratory.

Chemical analysis

Dry matter, fat and protein content were determined according to the Italian Official Methods for cheese analysis (D.M., 1986).

Organic acids (citric, orotic, pyruvic, lactic, oxalic, hippuric, formic, acetic, propionic, butyric, isobutyric, valeric and isovaleric), sugars (lactose, glucose and galactose), diacetyl and acetoin were determined by high performance liquid chromatography according to ZEPPA *et al.* (2001). Five grams of cheese were added to 25 mL of 0.013 N H₂SO₄ (mobile phase) and extracted for 10 min with a Stomacher blender (PBI,

Milano, Italy). The extract was subsequently centrifuged for 5 min (2,500×g) and the supernatant was filtered through a 0.20 µm disposable syringe membrane filter (Sartorius AG, Göttingen, Germany). The HPLC system (Thermo Electron Corporation, Waltham, MA, USA) was equipped with an isocratic pump (P1000), a multiple autosampler (AS3000) fitted with a 20 µL loop, a UV detector (UV100) set at 210 and 290 nm and a refractive index detector (RI-150). The detectors were connected in series. Data were collected on an EZChrom ver. 6.6 system (Thermo Electron Corporation, Waltham, MA, USA). The analyses were performed isocratically at 0.8 mL/min and 65°C with a 300x7.8 mm i.d. cation exchange column Aminex HPX-87H (Bio-Rad Laboratories, Hercules, CA, USA) equipped with a Cation H⁺ Microguard cartridge (Bio-Rad Laboratories, Hercules, CA, USA). Mobile phase was 0.013 N H₂SO₄ prepared by diluting reagent grade sulfuric acid with distilled water, filtered through a 0.45 µm membrane filter (Sartorius, AG, Göttingen, Germany) and degassed under vacuum. Each sample was analysed three

times. Analytical grade reagents were used as standards (Sigma-Aldrich Corporation, Milano, Italy).

Statistical analysis

Conventional statistical methods were used to evaluate the chemical data. Analysis of variance and Duncan's multiple mean comparison test were applied using STATISTICA for Windows Release 7.1 (StatSoft Inc., Tulsa, OK, USA) to determine statistical differences between the cheese varieties.

RESULTS AND DISCUSSION

The gross composition of the 17 different varieties of cheeses is reported in Table 2. The concentration of organic acids, sugars and ketones is shown in Table 3. A great variability was observed within each cheese which was a consequence of differences in milk composition, starter cultures used, adventitious non-starter bacteria in milk, cheese-making and ripening conditions.

In spite of this variability the analysis

Table 1 - Identification code, milk generally used for cheese-making and ripening time for the cheeses.

Cheese variety	Number of samples	Identification code	Milk used	Ripening time (days)
Bra duro PDO	14	BD	Cow	90
Bra tenero PDO	12	BT	Cow	60
Castelmagno PDO	20	CA	Cow	60
Cevrin di Coazze	10	CE	Goat/Cow	60
Maccagno	16	MA	Cow	60
Murazzano PDO	18	MU	Sheep/Cow	10
Murianengo	12	MUR	Cow	90
Plaisentif	16	PL	Cow	90
Raschera PDO	19	RA	Cow	60
Robiola d'Alba	18	RdA	Cow	10
Robiola di Roccaverano PDO	44	RR	Sheep	10
Saras del Fen	24	SA	Goat/Sheep/Cow	20
Toma del lait brusc	23	TLB	Cow	90
Toma Piemontese PDO	174	TP	Cow	60
Tomino di Melle	12	TMELL	Cow	5
Tomino di Talucco	18	TAL	Goat/Cow	10
Tuma 'd Trausela	10	TRA	Cow	1

of variance showed significant differences among the tested cheese varieties for organic acids, sugars and ketones (Table 4). No significant differences among products were observed for orotic acid.

The mean values determined for these compounds are similar to those reported for similar products such as Pickled White cheese (AKALIN *et al.*, 2002), Greek cheeses (PAPADAKIS and POLYCHRONIA-DOU, 2005), Cheddar (KEEN and WALKER, 1974; LUES *et al.*, 1998), Canestrato Pugliese (FACCIA *et al.*, 2004), different goat cheeses (PARK and DRAKE, 2005; PARK and LEE, 2006), Feta (MANOLAKI *et al.*, 2006) and ricotta cheese (KINIK and GÜRISOY, 2004).

Lactose concentrations varied from 0 to 73.9 mg/g of cheese (Table 3) but lactose was absent in 207 samples. Only 61 of the analyzed cheeses had lactose concentrations that were higher than 1%. Lactose is metabolised by lactic acid bacteria (LAB) during cheese-making and ripening at a rate that is largely determined by temperature and salt-in-moisture levels of curd (FOX *et*

al., 2004). Lactose was usually detected only in fresh unsalted cheeses such as Toma 'd Trausela, Tomino di Melle, Robiola d'Alba, Robiola di Roccaverano PDO, Tomino del Talucco and Saras del Fen and the quantity was higher in cheeses with shorter ripening times. Lactose was generally absent in all the other samples with longer ripening times. The concentrations of glucose and galactose, which are the two hydrolysis products of lactose, were also low. Glucose was absent in 309 samples since it is rapidly metabolized by LAB (FOX *et al.*, 2004) and the concentrations in twelve samples were about 0.1 mg/g of cheese. The maximum glucose concentration was about 4 mg/g in Saras del Fen cheese. The galactose concentration varied widely among samples with a maximum value of about 9.4 mg/g in Tomino del Talucco cheese. Some LAB can use galactose, but also the adventitious non-starter lactic acid bacteria have the potential to use this substrate. In fact a close relationship between the increase in this flora and

Table 2 - Basic composition (mean \pm standard deviation) of the cheeses.

	Dry matter (%)	Protein (% dm)	Fat (% dm)	Ash (% dm)
Bra duro PDO	63.1 \pm 7.5	55.3 \pm 6.9	37.4 \pm 6.6	4.1 \pm 2.8
Bra tenero PDO	56.2 \pm 6.9	49.8 \pm 8.1	44.5 \pm 7.2	4.1 \pm 2.8
Castelmagno PDO	65.4 \pm 7.2	41.2 \pm 6.9	48.5 \pm 5.7	5.1 \pm 1.4
Cevrin di Coazze	48.4 \pm 4.6	48.7 \pm 8.8	31.4 \pm 7.2	7.6 \pm 1.7
Maccagno	53.1 \pm 4.6	43.5 \pm 3.1	46.5 \pm 5.7	4.1 \pm 2.1
Murazzano PDO	50.2 \pm 14.1	38.7 \pm 5.7	56.2 \pm 8.5	5.1 \pm 1.4
Murianengo	56.1 \pm 17.4	40.2 \pm 13.9	41.3 \pm 13.8	5.8 \pm 4.2
Plaisentif	57.1 \pm 2.9	45.2 \pm 3.7	47.3 \pm 4.6	7.5 \pm 1.7
Rashera PDO	55.6 \pm 7.2	48.5 \pm 7.6	48.7 \pm 6.8	4.3 \pm 2.1
Robiola d'Alba	44.3 \pm 4.6	36.1 \pm 5.2	49.8 \pm 9.4	3.4 \pm 0.8
Robiola di Roccaverano PDO	51.2 \pm 4.2	38.9 \pm 5.7	55.2 \pm 7.1	3.5 \pm 0.7
Saras del Fen	53.5 \pm 13.4	29.7 \pm 15.6	57.5 \pm 17.7	8.4 \pm 4.2
Toma del lait brusc	60.9 \pm 10.4	46.4 \pm 7.1	37.8 \pm 10.2	5.8 \pm 2.6
Toma Piemontese PDO	51.5 \pm 7.8	48.5 \pm 16.3	42.5 \pm 17.7	4.5 \pm 2.8
Tomino di Melle	52.4 \pm 6.4	39.5 \pm 7.8	47.9 \pm 9.5	4.1 \pm 3.1
Tomino di Talucco	40.8 \pm 1.1	47.1 \pm 1.9	42.1 \pm 2.5	5.7 \pm 0.7
Tuma 'd Trausela	37.3 \pm 2.2	48.4 \pm 2.6	38.8 \pm 3.5	5.1 \pm 0.3
(dm – dry matter).				

Table 3 - Concentration (mg/g of cheese) of organic acids, sugars and ketones in the cheeses (m – minimum value; M – maximum value; SD – standard deviation). See Table 1 for cheese codes.

	BD				BT				CA			
	m	M	Mean	SD	m	M	Mean	SD	m	M	Mean	SD
Acetic acid	0.323	1.426	0.835	0.352	0.045	0.886	0.361	0.262	0.193	1.822	0.737	0.386
Butyric acid	0.168	0.332	0.215	0.068	0.065	0.300	0.142	0.069	0.048	1.245	0.377	0.286
Citric acid	0.016	1.745	0.544	0.808	0.030	3.302	1.419	1.219	0.033	0.227	0.114	0.101
Formic acid	-	-	-	-	-	-	-	-	0.014	0.116	0.065	0.072
Hippuric acid	-	-	-	-	0.002	0.004	0.003	0.001	-	-	-	-
Iso-butyric acid	-	-	-	-	-	-	-	-	-	-	-	-
Iso-valeric acid	0.289	0.326	0.303	0.014	0.050	0.474	0.205	0.149	0.078	1.454	0.472	0.436
Lactic acid	19.146	39.099	26.311	6.729	9.177	28.327	17.934	4.946	5.120	24.452	14.832	3.870
n-Valeric acid	0.572	4.108	1.862	1.952	0.775	1.716	1.293	0.413	0.330	3.643	1.971	1.077
Orotic acid	0.001	0.020	0.012	0.007	0.003	0.036	0.021	0.011	0.000	0.020	0.012	0.006
Oxalic acid	0.309	0.336	0.322	0.014	0.035	0.210	0.129	0.072	0.127	0.155	0.140	0.012
Propionic acid	0.590	3.568	1.757	1.055	0.031	1.021	0.435	0.281	0.034	1.471	0.433	0.401
Pyruvic acid	0.006	0.195	0.117	0.071	0.007	0.050	0.025	0.012	0.002	0.257	0.093	0.083
Uric acid	0.000	0.003	0.001	0.001	0.001	0.004	0.003	0.001	0.000	0.004	0.001	0.001
Lactose	-	-	-	-	0.010	0.015	0.013	0.004	0.014	0.018	0.015	0.002
Galactose	0.002	0.057	0.025	0.029	0.015	7.158	2.894	2.634	0.002	0.098	0.037	0.043
Glucose	-	-	-	-	-	-	-	-	-	-	-	-
Acetoin	0.004	0.008	0.006	0.001	0.001	0.027	0.009	0.008	0.002	0.010	0.007	0.003
Diacetyl	0.312	0.980	0.512	0.260	0.198	1.883	0.765	0.539	0.047	1.304	0.536	0.327

	CE				MA				MU			
	m	M	Mean	SD	m	M	Mean	SD	m	M	Mean	SD
Acetic acid	0.050	0.450	0.283	0.196	0.017	0.638	0.390	0.198	0.038	1.248	0.352	0.507
Butyric acid	0.040	0.220	0.110	0.096	0.079	0.816	0.258	0.233	0.083	0.203	0.115	0.051
Citric acid	0.010	0.050	0.023	0.018	0.342	2.052	1.370	0.768	0.241	2.448	1.305	1.076
Formic acid	-	-	-	-	0.035	0.040	0.038	0.002	0.087	0.092	0.089	0.003
Hippuric acid	-	-	-	-	0.010	0.011	0.010	0.001	-	-	-	-
Iso-butyric acid	0.093	0.097	0.095	0.002	-	-	-	-	-	-	-	-
Iso-valeric acid	0.060	0.445	0.230	0.160	0.062	0.481	0.243	0.174	0.040	2.260	0.429	0.813
Lactic acid	2.895	21.105	11.743	8.632	14.895	25.001	19.965	3.131	20.301	30.621	24.618	3.841
n-Valeric acid	0.004	0.006	0.005	0.001	0.896	2.799	1.836	0.851	0.256	1.669	0.869	0.596
Orotic acid	0.005	0.020	0.015	0.007	0.001	0.038	0.011	0.013	0.000	0.029	0.016	0.010
Oxalic acid	0.010	0.175	0.064	0.076	0.071	0.268	0.163	0.078	0.149	0.638	0.307	0.153
Propionic acid	0.034	1.455	0.424	0.689	0.052	0.692	0.308	0.202	0.013	2.907	0.380	0.948
Pyruvic acid	0.005	0.055	0.024	0.019	0.005	0.058	0.035	0.017	0.030	0.493	0.157	0.163
Uric acid	0.001	0.002	0.001	0.001	0.001	0.003	0.001	0.001	0.001	0.004	0.002	0.001
Lactose	0.045	4.100	1.113	1.992	-	-	-	-	0.205	3.500	1.409	1.302
Galactose	0.147	0.307	0.227	0.113	0.020	6.357	2.423	3.072	0.145	2.141	0.769	0.862
Glucose	0.015	0.125	0.053	0.062	-	-	-	-	-	-	-	-
Acetoin	0.001	0.004	0.002	0.001	0.000	0.005	0.003	0.002	0.006	0.032	0.013	0.010
Diacetyl	0.225	3.470	1.178	1.303	0.633	1.798	1.124	0.461	0.208	0.690	0.409	0.179

Table 3 - (continued).

	MUR				PL				RA			
	m	M	Mean	SD	m	M	Mean	SD	m	M	Mean	SD
Acetic acid	0.100	0.210	0.155	0.078	0.455	1.975	1.093	0.526	0.006	3.030	0.583	0.657
Butyric acid	0.140	0.410	0.274	0.131	0.135	0.925	0.513	0.235	0.106	0.302	0.177	0.061
Citric acid	0.005	0.070	0.047	0.036	0.025	0.870	0.444	0.338	0.200	2.244	1.322	0.720
Formic acid	-	-	-	-	0.150	2.991	0.681	0.945	0.158	0.580	0.369	0.298
Hippuric acid	-	-	-	-	-	-	-	-	-	-	-	-
Iso-butyric acid	-	-	-	-	0.025	0.130	0.048	0.041	-	-	-	-
Iso-valeric acid	0.390	1.005	0.794	0.282	0.025	0.445	0.235	0.138	0.072	0.744	0.236	0.186
Lactic acid	3.660	15.310	9.278	5.374	2.825	30.050	17.381	7.541	13.593	31.364	22.773	5.306
n-Valeric acid	0.003	0.007	0.005	0.002	0.145	3.035	0.855	1.228	0.401	2.003	1.252	0.772
Orotic acid	0.015	0.020	0.017	0.003	0.005	0.100	0.024	0.034	0.007	0.156	0.031	0.038
Oxalic acid	0.085	0.205	0.160	0.065	0.005	0.250	0.092	0.093	0.000	0.718	0.153	0.197
Propionic acid	0.064	0.770	0.472	0.366	0.677	3.896	1.411	1.013	0.065	1.813	0.473	0.481
Pyruvic acid	0.090	0.320	0.171	0.102	0.020	0.420	0.159	0.159	0.005	0.384	0.101	0.127
Uric acid	0.010	0.050	0.026	0.018	0.005	0.020	0.012	0.006	0.000	0.008	0.002	0.002
Lactose	0.005	0.015	0.010	0.007	1.572	1.596	1.581	0.011	0.090	0.180	0.135	0.064
Galactose	0.055	0.068	0.061	0.009	0.014	1.437	0.330	0.563	0.014	6.576	1.323	2.017
Glucose	0.005	0.125	0.053	0.052	0.020	0.645	0.333	0.442	0.015	0.025	0.020	0.005
Acetoin	0.001	0.010	0.006	0.005	0.005	0.210	0.058	0.102	0.003	0.017	0.009	0.005
Diacetyl	0.550	4.315	1.898	2.098	0.015	0.880	0.341	0.310	0.016	1.162	0.677	0.361

	RdA				RR				SA			
	m	M	Mean	SD	m	M	Mean	SD	m	M	Mean	SD
Acetic acid	0.114	0.984	0.450	0.255	0.003	0.634	0.183	0.137	0.033	0.759	0.237	0.206
Butyric acid	0.067	0.218	0.126	0.054	0.023	0.197	0.113	0.074	0.051	2.598	0.768	1.067
Citric acid	0.366	0.516	0.442	0.060	0.019	0.882	0.177	0.260	0.008	2.305	0.450	0.670
Formic acid	0.043	0.193	0.118	0.087	0.008	0.648	0.176	0.221	0.037	0.845	0.252	0.200
Hippuric acid	-	-	-	-	0.010	0.029	0.019	0.010	0.006	0.117	0.025	0.027
Iso-butyric acid	-	-	-	-	-	-	-	-	0.077	1.880	0.719	0.461
Iso-valeric acid	0.039	0.151	0.080	0.055	0.015	0.224	0.079	0.066	0.085	1.105	0.376	0.322
Lactic acid	12.795	27.832	20.553	4.947	6.046	13.656	9.794	1.998	0.378	15.995	3.221	3.452
n-Valeric acid	0.603	3.501	1.657	1.433	0.210	2.332	1.272	0.595	0.269	0.685	0.518	0.220
Orotic acid	0.004	0.043	0.025	0.015	0.003	0.200	0.028	0.036	0.001	0.042	0.008	0.009
Oxalic acid	0.076	0.343	0.182	0.094	0.023	0.519	0.152	0.113	0.005	0.143	0.043	0.035
Propionic acid	0.021	0.074	0.045	0.019	0.002	0.091	0.045	0.022	0.110	0.489	0.232	0.092
Pyruvic acid	0.009	0.109	0.050	0.033	0.006	0.191	0.060	0.044	0.014	2.288	0.474	0.716
Uric acid	0.000	0.002	0.001	0.001	0.000	0.002	0.001	0.001	0.451	12.395	2.751	2.515
Lactose	2.430	13.285	7.744	3.539	0.655	22.855	11.216	4.838	0.145	73.975	10.250	15.487
Galactose	0.114	4.070	0.644	1.251	0.006	0.848	0.256	0.182	0.015	0.471	0.103	0.113
Glucose	-	-	-	-	0.010	0.400	0.096	0.098	0.174	4.370	1.336	1.112
Acetoin	0.001	0.025	0.007	0.008	0.000	0.013	0.005	0.004	-	-	-	-
Diacetyl	0.394	0.731	0.598	0.124	0.196	0.348	0.275	0.067	-	-	-	-

Table 3 - (continued).

	TAL				TLB				TMELL			
	m	M	Mean	SD	m	M	Mean	SD	m	M	Mean	SD
Acetic acid	0.035	0.314	0.109	0.137	0.005	0.620	0.228	0.199	0.215	0.235	0.225	0.010
Butyric acid	-	-	-	-	0.005	0.895	0.252	0.291	-	-	-	-
Citric acid	0.545	1.615	1.230	0.458	0.010	0.840	0.141	0.244	0.005	1.255	0.427	0.717
Formic acid	-	-	-	-	-	-	-	-	-	-	-	-
Hippuric acid	0.005	0.040	0.023	0.025	0.005	0.050	0.016	0.014	0.003	0.008	0.005	0.003
Iso-butyric acid	0.847	0.863	0.855	0.008	0.002	0.009	0.005	0.004	0.003	0.008	0.005	0.003
Iso-valeric acid	0.005	0.375	0.190	0.262	0.015	1.215	0.469	0.365	0.014	0.026	0.020	0.006
Lactic acid	1.195	15.155	7.527	5.006	0.075	23.865	11.621	6.556	11.470	14.355	12.943	1.443
n-Valeric acid	0.005	0.340	0.100	0.161	0.005	0.570	0.073	0.187	0.002	0.008	0.005	0.003
Orotic acid	0.010	0.110	0.038	0.038	0.005	0.075	0.024	0.019	0.010	0.050	0.030	0.028
Oxalic acid	0.260	0.450	0.312	0.070	0.005	0.205	0.053	0.067	0.030	0.170	0.095	0.071
Propionic acid	0.012	0.033	0.022	0.011	0.003	0.400	0.171	0.195	0.002	0.004	0.003	0.001
Pyruvic acid	0.010	0.100	0.037	0.036	0.005	0.905	0.118	0.207	0.055	0.175	0.107	0.062
Uric acid	0.001	0.003	0.001	0.001	0.001	0.012	0.004	0.003	0.001	0.002	0.001	0.001
Lactose	6.080	23.810	19.488	6.766	0.005	1.080	0.287	0.445	0.475	2.730	1.457	1.155
Galactose	0.030	9.447	1.920	3.696	0.004	0.280	0.058	0.086	0.099	9.536	3.334	5.372
Glucose	0.005	0.615	0.293	0.306	0.005	0.135	0.060	0.038	0.092	0.098	0.095	0.003
Acetoin	0.002	0.027	0.009	0.009	0.001	0.035	0.006	0.009	0.001	0.005	0.003	0.002
Diacetyl	0.010	0.330	0.136	0.132	0.065	4.020	1.176	0.946	0.540	0.740	0.640	0.141

	TP				TRA			
	m	M	Mean	SD	m	M	Mean	SD
Acetic acid	0.023	3.290	0.557	0.531	0.023	0.047	0.035	0.012
Butyric acid	0.010	1.380	0.374	0.310	-	-	-	-
Citric acid	0.001	4.035	0.876	1.122	0.755	1.430	1.178	0.296
Formic acid	0.016	0.916	0.227	0.189	-	-	-	-
Hippuric acid	0.001	0.014	0.007	0.004	0.005	0.090	0.047	0.043
Iso-butyric acid	0.001	0.580	0.038	0.089	0.005	0.855	0.430	0.601
Iso-valeric acid	0.001	1.102	0.140	0.167	0.365	0.385	0.375	0.010
Lactic acid	1.986	35.530	16.629	8.496	2.755	7.035	4.600	2.200
n-Valeric acid	0.006	5.382	0.973	1.145	0.002	0.008	0.005	0.003
Orotic acid	0.001	0.302	0.020	0.026	0.010	0.065	0.044	0.025
Oxalic acid	0.006	0.760	0.178	0.185	0.040	0.450	0.250	0.169
Propionic acid	0.015	7.098	0.455	0.753	0.099	0.107	0.103	0.004
Pyruvic acid	0.003	0.595	0.064	0.073	0.005	0.060	0.023	0.032
Uric acid	0.000	0.110	0.010	0.015	0.001	0.002	0.001	0.001
Lactose	0.005	11.125	3.018	3.198	24.370	30.370	27.035	2.488
Galactose	0.000	7.782	1.419	1.943	0.025	0.368	0.225	0.146
Glucose	0.010	4.065	0.470	0.785	-	-	-	-
Acetoin	0.001	0.105	0.015	0.019	0.001	0.033	0.010	0.015
Diacetyl	0.003	4.198	0.460	0.772	0.010	0.130	0.083	0.064

Table 4 - Results of variance analysis and Duncan's test performed on all parameters determined by HPLC for all cheese varieties. Mean values (mg/g of cheese) in the same column followed by the same letter are not significantly different ($p \geq 0.05$). See Table 1 for cheese codes.

Cheese	Acetic acid	Butyric acid	Citric acid	Formic acid	Hippuric acid	Iso-butyric acid	Iso-valeric acid	Lactic acid	n-Valeric acid	Orotic acid
BD	0.835 cd	0.215 a	0.544 a	-	-	-	0.303 abc	26.311 i	1.862 c	0.012
BT	0.361 abc	0.142 a	1.419 ab	-	0.003 a	-	0.205 abc	17.934 efgh	1.293 ab	0.021
CA	0.737 bcd	0.377 a	0.114 a	0.065 a	-	-	0.472 c	14.832 cdef	1.971 c	0.012
CE	0.283 abc	0.110 a	0.023 a	-	-	0.095 ab	0.230 abc	11.743 bcde	0.005 a	0.015
MA	0.390 abc	0.258 a	1.370 a	0.038 a	0.010 a	-	0.243 abc	19.965 fgh	1.836 c	0.011
MU	0.352 abc	0.115 a	1.305 a	0.089 a	-	-	0.429 bc	24.618 hi	0.869 abc	0.016
MUR	0.155 a	0.274 a	0.047 a	-	-	-	0.794 d	9.278 abcd	0.005 a	0.017
PL	1.093 d	0.513 ab	0.444 a	0.681 b	-	0.048 a	0.235 abc	17.381 efgh	0.855 abc	0.024
RA	0.583 abcd	0.177 a	1.322 a	0.369 ab	-	-	0.236 abc	22.773 ghi	1.252 abc	0.031
RdA	0.450 abc	0.126 a	0.442 a	0.118 a	-	-	0.080 ab	20.553 ghi	1.657 b	0.025
RR	0.183 ab	0.113 a	0.177 a	0.176 a	0.019 ab	-	0.079 ab	9.794 abcd	1.272 abc	0.028
SA	0.237 ab	0.768 b	0.450 a	0.252 ab	0.025 ab	0.719 cd	0.376 bc	3.221 a	0.518 abc	0.008
TAL	0.109 a	-	1.230 a	-	0.023 ab	0.855 d	0.190 abc	7.527 abc	0.100 ab	0.038
TLB	0.228 ab	0.252 a	0.141 a	-	0.016 a	0.005 a	0.469 c	11.621 bcde	0.073 ab	0.024
TMELL	0.225 ab	-	0.427 a	-	0.005 a	0.005 a	0.020 a	12.943 cdef	0.005 a	0.030
TP	0.557 abc	0.374 a	0.876 a	0.227 a	0.007 a	0.038 a	0.140 abc	16.629 abcd	0.973 abc	0.020
TRA	0.035 a	-	1.178 a	-	0.047 b	0.430 b	0.375 bc	4.600 ab	0.005 a	0.044
Significance	***	***	***	***	**	***	***	***	***	ns
(***) $p \leq 0.001$; ** $p \leq 0.01$; * $p \leq 0.05$; ns not significant).										
Cheese	Oxalic acid	Propionic acid	Pyruvic acid	Uric acid	Lactose	Galactose	Glucose	Acetoin	Diacetyl	
BD	0.322 d	1.757 b	0.117 a	0.001 a	-	0.025 a	-	0.006 a	0.512 ab	
BT	0.129 abc	0.435 a	0.025 a	0.003 a	0.013 a	2.894 bc	-	0.009 a	0.765 ab	
CA	0.140 abcd	0.433 a	0.093 a	0.001 a	0.015 a	0.037 a	-	0.007 a	0.536 ab	
CE	0.064 ab	0.424 a	0.024 a	0.001 a	1.113 a	0.227 a	0.053 a	0.002 a	1.178 bc	
MA	0.163 abcd	0.308 a	0.035 a	0.001 a	-	2.423 abc	-	0.003 a	1.124 bc	
MU	0.307 cd	0.380 a	0.157 a	0.002 a	1.409 a	0.769 ab	-	0.013 a	0.409 ab	
MUR	0.160 abcd	0.472 a	0.171 a	0.026 a	0.010 a	0.061 a	0.053 a	0.006 a	1.898 c	
PL	0.092 ab	1.411 b	0.159 a	0.012 a	1.581 a	0.330 a	0.333 a	0.058 b	0.341 ab	
RA	0.153 abcd	0.473 a	0.101 a	0.002 a	0.135 a	1.323 abc	0.020 a	0.009 a	0.677 ab	
RdA	0.182 abcd	0.045 a	0.050 a	0.001 a	7.744 ab	0.644 ab	-	0.007 a	0.598 ab	
RR	0.152 abcd	0.045 a	0.060 a	0.001 a	11.216 b	0.256 a	0.096 a	0.005 a	0.275 ab	
SA	0.043 a	0.232 a	0.474 b	2.751 b	10.250 ab	0.103 a	1.336 b	-	-	
TAL	0.312 cd	0.022 a	0.037 a	0.001 a	19.488 bc	1.920 abc	0.293 a	0.009 a	0.136 a	
TLB	0.053 a	0.171 a	0.118 a	0.004 a	0.287 a	0.058 a	0.060 a	0.006 a	1.176 bc	
TMELL	0.095 ab	0.003 a	0.107 a	0.001 a	1.457 a	3.334 c	0.095 a	0.003 a	0.640 ab	
TP	0.178 abcd	0.455 a	0.064 a	0.010 a	3.018 a	1.419 abc	0.470 ab	0.015 a	0.460 ab	
TRA	0.250 bcd	0.103 a	0.023 a	0.001 a	27.035 c	0.225 a	-	0.010 a	0.083 a	
Significance	***	***	***	***	***	***	***	***	***	
(***) $p \leq 0.01$; ** $p \leq 0.05$; ns not significant).										

the disappearance of galactose has been demonstrated (MICHEL and MARTLEY, 2001). The cheeses studied are generally produced with raw milk so differences in the galactose content could be explained by the different indigenous microflora present in each product.

Diacetyl and acetoin are produced from lactose and citric acid and are very important compounds for cheese aroma. The diacetyl and acetoin concentrations varied from 0 to 4.3 and from 0 to 0.2 mg/g of cheese, respectively. These values were not only directly correlated with ripening time but also with bacterial activity since higher values were recorded in cheeses like Toma del Lait brusc or Robiola di Roccaverano PDO which are produced with sour milk or in cheeses generally produced with a starter addition such as Bra tenero PDO or Bra duro PDO.

Among the organic acids, lactic acid was the most abundant ranging from 0.3 to 39 mg/g of cheese. It is produced by carbohydrate hydrolysis and its concentration is correlated with ripening time (PARK and DRAKE, 2005; PARK *et al.*, 2006); it increases in the initial phases and then diminishes during ripening (AKALIN *et al.*, 2002; FACCIA *et al.*, 2004). This reduction is probably due to the activity of lactate-fermenting bacteria as suggested by trends in the formic, propionic and butyric acids that are higher in long-ripening cheeses.

Citric acid is also quantitatively very important with a maximum of 4 mg/g in the Toma Piemontese PDO cheese. This acid is involved in the Krebs cycle where it acts as both substrate and product and its concentration is higher for cheeses with a short ripening time. Citrate can also be used by starter as a substrate to produce pyruvic and acetic acids. The concentrations of these compounds ranged from 0 to 2.2 mg/g of cheese and from 0 to 3.3 mg/g of cheese, respectively, and were higher in the longer ripened cheeses.

The presence of carboxylic acids like formic, acetic, propionic, butyric, valeric and iso-valeric acids is mainly related to the intensity of bacterial fermentation that takes place during ripening. These compounds are formed by the lipolytic activities of starters and secondary microflora of cheese. The highest concentration of formic acid (2.9 mg/g of cheese) was found in a Plaisentif sample, but this cheese also showed the highest mean values. This can be explained by the long ripening time of this cheese and by the fact that raw non-skimmed cow milk was used by all of the producers. The highest value for acetic acid (3.3 mg/g of cheese) was recorded in a Toma Piemontese PDO sample, while the highest values for propionic, butyric, iso-butyric, valeric and iso-valeric acids were 7.1, 2.6, 1.9, 5.4, 2.3 mg/g of cheese, respectively.

The maximum uric acid concentration (12 mg/g of cheese) was detected for Saras del Fen, a ripened ricotta cheese. Lower concentrations ranging from 0.002 to 0.14 mg/g of cheese were reported previously (LUES *et al.*, 1998; AKALIN *et al.*, 2002; TORMO and IZCO, 2004; PARK and DRAKE, 2005; MANOLAKI *et al.*, 2006; PARK and LEE, 2006), 0.67 mg/g of milk (TORMO and IZCO, 2004) or 0.005 mg/mL of whey (KINIK and GÜRSOY, 2004). Further studies may be needed to clarify the differences between the reported data and the uric acid concentration determined in Saras del Fen.

Orotic acid concentrations varied from 0 to 0.3 mg/g of cheese with a mean value of 0.019 mg/g of cheese; the concentration of this acid in the milk depends on the origin, diet and lactation of the cow (ANASTASI *et al.*, 2000), while in the cheese, its relationship with ripening has not been defined. For some authors, the concentrations are lower in ripened cheeses because orotic acid is readily used by various bacteria (BOUZAS *et al.*, 1991; PARK and DRAKE, 2005;

MANOLAKI *et al.*, 2006) but for other authors the orotic acid concentration is higher in ripened cheese (AKALIN *et al.*, 2002). Still other authors have reported that the orotic acid concentration is independent of ripening time (LUES *et al.*, 1998; CALIFANO and BEVILACQUA, 1999; PARK and DRAKE, 2005). In the present study, the orotic acid concentrations were similar among all the Piedmont cheeses, independent of the ripening time.

Oxalic acid concentrations varied from 0 to 0.76 mg/g of cheese with a mean value of 0.099 mg/g of cheese. These values are similar to those reported in the literature; no differences due to ripening time were noted (LUES *et al.* 1998; TORMO and IZCO, 2004).

CONCLUSIONS

The present study is the first to determine the organic acid, sugar and ketone contents in typical Piedmont cheeses. These compounds are very important for the flavour profiles of the cheeses. Variability was very high for all the examined cheeses due to the non-standardised home-made production methods. Nevertheless, statistical differences can be highlighted for all the parameters examined. All of the cheeses investigated can be defined by a unique chemical profile that can be used to support an eventual request for a PDO designation. According to the published data the sugar concentration is inversely correlated to ripening time. The reported concentrations of acids and ketones are probably due to differences in the cheese-making procedures used by the various producers to make the same cheese. Further studies will be carried out on each of these typical cheeses in order to better define the interactions between the cheese-making parameters and the concentration of these compounds in the final product.

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