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Evaluation of different salted governing liquids on shelf life extension of lacto-fermented mozzarella cheese

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Research highlights

Addition of salts to the brine can improve quality of mozzarella cheese

Calcium lactate contributed to slow down Pseudomonas *spp.* growth preserving mozzarella texture

0.6% Calcium lactate in water can improve mozzarella shelf life until 18 days

Abstract

The effect of different governing liquids on qualitative parameters of lacto-fermented mozzarella cheeses (LFM) were studied. 0.6% calcium lactate (CL) solution maintained the quality of mozzarella cheese for microbial, colour and textural properties and prolonged its shelf life up to 18 days, also improving its antioxidant activity by Trolox equivalent antioxidant capacity (TEAC) and Oxygen radical absorbance capacity (ORAC) assays.

Keywords: governing liquid; mozzarella cheese; quality; salts; shelf life

Introduction

Qualitative cheese properties are generally related to manufacturing practices, local environmental and storage conditions (Piscopo et al. 2015).

Lacto-fermented mozzarella (LFM) is a soft white cheese obtained after coagulation of milk by rennet and/or coagulant enzymes acidified by bacterial cultures (Faccia et al. 2013) with a shelf-life of 5 days whereas only for industrial products, obtained with direct acidification, is of 20 days at 5°C.

Several studies have established that fresh cheese spoilage can be attributed to a consortium of bacteria, commonly dominated by Pseudomonas *spp*, at different temperatures (-1 to 25 °C) producing pigments, also fluorescent, which cause colour changes (Carminati et al. 2019; Faccia et al. 2019). The relationship between microbiological growth and biochemical changes occurring during storage has been recognized as a potential indicator, useful for monitoring freshness and safety of mozzarella cheese. In fact, the contamination could occur during or after the remaining cheese-making process because if pathogen bacteria are present in the milk, they are likely inactivated during the mozzarella production process due to the heat treatment applied during the spinning step (Tirioni et al. 2019).

Usually LFM is stored under refrigerated conditions in a conditioning liquid (water, whey, stretching water, brine), which preserves its soft and springy texture, but its high moisture, a_w and pH values are not limiting factors for the microbial growth. NaCl is widely used in dairy products because it can control the quality and texture, but high levels of sodium are not recommended for human health. Many techniques have been studied to increase LFM shelf life, such as addition of coatings on mozzarella cheese (Angiolillo et al. 2014) and calcium lactate-based governing liquids (Falcone et al. 2017). According to Faccia et al. (2011), calcium chloride in the governing liquid gives a metallic and bitter off-flavour to mozzarella, so lactate can be considered as substitute, according to the research of Lawless et al. (2003).

The aim of this research was to evaluate the effects of different salted governing liquids on microbiological, physico-chemical, textural properties and antioxidant activities of lacto-fermented mozzarella and to investigate possible improvement of LFM quality.

Materials and methods

Sample processing

LFM was manufactured in a cheese factory (Delizie della Natura) located in Reggio Calabria (Southern Italy). During the cheesemaking the pasteurized cow's milk was acidified with lactic acid cultures and added with the liquid rennet. When the curd pH reached a value between 6.2-6.4., the whey was removed and the curd was cut and stretched. After the cooling in cold water, three mozzarella cheeses (55-60% of moisture,125 g) were packaged in polypropylene trays with different governing liquids: GL1 (tap water), GL2 (0.2% calcium lactate (CL) solution), GL3 (0.6% CL solution), GL4 (0.2% sodium chloride (SC) solution), GL5 (0.6% SC solution), GL6 (0.4% SC+ 0.2% CL solution). LFM were named respectively M1, M2, M3, M4, M5 and M6, stored at 5°C and monitored for qualitative parameters.

Microbiological analyses

Diluted samples were detected for Total bacterial count (TBC) after incubation in Plant Count Agar; Lactic acid bacteria (LAB) after anaerobic incubation in MRS Agar and Pseudomonas *spp.* after incubation in Pseudomonas Agar Base added of CFC Pseudomonas supplement. Results were expressed as Log_{10} cfu g^{-1} and as mean of four replicates.

Physico-chemical analyses

LFM were submitted to moisture (AOAC, 1990), pH (AOAC,1980a); titrable acidity (AOAC, 1980b) and chlorides (AOAC, 2016) determinations. Colour evaluation (ΔE^*) was performed according to Dukalska et al. (2011) and texture profile analysis (Hardness, Adhesiveness, Springiness, Cohesiveness, Gumminess, Chewiness, Resilience) was performed by TA-XT Plus Texture Analyzer at room temperature. Governing liquids were analyzed for total acidity, pH and chlorides. All the analyses were performed in triplicate.

Antioxidant capacity

About 10 g of mozzarella sample were mixed to 50 mL of methanol:water (80:20, v:v), and then centrifuged. The supernatant solution was filtered and submitted to antioxidant activity assays: Trolox equivalent antioxidant capacity (TEAC) according to Re et al. (1999) and Oxygen radical absorbance capacity assay (ORAC) assay according to Zulueta et al. (2009). All the analyses were performed in triplicate.

Statistical analyses

The results were expressed as mean±standard deviation. Experimental data were compared by statistical analysis of variance (one-way ANOVA and Multivariate analysis) and Tukey's multiple range test was used to determine significant differences among samples ($p < 0.05$) using SPSS Statistics 15.0 software.

Results and discussions

Microbiological analyses

At initial storage time significant ($p<0.01$) differences in all microbiological analyses were observed among M samples. The microbial charge generally increased during the storage. M2, M3 and M6 possessed after 18 days of storage also an acceptable TBC and were within the microbiological limit (10^6 log cfu g^{-1}) for Pseudomonas *spp*, as reported by Bishop and White (1986), denoting the positive preservation effect of calcium lactate (Table 1).

Physico-chemical analyses

Moisture content of M samples increased significantly during the storage time probably for a protein hydration and the water-binding capacity of the protein matrix of cheeses when salts are added: it was observed in particular with sodium chloride (M4, M5, M6), whereas calcium lactate probably controlled better the water diffusion from governing liquid to mozzarella and maintained the initial moisture of M2 and M3. (Table 2). ΔE^* values > 3 reveal that colour differences are clear for the human eye (Francis and Clydesdale,1975): the effect of different GL was so more evident in the inner layers of M4 and M5 samples (6.00 and 6.52, data not shown).

Negative correlation values were found between hardness and moisture after 12 days (r = -0.921). Hardness, springiness and gumminess significantly ($p < 0.01$) decreased during the monitoring time and differed among the samples (Table 2): M3 possessed the highest springiness from 0 to 12 days, denoting a good effect by CL to prevent a probable proteolysis: a negative correlation between springiness and Pseudomonas spp. microorganisms was in fact found (r = -0.921). M3 possessed also the highest gumminess, correlated to hardness values after 12 days (r = 0.). Titrable acidity of M2, M3, M5 and M6 increased with a corresponding slowdown of pH (Table 1, $p < 0.01$). Chlorides content in M samples varied during the storage time for the movement of ions from the governing liquid into the mozzarella. The evolution of acidity, pH and NaCl % in governing liquids was significantly ($p < 0.01$) affected by the salt type and storage time as shown in Table 1.

Antioxidant capacity

TEAC increased significantly ($p < 0.01$) after 12 days specially in M3, M4 and M5: it is related to the hydrolysis by microbial protease as confirmed by correlation between TEAC and TBC (r = 0.820 $p < 0.05$). An increase of ORAC values, after 12 storage days, was shown on M2 and M5 mozzarella cheese samples (Figure 1).

Conclusions

Governing liquids with calcium lactate successfully influenced the quality of LFM, in particular controlling the Pseudomonas *spp.* growth and promoting a better hardness up to18 days: the other samples beyond 12 days presented higher microbiological charge, a worse texture profile, visually observable as a collapse of structure. So, this suggested that CL alone or in mixture with SC in water can improve mozzarella shelf life. Mozzarella cheese samples had shown good results for TEAC when preserved in 0.6% CL and the both % of SC.

Acknowledgements

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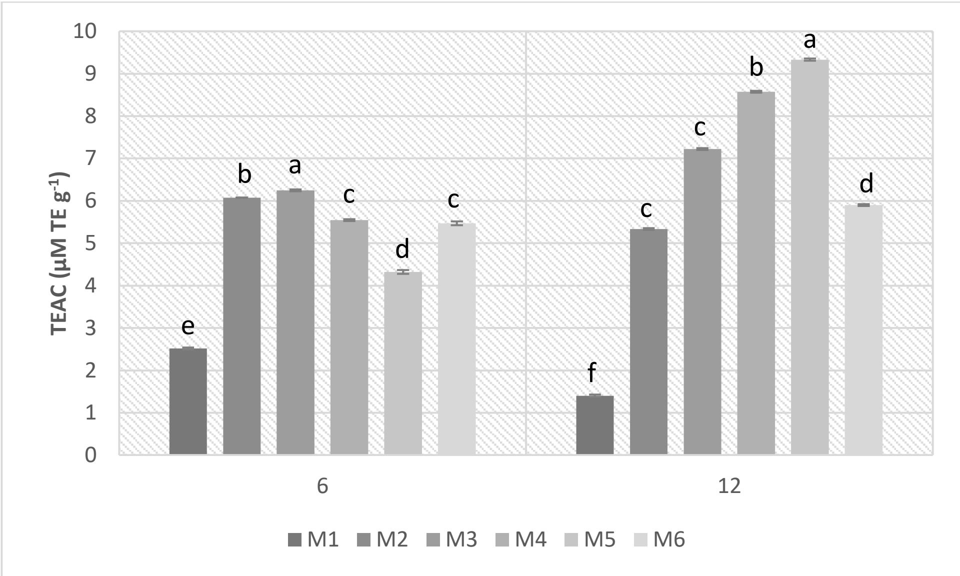
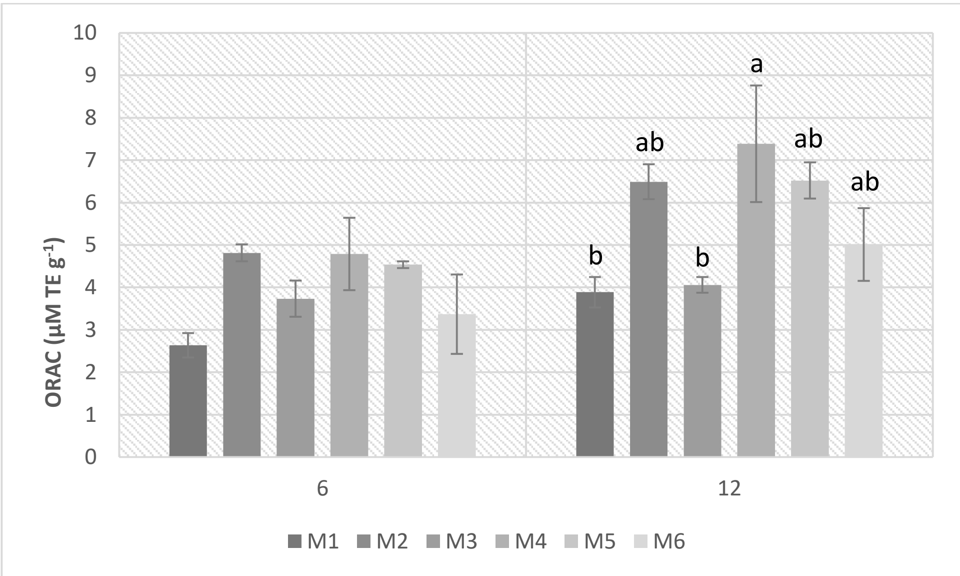
List of figures

Fig. 1 Trolox equivalent antioxidant capacity (TEAC) and Oxygen radical absorbance capacity (ORAC) antioxidant activity in lacto-fermented mozzarella cheeses after 6 and 12 storage days, expressed as µM TE g^{-1}. Different letters are significantly different by Tukey's post hoc test ($p < 0.05$)


TEAC (µM TE g-1)
10
9
8
7
6
5
4
3
2
1
0
e
b
a
c
d
c
f
c
c
b
a
d
6
12
M1
M2
M3
M4
M5
M6

a


ORAC (µM TE g-1)
10
9
8
7
6
5
4
3
2
1
0
b
ab
b
a
ab
ab
6
12
M1
M2
M3
M4
M5
M6

b

Table 1 Microbiological counts of different lacto-fermented mozzarella cheeses during the storage
Microbiological counts of different lacto-fermented mozzarella cheeses during the storage

		Storage time (days)				
Microorganisms	Samples[1]	0	6	12	18	Sig.
Total bacterial count (log_{10} cfu g^{-1})	M1	4,61^{bE}	6,03^{ab}	6,66^{aE}	/	**
	M2	6,03^{dB}	6,40^{c}	6,75^{bD}	7,03^{aA}	**
	M3	6,98^{aA}	6,51^{d}	6,92^{bBC}	6,72^{cC}	**
	M4	4,69^{cD}	6,59^{b}	6,90^{aC}	/	**
	M5	5,93^{cC}	6,44^{b}	7,25^{aA}	/	**
	M6	4,38^{dF}	6,68^{c}	6,95^{bB}	6,97^{aB}	**
	Sig.†	**	n.s.	**	**	
Total lactic acid bacteria (log_{10} cfu^{-1})	M1	4,21^{cF}	5,18^{bC}	6,34^{aE}	/	**
	M2	5,95^{dB}	6,19^{cB}	6,82^{bC}	7,00^{aB}	**
	M3	6,88^{dA}	6,30^{cB}	7,07^{bB}	7,86^{aA}	**
	M4	4,61^{cD}	6,31^{bB}	6,79^{aC}	/	**
	M5	5,86^{cC}	6,14^{bB}	6,53^{aD}	/	**
	M6	4,28^{dE}	6,68^{cA}	7,12^{aA}	6,87^{bC}	**
	Sig.†	**	**	**	**	
Pseudomonas spp. (log_{10} cfu/g)	M1	0,00^{bB}	4,09^{aA}	4,11^{aBC}	/	**
	M2	0,00^{dB}	3,64^{bB}	4,10^{aC}	2,46^{cC}	**
	M3	0,01^{dB}	3,28^{bE}	3,70^{aD}	3,26^{cB}	**
	M4	0,01^{cB}	3,32^{bD}	4,14^{aB}	/	**
	M5	0,47^{cA}	3,00^{bF}	4,55^{aA}	/	**
	M6	0,00^{cB}	3,39^{bC}	3,39^{bE}	3,86^{aA}	**
	Sig.†	**	**	**	**	

$^{A-D}$ Data (mean of three replicates) followed by different capital letters in a column are significantly different by Tukey's multiple range test ($p < 0.05$)

$^{a-d}$ Data (mean of three replicates) followed by different lowercase letters in a line are significantly different by Tukey's multiple range test ($p < 0.05$)

† $p > 0.05$ n.s. not significant, $p < 0.05$ *, $p < 0.01$ **

[1]Samples: M1 = control mozzarella cheeses in sanitised tap water, M2 = mozzarella cheeses in 0.2% (v/v) calcium lactate (CL), M3 = mozzarella cheeses in 0.6% (v/v) CL, M4 = mozzarella cheeses in 0.2% (v/v) sodium chloride (SC), M5 = mozzarella cheeses in 0.6% (v/v) SC, M6 = mozzarella cheeses in 0.4% (v/v) SC

Table 2 Physico-chemical parameters of different lacto-fermented mozzarella cheeses (M) and governing liquids (GL) during the storage

	Samples[1]	0	6	12	18	Sig.†		Samples[1]	0	6	12	18	Sig.†
Moisture (%)	M1	59^{bB}	64^{aC}	64aC	/	**	**Hardness (g)**	M1	6315,80aB	2687,06bC	2473,21bC	/	**
	M2	64^{bcAB}	65^{bC}	63cC	68aB	**		M2	6423,64aB	2768,80bB	2666,10cB	1978,46d	**
	M3	64^{A}	65^{C}	64C	65C	n.s.		M3	5309,05aD	2620,82bD	2879,39bA	2606,61b	**
	M4	66^{bA}	69^{aB}	68abB	/	*		M4	6002,01aC	3189,14bA	2125,29cE	/	**
	M5	63^{bAB}	74^{aA}	71bA	/	**		M5	7509,55aA	1829,76bF	1562,01cF	/	**
	M6	64^{cA}	69^{bB}	68bB	71aA	**		M6	6445,82aB	1912,83dE	2196,30bD	2106,68c	**
	Sig.†	*	**	**	**			Sig. †	**	**	**	n.s.	
Springiness (mm)	M1	0,802bA	0,885aAB	0,790bC	/	**	**Gumminess (g)**	M1	5026,39aC	2075,07cB	2518,08bA	/	**
	M2	0,820abAB	0,861aB	0,803abB	0,768b	**		M2	5007,00aC	2026,38bC	1885,77cC	1304,69dC	**
	M3	0,866bA	0,894aA	0,855cA	0,726d	**		M3	4287,00aE	2006,30cD	2162,84bB	1766,73dA	**
	M4	0,855aA	0,826bD	0,761cD	/	**		M4	4752,54aD	2341,42bA	1368,43cE	/	**
	M5	0,838aAB	0,830aCD	0,681bE	/	**		M5	6091,56aA	1364,69bF	871,75cF	/	**
	M6	0,854aA	0,858aBC	0,803abB	0,739b	**		M6	5063,27aB	1462,42bE	1474,41bD	1478,30bB	**
	Sig.†	**	**	**	n.s.			Sig.†	**	**	**	**	
TA(%)	M1	0,17BC	0,23A	0,19AB	/	n.s.	**TA(%)**	GL1	0,01c	0,09bB	0,20aA	/	**
	M2	0,20aB	0,18abB	0,14bB	0,21a	*		GL2	0,03d	0,12cA	0,20bAB	0,25a	**
	M3	0,16bC	0,11cD	0,17abB	0,21a	**		GL3	0,03d	0,12cA	0,21bA	0,24a	**
	M4	0,18BC	0,14BCD	0,18AB	/	n.s.		GL4	0,02c	0,08bB	0,17aC	/	**
	M5	0,16bC	0,13bCD	0,23aA	/	**		GL5	0,02c	0,07bC	0,18aBC	/	**
	M6	0,24aA	0,19bAB	0,19bAB	0,22ab	*		GL6	0,02d	0,11cA	0,18bBC	0,24a	**
	Sig.†	**	**	**	n.s.			Sig.†	n.s.	**	**	n.s.	
pH	M1	5,98aA	5,88bBC	5,76cA	/	**	**pH**	GL1	6,92aA	5,85bA	5,06cAB	/	**
	M2	5,94aA	5,94aB	5,61cD	5,78bA	**		GL2	6,36aBC	5,73bBC	5,03dBC	5,19c	**
	M3	6,01aA	5,86bC	5,63dCD	5,70cB	**		GL3	6,28aC	5,72bC	5,08dA	5,20c	**
	M4	5,90bAB	6,02aA	5,74cA	/	**		GL4	6,40aB	5,80bAB	5,03cBC	/	**
	M5	6,00aA	6,02aA	5,67bB	/	**		GL5	6,41aB	5,80bAB	5,00cC	/	**
	M6	5,75abA	5,87aC	5,65bBC	5,75abA	*		GL6	6,43aB	5,70bC	5,00dC	5,21c	**
	Sig.†	**	**	**	*			Sig.†	**	**	**	n.s.	
Chlorides (%)	M1	$1,53^{aB}$	$1,65^{aAB}$	$1,16^{bD}$	/	**	**Chlorides (%)**	GL1	0,15cE	0,15bE	0,42aE	/	**
	M2	$1,56^{abB}$	$1,73^{AB}$	$1,72^{aC}$	1,66c	n.s.		GL2	0,18cDE	0,30bD	0,60aD	0,54aC	**
	M3	$1,96^{aA}$	$1,19^{cC}$	1,80abBC	1,51bc	**		GL3	0,21cD	0,27bD	0,60aD	0,61aB	**
	M4	$1,87^{aA}$	$1,56^{bB}$	1,81aBC	/	*		GL4	0,30cC	0,38bC	0,83aC	/	**
	M5	$1,86^{abA}$	$1,66^{bAB}$	2,19aA	/	*		GL5	0,46cA	0,52bA	1,07aA	/	**
	M6	$2,13^{aA}$	$1,95^{abA}$	2,11abAB	1,86b	*		GL6	0,36dB	0,45cB	0,94bB	0,95aA	**
	Sig.†	**	**	**	n.s.			Sig.†	**	**	**	**	

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$^{a-d}$ Data (mean of three replicates) followed by different lowercase letters in a line are significantly different by Tukey's multiple range test ($p < 0.05$)

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[1]Samples: M1 = control mozzarella cheeses in sanitised tap water, M2 = mozzarella cheeses in 0.2% (v/v) calcium lactate (CL), M3 = mozzarella cheeses in 0.6% (v/v) CL, M4 = mozzarella cheeses in 0.2% (v/v) sodium chloride (SC), M5 = mozzarella cheeses in 0.6% (v/v) SC, M6 = mozzarella cheeses in 0.4% (v/v) SC; GL1 = control mozzarella cheeses in sanitised tap water, GL2 = mozzarella cheeses in 0.2% (v/v) calcium lactate (CL), GL3 = mozzarella cheeses in 0.6% (v/v) CL, GL4 = mozzarella cheeses in 0.2% (v/v) sodium chloride (SC), GL5 = mozzarella cheeses in 0.6% (v/v) SC, GL6 = mozzarella cheeses in 0.4% (v/v) SC + 0.2 % CL