Effect of a new sustainable cooling system used during firming and brining on the microbiological, chemical, and sensory characteristics of buffalo mozzarella cheese

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Key words: ammonia chiller, buffalo dairy supply chain, food industry, sustainable innovation.

Contributions: RM, AA, conceptualization; MDP, VV, MDS, methodology; MDP, GP, software; AA, AMLS, RM, validation; MDP, MDS, GP, VV, formal analysis; MDP, MDS, GP investigation; AMLS, AA, resources; MDP, VV, data curation; MDP, MDS, writing - original draft preparation; VV, RM, AA, writing - review and editing; GP, AMLS, MDS, visualization; RM, AA, supervision, project administration; RM, AMLS, funding acquisition. All authors have read and agreed to the published version of the manuscript.

Conflict of interest: the authors declare no potential conflict of interest.

Funding: none.

Availability of data and materials: data and materials are available from the corresponding author upon request.

Conference presentation: this paper was presented at the XXXI National Conference of the Italian Association of Veterinary Food Hygienists (AIVI), 2022 September 22-24, Italy.

Acknowledgments: the authors thank Spinosa S.p.a. for providing expertise that greatly assisted the research.

Received: 2 March 2023.
Accepted: 24 May 2023.
Early view: 11 July 2023.

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Licensee PAGEPress, Italy
Italian Journal of Food Safety 2023; 12:11290
doi:10.4081/ijfs.2023.11290

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Abstract

The cooling applied during the firming and brining processes represents an important production step in mozzarella cheese-making. The temperature fluctuations of the cooling water can negatively affect the hygiene, composition, and quality of mozzarella. Some sustainable cooling systems can minimize this problem by using hot process fluids as heat sources to generate refrigerated energy. This study aimed to evaluate the effects of a new cooling system equipped with a water-ammonia absorption chiller (MA) on the characteristics of buffalo mozzarella through a comparative study with products cooled with a traditional ice water chiller (MT). The buffalo mozzarella cheese manufacture was monitored, and the samples were analyzed for chemical, nutritional, microbiological, and sensory characteristics. The MT samples showed an overall weight loss of 7.4% compared to an average of 2.8% for the MA samples. The MT samples were characterized by greater sapidity than the MA ones, which instead showed a higher moisture content that increased juiciness. The microbiological analysis showed a lower concentration of mesophilic bacterial load in the MA samples than in the MT ones [difference of 1 Log (CFU/g)], which is probably due to the low and constant temperatures that reduced the permanence time of the mozzarella in the vats (firming and brining). This study represents a preliminary positive evaluation of the use of this sustainable cooling system for mozzarella cheese, which is useful for dairy plants with an annual cheese production volume sufficient to justify the operating cost of the plant and the annual energy cost.

Introduction

World buffalo milk production is ranked second after that of bovine milk and represents an important source of nutrients. It is characterized by high fat contents, lactose, casein, whey proteins, and minerals (OECD/FAO, 2022). Buffalo milk is consumed in liquid form or processed into numerous cheeses (Di Paolo *et al.*, 2023). Mozzarella cheese is the main and most typical buffalo dairy product (Sales *et al.*, 2018) that is certified with a European Protected Designation of Origin (PDO) as *Mozzarella di Bufala Campana* (European Commission, 2008). Its production meets the growing trend for healthy and high-nutrient foods (Ahmad *et al.*, 2013). The propriety characteristics of mozzarella cheese (viscoelasticity, flowability, meltability, stretchability, and free oil release) are dependent on the making processes because they affect the microstructure and composition (Ma *et al.*, 2013). In fact, the thermomechanical treatments influence fat and protein structures, changing the structure of mozzarella cheese itself (Gonçalves and Cardarelli, 2021). In mozzarella cheese-making, the cooling process represents an important production step that could influence the characteristics of the final product, such as its weight and organoleptic and microbiological characteristics. These features are mainly linked to the temperature fluctuations of the cooling water that influence the permanence time of mozzarella cheese in the vats (firming and brining) and, consequently, the final quality and manufacturing capacity. New cooling systems are capable of minimizing the temperature fluctuations of the cooling water in the vats and could lead to greater manufacturing capacity and an increase in the competitiveness of companies with effective economic advantages, saving time and energy. A water-ammonia absorption chiller guarantees a water flow into the vat expressly studied to avoid thermal shocks that cause peeling and damage to the structure of the mozzarella cheese. The novelty of the system is represented by the absorbers that exploit heat sources (hot process fluids, industrial waste gases, and waste of congeners) to generate refrigerated energy, creating a sustainable system (Beccali *et al.*, 2012). Mozzarella cheese manufacturers need to understand the importance of process conditions to better control the stability of the cheese properties, increase shelf life, improve product performance, and work sustainably. The impact of some cheese-making steps on the cheese properties still needs to be clarified, and contributions to the improvement of the industrial processes should be continuously investigated.

Thus, this study suggests the evaluation of how the use of a cooling system equipped with a water-ammonia absorption chiller influences the organoleptic, nutritional, microbiological, and sensorial characteristics of the mozzarella through a comparative study with products cooled by using a traditional ice water chiller.

Materials and Methods

Cheese manufacturing and sample collection

Two cheese-making processes were carried out for buffalo mozzarella using two different cooling systems [traditional cooling system (MT) and water-ammonia absorption chiller (MA)]. The new system used in this study was a cooling structure (Zudek S.r.l., Muggia, Trieste, Italy) equipped with a water-ammonia absorption chiller (Enermatik®, Zudek S.r.l., Muggia, Trieste, Italy) that exploits heat sources (hot process fluids, industrial waste gases, and waste of congenators) to generate energy refrigerator. For both buffalo mozzarella productions, raw buffalo milk was pasteurized (72°C for 15 seconds, pH 6.8) and added with natural whey starter culture from the previous day's manufacture and liquid rennet (Caglificio Clerici S.p.a., Codrago, Como, Italy). At curd formation, the coagulum was reduced to particles of 2-3 cm and held under whey until pH 4.9-5.2, a value suitable for manual stretching into hot water (80-90°C). Thereafter, the stretched curd was mechanically formed into 125 g balls that were placed in firming and brine vats (2% NaCl), as reported in Figure 1. During the two different studied buffalo mozzarella cheese manufacturing processes, the following findings were collected on semi-finished cheese products: i) weight and temperature at the outlet of the molder; ii) weight and temperature in the firming vats; iii) weight and temperature in the brine vats. At the end of the processes, 3 samples of MT and 3 samples of MA were collected, sent to the laboratory in refrigerated boxes, and immediately analyzed to determine physicochemical, microbiological, and sensorial parameters.

Physicochemical analysis

The pH measurements were carried out using a digital pH meter (Crison-Micro TT 2022, Crison Instruments, Barcelona, Spain). Water activity (a_w) was measured using Aqualab 4 TE (Decagon Devices Inc., Pullman, USA). Moisture (%) was determined by oven drying for 24 hours at 105°C (AOAC International, 2005). For the determination of nutritional value and labeling purposes, according to Regulation 1169/11 (European Parliament, 2011), total fat content, total saturated fat, salt content (% NaCl), sugars, carbohydrates, proteins, and energy were determined (AOAC International, 2005). Primary and secondary lipid oxidation were evaluated by thiobarbituric acid test [(TBARs test), mg of malondialdehyde per kg of sample (mg of MDA/Kg)], and peroxide value [(PV) mgO2/Kg fat] measurement, respectively. Measurements were performed according to the methods proposed by Ambrosio *et al.* (2021) and Di Paolo *et al*. (2023), respectively.

Microbiological analysis

Ten grams of each sample were aseptically transferred in a sterile stomacher bag with 90 mL [1:10 (w/v)] of sterilized peptone water (Oxoid, Madrid, Spain) and homogenized for 3 minutes at 230 rpm using a peristaltic homogenizer (BagMixer®400 P, Interscience, Saint Nom, France). Appropriate serial decimal dilutions of each homogenate were prepared for the following microorganism counts: total aerobic bacteria (TAB 30°C) were performed on plate count agar (Oxoid, Madrid, Spain) incubated at 30°C for 48/72 hours for 10 days (ISO, 2013); total coliforms on violet red bile lactose agar (Oxoid, Madrid, Spain) incubated at 37°C for 48 hours (ISO, 2006); *Enterobacteriaceae* on violet red bile glucose agar (Oxoid, Madrid, Spain) incubated at 37°C for 48 hours (ISO, 2017); lactic acid bacteria (LAB 30°C) on de man, rogosa and sharpe agar with tween 80 (Oxoid, Madrid, Spain), incubated at

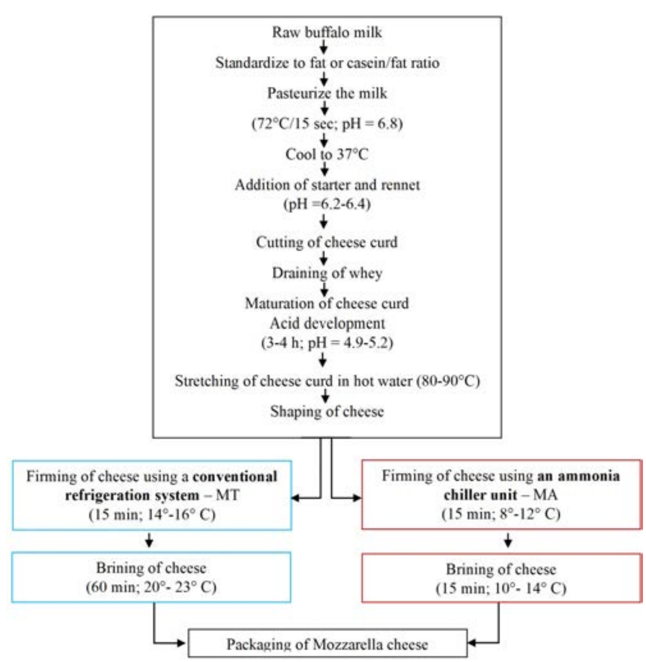

Figure 1. Flow diagram of buffalo mozzarella cheese. After shaping, the firming and brining conditions of mozzarella obtained with a conventional refrigeration system (MT) and mozzarella obtained with an ammonia chiller unit (MA) are reported in blue and red squares, respectively.

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30°C for 72 hours (ISO, 1998); *Pseudomonas* spp. on pseudomonas agar base with PP supplement (Oxoid, Madrid, Spain) incubated at 25°C for 48 hours (ISO, 2010); β-glucuronidase-positive *Escherichia coli* (ISO, 2018) on triptone bile x-glucoronide agar (Oxoid, Madrid, Spain) at 44°C for 24 hours; yeasts and molds on dichloran rose-bengal chloramphenicol Agar (Oxoid, Madrid, Spain) incubated at 25°C for 120/168 hours (ISO, 2008). After counting, the data were expressed as logarithms of the number of colony-forming units (CFU/g), and the means and standard error were calculated.

Sensory analysis

Sensory testing of the mozzarella cheese was performed by 7 trained panel members (average age 25 years) who performed two separate quantitative descriptive sensory analyses of the products obtained in each trial. The panelists were trained on the use of the scale (Stone *et al.*, 2004). The sensory evaluation was performed in a food-grade lab at room temperature, and mozzarella samples with cut surface dimensions 5.8×2.7 cm were placed on a tray, labeled with a randomly generated three-digit code, and served to the assessors. Based on the available literature (Uzun *et al.*, 2018), the panelists developed a specific vocabulary for mozzarella cheese and agreed on a list of 19 attributes consensus concerning the external and internal appearance, odor/flavor, structure, and taste (3, 3, 5, 4 and 4 attributes were identified, respectively). Moreover, texture profile scores were evaluated, identifying 5 attributes (tenderness, chewiness, springiness, juiciness, and cohesion). To assign a score, 4 points of an acceptance scale (absent=0, low=1, medium=2 and high intensity=3) were considered. Randomly numbered samples were served on a tray.

Statistical analyses

Data were statistically analyzed using SPSS version 28 (IBM Analytics, Armonk, NY, US). The generalized linear model was adopted to study mozzarella cheese parameters, including the fixed effects of the cooling systems (MT and MA) used in an experimental trial. All data were presented as the mean±standard error.

Results and Discussion

Cooling process monitoring of mozzarella cheese-making

Mozzarella weights were recorded during the cooling process, from shaping (the starting point for both experimental trials) to brining (Table 1). Cooling is the final stage of the cheese-making process and allows mozzarella to consolidate its structure and color (Calandrelli, 2007). The MT samples showed greater weight loss than the MA ones during the post-shaping phases. At the end of the cheese-making process, the MT samples showed an average weight loss of 7.4% compared to an average of 2.8% for the MA samples. The weight loss data could be explained if it is assumed that the brining phase that took place in the vats using the water-ammonia absorption chiller (MA samples) allowed the mozzarella cheeses to settle for less time in the vats (15 min) in order to reach the final temperature. In mozzarella cheese-making, the cooling should rather quickly reach a temperature below 20°C and then complete it (6-12°C). In the study, the MA samples left the shaping phase at a temperature of about 64°C, consolidated their structure during the firming phase (15 minutes), reaching a temperature of about 32°C, and then passed to the brining phase, reaching a temperature of about 17°C in 15 minutes. Instead, the MT samples, after the shaping phase (cheese internal temperature of 64°C), reached a temperature of about 43°C in the firming vats (15 minutes) and then moved on to the brining phase and reached a temperature of about 21°C in 60 minutes. The temperature fluctuations, using a traditional cooling system, determined a significantly high temperature of MT mozzarella cheeses (43.05±0.47 of MT *vs* 32.57±0.88 of MA; $P<0.01$) after the firming phase, although the permanence time in these vats was the same as that of MA samples (15 minutes). This aspect caused a longer permanence time (60 minutes) for the mozzarella cheese in the brine vats to reach the final cooling temperature (~20°C). According to Fucà *et al*. (2012), during the brining phase, a contraction of the cheese protein matrix that expels whey as well as moisture by osmosis takes place. For this reason, the MT mozzarella cheeses reduced the moisture content (60.64±0.62 of MT *vs* 63.10±1.50 of MA; $P<0.01$), and consequently the final weight (122.60±0.60 of MT *vs* 128.70±0.44 of MA; $P<0.01$).

Physicochemical parameters

Table 2 shows the nutritional values of 125 g of mozzarella cheese according to Regulation 1169/11 (European Parliament, 2011). The results indicated a difference in fat and protein content that was significantly ($P<0.01$) higher in MT than in MA samples, probably due to the low moisture content in MT (60.64±0.62 of MT *vs* 63.10±01.50 of MA; $P<0.01$) that concentrated the nutritional components. For the same reason, the content of salt in MT was higher than in MA (0.43±0.10 of MT *vs* 0.39±0.00 of MA; $P<0.05$). These findings could be explained by the short permanence time of the MA samples in brine vats (15 minutes) compared to the MT samples (60 minutes), which reduced the osmosis phenomenon (Salek *et al.*, 2022). The effects of different cooling systems were also shown by the fat alteration index results (Table 3). PV measures the concentration of hydroperoxides formed in the first step of oxidation (Nadeem *et al.*, 2015) and in this study, PV values of the MT samples were higher than those of the MA samples due to the longer permanence time and consequently light exposure in the cooling vats that had an influence on mozzarella

Table 1. Weights and temperatures detected during the cooling process monitoring of mozzarella cheese production.

Items		Shaping	Firming	Brining
Weight (g)	MT	132.53±0.58^{A}	127.73±0.40^{B,X}	122.60±0.60^{C,X}
	MA	132.53±0.58^{A}	132.60±0.51^{A,Y}	128.70±0.44^{B,Y}
Temperature (°C)	MT	64.28±0.30^{A}	43.05±0.47^{B,X}	21.42±0.28^{C,X}
	MA	64.28±0.30^{A}	32.57±0.88^{B,Y}	17.68±0.57^{C,Y}

MT, mozzarella cheese obtained with a conventional refrigeration system; MA, mozzarella cheese obtained with an ammonia chiller unit. All data were presented as the mean±standard error. Different superscript uppercase letters indicate a significant difference at $P<0.01$. $^{A-C}$Mean values in the same row with different letters presented significant differences; ^{X,Y}mean values in the same column with different letters presented significant differences.

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cheese for more time (Kristensena *et al.*, 2001). If exposed to further oxidation conditions, lipid primary oxidation products can generate secondary oxidation products, such as MDA, which is most commonly used as an oxidation marker (Nadeem *et al.*, 2015). No significant difference was found between the MA and MT samples for TBARs values.

Microbiological parameters

Figure 2 shows the results of microbiological analyses and the effect of the cooling system used on mozzarella cheeses. It is known that the first limiting factor for the growth of mesophilic bacteria is temperature, and its continuous management guarantees the control and slowing down of its growth (Price and Sowers, 2004). In this study, the cooling system equipped with a water-ammonia absorption chiller, guaranteeing low and constant temperatures of the cooling water in the vats, allowed the achievement of low temperatures at the core of the product in a short time (30 minutes overall compared to the 75 minutes required by traditional cooling), ensuring control of bacterial growth. In fact, the levels of TAB 30°C appeared to be affected by the cooling system used, showing a concentration of 3.9 Log (CFU/g) in MA and 5 Log (CFU/g) in MT samples. Although remaining within the acceptability limits, TAB 30°C levels appeared not to be correlated to those of *Lactobacilli* (Faccia *et al*., 2012). Even though pH and a_w are important parameters influencing the growth rate of microbiological communities (Di Paolo *et al.,* 2023), the low presence of LAB 30°C was unable to produce significant quantities of lactic acid capable of lowering the pH and altering the growth of other bacterial populations. In fact, in the MA samples, pH values are slightly lower than in the MT samples (5.28±0.02 in MA *vs* 5.34±0.00 in MT; P<0.05), while there is no significant difference for the a_w values (0.986±0.000 in MA *vs* 0.9874±0.001 in MT; P>0.05). Concerning *Pseudomonas* spp., the results are superimposable and lower than 2 Log (CFU/g); the low concentration of this spoilage bacteria in both samples underlines that there was a correct application of good hygiene practices. The other bacterial populations included in the experimental study were always below the minimum detectable limit. This data confirms and highlights the correct application of good practices, especially if we consider that the concentration of *E. coli* and *Enterobacteriaceae* has always been lower than 1 Log (CFU/g).

Sensory evaluation

A panel test was performed to verify the sensory characteristics of the MA and MT samples. No appreciable significant differences were found between the MA and MT samples for external and internal aspects, odor/flavor, structure, and taste. A significant difference (data not shown) was found in the MT samples character-

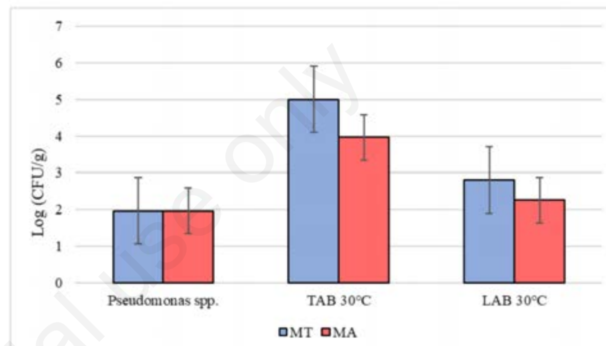

Figure 2. Microbiological [Log (CFU/g)] results of mozzarella cheese. MT, mozzarella cheese obtained with a conventional refrigeration system; MA, mozzarella cheese obtained with an ammonia chiller unit; TAB, total aerobic bacteria; LAB, lactic acid bacteria.

Table 2. Nutritional values and fat alteration index of mozzarella cheese in two different cooling systems.

Nutritional values	MT	MA
Energy (Kcal)	268.15±1.36[a]	252.63±3.41[b]
Fat (%)	22.37±0.15[a]	21.37±0.26[b]
of which saturates	14.63±0.17	14.30±0.26
Carbohydrate (%)	0.80±0.06	0.83±0.03
of which sugar	0.80±0.06	0.83±0.03
Protein (%)	15.90±0.02[a]	14.25±0.30[b]
Salt (%)	0.43±0.10[a]	0.39±0.00[b]

MT, mozzarella cheese obtained with a conventional refrigeration system; MA, mozzarella cheese obtained with an ammonia chiller unit. All data were presented as the mean±standard error. Different superscript uppercase letters indicate a significant difference at P<0.01. Different superscript lowercase letters indicate a significant difference at P<0.05. [a-b]Mean values in the same row with different letters presented significant differences.

Table 3. Fat alteration index of mozzarella cheese in two different cooling systems.

Fat alteration index	MT	MA
PV (mgO2/Kg fat)	0.85±0.03[a]	0.70±0.02[b]
TBARs (mgMDA/kg)	0.01±0.00	0.01±0.00

MT, mozzarella cheese obtained with a conventional refrigeration system; MA, mozzarella cheese obtained with an ammonia chiller unit; MDA, malondialdehyde. All data were presented as the mean±standard error. Different superscript lowercase letters indicate a significant difference at P<0.05. [a-b]Mean values in the same row with different letters presented significant differences.

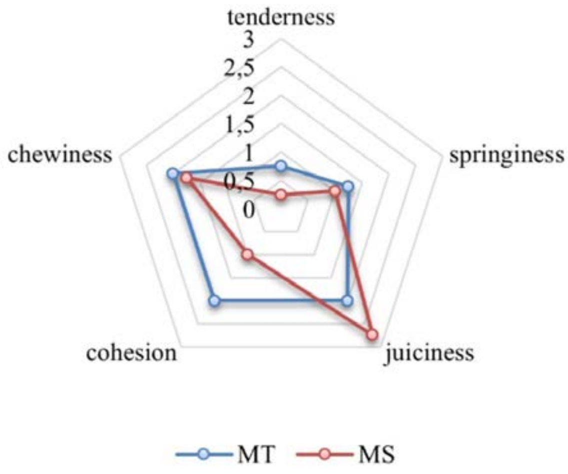

Figure 3. Influence of different cooling systems on texture profile scores. MT, mozzarella cheese obtained with a conventional refrigeration system; MA, mozzarella cheese obtained with an ammonia chiller unit.

ized by a greater sapid flavor, which is also confirmed by the chemical analyses that showed a higher salt content in MT than MA (0.43±0.10 in MT vs 0.39±0.00 in MA; $P<0.05$). However, this result could be explained by the water loss of the MT mozzarella due to its long permanence in the brine vats. In fact, the removal of water slows down the gustatory perception of the palate. This aspect also affected the panelists' judgment regarding the texture profile during chewing, highlighting significant differences between MA and MT samples (Figure 3). In particular, MA samples reported higher scores for the juiciness parameter and lower scores for the cohesion parameter than MT samples. These results could be explained by the lower water content retained by the MT mozzarella, which affected its structure, determining greater compactness and reducing the moisture released during chewing.

Conclusions

This study highlights the impact of the cooling phase (firming and brining) on the properties of buffalo mozzarella cheese, clarifying the possibility of improving the industrial processes investigated. The use of a cooling system equipped with a water-ammonia absorption chiller influenced the organoleptic, microbiological, and, in particular, sensorial characteristics (more juiciness) of the buffalo mozzarella, even if the nutritional quality was not particularly altered. A new cooling system that keeps the cooling water temperature constant makes the reduction and standardization of the permanence time of mozzarella cheeses in firming and brine vats possible. This aspect guarantees the finished product, avoiding additional time and reducing the inevitable weight loss. However, the new cooling system has controlled mesophilic bacterial growth, which is probably positively affecting the products' shelf life. The system's novelty is represented by the absorbers that exploit heat sources (hot process fluids, industrial waste gases, and waste from congeners) to generate energy refrigerator, creating a sustainable system that avoids temperature fluctuations. The study represents a first positive evaluation of the use of this sustainable cooling system in mozzarella cheese-making, which is mostly useful for dairy plants with an annual cheese production volume that justifies the operating cost of the plant and the annual energy cost.

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