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Study of the chemical composition, proteolysis, lipolysis and volatile compounds profile of commercial Reggianito Argentino cheese: Characterization of Reggianito Argentino cheese

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ABSTRACT

Reggianito is a typical variety of grana-type hard cheese produced in Argentina. It is the most exported and due to its organoleptic characteristics is very appreciated by the consumers. The objective of this study was to characterise the global composition, lipolysis, proteolysis and volatile compound profiles of commercial Reggianito cheeses from different dairy plants.

Statistical differences ($P \leqslant 0.05$) in some physicochemical parameters, nitrogen fractions and FFA levels among commercial brands were detected. The volatile profiles were studied by SPME–GC–MS/FID. A total of 53 compounds were identified, the majority belonging to the groups of ketones, alcohols, acids, esters and aldehydes. All these compounds have been reported in Italian grana-type cheeses. Visualization of the analytical results was performed by principal component analysis. This analysis clustered cheese samples according to dairy plants. This fact could be, among other factors, consequences of differences in technologies and ripening time of different manufacturers.

1. Introduction

Grana-type cheeses are a variety of a few hard and extra-hard cheeses characterised by *grainy* and *brittle* texture, with Parmigiano Reggiano and Grana Padano from Italy being the best expressions of this category. Reggianito is a popular Argentinean grana-type cheese developed by immigrant in late XIX and early XX centuries and inspired in the Italian hard cheeses. Through the years it was modified and adapted to raw materials and environmental conditions to give a distinctive product (Candioti et al., 2002; Zalazar, Meinardi C., & E., 1999). Some features such as the use of pasteurized cow milk, a lesser time of ripening and a smaller size distinguish it from the Italian cheeses (Montero et al., 2002).

The cheese is produced in several dairy plants of a wide geographical area of our country, comprising the central and north of Buenos Aires, east of La Pampa and south of Santa Fe and Cordoba provinces, with an area of about 500,000 Km^2. Today, Reggianito is the more exported cheese, and for this reason in recent years efforts to improve and to standardize its quality were made.

The manufacture process of Reggianito cheese is widely described in the "*Protocolo de Calidad para Queso Reggianito*" (Resolución SAGPyA No. 16/2008) and in other publications (Candioti et al., 2002; Gallino, 1994; Zalazar et al., 1999). On the other hand, the *Código Alimentario Argentino* (CAA) establishes in the Art. 635 (Res Conj. SPyRS and SAGPA Nos. 33/2006 and 563/2006) for Reggianito cheese a minimum fat content of 30% w/w (dry basis) and a moisture content of 27–35%w/w. Its body is compact, firm with brittle texture and fine grain, and it has a slight salty taste with mild and well-developed flavour. The color is white or yellow and uniform. Generally, these cheeses have a cylindrical shape, between 5 and 10 kg and have a minimum ripening period of 6 months.

In the last years, different aspects of Reggianito cheese related to rheological and sensory properties and their correlations with instrumental parameters (Hough et al., 1996; Montero et al., 2002); the effect of plastic film packaging on texture and certain ripening parameters (Bertola, Bevilacqua, & Zaritzky, 1995) and the incidence of elevated temperature on ripening (Sihufe et al., 2007; Sihufe et al., in press) have been evaluated. Besides, our working group has also made an important contribution. In this regard, the microbiological and technological characteristics of microorganisms involved in the manufacture and ripening (Reinheimer, Quiberoni, Tailliez, Binetti, & Suárez, 1996; Reinheimer, Suárez,

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Bailo, & Zalazar, 1995) and the influence of selected strains of lactobacilli isolated from natural whey on physicochemical, free fatty acid profiles, proteolysis and sensorial characteristics (Candioti et al., 2002; Hynes, Bergamini, Suárez, & Zalazar, 2003; Perotti, Bernal, Meinardi, & Zalazar, 2005; Perotti, Bernal, Wolf, & Zalazar, 2008) have been studied.

Although these last works have contributed to the understanding of the ripening process, they were performed on cheeses made at pilot plant, lacking studies on commercial Reggianito cheeses. For these reasons, the present work was carried out to improve the knowledge of the compositional, proteolysis, lipolysis and the flavour profile of commercial Reggianito cheeses from different producers. Particularly, the flavour compound profile is an important aspect, since it is a real fingerprint of cheese.

With this information we hope to make a contribution for the best characterization of Reggianito cheese in order to improve the quality and to establish standardization parameters.

2. Materials and methods

2.1. Cheese samples

Over a period of 2 years, a total of eighteen samples from six leader commercial brands of Reggianito cheeses (RA, RB, RC, RD, RE, RF) were purchased in local markets of the Santa Fe city (from the area of Reggianito cheese production) in three opportunities (I, II, III) for each brand.

For physicochemical, proteolysis and lipolysis analyses, the cheeses were sampled according to standard method (IDF, 1995: 50C) and kept at −18 °C until analysis.

For volatile compounds analysis, the cheeses (c.a 200 g) were sliced as wedges, wrapped in aluminium foil and stored at −18 °C. Prior to analysis, samples were finely grated and homogenized using a 600 W food processor.

2.2. Gross composition and proteolysis of cheeses

Cheeses were analysed for moisture (oven drying at 102 °C; IDF, 1982: 4A), protein content (macro-Kjeldahl; IDF, 1993: 20B), fat content (Gerber-Van Gulick method, ISO 3433, 1975) and salt (AOAC method, 1990). For pH measurement, grated cheese (10 g) was macerated with 10 ml of distilled water and the value was obtained by direct insertion of a pH electrode into the slurry cheese (Bradley et al., 1993).

Proteolysis was assessed by determination of soluble nitrogen (SN) at pH 4.6, in trichloroacetic acid (TCA) 12% and in phosphotungstic acid (PTA) 2.5% according to Gripon, Desmazeaud, Le Bars, and Bergère (1975). The nitrogen content was determined by the macro-Kjeldahl method (IDF, 1993: 20B) and the values were expressed as percentage of total nitrogen.

All analyses were performed in duplicate.

2.3. Assessment of lipolysis

Extraction of cheese lipids, isolation of free fatty acids (FFA), derivatization to ethyl esters, and determination of their concentrations by gas–liquid chromatography were performed as described by Perotti et al. (2005), with some modifications. A Perkin–Elmer model GC-9000 series gas chromatograph (Perkin–Elmer Corp., Waltham, Massachusetts, USA) equipped with a flame ionization detector (FID), and with a split/splitless injector was used. FFA were separated on a fused-silica capillary column (PE-Wax, 60 m × 0.25 mm) coated with a bonded polyethylene glycol stationary phase (0.25 μm layer thickness). Chromatographic conditions were as follows: initial 75 °C (1.5 min), up to 150 °C (3 min) at 8 °C/min, up to a final temperature of 230 °C (15 min) at 10 °C/min. Nitrogen flow was set at 3 ml/min and the split ratio at 1:50. The injector and detector temperatures were 250 °C and 300 °C, respectively. The quantification ($C_{4:0}$–$C_{18:2}$) was performed using the internal standards method, with enantic ($C_{7:0}$) and margaric acids ($C_{17:0}$) (Sigma–Aldrich, St. Louis, USA) as standards. Calibration curves were prepared by combining increasing concentrations of a mixture of butyric ($C_{4:0}$), caproic ($C_{6:0}$), caprylic ($C_{8:0}$), capric ($C_{10:0}$), lauric ($C_{12:0}$), myristic ($C_{14:0}$), palmitic ($C_{16:0}$), stearic ($C_{18:0}$), oleic ($C_{18:1}$) and linoleic ($C_{18:2}$) fatty acids, with fixed concentrations of enantic ($C_{7:0}$) and margaric ($C_{17:0}$) fatty acids. Both standards were added to the cheese sample before starting extraction step. The FID outsignal was recorded, and the chromatograms were processed using Turbochrom v.4. Software (Perkin–Elmer Corp., Waltham, Massachusetts, USA). The results were expressed as mg of FFA per kg of cheese.

2.4. Isolation and analysis of the volatile compounds

A portion of 5 g of cheese was placed in 30-ml glass vials. The vessels were hermetically sealed with an aluminium seal and butylteflon septa. The vials were thermostatized at 40 ± 1 °C for 10 min. A SPME holder manual (Supelco, Bellafonte, PA, USA) equipped with a 1 cm × 50/30 μm Stable-Flex DVB/CAR/PDMS (Supelco, Bellafonte, PA, USA) was used, and the fiber was exposed into the headspace for 15 min at 40 ± 1 °C. Volatile compounds adsorbed on the fiber were immediately thermally desorbed at 250 °C during 5 min in the injector port of gas chromatograph (mode splitless).

The conditions for headspace sampling and desorption of compounds were optimized from analysis of some cheese samples. Particularly, the fiber exposure time was selected for to avoid the analyte competition and/or displacement phenomenas onto fiber and to reach an adequate sensibility (results not shown).

A GC–FID (Perkin–Elmer Model 9000) was used for to obtain information of peak areas (arbitrary units) and to compare the profile of different cheeses. Separation was carried out with the same column as that used in the free fatty acids analyses (PE-Wax). The oven was programmed as follows: 45 °C/min (4 min), 8 °C/min to 150 °C (3 min), 10 °C/min to 250 °C (5 min).

A GC–MS (Shimadzu QP-5000) coupled to an ion trap mass spectrometer was used to identify the compounds. The chromatographic conditions and the column employed were the same as those used in the GC–FID analyses. Mass spectra were obtained with 70-eV electron impact ionization, and the mass spectrometer scanning from 42 m/z to 300 m/z (scan rate 250 amu/seg). Compounds were identified by matching mass spectra with Nist-62 library of standard compounds. Both mass spectrometric identifications and chromatographic peaks of FID were further confirmed by comparing retention times with reference standards (Sigma–Aldrich, Italy) or bibliographical data.

All analyses were conducted in duplicate.

2.5. Statistical analysis

Data from gross composition, proteolysis and lipolysis analysis were processed by one way ANOVA with Statistix 7 (Analytical Software, Tallahasse, USA) in order to detect significant statistical differences among commercial brands of cheeses. When differences were found, means were compared by the least significant difference test (LSD) using the same tool.

Principal component analysis (PCA) was applied on the correlation matrix (variables are standardized to mean = 0 and SD = 1) to help interpret the results of all analysis performed on cheese samples using the SPSS version 10.0 (SPSS Inc., Chicago, USA).

3. Results and discussion

3.1. Gross composition and proteolysis of cheeses

The average values of all cheeses for pH, moisture, NaCl content (S/M), fat (dry matter) and protein (dry matter) were 5.4 ± 0.1%, 32.9 ± 1.6%, 6.5 ± 1.9%, 40.9 ± 3.5% and 48.1 ± 3.6%, respectively. The values of moisture and fat content were within the range established by CAA for Reggianito cheese. The average degree of ripening (SN-pH 4.6/NT) was 18.5 ± 3.8%, and the levels of SN–TCA/NT and SN–PTA/NT reached values of 16.0 ± 3.6% and 9.8 ± 2.4%, respectively.

Except for NaCl content, the remaining physicochemical parameters were similar to those reported for Italian grana cheeses. The levels of nitrogen fractions reported for these cheeses are clearly higher than Reggianito cheeses (Battistotti & Corradini, 1993; Careri, Spagnoli, Panari, Zannoni, & Barbieri, 1996; Gobbetti & Di Cagno, 2002; Malacarne, Summer, Panari, Pecorari, & Mariani, 2006).

Within some commercial brands, a noticeable variability (according to standard error value) for moisture (RF), protein (RA), salt content (RB, RC, RF) and proteolysis levels (RB, RF) was observed (Table 1). Overall, the main differences were found in salt content and in SN–PTA fraction. These parameters are associated because a high concentration of NaCl may have a negative influence on the rate of proteolysis during ripening (Di Cagno et al., 2003; Malacarne et al., 2006).

Statistical analysis (ANOVA) indicated significant differences ($P \leqslant 0.05$) among commercial brands (Table 1) for moisture, NaCl, fat matter, protein, SN-pH4.6/TN, SN–TCA/TN and SN–PTA/TN.

The nitrogen fractions are very important parameters to determine the extent of proteolysis. Particularly, SN-pH 4.6 is frequently used as index of cheese ripening and SN–PTA is a rough indicator of the free aminoacid levels, which are precursors of flavour compounds (Malacarne et al., 2006). As can be seen in Table 1, cheese samples from RD and RE had the higher proteolysis levels.

The geographical zone of cheese making, season of production, ripening temperature and duration, and type of dairy are all factors which influence the proteolysis levels in cheeses (Di Cagno et al., 2003). Different studies of the evolution of the nitrogen fractions during the ripening indicate an increase of the proteolysis for grana-type cheeses and other cheese varieties (Gobbetti, Burzigotti, Smacchi, Corsetti, & De Angelis, 1997; Malacarne et al., 2006; Pecorari, Fossa, Sandri, & Mariani, 1997; Prieto, Franco, Fresno, Bernardo, & Carballo, 2000).

3.2. Lipolysis

FFA levels accumulated during ripening are considered as an overall measure of lipolysis (Svensson, Hernández, Virto, & de Renobales, 2006). The free fatty acids play an important role as precursors of volatile compounds such as ketones, lactones, alcohols, esters and aldehydes. Some biochemical pathways of formation of fat-derived flavour compounds are proposed by Alewijn (2006).

The ten single free fatty acid concentrations ($C_{4:0}$–$C_{18:2}$) and the degree of lipolysis, represented by the total concentrations of FFA (sum of individual FFA), were determined. The average level of lipolysis for Reggianito cheeses ($n = 18$) was 2069 ± 684 mg/kg of cheese, and the values of individual samples varied from 1187 mg/kg up to 3810 mg/kg. The global lipolysis was similar to those reported by Perotti et al. (2005, 2008) and Sihufe et al. (2007) for Reggianito cheeses at 180 days of ripening but considerably lower than those reported for Grana Padano and Parmigiano Reggiano cheeses (Caboni, Zannoni, & Lercker, 1990; Pecorari et al., 1995; Sandri, Fossa, Pecorari, Summer, & Mariani, 1997; Malacarne et al., 2009).

The analysis of variance detected significant differences for lipolysis level ($P \leqslant 0.05$) among dairy factories (Table 1). Particularly, the samples of RF commercial brand had the higher values of total FFA.

Several factors such as type of cheese, ripening time, milk quality and production technology have an influence on lipolysis levels (Caboni et al., 1990; Svensson et al., 2006). In general, the total FFA concentration increases throughout ripening period. In Italian grana cheeses, an increase in lipolysis levels during ripening has been reported (Caboni et al., 1990; Malacarne et al., 2006; Sandri et al., 1997). Studies carried out on Reggianito cheeses showed that ripening time and temperature significantly affected the FFA concentrations (Perotti et al., 2005; Sihufe et al., 2007).

Fig. 1 shows the average profile ($n = 18$) of FFA from $C_{4:0}$ to $C_{18:2}$ of Reggianito cheeses. The results obtained were similar to those published for Reggianito (Perotti et al., 2005, 2008; Sihufe et al., 2007) and for Italian cheeses (Pecorari et al., 1995). The major fatty acids were palmitic ($C_{16:0}$) and oleic ($C_{18:1}$), and the following acids in decreasing order of concentration were myristic ($C_{14:0}$) and stearic ($C_{18:0}$). This observation is consistent with the literature not only in these types of cheese but also in other varieties (Collins, McSweeney, & Wilkinson, 2003).

To investigate whether certain FFA were generated preferentially, the individual FFA were arbitrarily classified into short ($C_{4:0}$–$C_{8:0}$, SCFA), medium ($C_{10:0}$–$C_{12:0}$, MCFA) and long-chain fatty acids ($C_{14:0}$–$C_{18:2}$, LCFA) and were expressed as percentages of total FFA. While the long-chain fatty acids are those that are in greater proportion, do not intrinsically contribute to cheese flavour, as they have a higher perception threshold. However, short and medium-chain, even-numbered fatty acids have considerably lower perception thresholds and each gives a characteristic flavour note (Collins et al., 2003; Molimard & Spinnler, 1996).

Fig. 2 shows the mean percentages of SCFA and MCFA for Reggianito cheeses ($n = 18$) and for two samples of Italian grana cheeses

Table 1
Physicochemical parameters, nitrogen fractions and global lipolysis in Reggianito cheeses, grouped according to six commercial brands (mean ± SD; $n = 3$).

Parameters	Commercial brands of Reggianito cheeses					
	RA	RB	RC	RD	RE	RF
pH	5.4 ± 0.1^{a}	5.4 ± 0.1^{a}	5.3 ± 0.1^{a}	5.4 ± 0.1^{a}	5.4 ± 0.1^{a}	5.4 ± 0.1^{a}
Moisture, %	31.3 ± 1.3^{ac}	32.8 ± 0.7^{abc}	34.8 ± 0.3^{b}	33.1 ± 0.6^{abc}	33.7 ± 1.4^{b}	30.8 ± 2.0^{c}
NaCl (S/M)[e], %	5.8 ± 1.3^{a}	5.7 ± 1.9^{a}	5.3 ± 2.0^{a}	7.0 ± 0.7^{ac}	6.3 ± 0.3^{a}	9.6 ± 2.0^{bc}
Fat (dry matter), %	43.5 ± 2.9^{a}	37.0 ± 1.5^{b}	43.7 ± 2.3^{a}	41.8 ± 0.6^{a}	37.6 ± 2.1^{b}	44.0 ± 0.8^{a}
Protein (dry matter), %	46.8 ± 4.1^{cd}	53.5 ± 0.9^{a}	46.2 ± 1.4^{cd}	46.3 ± 0.8^{cd}	49.4 ± 1.5^{ac}	43.6 ± 0.2^{bd}
SN-pH 4.6/TN, %	15.6 ± 0.7^{a}	16.7 ± 4.4^{a}	17.2 ± 2.1^{a}	23.0 ± 0.3^{a}	22.2 ± 2.2^{a}	15.7 ± 3.2^{a}
SN–TCA/TN, %	13.2 ± 0.6^{a}	15.1 ± 4.6^{a}	14.8 ± 1.7^{a}	19.9 ± 1.2^{a}	19.6 ± 2.6^{a}	12.5 ± 2.7^{a}
SN–PTA/TN, %	8.2 ± 0.2^{a}	9.5 ± 2.8^{a}	8.6 ± 0.9^{a}	12.8 ± 0.2^{b}	12.1 ± 1.6^{b}	7.5 ± 2.0^{a}
TFFA mg kg^{-1} of cheese	2168 ± 241^{a}	2053 ± 470^{a}	1421 ± 248^{a}	1943 ± 155^{a}	1712 ± 205^{a}	3072 ± 998^{b}

$^{a-d}$ Average values in the same rows with different letters are significantly different between commercial brands of Reggianito cheeses ($P \leqslant 0.05$).
[e] S/M: salt in moisture.

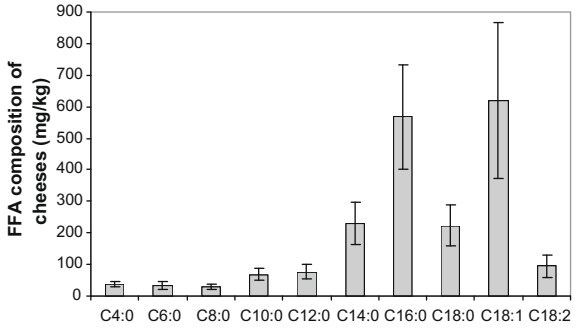

Fig. 1. Levels of individual free fatty acid (expressed as mg kg^{-1} of cheese) in Reggianito cheeses (mean values of 18 samples).

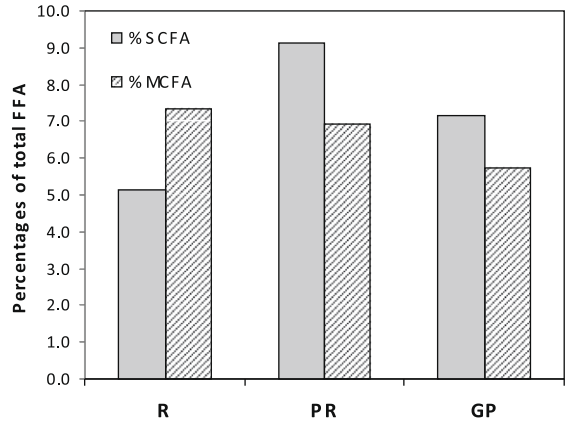

Fig. 2. Relative concentrations of short and medium FFA (% of total) in Reggianito and Italian grana cheeses. References: SCFA: short-chain fatty acids ($C_{4:0}$–$C_{8:0}$); MCFA: medium-chain fatty acids ($C_{10:0}$–$C_{12:0}$). R: Reggianito cheeses (mean value of 18 samples); PR: Parmigiano Reggiano cheese; GP: Grana Padano cheese.

analysed in our laboratory (unpublished data). In Reggianito cheeses, the fraction of MCFA is higher than the SCFA. This situation is opposite to that found in Parmigiano Reggiano and Grana Padano. The process of lipolysis that occured in Italian cheeses had preferential release of short-chain acids. Malacarne et al. (2009) and Sandri et al. (1997) found values of 6% and 9% for SCFA, and 4% and 7% for MCFA in Parmigiano Reggiano. They suggested that this behavior may be associated probably with the increasing release of NSLAB esterases/lipases by cell lysis that occurs during the long ripening period of these cheeses.

3.3. Volatile compound profiles

The 53 volatile components identified in the headspace of Reggianito cheeses and classified by chemical families (ketones, alcohols, aldehydes, esters, acids and others) are listed in Table 2. In this table are also showed, for each compound, the average peak areas (mean ± SD; $n = 18$), the range of area values (minimum and maximum values) and the occurrence of the volatile compounds.

A first analysis of the data revealed that the volatile profiles from both semi-quantitative (peak areas) and qualitative viewpoint were different. The peak areas varied in a wide range, and a great number of compounds were not found in all samples. This variability is frequently observed when different samples from one same variety of cheese are analysed (Bellesia et al., 2003). The flavour compounds in cheeses are generated from multiple enzymatic activities and these reactions are highly conditionated for physicochemical parameters and ripening conditions (Singh, Drake, & Cadwallader, 2003).

Table 2
Results of the volatile compositional analysis on Reggianito cheeses showing the averaged area values, standard deviation (mean of 18 samples) and occurence of compounds.

Compounds	*Reggianito cheeses*		*n*[c]
	Range[a]	Mean ± SD[b]	
Ketones			
Propanone	53–1042	356 ± 319	18
2-Butanone	n.d–228	94 ± 70	17
2-Pentanone + diacetyl	53–1410	464 ± 461	18
2-Hexanone	8–157	49 ± 42	18
2-Heptanone	82–651	278 ± 173	18
3-Hydroxy 2-butanone or acetoin	n.d–700	238 ± 242	15
2-Nonanone	n.d–163	33 ± 42	17
2-Undecanone	n.d–8	1 ± 2	2
Alcohols			
2-propanol	n.d–100	34 ± 29	17
Ethanol	301–3655	1474 ± 1163	18
2-Butanol	n.d–230	47 ± 67	12
1-Propanol	n.d–585	55 ± 155	4
2-Methyl 1-propanol	n.d–159	16 ± 41	5
2-Pentanol	16–180	77 ± 53	18
1-Butanol	23–167	53 ± 39	18
3-Methyl 1-butanol	n.d–285	68 ± 69	17
2-Hexanol	n.d–110	7 ± 28	1
1-Pentanol	12–206	64 ± 60	18
2-Heptanol	13–70	34 ± 18	18
1-Hexanol	n.d–80	15 ± 24	9
2-Ethyl 1-hexanol	n.d–55	6 ± 15	4
2,3-Butanediol	n.d–46	16 ± 17	12
Benzenmethanol	n.d–25	8 ± 10	8
Esters			
Ethyl acetate	n.d–73	38 ± 20	17
Methyl butanoate	n.d–131	16 ± 43	3
Ethyl butanoate	62–2506	568 ± 690	18
Methyl hexanoate	n.d–60	4 ± 15	2
Ethyl hexanoate	n.d–58	10 ± 17	9
Isopropyl hexanoate	n.d–176	42 ± 45	17
Isoamyl hexanoate	n.d–91	22 ± 34	7
Aldehydes			
Acetaldehyde	48–934	332 ± 309	18
2-Methyl butanal	19–436	106 ± 102	18
3-Methyl butanal	23–517	179 ± 120	18
2-Butenal	n.d–570	108 ± 172	7
Benzaldehyde	n.d–132	38 ± 40	17
Acids			
Acetic acid	275–1967	1086 ± 557	18
2-Methyl propanoic acid	n.d–434	53 ± 115	10
Butanoic acid	524–3261	1289 ± 823	18
3-Methyl butanoic acid	7–1697	238 ± 492	18
Pentanoic acid	n.d–21	7 ± 7	10
Hexanoic acid	147–972	406 ± 227	18
Heptanoic acid	n.d–26	3 ± 8	4
Octanoic acid	35–294	111 ± 64	18
2,4-Hexadienoic or sorbic acid	n.d–410	65 ± 109	10
Decanoic acid	16–56	28 ± 16	18
Dodecanoic acid	n.d–50	8 ± 16	5
Other compounds			
1,3-Pentadiene	10–196	61 ± 46	18
p-Xylene	n.d–107	17 ± 28	8
m-Xylene	n.d–285	34 ± 74	10
D-Limonene	n.d–94	15 ± 25	11
2,6-Dimethyl pyrazine	n.d–153	40 ± 59	13
γ-Hexanolactone	n.d–20	11 ± 6	17

n.d: Not detected.
[a] Range of area values ($\times 10^3$) in arbitrary units for each compound.
[b] Average peak areas ($\times 10^3$) in arbitrary units ± standard deviations (mean of 18 samples).
[c] Ocurrence of compounds (n).

Ketones: A total of nine ketones were identified, the majority of them belonging to methylketones group. With the exception of 2-undecanone, the rest of the methylketones from C_3 to C_9 were detected in the majority of samples. Propanone, 2-pentanone +

diacetyl (unresolved peak), 2-heptanone and acetoin have the highest area values, particularly in RBI, RBII and RFII samples.

Ketones are considered as an important fraction of volatile profile of grana cheeses, principally methylketones from C_3 to C_{15} (2-pentanone, heptanone and 2-nonanone prevailing), diacetyl and acetoin (Barbieri et al., 1994; Bellesia et al., 2003; Careri, Manini, Spagnoli, Barbieri, & Bolzoni, 1994; Lee, Diono, Kim, & Min, 2003; Qian & Reineccius, 2002a). The majority of them are associated with butter, fruity, floral and musty notes (Boscaini, van Ruth, Biasioli, Gasperi, & Märk, 2003; Curioni & Bosset, 2002; Frank, Owen, & Patterson, 2004; Qian & Reineccius, 2002a; Qian & Reineccius, 2002b).

Methylketones are considered to derive from acyl lipids through a microbial degradation by a β-oxidation pathway mechanism (Barbieri et al., 1994; McSweeney & Sousa, 2000). Propanone can be derived from butanoic acid but is also probably formed in mammarian gland and then is transferred to the milk (Castillo, Calvo, Alonso, Juárez, and Fontecha, 2007). On the other hand, diacetyl is produced as a consequence of lactococcal lactose and citrate metabolism, but it can be also produced from aspartic acid (Yvon, 2006). Reduction of diacetyl results in formation of acetoin and further reduction of acetoin results in formation of 2,3-butanediol, then 2-butanone and finally 2-butanol (Izco & Torre, 2000).

Alcohols: Numerous alcohols (15) were found in Reggianito cheese: five linear-chain primary alcohols (from C_2 to C_6), six linear-chain secondary alcohols (from C_3 to C_7), three branched-chain primary alcohols (2-methyl 1-propanol, 3-methyl 1-butanol, 2-ethyl 1-hexanol) and one aromatic alcohol (benzenmethanol). In the majority of samples, ethanol, 2-propanol, 2-pentanol, 2-heptanol, 1-butanol, 1-pentanol and 3-methyl 1-butanol were detected, but the alcohol present at the greatest level (taking into account the peak areas) was ethanol. Particularly, samples of RC brand had higher ethanol contents. Ethanol is produced by the fermentation of lactose or citrate (Marilley & Casey, 2004) or from alanine catabolism (Castillo et al., 2007; Izco & Torre, 2000), and the rest of primary alcohols are considered to originate from the corresponding aldehydes produced from fatty acids and amino acid metabolism (Barbieri et al., 1994; McSweeney & Sousa, 2000). The majority of secondary alcohols are formed by enzymatic reduction of the corresponding methylketones (Collins et al., 2003) and methyl-branched primary alcohols such as 3-methyl 1-butanol may be derived through reduction of 3-methyl butanal formed via Strecker degradation from leucine (Marilley & Casey, 2004; McSweeney & Sousa, 2000; Urbach, 1995).

The alcohols identified in our study have been previously reported in Italian grana cheeses (Barbieri et al., 1994; Bellesia et al., 2003; Careri et al., 1994; Qian & Reineccius, 2002a) and 1-butanol, 2-butanol, 2-pentanol and 2-heptanol have been found to dominate the alcohol profile of these cheeses (Barbieri et al., 1994; Moio & Addeo, 1998). On the other hand, the olfactometric analyses carried out on Parmigiano Reggiano samples have suggested that the alcohols probably make minor, if any, contributions to the aroma of this cheese (Qian & Reineccius, 2002a; Qian & Reineccius, 2003a; Qian & Reineccius, 2003b), whereas in Grana Padano 2-heptanol and 1-octen 3-ol have been identified as key odorants (Moio & Addeo, 1998).

Esters: Seven esters were identified in Reggianito cheeses and particularly, esters formed by butanoic and hexanoic acids with ethanol, 2-propanol and 3-methyl 1-butanol. Ethyl acetate, ethyl butanoate and isopropyl hexanoate were found in the majority of samples but only ethyl butanoate presented higher area values. Some samples like REII and RBIII had the greatest levels of esters, while others such as RDI and RFIII presented the lowest values.

Different authors have remarked the majority presence of ethyl esters of even-number fatty acids (from $C_{2:0}$ to $C_{16:0}$) in Parmigiano Reggiano cheeses, being ethyl acetate, ethyl butanoate, ethyl hexanoate and ethyl octanaote, the most abundant (Barbieri et al., 1994; Bellesia et al., 2003; Careri et al., 1994; McSweeney & Sousa, 2000; Qian & Reineccius, 2002a). Moio and Addeo (1998) reported ethyl butanoate and ethyl hexanoate as predominant esters in Grana Padano.

It is well known that esters are important contributors to flavour cheeses, and ethyl esters play an important role in the formation of the fruity character of Italian grana cheeses (Fenster, Rankin, & Steele, 2003; Liu, Holland, & Crow, 2004). Particularly, ethyl butanoate and ethyl hexanoate are considered as key odorants (Boscaini et al., 2003; Frank et al., 2004; Moio & Addeo, 1998; Qian & Reineccius, 2002a; Qian & Reineccius, 2002b; Qian & Reineccius, 2003a; Qian & Reineccius, 2003b). In addition, other esters have been identified as odour-active compounds making probably a minor contribution to fruity flavour (Boscaini et al., 2003; Frank et al., 2004; Qian & Reineccius, 2002a; Qian & Reineccius, 2003a; Qian & Reineccius, 2003b).

The presence of esters is probably linked to the esterase activity of lactic acid bacteria. The dogma of ester synthesis via esterification in cheese has been challenged by recent studies (Holland et al., 2005). It have been demonstrated that esterases of LAB are the enzymes responsible for ester synthesis via alcoholysis in aqueous systems (Liu, Holland, & Crow, 2003). Particularly, the ability of esterases from *Lactobacillus helveticus*, *Lactobacillus casei* and *Lactococcus lactis* to accumulate esters in a model systems simulating Parmesan cheese ripening conditions was demonstrated by Fenster et al. (2003).

Fatty acids: A total of 11 acids were identified in Reggianito cheeses. Saturated fatty acids of even number of carbon atoms from C_2 to C_{10} were present in all samples, being acetic and butanoic acids the acidic compounds with the highest area values, especially in RAI and RFIII cheeses. Odd numbered linear-chain fatty acids such as pentanoic and heptanoic acids were found only in some samples and they represented a minority fraction. Among the branched-chain fatty acids only 2-methyl propanoic and 3-methyl butanoic were found, the latter being more abundant. Particularly, 2,4-hexadienoic or sorbic acids was detected in a few samples. According to CAA, this compound can be added during manufacture of cheese in order to avoid the growth of moulds. Propionic acid was not detected in analysed samples. Some studies have indicated the presence of this compound in Reggianito cheeses and have also suggested a close relationship between total flavour and aroma intensity and propionic content (Hough et al., 1996; Lombardi, Bevilacqua, & Califano, 1994). However, in these works other methodology for the extraction of organic acids was employed. For organic acid analysis by SPME, stationary phases of higher polarity than DVB/CAR/PDMS such as CW/PDMS or polyacrylate, are preferred (González-Córdova & Vallejo-Córdoba, 2001; Pinho, Ferreira, & Ferreira, 2003; Roberts, Pollien, & Milo, 2000). Particularly, a study carried out on volatile profile of Terrincho cheeses employing different fiber types, the propionic acid was not detected with the DVB/CAR/PDMS fiber used in our work (Pinho, Pérèz, & Ferreira, 2003).

Fatty acids have a key role on profile aroma of grana type-cheeses. They are very important both quantitatively and for its contribution to flavour, especially short-chain acids. A wide range of these compounds has been reported in this variety, being ethanoic, butanoic, hexanoic and octanoic acids the most abundant (Barbieri et al., 1994; Bellesia et al., 2003; Careri et al., 1994; Frank et al., 2004; Lee et al., 2003; Moio & Addeo, 1998; Qian & Reineccius, 2002a). Butanoic and hexanoic acids have been recognized as key odorants in Italian grana cheeses (Frank et al., 2004; Moio & Addeo, 1998; Qian & Reineccius, 2002a; Qian & Reineccius, 2003a; Qian & Reineccius, 2003b), whereas the acids such as ethanoic, octanoic, decanoic, 2-methyl propanoic and 3-methyl butanoic (Boscaini et al., 2003; Frank et al., 2004; Qian & Reineccius, 2002a; Qian & Reineccius, 2003a; Qian & Reineccius, 2003b) are considered odour-active compounds, with a minor contribution to the global flavour.

Several biochemical pathways have been proposed for the fatty acids formation: lipolysis, proteolysis and lactose fermentation. Enzymes with lipolytic activity (esterases and lipases) can release linear-chain fatty acids (from C_4 to C_{18}) (Barbieri et al., 1994). Proteolytic enzymes are responsible for the formation of branched-chain fatty acids (isobutyric and isovaleric acids) from the catabolism of valine, leucine and isoleucine (Barbieri et al., 1994; Marilley & Casey, 2004; Urbach, 1995). Acetic acid originates from different processes including the lactose fermentation by heterofermentative lactic acid bacteria (Di Cagno et al., 2003), the catabolism of alanine, glycine, aspartic and serine by lactic acid bacteria, or from citrate metabolism (McSweeney & Sousa, 2000; Urbach, 1995).

Aldehydes: Five aldehydes were identified in cheese samples, and acetaldehyde, 2-methyl butanal and 3-methyl butanal presented the highest area values, especially in some samples such as RAI, RBII, RBIII and RFII. Acetaldehyde is considered to provide a sharp, penetrating, and fruity note to grana cheeses (Barbieri et al., 1994), whereas 2-methyl butanal, 3-methyl butanal and 2-butenal are responsible for malty and green notes (Qian & Reineccius, 2003b). Acetaldehyde is produced by the lactose metabolism or by the oxidation of ethanol (McSweeney & Sousa, 2000) but also by breakdown of threonine. Branched-chain aldehydes such as 2-methyl butanal and 3-methyl butanal are generated from isoleucine and leucine metabolism, respectively (Urbach, 1995).

In cheeses such as Parmigiano Reggiano and Grana Padano, this group of compounds represents a minority fraction. The low level of aldehydes compared to other volatile compounds could indicate an optimal maturation of cheeses (Di Cagno et al., 2003). The strong reducing environment observed in hard cheeses with longer ripening time and the presence of reductases from the wild microbiota could be responsible for the lower aldehyde contents (Mallia, Fernández-García, & Bosset, 2005).

Although a lot of aldehydes have been identified in grana cheeses (Barbieri et al., 1994; Bellesia et al., 2003; Careri et al., 1994; Qian & Reineccius, 2002a), only some of them such as 2-methyl propanal, 2-methyl butanal, 3-methyl butanal, 2-butenal, acetaldehyde, nonanal and phenylacetaldehyde (Boscaini et al., 2003; Moio & Addeo, 1998; Qian & Reineccius, 2002a; Qian & Reineccius, 2002b; Qian & Reineccius, 2003a; Qian & Reineccius, 2003b) are the major odour-active compounds.

Compounds of other chemical families: Other compounds identified in Reggianito cheeses belonging to hydrocarbons (1,3-pentadiene and xylenes), terpenes (limonene), nitrogen compounds (2,6-dimethyl pyrazine) and lactones (γ-hexanolactone) groups. These compounds were detected in some samples and they presented lower area values. Particularly, 1,3-pentadiene and γ-hexanolactone were identified in the majority of samples.

A wide number of hydrocarbons have been isolated in grana cheeses but they did not contribute significantly to the aroma (Barbieri et al., 1994; Careri et al., 1994; Frank et al., 2004; Qian & Reineccius, 2002a). Limonene has been detected together with other terpenes in Parmigiano Reggiano (Barbieri et al., 1994; Bellesia et al., 2003; Careri et al., 1994). Alkylpyrazines such as 2,6-dimethylpyrazine have been isolated in Italian grana cheeses (Barbieri et al., 1994; Careri et al., 1994; Frank et al., 2004; Qian & Reineccius, 2002a), having a key role on cheese aroma (Boscaini et al., 2003; Frank et al., 2004; Moio & Addeo, 1998; Qian & Reineccius, 2002a).

The presence of alkenes and terpenes in cheeses is probably not related to the ripening process but to the animal feed (Di Cagno et al., 2003) whereas aromatic hydrocarbons such as xylenes have a not dilucidate origin, may be due to the freezer storage of samples or ambiental contaminants (Bosset, Gubler, Bütikofer, & Gauch, 2000). Lactones are considered to contribute to the buttery sensory character in cheeses and seem to be related to lipid degradation (Di Cagno et al., 2003).

3.4. Statistical analysis

The values of physicochemical parameters, the extent of proteolysis and lipolysis, and volatile compound production in cheeses, are aspects closely related with technology, raw material and type of ingredients employed in dairy industries. Besides, it is common that every dairy plant adopts its own scheme of production. For this reason, in spite of few number of samples, it resulted of interest to determine similarities and differences among cheeses from different dairy plants by PCA analysis. This method analyses simultaneously all the parameters allowing to detect the most important variables and to establish differences among commercial brands. The matrix data was made with 18 cheese samples and 42 input

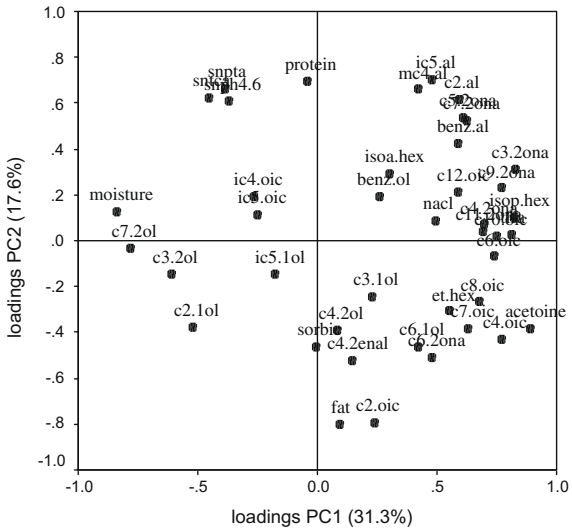

Fig. 3. Loading plot of the first two principal components (PC) obtained by PCA analysis of Reggianito samples. References: C3.2one (2-propanone); C4.2one (2-butanone); C5.2one (2-pentanone); C6.2one (2-hexanone); C7.2one (2-heptanone); C9.2one (2-nonanone); C11.2one (2-undecanone); C2.1ol (ethanol); C3.1ol (1-propanol); C6.1ol (1-hexanol); C3.2ol (2-propanol); C4.2ol (2-butanol); C7.2ol (2-heptanol); iC5.1ol (3-methyl 1-butanol); benz.ol (benzenmethanol); et.hex (ethyl hexanoate); isop.hex (isopropyl hexanoate); isoa.hex (isoamyl hexanoate); C2.al (acetaldehyde); mC4.al (2-methyl butanal); iC5.al (3-methyl butanal); C4.2enal (2-butenal); benz.al (benzaldehyde); C2.oic (acetic acid); C4.oic (butyric acid); C6.oic (hexanoic acid); C7.oic (heptanoic acid); C8.oic (octanoic acid); C10.oic (decanoic acid); C12.oic (dodecanoic acid); iC4.oic (2-methyl propanoic acid); iC5.oic (3-methyl butanoic acid); sorbic (sorbic acid or 2,4-hexadienoic acid).

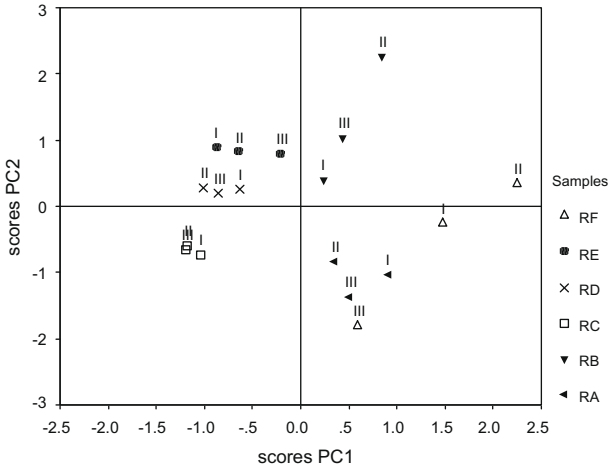

Fig. 4. Score plot of the first two principal components (PC) obtained by PCA analysis of Reggianito samples.

Table 3
Factor scores (means ± SD; $n = 3$) from PC analysis on physicochemical parameters and volatile compounds for Reggianito cheeses.

	RA	RB	RC	RD	RE	RF
PC1	0.56 ± 0.33^{a}	0.54 ± 0.35^{a}	-1.14 ± 0.09^{b}	-0.83 ± 0.21^{b}	-0.55 ± 0.34^{b}	1.41 ± 0.87^{c}
PC2	-1.19 ± 0.19^{a}	1.15 ± 0.96^{d}	-0.65 ± 0.09^{ab}	0.33 ± 0.06^{bd}	0.85 ± 0.08^{d}	-0.50 ± 1.05^{ac}
PC3	1.06 ± 0.85^{a}	1.09 ± 0.84^{b}	-0.12 ± 0.25^{b}	-0.59 ± 0.06^{bc}	-7.50 ± 0.35^{b}	$-1.37 \pm \pm 0.15^{c}$

a–d: Means within rows without a common superscript are significantly different ($P \leqslant 0.05$).

variables (loadings $\geqslant 0.6$). Taking into account that input variables differed in magnitude, a correlation matrix was used.

Three principal components accounted for 64% of the total variance (TV). Figs. 3 and 4 show the variable loadings and the cheese sample distribution in the two-dimensional coordinate system defined by PC1 and PC2 (accounting for 31% and 18% of TV, respectively).

Volatile compounds such as linear short- and medium acids (butanoic, hexanoic, octanoic, decanoic), methyl ketones (propanone, butanone, nonanone, undecanone), acetoin, isopropyl hexanoate together with TFFA showed high positive loadings ($\geqslant 0.7$) with PC1. By contrast, secondary alcohols such as 2-heptanol together with the moisture content were negatively correlated with PC1 ($\geqslant |0.7|$).

Positive loadings ($\geqslant 0.65$) with PC2 were found for nitrogen fractions and protein content, whereas the variables that had the negative loadings were fat content together with acetic acid ($\geqslant |0.7|$) (Fig. 3).

PC1 distinguished two groups of cheeses (Fig. 4). The samples of RA, RB and RF were characterised by important levels of TFFA, acids, methyl ketones and acetoin, whereas the second group of cheeses (RC, RD and RE) presented high levels of the moisture content and alcohols such as 2-propanol, 2-heptanol and ethanol.

In relation to PC1 versus PC3 (data not shown), PC3 described further differences between RF and the other two brand cheeses (RA and RB), while the remaining commercial brands samples were not modified with respect to the location that presented in PC1 versus PC2. The sodium chloride content had an important loading on PC3 (accounting for 15% of TV) ($\geqslant |0.7|$), so this variable was allowed to differentiate the brand RF of RA. In PC2 versus PC3 (data not shown), the cheeses RC, RD and RE showed the same tendency to group together; however, the cheeses that belonged to RA, RB and RF brands were distinguished among them.

In order to validate the previous conclusion based on the visual observation of scores, ANOVA to the PC1, PC2 and PC3 factor scores was applied. Table 3 shows the factor score mean values calculated for each brand of cheese. As observed, significant differences ($P \leqslant 0.05$) among dairy factories were recorded.

The results found are not surprising. Besides the wide geographical zona of production of Reggianito cheeses, the samples could have different ripening times, and since several brands were sampled, it is well known that there are differences in the manufacturing procedures. Particularly, in relation to the ripening time, a study carried out in our institute about the application of statistical tools to predict the ripening time in commercial Reggianito cheese indicated that some cheeses found in the market had ripening times lower than those established for Argentinean legislation (Ramonda, 2009).

4. Conclusions

In the present work, different aspects in relation to the ripening of commercial Reggianito cheeses were studied. Physicochemical parameters, proteolysis, lipolysis and volatile profiles were analysed. Gross composition was similar compared to those of Italian grana cheeses, except the NaCl content which resulted higher in Reggianito cheeses. The lipolysis and proteolysis levels were lower than that reported for other grana type-cheeses. The analysis of the volatile fraction allowed identifying the more characteristic compounds of this variety. In spite of the noticeable variability found in volatile profiles and some physicochemical parameters within of commercial brands, univariate and multivariate analysis clustered cheese samples according to dairy plants. This fact could be due to differences in technology, additives and ripening times among different manufacturers.

Based on these promising results arises the necessity of more studies in this aspect including a higher number of samples and establishing relationships with sensory attributes in order to increase the knowledge about this typical Argentinean cheese.

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