LWT - Food Science and Technology 140 (2021) 110845

Contents lists available at ScienceDirect

LWT

journal homepage: www.elsevier.com/locate/lwt

Comparison of lactose free and traditional mozzarella cheese during shelf-life by aroma compounds and sensory analysis

Fabrizio Cincotta [a], Concetta Condurso [a,*], Gianluca Tripodi [b], Maria Merlino [a], Ottavia Prestia [a], Catherine Stanton [c,d], Antonella Verzera [a]

[a] Department of Veterinary Sciences, University of Messina, Polo Universitario Dell'Annunziata, 98168, Messina, Italy
[b] Department of Human Sciences and Promoting of the Quality of Life, San Raffaele Telematic University, 00166, Rome, Italy
[c] APC Microbiome Ireland, University College Cork, Western Road, T12 YN60, Cork, Ireland
[d] Teagasc Food Research Centre, Food Biosciences Department, Moorepark, Fermoy, Co Cork, P61 C996, Ireland

ARTICLE INFO

Keywords:
Mozzarella cheese
Lactose free
Shelf-life
Aroma compounds
Sensory features

ABSTRACT

Aroma compounds and sensory features of lactose free (LFM) and traditional (TM) Mozzarella cheese have been investigated during their labeled shelf-life. Acetoin and 2-heptanone characterized both types of cheese at the production time. During the shelf-life, a statistically significant increase in the amount of the volatiles coming from amino acid and fatty acid metabolism occurred in the LFM samples after 8 days of storage and, to a lesser extent, in TM cheese after 13 days of storage. As regard sensory analysis, milk odor and milk flavor descriptors characterized TM and LFM in the early stage of their shelf-life; bitter and acid taste and yoghurt odor descriptors characterized LFM after 8 days and TM after 13 days. The differences between the two cheese types can be attributed to the proteolytic activity of the lactase enzyme. As a result, the volatile aroma profile and the sensory quality should be taken into account for a proper shelf-life definition of Mozzarella cheese and a shorter shelf-life should be suggested for LFM than TM cheese.

1. Introduction

The shelf-life extension of Mozzarella Cheese is a subject of great interest and recently it has been largely investigated due to the high foreign demand and the increase exports (Braghieri et al., 2018; Faccia, Gambacorta, Natrella, & Caponio, 2019; Gorrasi et al., 2016; Luz, Torrijos, Quiles, Mañes, & Meca, 2019).

Mozzarella cheese is a traditional Italian Pasta-filata cheese mainly produced with bovine milk. On the basis of the moisture content two different types of mozzarella cheese can be defined: 1) low-moisture mozzarella (47–48% water content) typically used for cooking procedures such as dressing pizza; 2) high moisture mozzarella (60–65% water content) mainly used as table cheese. High moisture Mozzarella is particularly appreciated for its freshness and fresh milk flavors and it remains one of the most consumed dairy products worldwide (Faccia et al., 2019; Francolino, Locci, Ghiglietti, Iezzi, & Mucchetti, 2010; Jana & Mandal, 2011). To preserve the freshness characteristics during the shelf-life, high moisture Mozzarella is packaged in brine. This condition increases the probability of deterioration in terms of microbial growth, chemical reactions and mass transfer between the product and the preserving liquid leading to the generation of off-flavors, chromatic alteration and changes in structure (Faccia, Mastromatteo, Conte, & Del Nobile, 2012).

Since lactose intolerance affects approximately 75% of the world population, many dairy companies produce lactose free Mozzarella cheeses which are now widely present on the market.

The shelf-life of high moisture Mozzarella, including the lactose free one, commonly ranges from 1 to 2 weeks (Gammariello, Conte, Attanasio, & Del Nobile, 2010; Ricciardi et al., 2015). Researches for the shelf-life extension are based on the addition of antimicrobial compounds in the storage liquid (called conditioning brine), use of specific starter cultures, conditioning brines with different compositions and freezing (Alinovi, Corredig, Mucchetti, & Carini, 2020; Alinovi & Mucchetti, 2020; Braghieri et al., 2018; Facciaet al., 2019; Gorrasi et al., 2016). However, very little is known about the stability of lactose free products and no research has been performed on lactose free Mozzarella cheese. Some researchers suggest that lactose free products are more likely to undergo to Maillard reaction due the presence of a higher

* Corresponding author.
E-mail address: cconcurso@unime.it (C. Condurso).

https://doi.org/10.1016/j.lwt.2020.110845
Received 17 August 2020; Received in revised form 16 December 2020; Accepted 29 December 2020
Available online 2 January 2021

amount of reducing sugars and an increased level of free amino acids than a product containing unhydrolyzed lactose (Jansson et al., 2014; Troise et al., 2016).

Microbiological and sensory parameters are generally used to define shelf-life of Mozzarella cheese. To the best of our knowledge, no research has taken into account the aroma volatile compounds for the shelf-life monitoring of Mozzarella cheese although it is common for other types of cheese (Condurso, Verzera, Romeo, Ziino, & Conte, 2008; Nzekoue et al., 2019). Studies which have focused on Mozzarella cheese aroma compounds only deal with the influence of different calves' diet or the use of different acidification methods (Natrella, Faccia, Lorenzo, De Palo, & Gambacorta, 2020; Sabia, Gauly, Napolitano, Cifuni, & Claps, 2020; Sacchi et al., 2020).

In this context, this study aimed to verify the stability of Traditional Mozzarella (TM) and Lactose Free Mozzarella (LFM) cheese and the importance of volatile aroma compounds and sensory features in the shelf-life definition of these products.

2. Materials and methods

2.1. Preparation of mozzarella cheese

TM and LFM cheese samples were produced by a local dairy industry, manufactured using the same standardized cow's milk (3.20 g/100 g protein, 3.50 g/100 g fat). After pasteurization (74 °C for 25 s), half of the milk was subjected to the enzymatic process for lactose breakdown using 8000 NLU/L milk of a commercial lactase 5200 NLU/g (HA-Lactase™ 5200, Chr. Hansen Italia S. p.A, Parma, Italy). Citric acid (1.2 g/100 g) and 40 IMCU/L milk of liquid rennet 200 IMCU/mL (CHY -MAX® plus, Chr. Hansen Italia S. p.A, Parma, Italy) were added for the acidification and coagulation of the milk. Both types of cheese were prepared using a highly standardized technology.

Mozzarella was mechanically stretched in hot water (90–95 °C), molded in ~100 g units and then cooled in unsalted water (4 °C). Each Mozzarella was individually packaged in polyethylene plastic bags with a preservation liquid made from potable water, calcium chloride (6.7 g/L) and sodium chloride (4 g/L). Samples were kept at + 4 °C for all the shelf-life and analyzed at five different storage times, namely at production day (0) and after 4, 8, 13 and 20 days. At any set time volatile and sensory analyses were carried out in triplicate within the same day. Production was repeated three time on three different days.

2.2. Volatile extraction: HS-SPME

For the isolation and concentration of volatiles, the headspace solid phase microextraction (HS-SPME) technique was used. In particular, a 40 mL vial equipped with a "mininert" valve (Supelco, Bellefonte, PA, USA) was filled with 10 g, exactly weighed, of each chopped and homogenized sample, and 10 mL of NaCl saturated aqueous solution were added. Extraction was performed in the headspace vial kept at 40 °C using a 50/30 μm Divinylbenzene/Carboxen/Polydimethylsiloxane (DVB/CAR/PDMS) fiber (Supelco, Bellefonte, PA, USA), housed in its manual holder (Supelco, Bellefonte, PA, USA). The fiber was activated according to the manufacturer's instructions. The sample was equilibrated for 20 min and then extracted for 30 min; during the extraction, the sample was continuously stirred. After sampling, the SPME fiber was introduced onto the splitless injector of the GC/MS and kept there for 3 min at 260 °C for the thermal desorption of the analytes onto the capillary GC column.

2.3. Volatile analysis: GC-MS analysis

A Shimadzu GC 2010 Plus gas chromatograph directly interfaced with a TQMS 8040 triple quadrupole mass spectrometer (Shimadzu, Milan, Italy) was used. The conditions were: injector temperature, 260 °C; injection mode, splitless; capillary column, VF-WAXms, 60 m × 0.25 mm i. d. × 0.25-μm film thickness (Agilent, S. p.a. Milan, Italy); oven temperature, 45 °C held for 5min, then increased to 80 °C at a rate of 10 °C/min and to 240 °C at 2 °C/min held for 5min; carrier gas, helium at a constant flow of 1 mL/min; transfer line temperature, 250 °C; acquisition range, 40–400 m/z; scan speed, 1250 amu/s. Each compound was identified using mass spectral data, NIST' 18 (NIST/EPA/NIH Mass Spectra Library, version 2.0, USA) and FFNSC 3.0 database, linear retention indices (LRI), literature data and the injection of the available standards, as previously reported (Cincotta, Verzera, Tripodi, & Condurso, 2018). The volatile compounds were quantified using the method of standard additions as previously reported by Condurso, Cincotta, Merlino, Stanton, and Verzera (2020). A mother solution was prepared using 2-heptanone (≥99.0%), acetoin (monomer, 99.0%), benzaldehyde (≥99.5%) ethanol (≥99.9%), 3-methyl-1-butanol (≥98.5%), 2-heptanol (≥98.0%), 2,3-butanediol (≥97.0%), phenylethyl alcohol (≥99.0%), acetic acid (≥99.99%), 3-methylbutanoic acid (≥98.5%), octanoic acid (≥99.5%), δ-octalactone (≥98.0%), δ-decalactone (≥98.0%), γ-dodecalactone (≥98.0%) analytical standards (Merk Life Science S. r.l., Milan, Italy) each at a concentration twenty times that one present in the cheese samples.

Four working solutions were prepared by 1:3, 1:2. 1:1 and 2:3 dilutions of the mother one and added (1,0 mL) to four aliquots of each cheese sample. The spiked cheese samples and sample alone (not spiked) were extracted and analyzed in triplicate by HS-SPME–GC–MS as previously described. Quantitation was based on a five-point calibration curve generated by plotting detector response versus the amount spiked of each standard.

2.4. Sensory analysis

Qualitative Descriptive Sensory Analysis (QDA) was performed according to ISO 13299 (ISO, 2003) using a trained sensory panel consisting of 8 assessors, 4 males and 4 females, between 21 and 30 years old recruited among the students of the Department of Veterinary Science at Messina University. The assessors were selected among who habitually consumed mozzarella cheese and trained according to ISO 8586-1 (ISO, 1993); the analyses were carried out in a sensory laboratory according to ISO 8589 (ISO, 1988).

In details the panel was subjected to a 6-week training period. During this period, TM and LFM cheeses of different brands were used to validate the assessors, to familiarize them with the product and procedures and to develop a common vocabulary to describe unequivocally their perceptions; assessors were asked to taste mozzarella cheese samples and to describe their taste, odor, flavor, appearance and texture. At that time, a list of attributes and their definitions were developed. Then, standard reference products were settled for each previously identified attribute according to Braghieri et al. (2018). A set of fifteen descriptive terms was developed: white color, smooth surface, milk odor, butter odor, yoghurt odor, acid, bitter, sweet, salty, milk flavor, firmness, elasticity, cohesiveness, gumminess, juiciness. The descriptors were quantified using a nine-point intensity scale, where 1 = not perceptible and 9 = strongly perceptible, on a direct computerized registration system (FIZZ Biosystemes. ver. 2.00 M, Couternon, France). The results were expressed as the average for each sensory attribute.

The work plan provided the evaluation of TM and LFM at five different times of storage in five different sessions, one session per storage time. For each storage time, three replicate measurements were performed in the same session, with a 10 min break between each sample and a total time of 170 min per session. All samples were supplied on polyethylene white dishes labeled with a three-digit random number and served one at a time, in randomized order at a serving temperature of 13 °C. To avoid any effect of color on odor/flavor and taste evaluation, assessors evaluated firstly cheese under red light for odor/flavor, taste and texture attributes, and a second cheese under white fluorescent lighting, for appearance attributes. Unsalted crackers and water were served for cleansing the palate between samples.

2.5. Statistical analysis

Data were statistically analyzed using XLStat software, version 2014.5.03 (Addinsoft, Damremont, Paris, France). Two-way ANOVA (storage time and cheese type), Duncan's test and Principal Component Analysis (PCA) were performed on volatile and sensory data to investigate the differences among samples of different types (TM and LFM) and at different storage times during the shelf-life. The model was statistically significant with a P-value < 0.05.

3. Results and discussion

3.1. Volatile aroma compounds

Table 1 reports the volatile compounds identified in the TM and LFM cheeses along with their linear retention indices (LRI), the method of identification and the references of the earlier identified volatiles in Mozzarella cheese. In total, 54 volatile compounds have been identified belonging to the following classes of substances: ketones, aldehydes, alcohols, esters, acids, terpenes, lactones and hydrocarbons. Most of the identified compounds were present in both types of Mozzarella cheese here analyzed and the majority of them has been previously reported in various studies on Mozzarella cheese (Natrella, Faccia, et al., 2020; Natrella, Gambacorta, De Palo, Lorenzo, & Faccia, 2020; Sabia et al., 2020; Sacchi et al., 2020) but some were found here for the first time. All of the identified volatiles arise from lipolysis, proteolysis, catabolic reactions of free amino acids (FAA) and free fatty acids (FFA), metabolism of residual lactose, lactate and citrate (McSweeney & Sousa, 2000). The biochemical processes which lead to the synthesis of cheese volatile compounds are very complex and are related to the enzymatic activity of the complex microbial populations of the cheeses.

Table 2 reports the quantitative data of the compounds which showed statistically significant differences ($P < 0.05$) during the shelf-life in TM and LFM samples. Among them, acetoin was the quantitatively most represented volatile compound both in TM and LFM samples at production time. At the end of shelf-life, ethanol, acetic acid and octanoic acid prevailed in TM and LFM samples, while 3-methyl-1-butanol only in LFM samples.

In order to better understand the impact of each single compound on TM and LFM shelf-life, Principal Component Analysis (PCA) was applied to the data from Table 2. Fig. 1 reports the PCA loading and score plot: PC1 explains 65.57% of the total variability whereas PC2 explains 17.96%. LFM samples were separated from TM samples along PC2 with LFM in the positive region of PC2 and TM samples in the negative one. Instead, PC1 allowed to differentiate cheese samples at different storage time: TM samples were close to each other in the negative region of PC1 until day 13 and in the positive region of PC1 after 20 days of refrigerated storage; LFM samples were in the negative region until day 8 and in the positive after 13 and 20 days of storage.

The separation by PCA associated with the loadings identified the volatile aroma compounds responsible for this separation. The variables that mostly weighted on the negative region of PC1 were 2-heptanone and acetoin that are considered the main compounds responsible for fresh cheese aroma. 2-Heptanone and, generally, 2-ketones come from β-oxidation of saturated fatty acids and successive decarboxylation of β-ketoacids (Dursun, Güler, & Şekerli, 2017). Acetoin is the main compound associated with citrate metabolism of lactic acid bacteria (McSweeney, Fox, & Ciocia, 2017) and it has a central role in determining the flavor of immature fresh cheese (Curioni & Bosset, 2002); in fact, its amount exceeded its odor threshold of 800 μg/kg (Natrella, Faccia, et al., 2020) in both mozzarella type at production day and, limited to LFM, also at the early stage of storage (4 days). According to Moio, Langlois, Etievant, and Addeo (1993) acetoin is the main ketone in mozzarella and it is characterized by buttery and woody sensory notes.

The variables that mostly weighted on the positive regions of PC1

Table 1
Volatile aroma compounds identified in TM and LFM cheese.

Compounds	LRI[a]	TM	LFM	Literature[b]	Identification[c]
Ketones					
2-Heptanone	1188	x	x	1,2	LRI, MS, St
Acetoin	1299	x	x	1,2,4	LRI, MS, St
1-Hydroxy-2-propanone	1318	-	x	-	LRI, MS, St
6-Methyl-5-hepten-2-one	1343	-	x	1,2	LRI, MS, St
2-Nonanone	1394	x	x	1,2,3	LRI, MS, St
2-Undecanone	1602	x	x	1,2	LRI, MS, St
Acetophenone	1657	x	-	4	LRI, MS, St
Aldehydes					
Hexanal	1093	x	-	1,2,3,4	LRI, MS, St
Nonanal	1400	x	x	1,2,3,4	LRI, MS, St
Furfural	1473	x	-	-	LRI, MS, St
Decanal	1504	x	x	1,2,4	LRI, MS, St
Benzaldehyde	1532	x	x	2,4	LRI, MS, St
Dodecanal	1714	x	x	-	LRI, MS, St
Tetradecanal	1924	x	x	-	LRI, MS, St
Alcohols					
Ethanol	944	x	x	1,2	LRI, MS, St
3-Methyl-1-Butanol	1210	x	x	1,2	LRI, MS, St
2-Heptanol	1321	x	x	-	LRI, MS, St
1-Hexanol	1354	x	x	2	LRI, MS, St
1-Heptanol	1457	-	x	-	LRI, MS, St
1-Octanol	1560	-	x	4	LRI, MS, St
2,3-Butanediol	1584	x	-	4	LRI, MS, St
1-Nonanol	1663	-	x	-	LRI, MS, St
2-Furanmethanol	1670	x	-	-	LRI, MS, St
Phenylethyl alcohol	1918	x	x	1	LRI, MS, St
1-Dodecanol	1969	x	x		LRI, MS, St
Esters					
Isoamyl acetate	1124	-	x	-	LRI, MS, St
Ethyl octanoate	1436	x	x	-	LRI, MS, St
Ethyl decanoate	1641	-	x	-	LRI, MS, St
2-Phenylethyl acetate	1821	x	x	-	LRI, MS, St
Acids					
Acetic acid	1467	x	x	1,2,4	LRI, MS, St
Propanoic acid	1553	x	-	-	LRI, MS, St
Butanoic acid	1644	x	x	1,2,3,4	LRI, MS, St
2-Methylbutanoic acid	1682	x	-	-	LRI, MS, St
3-Methylbutanoic acid	1684	-	x	2	LRI, MS, St
Hexanoic acid	1856	x	x	1,2,3	LRI, MS, St
2-Ethyl hexanoic acid	1960	-	x	-	LRI, MS, St
Heptanoic acid	1964	x	x	1,2	LRI, MS, St
(*E*)-2-Hexenoic acid	1980	x	-	-	LRI, MS
Octanoic acid	2071	x	x	1,2,3	LRI, MS, St
Nonanoic acid	2177	x	x	1,2	LRI, MS, St
Decanoic acid	2280	x	x	1,2	LRI, MS, St
(*E*)-9-Decenoic acid	2345	x	x	-	LRI, MS
(*E*)-2-Decenoic acid	2408	x	x	-	LRI, MS
Dodecanoic acid	2494	x	x	-	LRI, MS, St
Tridecanoic acid	2599	-	x	-	LRI, MS, St
Tetradecanoic acid	2705	x	x	-	LRI, MS, St
Terpenes					
β-Pinene	1110	-	x	-	LRI, MS, St
Limonene	1203	-	x	1,3	LRI, MS, St
o-Cymene	1276	x	x	-	LRI, MS, St
p-Cymene	1279	-	x	-	LRI, MS, St
Lactones					
δ-Octalactone	1972	-	x	-	LRI, MS, St
δ-Decalactone	2198	x	x	-	LRI, MS, St
γ-Dodecalactone	2430	x	x	-	LRI, MS, St
Hydrocarbons					
Toluene	1049	-	x	3,4	LRI, MS, St

[a] Linear retention index calculated on a VF-WAXms, 60 m × 0.25 mm i. d. × 0.25-μm film thickness.
[b] 1) Natrella, Faccia, Lorenzo, De Palo, and Gambacorta (2020); 2) Natrella, Gambacorta, et al. (2020); 3) Sabia et al. (2020); 4) Sacchi et al. (2020).

[c] Identification method: LRI = Linear retention index; MS = mass spectrum; St = Standard.

were 2,3-butanediol, ethanol, acetic acid and 2-heptanol on the negative side of PC2, whereas benzaldehyde, phenylethyl alcohol, 3-methylbutanoic acid, 3-methyl-1-butanol and lactones on the positive side of PC2. These volatile compounds are responsible for the separation of TM_20 samples (20 days of refrigerated storage) and LFM_13 and LFM_20 samples (13 and 20 days of refrigerated storage, respectively) from the corresponding samples with a shorter storage time.

These compounds could be the result of the microbiological spoilage that takes place during the storage of Mozzarella cheese, and that is facilitated by the traditional way of packaging this cheese in brine. It has been demonstrated by different authors that Mozzarella cheese spoilage is mainly due to coliforms and Pseudomonas spp. and/or psychotropic bacteria, that grow on the cheese surface (Cabrini & Neviani, 1983; Rondinini & Garzaroli, 1990; Sinigaglia, Bevilacqua, Corbo, Pati, & Del Nobile, 2008). Coliforms can grow rapidly at the storage conditions of

Table 2
Quantitative average amount (μg Kg^{-1}) for volatile compounds with statistically significant differences in TM and LFM during the shelf-life.

Compounds	TM					LFM					TM	Odor descriptor
	Storage time (days)					Storage time(days)					vs	
	0	4	8	13	20	0	4	8	13	20	LFM	
2-Heptanone	2.88^{a}	2.80^{a}	2.89^{a}	2.69^{a}	1.81^{b}	3.56^{a}	3.69^{a}	3.79^{a}	3.79^{a}	2.49^{b}	ns	Cheese, fruity, ketonic, green banana
Acetoin	975.92^{a}	671.36^{b}	611.34^{b}	412.75^{c}	134.87^{d}	1281.22^{a}	815.12^{b}	683.22^{b}	380.88^{c}	226.01^{c}	ns	Sweet, buttery, creamy, dairy, milky
Benzaldehyde	tr^{e,a}	tr^{a}	-^{f,b}	-^{b}	-^{b}	0.30^{c}	0.53^{c}	0.88^{b}	1.89^{a}	1.93^{a}	*	Almond, fruity, nutty
Ethanol	4.39^{d}	7.62^{d}	17.06^{c}	116.40^{b}	482.97^{a}	13.52^{d}	59.31^{c}	76.56^{c}	243.81^{b}	469.98^{a}	ns	Pleasant, weak, ethereal, vinous
3-Methyl-1-Butanol	-^{c}	5.58^{b}	5.77^{b}	18.99^{a}	18.16^{a}	3.99^{c}	7.06^{c}	9.46^{c}	76.80^{b}	128.00^{a}	*	Banana, alcohol, fruity
2-Heptanol	-^{b}	-^{b}	-^{b}	-^{b}	3.58^{a}	-^{b}	-^{b}	-^{b}	-^{b}	4.87^{a}	ns	Fresh, lemon, grass, herbal
2,3-Butanediol	-^{c}	-^{c}	1.64^{b}	2.32^{b}	13.78^{a}	-^{c}	-^{c}	-^{c}	8.88^{b}	24.39^{a}	*	Fruity, creamy, buttery
Phenylethyl alcohol	-^{d}	-^{d}	2.19^{c}	4.97^{b}	10.85^{a}	4.57^{c}	4.09^{c}	5.48^{c}	9.03^{b}	13.75^{a}	*	Sweet, floral, rose-like
Acetic acid	37.58^{c}	47.95^{c}	62.13^{c}	237.33^{b}	820.13^{a}	9.32^{d}	37.90^{c}	74.70^{b}	64.28^{b}	727.59^{a}	ns	Vinegar, sharp, pungent
3-Methylbutanoic acid	40.51^{a}	-^{b}	-^{b}	-^{b}	-^{b}	-^{d}	2.83^{c}	6.49^{b}	20.83^{a}	17.80^{a}	*	Pungent, rancid, stinky, ripe fatty acid
Octanoic acid	34.44^{c}	67.11^{c}	125.05^{b}	224.40^{a}	265.60^{a}	50.83^{c}	60.67^{c}	140.96^{b}	184.20^{b}	292.08^{a}	ns	Waxy, musty, rancid, unpleasant, fatty
δ-Octalactone	-	-	-	-	-	tr^{c}	0.12^{b}	0.19^{b}	0.16^{b}	0.39^{a}	*	Sweet, coconut, creamy
δ-Decalactone	0.27^{c}	0.31^{c}	0.62^{b}	0.90^{a}	1.00^{a}	0.39^{b}	0.55^{b}	0.91^{a}	1.06^{a}	1.15^{a}	*	Sweet, creamy, coconut, milky
γ-Dodecalactone	-^{d}	-^{d}	tr^{c}	0.10^{b}	0.22^{a}	0.10^{c}	0.13^{c}	0.21^{b}	0.22^{b}	0.47^{a}	*	Fatty, peach, sweet, metallic, fruity

[a-d] Different uppercase letters in the same row, for each Mozzarella cheese type, indicate statistically significant differences ($P < 0.05$) from Duncan test during the storage time; *volatile compounds that exhibited statistically significant differences ($P < 0.05$) depending on cheese type; ns = not significant ($P > 0.05$); [e] inferior to 0.10 μg kg^{-1}; [f] not detected.

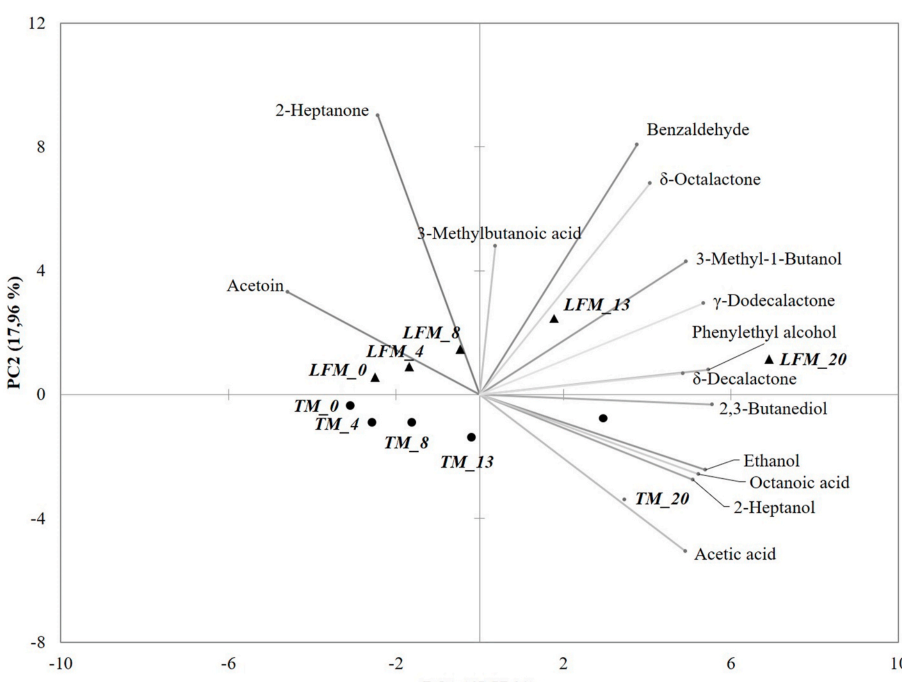

Fig. 1. Two dimensional PCA centroid (average scores) and loading plot performed on volatile data with a $P < 0.05$ of TM and LFM cheese sample during the shelf-life.

Mozzarella cheese, and they are responsible for the production of acetic acid, formic acid, succinic acid, lactic acid, ethanol, 2,3-butyleneglycol, H_2 and CO_2 (Sinigaglia et al., 2008). The increasing amounts of acetic acid and ethanol observed in both TM and LFM cheese samples during the shelf life and the high amounts found after 20 days of storage are presumably attributable to coliform metabolism.

Pseudomonas spp. and psychotropic bacteria have both lipolytic and proteolytic activity; these bacteria, being capable of growing at refrigeration temperatures, can rapidly prevail over lactic flora and could be responsible for the increasing amount of 2-heptanol, δ-octalactone, δ-decalactone, γ-dodecalactone, 3-butanediol, benzaldehyde, phenylethyl alcohol, 3-methylbutanoic acid and 3-methyl-1-butanol in the TM and LFM samples during the storage time.

2-Heptanol and lactones originates from lipolytic processes; in particular, 2-heptanol is formed during the shelf-life from 2-heptanone reduction, whereas lactones are produced by transesterification of hydroxylated free fatty acids incorporated in milk fat triglycerides and released by enzymatic lipolytic activity or by any heating process (Alewijn, Smit, Sliwinski, & Wouters, 2007). Lactones are typically found in ripened cheeses and are important aroma compounds in Blue cheese (Gallois & Langlois, 1990), Cheddar (Wong, Ellis, & LaCroix, 1975) and Parmigiano Reggiano cheese (Meinhart & Schreier, 1986). Lactones are here identified for the first time in Mozzarella cheese with a higher ($P < 0.05$) content in LFM than in TM.

Finally, 2,3-butanediol, 3-methylbutanoic acid, 3-methyl-1-butanol, benzaldehyde and phenylethyl alcohol arise from FAA catabolism: 2,3-butanediol originates from transamination of aspartic acid (Ardö, 2006); 3-methylbutanoic acid derives from leucine and it is responsible for the rancid, cheesy and sweety odor in cheese (Thierry, Maillard, Richoux, Kerjean, & Lortal, 2005); benzaldehyde (bitter, fruity and nutty flavors) and phenylethyl alcohol (floral, rose-like) arise from phenylalanine following transamination of phenylpyruvate by nonenzymatic breakdown (Kong, Strickland, & Broadbent, 1996).

Excluding 2-heptanol, all the volatile compounds formed by FFA and FAA catabolism were present at higher levels ($P < 0.05$) in the LFM cheese samples. This difference could be related to the hydrolytic processes to which the milk has been subjected for the production of LFM cheese. In addition to break down the natural sugar present in milk into glucose and galactose, the enzyme lactase used in this research has also a proteolytic activity that could determine the degradation of protein during processing and storage (Troise et al., 2016). Further, heating treatments during cheesemaking, especially during the spinning processes, may have favored the release of hydroxylated free fatty acids from triglycerides and thus the formation of higher levels of lactones in LFM cheese.

3.2. Sensory analysis

Table 3 reports the results of sensory descriptive analyses for TM and LFM during the shelf-life. Statistically significant variations occurred during the shelf-life of both cheese types for all the sensory descriptors evaluated by the panel. Moreover, TM and LFM cheese differed ($P < 0.05$) with respect to yoghurt odor, bitter and salty taste, elasticity and juiciness. Fig. 2 reports the PCA loading and score plot of sensory data; comparing it with that of volatile aroma compounds (Fig. 1), an interesting and clear similarity emerges between the way the two methods describe the relative spatial positioning in the multivariate model. TM cheese samples from 0 to 13 storage days and LFM cheese samples from 0 to 8 days were grouped in the positive side of PC1 (73.25% of variability), indicating a sensory stability of the two products until this time. Sensory descriptors which most influenced this separation were sweet taste, white color, firmness, juiciness, and gumminess for LFM, milk flavour, milk odor, elasticity, cohesiveness and smooth surface for TM. All these descriptors are associated with positive sensory characteristics

Table 3
Mean values and standard deviation for the QDA during the shelf-life for TM and LFM cheese.

	TM Days					LFM Days					TM vs LFM
Descriptors	**0**	**4**	**8**	**13**	**20**	**0**	**4**	**8**	**13**	**20**	
White color	$8.63^{a}\pm0.75$	$7.89^{a}\pm1.31$	$7.67^{a}\pm0.46$	$7.20^{a}\pm0.94$	$6.75^{b}\pm0.83$	$8.17^{a}\pm0.93$	$7.60^{a}\pm1.26$	$7.40^{a}\pm0.86$	$7.17^{a}\pm0.74$	$4.40^{b}\pm0.51$	ns
Smoot surface	$6.74^{a}\pm0.32$	$6.48^{a}\pm0.34$	$6.17^{a}\pm0.49$	$6.00^{a}\pm0.42$	$4.25^{b}\pm0.26$	$6.77^{a}\pm0.54$	$6.80^{a}\pm0.41$	$6.80^{a}\pm0.53$	$5.27^{b}\pm0.49$	$5.50^{b}\pm0.49$	ns
Milk odor	$7.43^{a}\pm0.94$	$7.02^{a}\pm1.23$	$6.17^{a}\pm0.64$	$5.60^{b}\pm0.31$	$4.50^{b}\pm0.39$	$6.25^{a}\pm0.37$	$6.20^{a}\pm0.83$	$5.80^{a}\pm0.67$	$5.83^{a}\pm0.63$	$4.80^{b}\pm0.53$	ns
Butter odor	$5.20^{b}\pm0.29$	$5.40^{b}\pm0.76$	$5.50^{b}\pm0.76$	$6.00^{a}\pm0.36$	$6.00^{a}\pm0.72$	$6.03^{b}\pm0.43$	$5.80^{b}\pm0.47$	$5.80^{b}\pm0.53$	$6.17^{b}\pm0.73$	$7.40^{a}\pm0.64$	ns
Yoghurt odor	$2.87^{b}\pm0.08$	$3.10^{b}\pm0.59$	$3.00^{b}\pm0.16$	$3.60^{a}\pm0.49$	$4.00^{a}\pm0.38$	$4.17^{b}\pm0.34$	$4.40^{b}\pm0.36$	$5.40^{a}\pm0.39$	$5.42^{a}\pm0.53$	$5.80^{a}\pm0.37$	*
Acid taste	$2.80^{c}\pm0.13$	$2.74^{c}\pm0.24$	$3.17^{c}\pm0.37$	$3.80^{b}\pm0.73$	$4.00^{b}\pm0.61$	$1.83^{c}\pm0.31$	$2.80^{c}\pm0.31$	$4.20^{b}\pm0.28$	$5.17^{b}\pm0.46$	$7.20^{a}\pm0.83$	ns
Bitter taste	$1.80^{c}\pm0.02$	$2.54^{c}\pm0.36$	$3.00^{b}\pm0.42$	$3.80^{b}\pm0.67$	$4.50^{b}\pm0.75$	$2.00^{d}\pm0.14$	$3.20^{c}\pm0.42$	$3.40^{c}\pm0.13$	$4.17^{b}\pm0.56$	$5.60^{a}\pm0.62$	*
Sweet taste	3.45 ± 0.47	3.67 ± 0.43	3.33 ± 0.14	3.60 ± 0.15	3.50 ± 0.24	$4.67^{a}\pm0.29$	$4.00^{a}\pm0.36$	$3.00^{b}\pm0.41$	$3.00^{b}\pm0.25$	$2.60^{c}\pm0.18$	ns
Salty taste	$2.24^{c}\pm0.34$	$2.50^{bc}\pm0.51$	$2.83^{b}\pm0.34$	$4.60^{a}\pm0.29$	$4.75^{a}\pm0.68$	3.50 ± 0.14	3.40 ± 0.29	3.20 ± 0.27	3.17 ± 0.18	3.12 ± 0.38	*
Milk flavor	$7.28^{a}\pm0.91$	$6.45^{a}\pm0.97$	$5.80^{b}\pm0.64$	$4.80^{c}\pm0.76$	$4.50^{c}\pm0.36$	$6.33^{a}\pm0.75$	$6.40^{a}\pm0.53$	$5.80^{a}\pm0.43$	$4.17^{b}\pm0.51$	$4.20^{b}\pm0.31$	ns
Firmness	$8.06^{a}\pm1.15$	$8.10^{a}\pm1.26$	$7.80^{a}\pm0.45$	$6.00^{b}\pm0.42$	$4.50^{c}\pm0.24$	$7.50^{a}\pm0.64$	$7.00^{a}\pm0.61$	$5.80^{b}\pm0.52$	$3.00^{c}\pm0.27$	$2.40^{c}\pm0.29$	ns
Elasticity	$7.10^{a}\pm0.68$	$6.36^{a}\pm0.72$	$6.40^{a}\pm0.73$	$5.20^{b}\pm0.61$	$3.75^{c}\pm0.48$	$5.50^{a}\pm0.39$	$5.30^{a}\pm0.46$	$5.20^{a}\pm0.34$	$2.83^{b}\pm0.19$	$2.80^{b}\pm0.17$	*
Cohesiveness	$5.10^{a}\pm0.49$	$5.20^{a}\pm0.43$	$5.00^{a}\pm0.27$	$4.40^{a}\pm0.38$	$2.00^{b}\pm0.15$	$5.17^{a}\pm0.43$	$5.20^{a}\pm0.13$	$5.12^{a}\pm0.29$	$3.17^{b}\pm0.42$	$2.20^{b}\pm0.13$	ns
Gumminess	$6.60^{a}\pm0.74$	$6.40^{a}\pm0.81$	$5.80^{a}\pm0.71$	$5.80^{a}\pm0.61$	$3.75^{b}\pm0.46$	$6.50^{a}\pm0.73$	$6.40^{a}\pm0.91$	$6.20^{a}\pm0.73$	$3.00^{b}\pm0.29$	$2.60^{b}\pm0.24$	ns
Juiciness	$7.03^{a}\pm0.87$	$6.78^{a}\pm0.57$	$6.80^{a}\pm0.94$	$6.40^{a}\pm0.73$	$4.75^{b}\pm0.53$	$6.00^{a}\pm0.45$	$5.40^{a}\pm0.36$	$4.80^{b}\pm0.31$	$4.50^{b}\pm0.36$	$4.40^{b}\pm0.53$	*

[a-d] Different uppercase letters in the same row, for each Mozzarella cheese type, indicate statistically significant differences ($P < 0.05$) from Duncan test during the storage time; *sensory descriptors that exhibited statistically significant differences ($P < 0.05$) depending on cheese type; ns = not significant ($P > 0.05$); [e] not detected.

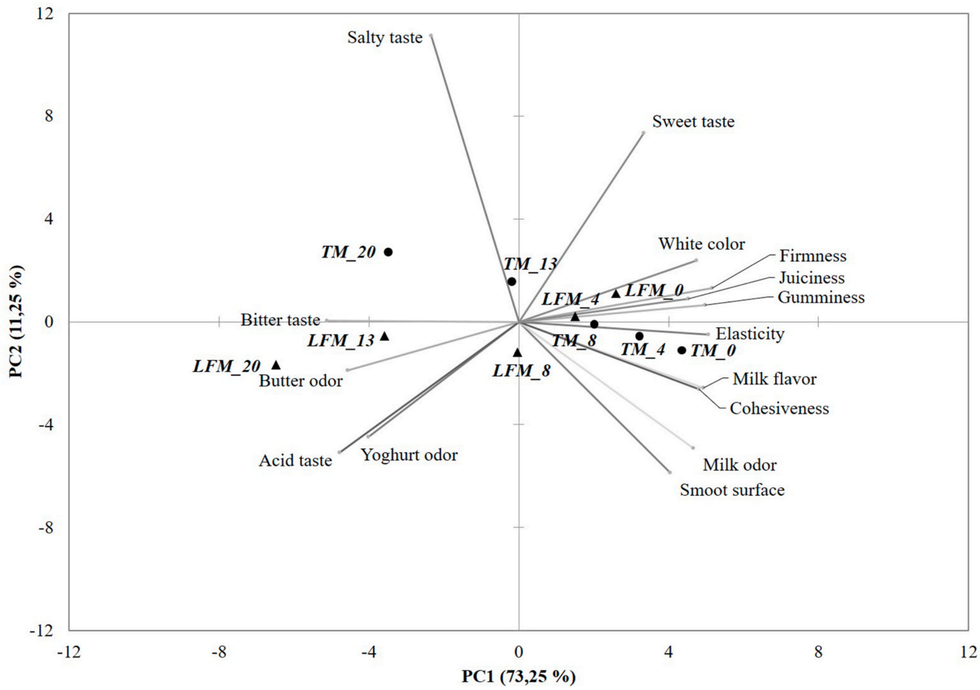

Fig. 2. Two dimensional PCA centroid (average scores) and loading plot performed on sensory data of TM and LFM cheese sample during the shelf-life.

of fresh cheese products.

TM_20 samples were separated from the others and characterized by a salty and bitter taste, whereas LFM_13 and LFM_20 samples were in the negative side of PC1 and PC2, characterized by the negative descriptors of butter odor, yoghurt odor, acid taste and bitter taste. Interestingly, the development of bitter taste occurred at the end of the shelf-life that could be associated with release of bitter tasting peptides due to the proteolytic activity of spoilage microorganisms plus that of lactase enzyme limited to LFM cheese samples (Jansson et al., 2014; Nielsen et al., 2017; Troise et al., 2016). A correlation between aroma volatile compounds and odor and flavor descriptors are presented in Fig. 3. As shown in section 3.1, the samples from 0 to 8 days are characterized by a higher content of acetoin mainly associated with milk odor and flavor, typical sensory properties that characterize fresh mozzarella cheese. As the storage time increased, the levels of compounds associated with fatty acid and amino acid metabolism increased too, determining a detrimental increase of yoghurt odor, correlated with benzaldehyde and δ-octalactone, and butter odor correlated with 3-methyl-1-butanol and γ-dodecalactone.

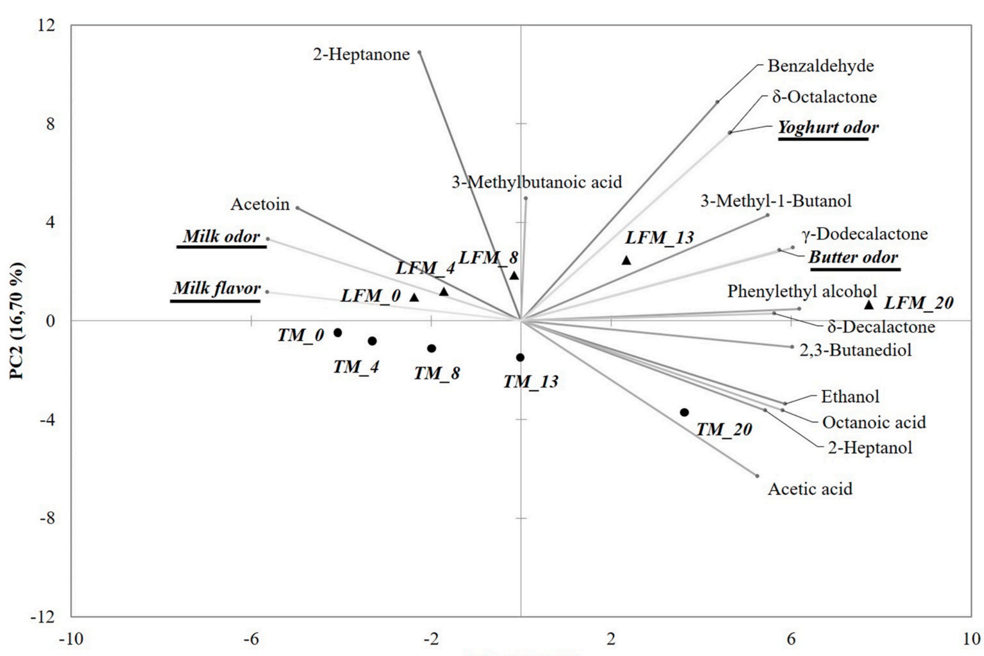

Fig. 3. Two dimensional PCA centroid (average scores) and loading plot correlation performed on volatile and odor and flavor sensory data of TM and LFM cheese sample during the shelf-life.

4. Conclusions

Food shelf-life studies are an essential part of food product development determined routinely by the manufacturer. As regard TM and LFM cheese samples, the manufacturer established a shelf-life of 20 days at refrigerated conditions (+6 °C) on the basis of their microbiological stability.

Data here obtained indicate that during the shelf-life the volatile profile of TM and LFM significantly changed: an increase in the amount of the aroma compounds coming from amino acid and fatty acid metabolism occurred in the LFM samples after 8 days of storage and, to a lesser extent, in TM cheese after 13 days of storage. This resulted in a sensory decay perceived by the panel which assigned lower score values to positive descriptors associated with fresh cheese products and higher score values to the negative ones at the end of the shelf life.

In conclusion this study highlights the importance of considering the volatile aroma profile and the sensory quality of Mozzarella cheese for its shelf-life definition and therefore it indicates that, despite the microbiological stability, a shorter shelf-life should be established for LFM than TM cheese.

Funding

This research was accomplished within the framework of the PON project "Attraction and International Mobility" (AIM) Line 1. Researcher Mobility, financed by the Italian Ministry for Education, University and Research (MIUR) on FSE funds. AIM 1823923-3 - CUP J44I18000190006.

CRediT authorship contribution statement

Fabrizio Cincotta: Research design, Writing - original draft. **Concetta Condurso:** Supervision, Methodology. **Gianluca Tripodi:** Research design, Methodology. **Maria Merlino:** Formal analysis, Data Formal analysis. **Ottavia Prestia:** Formal analysis, Data Formal analysis. **Catherine Stanton:** Supervision, Writing - review & editing. **Antonella Verzera:** Conceptualization, Writing - review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

Alewijn, M., Smit, B. A., Sliwinski, E. L., & Wouters, J. T. M. (2007). The formation mechanism of lactones in Gouda cheese. *International Dairy Journal, 17*(1), 59–66. https://doi.org/10.1016/j.idairyj.2006.01.002

Alinovi, M., Corredig, M., Mucchetti, G., & Carini, E. (2020). Water status and dynamics of high-moisture Mozzarella cheese as affected by frozen and refrigerated storage. *Food Research International*, 109415. https://doi.org/10.1016/j.foodres.2020.109415

Alinovi, M., & Mucchetti, G. (2020). Effect of freezing and thawing processes on high-moisture Mozzarella cheese rheological and physical properties. *LWT, 124*, 109137. https://doi.org/10.1016/j.lwt.2020.109137

Ardö, Y. (2006). Flavour formation by amino acid catabolism. *Biotechnology Advances, 24*(2), 238–242. https://doi.org/10.1016/j.biotechadv.2005.11.005

Braghieri, A., Zotta, T., Morone, G., Piazzolla, N., Majlesi, M., & Napolitano, F. (2018). Starter cultures and preservation liquids modulate consumer liking and shelf life of mozzarella cheese. *International Dairy Journal, 85*, 254–262. https://doi.org/10.1016/j.idairyj.2018.06.013

Cabrini, A., & Neviani, E. (1983). Il genere Pseudomonas causa di sapore amaro e di odore putrido sulla superficie di formaggio Mozzarella. *Il Latte, 8*, 90.

Cincotta, F., Verzera, A., Tripodi, G., & Condurso, C. (2018). Non-intentionally added substances in PET bottled mineral water during the shelf-life. *European Food Research and Technology, 244*(3), 433–439. https://doi.org/10.1007/s00217-017-2971-6

Condurso, C., Cincotta, F., Merlino, M., Stanton, C., & Verzera, A. (2020). Stability of powdered infant formula during secondary shelf-life and domestic practices. *International Dairy Journal*, 104761. https://doi.org/10.1016/j.idairyj.2020.104761

Condurso, C., Verzera, A., Romeo, V., Ziino, M., & Conte, F. (2008). Solid-phase microextraction and gas chromatography mass spectrometry analysis of dairy product volatiles for the determination of shelf-life. *International Dairy Journal, 18*(8), 819–825. https://doi.org/10.1016/j.idairyj.2007.12.005

Curioni, P. M. G., & Bosset, J. O. (2002). Key odorants in various cheese types as determined by gas chromatography-olfactometry. *International Dairy Journal, 12*(12), 959–984. https://doi.org/10.1016/S0958-6946(02)00124-3

Dursun, A., Güler, Z., & Şekerli, Y. E. (2017). Characterization of volatile compounds and organic acids in ultra-high-temperature milk packaged in tetra brik cartons. *International Journal of Food Properties, 20*(7), 1511–1521. https://doi.org/10.1080/10942912.2016.1213280

Faccia, M., Gambacorta, G., Natrella, G., & Caponio, F. (2019). Shelf life extension of Italian mozzarella by use of calcium lactate buffered brine. *Food Control, 100*, 287–291. https://doi.org/10.1016/j.foodcont.2019.02.002

Faccia, M., Mastromatteo, M., Conte, A., & Del Nobile, M. A. (2012). Influence of the different sodium chloride concentrations on microbiological and physico-chemical characteristics of mozzarella cheese. *Journal of Dairy Research, 79*(4), 390–396. https://doi.org/10.1017/S0022029912000209

Francolino, S., Locci, F., Ghiglietti, R., Iezzi, R., & Mucchetti, G. (2010). Use of milk protein concentrate to standardize milk composition in Italian citric Mozzarella cheese making. *LWT-Food Science and Technology, 43*(2), 310–314. https://doi.org/10.1016/j.lwt.2009.08.007

Gallois, A., & Langlois, D. (1990). New results in the volatile odorous compounds of French cheeses. *Le Lait, 70*(2), 89–106. https://doi.org/10.1051/lait:199028

Gammariello, D., Conte, A., Attanasio, M., & Del Nobile, M. A. (2010). Study on the combined effects of essential oils on microbiological quality of Fior di Latte cheese. *Journal of Dairy Research, 77*(2), 144–150. https://doi.org/10.1017/S0022029909990574

Gorrasi, G., Bugatti, V., Tammaro, L., Vertuccio, L., Vigliotta, G., & Vittoria, V. (2016). Active coating for storage of Mozzarella cheese packaged under thermal abuse. *Food Control, 64*, 10–16. https://doi.org/10.1016/j.foodcont.2015.12.002

ISO. (1988). ISO 8589. *Sensory analysis - general guidance for the design of test rooms*. Geneva, Switzerland: International Standardization Organization.

ISO. (1993). *ISO 8586-1. Sensory analysis: General guidance for the selection, training and monitoring of assessors. Part 1. Selected assessors*. Geneva, Switzerland: International Standardisation Organisation.

ISO. (2003). *ISO 13299. General guidance for establishing a sensory profile*. Geneva, Switzerland: International Organization for Standardization.

Jana, A. H., & Mandal, P. K. (2011). Manufacturing and quality of mozzarella cheese: A review. *International Journal of Dairy Science, 6*(4), 199–226. https://doi.org/10.3923/ijds.2011.199.226

Jansson, T., Jensen, H. B., Sundekilde, U. K., Clausen, M. R., Eggers, N., Larsen, L. B., et al. (2014). Chemical and proteolysis-derived changes during long-term storage of lactose-hydrolyzed ultrahigh-temperature (UHT) milk. *Journal of Agricultural and Food Chemistry, 62*(46), 11270–11278. https://doi.org/10.1021/jf504104q

Kong, Y., Strickland, M., & Broadbent, J. R. (1996). Tyrosine and phenylalanine catabolism by *Lactobacillus casei* flavor adjuncts: Biochemistry and implications in cheese flavor. *Journal of Dairy Science, 79*(1), 101.

Luz, C., Torrijos, R., Quiles, J. M., Mañes, J., & Meca, G. (2019). Shelf life extension of mozzarella cheese contaminated with Penicillium spp. using the antifungal compound ϵ-polylysine. *Food Science and Technology International, 25*(4), 295–302. https://doi.org/10.1177/1082013218823136

McSweeney, P. L., Fox, P. F., & Ciocia, F. (2017). Metabolism of residual lactose and of lactate and citrate. In *Cheese: Chemistry, physics and microbiology* (4th ed., pp. 411–421). Academic Press.

McSweeney, P. L., & Sousa, M. J. (2000). Biochemical pathways for the production of flavour compounds in cheeses during ripening: A review. *Le Lait, 80*(3), 293–324. https://doi.org/10.1051/lait:2000127

Meinhart, E., & Schreier, P. (1986). Study of flavour compounds from Parmigiano Reggiano cheese. *Milchwissenschaft, 41*(11), 689–691.

Moio, L., Langlois, D., Etievant, P. X., & Addeo, F. (1993). Powerful odorants in water buffalo and bovine Mozzarella cheese by use of extract dilution sniffing analysis. *Italian Journal of Food Science, 5*(3), 227–237.

Natrella, G., Faccia, M., Lorenzo, J. M., De Palo, P., & Gambacorta, G. (2020). Sensory characteristics and volatile organic compound profile of high-moisture mozzarella made by traditional and direct acidification technology. *Journal of Dairy Science, 103*(3), 2089–2097. https://doi.org/10.3168/jds.2019-17059

Natrella, G., Gambacorta, G., De Palo, P., Lorenzo, J. M., & Faccia, M. (2020). Evolution of volatile compounds from milk to curd during manufacturing of Mozzarella. *Mljekarstvo: Časopis Za Unaprjeđenje Proizvodnje I Prerade Mlijeka, 70*(1), 50–58. https://doi.org/10.15567/mljekarstvo.2020.0105

Nielsen, S. D., Jansson, T., Le, T. T., Jensen, S., Eggers, N., Rauh, V., et al. (2017). Correlation between sensory properties and peptides derived from hydrolysed-lactose UHT milk during storage. *International Dairy Journal, 68*, 23–31. https://doi.org/10.1016/j.idairyj.2016.12.013

Nzekoue, F. K., Caprioli, G., Fiorini, D., Torregiani, E., Vittori, S., & Sagratini, G. (2019). HS-SPME-GC-MS technique for FFA and hexanal analysis in different cheese packaging in the course of long term storage. *Food Research International, 121*, 730–737. https://doi.org/10.1016/j.foodres.2018.12.048

Ricciardi, A., Guidone, A., Ianniello, R. G., Cioffi, S., Aponte, M., Pavlidis, D., et al. (2015). A survey of non-starter lactic acid bacteria in traditional cheeses: Culture dependent identification and survival to simulated gastrointestinal transit. *International Dairy Journal, 43*, 42–50. https://doi.org/10.1016/j.idairyj.2014.11.006

Rondinini, G., & Garzaroli, C. (1990). Mozzarelle prodotte per acidificazione chimica: Aspetti microbiologici e alterativi. *Industrie Alimentari, 29*, 329–334.

Sabia, E., Gauly, M., Napolitano, F., Cifuni, G. F., & Claps, S. (2020). The effect of different dietary treatments on volatile organic compounds and aromatic characteristics of buffalo Mozzarella cheese. *International Journal of Dairy Technology, 73*(3), 594–603. https://doi.org/10.1111/1471-0307.12696

Sacchi, R., Marrazzo, A., Masucci, F., Di Francia, A., Serrapica, F., & Genovese, A. (2020). Effects of inclusion of fresh forage in the diet for lactating buffaloes on volatile organic compounds of milk and mozzarella cheese. *Molecules, 25*(6), 1332. https://doi.org/10.3390/molecules25061332

Sinigaglia, M., Bevilacqua, A., Corbo, M. R., Pati, S., & Del Nobile, M. A. (2008). Use of active compounds for prolonging the shelf life of mozzarella cheese. *International Dairy Journal, 18*(6), 624–630. https://doi.org/10.1016/j.idairyj.2007.11.022

Thierry, A., Maillard, M. B., Richoux, R., Kerjean, J. R., & Lortal, S. (2005). *Propionibacterium freudenreichii* strains quantitatively affect production of volatile compounds in Swiss cheese. *Le Lait, 85*, 57–74. https://doi.org/10.1051/lait:2004036

Troise, A. D., Bandini, E., De Donno, R., Meijer, G., Trezzi, M., & Fogliano, V. (2016). The quality of low lactose milk is affected by the side proteolytic activity of the lactase used in the production process. *Food Research International, 89*, 514–525. https://doi.org/10.1016/j.foodres.2016.08.021

Wong, N. P., Ellis, R., & LaCroix, D. E. (1975). Quantitative determination of lactones in Cheddar cheese. *Journal of Dairy Science, 58*(10), 1437–1441. https://doi.org/10.3168/jds.S0022-0302(75)84734-5