Short communication: Sensory characteristics and volatile organic compound profile of high-moisture mozzarella made by traditional and direct acidification technology

G. Natrella,[1] M. Faccia,[1]* J. M. Lorenzo,[2] P. De Palo,[3] and G. Gambacorta[1]
[1]Department of Soil, Plant and Food Science, University of Bari, via G. Amendola 165/a, 70126 Bari, Italy
[2]Centro Tecnológico de la Carne de Galicia, Rúa Galicia No 4, ParqueTecnológico de Galicia, San Cibrán das Viñas, 32900 Ourense, Spain
[3]Department of Veterinary Medicine, University of Bari A. Moro, S.P. per Casamassima km 3, 70010, Valenzano, Bari, Italy

ABSTRACT

In the present study, the sensory characteristics and the volatile organic compound (VOC) profiles of high-moisture mozzarella made by different acidification techniques were compared. The cheeses were manufactured at the same dairy by fermentation by autochthonous natural whey starter (traditional backslopping method) and direct acidification with citric acid (the most used industrial technology). Three cheesemaking trials were performed from February to June using raw milk from a single farm. The mozzarella samples were subjected to assessment of the chemical, microbiological, and sensory characteristics and to VOC analysis by headspace solid-phase microextraction coupled to gas chromatography-mass spectrometry. The relevant microbiological differences found between the 2 types of products were reflected in different sensory and VOC profiles. The cheeses were clearly discriminated by the panel, and traditional mozzarella had higher intensity attributes. The most discriminating descriptors were elasticity, overall odor and taste intensity, sour milk and fruity/vegetable odors, sour taste, and aftertaste. Even though some variability was observed among the trials, the VOC profile of traditional product was always much more complex than that obtained by direct acidification. In both products the carbonyl compounds were the most abundant chemical class, accounting for about 50% of the total. In detail, 51 compounds were identified in the entire set of samples, and their contribution to cheese aroma was roughly estimated by calculating the odor activity values on the basis of the odor thresholds available in the literature. The results allowed hypothesizing that only 12 of them could play a primary role. The most important among the odor-active compounds was 3-methyl-butanal that can both

derive from metabolism of lactic acid bacteria and yeasts. The results of the study may be very useful in view of European Union PDO labeling of the traditional product, in terms of protecting it from imitations.

Key words: mozzarella, acidification, volatile organic compound, 3-methyl-butanal

Short Communication

High-moisture mozzarella (**HMM**) is a fresh pasta filata cheese that originated in Italy in the 17th century. The production technology is based on acidification of the curd that determines casein demineralization and makes it stretchable in hot water. Curd acidification can be obtained by starter fermentation (traditional method) or by direct acidification of milk (industrial method). Direct acidification by addition of citric acid (**CA**) to milk is very popular in the dairy industry because it is cheaper and allows better standardization than starter fermentation (Faccia et al., 2009). In contrast, the traditional method is adopted in artisanal dairies, where an autochthonous whey starter called sieroinnesto (**SI**) is commonly used (backslopping method). Sieroinnesto is obtained by spontaneous fermentation of the whey kept warm (38–42°C) for 12 to 24 h and represents one of the characterizing points for the Protected Designation of Origin (**PDO**) label that is currently under approval by the European Commission (EU, 2017). Unfortunately, the appearance of the cheese is independent of the acidification method used and it is very difficult for consumers to distinguish between the products. Research on the flavor of HMM is very scarce, and in view of the upcoming adoption of the PDO trademark, information is strongly needed. It is well known that aroma plays a primary role in cheese flavor: it can be defined as the odor-active part of the volatile fraction (Curioni and Bosset, 2002) containing several volatile organic compounds (**VOC**). The most used technique for investigating VOC in cheese is headspace solid-phase microextraction coupled to GC-MS.

This has been applied to almost all popular cheeses but, to our knowledge, not yet to HMM. The few studies regarding the volatile compounds of this cheese have been carried out by different techniques: Moio et al. (1993a) and Pisano et al. (2016) used liquid-liquid partition, whereas Gasperi et al. (2001) directly applied proton transfer reaction MS. The present research aimed to assess the chemical-sensory characteristics of the upcoming PDO-labeled cow mozzarella in comparison to the industrial one, with particular emphasis on the VOC profile.

The study was conducted on mozzarella samples manufactured by traditional (SI fermentation) and direct acidification (addition of CA) in a local dairy. The manufacturing protocols adopted were those commonly used in Southern Italy (Figure 1). Overall, 12 cheesemaking trials were carried out from February to June (3 trials × 2 different technologies × 2 replicates on the same day) using raw milk from a single farm. Milk was subjected to determination of pH, fat, and protein (infrared analysis by Milko Scan FT1, Foss, Hillerød, Denmark), total viable counts (IDF, 1991), and SCC (Fossomatic FC, Foss). During processing, titratable acidity of the starter and pH of the curd samples were measured. The fresh cheese samples underwent analyses of pH, moisture (oven drying), fat (Soxhlet method), and total protein (Kjeldahl method). The following microbial counts were done on starter and fresh cheese samples: mesophilic and thermophilic lactobacilli on de Man, Rogosa and Sharpe medium at 30 and 42°C for 48 h, respectively; lactococci and streptococci on M17 agar at 30 and 42°C for 48 h, respectively; enterococci on Slanetz and Bartley agar at 37°C for 48 h; *Pseudomonas* spp. on Pseudomonas Agar Base with added CFC selective supplement, incubated at 25°C for 48 h; *Enterobacteriaceae* on Violet Red Bile Glucose Agar incubated at 37°C for 24 h; yeasts and molds on Yeast Extract Dextrose Chloramphenicol agar incubated at 30°C for 48 h. All media were purchased from Oxoid Limited (Basingstoke, UK). The cheese sensory characteristics were evaluated by a panel composed of 8 trained assessors aged 25 to 58 yr, selected following the international standard (ISO, 1993). They were trained for a period of 3 mo (one session a week) during which they tasted 36 samples of table mozzarella taken from 5 different producers. During training, each panelist indicated a series of sensory attributes and quantified them using a 5-point numerical category scale (from 0 = not perceived to 4 = highly perceived). Only attributes with a weight percentage (frequency of citations × perceived intensity) greater than 30% were selected for evaluating the samples of the experimentation (Trani et al., 2016). Descriptive analysis was conducted on the samples during experimentation using the same scale used during training sessions. The CA and SI mozzarella samples (1 whole cheese weighing about 100 g) were presented at 20°C within 15 min of each other, on a white dish marked with a 3-digit code. The analysis was repeated 3 times for each cheesemaking trial, and the panelists received the samples in a random and balanced way so that each member evaluated the 2 mozzarella samples the same number of times. The VOC were investigated by headspace solid-phase microextraction coupled to GC-MS. One gram of cheese sample was transferred into a 15-mL glass vial (Supelco, Bellefonte, PA), added with 10 µL of internal standard (3-pentanone) in water at 8.15 µg/mL and 0.2 g of sodium chloride, and sealed with a screw cap with a silicone pierceable septum. The vials were kept at 37°C for 10 min for equilibration, then a triphasic fiber 50/30 µm of DVB/Carboxen/PDMS (Supelco) was inserted through the cap septum and exposed to the sample headspace for 15 min at the same temperature. Thereafter, the volatile compounds were desorbed by inserting the fiber for 2 min in the split/splitless injector port of the GC set at 220°C (split ratio 1:18). The GC system was a Trace 1300 coupled with a single quadrupole ISQ mass spectrometer (Thermo Fisher Scientific, Rodano, Italy). The column used for separation was a TR-WAX MS (20 m length × 0.1 mm i.d. × 0.1 µm film thickness, Agilent, Santa Clara, 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, held for 1.5 min; source and transfer line temperature 250°C, and carrier gas helium at a 0.4 mL/min constant flow rate. The impact energy was 70 eV. Data were acquired in full-scan mode in the range from 33 to 250 m/z (dwell time 0.1 s/scan), and processed by Xcalibur v2.0 software (Thermo Fisher Scientific). Tentative identification of the compounds was done by comparison with standard reference mass spectra of the National Institute of Standards and Technology library (Gaithersburg, MD), and when available, it was confirmed by comparison to pure standards. Finally, the odor activity value (**OAV**) of the identified compounds was calculated by the ratio between concentration in the headspace/odor threshold in water. All analytical determinations were carried out in duplicate. Mean, standard deviation, and standard error of the mean were calculated and compared by the Tukey's test using Excel 2017 software (Microsoft Corp., Redmond, WA). The ANOVA and principal component analysis (**PCA**) were carried out by XL Stat software (Addinsoft SARL, New York, NY).

Besides the acidification technique, the cheeses also differed in the amount of rennet used, size of the curd grains, and whey drainage (Figure 1). Such variations are related to the different chemical and microbiologi-

cal conditions under which cheesemaking is performed. In particular, (1) the amount or rennet is adapted to the pH value of milk for getting the required coagulation time (15–20 min). In direct acidification a reduced dose is needed, since the lower pH of milk tends to ac-celerate coagulation. (2) In traditional technology a greater size of curd grains is required to counterbalance the moisture losses caused by syneresis during fermentation; and (3) the whey is only partial removed in the traditional process for keeping the curd warm and fa-
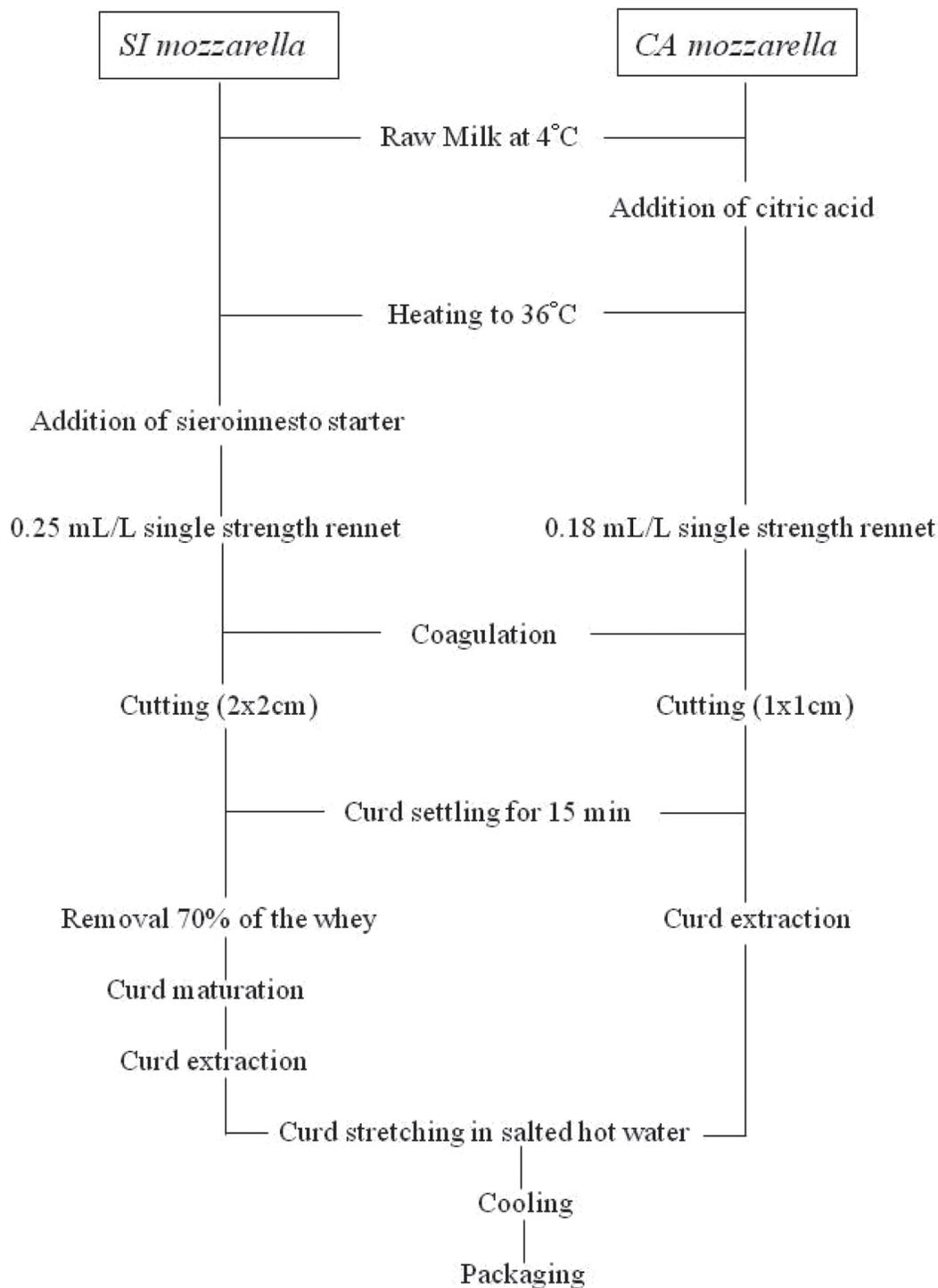
Figure 1. Technological scheme for mozzarella manufacturing. SI = sieroinnesto; CA = citric acid.

Table 1. Gross composition (mean $\pm$ SD) of mozzarella samples[1]

Trial	pH	Moisture (%)	Protein (%)	Fat (%)
CA1	6.00 ± 0.03^A	58.61 ± 1.26^A	18.28 ± 0.89	20.92 ± 0.37^A
SI1	5.56 ± 0.05^B	56.45 ± 0.85^B	18.54 ± 0.92	21.03 ± 0.44^A
CA2	5.91 ± 0.02^A	61.26 ± 4.03^A	17.56 ± 2.21	14.70 ± 2.07^B
SI2	5.37 ± 0.04^C	53.74 ± 2.07^C	21.68 ± 2.40	18.53 ± 2.27^A
CA3	6.01 ± 0.01^A	59.34 ± 2.20^A	17.69 ± 2.28	15.27 ± 0.75^B
SI3	5.61 ± 0.02^B	55.20 ± 1.12^{BC}	19.90 ± 1.28	16.65 ± 0.65^B
Mean CA (SEM)	6.0 (0.03)	59.7 (1.02)	17.8 (0.73)	17.0 (0.44)
Mean SI (SEM)	5.5 (0.05)	55.1 (0.69)	20.0 (0.63)	18.7 (0.46)

A–CValues in the same column with different superscripts are different at $P < 0.05$.

[1]CA1,2,3 = mozzarella made by direct acidification (citric acid); SI1,2,3 = mozzarella made by sieroinnesto fermentation (natural whey starter).

voring microbial activity. The total processing times were very different: the average time for curd maturation (from end of curd settling to stretching) was 17 min for direct acidification and 205 min for SI. The chemical and microbiological characteristics of the milk used in the trials were rather constant: the mean values (SD) were 3.71% (0.13) fat, 3.18% (0.04) protein, 8.3 (0.34) $\times 10^4$ cfu/mL total viable counts, 2.5 (0.54) $\times 10^5$ SCC/mL. Some variability was found in the characteristics of the starter: acidity varied from 38 to 52 Soxhlet-Henkel degrees/100 mL, and the counts of the different microbial groups roughly varied in the order of a maximum of 0.5 log units. Variability in the composition of autochthonous starter for mozzarella is normal and is connected with periodic changes of the microbiological characteristics of the milk used and of the environmental conditions during preparation (Morea et al., 1999). On the whole, the starters used in the 3 trials had the following common traits: (1) lactobacilli were the dominant microorganisms ($\approx 1.5 \times 10^7$ cfu/mL), followed by lactococci ($\approx 5.0 \times 10^6$ cfu/mL), enterococci ($\approx 1.3 \times 10^6$ cfu/mL), and streptococci ($\approx 1.0 \times 10^6$ cfu/mL); (2) the spoilage bacteria were poorly present; and (3) yeasts and molds always represented important secondary components ($\approx 0.9 \times 10^6$ cfu/mL). The gross composition of the cheeses is shown in Table 1. The main differences between the 2 groups regarded pH: it depends on the fact that the 2 acidification methods follow different kinetic of casein demineralization. Direct acidification is more effective at sequestering calcium from the casein micelle, and allows stretching to be performed at higher pH (Mucchetti and Neviani, 2006). This depends on the fact that when the acid is added to milk it immediately acts on the micelles suspended in the water medium, sequestering part of the intra-micellar colloidal calcium phosphate. In contrast, in traditional technology lactic acid is slowly released in the curd by starter fermentation and reaches the casein micelles after coagulation. In this case the micelles are concentrated into the paracaseinate network, connected among them by inter-micellar calcium bridges: the colloidal calcium/casein ratio is higher, and more acid is needed to perform the sequestering reaction. Table 2 shows the microbial counts in the cheese samples. The microbiota of the CA cheese was mainly composed of adventitious spoilage microorganisms, whereas lactic acid bacteria dominated the profiles of SI mozzarella. Such differences gave rise to well-differentiated sensory characteristics, as shown in Table 3. During the training sessions, 14 attributes were selected by the panelists: 3 regarded texture (appearance, elasticity, hardness), 5 odor (odor intensity, fresh and sour milk, buttery, fruit/vegetable), and 6 taste (taste intensity, sweet, savory, bitter, sour, and aftertaste). The panel decided to merge the fruit and vegetable descriptors into a single attribute to make a

Table 2. Mean values (log cfu/g) for microbial counts in the cheese samples[1]

Item	MLAB	TLAB	LAC	STR	EC	ENT	Y&M
CA1	3.2 ± 0.1^A	3.1 ± 0.1^A	ND[2]	1.9 ± 0.1^A	ND	3.2 ± 0.1^C	1.0 ± 0.3^A
SI1	5.6 ± 0.2^B	6.4 ± 0.3^B	2.3 ± 0.2	6.0 ± 0.3^C	2.9 ± 0.2	1.8 ± 0.1^B	2.7 ± 0.2^B
CA2	3.1 ± 0.1^A	2.9 ± 0.3^A	ND	3.0 ± 0.1^B	ND	2.8 ± 0.3^C	1.5 ± 0.2^A
SI2	6.3 ± 0.3^C	6.4 ± 0.1^B	2.1 ± 0.1	7.3 ± 0.3^D	2.5 ± 0.3	1.3 ± 0.1^A	3.5 ± 0.1^C
CA3	3.0 ± 0.1^A	3.2 ± 0.1^A	ND	2.0 ± 0.1^A	ND	3.7 ± 0.1^D	1.5 ± 0.2^A
SI3	5.6 ± 0.1^B	6.2 ± 0.2^B	1.9 ± 0.3	6.2 ± 0.2^C	2.9 ± 0.1	1.3 ± 0.1^A	2.9 ± 0.1^B

A–DValues in the same column with different superscripts are different at $P < 0.05$.

[1]CA1,2,3 = mozzarella made by direct acidification (citric acid); SI1,2,3 = mozzarella made by sieroinnesto fermentation (natural whey starter); MLAB = mesophilic lactobacilli; TLAB = thermophilic lactobacilli; LAC = lactococci; STR = streptococci; EC = enterococci; ENT = enterobacteria; Y&M = yeasts and molds.

[2]Not detected.

Table 3. Sensory profile of traditional (SI) and industrial (CA) high-moisture mozzarella (mean values of 3 cheesemaking trials)[1]

Attribute	SI	CA	Sig
Appearance	4	3	
Hardness	1	1	
Elasticity	3	2	*
Odor intensity	3	1	*
Fresh milk	1	2	
Sour milk	1	0	*
Butter	2	1	*
Fruit/vegetable	2	0	*
Taste intensity	3	1	*
Sweet	1	2	*
Savory	2	1	
Bitter	1	0	
Sour	2	0	*
Aftertaste	3	1	*

[1]0 = not perceived; 4 = highly perceived; Sig = statistical significance of the difference (*$P < 0.05$).

Table 4. Abundance of the volatile organic compound chemical classes in high-moisture mozzarella, expressed as µg/kg headspace volume (mean values ± SD)[1]

Chemical class	CA	SI
Ketones	284.7 ± 8.3^{B}	$1{,}041.6 \pm 70.2^{A}$
Aldehydes	179.4 ± 16.1^{B}	370.6 ± 9.3^{A}
Alcohols	148.8 ± 3.1^{B}	595.6 ± 72.9^{A}
Acids	129.3 ± 11.3^{B}	312.7 ± 33.3^{A}
Terpenes	51.1 ± 4.45	35.3 ± 5.75
Esters	8.8 ± 2.2^{B}	348.0 ± 71.9^{A}
Sulfur compounds	9.0 ± 0.6^{A}	2.9 ± 0.4^{B}
Miscellaneous	118.1 ± 15.5^{B}	151.9 ± 19.6^{A}
Total	929.2^{B}	$2{,}858.6^{A}$

[A,B]Values in the same row with different superscripts are different at $P < 0.05$.
[1]CA = mozzarella made by direct acidification (citric acid); SI = mozzarella made by sieroinnesto fermentation (natural whey starter).

synthesis of different opinions. The results obtained demonstrated that the cheeses were clearly discriminated in all of the 3 cheesemaking trials. Traditional mozzarella received higher scores for almost all attributes and was described as elastic, with butter, sour milk and fruity/vegetable odor, and slight sour taste. In contrast, direct set mozzarella was less elastic, with delicate/fresh milk odor, and sweetish notes. This finding suggests that sensory analysis allows one to easily differentiate between traditional mozzarella and the industrial mozzarella, and should be recommended for official checking of commercial products. As far as VOC

are concerned, 51 volatile compounds were identified in the entire set of samples, grouped in 8 chemical classes (Table 4). A 3 times higher concentration of volatiles was observed in the traditional product, with carbonyl compounds representing a relevant part of the volatile fraction in both types of cheese. Several pathways can be hypothesized for aldehyde and ketone formation in HMM: apart some transfer from milk, the primary source should be lactose and CA fermentation (Rodriguez et al., 2009; Cheng, 2010), whereas the lipolytic way should be negligible because mozzarella is a fresh cheese. This hypothesis was supported by the concentrations found in CA, which roughly corresponded to
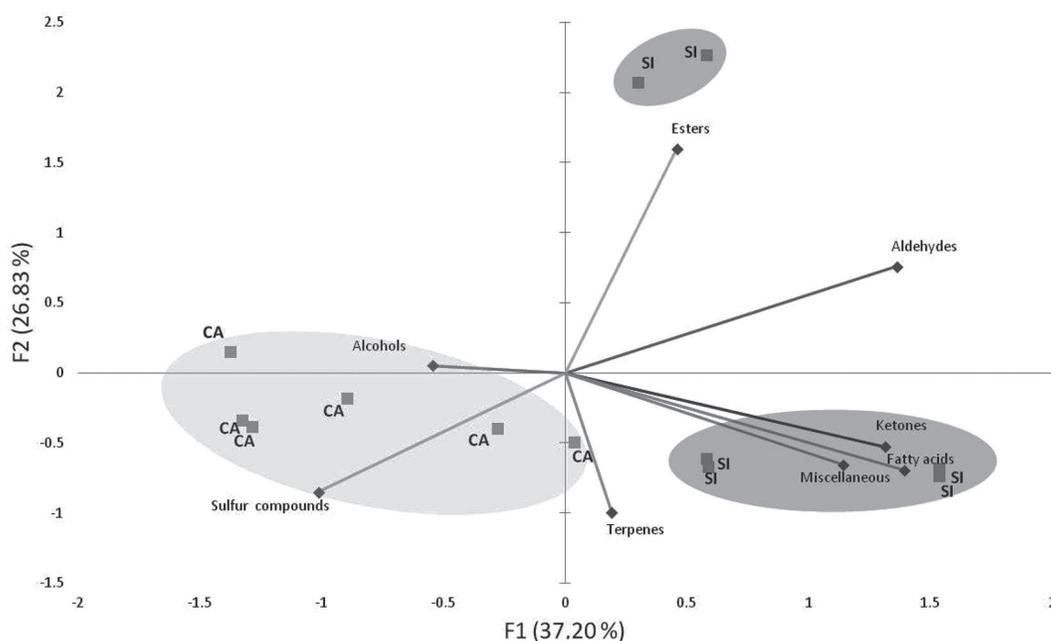
Figure 2. Principal component analysis of volatile organic compounds of mozzarella samples. SI = sieroinnesto; CA = citric acid; F1 = principal component 1; F2 = principal component 2.

Table 5. Volatile organic compounds (VOC) in mozzarella samples from the cheesemaking trials[1]

VOC	LRI Lit.	LRI Exp.	R	Trial 1 CA	Trial 1 SI	Trial 2 CA	Trial 2 SI	Trial 3 CA	Trial 3 SI	OT[2] (μg/L)
Aldehyde										
3-Methyl butanal	920	919	MS, LRI	3.46[B]	7.90[A]	2.78[B]	10.90[A]	5.75[B]	47.28[A]	0.2 [1]
Hexanal	1,085	1,083	MS, LRI	4.34[B]	19.65[A]	4.62[A]	3.03[B]	3.53[A]	2.98[A]	4.5 [1]
Heptanal	1,188	1,185	MS, LRI	ND	ND	3.59[A]	2.31[A]	1.36[B]	6.55[A]	5.87 [1]
Octanal	1,292	1,296	MS, LRI	1.14[A]	4.60[A]	3.56[A]	2.89[A]	ND	ND	1.4 [2]
Nonanal	1,397	1,394	MS, LRI	9.57[B]	22.87[A]	27.48[A]	32.58[A]	10.24[A]	16.17[A]	1.0 [2]
Furfural	1,478	1,476	MS, LRI	0.17[B]	0.46[A]	ND	ND	ND	ND	3,000 [3]
Decanal	1,509	1,500	MS, LRI	ND	ND	7.52[A]	3.63[B]	ND	ND	0.1 [1]
2-Undecanal			MS, LRI	ND	1.51[A]	0.63[A]	ND	ND	ND	7 [1]
Ketone										
Acetone	812	815	MS, LRI	21.65	22.07	17.78	19.75	22.04	25.30	840 [4]
2-Butanone	908	906	MS, LRI	3.19[B]	8.58[A]	4.38[B]	6.33[A]	2.57[A]	3.97[A]	50,000 [5]
Diacetyl			MS	ND	5.30[A]	ND	15.09[A]	ND	ND	2.3 [3]
2,3 Pentanedione	1,070	1,069	MS, LRI	ND	9.31[A]	0.86[B]	7.18[A]	ND	1.05[A]	30 [5]
2-Heptanone	1,189	1,183	MS, LRI	7.52[B]	21.09[A]	4.00[B]	9.98[A]	5.50[A]	4.43[A]	140 [1]
Acetoin	1,299	1,292	MS, LRI	3.18[B]	157.02[A]	14.17[B]	130.76[A]	18.73[B]	74.39[A]	800 [6]
6-Methyl-5-hepten-2-one	1,345	1,342	MS, LRI	1.34[A]	2.18[A]	3.87[A]	3.35[A]	1.55[A]	ND	50 [6]
2-Hydroxy-3-pentanone			MS	ND	2.80[A]	0.64[B]	2.66[A]	ND	ND	—
2-Nonanone	1,394	1,384	MS, LRI	1.19[B]	4.58[A]	1.37[A]	3.19[A]	2.60[A]	1.31[B]	5 [1]
2-Undecanone	1,606	1,603	MS, LRI	ND	0.72[A]	0.47[A]	0.60[A]	ND	ND	7 [1]
6,10-Dimethyl-5,9-undecadien-2-one			MS	ND	ND	3.71[A]	3.10[A]	ND	ND	60 [2]
Alcohol										
Ethanol	937	932	MS, LRI	ND	3.87[A]	7.74[B]	27.11[A]	23.49[B]	132.67[A]	8 [2]
3-Methyl,1-butanol	1,222	1,216	MS, LRI	1.34[B]	5.38[A]	9.23[B]	22.57[A]	ND	56.99[A]	250 [6]
1-Pentanol	1,261	1,266	MS, LRI	ND	ND	ND	0.41[A]	ND	ND	4,000 [6]
3-Methyl-3-buten-1-ol	1,263	1,268	MS, LRI	ND	ND	ND	0.52[A]	ND	ND	—
3-Methyl-2-buten-1-ol			MS	ND	1.05[A]	ND	0.84[A]	ND	ND	140 [2]
Hexanol	1,359	1,355	MS, LRI	ND	ND	1.23[B]	3.37[A]	ND	ND	2,500 [7]
2-Methoxy-1-methylethoxy,2-propanol			MS	2.21[A]	ND	ND	0.41[A]	ND	ND	—
2-Methoxypropoxy,2-propanol			MS	3.39[A]	0.37[A]	0.81[A]	1.71[A]	ND	ND	—
2-Furanmethanol			MS	2.66[A]	3.24[A]	ND	ND	ND	ND	1,000 [2]
2-Octyloxy-ethanol			MS	ND	ND	7,212[A]	12,170[A]	ND	ND	—
Phenylethyl alcohol			MS	1.36[A]	1.76[A]	0.59[B]	1.42[A]	ND	1.12[A]	1.2 [2]
Sulfur compound										
Dimethyl sulfide			MS	0.76[A]	0.94[A]	1.04[A]	ND	1.50[A]	ND	0.3 [6]
Dimethyl sulfoxide			MS	ND	ND	ND	ND	0.45[A]	ND	—
Dimethyl sulfone			MS	ND	ND	0.54[A]	0.50[A]	0.20[A]	ND	—
Fatty acid										
Formic acid			MS	1.20[A]	2.81[A]	ND	ND	ND	ND	520 [8]
Acetic acid	1,480	1,468	MS, LRI	5.56[B]	23.85[A]	17.05[B]	69.28[A]	3.19[A]	5.41[A]	240 [5]
Butanoic acid	1,630	1,625	MS, LRI	1.42[A]	3.18[B]	2.89[B]	4.52[A]	1.41[A]	1.68[A]	93 [6]
Hexanoic acid			MS	3.08[A]	5.94[A]	5.72[B]	10.64[A]	6.23[A]	4.02[A]	640 [2]
Heptanoic acid			MS	ND	ND	ND	0.51[A]	ND	1.77[A]	910 [2]
Octanoic acid			MS	2.65[A]	4.04[A]	3.18[B]	4.55[A]	2.99[A]	4.41[A]	—
Nonanoic acid			MS	ND	ND	4.69[A]	3.07[A]	0.77[B]	3.03[A]	2,200 [1]
Decanoic acid			MS	0.64[B]	1.02[A]	1.37[A]	1.14[A]	0.57[B]	1.48[A]	—
Ester										
Ethyl acetate	891	894	MS, LRI	ND	9.65[A]	ND	14.16[A]	4.38[B]	124.90[A]	5 [2]

Continued

Table 5 (Continued). Volatile organic compounds (VOC) in mozzarella samples from the cheesemaking trials[1]

VOC	LRI Lit.	LRI Exp.	R	Trial 1 CA	Trial 1 SI	Trial 2 CA	Trial 2 SI	Trial 3 CA	Trial 3 SI	OT[2] (μg/L)
Miscellaneous										
α-Pinene	1,017	1,011	MS, LRI	5.65A	8.46A	1.04A	ND	ND	ND	2.5 [2]
Ethylbenzene	1,122	1,126	MS, LRI	ND	ND	ND	0.62A	ND	ND	—
p-Xylene	1,125	1,120	MS, LRI	ND	ND	0.73A	0.86A	ND	ND	—
D-Limonene	1,192	1,189	MS, LRI	ND	ND	2.05A	1.13A	ND	ND	10 [5]
Heucaliptol			MS	4.31A	4.36A	0.84A	0.57A	ND	ND	—
Styrene	1,263	1,260	MS, LRI	ND	ND	0.84A	1.66A	ND	ND	730 [5]
Hexane			MS	3.02A	3.98A	14.40B	17.93A	4.68A	4.30A	—
Heptane			MS	6.52A	4.76A	18.45A	24.23A	5.43A	2.20A	950 [9]
Trichloromethane			MS	ND	1.72A	ND	ND	ND	9.03A	—
3-Methyl-pyridine			MS	ND	ND	1.56A	1.47A	ND	ND	—

A,BValues in the same row for each trial with different superscripts are different at $P < 0.05$.

[1]LRI = linear retention index; Lit. = LRI taken from Bianchi et al. (2007); Exp. = LRI on TR-WAX MS column (Agilent, Santa Clara, CA), experimentally determined using homologous series of C8-C30 alkanes; R = identification method; CA = trial by direct acidification (citric acid); SI = trial by sieroinnesto fermentation (natural whey starter). ND = not detected.

[2]OT = odor threshold in water. References: [1] Buttery et al., 1988; [2] Burdock, 2005; [3] Fors, 1988; [4] Cometto-Muñiz et al., 2004; [5] Blank et al., 1992; [6] Buttery et al., 1990; [7] Takeoka et al., 1990; [8] Cometto-Muñiz and Abraham, 2010; [9] Nagata, 2003.

those reported by Toso et al. (2002) in refrigerated raw milk. Alcohols and acids represented another important part of the VOC mixture, followed by esters that derive from their condensation. Esters are powerful odorants in milk and cheeses, having a low odor threshold (Moio et al., 1993b; Curioni and Bosset, 2002). Their concentration in the SI samples corresponded to about 38% of the sum of the above precursors, against only 3% in CA samples, suggesting that their formation should be microbiologically mediated. Elaboration of the data by PCA allowed clear separation of the samples (Figure 2). The PCA explained 64% of variance, 37% of which was along the first component and 27% along the second component. Along principal component 1, all SI samples were positioned in the positive quadrant due to higher richness of compounds, whereas the CA samples were in the negative quadrant. Principal component 2 allowed clustering of the SI samples into 2 subgroups, one of which laid in the positive area due to higher level of esters, indicating some heterogeneity for the traditional cheese samples. As to the single VOC (Table 5), the only qualitative differences between technologies regarded diacetyl and 3-methyl-2-buten-1-ol, which were only detected in traditional mozzarella. Besides these 2 compounds, the SI samples were discriminated from CA by higher concentrations of acetoin and related conversion product 2-butanone, 3-methyl butanal, 2–3 pentanedione, and 2-nonanone. Other discriminative compounds were ethanol, 3-methyl-1-butanol, acetic, butanoic, and decanoic acids. Also ethyl acetate was a good discriminant compound, which is predictable considering the high concentration of its precursors in traditional mozzarella. To estimate the role of VOC on flavor, previously published threshold data were applied with relative abundance results to generate estimated OAV. Table 6 shows the possible aromatic backbone of the cheeses, composed of the aroma-active compounds with OAV > 1. Some of them exceeded the value in all cheesemaking trials, others only in 2 or just 1. In particular, 3-methyl butanal, nonanal, diacetyl, and ethyl acetate always acted as aroma-active compounds, whereas hexanal, heptanal, octanal, ethanol, and phenylethyl alcohol exceeded value 1 in 2 trials. The results agree with those reported by other authors in studies on fresh or model cheeses (Urbach, 1997; Ruggirello et al., 2018), but a specific outcome of the present study regarded 3-methyl-butanal. It was found to be the most relevant key compound in mozzarella: it has malty/nutty/fresh cheese odor and is much more present in the traditional product, in which can be formed both by lactic acid bacteria and yeast metabolism (Afzal et al., 2017). The other key compounds, hexanal, eptanal, and nonanal, are responsible for green/grass odor; ethyl acetate has ethereal and

Table 6. Volatile organic compounds (VOC) exceeding value 1 for odor activity value in traditional (SI) and direct acidification (CA) mozzarella, odor threshold in water (OT), and relative odor description[1]

VOC	Trial 1			Trial 2			Trial 3			OT (μg/L)	Odor description
	SI	CA	Sig	SI	CA	Sig	SI	CA	Sig		
3-Methyl butanal	39.5	17.3	*	54.5	13.9	*	236.4	28.5	*	0.2	Fruity, apple
Hexanal	4.4	1.0	*	—	1.0	*	—	—		4.5	Green, fatty, fruity
Heptanal	—	—		—	1.2		2.2	—	*	5.87	Fatty, fruity
Octanal	3.3	—	*	2.1	2.5		—	—		1.4	Fruity
Nonanal	22.9	9.6	*	32.6	27.5		16.2	10.2		1.0	Fatty, citrus, green
Decanal	—	—		36.3	75.2	*	—	—		0.1	Flower, fatty
Diacetyl	2.3	—	*	6.6	—		1.7	—	*	2.3	Sweet, buttery, creamy
Ethanol	—	—		3.4	1.0	*	16.6	2.9	*	8.0	Pleasant, fragrant, alcohol
Phenylethyl alcohol	1.5	1.1		1.2	—	*	—	—		1.2	Rose
Dimethyl sulfide	—	—		—	3.5	*	—	5.0	*	0.3	Unpleasant wild radish, cabbage
Ethyl acetate	1.9	—		2.8	—	*	25.0	—	*	5.0	Pineapple
α-Pinene	3.4	2.3		—	—		—	—		2.5	Pine, turpentine

[1]Sig = significantly different at *$P < 0.05$. For OT details, see Table 5.

fruity notes; diacetyl is well known as being responsible for butter odor; and phenylethanol has a rose/flowery odor. This latter derives from phenylalanine metabolism (Smit et al., 2005) and has been reported to be an important aromatic compound of buffalo mozzarella, in which it is produced by some lactococcal and *Streptococcus thermophilus* strains and yeasts (Moio et al., 1993b; Mauriello et al., 2003).

In conclusion, the present study suggested that traditional mozzarella can be discriminated both by sensory and SPME-GC/MS analysis. The results obtained can be very useful in view of European Union PDO labeling of the traditional product, when the question of protecting it from imitations will arise. Further research is needed to validate the results on commercial samples.

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ORCIDS

M. Faccia ⓘ https://orcid.org/0000-0001-9496-9151
P. De Palo ⓘ https://orcid.org/0000-0002-5612-1691