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Short communication: Chemical-sensory and volatile compound characterization of ricotta forte, a traditional fermented whey cheese

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ABSTRACT

Ricotta forte is a traditional whey cheese, obtained through natural fermentation of fresh ricotta, that is getting increasing attention by food traders. In view of possible initiatives for its valorization, the chemical and sensory characteristics were investigated. Samples were obtained from 14 different manufacturer, and were subjected to chemical, biochemical, volatile organic compound, and sensory analyses. All samples presented low pH with high moisture (62–66%) and fat content (57–60% on dry matter). From a biochemical point of view, the electrophoretic patterns evidenced that β-lactoglobulin was the main protein present at all sample ages. Only intermediate levels of proteolysis (20.69% ripening index) took place during aging, whereas the main biochemical event in this dairy product was lipolysis (2.10 mEq/g of acid degree value). Accordingly, free fatty acids dominated the volatile organic compound profile and strongly influenced the sensory characteristics with flavor described as rancid, pungent, acrid, and smelly feet: all associated with short-chain fatty acids such as acetic, propionic, butyric, and caproic. Finally, the sample age did not influence chemical composition, whereas it had significant effect on lipolysis and flavor intensity.
Key words: fermented whey cheese, chemical characterization, sensory evaluation, volatile organic compound profile

Short Communication

Ricotta forte is an Italian traditional dairy product manufactured through natural fermentation of ricotta, the whey cheese "par excellence." It appears as a spreadable ivory-colored cream, with piquant taste and strong aroma. Besides having unique sensory characteristics, it has historical and socio-ethical importance, as it is an example of food by-product recovery. In fact, its production technology was developed in ancient times to recover leftover ricotta by converting it into a shelf-stable new product. Nowadays, harvesting of food by-products is not always in line with the modern approach to food safety, and the production technology has thus been modified. The modern manufacturing protocol has been recently described by Mascaro et al. (2010): briefly, freshly produced ricotta is put into a small tank and thoroughly mixed, then the tank is covered and kept in a cool place for at least 6 mo in a cool place at room temperature. During this time natural fermentation takes place (no starter is added), and the only operations that are performed are daily mixing to avoid formation of molds and draining off whey that is progressively released. At the end of the ripening period salt is added (20–40 g/kg), and the product, which has become shelf-stable at room temperature, is packaged in glass jars, with normal shelf-life being 2 yr or more. After being sold exclusively in local markets, ricotta forte is now receiving the appreciation of gourmets, restaurant owners, and experts in food tourism, who are boosting marketing on broader areas. Despite increased interest, scientific information about the chemical, nutritional, microbiological, and sensory features of this dairy product is very scarce. A small amount of information about the chemical and nutritional characteristics has been reported by the Italian National Research Council (CNR, 1996), Mascaro et al. (2010), and Rea et al. (2010). A microbiological study of Baruzzi et al. (2000) described the evolution of the *Lactobacillus* community during manufacturing and concluded that safety of the product is guaranteed by the long ripening period at low pH values. No information is available about the sensory characteristics and volatile compound profile of ricotta forte. The aim of the present work was to close this information gap by exploring the chemical-sensory characteristics and volatile compounds associated with ricotta forte samples taken from a broad range of different producers.

Samples were collected in duplicate from 14 different manufacturers and were grouped into 3 classes according to age: young (4–6 mo, group A, samples no. 3, 6, 7, 8, and 14), matured (8–9 mo, group B, samples no. 5, 9,

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and 11), and long-stored (13–15 mo, group C, samples no. 1, 2, 4, 10, 12, and 13). Gross composition was assessed by determining pH (glass electrode-pH meter, Hanna Instruments, Woonsocket, RI), moisture (IDF, 1986), total protein (total N × 6.38, AOAC International, 1995), fat (IDF, 1988), and lactose (enzymatic assay, Trani et al., 2017). The protein fraction was characterized by SDS-PAGE according to the procedure reported by Harper et al. (1989), and proteolysis was measured by calculation of the ripening index (**RI**; water-soluble N/total N × 100). The fat fraction was characterized by GC determination of the total fatty acid profile (Trani et al., 2010), and lipolysis was estimated by measuring the acid degree value (**ADV**) as reported by Park (2001). The volatile compounds were studied by head space solid-phase microextraction coupled to GC/MS. The fiber used for microextraction was a 50/30 μm of divinylbenzene/carboxen/polydimethylsiloxane (DVB-CAR-PDMS, Supelco, Bellefonte, PA). It was maintained for 30 min at 37°C into the headspace of a 12-mL vial containing a 1-g cheese sample, tightly capped with a PTFE-silicon septum and previously conditioned for 10 min. Desorption of the volatile compounds was obtained into the split-splitless injector of the GC system set at 220°C (split ratio 1:70). The GC system was a Trace 1300 coupled with a single quadrupole ISQ MS (Thermo Scientific, Waltham, MA), equipped with a VF-WAXms column (20 m length × 0.1 mm i.d. × 0.1 μm film thickness, Agilent J&W, Folsom, CA). The chromatographic conditions were oven temperature 50°C for 0.1 min, then to 180°C at 13°C/min and to 220°C at 18°C/min, hold for 3 min; source temperature 250°C; transfer line temperature 250°C; carrier gas helium at 0.4 mL/min constant flow rate. The impact energy was standardized at 70 eV. Data were acquired in full-scan mode in the range of 33 to 200 m/z, dwell time of 0.1 s/scan. The volatile components were identified using computer matches to standard reference mass spectra of the National Institute of Standards and Technology library (NIST, Gaithersburg, MD), and when possible, identification was confirmed by comparison to reference compounds. Their relative abundance in the chromatograms was calculated by considering the relative peak area. Sensory evaluation was performed by 9 trained panelists (5 women and 4 men, aged 35–58 yr) selected following international standards (ISO, 1993). They carried out a quantitative descriptive analysis according to the protocol reported in a previous paper (Trani et al., 2016). The panel had 2 open training sessions on 4 samples of ricotta forte purchased from a supermarket and created a series of sensory descriptors. All descriptors were quantified on a 6-point scale from 0 (low) to 5 (high) and were selected based on weight percentage (frequency of citations × perceived intensity; AFNOR, 1994). Only descriptors with a weight percentage greater than 30% were considered. The panelists then performed 4 sessions on different days for evaluating the samples under study. For sensory analysis, the discrete variables were described by their mode value and compared using the Kruskal–Wallis test. For chemical parameters, the means and standard deviations were calculated and compared using *t*-test. Analytical data of the volatile organic compounds (**VOC**) were processed by 1-way ANOVA. All data were statistically processed using SPSS 19 software (IBM, Armonk, NY).

The mean chemical characteristics of ricotta forte are reported in Table 1, with rather high standard deviations observed for most of the parameters, suggesting that the production technology and compositional targets are not well standardized. The mean values of pH (4.69) and moisture (64.1%) were in accordance with the data reported by CNR (1996) and Baruzzi et al. (2000), fat content was approximately 59.7% on DM, protein averaged 12.2%, and lactose was present in negligible amounts. We did not find any correlations between gross composition and sample age. Indices of proteolysis and lipolysis showed wide variability, but in contrast to chemical composition, we observed a significant correlation with cheese age. The RI values ranged from a minimum of 13.59% in a 6-mo-old sample, to a maximum of 26.11% in a 15-mo-old sample, with a mean value of 20.69%. Such results indicate only a moderate level of proteolysis and were unexpected, considering the high moisture content, ripening under uncontrolled conditions, and storage at room temperature. Comparison of the means revealed that the RI value was highest in sample group C, suggesting that the rate of proteolysis significantly increases after at least 8 to 9 mo of ripening. This result is probably connected with the composition of the protein matrix (Figure 1), which is mainly composed of β-LG, a protein that is highly resistant to enzymatic hydrolysis (Reddy et al., 1988; Santoro and Faccia, 1996). Lipolysis was measured by

Table 1. Gross composition (% except pH) of ricotta forte samples grouped per age: A (4–6 mo), B (8–9 mo), and C (13–15 mo)

Item	A	B	C	Mean	SD
pH	4.69	4.75	4.65	4.69	0.32
Moisture	62.06	63.00	66.30	64.08	5.84
Fat	22.82	21.20	20.39	21.43	5.69
Protein	11.64	10.94	13.35	12.22	3.75
Lactose	0.28	0.20	0.13	0.20	0.12
RI	19.72	17.07	23.30[a]	20.69	3.43
ADV	1.50[a]	2.53[b]	2.39[b]	2.10	1.22

[a,b]Values in the same row with different superscripts are different at $P < 0.05$.

[1]RI = ripening index (% water-soluble N on total N); ADV = acid degree value (mEq/g of fat).

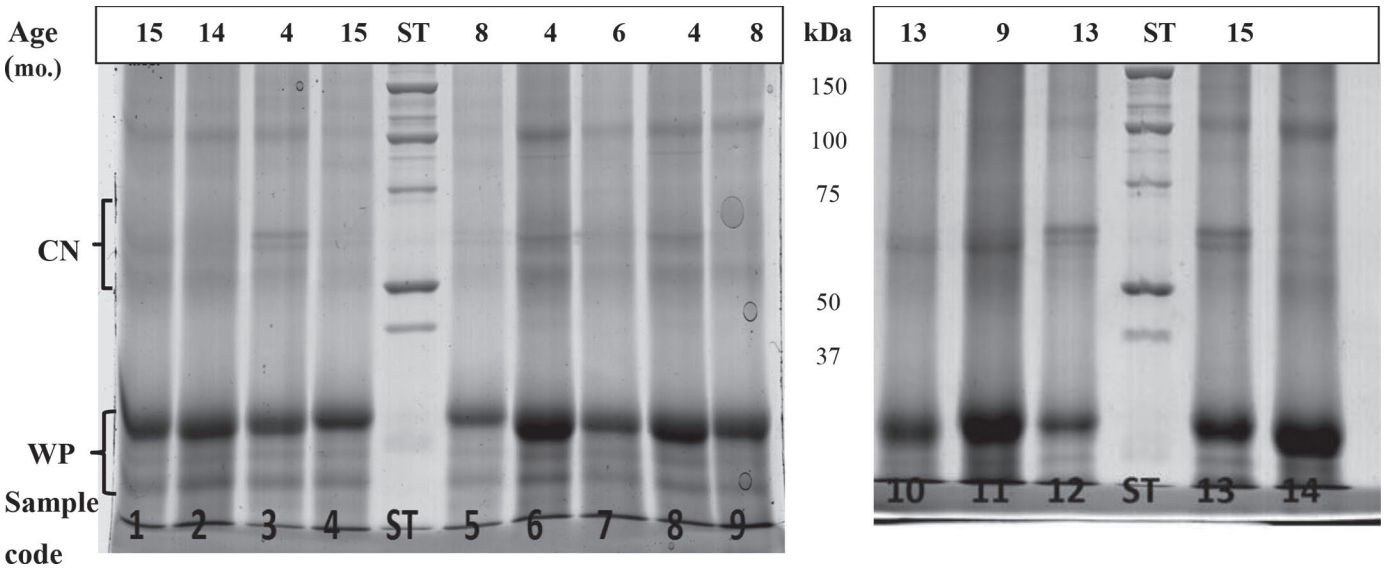

Figure 1. Sodium dodecyl sulfate-PAGE electropherograms of ricotta forte whey cheese. CN = caseins; WP = whey proteins; ST = molecular weight protein standard.

determination of ADV of fat, and even though this method could underestimate the contribution of short-chain free fatty acids (Duncan and Christen, 1991), it is fast and applies very well to products having high fat content. The ADV mean was 2.10 mEq/g, much higher than reported in the literature for long ripened and whey cheeses (Yong and Park, 1995; Mallatou et al., 2003; Pappa et al., 2016). It indicates a dramatic level of lipolysis, and therefore ricotta forte should be considered a rancid dairy product. Excessive rancidity is not a healthy characteristic, as it is associated with the presence of free radicals (Lobo et al., 2010); this aspect, together with the possible presence of biogenic amines reported by Rea et al. (2010), suggests that consumption of this product should be moderated. The level of free fatty acids probably gives support to food safety because both medium- and short-chain compounds exhibit intrinsic broad-spectrum antimicrobial activity (Tan et al., 2014). As to the effect of sample age on lipolysis, comparison of the means revealed that the

Table 2. Total fatty acid composition (% of total peak area) of ricotta forte samples grouped per age: A (4–6 mo), B (8–9 mo), and C (13–15 mo)

Item	A	B	C	Mean	SD	Mean in cow milk fat[1]
C4:0	0.95[a]	0.41[b]	0.31[b]	0.56	0.26	3.0–4.0
C6:0	1.53[a]	1.06[b]	0.61[c]	1.03	0.31	1.7–5.0
C8:0	1.75[a]	1.39[b]	0.85[c]	1.10	0.43	0.9–1.5
C10:0	5.85	4.93	3.22[a]	4.52	0.95	1.9–3.0
C11:0	0.26	0.25	0.16	0.21	0.17	—
C12:0	4.09	3.74	4.33	4.12	0.41	2.1–4.0
C14:0	11.78	11.61	13.79[a]	12.60	0.66	7.9–11.0
C15:0	2.04	2.71	3.17	2.67	0.72	1.0–1.5
C16:0	28.29	30.85	31.54	30.23	2.11	20.0–30.0
C16:1	1.90	1.08	1.36	1.49	0.61	2.0–2.3
C18:0	12.68	13.10	13.07	12.94	0.68	9.0–14.0
C18:1 *trans*	2.64	2.01	1.83	2.16	0.55	2.0–3.0
C18:1	20.73	22.75	20.37	21.87	2.77	23.0–29.8
C18:2	1.84	2.09	2.54	2.19	0.81	2.0–2.4
C18:2 (9,11) CLA	0.89	0.46	0.91	0.81	0.31	0.80–2.2
C18:3n-3	0.92	0.69	0.42	0.66	0.31	0.5–1.1
C20:4	0.15	0.22	0.04	0.12	0.12	0.1–0.2
Others	1.71	0.65	1.48	1.15	—	—

[a–c]Values in the same row with different superscripts are different at $P < 0.05$.

[1]Collomb et al., 2002; Mucchetti and Neviani, 2006.

Table 3. Relative concentration (integrated area counts) of volatile compounds of ricotta forte samples grouped per age: A (4–6 mo), B (8–9 mo), and C (13–15 mo)

VOC	A	B	C	Significance
Acids	1.15E+09	1.23E+09	1.39E+09	*
Acetic acid	6.29E+07	7.09E+07	8.45E+07	*
Propanoic acid	5.54E+07	2.97E+07	4.79E+07	
Isobutyric acid	4.76E+06	2.46E+06	2.35E+06	
Butanoic acid	8.07E+08	8.49E+08	8.29E+08	
Valeric acid-3-methyl	1.26E+07	1.04E+07	4.96E+06	*
Valeric acid	4.96E+06	1.56E+07	5.49E+06	*
Valeric acid-4-methyl	5.20E+06	7.49E+05	5.21E+05	*
Hexanoic acid	1.66E+08	3.64E+08	3.68E+08	*
4-Hexenoic acid	3.42E+05	1.50E+08	—	
3-Hexenoic acid	—	3.11E+06	—	
trans-Hexenoic acid	—	8.51E+05	—	
Heptanoic acid	7.19E+05	1.21E+06	9.49E+05	
Octanoic acid	2.56E+07	2.99E+07	3.96E+07	
Sorbic acid	—	3.16E+07	—	
Decanoic acid	3.54E+06	5.15E+06	5.62E+06	*
Alkane	5.02E+05	0.00E+00	5.22E+04	*
Octane	2.41E+05	—	2.61E+05	
Decane	7.63E+05	—	—	
Alcohol	4.96E+07	1.75E+07	2.66E+07	*
Isopropyl alcohol	4.71E+05	3.20E+05	3.20E+05	
Ethanol	8.89E+06	6.45E+06	1.44E+07	
2-Butanol	2.77E+07	3.77E+06	1.26E+07	*
3-Pentanol	—	2.23E+06	—	
2-Propen-1-ol	2.08E+06	3.50E+06	3.46E+06	
1-Butanol	9.42E+06	2.62E+06	1.55E+06	*
1-Pentanol	9.56E+05	1.69E+06	3.64E+05	*
Isopentanol	—	6.10E+05	—	
2-Penten-1-ol	—	4.64E+05	—	
1-Hexanol	1.11E+06	2.33E+06	8.02E+05	*
2-Nonanolo	4.21E+05	3.22E+05	—	
2,3-Butandiol	—	1.03E+06	2.76E+06	
Ketones	2.05E+07	1.07E+08	1.40E+07	*
Acetone	7.64E+05	7.83E+05	4.06E+05	
2-Butanone	1.65E+07	3.34E+06	3.86E+06	*
3-Pentanone	4.14E+06	3.66E+06	7.19E+06	
2-Heptanone	2.11E+06	3.72E+06	3.50E+06	
2-Nonanone	1.10E+06	3.31E+06	1.66E+06	
8-Nonen-2-one	—	1.08E+06	—	
2-Undecanone	—	4.90E+08	3.98E+05	
Esters	1.03E+08	1.21E+08	2.14E+08	*
Ethyl acetate	9.71E+05	1.18E+06	2.61E+06	*
Ethyl propanoate	8.64E+05	7.44E+05	8.50E+05	
Propyl acetate	—	8.07E+06	8.19E+05	
Ethyl butanoate	3.47E+07	2.12E+07	4.22E+07	
Propyl propanoate	—	—	2.88E+06	
Ethtyl isobutanoate	1.94E+06	—	—	
Methyl thiolacetate	6.56E+05	—	—	
Butyl acetate	1.63E+06	7.25E+05	5.27E+05	*
Propyl butyrate	1.84E+07	9.62E+06	2.45E+07	
Ethyl pentanoate	1.04E+07	8.38E+05	5.32E+05	
Propyl isovalerate	3.25E+05	—	—	
2-Propenyl butyrate	6.06E+05	4.80E+05	1.27E+06	*
Ethyl isovalerate	1.77E+06	—	—	
Butyl isovalerate	5.74E+06	2.33E+06	2.88E+06	
Ethyl caproate	1.79E+07	4.32E+07	7.89E+07	*
Isopentyl butyrate	4.93E+05	1.20E+06	2.25E+05	*
Pentyl isohexanoate	1.33E+06	—	—	
Propyl caproate	1.33E+07	1.12E+07	2.38E+07	
Ethyl eptanoate	4.16E+05	1.41E+06	9.43E+05	
Ethyl lactate	—	5.64E+05	1.06E+06	
2-Propenyl hexanoate	—	—	1.18E+06	
Butyl caproate	7.27E+05	9.60E+05	1.25E+06	
Isopropyl lactate	—	5.59E+05	1.50E+06	
Ethyl caprylate	5.39E+06	1.91E+07	2.67E+07	*

Continued

Table 3 (Continued). Relative concentration (integrated area counts) of volatile compounds of ricotta forte samples grouped per age: A (4–6 mo), B (8–9 mo), and C (13–15 mo)

VOC	A	B	C	Significance
Propyl octanoate	2.86E+06	1.54E+06	6.23E+06	
Butyl caprylate	—	—	4.15E+05	
Methyl isobutyrate	—	1.00E+06	1.96E+06	
Propyl decanoate	—	—	1.36E+06	
Butyl butyrate	4.44E+05	7.21E+06	1.31E+06	
Phenols	1.51E+06	6.32E+05	5.16E+05	*
Benzaldehyde	—	4.10E+05	—	
Phenol	3.03E+06	—	4.25E+05	
p-Cresol	—	9.17E+05	8.65E+05	
Pyrazine	0.00E+00	1.63E+06	0.00E+00	*
Trimethyl pyrazine	—	6.44E+05	—	
Tetramethyl pyrazine	—	2.50E+06	—	
Others	2.30E+05	1.58E+06	8.14E+06	*
α-Myrcene	—	1.31E+06	—	
2-Butanone, 3-hydroxy (acetoin)	7.40E+05	1.94E+06	4.02E+07	*
2-Methylfuran	—	7.81E+05	—	
Dimethyl sulfide	1.81E+05	—	—	
2-Acetoxy-3-butanone	—	—	5.62E+05	
Total	1.32E+09	1.48E+09	1.65E+09	

*Different at $P < 0.05$; only compounds detected in all of the 3 groups were compared.

young samples had the lowest ADV, which significantly differed from samples in the other 2 groups, which had similar values. This result indicates that formation of free fatty acids reaches a maximum level at around 8 to 9 mo, after which ADV stops or starts to decrease, probably due to degradation of free fatty acids into other compounds. Table 2 shows the total fatty acid composition, expressed as relative percentage of total peak area. The most significant differences detected among cheese samples were the short-chain fatty acids (from C4 to C8): in particular, we found their relative concentration decreased as sample age increased. In addition, the concentration of these compounds was always below the ranges commonly reported in the literature for cow milk; the same phenomenon was also observed for several unsaturated fatty acids (Collomb et al., 2002; Mucchetti and Neviani 2006). A possible explanation is that, after being released by lipolytic enzymes, the short-chain fatty acids can partially volatilize, whereas the unsaturated compounds can be oxidized or subjected to other degradation patterns.

Regarding the VOC profile, 76 compounds were identified from the entire set of samples: 29 esters, 16 carboxylic acids, 12 alcohols, 7 ketones, 3 phenols, 2 pyrazines, 2 alkanes, and 5 classified as other (Table 3). The most abundant class was that of carboxylic acids, in accordance with the strong lipolysis levels detected. This class represented about 84% of total peak area in the chromatograms, and was composed of acids from C2, acetic, to C10, decanoic. One sample also contained sorbic acid, but it was likely added as a preservative by the producer because it is not found in milk fat triglycerides. The most abundant acids were butanoic and hexanoic, followed by acetic and propionic. Butanoic can originate from both lipolysis of triglycerides and fermentation of lactate by clostridia, whereas hexanoic can only originate from lipolysis; acetic acid can derive from catabolism of lactose, citrate, and AA, and from propionic fermentation, which also gives rise to propionic acid (McSweeney and Sousa, 2000; Beuvier and Buchin, 2004). Butanoic acid is considered to be the primary cause of cheesy/rancid flavor and, together with hexanoic, of smelly feet and sweat odors, whereas acetic and propionic acids are associated with pungent, vinegary, and acidic notes (Qian and Reineccius, 2002; Carunchia Whetstine et al., 2003; Zabaleta et al., 2016). The second VOC group detected and sorted by order of importance, was that of esters. These compounds are formed in cheeses by spontaneous or esterase-mediate condensation of an acid and an alcohol, and have fruity odors (Beuvier and Buchin, 2004). They represented 10% of the total chromatographic area, and even though none of them reached the same levels as the more abundant acids, they can provide contributions to the aroma of the cheese as a consequence of their low odor thresholds. Finally, the sum of ketones and alcohols accounted for slightly more than 5% of relative concentration; however, these compounds are mainly formed by molds and play a key role in aroma of blue cheeses. Their formation involves lysis of triglycerides, oxidation of saturated free fatty acids and decarboxylation of the resulting ketoacids to ketones, which can be finally reduced to alcohols (Urbach, 1997). The low level of ketones found should be linked to the fact that molds are absolutely unwanted and strongly countered in ricotta forte manufacturing. The only ketone found

Table 4. Sensory profile of ricotta forte (scale from 0 = absence of perception to 5 = maximum intensity of perception)

Attribute	Modal value	Maximum	Minimum	Significance	Cheese group involved[1]
Soluble	5	5	4		All
Rancid	5	5	4		All
Smelly feet	4	5	3		All
Piquant	4	5	3		All
Creamy	4	4	3		All
Fermented	3	4	2		All
Acrid	3	3	2		All
Adhesive	3	3	2		All
Astringent	2	3	1		All
Onion/sulfuric	2	3	1	*	All
Ammonia	2	3	1	*	All
Sweat	2	3	0	*	B, C
Greasy	2	3	0	*	A, B
Salty	2	2	1		All
Sheep barn	1	2	0	*	B, C
Boiled potato	1	2	0	*	A, B

[1]A (4–6 mo), B (8–9 mo), and C (13–15 mo).

*Different among the cheese groups at $P < 0.05$.

at significant level was 2-undecanone: it has green/floral notes and was detected in just 2 samples. Several other compounds were found, but only at insignificant levels. Application of ANOVA to VOC data analysis allowed correlation of the age effect of the samples on the volatiles profile. The levels of acids and esters increased along with sample age, but although statistically significant, the differences mostly were small. Ketones and alcohols were not closely related to cheese age and strong heterogeneity was observed for the other chemical groups. For instance, some pyrazines were only found in middle-aged samples; they impart roasted nutty, raw potato, and savory broth-like notes to cheese (Frank et al., 2004).

Sixteen terms were selected for describing the sensory characteristics of ricotta forte (Table 4): 4 regarding body, 9 regarding odor, and 3 regarding taste. Ten of them were perceived in all samples, had the highest scores, and were not related to age of the cheese. The most important of them were soluble, rancid, smelly feet, piquant, and creamy. Solubility and creaminess are structural characteristics that are consistent with the high fat content and level of proteolysis observed in the cheese, whereas rancid, smelly feet, and piquant are consistent with the sensory features of short-chain fatty acids. Considering the high level of lipolysis, this observation was expected because free fatty acids have strong effects on cheese flavor and act as precursors to other flavor compounds (Collins et al., 2003; Fox et al., 2017). The other 6 descriptors (onion/sulfuric, ammonia, sweat, greasy, sheepfold, and boiled potato) were detected at lower levels and were not present in all samples, but were significantly linked to cheese age. In particular, onion/sulfuric and ammonia were more highly perceived in long ripened samples, whereas boiled potato was only detected in young and matured samples.

In conclusion, this study provides the first detailed description of the chemical and sensory characteristics of ricotta forte whey cheese. The results demonstrate that this dairy product, even though poorly standardized, possesses unique features that are mainly linked to high fat content and high lipolysis. Free fatty acids play a key role in determining the flavor characteristics, and together with the low pH value, should contribute to its microbiological stability.

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