

Evolution of volatile compounds from milk to curd during manufacturing of Mozzarella

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Abstract

The aim of this study was to investigate the evolution of the volatile compounds (VOC) from milk to curd during mozzarella manufacturing, in connection with the technique used for curd acidification (traditional = natural whey starter fermentation; industrial = direct acidification by citric acid addition). Overall, 40 compounds were identified from the entire set of samples, belonging to different chemical classes. All compounds detected in milk were also found in the curd, but at much higher concentration. In addition, many other compounds formed during curd production. By comparison of the samples from the 2 acidification techniques, and analysing the scientific literature to explain the source of the volatile compounds, it was found that microbial activity played a main role in VOC formation. In fact, the curd obtained by natural whey starter fermentation showed the most complex profile, whereas that of the curd obtained by direct acidification was much simpler. The most important odour-active compounds that could contribute to flavour of the curd made by traditional technology were 3-methylbutanal, ethyl acetate and 2,3-butanedione, responsible for ethereal, fruity and buttery odour. For direct acidification they were 3-methylbutanal (at lower level), nonanal and decanal (herbal/fruity odour). The PCA analysis showed clear clusterization of the three types of samples: milks and direct acidified curds were rather close in the bi-plot graph, whereas traditional curds were spread all along the space. The research is currently in progress, for assessing the evolution of the volatile compounds during the next technological steps of mozzarella manufacturing.

Key words: volatile organic compounds, milk, curd, mozzarella, acidification

Introduction

Table mozzarella (high moisture type, 60-65 % water content) is the most popular among the “pasta filata” cheeses, a group of dairy products character-

ised by rearrangement of the curd by plasticization and stretching process using hot water (McMahon and Oberg, 2017). It is traditionally manufactured from cow's or buffalo milk, even though the procedure for making it from sheep and goat milk has

been recently reported (Faccia et al., 2015). For plasticization the curd needs to be suitably acidified, in order to cause partial casein demineralization. For cow's mozzarella, acidification can be performed by two different ways: direct addition of organic acids to milk or fermentation of the curd by lactic acid bacteria (Guidone et al., 2016). Fermentation by natural autochthonous whey culture represents the original method, but currently it is only used in traditional dairies due high costs and difficulty in standardization. Differently, direct acidification by adding citric or lactic acid is mostly used in industrial dairies. Despite of the worldwide manufacturing of mozzarella, scientific information on aroma of this cheese are very scarce (Moio et al., 1993; Pisano et al., 2016). The acidification step should be an important phase for the development of aroma in mozzarella, since the product does not undergo the ripening process and is kept under refrigeration until consumption (Faccia et al., 2014; 2019). In general, only few studies have dealt with the formation of VOCs from milk to curd (Partidário et al., 1998). However, the VOCs profile of the curd is expected to depend on the type of cheese, since the processes for curd production strongly vary. The aim of the present study was to investigate the evolution of VOCs from milk to curd during the manufacturing process of table cow's mozzarella. In particular, acidification by direct addition of citric acid and by whey starter fermentation were compared by using the Solid Phase Microextraction technique (SPME) coupled to mass-spectrometry gas chromatography (GC-MS).

Materials and methods

Milk and curd samples

The samples were obtained by 3 cheese making trials performed at a traditional dairy located in the town of Gioia del Colle (Puglia, Italy). Each trial was composed of two vats containing 100 L raw milk taken from the same farm, which were processed by direct acidification and whey starter fermentation, respectively. For direct acidification curd (DAC), 10 % citric acid solution was added to cold milk until pH reached about 5.7, then rennet was added for coagulation. For the whey starter curd

(WSC), 3 % v/v autochthonous whey starter culture was added to milk at room temperature, and after about 10 minutes, rennet was added. In this case, milk coagulation took place at pH 6.1-6.2. Autochthonous starter derived from the whey obtained from cheese making of the previous day, heated to 44 °C and left to ferment at room temperature for about 24 h. In both cases, after acid or starter addition the milk was coagulated at 36 °C by addition of calf rennet at the doses normally used in the factory (18 and 25 g 100 L⁻¹ of milk for DAC and WSC, respectively. The difference depended on the different pH value in milk). After coagulation, the curd was cut to the size of a hazelnut grain and left to rest. Then, in the direct acidification process the whey was removed after 10 min since the curd was already demineralised and ready to be stretched. Differently, for the whey starter process, the whey was drained after 3 hours in order to allow the curd maturation to reach the suitable "stretching pH" (about 5.2-5.3). In both cases, after whey removal, samples of curd were taken, put into plastic bags under refrigerated conditions and transported to the laboratories for VOCs analyses.

Chemical and VOCs analysis

Milk and curds underwent to the following chemical analyses: for milk, pH (pH meter with glass electrode, Hamilton, USA), fat, protein and lactose (infrared analysis by Milko Scan FT1, Foss, Denmark); for curd, pH (pH meter with Polyplast penetration electrode, Hamilton, USA), moisture (IDF, 1986), fat (Soxhlet method), total protein (Kjeldhal method), lactose (HPLC with refractive index detection as indicated by Trani et al., 2017). The volatile compounds were extracted from milk and curd samples by solid phase micro-extraction (SPME). The samples were weighed (1±0.05 g) into 20 mL vials, closed by a rubber septum and an aluminium cap; for milk sample 0.2 g NaCl was added to increase the ionic strength. Both samples were added with internal standard (81.3 ng 3-pentanone) to perform a semi-quantitation. Before extraction, stabilization of the headspace in the vial was obtained by equilibration for 10 min at 37 °C. The extraction was carried out using a divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) 50/30 mm SPME fibre assembly (Supelco,

Bellefonte, PA, USA) at 37°C for 15 min. The fibre was desorbed at 220 °C for 2 min in the injection port of the gas chromatograph, operating in splitless mode. The GC-MS analyses were performed using a Trace1300 gas chromatograph equipped with a mass spectrometer ISQ Series 3.2 SP1. The compounds were separated on a Thermo capillary column TR-WAX MS (20 m, 0.1mm, 0.1 mm), under the following conditions: injection port temperature, 220 °C; oven temperatures, 50 °C for 0.1 min then 13 °C min⁻¹ to 180 °C, 18 °C min⁻¹ to 220 °C final isothermal for 1.5 min for curd samples and 3 min for milk samples. Mass detector was set at the following conditions: detector voltage, 1700 V; source temperature, 250 °C; ionization energy, 70 eV; scan range 33-200 amu. Peak identification was done by means of Xcalibur V2.0 software, in particular Qual Browse, by matching with the reference mass spectra of NIST library.

Statistical analysis

Statistical treatment of the data was carried out using XLSTAT software (Microsoft) for one-way analysis of variance (ANOVA), and OriginPro2017 (OriginLab Corporation) for PCA analysis.

TABLE 1. Chemical gross composition (%) of the milk and curds from the experimentation (average of 3 cheese making trials)

	Milk	DAC curd	WSC curd
pH	6.74 (±0.02)	5.65 (±0.03) ^a	5.23 (±0.05) ^b
Dry matter	86.97 (±0.16)	55.4 (±2.2)	53.78 (±1.4)
Protein	3.33 (±0.04)	20.51 (±0.9)	21.11 (±0.6)
Fat	3.72 (±0.11)	22.52 (±1.8)	23.33 (±0.9)
Lactose	4.86 (±0.02)	1.52 (±0.2) ^a	0.51 (±0.11) ^b

Values bearing different superscripts are different at P<0.05

Results and discussion

The average chemical gross composition of milk and curds from the experimentation is shown in Table 1. The milk used in the three cheese making trials had only slight variations, whereas significant differences were observed in pH and lactose concentration in the curds. They were expected, since these two chemical parameters are

closely related to the method of acidification. The difference in pH depends on the kinetic of casein demineralization: DAC method is more effective in sequestering calcium from the casein micelle than WSC, and curd stretching takes place at higher pH (Mucchetti and Neviani, 2006). However, such differences are not so high to expect deep variations in VOC formation (5.65 versus 5.23, respectively). The difference in lactose are connected to different microbial activity: DAC does not involve lactic fermentation, and lactose is poorly degraded; differently, the WSC method is based on lactic fermentation, and part of the sugar is metabolized by the starter microflora. Table 2 shows the volatile compounds identified in the entire set of samples. Overall, 40 compounds were found: 7 aldehydes, 10 methyl ketones, 8 alcohols, 2 sulphur compounds, 8 acids, 2 esters, 1 terpene, 2 aliphatic hydrocarbons. Figure 1 resumes the evolution of the volatile compounds during processing. None of the milk compounds disappeared, however, they represented a very low percentage of total VOCs in the curds. As expected, the volatile profile of milk was poorer than that of the curd, both under the qualitative and quantitative point of view. Several compounds were found both in milk and curd, others were newly formed. Among the former, acetic acid had huge increase during processing: it was 42 and 13 times higher in WSC and DAC, respectively than in milk. According to the literature, in fresh dairy products it mainly derives from bacterial metabolism of citrate and sugars, whereas in ripened cheese it is also formed by oxidative deamination of glycine, alanine and serine (Partidário et al., 1998). Another important compound was nonanal; its concentration was about 9 times higher in WSC and 5 times higher in DAC than milk. It should derive from β-oxidation of unsaturated fatty acids (McSweeney and Sousa, 2000). Concentration of hexanoic and butanoic acids almost triplicated in WSC samples, whereas a less significant increase was found in DAC. Since these compounds mainly derive from enzymatic degradation of milk triglycerides, the differences between the 2 types of sample should be ascribed to some lipolytic activity by the starter microflora. Other compounds had slight increase, but with low or no statistical significance, such as heptanal, 6-methyl-5-hepten-2-one, dimethyl sulfone and nonanoic acid. Among the newly formed

TABLE 2. Total volatile organic compounds detected in milk (M), citric acid curd (DAC) and whey starter curd (WSC), expressed as ppb

	M	DAC	WSC
Aldehydes			
3-methyl butanal	-	71.07 ^B	215.66 ^A
hexanal	1.04 ^A	2.58 ^A	12.08 ^A
heptanal	0.20 ^A	1.05 ^A	2.48 ^A
octanal	0.49 ^B	1.43 ^{AB}	4.08 ^A
nonanal	3.52 ^B	14.84 ^{AB}	27.98 ^A
decanal	2.01 ^A	2.03 ^A	2.62 ^A
benzaldehyde	-	-	17.08
Ketones			
acetone	67.18 ^A	52.25 ^A	82.27 ^A
2-butanone	4.50 ^A	4.35 ^A	13.92 ^A
Diacetyl (2,3-butanedione)	-	30.35 ^B	159.42 ^A
2,3 pentanedione	-	0.69 ^B	28.48 ^A
2-heptanone	-	4.62 ^B	18.46 ^A
acetoin	-	207.24 ^B	865.79 ^A
6-methyl-5-hepten-2-one	0.88 ^A	1.72 ^A	2.01 ^A
2-hydroxy-3-pentanone	-	-	5.71
2-nonanone	-	1.06 ^B	4.37 ^A
2-undecanone	-	-	0.85
Alcohols			
ethanol	-	6.48 ^B	461.09 ^A
3-methyl,1-butanol	-	26.88 ^B	538.68 ^A
3-methyl-2-buten-1-ol	-	-	1.26
2-methoxy-1-methylethoxy,2-propanol	0.28 ^A	1.45 ^A	0.76 ^A
2-propyl-1-pentanol	1.48 ^A	-	0.30 ^A
2-methoxypropoxy, 2-propanol	-	2.35 ^A	0.68 ^A
2-octyloxy-ethanol	-	20.58 ^A	6.31 ^A
phenylethyl alcohol	-	1.04 ^B	4.37 ^A
Sulfur compounds			
dimethyl sulfide	0.12 ^B	2.64 ^A	-
dimethyl sulfone	1.97 ^A	1.29 ^A	2.06 ^A
Acids			
acetic acid	2.69 ^B	35.66 ^B	109.91 ^A
butanoic acid	5.09 ^B	6.82 ^B	15.57 ^A
3-methyl-butanoic acid	-	0.47 ^A	0.69 ^A
hexanoic acid	7.87 ^B	9.41 ^B	20.79 ^A
heptanoic acid	-	0.60 ^A	1.11 ^A
octanoic acid	4.96 ^{AB}	3.75 ^B	8.25 ^A
nonanoic acid	1.37 ^A	2.12 ^A	2.08 ^A
decanoic acid	3.37 ^A	0.95 ^B	2.65 ^{AB}
Esters			
ethyl acetate	-	3.71 ^B	685.36 ^A
1-butanol-3-methyl-acetate	-	-	1.945
Miscellaneous			
styrene	-	0.71	-
hexane	1.27 ^A	2.06 ^A	4.24 ^A
heptane	0.76 ^B	3.54 ^{AB}	26.31 ^A

compounds during processing, 3-methylbutanal was much more abundant in WSC (3 times higher) than DAC. In foods, it is generated by non-enzymatic browning reaction of leucine induced by heating (Contarini et al., 1997), but in our study the heating level during processing was too low for

allowing significant Maillard reaction. As a consequence, it should derive by bacteria and yeast activity on leucine or isoleucine (Valero et al., 2001; Afzal et al., 2017). 2-heptanone and 2-nonanone were 4 times higher in WSC: they should come from β -oxidation of saturated fatty acids followed by a

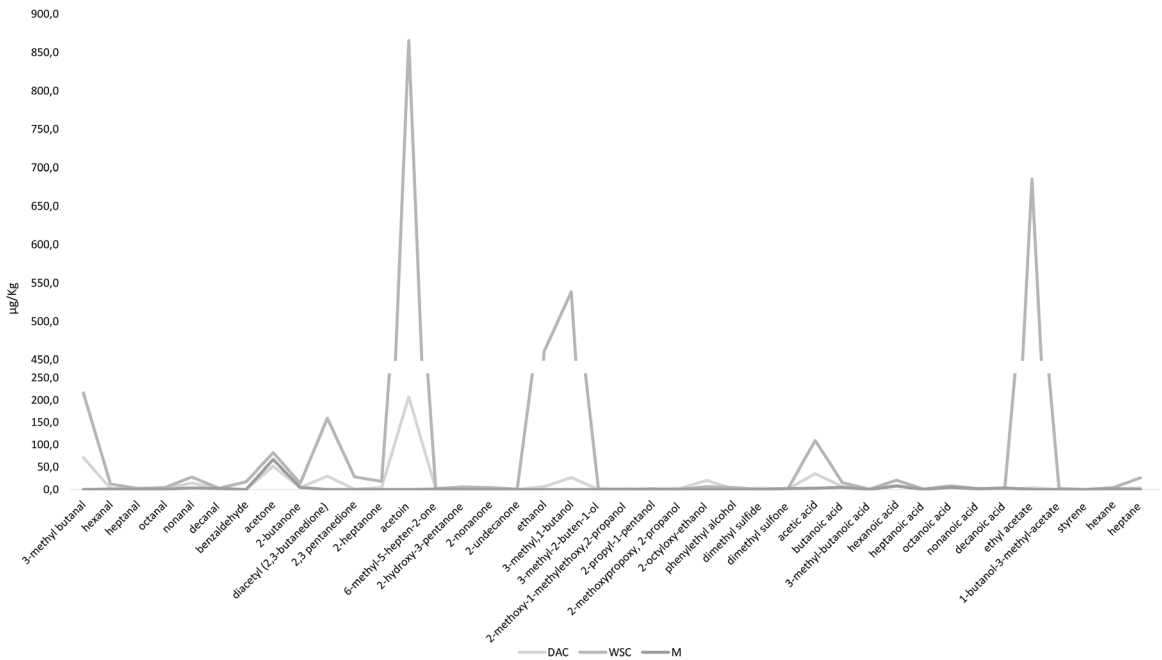


FIGURE 1. Evolution of the volatile compounds in milk (M), direct acidified curd (DAC) and whey starter curd (WSC)

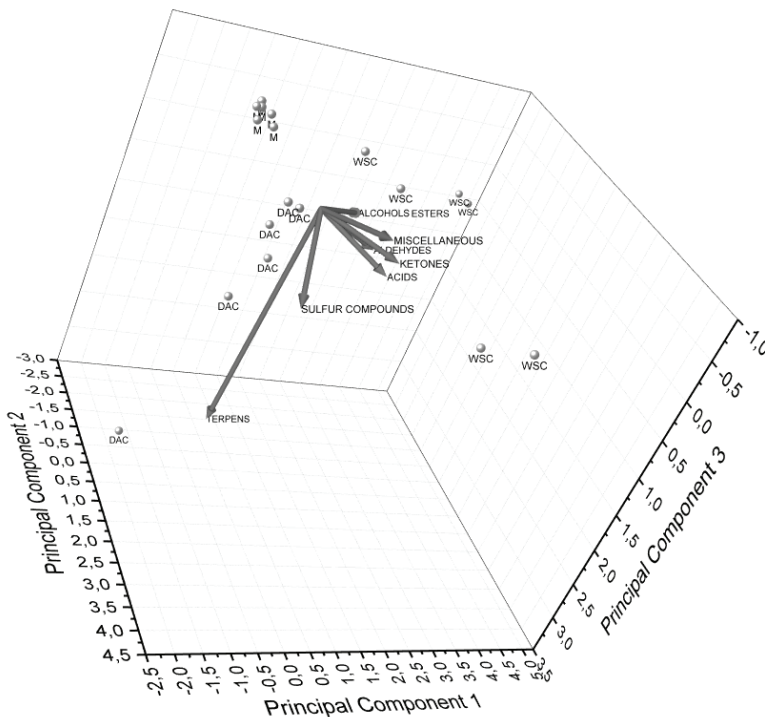


FIGURE 2. Principal Component Analysis of the volatile composition of milk (M), direct acidified curd (DAC) and whey starter curd (WSC)

first decarboxylation and successive β -ketoacids decarboxylation (Dursun et al., 2017). Diacetyl, acetoin and ethanol are known to be primary compounds of volatilome of lactic acid bacteria and yeasts fermentation, respectively (Bassette et al., 1966; Zeppa et al., 2008; Bertuzzi et al., 2018). As expected, their concentration was much higher in WSC than DAC: diacetyl and acetoin were 4-5 times higher, whereas ethanol was about 76 times higher. This latter result suggests an important role of yeasts: in fact, these microorganisms are common compounds of microbiota of the natural whey starters for mozzarella (Binetti et al., 2013; Trani et al., 2016). A second alcohol, 3-methylbutanol, should be a conversion product of 3-methylbutanal:

it was detected at very high level in WSC (20 times higher than DAC). Ethyl acetate was another characterizing compound of WSC samples: on the average, it was 28 times higher than in DAC: it could have been generated by heat-catalysed esterification reactions between ethanol and acetic acid (Vazquez Landaverde et al., 2005). Some compounds were only detected in WSC: benzaldehyde, 2-hydroxy-3-pentanone, 2-undecanone, 3-methyl-2-buten-1-ol, 1-butanol-3-methyl-acetate. They are mostly generated during curd maturation due to bacterial fermentations, proteolysis or lipolysis (Bassette et al., 1966; Yvon et al., 2001; Bertuzzi et al., 2018).

TABLE 3. "Aromatic backbone" of milk (M), direct acidified curd (DAC) and whey starter curd (WSC) and VOCs odour description

	Aroma value			OT	Odour description
	M	CCA	CWS		
Aldehydes					
3-methyl butanal*	0	355	1078	0,2[1]	Powerful penetrating, acrid odour, apple-like odour
hexanal	0	1	3	4,5[2]	Green, fatty, fruity
octanal*	0	1	3	1,4[3]	Aldehydic, waxy, citrus orange with a green peely nuance
nonanal*	4	15	28	1[3]	Sweet, orange, orange peel
decanal	20	20	26	0,1[2]	Sweet, aldehydic, orange, waxy and citrus rind
Ketones					
2,3-butanedione*	0	13	69	2,3[4]	strong buttery
2,3-pentanedione*	0	0	1	30[5]	buttery diacetyl-like, fermented dairy and creamy, popcorn buttery
acetoin*	0	0	1	800[1]	Intense buttery, creamy
2-nonanone*	0	0	1	5[2]	Fruity, sweet, waxy, soapy, cheese, green herbaceous, coconut like
Alcohols					
ethanol	0	1	58	8[3]	Pleasant, weak, ethereal, vinous odour
3-methyl,1-butanol*	0	0	8	71[6]	Banana, alcohol, fruity
phenylethyl alcohol*	0	1	4	1,2[3]	Characteristic rose-like odour
Sulfur compounds					
dimethyl sulfide*	0	9	0	0,3[1]	Unpleasant odour of wild radish, cabbage-like
Esters					
ethyl acetate*	0	1	137	5[3]	Ethereal, fruity, green

* = significantly different at $p < 0.05$. OT= Odour threshold ($\mu\text{g/L}$) in water

[1] Buttery, 1990; [2] Buttery, 1988; [3] Burdock, 2005; [4] Fors, 1998; [5] Blank, 1992; [6] Czerny, 2008

Table 3 shows the “aromatic backbone” of the two types of mozzarella. It is composed of the VOCs that exceeded the aroma threshold concentration (Aroma Value >1), calculated on the basis of bibliographic data. The milk samples had just 2 compounds that exceeded such value: decanal and nonanal, which presents herbal/fruity odour. These two compounds were also among the most important odour-active VOCs in DAC samples, in which 10 compounds exceeded AV>1. The most important of them was 3-methylbutanal (AV=355), which is responsible of acrid or apple-like odour, depending on the concentration. The WSC samples presented the most complex aromatic backbone, with 13 compounds potentially involved into aroma perception. The most important were 3-methylbutanal (AV=1078), ethyl acetate (AV=137) and 2,3-butanedione (AV=69), responsible for ethereal, fruity and buttery odour. These results point out the huge importance of fermentations on the development of aroma in mozzarella and confirm that the “primary aroma” (i.e. odour-active compounds already present in milk) can play a role only in the case of direct acidification technology. This consideration is further supported by Figure 2, which reports the Principal Component Analysis of the data set. As it can be seen, the milk samples are all gathered together, and the DAC ones are near them (except for one that is characterized by characteristic terpenes content). Differently, the WSC samples are spread all along the space. Being the graph a bi-plot, it is

possible to observe that the WSC scores (dots) and loadings (arrow, variable correlation) are mostly on the same direction, which means that such samples are richer in esters, methyl ketones, acids and alcohols than the other samples.

Conclusions

The main outcome of the present experimentation was the description of the differences in the VOC profile of the two types of curds. Considering that the two acidification techniques only differed in the presence/absence of starter added, the most part of the differences observed should be connected to different microbial activity. This conclusion is also supported by the fact that, according to the scientific literature, the most abundant compounds detected derives from microbial metabolism. Among the possible odour-active compounds, only 2 were also detected in milk, suggesting scarce contribution of milk VOC to curd aroma. It was concluded that the VOC profile in curd from making mozzarella is mainly driven by microbial metabolism rather than to a concentration effect consequent to coagulation and whey drainage. The research is in progress, for assessing the evolution of the volatile compounds during the next technological steps: curd stretching, mozzarella cooling, and packaging.

Razvoj hlapivih mliječnih spojeva u grušu tijekom proizvodnje sira Mozzarella

Sažetak

Cilj ovog rada bio je istražiti razvoj hlapivih mliječnih spojeva (VOC) u grušu tijekom proizvodnje sira Mozzarella, ovisno o načinu zakiseljavanja sirnog gruša (tradicionalno = prirodno zakiseljavanje sirutke starter kulturama; industrijski = direktno zakiseljavanje dodatkom limunske kiseline). Iz svih testiranih uzoraka identificirano je ukupno oko 40 spojeva različitog kemijskog sastava. Svi spojevi pronađeni u mlijeku bili su prisutni i u grušu, no u puno višim koncentracijama. Osim toga, u gruševima su bili prisutni i mnogi drugi spojevi. Usporedbom uzoraka zakiseljenih na 2 različita načina utvrđeno je kako je mikrobna aktivnost igrala presudnu ulogu u formiranju VOC. Točnije, gruš zakiseljen prirodnim putem (starter kulturom) imao je najsloženiji profil VOC, dok je gruš zakiseljen dodatkom limunske kiseline bio puno jednostavnijeg sastava. Spojevi koji su u prirodno zakiseljenom grušu najviše doprinosili formiranju okusa i mirisa bili su 3-metilbutanal, etil acetat i 2,3-butandion, a koji su inače nosioci mirisa po

eteru, voću i maslacu. U slučaju gruša zakiseljenog dodatkom limunske kiseline, glavni nosioci okusa i mirisa bili su 3-metilbutanal (niska koncentracija), nonanal i dekanal (biljno/voćni miris). PCA analiza je pokazala da dolazi do klasterizacije tri uzorka - mlijeka i gruševa zakiseljenih dodatkom limunske kiseline, dok su prirodno zakiseljeni gruševi bili raspoređeni u širem području. U tijeku je istraživanje kojem je cilj odrediti razvoj hlapivih spojeva tijekom preostalih tehnoloških koraka u procesu proizvodnje sira Mozzarella.

Ključne riječi: hlapivi organski spojevi, mlijeko, gruš, Mozzarella, zakiseljavanje

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