Original Article

Mentha Leaf Extracts and Oils: A Novel Approach to Enhance the Flavor and Shelf Life of Soft Cheese

Ammar Abdulhasan Aldhalemi[1], Murtadha Abdulhasan Aldhalemi[1], Sadiq Jaafir Aziz Alneamah[1], Maeeda Mohammed Jawad Almaghanichy[1], Ghadeer Sabah Bustani[2*]

1. Department of Food Science, Faculty of Agriculture, University of Kufa, Kufa, Iraq.
2. Department of Anesthesia, Faculty of Medical Technologies, The Islamic University, Najaf, Iraq.

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ABSTRACT

Background: The culinary world is dynamic, in which flavors, textures, and preservation techniques are vital to creating pleasing and sustainable food offerings. However, food spoilage is a complex issue resulting from various factors across the food production chain. Mint, a plant known for its aromatic leaves and diverse applications, contains volatile oils and bioactive compounds with potential benefits in food preservation and enhancement.

Objectives: This study explores the potential of Mentha leaf extracts and oils to enhance the flavor and extend the shelf life of soft cheese.

Methods: Three extraction techniques (alcoholic, aqueous, and essential oil distillation) were applied to fresh mint leaves collected in Najaf City, Iraq. With Mentha leaf extract integration, soft cheese production involves traditional and innovative processes. The quality assessment included pH analysis, sensory evaluation, protein profiling, lipid, ash, carbohydrate, and moisture content determination.

Results: The results indicated that mint oil extracts exhibited significant antioxidant activity, surpassing previous studies. The sensory evaluation demonstrated improved texture and color in cheese samples with mint oil extracts. Furthermore, mint oil extracts inhibited the growth of bacterial (*Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Enterococcus faecalis*) and fungal (*Aspergillus flavus*, *Penicillium italicum*) contaminants.

Conclusion: This study highlights the potential of Mentha leaf extracts and oils to improve soft cheese quality and shelf life. The findings contribute to a greater understanding of natural preservatives in food production and open avenues for innovative culinary applications.

Keywords: Culinary innovation, Food preservation, Mentha leaf extracts, Soft cheese enhancement, Antioxidant properties, Microbial inhibition

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*** Corresponding Author:**
Ghadeer Sabah Bustani, Associate Professor.
Address: *Department of Anesthesia, Faculty of Medical Technologies, The Islamic University, Najaf, Iraq.*
Phone: *+964 (782) 7076417*
E-mail: *bustani@iunajaf.edu.iq*

Introduction

The culinary realm represents a dynamic and continually evolving sphere in which flavors, textures, and preservation methodologies are central to developing pleasurable and ecologically sound food offerings (Dwivedi et al., 2019). Food spoilage, on the contrary, constitutes a multifaceted predicament underpinned by an array of diverse determinants, manifested as a consequence of contamination throughout raw material procurement, preparation, handling, transportation, food processing, and packaging. Due to the contamination, the color had attributes and qualities in tactile and nutritional composition. In addition, a proliferation of microorganisms adversely impacts food quality, culminating in its unsuitability for human consumption (Bashir et al., 2022; Machado et al., 2020; Mokoena et al., 2021).

The mint is a plant part of the Lamiaceae family group, which includes more than 70 species (Tofan-Dorofeev & Ioniţă, 2021). Mint is native to Europe, Asia, and North America and is characterized by its aromatic shape and flowers. It is used for different purposes, including changing and improving the flavoring of food and drinks, pharmacy, and aromatherapy. *Mentha piperita* (peppermint) is the most common species of mint (Harismah et al., 2023; Saqib et al., 2022; Soleimani et al., 2022). The mint leaves had various volatile oils with distinctive aromas (El Menyiy et al., 2022; Soleimani et al., 2022). Menthol is the most critical volatile oil scenic and is responsible for the cooling sensation. The other volatile oils include menthone, isomenthone, and pulegone. Generally, the flavonoids, tannins, and sterols found in mint have antioxidant, antibacterial, and antifungal properties (Chatterjee et al., 2022; Nawal et al., 2022). Different studies illustrated that adding different concentrations of mint extract to soft cheese slows down the growth of bacteria and protects soft cheese from oxidation (Jafari et al., 2020) and other chemical reactions that change color and produce off-flavors. Furthermore, the leaf extracts contain antioxidants that prevent oxidation (Shahdadi et al., 2023). The present study aimed to investigate the potential of leaf extracts and oils to enhance the flavor of soft cheese and increase its shelf life.

Materials and Methods

Study design

In the present study, mint leaves were carefully cleaned, dried, and powdered. Then, they underwent three extraction methods: Alcoholic, aqueous, and essential oil distillation, each at concentrations of 1%, 0.6%, and 0.3%. Concurrently, white soft cheese was produced from pasteurized raw cow's milk. Next, a series of processes that involved microbial fermentation, curd formation, maturation, and eventual integration of the Mentha leaf extract were done. Finally, using the Clevenger apparatus at 40 ˚C, the alcoholic extraction tapped into ethanol to capture bioactive compounds from mint, while the aqueous method relied on water to derive hydrophilic compounds. The essential oil distillation followed the guidelines of the British Pharmacopoeia. Quality assessment of the produced cheese was conducted by analyzing its pH, sensory acidity, protein, lipid, and ash content. Physicochemical properties like moisture and lipid concentrations were determined, and a detailed sensory exploration encompassed a multidimensional assessment of flavor, aroma, and texture.

Collection of fresh mint

The mint was purchased from a market in Najaf City, Iraq, and the Mentha leaves were washed, cleaned, dried, and stored (Díaz-Maroto et al., 2003; Kripanand & Guruguntla, 2015). After storage, the mint was extracted by three techniques: Alcoholic extraction, aqueous extraction, and essential oil distillation, by Clevenger apparatus at an unconventional temperature of 40 ˚C (Mojally et al., 2022; Shrigod et al., 2017; Taher, 2012).

Cheese preparation

To prepare the cheese, raw cow's milk was used, which was pasteurized at 65 ˚C for 30 minutes and then rapidly cooled to 40-42 ˚C; then that sample was fermented with a microbial culture at a concentration of 0.04% per liter between 35 ˚C and 45 ˚C for 30 minutes (Al-Dahhan, 1983).

Extraction techniques using the clevenger apparatus

Alcoholic extraction

Ethanol was used as a solvent in an alcoholic extraction process with a Clevenger apparatus to extract medicinal compounds from mint leaves for better efficiency. The mint leaves were soaked in ethanol and heated to 40 ˚C. At this temperature, the volatile compounds from the leaves are mixed with the solvent, creating a concentrated alcohol extract rich in bioactive ingredients. Fatiha et al. (2012) showed that this method successfully extract-

ed phenolic compounds, terpenoids, and other volatile elements from the mint leaves.

Aqueous extraction

A specially modified Clevenger apparatus was used for this method, where the extraction process was carried out precisely at a constant temperature of 40 ˚C. The mint leaves were immersed in water inside the apparatus and subjected to controlled heating, which enhanced the extraction efficiency. As a universal solvent, water successfully extracted a wide range of compounds, including water-soluble polyphenols, carbohydrates, and hydrophilic elements present in the mint leaves (Jafari-Sales et al., 2019; Mahmoodabad et al., 2014).

Essential oil distillation

The essential oil extraction was done using a methodology described by previous studies (Guenther et al., 1959; Saharkhiz et al., 2012; Salim, 2017). About 15 g of dried peppermint samples were placed in a volumetric flask, to which 400 mL of deionized water was added. This mixture was subjected to an extraction process lasting 36 hours, maintaining a temperature of 40 ˚C to ensure it was not exceeded. The temperature was then reduced to 15 ˚C, and a Clevenger tube was used to isolate the oil. This extracted oil was carefully stored in opaque, airtight containers and refrigerated at 4 ˚C.

Quality assessment

The pH analysis

A designated 1-g cheese sample was taken, coupled with the introduction of 9 mL of distilled water, followed by comprehensive mixing. A pH meter gauged the sample's pH, elucidating the cheese's acidity or alkalinity and, thus, its intrinsic quality (Chen et al., 1979).

Sensory acidity

A 1-g cheese sample was employed, amalgamated with 9 mL of distilled water. After adding 3 drops of phenolphthalein indicator, titration with 0.1 N standardized sodium hydroxide (NaOH) solution ensued. The acid percentage was subsequently determined by invoking Hooi et al.'s (2004) formula (Equation 1):

1. Total acidity (TA) %=Volume of NaOH×0.09×0.1.

Protein profiling

For evaluation of the protein profiling, 10 mL of sample was placed in the Kjeldahl flask, digestion and titration procedures were applied, and nitrogen and protein percentages were calculated using Equations 2 and 3:

2. Nitrogen percentage=(V×0.14×100)/Weight of the sample

3. Protein percentage=Nitrogen percentage×5.38 (Yasmin et al., 2013).

Peptide identification (peptidomics)

The Ferber method was used to evaluate the lipids (Bai et al., 2010; Penner & Nielsen, 2010). Precisely 3 g of cheese were weighed and homogeneously blended with 10 mL of distilled water in a Ferber tube, then 10 mL of concentrated sulfuric acid and 1 mL of amyl alcohol were added to the flask.

Ash content

After achieving a light gray or bright white ash consistency, the resultant ash content was quantified via a precise formula that considers both the sample and the container's weight (Semeniuc et al., 2015).

Moisture chronicle

For analyzing moisture, we used the methodology described by Bradley (2010) and Penner and Nielsen (2010), with a modification in the drying duration (1.5 h). Precision was maintained by meticulously cleaning and recording the weights of both the dishes and their corresponding lids. About 5 g of the cheese was placed within the dishes, and after weighing, they were dried in an oven maintained at 85 ˚C (Lee et al., 2004). The moisture content was calculated by Equation 4:

4. Moisture=(Weight before drying-Weight after drying)/Initial weight of the sample)×100 (Bradley Jr & Vanderwarn, 2001; Fox & McSweeney, 2017).

DPPH

DPPH (2,2-diphenyl-1-picrylhydrazyl) was prepared by adding 4.4 U/mL of peroxidase to 50 µL of H_2O_2 and 100 µL of 2,2-azino-bis(3-ethylbenz-thiazoline-6-sulfonic acid) in 1 mL of water (Mukherjee et al., 2017). For evaluation, 1 mL of the methanol extract was added to 5 mL of freshly prepared methanolic DPPH solution, and the mixture was stored in the dark for 60 min and

measured in 514 nm of spectrophotometer. The blank sample was prepared by replacing the extract with methanol (1 mL). The percentage of free radical scavenging activity was calculated as follows Equation 5:

5. Radical scavenging %=[(A) control-(A) sample]/(A) control×100,

where (A) control is the absorbance for the control, and (A) sample is the absorbance of the essential oil/extracts or BHT (butylated hydroxytoluene). The IC_{50} value was calculated as the concentration sufficient to achieve 50% maximum scavenging capacity, determined from the inhibition percentage against concentration. All tests and analyses were performed in triplicate, and the mean values were calculated. The reduction capacity was measured according to the method described by Dun and Yen (1997) and Parejo et al. (2000). One milliliter of essential oil or plant extracts from *M. piperita* was mixed with phosphate buffer solution (0.2 M, pH 6.6, 0.5 mL) and potassium hexacyanoferrate solution (1% v/v) in a test tube and heated at 50 ˚C for 20 minutes. After cooling the tube on ice, 0.5 mL of 10% trichloroacetic acid was added. After centrifugation for 10 minutes, 1 mL of distilled water was added, 0.1 mL of ferric chloride (0.1%) was mixed with 1 mL of the solution, and the absorbance was measured at 514 nm. The increase in absorbance of the reaction mixture indicates the reducing power (Equation 6):

6. Radical scavenging %=(A) control-(A) sample/(A) control×100

Gas chromatography-mass spectrometry (GC-MS) overture

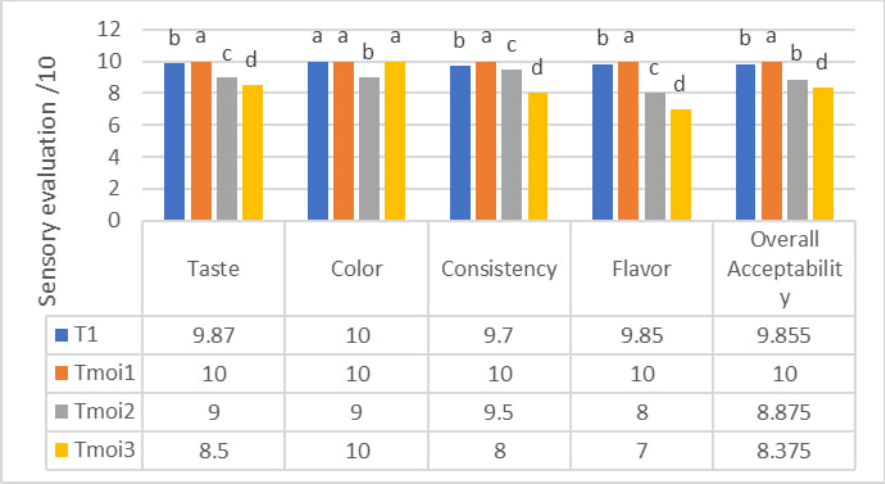
The esterification process of the oil extract was conducted before injection into the Clevenger apparatus. To achieve this goal, 0.5 mL of the oil was taken, and the acidic sample was placed in a capped test tube. Then, 2 mL of methanol was added, and the mixture was vigorously swirled. In the next step, 0.5 mL of concentrated sulfuric acid was added dropwise during stirring. The mixture was brought to a boil and then cooled to room temperature. To the test tube, 2 mL of hexane and 2 mL of water were added. The mixture was thoroughly shaken, and the layers were allowed to separate. The upper layer comprised approximately 20% of methyl esters of fatty acids and could be directly injected into gas chromatography without prior concentration. Subsequently, GC-MS analysis was performed at the Basra Oil Company laboratory using an Agilent Technologies system, 7890B GC, coupled with Agilent Technologies 5977A MSD equipped with an El signal detector. A 5% phenyl, 95% methyl silicone (30 m × 250 µm × 0.25) HP-5ms capillary column was used. The oven temperature was initially set at 40 ˚C for 5 minutes, then ramped up at a rate of 8 ˚C per minute to 300 ˚C for 20 minutes. The carrier gas flow rate was 1 mL per minute, and the purge flow was 3 mL per minute. The injections were made in the spitless mode with an injection temperature of 290 ˚C, and the injection volume was 0.5 µL. Mass spectrometry was conducted at 750-44 m/z. Data analysis was performed using the NIST 2014 and 2020 database libraries as an additional tool for compound identification (Holland et al., 2005).

The antimicrobial activity of peppermint extracts

The current study evaluated the antimicrobial efficacy of peppermint extracts (water, alcohol, and oil) against four bacterial strains commonly found in cheese: *Escherichia Coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, and *Enterococcus faecalis*. These bacteria were selected based on their prevalence in the mentioned cheese types and were isolated and purified by the Amin Laboratory in 2022. The antimicrobial activity was assessed using the agar well diffusion assay method, following the protocol established by Bauer et al. (1966) with some modifications. Muller Hinton agar plates were prepared and inoculated with a bacterial suspension containing approximately 1.5×10^6 CFU/mL using a glass spreader. The tested concentrations of peppermint extracts were 100 µL for Tmoi1, 60 µL for Tmoi2, and 30 µL for Tmoi3, with each volume being adjusted to a final volume of 1000 µL with appropriate diluents. The prepared dilutions (Tmoi1, Tmoi2, Tmoi3) were then transferred into 10-mm wells in the agar plates. The wells were filled with distilled water instead of the extract dilutions for the control treatment. After 24 hours of incubation at 37 ˚C, the effectiveness of the extracts was evaluated by measuring the diameter of the transparent zones (inhibition zones) surrounding each well (Bauer et al., 1966).

Assessing the antifungal activity of mint extracts

The method involved the addition of the extract to the culture medium after lowering and sterilizing the temperature under 40 ˚C. This modified approach allowed for the assessment of the antifungal potential of the extracts against *Penicillium italicum* and *Aspergillus flavus*, providing valuable insights into their effectiveness as antifungal agents under specific conditions (Zaker & Mosallanejad, 2010; Selim et al., 2024).



	Taste	Color	Consistency	Flavor	Overall Acceptability
T1	9.87	10	9.7	9.85	9.855
Tmoi1	10	10	10	10	10
Tmoi2	9	9	9.5	8	8.875
Tmoi3	8.5	10	8	7	8.375

Figure 1. Sensory evaluation results for soft white cheese and cheese supplemented with peppermint oil extract

Note: T1: Control; Tmoi1: 1% of oil extract; Tmoi2: 0.6% of oil extract; Tmoi3: 0.3% of oil extract.

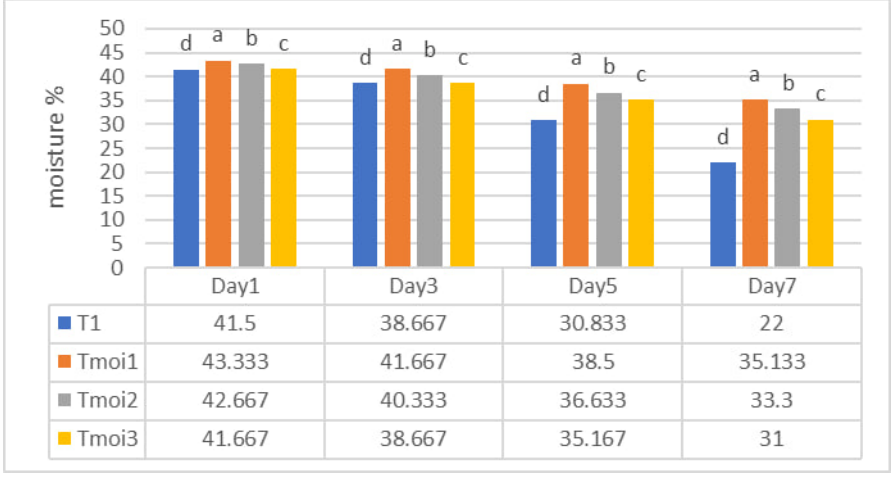
Statistical analysis

The experiment was conducted from February 10, 2022, to May 3, 2023, with three replicates for each concentration, considering the type of extract or oil in a complete randomized design. The means were compared using the least significant difference (LSD) method at a significance level of 0.05, following the approach described by Al-Amery et al. (2020) and Sadeq et al. (2022).

Results

Figure 1 illustrates the sensory evaluation results for soft white cheese and cheese supplemented with peppermint oil extract. The statistical analysis of the results showed significant differences among the treatments for soft white cheese. Regarding color, only treatment Tm3 showed no significant difference compared to the control sample. However, for texture, cheese samples supplemented with peppermint oil extract Tmoi1 and Tmoi2 performed better than the control sample, consistent with the findings of Rais Lima (2019). It is worth noting that the results of the alcoholic and aqueous extracts were excluded due to unsatisfactory outcomes for sensory evaluation.

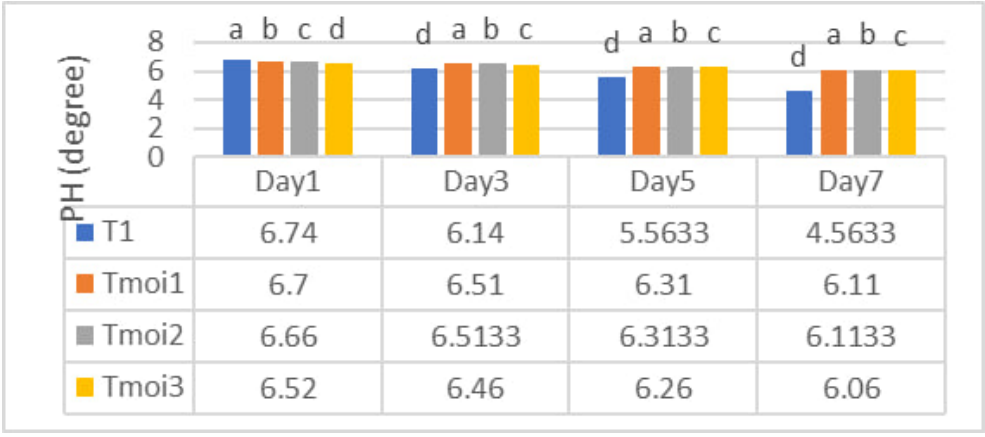
Based on Figure 2, the moisture content of samples of soft white cheese and cheese supplemented with peppermint leaf extracts was determined at different time intervals. The results for soft white cheese for treatment T1 on the first day showed a moisture content of 41.5, while treatment Tmoi1 had a moisture content of 43.333. On the first day, Tmoi2 and Tmoi3 had similar moisture



	Day1	Day3	Day5	Day7
T1	41.5	38.667	30.833	22
Tmoi1	43.333	41.667	38.5	35.133
Tmoi2	42.667	40.333	36.633	33.3
Tmoi3	41.667	38.667	35.167	31

Figure 2. Moisture content of soft white cheese and cheese supplemented with peppermint leaf extracts at different time intervals

Notes: T1: Control; Tmoi1: 1% of oil extract; Tmoi2: 0.6% of oil extract; Tmoi3: 0.3% of oil extract.



	Day1	Day3	Day5	Day7
T1	6.74	6.14	5.5633	4.5633
Tmoi1	6.7	6.51	6.31	6.11
Tmoi2	6.66	6.5133	6.3133	6.1133
Tmoi3	6.52	6.46	6.26	6.06

Figure 3. pH values of cheese samples on different days for various treatments

Notes: T1: Control; Tmoi1: 1% of oil extract; Tmoi2: 0.6% of oil extract; Tmoi3: 0.3% of oil extract.

contents of 42.667 and 41.667, respectively. However, on the third day, the results were lower than the first day, with treatment T1 at 38.667, treatment Tmoi1 at 41.667, treatment Tmoi2 at 40.333, and treatment Tmoi3 at 38.667. On the fifth day, the results showed the lowest moisture content for treatment T1 at 30.833, while treatment Tmoi1 had a moisture content of 38.5, treatment Tmoi2 had a moisture content of 36.633, and treatment Tmoi3 had a moisture content of 35.167. Finally, on the seventh day, treatment T1 had the lowest moisture content of 30.833, while treatment Tmoi1 had a moisture content of 38.5. Treatments Tmoi2 (36.633) and Tmoi3 (35.167) were somewhat similar. It is worth noting that the results of this study were better than those of Darwish and Habtan in 2010, even though a different extract (black seed oil) was used. It is worth noting that the results of the alcoholic and aqueous extracts were excluded due to unsatisfactory outcomes for moisture content evaluation.

Figure 3 illustrates the pH results for samples of cheese. On the first day, the results for treatment T1 showed a pH of 6.74, while treatment Tmoi1 had a pH value very close to 6.7. Treatment Tmoi2 had a pH of 6.66, and treatment Tmoi3 had the lowest pH at 6.52. However, on the third day, the results showed a decrease in pH for all treatments. Treatment T1 had the lowest pH at 6.14, treatment Tmoi1 had a pH of 6.51, treatment Tmoi2 had a pH of 6.5133, and treatment Tmoi3 had a pH of 6.46. Moving on to the fifth day, the pH values for the treatments were as follows: Treatment T1 at 5.5633, treatment Tmoi1 at 6.31, treatment Tmoi2 at 6.5133, and treatment Tmoi3 at 6.26. On the seventh day, treatment T1 had the lowest pH at 4.5633, treatment Tmoi1 had a pH of 6.11, treatment Tmoi2 had a pH of 6.1133, and treatment Tmoi3 had a pH of 6.06. It is worth noting that the results of this study showed variations compared to the results of Li et al. (2015).

Figure 4 displays the results of the titratable acidity assessment for cheese samples. Treatment Tmoi2 had an acidity level of 1, and treatment Tmoi3 had an acidity level of 0.98. However, on the third day, the acidity levels increased for all treatments. Treatment T1 had the highest acidity at 1.5, treatment Tmoi1 had an acidity of 1.1333, treatment Tmoi2 had an acidity of 1.2, and treatment Tmoi3 had the highest acidity at 1.2933. Moving on to the fifth day, the acidity values for the treatments were as follows: Treatment T1 at 1.8333, treatment Tmoi1 at 1.2167, treatment Tmoi2 at 1.3833, and treatment Tmoi3 at 1.58. On the seventh day, treatment T1 had the highest acidity at 2.0333, while treatment Tmoi1 had an acidity of 1.3767, treatment Tmoi2 had an acidity of 1.6167, and treatment Tmoi3 had an acidity of 1.7133. It is worth noting that this study's results showed better outcomes than the results of Gorissen et al. (2011).

Figure 5 illustrates the estimation of protein content in the soft white cheese. The results showed that for treatment T1, the protein content was 29.4168, while treatment Tmoi1 had a higher protein content at 35.4552. Treatment Tmoi2 had a similar protein content of 35.2308, and treatment Tmoi3 had a protein content of 32.9664. It is worth noting that this study's results are better than those presented in the studies by Fezea et al. (2017), Hamouda et al. (2015), and Khaji et al. (2023).

Figure 6 shows the soft white cheese's lipid (fat) content. The results showed that for treatment T1, the lipid content was the lowest at 14.37%, while treatment Tmoi1 had a higher lipid content of 19.91%. Treatment Tmoi2 had a lipid content of 19.03%, and treatment Tmoi3 had

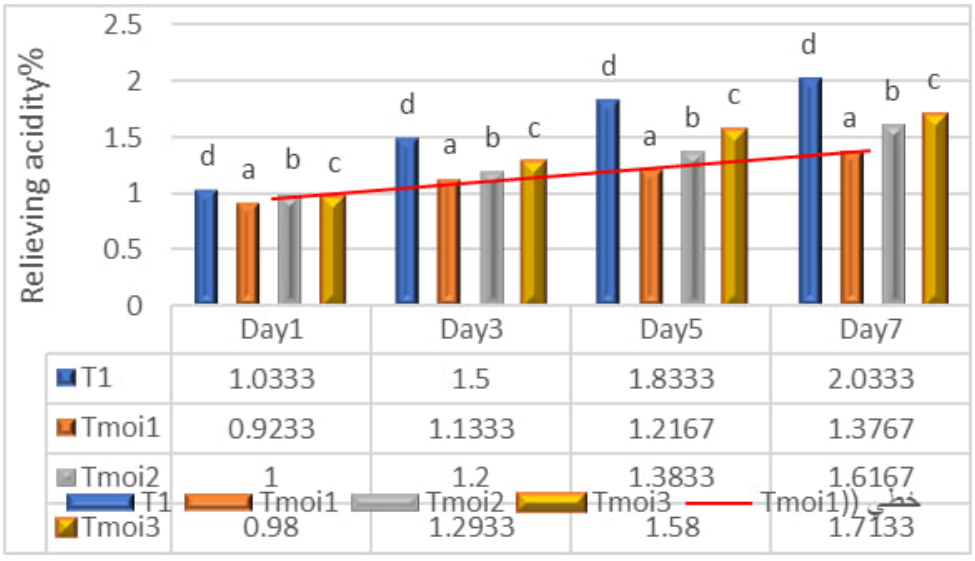


Figure 4. Titratable acidity assessment of cheese samples on different days for various treatments

Notes: T1: Control; Tmoi1: 1% of oil extract; Tmoi1: 0.6% of oil extract; Tmoi1: 0.3% of oil extract.

a lipid content of 18.89%. These results indicate significant differences in lipid content among the treatments. It is important to note that the results of this study differ from those reported in the study by Rabeea et al. (2023), suggesting that the use of mint leaf extracts may have a different impact on lipid content in soft white cheese compared to the previous research (Soltani et al., 2023; Fetouh et al., 2023).

Table 1 presents the estimation of carbohydrate content for different treatments. The results showed that treatment T1 had the highest carbohydrate content, reaching 13.7399%. In contrast, treatment Tmoi1 had the lowest carbohydrate content at 0.1851%, followed by treatment Tmoi2 at 2.0755% and treatment Tmoi3 at 5.7603%. These results indicate significant differences in carbohydrate content among treatments, as treatment T1 had the highest, while treatments containing mint leaf extracts (Tmoi1, Tmoi2, Tmoi3) had much lower carbohydrate content. (Al-Sahlany, 2016; Fezea et al., 2017).

Figure 7 shows the estimation of ash content in the cheese samples. The results showed that treatment T1 had an ash content of 0.9733%, while treatment Tmoi1 had the highest ash content of 1.1167%. Treatment Tmoi2 had an ash content of 0.9967%, and treatment Tmoi3 had an ash content of 0.9833%. These results indicate variations in ash content between treatments, as treatment Tmoi1 had the highest, while treatments Tmoi2 and Tmoi3 had slightly lower percentages. It is worth noting that these results differ from those reported by Kirk et al. (1998).

The results of the current study demonstrated remarkable antioxidant activity (oil, alcohol, and water extracts). These results outperformed similar studies (Aggarwal et al., 2001; Foda et al., 2010; Mikaili et al., 2013). Notably, treatment Tmoil exhibited significantly higher antioxidant capacity with an oxygen radical absorbance capacity (ORAC) value of 98.4294+1.18, followed by followed by treatment with methanol extract (Tmeth) with a value of 96.6678±1.12, and aqueous ex-

Table 1. Estimation of carbohydrate content for different treatments

Group	Carbohydrates (%)	Ash (%)	Lipids (%)	Proteins (%)	Moisture (%)
T1	13.73799	0.9733	14.37	29.4168	41.5
Tmoi1	0.1851	1.1167	19.91	35.4552	43.333
Tmoi2	2.0755	0.997	19.03	35.2308	42.667
Tmoi3	5.763	0.9833	18.89	32.6994	41.667

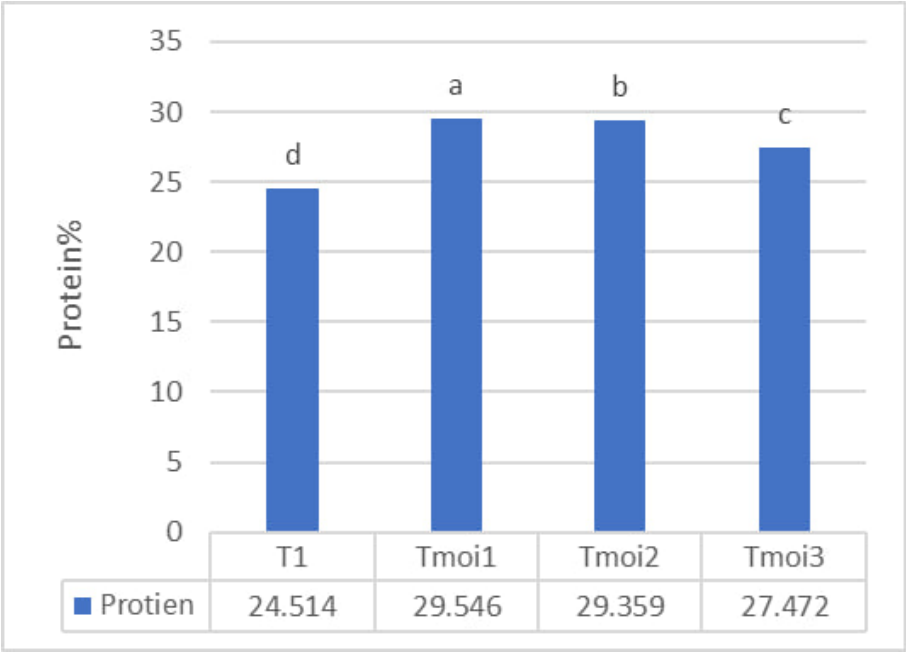


Figure 5. Estimation of protein content in soft white cheese for various treatments

Notes: T1: Control; Tmoi1: 1% of oil extract; Tmoi2: 0.6% of oil extract; Tmoi3: 0.3% of oil extract.

tract (Tmext) with a value of 76.8498±0.96. In terms of DPPH radical scavenging activity, treatment with peppermint oil extract (Tmoil) showed the highest activity with a value of 98.9526±1.2036, followed by treatment with Tmeth with a value of 93.4749±1.1424, and Tmext with a value of 77.4003±0.9792. However, regarding reducing power, treatment with peppermint Tmoil had the highest activity with a value of 0.9909±0.059, followed by treatment with Tmeth with a value of 0.8808±0.059, while Tmext exhibited lower reducing power with a value of 0.4404±0.048 (Table 2). It is important to note that these findings differ from those reported in the study by Singh et al. (2002).

Table 3 presents the results of the analysis of active compounds in mint oil, with the most significant compounds being methyl cyclohexene-1,4-acetyl (29.1983%), D-limonene (9.6097%), caryophyllene (8.9396%), and cyclohexanone, 2-methyl-5-(1-methylethenyl)-, trans (8.2958%). On the other hand, the alcohol extract contained significant active compounds, including 1-(4-hydroxy-3-methoxyphenyl)dec-4-en-3-one (15.1803%), butan-2-one,4-(3-hydroxy-2-methoxy-

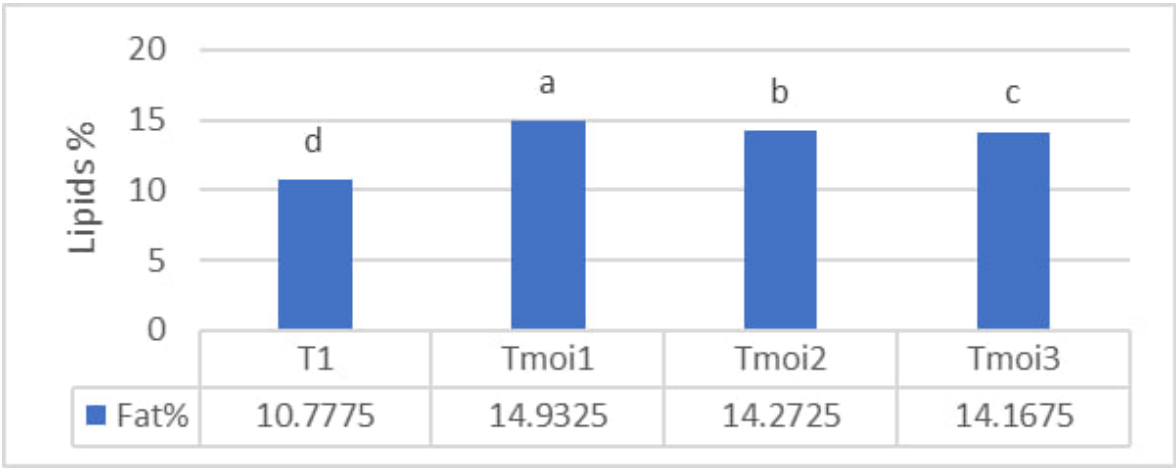


Figure 6. Estimation of lipid (fat) content in soft white cheese for various treatments

Notes: T1: Control; Tmoi1: 1% of oil extract; Tmoi2: 0.6% of oil extract; Tmoi3: 0.3% of oil extract.

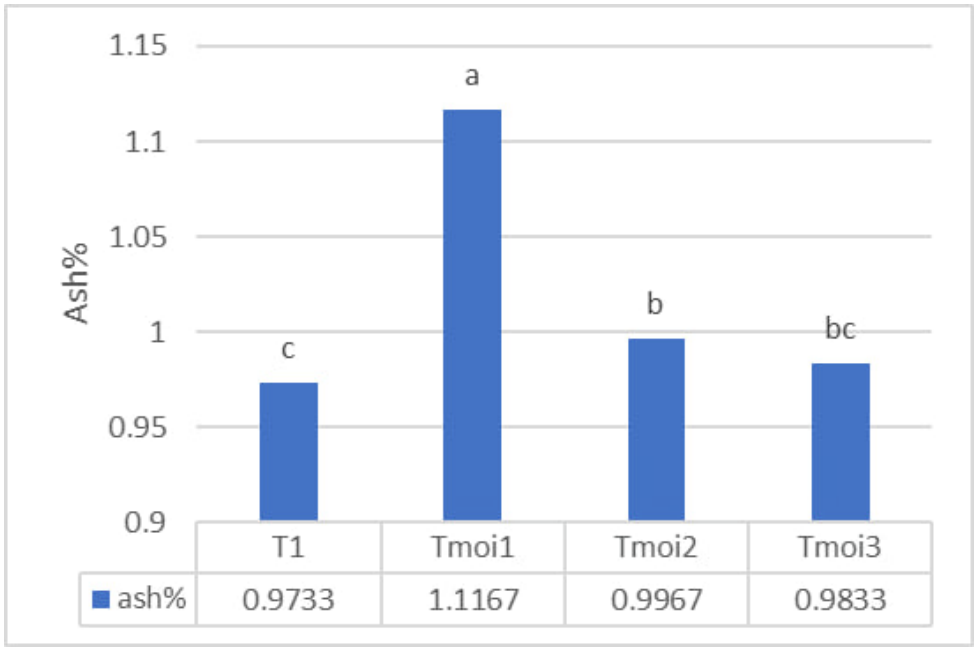


Figure 7. Ash content estimation in cheese samples for various treatments

phenyl) (10.6348%), and benzene, 1-(1,5-dimethyl)-4-methyl (4.3452%). It is worth noting that these results differ from those reported in the study by Abdul-Jalil (2014).

The results presented in Figures 8, 9, 10, 11 and 12 demonstrate the positive effect of mint oil extracts, ethanol extracts, and water extracts on inhibiting bacterial growth, particularly for *E. coli*, *K. pneumoniae*, *S. aureus*, and *E. faecalis*.

E. coli

Treatment Tm1.1 oil showed a moderate positive effect with a zone of inhibition of 26 mm.

Treatment Tm2.1 oil exhibited a slightly lower effect with a zone of inhibition of 25 mm.

Treatment Tm3.1 oil had a similar effect to Tm2.1 oil, with a zone of inhibition of 24 mm.

The ethanol extracts (Tm1.1 ethanol, Tm2.1 ethanol, Tm3.1 ethanol) and water extracts (Tm1.1 water, Tm2.1 water, Tm3.1 water) showed varying degrees of inhibition.

K. pneumoniae

Treatment Tm1.1 oil demonstrated the highest inhibition zone, measuring 29 mm.

Treatment Tm2.1 oil had a slightly lower zone of inhibition at 26 mm.

Treatment Tm3.1 oil showed a similar effect to Tm2.1 oil, with an inhibition zone of 24 mm.

Ethanol and water extracts also exhibited varying degrees of inhibition.

Table 2. Antioxidant activity assessment for different treatments

Type of Extraction	Antioxidant Capacity	DPPH	Reducing Power
Peppermint oil	98.4294+1.18	98.9526+1.2036	0.9909+0.059
Ethyl acetate	96.6678+1.12	93.4749+1.1424	0.8808+0.056
Aqueous extract	76.8498+0.96	77.4003+0.9792	0.4404+0.048

DPPH: 2,2-diphenyl-1-picrylhydrazyl.

Table 3. Analysis of active compounds in alcohol extract

Library/ID	%
1-(4-Hydroxy-3-methoxyphenyl)dec-4-en-3-one	15.1803
Butan-2-one, 4-(3-hydroxy-2-methoxyphenyl)-	10.6348
1,3-Cyclohexadiene, 5-(1,5-dimethyl-4-hexenyl)-2-methyl-, [S-(R*,S*)]-	4.6353
Cyclohexene, 3-(1,5-dimethyl-4-hexenyl)-6-methylene-, [S-(R*,S*)]-	4.3926
5-Hydroxy-1-(4-hydroxy-3-methoxyphenyl)decan-3-one	4.3556
Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl-	4.3452
1-(4-Hydroxy-3-methoxyphenyl)tetradec-4-en-3-one	4.3266
5-Hydroxy-1-(4-hydroxy-3-methoxyphenyl)dodecan-3-one	3.1235
1-(4-Hydroxy-3-methoxyphenyl)dodec-4-en-3-one	3.0073
β-Bisabolene	2.4441
α-Farnesene	2.1498
(3R,5S)-1-(4-Hydroxy-3-methoxyphenyl)decane-3,5-diyl diacetate	1.7262
(E)-1-(4-Hydroxy-3-methoxyphenyl)dec-3-en-5-one	1.6595
(E)-1-(4-hydroxy-3-methoxyphenyl)dec-1-ene-3,5-dione	1.5901
n-Hexadecanoic acid	1.5628
γ-Sitosterol	1.5149
9,12-Octadecadienoic acid (Z,Z)-	1.3811
(E)-4-(2-(2-(2,6-Dimethylhepta-1,5-dien-1-yl)-6-pentyl-1,3-dioxan-4-yl)ethyl)-2-methoxyphenol	1.3342
1-(4-Hydroxy-3-methoxyphenyl)tetradecane-3,5-dione	1.3342
1-(4-Hydroxy-3-methoxyphenyl)decane-3,5-dione	1.3253
1-(4-Hydroxy-3-methoxyphenyl)dodec-4-en-3-one	1.3174

S. aureus

Treatment Tm1.1 oil showed the highest inhibition zone at 34 mm.

Treatment Tm2.1 oil had a slightly lower inhibition zone at 28 mm.

Treatment Tm3.1 oil exhibited an inhibition zone of 25 mm.

E. faecalis

Treatment Tm1.1 oil demonstrated a significant inhibition zone of 30 mm.

Treatment Tm2.1 oil had an inhibition zone of 27 mm.

Treatment Tm3.1 oil exhibited an inhibition zone of 24 mm.

Overall, these results indicate that mint oil extracts, especially Tm1.1 oil, have a positive effect on inhibiting bacterial growth, and the effectiveness varies for different bacterial strains. These findings are consistent with the study by Mahmoud et al. (1989).

The current results demonstrate the positive impact of mint oil extracts, ethanol extracts, and water extracts on inhibiting fungal growth, particularly for the *A. flavus* and *P. italicum* strains.

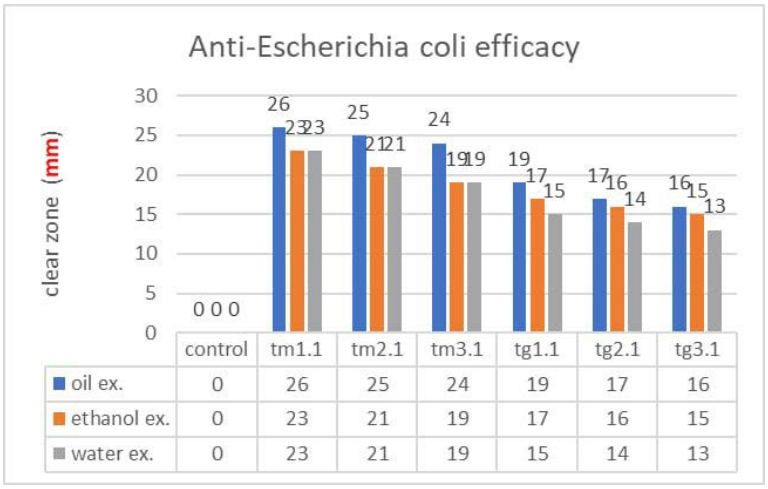


Figure 8. Inhibitory effects of mint oil, ethanol extracts, and water extracts on *E. coli* growth

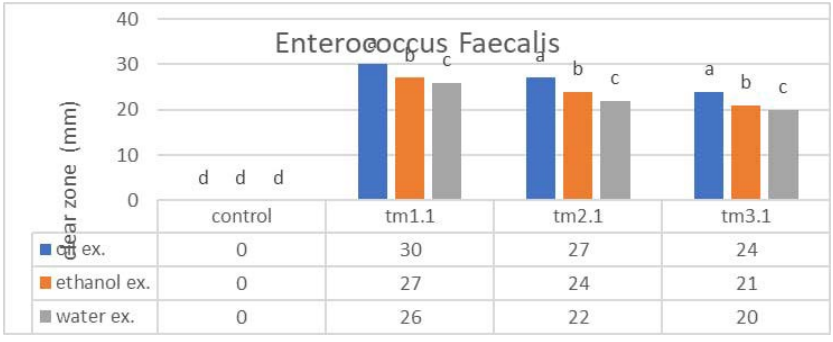


Figure 9. Inhibitory effects of mint oil, ethanol extracts, and water extracts on *E. faecalis* growth

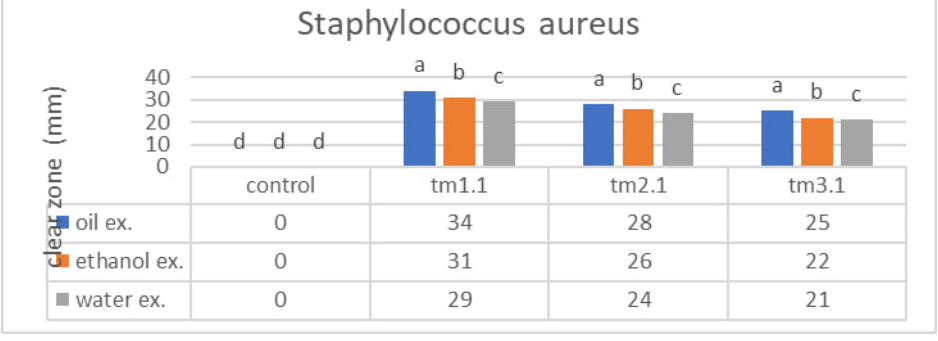


Figure 10. Inhibitory effects of mint oil, ethanol extracts, and water extracts on *S. aureus* growth

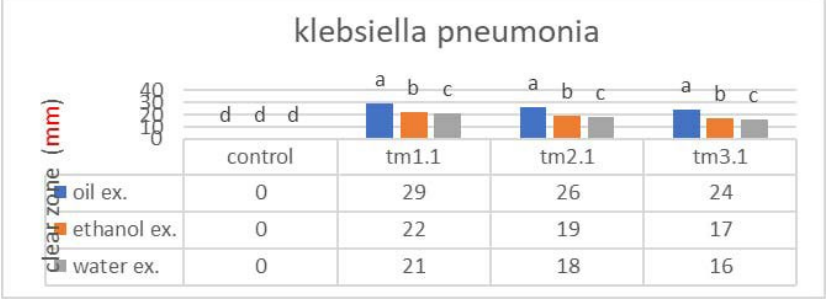
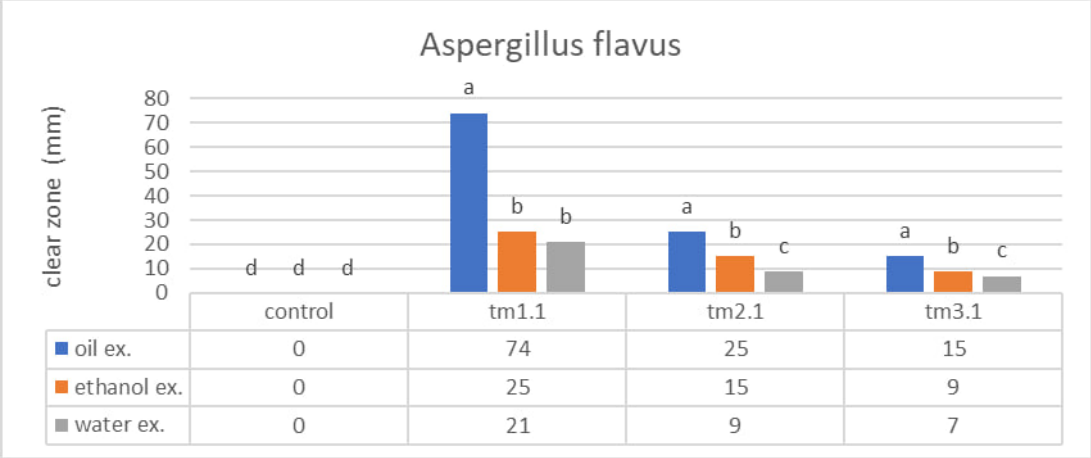


Figure 11. Inhibitory effects of mint oil, ethanol extracts, and water extracts on *K. pneumonia* growth



	control	tm1.1	tm2.1	tm3.1
oil ex.	0	74	25	15
ethanol ex.	0	25	15	9
water ex.	0	21	9	7

Figure 12. Inhibitory effects of mint oil, ethanol extracts, and water extracts on *A. flavus* growth

A. flavus

Treatment Tm1.1 oil showed the highest inhibition zone, with a diameter of 74 mm.

Treatment Tm2.1 oil had a lower inhibition zone of 25 mm.

Treatment Tm3.1 oil exhibited the lowest inhibition zone at 15 mm.

Ethanol and water extracts also showed varying degrees of inhibition, with Tm1.1 ethanol having an inhibition zone of 25 mm and Tm3.1 ethanol having an inhibition zone of 9 mm.

Tm1.1 water had an inhibition zone of 21 mm, while Tm2.1 water and Tm3.1 water did not show significant inhibition against *A. flavus*.

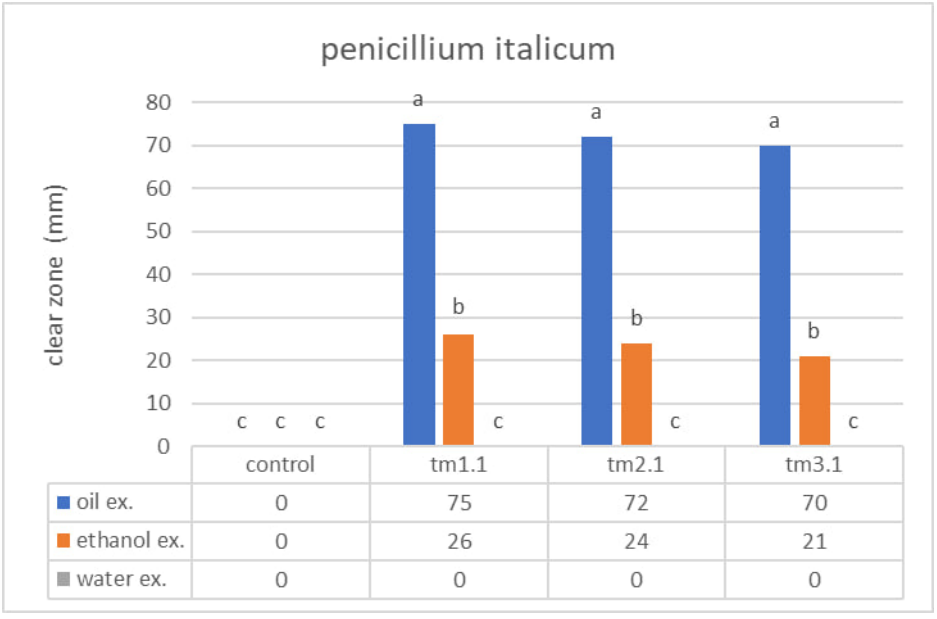
P. italicum

Treatment Tm1.1 oil exhibited the highest inhibition zone, measuring 75 mm.

Tm2.1 oil had a slightly lower inhibition zone of 72 mm.

Treatment Tm3.1 oil had an inhibition zone of 70 mm.

Ethanol extracts showed moderate inhibition, with Tm1.1 ethanol having an inhibition zone of 26 mm,



	control	tm1.1	tm2.1	tm3.1
oil ex.	0	75	72	70
ethanol ex.	0	26	24	21
water ex.	0	0	0	0

Figure 13. Inhibitory effects of mint oil, ethanol extracts, and water extracts on *P. italicum* growth

Tm2.1 ethanol with 24 mm, and Tm3.1 ethanol with 21 mm.

Water extracts (Tm1.1 water, Tm2.1 water, and Tm3.1 water) did not show significant inhibition against *P. italicum*.

These results indicate that mint oil extracts, especially Tm1.1 oil, have a substantial positive effect on inhibiting the growth of *A. flavus* and *P. italicum* fungi. These findings align with the study by Mahmoud et al. (1989).

Discussion

The findings of this study highlight the remarkable potential of Mentha leaf extracts and oils as natural additives for improving the quality, sensory attributes, and shelf life of soft cheese. The results demonstrated that different extraction techniques—alcoholic, aqueous, and essential oil distillation—successfully obtained bioactive compounds, including polyphenols, menthol, and volatile oils, known for their potent antioxidant and antimicrobial properties. These compounds contributed significantly to inhibiting the growth of bacterial species such as *E. coli*, *K. pneumoniae*, *S. aureus*, and *E. faecalis*, as well as fungal contaminants such as Aspergillus flavus and Penicillium italicum. This antimicrobial effect can be attributed to the disruption of microbial cell membranes caused by the bioactive components, particularly menthol and flavonoids, which interfere with enzymatic activities and lipid peroxidation in microbial cells, as previously reported by Mahmoud et al. (1989) and Jafari et al. (2020).

The sensory evaluation results in this study revealed significant improvements in cheese samples treated with 1% oil extract (Tmoi1), where texture, color, and flavor were markedly enhanced compared to the control group. These findings align with those of Foda et al. (2010) and Fezea et al. (2017), who reported that mint extracts improved sensory qualities in dairy products. This improvement can be explained by the antioxidant activity of mint-derived polyphenols, which stabilize lipids and proteins, preventing oxidative rancidity and maintaining the integrity of cheese matrix components.

A key observation in this study was the progressive reduction in the pH values over the storage period for all treatments, where mint-treated samples exhibited a slower decline compared to the control. This indicates that the antimicrobial properties of mint extracts probably reduced microbial metabolic activities responsible for acid production. Similar findings were reported by Darwish and Habtan (2010), who observed delayed pH changes in cheese treated with black seed oil. The slower pH reduction in mint-treated samples suggests that the essential oils not only inhibit spoilage microorganisms but may also selectively preserve beneficial starter cultures.

The moisture content results showed that mint-treated samples retained higher levels of moisture over time compared to the control. The hydrophilic nature of certain mint compounds, such as polyphenols and tannins, likely contributed to better water retention within the cheese matrix, enhancing texture and overall quality. Additionally, the antioxidant properties of the extracts helped reduce oxidative water loss, a phenomenon similarly observed in the study by Jafari et al. (2020).

In terms of protein and lipid content, cheese samples treated with mint extracts (Tmoi1 and Tmoi2) showed significantly higher values compared to the control. This can be attributed to the ability of mint polyphenols to inhibit protein and lipid oxidation. For example, menthol and menthone are known to scavenge free radicals, protecting proteins and lipids from degradation during storage (Singh et al., 2002). The higher protein content may also be related to the interaction between bioactive compounds and milk proteins, which stabilize the cheese matrix, as noted by Fezea et al. (2017).

The study also demonstrated the exceptional antioxidant activity of mint oil extracts, as evidenced by DPPH radical scavenging and reducing power assays. The higher activity observed in oil extracts compared to aqueous or alcoholic extracts suggests that hydrophobic compounds, such as menthol and menthone, contribute significantly to the antioxidant potential. These results surpass findings by Singh et al. (2002) and Foda et al. (2010), underscoring the efficiency of essential oil distillation in capturing active components. This strong antioxidant activity plays a pivotal role in delaying lipid perpetration and oxidative spoilage, thereby extending the shelf life of cheese.

The antimicrobial activity of mint extracts was another important finding, with oil extracts showing the highest inhibition zones against bacterial and fungal strains. The primary mechanism involves the disruption of microbial cell walls and inhibition of enzymatic pathways essential for microbial growth. Menthol, in particular, has been reported to interfere with ATP synthesis and membrane integrity in bacteria, which explains the effectiveness of mint oil extracts against *E. coli*, *S. aureus*, and other pathogens (Mahmoud et al., 1989). These results are

consistent with the findings of Jafari et al. (2020) and emphasize the role of mint extracts as potent natural preservatives.

Conclusion

The current study shows promising results, indicating the potential of mint extracts and oil as versatile natural resources. Through specific extraction methods, oil and alcoholic and aqueous extracts were efficiently obtained from mint plants, surpassing traditional techniques. These mint-derived products exhibited improved sensory and qualitative attributes and demonstrated potent antioxidant and antimicrobial properties against various contaminants, including bacteria and fungi. This finding suggests their applicability as natural preservatives in food and industrial products, marking them as valuable resources for various applications prioritizing quality, safety, and cleanliness.

Ethical Considerations

Compliance with ethical guidelines

There were no ethical considerations to be considered in this research.

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Authors' contributions

All authors contributed equally to the conception and design of the study, data collection and analysis, interception of the results and drafting of the manuscript. Each author approved the final version of the mnuscript for submission.

Conflict of interest

The authors declared no conflict of interest.

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