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The impact of medicinal and aromatic plant addition on antioxidant, total phenolic, antimicrobial activities, and microbiological quality of Mozzarella cheese

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Abstract

In the present work, the antioxidant, antimicrobial activities, total phenolic content, and microbiological quality of Mozzarella cheeses added with medicinal and aromatic plants namely rosemary (*Rosmarinus officinalis* L.), basil (*Ocimum basilicum* L.), peppermint (*Mentha piperita* L.), and Turkish oregano (*Origanum onites* L.) were investigated. Results demonstrated that peppermint and Turkish oregano increased antioxidant and antimicrobial activities, while Turkish oregano and basil increased the total phenolic content of the Mozzarella cheese samples. Mozzarella cheese samples added with Turkish oregano exhibited antimicrobial activity against *Escherichia coli, Listeria monocytogenes*, and *Enterococcus faecalis* during 30 days of storage. Mozzarella cheese sample added with rosemary and peppermint exhibited antimicrobial activity against *Staphylococcus aureus* in early storage, and Mozzarella cheese sample added with rosemary and Turkish oregano exhibited antimicrobial activity against *Bacillus cereus* on the 10th day of storage. It was determined that the highest activity against the tested microorganisms was observed in Mozzarella cheese sample added with Turkish oregano and peppermint. It was also determined that *Lactobacillus* spp. and *Streptococcus thermophilus* counts were above log 4 CFU/g for 30 days. The addition of medicinal and aromatic plants to the Mozzarella cheese samples did not affect yeast and mould development, and the yeast and mould counts increased during the storage period.

Introduction

Mozzarella cheese is a famous Italian fibrous cheese, and usually consumed fresh. It is originally produced from buffalo milk; however, a mixture of buffalo and cow milk or only cow milk has also been employed due to the insufficient buffalo milk supply. Mozzarella cheese is the main ingredient for pizza and other Italian dishes. Mozzarella is mostly spherical or egg-shaped, 10 - 15 cm in diameter, and each piece weighs around 125 - 350 g. It is known that Mozzarella cheese produced from buffalo milk is more delicious and aromatic as compared to those produced from cow milk (Jana and Mandal, 2011; Jana and Tagalpallewar, 2017).

Medicinal and aromatic plants, which are utilised for various purposes, are commonly used in food industry to add flavour and taste to food products. The increase in the utilisation of these medicinal and aromatic plants in the food industry has been due to their antioxidant, antidiabetic, and antihypertensive properties, phenolic content, and antimicrobial activities. Their aromatic properties also play a particularly significant role in food industry. There are several plants that are rich in phenolic acids, flavonoids, and aromatic compounds; and these compounds are known to have antioxidant properties. Furthermore, plants also have diverse antimicrobial properties that vary based on the plant species, microorganism types, and the essential oil concentrations of the plant. The antimicrobial properties of plants are mostly due to their essential oil content. Essential oils of thyme, rosemary, curcumin, pepper, and sage have been found to exhibit antimicrobial activities against pathogens and degradation of microorganisms in *in vitro* (Viuda-Martos *et al.,* 2010; Oraon *et al.,* 2017).

Several studies showed that the use of natural preservatives could extend the shelf life of several cheese varieties and minimise the consumer health risks associated with cheese consumption. Medicinal and aromatic plant extracts, and also essential oils have been shown to be natural preservatives with significant

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inhibitory activities against harmful microorganisms and pathogens that could lead to significant problems in cheeses and threaten human health. The addition of medicinal and aromatic plants and their extracts to cheeses aims to obtain different cheese flavour, reduce microbial content, and improve the attractiveness of cheese to increase cheese consumption. Furthermore, medicinal and aromatic plants could reduce salt requirement and structural defects in the cheese (Gouvea *et al.,* 2017; Ritota and Manzi, 2020).

It is crucial to conduct research on the use of different medicinal and aromatic plant extracts in food products such as cheese. Their possible combinations and interactions with the food matrix give effects on product quality. Furthermore, the acceptance of the sensory properties of cheese products by the consumers also has great significance. In recent years, several studies have been conducted on the use of medicinal and aromatic plants and also related products as preservatives (Granato *et al.,* 2018; El-Sayed and Youssef, 2019). The present work thus aimed to improve the functional properties of Mozzarella cheese added with certain medicinal and aromatic plants during its production. For this purpose, the antioxidant, total phenolic content, antimicrobial activity, and microbiological quality of the Mozzarella cheese added with dried Turkish oregano, rosemary, basil, and peppermint were investigated for 30 days.

Materials and methods

Materials

In the production of Mozzarella cheese, the curd produced from cow milk was provided by commercial milk producer (Altınköy Food Industry and Trade Limited Company, Izmir, Turkey); and the cheese starter culture which contained *Streptococcus thermophilus, Lactobacillus bulgaricus*, and *L. helveticus* combination was obtained from Chr. Hansen (Hoershom, Denmark). Chymosin (TSI® Inc. Lakeville, USA) was used as cheese starter, and NaCl (Billur Salt, Turkey) was used to prepare the brine. Turkish oregano (*Origanum onites* L.), rosemary (*Rosmarinus officinalis* L.), basil (*Ocimum basilicum* L.), and peppermint (*Mentha piperita* L.) were provided from Kimbiotek Chemicals Industry and Joint Stock Company (Istanbul, Turkey).

Method

Mozzarella cheese production and addition of medicinal and aromatic plants

The conventional curd that was used in the production of the Mozzarella cheese was kept in 80 - 85°C water for 15 - 30 s. Medicinal and aromatic plants were added during the kneading of the boiled curd (0.5%, w/w). The cheese was moulded into round Mozzarella shape, vacuum-packed in brine, and stored for 30 days at cold temperature. The analyses were performed on the 1st, 10th, 20th, and 30th days of storage. The experiment was conducted in duplicate, and the analyses were conducted twice.

Preparation of samples for total antioxidant activity and total phenolic content analyses

To begin, 5 mL of methanol (Sigma Aldrich, St Louis, USA) was added to 5 g of cheese sample, and vortexed for 5 min. Then, the extracts were stored in the refrigerator for 30 min, and centrifuged at 8,602 *g* at 4°C for 30 min. The supernatant was filtered through Whatman No. 42 filter.

Total antioxidant activity

The free radical scavenging activity of the cheese extracts was determined with the modified method of Pavithra and Vadivukkarasi (2015). Briefly, 100 µL of 0.2 mM DPPH solution (Sigma Aldrich, St Louis, USA) (prepared in methanol) was added to 100 µL of extract. After incubation of 30 min in the dark at room temperature, the absorbance was measured with a 96-well microplate reader (Thermo Scientific, Multiskan Sky, Waltham, Massachusetts, USA) at 517 nm. Next, 100 µL of methanol and 100 µL of DPPH solution were added to the control sample. The radical scavenging activity was calculated using Eq. 1

$$\% \text{ radical scavenging activity} = [(Abs_{control} - Abs_{sample}) / Abs_{contol} \times 100 \quad \text{(Eq. 1)}$$

Total phenolic content

The total phenolic content was determined based on the method proposed by Kocadag Kocazorbaz *et al.* (2017). Briefly, 100 µL of Folin (Merck, Darmstadt, Germany) reagent (1:10 diluted) was added to 20 µL of cheese extract, and incubated for 5 min at room temperature. Then, 80 µL of 7.5% sodium carbonate (Merck, Darmstadt, Germany) solution was added, and incubated at room temperature for 1 h in the dark. Absorbance was measured at 760 nm with a 96-well microplate reader (Thermo Scientific, Multiskan Sky, Waltham, Massachusetts, USA). For blank solution, the sample was substituted with methanol. The total phenolic content of the samples was calculated as gallic acid (Sigma Aldrich, St Louis, USA) equivalent.

Antimicrobial activity

Escherichia coli CECT 4267, *Staphylococcus aureus* ATCC 12600, *Listeria monocytogenes* ATCC

13932, *Bacillus cereus* CECT 131, and *Enterococcus faecalis* ATCC 29212 strains were employed as test microorganisms. Overnight cultures were grown by transferring the test microorganisms twice to 1% sterilised Trypticase Soy Agar (Oxoid, UK). The test microorganism load was adjusted to log 10^8 – 10^9 CFU/mL by measuring the optic density (OD) at 340 nm.

Next, 1 mL of each test microorganism was cultured in Trypticase Soy Agar (Oxoid, UK). Under aseptic conditions, 10 g of Mozzarella cheese was weighed in a sterile bag with filter, and 90 mL of peptone water was added prior to homogenisation with a stomacher (BagMixer, Interscience, France). Later, 20 µL of sample was impregnated onto 6 mm diameter Whatman AAA discs, and placed on the solidified medium surface in Petri dishes. The zones that developed around the discs in Petri dishes, which were incubated at the temperature and for a period adequate to the selected bacteria, were measured as mm (Zaidan *et al.,* 2005; Balouiri *et al.,* 2016).

Microbiological counts

To determine the *Lactobacillus* spp. count in cheese samples, MRS agar (Merck, Darmstadt, Germany) medium was employed and incubated anaerobically for 72 h at 42°C after the adequate dilutions were cultured. Anaerocult-A (Merck, Darmstadt, Germany) was placed in 2 L jars for anaerobic environment. For *Streptococcus thermophilus* count, M17 agar (Merck, Darmstadt, Germany) medium was used, and incubated for 48 h anaerobically at 37°C (Tharmaraj and Shah, 2003). Yeast Glucose Chloramphenicol Agar was used to determine the yeast and mould count, and the cultured Petri dishes were incubated at 25°C for 3 - 5 d. The yeast and mould colonies that developed on the medium were counted separately and presented as total yeast and mould count (Welthagen and Viljoen, 1997).

Statistical analysis

SPSS 20.00 statistics software was used to determine the impact of medicinal and aromatic plants on Mozzarella cheese. Data are presented as mean ± standard deviation (SD). Analysis of variance (ANOVA) was applied, and the obtained means were compared to determine the differences at $p < 0.05$ confidence level with the Duncan multiple comparison test.

Results and discussion

Total antioxidant activity

DPPH method was used to analyse the antioxidant activities of medicinal and aromatic plant-added Mozzarella cheese samples. DPPH radical scavenging activities of Mozzarella cheeses are given in Table 1. The use of medicinal and aromatic plants in Mozzarella cheeses and storage period significantly affected the DPPH radical scavenging activities ($p < 0.05$).

The highest DPPH radical scavenging activity was determined in the T sample on the 1st day (96.79%), in the B sample on the 10th day (97.29%), in the T sample on the 20th day (93.87%), and in the T sample on the 30th day (91.38%) ($p < 0.05$). The antioxidant activities of the C and R samples were quite low as compared to other samples during storage. The highest antioxidant activity was also found in the M sample on 20th and 30th days of storage. Medicinal and aromatic plant-added Mozzarella cheese samples exhibited higher antioxidant activity as compared to the control group due to the plant-specific phytochemical content and microbial metabolic activities. Peppermint, basil, Turkish oregano, and rosemary are rich in phytochemical compounds. In addition, high DPPH inhibition capacity could be associated with lactic acid bacteria that are active even at low temperatures. Sustenance of the microbial development during cold storage may have been triggered by phenolic compounds. For

Table 1. DPPH radical scavenging activities (%) of Mozzarella cheese samples during storage period ($n = 2$).

Storage day	DPPH radical scavenging activity (%)				
	C	R	B	M	T
1	34.38 ± 1.62^{Cc}	34.73 ± 4.04^{Cc}	97.41 ± 0.86^{Aa}	91.57 ± 3.60^{Bb}	96.79 ± 2.25^{Aa}
10	47.02 ± 8.14^{Dbc}	38.19 ± 5.76^{Ebc}	97.29 ± 1.56^{Aa}	87.95 ± 3.53^{Bc}	84.94 ± 2.56^{Cc}
20	51.96 ± 5.87^{Da}	43.19 ± 2.44^{Eb}	86.84 ± 2.81^{Cc}	93.87 ± 2.79^{Aa}	91.12 ± 8.37^{Bb}
30	45.68 ± 6.10^{Db}	61.77 ± 0.99^{Ca}	89.28 ± 3.86^{ABb}	91.38 ± 1.72^{Ab}	90.43 ± 5.69^{bB}

C = plain Mozzarella cheese; R = rosemary-added Mozzarella cheese; B = basil-added Mozzarella cheese; M = peppermint-added Mozzarella cheese; and T = Turkish oregano-added Mozzarella cheese. Values are mean ± SD. Means followed by different uppercase letters within a row are statistically significant ($p < 0.05$). Means followed by different lowercase letters within a column are statistically significant ($p < 0.05$).

example, carvacrol, which is a significant part of the oil content in thyme, is a phenolic monoterpene and has antioxidant properties. Gupta *et al.* (2009) reported that the antioxidant activity of Cheddar cheese increased during maturation, and the antioxidant activity was associated with proteolysis degree. The antioxidant capacity of milk and dairy products is mainly due to amino acids which contained sulphur such as cysteine, phosphate, vitamins A and E, carotenoids, zinc, selenium, enzyme systems, superoxide dismutase, catalase, glutathione peroxidase, milk oligosaccharides, and peptides. Several studies were conducted on the augmentation of the antioxidant activity of milk and dairy products with phytochemical supplements (Khan *et al.* 2019a; 2019b). Josipović *et al.* (2015) added various medicinal and aromatic plants to Cottage cheese, and reported that dried rosemary had the highest antioxidant effect due to its ingredients such as caffeine, rosmarinic acid, flavones, and phenolic diterpenes. Unlike the present work, Santos *et al.* (2012) confirmed higher antioxidant activity in rosemary extract. Madsen and Bertelsen (1995) attributed the antioxidant activity of rosemary to the presence of phenolic diterpenes such as carnosol and carnosic acid. In a study that investigated the antioxidant properties of 31 medicinal and aromatic plant species cultivated in Turkey (Akgul and Ayar, 1993), it was determined that rosemary had the highest antioxidant property, followed by sage, sumac, and oregano. Altiok *et al.* (2010) reported that thyme oil addition increased antioxidant capacity of thyme oil-added chitosan-based films. Flavonoids such as caffeic acid, rosmarinic acid, hispidulin, apigenin, and phenolic acids are the most common antioxidants found in thyme (Zheng and Wang, 2001).

Total phenolic content

Phenolic substances are bioactive components with significant effects on human health. In the present work, the highest TPC was determined in the B sample on the 20th day of storage (122.00 ± 5.57). The lowest TPC was determined in the control sample during the storage period. Although the TPC in the R, M, and T samples exhibited a constant increase during storage, TPC varied in control and B samples during storage (Table 2). In the final days of storage (20th and 30th days), the TPC was lower in the control and R samples as compared to the other samples, similar to the antioxidant activity findings discussed earlier. Results showed that there was a positive correlation between TPC and antioxidant activity.

It was also determined that the addition of various medicinal and aromatic plants and storage period significantly affected the TPC of samples ($p < 0.05$). Although the control sample was not added with any medicinal and aromatic plants, the TPC of the control sample could be associated with amino acids with phenolic rings due to the breakdown of milk proteins. It could also be associated with the microbial use of phenolic acids, such as ferulic and p-coumaric acid, formed during the fermentation and post-acidification that led to the production of other phenolic acids such as vanillic and *p*-hydroxybenzoic acids (Kumar and Goel, 2019; Valanciene *et al.,* 2020). The increase in TPC in medicinal and aromatic plant-added cheese samples could be explained with plant-specific phytochemical compounds (such as flavonoids and phenolic components) (Amirdivani and Baba, 2011). Josipović *et al.* (2015) used fresh or dried plants in Cottage cheese, and it was determined that the plant addition led to higher TPC as compared to the control sample, and the highest TPC (34.54 mg GAE/100 g) was observed with rosemary-added cheese sample. Similar to the present work, Alezandro *et al.* (2011) reported that rosemary extract exhibited high TPC. Branciari *et al.* (2015) reported that rosemary extract added to pecorino cheese also increased the TPC.

Table 2. Total phenolic contents of Mozzarella cheese samples during storage period ($n = 2$).

Storage day	Total phenolic content (mg gallic acid/L)				
	C	R	B	M	T
1	23.02 ± 9.83^{Dc}	56.16 ± 5.92^{Bb}	69.09 ± 3.92^{Ac}	48.64 ± 7.76^{Cd}	50.31 ± 6.67^{Cd}
10	68.46 ± 3.02^{Ba}	58.35 ± 2.95^{Cb}	48.06 ± 5.59^{Dd}	56.06 ± 1.71^{Cc}	92.70 ± 7.74^{Ac}
20	66.97 ± 3.00^{Ea}	79.72 ± 7.58^{Da}	122.00 ± 5.57^{Aa}	84.29 ± 8.37^{Cb}	90.32 ± 5.63^{Bb}
30	50.74 ± 4.52^{Db}	81.14 ± 4.74^{Ca}	89.00 ± 2.65^{Bb}	87.7 ± 5.59^{Ba}	105.32 ± 13.72^{Aa}

C = plain Mozzarella cheese; R = rosemary-added Mozzarella cheese; B = basil-added Mozzarella cheese; M = peppermint-added Mozzarella cheese; and T = Turkish oregano-added Mozzarella cheese. Values are mean ± SD. Means followed by different uppercase letters within a row are statistically significant ($p < 0.05$). Means followed by different lowercase letters within a column are statistically significant ($p < 0.05$).

Antimicrobial activity

Antimicrobial compounds have been observed in many plant species. Spices, medicinal, and aromatic plants are used in food products to add flavour and prolong the shelf life. The antimicrobial activity findings from medicinal and aromatic plant-added Mozzarella cheese samples are presented in Table 3.

No antimicrobial activity was observed in the control sample on the 1st day of storage, while the antimicrobial activity observed in the following days was due to the release of intracellular metabolites with antimicrobial effect as a result of the autolysis of starter cultures employed in the production. This effect was at a maximum on the 30th day. Among the Mozzarella cheese samples, the T sample exhibited the highest antimicrobial activity as compared to the other samples. The T sample exhibited antimicrobial activity against *Escherichia coli, Listeria monocytogenes*, and *Enterococcus faecalis* during storage, and the activity reached the maximum on the 10th day of storage. Antimicrobial activity of other medicinal and aromatic plants against the test microorganisms remained limited. While antimicrobial activity was observed against *Escherichia coli, Listeria monocytogenes*, and *Enterococcus faecalis* in R, B, M, and T samples, it was observed against *Staphylococcus aureus* on the 1st day of storage in R and M samples, and against *Bacillus cereus* on the 10th day of storage in T and R samples. Deans and Ritchie (1987) studied the antibacterial properties of 25 essential oils against 25 bacterial species, and reported that thyme essential oil exhibited the highest inhibition. Marino *et al.* (1999) reported the bacteriostatic activities of thyme on Gram-negative and Gram-positive bacteria. Similar to these studies, the antimicrobial activities of the T sample against *Escherichia coli, Listeria monocytogenes*, and *Bacillus cereus* were significantly higher during the storage as compared to other samples. Due to the high phenolic content, *Origanum onites* L. is known for its antibacterial and antiseptic properties.

Qian *et al.* (2016) investigated the antipathogenic activities of 30 plant extracts obtained from different sources. They determined that 15 plants exhibited antibacterial effects against *Escherichia coli, Salmonella* Enteritidis, *Salmonella*

Table 3. Antimicrobial activities of Mozzarella cheese samples during storage period (n = 2; diameter of inhibition zone [mm]).

Sample	Storage day	Indicator microorganism				
		E. coli **CECT 4267**	***L. monocytogenes*** **ATCC 13932**	***S. aureus*** **ATCC 12600**	***E. faecalis*** **ATCC 29212**	***B. cereus*** **CECT 131**
C	1	-	-	-	-	-
	10	-	14.00	-	-	-
	20	-	13.00	-	11.00	-
	30	-	12.00	-	13.00	-
R	1	-	10.00	10.50	10.00	-
	10	-	10.00	-	10.00	10.00
	20	-	-	-	10.00	-
	30	-	-	-	10.00	-
B	1	10.00	-	-	-	-
	10	-	10.00	-	-	-
	20	-	13.00	-	-	-
	30	10.00	10.00	-	13.00	-
M	1	-	-	10.00	11.00	-
	10	-	10.00	-	12.00	-
	20	-	12.00	-	11.00	-
	30	-	12.00	-	11.00	-
T	1	12.00	15.00	-	11.00	-
	10	10.00	11.00	-	17.00	10.00
	20	10.00	10.00	-	13.00	-
	30	10.00	12.00	-	12.00	-

C = plain Mozzarella cheese; R = rosemary-added Mozzarella cheese; B = basil-added Mozzarella cheese; M = peppermint-added Mozzarella cheese; and T = Turkish oregano-added Mozzarella cheese. (-) = diameter of inhibition zone lower than 10 mm.

Typhimurium, and *Staphylococcus aureus*. Smith-Palmer *et al.* (1998) reported that essential oils and two essences of 21 medicinal and aromatic plants exhibited antimicrobial effects against food pathogens such as *Campylobacter jejuni, Salmonella* Enteritidis, *Escherichia coli, Staphylococcus aureus*, and *Listeria monocytogenes*; with bay leaf, cumin, and thyme exhibiting the highest inhibitory effects. Josipović *et al.* (2015) added several medicinal and aromatic plants to Cottage cheese, and reported that dried rosemary-added sample exhibited the highest antimicrobial activity, and effectively reduced the number of food-borne pathogens such as *Salmonella* Typhimurium, *Escherichia coli*, *Staphylococcus aureus*, and *Listeria monocytogenes*, and suggested its use as natural preservative and antioxidant. Con *et al.* (1998) tested the antimicrobial activities of thyme, allspice, cumin, peppermint, black pepper, and chive essential oils against *L. monocytogenes, S. aureus, L. sake, L. plantarum, Y. enterocolitica, P. acidilactici, P. pentosaceus,* and *M. luteus*, and determined that thyme essential oil exhibited the highest antimicrobial activity. Zhang *et al.* (2016) emulsified thyme essential oil with whey, Arabic gum, lecithin, and their equal mixtures, and investigated the antimicrobial activities of these emulsions against *Escherichia coli* O157:H7, *Salmonella enterica*, and *Listeria monocytogenes*. They determined that the minimum inhibitory and minimum bactericidal concentrations of all emulsions were lower than plain thyme oil dissolved in ethanol. Further studies are required on the use of medicinal and aromatic plants with natural antimicrobials in food processes (Tajkarimi *et al.,* 2010).

Microbiological quality

The *Lactobacillus* spp., *Streptococcus thermophilus*, and yeast and mould counts determined in medicinal and aromatic plant-added Mozzarella cheese samples during storage are presented in Table 4. It was observed that the addition of various medicinal and aromatic plants to

Table 4. Microbial loads of Mozzarella cheese samples during storage period (n = 2; log CFU/g).

Sample	Storage day	*Lactobacillus* spp.	*Streptococcus thermophilus*	Yeast and mould
C	1	5.86 ± 0.23^{Dbc}	6.69 ± 0.22^{Ad}	2.24 ± 0.09^{Bd}
	10	6.24 ± 0.05^{Da}	7.00 ± 0.00^{Ac}	2.48 ± 0.01^{Cc}
	20	6.29 ± 0.05^{Da}	8.94 ± 0.02^{Aa}	3.58 ± 0.02^{Eb}
	30	6.04 ± 0.06^{Db}	8.57 ± 0.04^{Bb}	4.62 ± 0.05^{Da}
R	1	4.12 ± 0.40^{Ed}	4.40 ± 0.11^{Dd}	1.09 ± 0.12^{Dd}
	10	5.28 ± 0.19^{Ec}	6.03 ± 0.04^{Bc}	2.79 ± 0.01^{Ac}
	20	6.09 ± 0.07^{Eb}	8.27 ± 0.00^{Bb}	4.87 ± 0.04^{Ab}
	30	7.00 ± 0.00^{Ba}	8.47 ± 0.12^{Ca}	5.27 ± 0.05^{Aa}
B	1	6.60 ± 007^{Bb}	5.79 ± 0.05^{Bd}	2.67 ± 0.05^{Ac}
	10	6.51 ± 0.16^{Cc}	6.00 ± 0.00^{Bc}	2.60 ± 0.01^{Bc}
	20	6.50 ± 0.28^{Cc}	7.71 ± 0.15^{Cb}	3.95 ± 0.00^{Db}
	30	7.32 ± 0.07^{Aa}	7.96 ± 0.08^{Ea}	5.00 ± 0.01^{Ba}
M	1	7.22 ± 0.03^{Aa}	5.66 ± 0.06^{Cd}	2.22 ± 0.11^{Bc}
	10	6.74 ± 0.06^{Bb}	6.00 ± 0.00^{Bc}	2.04 ± 0.06^{Dd}
	20	6.78 ± 0.12^{ABb}	6.77 ± 0.07^{Db}	4.24 ± 0.01^{Cb}
	30	6.67 ± 0.02^{Cbc}	8.91 ± 0.02^{Aa}	4.30 ± 0.01^{Ea}
T	1	6.15 ± 0.02^{Cc}	5.75 ± 0.03^{Bd}	2.04 ± 0.37^{Cd}
	10	6.94 ± 0.11^{Ab}	6.00 ± 0.00^{Bc}	2.60 ± 0.01^{Bc}
	20	6.90 ± 0.08^{Aab}	7.61 ± 0.00^{Cb}	4.73 ± 0.00^{Bb}
	30	7.03 ± 0.05^{Ba}	8.33 ± 0.21^{Da}	4.76 ± 0.02^{Ca}

C = plain Mozzarella cheese; R = rosemary-added Mozzarella cheese; B = basil-added Mozzarella cheese; M = peppermint-added Mozzarella cheese; and T = Turkish oregano-added Mozzarella cheese. Values are mean ± SD. Means followed by different uppercase letters within a row are statistically significant ($p < 0.05$). Means followed by different lowercase letters within a column are statistically significant ($p < 0.05$).

the Mozzarella cheese samples and storage period significantly affected *Lactobacillus* spp., *Streptococcus thermophilus*, and yeast and mould counts (log CFU/mL) ($p < 0.05$). The *Lactobacillus* spp., *Streptococcus thermophilus*, and yeast and mould counts varied between log 4.12 and 7.32, log 4.40 and 8.94, and log 1.09 and 5.27 CFU/g during storage, respectively. *Lactobacillus* spp. count decreased only in the M sample, while increased in all the other samples during storage. *Streptococcus thermophilus* count increased in all samples during storage, and the highest viability was detected in the C sample. Yeast and mould count also increased during storage. Based on the increased yeast and mould counts, we can say that the type and composition of the Mozzarella cheese samples are suitable for the development of yeasts and moulds.

Hossain (2015) reported that use of various medicinal and aromatic plant with different concentrations (0.5, 1, 2, and 3%, w/w) (*Allium* sp., *Thymus* sp., *Anthriscus* sp., and *Ferule* sp.) supported acid production of *Streptococcus thermophilus* and *Lactobacillus delbrueckii*, and it was determined that an increase in medicinal and aromatic plant concentration increased the acid production. El-Nawawy *et al.* (1998) emphasised that the *Streptococcus thermophilus* and *Lactobacillus bulgaricus* bacteria decreased with the addition of thyme oil in Labneh cheese. Ayar and Akyuz (2003) determined that thyme extract prevented bacterial, yeast, and mould activities to a lesser extent in peppermint and thyme extract-added Feta cheese sample.

The yeast and mould count increased during the storage of medicinal and aromatic plant-added Mozzarella cheeses. This increase could be explained by the fact that the contribution of the medicinal and aromatic plants to the microbial stability of the Mozzarella cheese samples was insufficient. Ekici *et al.* (2019) reported that total lactic acid bacterial count in 100 medicinal and aromatic plant-added cheeses were between log < 10 and 7.0 CFU/g. Alemdar and Agaoglu (2016) reported that lactic acid bacterial count in medicinal and aromatic plant-added cheese was log 8.61 CFU/g, while mould growth was not observed in cheese samples. Contrary to our findings, Paksoy (2016) reported that yeast and mould count was negatively affected in medicinal and aromatic plant-added ultra-filtrated cheese samples. Olmedo *et al.* (2013) determined that essential oil-added samples had longer shelf life and lower peroxide content in thyme and rosemary essential oil-added cream cheeses.

Conclusion

In the present work, peppermint (*Mentha piperita* L.), rosemary (*Rosmarinus officinalis* L.), Turkish oregano (*Origanum onites* L.), and basil (*Ocimum basilicum* L.) were added in Mozzarella cheeses, and the antioxidant activity, total phenolic content, antimicrobial activity, and microbiological quality of the cheese samples were analysed during 30 days of storage. The antioxidant activities in basil-, peppermint-, and Turkish oregano-added cheese samples were quite higher as compared to the rosemary-added and control samples. Although the control sample was plain Mozzarella cheese, it was determined that it included a certain level of phenolic content. Antimicrobial activities in the Turkish oregano-added cheese against *Escherichia coli, Listeria monocytogenes*, and *Bacillus cereus* were significantly higher as compared to other samples. Furthermore, the medicinal and aromatic plant addition significantly affected *Lactobacillus* spp., *Streptococcus thermophilus*, and yeast and mould counts. It was also revealed that the functional properties of the Mozzarella cheese could be improved with medicinal and aromatic plants. Future *in vitro* studies could aim to determine some biological activities such as antihypertensive and antidiabetic activity of Mozzarella cheese samples added with various medicinal and aromatic plants.

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