

Research article

Malva neglecta essential oil: A promising approach for bio-preserving mayonnaise and assessing variable correlations through principal component analysis and heat map

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ABSTRACT

Consumers increasingly regard plant-derived biocompounds as a safe alternative to synthetic additives. To increase shelf life and enhance safety status, this study sought to determine how *Malva neglecta* essential oil (MNEO) might affect microbiological and chemical changes in mayonnaise samples at 4 °C. MNEO was extracted, and the primary chemical constituents were identified using a GC-MS. Based on the determined MIC/MBC, various concentrations of MNEO have been utilized in the preparation of mayonnaise and evaluated for microbial, chemical, and sensorial characteristics for 40 days at 4 °C. MNEO contains podocarpa-8,11,13-trien-3-one, 12-hydroxy-13-isopropyl, δ -elemene, γ -elemene, bicyclogermacrene, β -ylangene, viridiflorol, spathulenol, 6,10,14-trimethyl-2-pentadecanone, L-linalool, γ -curcumene, germacrene D, n-tetradecane, β -elemene, β -damascenone, δ -cadinene, α -muurolol, cis-muurola-3,5-diene, and α -cadinol with substantial antibacterial and antioxidant properties. The MNEO-based bio-preservation strategy efficiently diminished microbial growth (*S. aureus* > *E. coli* O157:H7 > *P. aeruginosa* > *S. enteritidis*) and augmented the mayonnaise sample's shelf life (>40 days). When MNEO was utilized at 5084 ppm, the oxidative stability of the specimens was also more effectively preserved at optimal levels (peroxide values of 1.76, 1.98, and 2.34 meq O₂/kg of oil for TBHQ, 5084 ppm, and 2542 ppm treatments, respectively) ($P < 0.05$). Furthermore, it was noted that the specimens exposed to 5084 ppm MNEO had the highest sensory evaluation scores regarding aroma, hue, flavor, consistency, and general appeal ($P < 0.05$). Using MNEO at the recommended concentration (5084 ppm) significantly reduces microbiological and chemical deterioration in mayonnaise throughout preservation (greater than 40 days) without causing any adverse sensory consequences.

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1. Introduction

Vinegar, egg yolk, and other components solidify the semi-solid emulsion of water and oil that is mayonnaise [1]. Mayonnaise is vulnerable to oxidative degradation because it contains a significant amount of fat (65–85 %), with a pH between 3.5 and 4, and contains iron from egg yolk. Lipid oxidation affects mayonnaise's organoleptic qualities, including flavor, texture, color, and odor, in addition to lowering its nutritional value. Consequently, this reduces the goods' shelf life. Pathogenic bacterial contaminants are an additional potential threat associated with mayonnaise. Although its elevated fat content and low pH provide some protection, mayonnaise is not completely pathogen-free. The type of materials, absence of heat treatment, and storage circumstances can all have an impact on the microbial safety of the product. Since mayonnaise is typically used as a condiment, it raises the risk of contaminating other foods [2]. Hence, to manage the process of oxidative degradation and microbiological action, artificial antioxidants like butyl-hydroxyanisole (BHA), butyl-hydroxytoluene (BHT), and t-butyl-hydroxyquinone (TBHQ), as well as antimicrobial agents like potassium sorbate and sodium benzoate, are used in mayonnaise formulations [3]. Considering the harmful impact of synthetic chemicals on the wellness of customers, organic antimicrobial and antioxidant substances have emerged as safe substitutes and a bio-preservation strategy [4]. The application of essential oils as organic preservatives has been the subject of previous investigations on a variety of plant-based substances, including basil [5], thyme [6], and rosemary [7].

Naturally occurring substances with certain antibacterial and antioxidant qualities are called essential oils. These are unstable, secondary compounds generated by medicinal plants that mostly consist of terpenes, phenolics, and aromatic substances [8]. Ten natural Iranian plant and shrub species belong to the genus *Malva*; these are known locally and in Persian as Panirak, a traditional remedy. Specifically, the seeds contain 15.2 % fat, and 21 % protein [9]. The *M. neglecta* plant has been identified as a native plant in the USA. It is frequently found in areas that have been disrupted, such as roadways, railways, wastelands, agricultural land, and nurseries [10]. Presently, there have been reports of encouraging phytochemical activity exhibited by many species within the *Malva* genus [11,12]. These results provide evidence for the feasibility of using this medicinal plant in place of chemicals with unfavorable adverse reactions.

Pathogenic microorganisms transmitted by food such as *Staphylococcus aureus*, *Escherichia coli*, *Salmonella* sp., and *Pseudomonas aeruginosa*, have been found in contaminated mayonnaise [13]. The aim of this study was to assess the antibacterial efficacy of *M. neglecta* essential oil (MNEO) toward these pathogenic bacteria. Regarding this matter, MNEO were incorporated into the oil component of mayonnaise. Over the course of storage, the antibacterial activity of MNEO in regard to *S. enteritidis*, *S. aureus*, *E. coli* O157:H7, and *P. aeruginosa*, as well as its antioxidant qualities, was assessed by evaluating the oil's peroxide, thiobarbituric acid, and total oxidation values (TOTOX values) in mayonnaise samples.

2. Materials and methods

2.1. Materials

Refined sunflower oil, vinegar, and eggs were procured from a local supermarket in Tabriz, Iran. Agar from Cetrimide, Brain Heart Infusion Broth, Eosin Methylene Blue (EMB) agar, Mueller Hinton Agar (MHA) agar, Salmonella-Shigella agar, and Nutrient Agar were all provided by Merck, Germany. *S. enteritidis*, *S. aureus*, *P. aeruginosa*, and *E. coli* O157:H7 are graciously supplied by the Microbiology Unit of the Drug Applied Research Center at Tabriz University of Medical Sciences. To generate operational cultures, frozen bacterial strains were thawed and then cultivated on NA plates. The plates underwent incubation for a duration of 18 h at a temperature of 37 °C.

2.2. Collection and botanical identification of plants

Geographically located at 36.8427° N and 54.4439° E, respectively, and at an elevation of 2031.4 m beyond sea level, the plant substance was harvested in April 2023 at the blossoming stage in the Gorgan region of Golestan Province, Iran. For additional authentication, a voucher specimen was placed at the Faculty of Agriculture Herbarium (Herbarium number 8894), Shahrood University of Technology, Shahrood, Iran. Subsequently, the substance was dehydrated under shade and pulverized using a mill (Mill 120, USA) before being kept in a refrigerator.

2.3. Essential oil extraction

By applying Cleveger (Glassco, India), the essential oil was obtained. Sodium sulfate was used to dry it, and the mixture was then kept in closed, darkened containers at 4 °C [14].

2.4. Gas chromatography–mass spectrometry

Analytical evaluation of the essential oil was conducted utilizing gas chromatography-mass spectrometry (GC-MS) with a flame ionization detector, utilizing a Model YL6100 instrument manufactured by Young Lin Company. The apparatus featured a BPX70 capillary column of 30 m in length and 0.25 mm in internal diameter, with a thickness of 0.25 µm. The initial temperature of the oven was set at 60 °C and was then raised gradually to 280/300 °C at a rate of 4 °C per minute. The carrier gas utilized was helium, with a flow rate set at 1 mL/min. The MS transfer line and injector temperatures were established at 290 °C and 220 °C, respectively. The column temperature started at 60 °C and was then steadily raised to 280/300 °C at a rate of 4 °C per minute. Manual injections of

essential oil were made. The compounds were identified through contrasting their respective retention indices (RI), retention time (RT), and mass spectral data with the library information from the GC-MS apparatus, relevant literature, and standards of the principal elements, as well as resources from the National Institute of Standards and Technology (NIST) and Wiley. With the use of retention periods of reference n-alkanes that were eluted prior to and following the portion in question and ran under the same circumstances, RI was manually determined by interpolation [14].

2.5. Antimicrobial assessments of MNEO

2.5.1. Disk diffusion assay

To confirm the purity of MNEO, a one hundred μL sample of MNEO was uniformly applied on nutrient agar utilizing a cleaned, curved glass rod. For the next 24 h, the combination underwent incubation at $37\text{ }^{\circ}\text{C}$. The antibacterial property of MNEO was investigated using the disc diffusion approach, following the protocol established by Sabahi and colleagues (2022) with a few alterations [11]. A total of one hundred μL of microbial solution was evenly distributed throughout the surface of the petri dishes with Mueller Hinton agar. Subsequently, discs that are aseptic and have a diameter of 6 mm were carefully positioned on the petri plates, utilizing sterile forceps. Subsequently, varying volumes of MNEO, specifically 20 and 40 μL , were individually applied to the discs. The samples consisting of dimethyl sulfoxide along with gentamicin solutions were designated as the negative and positive control discs, correspondingly. For 24 h, The Petri dishes were maintained in an incubation chamber regulated to a temperature of $37\text{ }^{\circ}\text{C}$. The diameter of the area where growth was inhibited (referred to as the inhibition zone, or IZ) was then measured and recorded in millimeters.

2.5.2. Minimum inhibitory/bactericidal concentration assay

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of MNEO versus *S. aureus*, *E. coli* O157:H7, *P. aeruginosa*, and *S. enteritidis* were established utilizing the approach outlined by the Clinical and Laboratory Standards Institute (CLSI) with a few alterations [15]. In summary, a concentrated preparation of MNEO (5084 mg/mL) was produced using ten percentage (v/v) dimethyl sulfoxide. Next, 1 mL of the given preparation was introduced to the initial container and subsequently dispersed with the BHI solution in a sequential manner. The amounts of MNEO in the specimens after the experiment were 2542, 1271, 635.5, 317.75, and 158.87 mg/mL. Subsequently, tubes with various levels of MNEO have been introduced with fifty μL of bacterial suspensions, each comparable in accordance with a 0.5 McFarland turbidity criterion (1.5×10^8 CFU/mL). A tube with Brain Heart Infusion medium and MNEO was designated as the positive control, while a tube holding only BHI media and a solution of microorganisms was employed as the negative control. Each tube was made with dilutions, and three sequential preparations were grown on Muller Hinton agar. Following this, the specimens underwent incubation at an ambient condition ($37\text{ }^{\circ}\text{C}$) for a duration of 24 h. The MIC was then established as the minimal amount of MNEO that entirely prevented the active development of bacteria. The MBC was evaluated by transferring 100 μL of the tube's contents (obtained from the MIC test) without any visible growth and sub-cultivating it.

2.6. Mayonnaise preparation

The preparation of mayonnaise followed the methodology outlined by Alizadeh and collaborators (2019), with certain adjustments. The initial mixture consisted of vinegar (6.5 wt percent), egg yolk (12 wt percent), as well as water (22 wt percent). Subsequently, the powdered substances, namely stabilizer (1 wt percent, carboxymethyl cellulose), salt (weight percent), and sugar (3.5 wt percent), were incorporated and combined for a duration of 1 min. Subsequently, the oil (53 wt percent) was incrementally introduced into the mixture with the aid of an Ultra-Turrax homogenizer (T 25 ULTRA-TURRAX, Iran) operating at 10,000 rpm til a homogeneous mixture was achieved.

2.7. Microbiological assessment of mayonnaise

MNEO was added to the mayonnaise's oil portion in increments of two and was equivalent to the MBC found for each bacterium. Next, 50 μL of bacterial preparation (1.5×10^8 CFU/mL, corresponding 0.5 McFarland) was added under aseptic conditions to mayonnaise samples in a sterile laminar flow hood. The positive control sample of mayonnaise had sodium benzoate at a concentration of 1000 ppm, while the negative control sample of mayonnaise did not contain any preservatives. Ultimately, all specimens were positioned in sterilized receptacles and maintained at an average temperature of $4 \pm 2\text{ }^{\circ}\text{C}$ for a period of 40 days. The bacterial count was measured at consistent time intervals (0, 5, 10, 15, 20, 25, 30, 35, and 40 days). Five grams of mayonnaise specimens were blended with 45 mL of sterile peptone water. Serial dilutions were then set up, ranging from 10^{-1} to 10^{-5} . Three subsequent dilutions, specifically from 10^{-3} to 10^{-5} , were cultured on specific agar media: *S. aureus*-MHA, *E. coli* O157:H7-EMB, *P. aeruginosa*-CA, and *S. enteritidis*-SS agar. The Miles-Misra approach was used for culturing, and the specimens were afterward incubated for 24–48 h at $37\text{ }^{\circ}\text{C}$. The colony count was established as the bacterial population in the mayonnaise specimens and expressed as log CFU/g [1].

2.8. Physicochemical assessment of mayonnaise

Mayonnaise was individually enhanced with amounts of MNEO at 2542 ppm (corresponding to the highest MBC) and 5084 ppm (double the highest MBC). The positive-controlled specimen was created by adding 75 ppm TBHQ into mayonnaise, while the negative control specimen did not include any antioxidants. Ultimately, the specimens were placed in containers and was kept for 60 days at 4

± 2 °C on average. Mayonnaise's physicochemical characteristics, including the pH, color, peroxide value (PV), thiobarbituric acid value (TBA), and total oxidation values (TOTOX values (2 P V TBA)), were assessed at 10-day intervals (0–60 days) throughout the storage period.

2.8.1. pH

At room temperature, the pH level of the mayonnaise specimens was measured with a pH meter (HI991003, Iran). Before measuring pH, the calibration of the instrument was conducted utilizing buffer mixtures with pH values of 4, 7, and 9 [16].

2.8.2. Thiobarbituric acid (TBA) value

The TBA value was evaluated using the AOCS approved technique [17].

2.8.3. Peroxide value

In order to determine the peroxide value, a solution was prepared by dissolving 5 g of oil in a solution of 25 mL of chloroform and acetic acid in a 2:3 ratio. Subsequently, a volume of 1 mL of a potassium iodide concentrated preparation was incorporated into the mixture, and the resulting solution was then kept in a dark environment for a duration of 10 min. Subsequently, 30 mL of purified water was introduced, and the combination was subjected to titration utilization a 0.01 M solution of sodium thiosulfate in the presence of starch solution [6].

2.8.4. Color

A colorimeter (YS6060, China) was used to measure the visual appearance of mayonnaise specimens throughout the preservation period utilizing the CIE L* a* b* system. Initially, the device was calibrated. Subsequently, the color parameters, namely L* (representing brightness), a* (indicating the range from green to red), and b* (representing the range from blue to yellow), were measured using D65/10° illumination conditions [18].

2.9. Sensory assessment of mayonnaise

A 5-point hedonic scale, with 1 denoting “strong dislike” and 5 denoting “strong liking,” was used for the sensory evaluation. In order to accomplish this, five newly produced samples of mayonnaise were created: one without any preservatives and the others having essential oil at concentrations of 2542 ppm and 5084 ppm. Next, 25 panelists with specialized training (12 females and 13 males, aged 20–60) assessed sensory aspects, including odor, color, taste, texture, and overall acceptability. To prevent residual flavors, they were instructed to consume water after every specimen [19].

2.10. Statistical analysis

The dataset underwent a one-way analysis of variance (ANOVA) utilizing SPSS software (version 26). The results were expressed as the mean \pm standard deviation. The Duncan test was applied to identify significant differences among the means at a 95 % confidence level ($P < 0.05$). The experiments were carried out at least three times. Pearson's method and principal component analysis (PCA) were employed to compute correlation coefficients, utilizing GraphPad Prism software (version 10.2.2.397).

3. Results and discussion

3.1. Chemical profile of MNEO

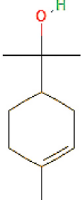
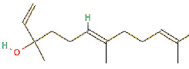
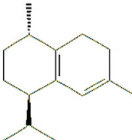
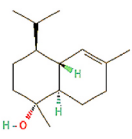
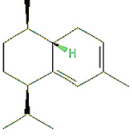
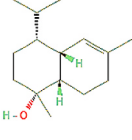
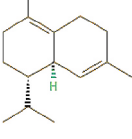
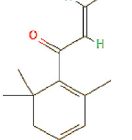
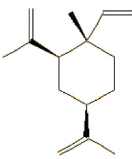

Based on dry weight, the essential oil yield of *Malva neglecta* was 1.43 % (w/w). According to the findings, the essential oil of *M. neglecta* contained a total of twenty-one identifiable components, as shown in Table 1. The hydrodistilled oil of *M. neglecta* primarily consists of podocarpa-8,11,13-trien-3-one, 12-hydroxy-13-isopropyl (41.8 %), δ -elemene (10.2 %), γ -elemene (6.4 %), bicyclogermacrene (5.5 %), and β -ylangene (4.6 %). In addition, the oil structure contained various components in relatively smaller amounts, such as viridiflorol (3.9 %), spathulenol (3.6 %), 6,10,14-trimethyl-2-pentadecanone (3.5 %), L-linalool (2.8 %), γ -curcumene (2.6 %), germacrene D (2.4 %), n-tetradecane (2.2 %), β -elemene (2.0 %), β -damascenone (1.4 %), δ -cadinene (1.3 %), α -muurolol (1.2 %), cis-muurola-3,5-diene (1.1 %), and α -cadinol (1.0 %). Conversely, the chemical profile revealed extremely low concentrations of natural chemicals with less than 1 % contribution, including cis-cadina-1 (6), 4-diene (0.8 %), (E)-nerolidol (0.7 %), and α -terpineol (0.2 %).

3.2. Antimicrobial activity of MNEO

3.2.1. Disc diffusion

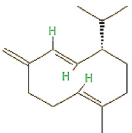
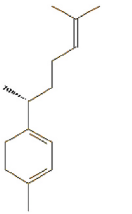
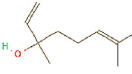
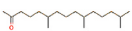
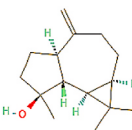
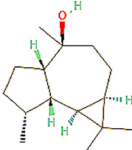
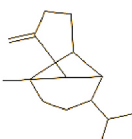
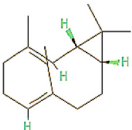
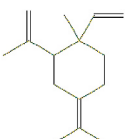
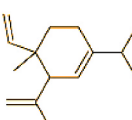
The method of disc diffusion was employed to initially investigate the antibacterial properties of MNEO against the target bacteria, as revealed in Table 2. The cultivation of MNEO yielded no colonies, suggesting an absence of bacteria-related cross-contamination in MNEO. Gentamicin, which was used as a positive control, showed the most potent inhibiting action on *P. aeruginosa* (IZ: 32), *S. aureus* (IZ: 29 mm), *E. coli* O157:H7 (IZ: 25 mm), and *S. enteritidis* (IZ: 19 mm). As anticipated, dimethyl sulfoxide did not demonstrate any inhibitory effect (IZ: 0 mm). Increasing the concentration of MNEO enhanced its antibacterial efficacy, as evidenced by larger

Table 1
Chemical composition of *Malva neglecta* essential oil (MNEO) identified and quantified by gas chromatography– mass spectroscopy.

No.	Molecular formula	Peak identity	2D Structure	Molecular weight (g/mol)	Kovats retention index	Area (%)
1	C ₁₀ H ₁₈ O	α-terpineol		154.25	1184.3	0.2
2	C ₁₅ H ₂₆ O	(E)-nerolidol		222.37	1564.7	0.7
3	C ₁₅ H ₂₄	cis-cadina-1 (6), 4-diene		204.35	1463.6	0.8
4	C ₁₅ H ₂₆ O	α-cadinol		222.37	1663.5	1
5	C ₁₅ H ₂₄	cis-muurolo-3,5-diene		204.35	1458	1.1
6	C ₁₅ H ₂₆ O	α-muurolool		222.37	1645.2	1.2
7	C ₁₅ H ₂₄	δ-cadinene		204.35	1534.5	1.3
8	C ₁₃ H ₁₈ O	β-damascenone		190.28	1392.6	1.4
9	C ₁₅ H ₂₄	β-elemene		204.35	1394.8	2
10	C ₁₄ H ₃₀	n-tetradecane		198.39	1448.3	2.2

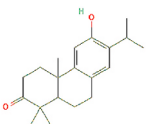
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Table 1 (continued)

No.	Molecular formula	Peak identity	2D Structure	Molecular weight (g/mol)	Kovats retention index	Area (%)
11	C ₁₅ H ₂₄	Germacrene D		204.35	1486.3	2.4
12	C ₁₅ H ₂₄	γ-curcumene		204.35	1482.3	2.6
13	C ₁₀ H ₁₈ O	L-linalool		154.25	1198.3	2.8
14	C ₁₈ H ₃₆ O	6,10,14-trimethyl-2-pentadecanone		268.5	1856.7	3.5
15	C ₁₅ H ₂₄ O	Spathulenol		220.35	1575.8	3.6
16	C ₁₅ H ₂₆ O	Viridiflorol		222.37	1590.2	3.9
17	C ₁₅ H ₂₄	β-ylangene		204.35	1422.7	4.6
18	C ₁₅ H ₂₄	Bicyclogermacrene		204.35	1522.4	5.5
19	C ₁₅ H ₂₄	γ-elemene		204.35	1451.2	6.4
20	C ₁₅ H ₂₄	δ-elemene		204.35	1327.6	10.2

(continued on next page)

Table 1 (continued)

No.	Molecular formula	Peak identity	2D Structure	Molecular weight (g/mol)	Kovats retention index	Area (%)
21	C ₂₀ H ₂₈ O ₂	Podocarpa-8,11,13-trien-3-one, 12-hydroxy-13-isopropyl		300.4	2550	41.8

inhibition zones (from 6.9 to 12.74 mm for *S. aureus*, from 6.7 to 11.5 mm for *E. coli* O157:H7, from 5.5 to 9.67 mm for *P. aeruginosa*, and from 4 to 8.9 mm for *S. enteritidis*) ($P < 0.05$). It is crucial to note that while the concentration was the same, the inhibitory impact of MNEO was detected in the following order: *S. aureus* > *E. coli* O157:H7 > *P. aeruginosa* > *S. enteritidis*.

An investigation carried out by Teneva and colleagues (2021) examined the suppressor impact of dill and basil essential oils on a collection of harmful and non-harmful microorganisms in the matrix of mayonnaise. Dill essential oil's inhibitory zone against *Salmonella* and *E. Coli* was found to be eight mm and nine mm, respectively. However, the basil essential oil's inhibitory zone was found to be ten mm for both bacteria. The essential oil used in this investigation had a major component of 39.88 % allyl isothiocyanate [5]. Prior research has demonstrated that allyl isothiocyanate effectively suppressed the proliferation of disease-causing microorganisms, potentially due to its capacity to hinder the activity of enzymes within cells, cause leakage of essential cellular components, and disrupt the metabolism of cells [20]. Olaimat and colleagues (2014) found that using at least 50 $\mu\text{L/g}$ of allyl isothiocyanate in a κ -chitosan/carrageenan wrapping mixture reduced the number of active *Campylobacter jejuni* in the vacuum-packaged matrix of chicken breasts [21]. Yang and colleagues, (2023) highlighted the antibacterial characteristics of chitosan/carboxymethyl gelatin gum film composites with *Malva* essential oil (containing 94 % allyl isothiocyanate) for preserving mangoes. Based on their findings, increasing the content of *Malva* essential oil resulted in a rise in the diameter of the inhibition zone for *E. coli*, *Bacillus anthracis*, and *S. aureus*. Hence, including *Malva* essential oil in the film could enhance the longevity of the coated mango. Furthermore, this particular essential oil demonstrated superior antibacterial efficacy against Gram-positive bacteria [22].

3.2.2. MIC and MBC

The minimum inhibitory/bactericidal concentrations were established in order to gauge the antimicrobial effectiveness of MNEO and identify the optimal concentration for its inclusion in mayonnaise specimens. The MIC and MBC of MNEO for *S. aureus* were found to be 158.87 ppm and 317.75 ppm, respectively. For *E. coli* O157:H7, the corresponding concentrations were 317.75 ppm and 635.5 ppm. *P. aeruginosa* showed MIC and MBC at 635.5 ppm and 1271 ppm, while *S. enteritidis* exhibited MIC and MBC at 1271 ppm and 2542 ppm ($P < 0.05$) (Table 2). The antibacterial efficacy of the MNEO was contingent upon the bacterial species, with Gram-positive bacteria exhibiting greater susceptibility compared to Gram-negative types. The changes in the structure of the bacterial cell wall have contributed to these findings, according to the MIC and MBC assays. The former group exhibits a more substantial mucopeptide layer compared to the latter group. Lipopolysaccharides and lipoproteins constitute the primary components of the wall framework in gram-negative bacteria, enhancing their tolerance to antibacterial agents [23].

The antibacterial action of MNEO is due to its components' propensity to hinder the manufacture of key enzymes in bacteria and/or induce damage to their cell walls [24]. In addition, it was observed that *S. enteritidis* exhibited greater resistance to MNEO compared to *P. aeruginosa* and *E. coli* O157:H7. This was further validated by the assessment of the inhibitory zone in the previous section. Research done by Clemente and colleagues (2016), it was discovered that the MIC and MBC values of *Malva* essential oil (MEO) comprising 90 % allyl isothiocyanate were 100 ppm and 200 ppm, correspondingly, versus both *E. coli* O157:H7 and *S. enterica*. The investigators discovered that MEO exhibited a more potent antibacterial action and more vapor activity compared to cinnamon essential oil against the pathogens examined. As a result, MEO was suggested as a viable choice for utilization in active containers within the realm of food production [25]. Peng and colleagues (2014) conducted a study to assess the minimum inhibitory/bactericidal concentrations of MEO, which contains 71.06 % allyl isothiocyanate, against various food pathogens like *Salmonella* sp. And *E. coli* sp. The outcomes were then compared to the properties of pure allyl isothiocyanate. The MIC and MBC of MEO against *Salmonella Lignieres* (ATCC14028) were detected to be 256 and 512 ppm, correspondingly. In the case of *E. coli* (ATCC 8739), the corresponding values were found to be 512 ppm. Additionally, it was mentioned that pure allyl isothiocyanate exhibited marginally greater antibacterial efficacy compared to

Table 2

Antimicrobial activity of *Malva neglecta* essential oil (MNEO) against mayonnaise associated pathogenic microorganisms. Data express mean \pm standard deviation (SD).

Microorganisms	Inhibition zone diameter (mm)				Minimum concentration (ppm)	
	MNEO (20 μL)	MNEO (40 μL)	Gentamicin	Dimethyl sulfoxide	MIC	MBC
<i>Staphylococcus aureus</i>	6.9 \pm 0.46 ^I	12.74 \pm 33 ^E	29.10 \pm 0.12 ^B	0.00 \pm 0.00 ^M	158.87	317.75
<i>Escherichia coli</i> O157:H7	6.7 \pm 0.37 ^J	11.5 \pm 0.37 ^F	25.32 \pm 0.45 ^C	0.00 \pm 0.00 ^M	317.75	635.5
<i>Pseudomonas aeruginosa</i>	5.5 \pm 0.28 ^K	9.67 \pm 0.64 ^G	32.76 \pm 0.33 ^A	0.00 \pm 0.00 ^M	635.5	1271
<i>Salmonella enteritidis</i>	4 \pm 0.85 ^L	8.9 \pm 0.81 ^H	19.45 \pm 57 ^D	0.00 \pm 0.00 ^M	1271	2542

•Means with different superscript for inhibition zone (IZ) indicate significant difference at $P < 0.05$.

MEO [26].

To include MNEO in mayonnaise formulations for microbiological examination, we determined the concentration of MNEO to be twice the minimum bactericidal level of the bacteria being studied. Therefore, taking into account the minimum bactericidal concentration of *S. enteritidis*, the amounts of 2542 ppm (equivalent to the MBC) and 5084 ppm (double the MBC) were used to formulate the mayonnaise specimens. Alternatively, for different bacteria, MNEO was added individually to mayonnaise based on their equal and double MBC concentration values. Specifically, *S. aureus* had MBC values of 317.75 ppm and 635.5 ppm, *E. coli* O157:H7 had values of 635.5 ppm and 1271 ppm, and *P. aeruginosa* had values of 1271 ppm and 2542 ppm.

3.3. Microbiological properties of mayonnaise

The impact of different levels of MNEO and the duration of storage on the survival rate of the selected bacteria was statistically significant ($P < 0.05$). Table 3 presentations the log CFU/g results of the pathogenic bacteria count at various time points throughout the storage period (0, 5, 10, 15, 20, 25, 30, 35, and 40 days). In order to gain a deeper comprehension of the antibacterial efficacy of MNEO, sodium benzoate (SB), a commercially available chemical additives, was employed as a control. The population of *S. aureus* and *E. coli* O157:H7 fell at a faster rate in the positive control and were completely eliminated after 5 days, demonstrating the potent bactericidal activity of SB ($P < 0.05$). Regarding the treatments of *S. aureus* and *E. coli* O157:H7 in preservative-free mayonnaise, the growth trend initially increased until the 20th day and then decreased. This could be attributed to the decrease in available nutrients and the low pH of the mayonnaise, which makes the bacteria more sensitive to these circumstances. The same pattern was observed for the treatments of *P. aeruginosa* and *S. enteritidis*, with the largest number of bacteria discovered on the 15th day. The utilization of MNEO has the potential to decrease the number of pathogens prevalent. The proliferation behavior of *S. aureus* was affected by MNEO, and in the 1271 ppm MNEO treatment, it showed a decrease of about four log CFU/g on the 10th day, and finally, no growth was detected on the 15th day. Likewise, increasing the concentration of MNEO (2542 ppm) led to the growth-inhibited response of *S. aureus* on the fifth day ($P < 0.05$).

Mayonnaise containing 2542 ppm essential oil exhibited a 3-log reduction in *E. coli* counts within 5 days, with complete elimination by day 10. Similarly, the utilization of 1271 ppm MNEO reduced the *E. coli* O157:H7 count over a longer period of time compared to the 2542 ppm concentration, reaching nearly undetectable levels by the 20th day ($P < 0.05$). The presence of MNEO in mayonnaise formulations also repressed the proliferation of *P. aeruginosa* and *S. enteritidis* (Table 3). This effect was more pronounced in specimens with a higher quantity of essential oil ($P < 0.05$). The main bioactive compounds found in the employed MNEO are podocarpa-8,11,13-trien-3-one, 12-hydroxy-13-isopropyl (41.8 %), δ -elemene (10.2 %), γ -elemene (6.4 %), bicyclogermacrene (5.5 %), and β -ylangene (4.6 %). These compounds are considered to be responsible for the strong antibacterial activity of MNEO. Research done by Mansouri and colleagues (2021) examined the use of a nanoemulsion form of *Thymus daenensis* L. essential oil as an organic additive in mayonnaise. Both the optimum treatment and sodium benzoate were effective in diminishing the count of *E. coli* and *S. typhimurium* in mayonnaise throughout preservation at 4 °C. However, no impact was seen on the count of *Listeria monocytogenes* [19]. Porter and colleagues (2020) revealed that white *Malva* essential oil has the capacity to act as an organic preservation agent versus *Salmonella* in chicken goods [27]. Furthermore, Turgis and collaborators (2008) noted that MEO has the capacity to improve the radiosensitivity of *E. coli* and *S. typhi* in ground beef. In addition, both MEO and allyl isothiocyanate showed comparable susceptibility to radiation, with a value of 0.072 kGy [28].

3.4. Physicochemical properties of mayonnaise

3.4.1. pH

Fig. 1A illustrates the variations in pH levels of mayonnaise specimens throughout a 60-day storage period at a temperature of 4 ± 2 °C. During the 60-day preservation period, a significant decrease in pH value was detected across all treatments ($P < 0.05$). The control sample experienced the most significant decline, going from 4.45 to 3.71. In contrast, the synthetic antioxidant (TBHQ) had the smallest decrease, going from 4.40 to 4.35. The decrease in pH level of mayonnaise, particularly in the additive-free control group, is likely caused due to the oil phase's oxidation and its dissolution of condensation mediators, such as liberated fatty acids [29]. Furthermore, a reduction in pH can augment the iron dissolution in egg yolks by disrupting the phosvitin-iron interaction, hence promoting oxidation in mayonnaise [30].

The existence of MNEO with a level of 5084 ppm was found to be more effective in slowing down the decrease in pH following the addition of the synthetic antioxidant TBHQ. Furthermore, Kishk and Elsheshetawy (2013) conducted a study that likewise documented the decrease in pH levels when mayonnaise specimens exposed to ginger powder were stored. The observation was linked to the proliferation of probiotics. It was shown that ginger powder, which has antibacterial capabilities, can slow down the lowering of pH, particularly at greater doses [31]. Khalid and colleagues (2021) observed a drop in the pH levels of the mayonnaise samples during preservation. The samples including varying amounts of apple peel extract, particularly at higher doses, exhibited a lesser decline compared to the control group and samples containing BHT [32].

3.4.2. Thiobarbituric acid (TBA) value

The primary concentration of TBA in every specimen was 0.04 mg per kg. Over the period of preservation, there was a significant rise ($P < 0.05$) in the subsequent sequence: TBHQ < 5084 ppm MNEO < 2542 ppm MNEO < control. The negative control specimen, which did not include any antioxidants, exhibited the greatest elevation in TBA value in comparison to the treatments with synthetic antioxidants and MNEO (Fig. 1B). Based on the findings, it appears that MNEO may be a viable method for safeguarding mayonnaise

Table 3Microbiological properties of mayonnaise samples over the storage period. Data express mean \pm standard deviation (SD).

Microorganisms	Treatment	Time (Day)									
		0	5	10	15	20	25	30	35	40	
<i>Staphylococcus aureus</i>	1271 ppm MNEO	6.51 \pm 0.23 ^D	4.64 \pm 0.42 ^H	2.76 \pm 0.51 ^I	ND*	ND	ND	ND	ND	ND	ND
	2542 ppm MNEO	6.11 \pm 0.41 ^E	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Positive control (SB)	5.96 \pm 0.73 ^{EF}	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Negative control	5.64 \pm 0.49 ^G	5.86 \pm 0.47 ^F	6.88 \pm 0.36 ^C	7.57 \pm 0.54 ^B	7.97 \pm 0.24 ^A	6.34 \pm 0.33 ^{DE}	6.14 \pm 0.21 ^E	5.74 \pm 0.49 ^G	5.53 \pm 0.73 ^{GH}	
<i>Escherichia coli</i> O157:H7	1271 ppm MNEO	6.45 \pm 0.34 ^D	5.36 \pm 0.28 ^H	4.64 \pm 0.44 ^I	3.11 \pm 0.35 ^J	ND	ND	ND	ND	ND	ND
	2542 ppm MNEO	6.34 \pm 0.45 ^{EF}	2.97 \pm 0.34 ^J	ND	ND	ND	ND	ND	ND	ND	ND
	Positive control (SB)	6.30 \pm 0.25 ^F	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Negative control	6.52 \pm 0.64 ^D	6.74 \pm 0.35 ^C	6.87 \pm 0.42 ^C	6.98 \pm 0.66 ^B	7.23 \pm 0.78 ^A	6.41 \pm 0.24 ^D	6.13 \pm 0.33 ^G	5.78 \pm 0.38 ^G	5.24 \pm 0.57 ^I	
<i>Pseudomonas aeruginosa</i>	1271 ppm MNEO	6.34 \pm 0.49 ^{CD}	5.41 \pm 0.23 ^E	4.37 \pm 0.67 ^G	3.33 \pm 0.42 ^I	2.32 \pm 0.74 ^J	ND	ND	ND	ND	ND
	2542 ppm MNEO	6.11 \pm 0.42 ^D	4.61 \pm 0.76 ^F	2.31 \pm 0.39 ^J	ND	ND	ND	ND	ND	ND	ND
	Positive control (SB)	6.10 \pm 0.64 ^D	3.16 \pm 0.25 ^I	ND	ND	ND	ND	ND	ND	ND	ND
	Negative control	6.47 \pm 0.78 ^C	6.76 \pm 0.24 ^B	6.89 \pm 0.66 ^A	7.25 \pm 0.49 ^A	6.79 \pm 0.77 ^B	6.62 \pm 0.24 ^{BC}	5.48 \pm 0.64 ^E	4.45 \pm 0.76 ^{FG}	4.12 \pm 0.16 ^H	
<i>Salmonella enteritidis</i>	1271 ppm MNEO	6.22 \pm 0.56 ^E	6.13 \pm 0.45 ^F	4.32 \pm 0.78 ^H	3.22 \pm 0.64 ^J	2.36 \pm 0.34 ^M	ND	ND	ND	ND	ND
	2542 ppm MNEO	6.10 \pm 0.44 ^F	5.44 \pm 0.64 ^G	2.55 \pm 0.34 ^K	ND	ND	ND	ND	ND	ND	ND
	Positive control (SB)	6.20 \pm 0.23 ^{EF}	2.41 \pm 0.33 ^{LM}	ND	ND	ND	ND	ND	ND	ND	ND
	Negative control	6.24 \pm 0.75 ^E	6.54 \pm 0.28 ^D	6.88 \pm 0.73 ^B	7.10 \pm 0.54 ^A	6.72 \pm 0.25 ^C	6.24 \pm 0.47 ^E	5.32 \pm 0.33 ^G	4.42 \pm 0.36 ^H	3.61 \pm 0.77 ^I	

* Not detected.

Means with different superscript for each bacterium indicate significant difference at $P < 0.05$.

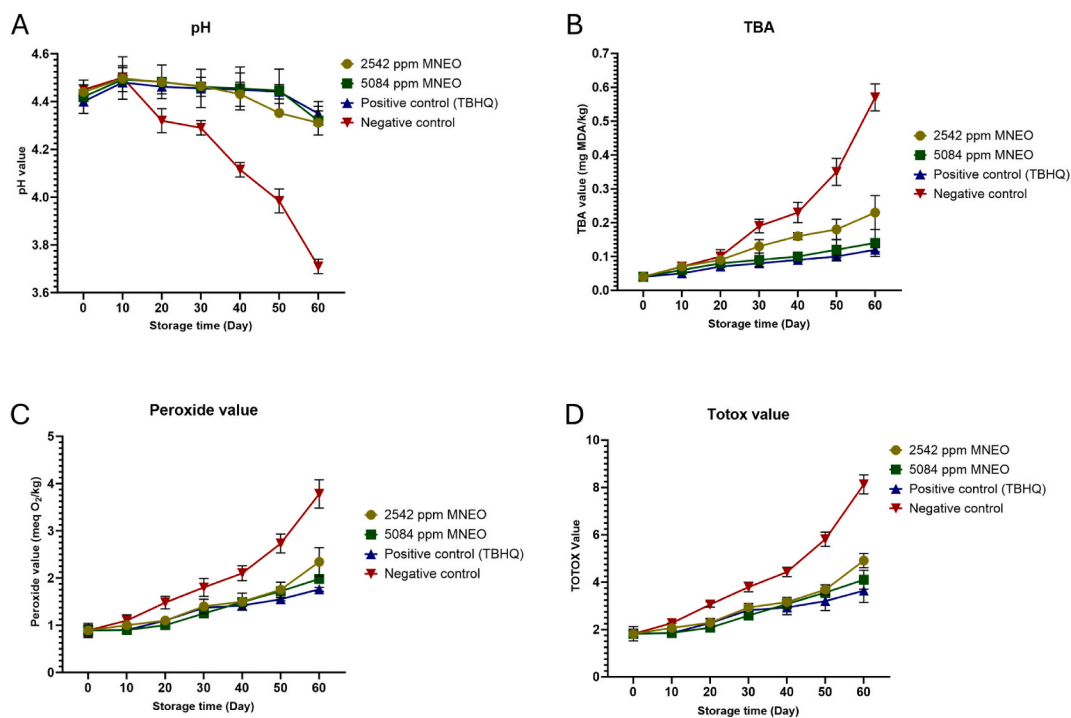


Fig. 1. Physicochemical characteristics of the of the mayonnaise samples. (A) Changes (mean \pm SD) of pH, (B) Mean values (\pm SD) of TBA value, (C) Mean values (\pm SD) of peroxide value, (D) Mean values (\pm SD) of totox value (2 P V + TBA) in mayonnaise samples treated with various concentrations (2542 ppm, and 5084 ppm) of Malve neglecta essential oil (MNEO), positive control (TBHQ), and negative control (without any preservatives) during 60 days of storage at 4 ± 2 °C.

oil from oxidative damage. This is supported by the fact that the sample containing 5084 ppm essential oil had an antioxidant power comparable to that of an artificial antioxidant. This establishes the antioxidant properties of MNEO, which are attributed to the attendance of viridiflorol, germacrene D, podocarpa-8,11,13-trien-3-one, 12-hydroxy-13-isopropyl, β -elemene, spathulenol, γ -elemene, β -damascenone, δ -elemene, 6,10,14-trimethyl-2-pentadecanone, β -ylangene, γ -curcumene, cis-muurolo-3,5-diene, bicyclo-germacrene, α -cadinol, δ -cadinene, and α -muurolo-3,5-diene in the MNEO chemical profile [33,34].

Dias and colleagues, (2013) performed a study that highlighted not only the antibacterial characteristics of allyl isothiocyanate but also its significance as an antioxidant. By combining carbon nanotubes with cellulose film, it is possible to control lipid oxidation in packaging for shredded cooked chicken meat contaminated with *S. choleraesuis* [35]. Ahmadi and collaborators (2022) discovered that thyme effectively postponed the oxidative deterioration of mayonnaise and exhibited comparable performance to synthetic antioxidants. The antioxidant activities of essential oils were ascribed to the existence of phenolic components such as terpinene, thymol, and carvacrol, as well as terpenes [6]. Amiri and colleagues (2020) found that mayonnaise specimens with six hundred ppm cumin essential oil, along with a artificial antioxidant, had the greatest effect in preventing the production of malondialdehyde [36].

3.4.3. Peroxide value

All mayonnaise specimens showed an increasing tendency in their peroxide values over the course of storage ($P < 0.05$) (Fig. 1C). Mayonnaise is prone to oxidation owing to multiple influences: the use of sunflower oil, which contains approximately eighty-five percent unsaturated fatty acids; an abundance of oxygen in the water sections; the emulsification process that increases contact between the oil and water sections; and the dissolution of egg yolk proteins-related iron in acidic conditions [37]. The negative control group exhibited the most significant rise in peroxide value, increasing from 0.89 to 3.78 meq O_2 /kg of oil. Conversely, the synthetic antioxidant (TBHQ) showed the smallest increase, going from 0.89 to 1.76 meq O_2 /kg of oil. As anticipated, the addition of MNEO culminated in a decrease in the rate of peroxide generation.

Increasing the level of essential oil in mayonnaise specimens resulted in a greater display of antioxidant characteristics. For instance, the peroxide content of the mayonnaise containing 5084 ppm of essential oil increased more gradually (from 0.89 to 1.98 meq O_2 /kg of oil) than the level of 2542 ppm (from 0.89 to 2.34 meq O_2 /kg of oil). Furthermore, it had the greatest impact on inhibiting the generation of peroxides ($P < 0.05$). In this context, the investigation of Hassanzadeh and colleagues (2023) exhibited that the inclusion of garlic extract in mayonnaise resulted in a decrease in peroxide value over time. This can be attributed to the extract's abundant polyphenolic substance as well as its capacity to counteract free radicals [38].

3.4.4. TOTOXTBA value

To assess the extent of progressive oxidative damage, the TOTOX value was determined by measuring the peroxide and TBA values (Fig. 1D) [1]. There was a noticeable rise in the TOTOX value in each of the specimens ($P < 0.05$). While TBHQ exhibited the highest antioxidant activity, MNEO was successful in suppressing the progression of both primary and secondary oxidation mediators. The mayonnaise sample with a level of 5084 ppm of MNEO exhibited the maximum level of oxidative stability among the naturally preserved specimens.

3.4.5. Color

The sensory parameter of color significantly influences consumers' inclination to select a good. The yellow hue of mayonnaise is mostly derived from the presence of carotenoids in the egg yolk, together with oil and various ingredients such as spice blends [18]. This factor's evaluation was conducted in order to determine the effect of applying MNEO and the storage duration on the color of the specimens. Fig. 2 depicts the alterations in color characteristics of mayonnaise specimens throughout the duration of preservation. The inclusion of MNEO led to a statistically significant ($P < 0.05$) elevation in the L^* index of mayonnaise in relation to the negative comparison group (Fig. 2A). This discovery is consistent with the outcomes of Wang and colleagues (2022), who noted a decline in the color intensity of mayonnaise specimens after preservation. They ascribed this phenomenon to the development of lipid oxidation,

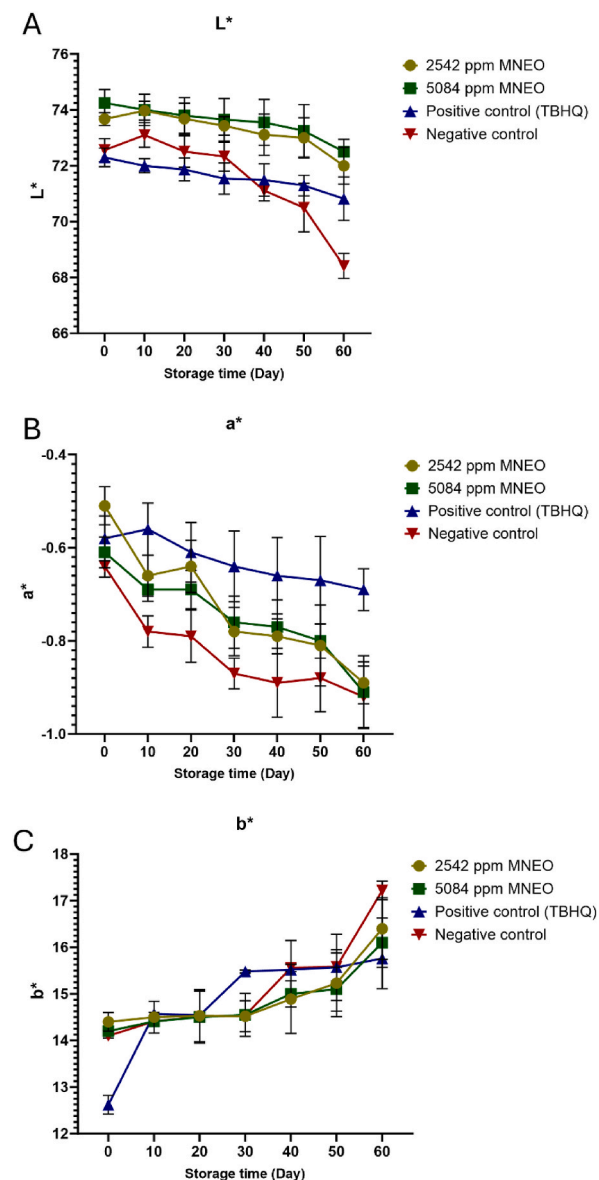


Fig. 2. Changes in L^* (A), a^* (B), and b^* (C) values of mayonnaise samples treated with various concentrations (2542 ppm, and 5084 ppm) of Malve neglecta essential oil (MNEO), positive control (TBHQ), and negative control (without any preservatives) during 60 days of storage at 4 ± 2 °C.

which led to a darkening of the hue [39]. Overall, the L^* score of every specimen dropped over time, with the negative control group showing the greatest decrease (from 72.56 to 68.41). Mayonnaise comprising TBHQ and 5084 ppm MNEO showed a reduced change in the L^* value. In a previous study, Flammini and colleagues (2020) found that mayonnaise with free olive leaf phenolic extraction had lower L^* scores in comparison to mayonnaise with alginate/pectin as a coating [18].

All mayonnaise specimens had a negative value of a^* , indicating their inclination towards a green color, which diminished as the storage time progressed (Fig. 2B). In contrast, the positive b^* scores revealed the yellow hue of the specimens, which exhibited a progressive increase as time passed ($P < 0.05$). The additive-free negative control revealed the biggest rise in the b^* score (from 14.10 to 17.22), while the minimal rise was observed in the sample containing TBHQ (from 14.62 to 15.76) (Fig. 2C).

3.5. Sensory properties of mayonnaise

The sensory features of mayonnaise specimens with MNEO concentrations of 2542 ppm, 5084 ppm, and control were assessed in this part (Fig. 3). The obtained findings indicated a notable disparity concerning the specimens in terms of color, aroma, flavor, and general acceptance ($P < 0.05$). Nevertheless, no substantial variation was noted regarding texture. The sample containing 5084 ppm essential oil was evaluated as the most appealing by the panelists in terms of all sensory aspects, including color (3.9), odor (4.3), taste (4.41), texture (4.15), and overall acceptability (4.35). In a similar vein, Ouattara and colleagues, (2001) found that using nearly two percent thyme as a covering for shrimp resulted in a drop in sensory features, specifically odor and taste scores [40]. In a study conducted by Moradi and colleagues (2023), the sensory characteristics of mayonnaise comprising the essential oil of *Cuminum cyminum* L. and associated nanoemulsion were examined. The researchers found that, compared to the nanoemulsion, the pure essential oil had an unpleasant odor and color, resulting in reduced acceptance [41]. Savaghebi and colleagues (2021) found that encapsulating brown algal extract in nanoliposomes exerted a positive influence on the sensory attributes of mayonnaise. The addition of brown algae to the mayonnaise formulation had a negative impact on the sensory properties. The outcome was a reduction in brightness, accompanied by a strong, bitter flavor, and a light green color. However, encapsulation of the extracts effectually improved the acceptance of the mayonnaise by masking its potent color and taste [37].

3.6. Principal component analysis

Principal Component Analysis (PCA) is a statistical technique used to simplify complex datasets by reducing their dimensionality while retaining the most critical information. PCA works by transforming the original variables into a new set of independent variables called principal components. These components are created as linear combinations of the original variables and are ranked based on the amount of variance they capture within the data. This technique is particularly useful for uncovering relationships between

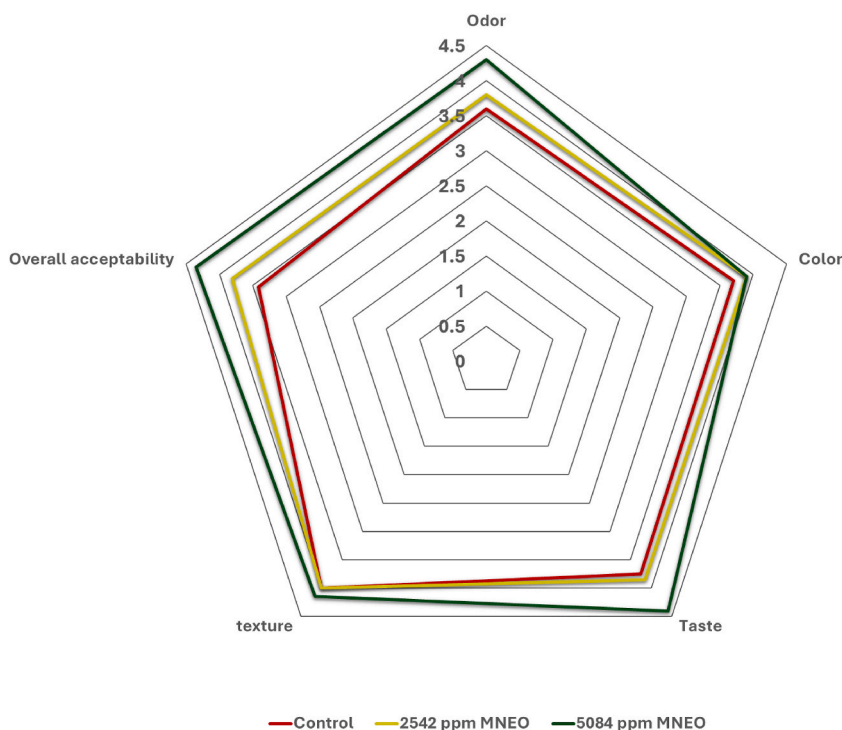


Fig. 3. Radar chart depicting the sensory scores of mayonnaise samples containing different concentrations (2542 ppm, and 5084 ppm) of Malve neglecta essential oil (MNEO), and positive control (TBHQ).

microbiological, physicochemical, and sensory characteristics in processed food products [42].

In this study, PCA was employed to discern correlations between effective dose parameters and potential antimicrobial activity among different MNEO treatments, as well as to demonstrate the effectiveness of numerous treatments on the microbiological, chemical, and sensory attributes of mayonnaise specimens during refrigeration. The scree plot, proportion of variance, loading plot, PC scores plot, biplot, contribution rate to variability, and cumulative contribution rate are presented in Fig. 4 and Table 4. A scree plot helps determine the ideal number of components that can be extracted from the dataset. The analysis of the scree plot indicates that the extraction of two components represents the most effective strategy (Fig. 4A and B). The total variability was elucidated by two main components, where the first principal component (PC1) represented 60.43 % and the second principal component (PC2) accounted for 25.66 %. Collectively, these two elements accounted for 86.09 % of the overall variability (Fig. 4C and Table 4). PC1 demonstrated a robust association among pH, TBA, PV, TV, L*, a*, odor, color, taste, texture, and overall acceptability. PC2 categorized the treatments according to their levels of *S. aureus*, *E. coli*, *P. aeruginosa*, *S. enteritidis*, and b* (Fig. 4D and Table 4). The scoring plots indicated that the treatments had similar effects on the qualities of the mayonnaise samples, as evidenced by their close proximity to one another. According to Fig. 4C–E, some doses of MNEO and positive control at 60 days of storage as well as all negative controls were situated in the first and second quadrants, respectively, representing a noteworthy effect on the establishment of b* and less delayed influence on the development of oxidative reactions (TBA, PV, TV), as well as all samples without any preservative agent exhibiting no growth-inhibitory effect on the investigated mayonnaise pathogens (*S. aureus*, *E. coli*, *P. aeruginosa*, *S. enteritidis*). Furthermore, the major-dose doses of MNEO and positive control treatments were situated in the third and fourth quadrants, revealing a significant growth-

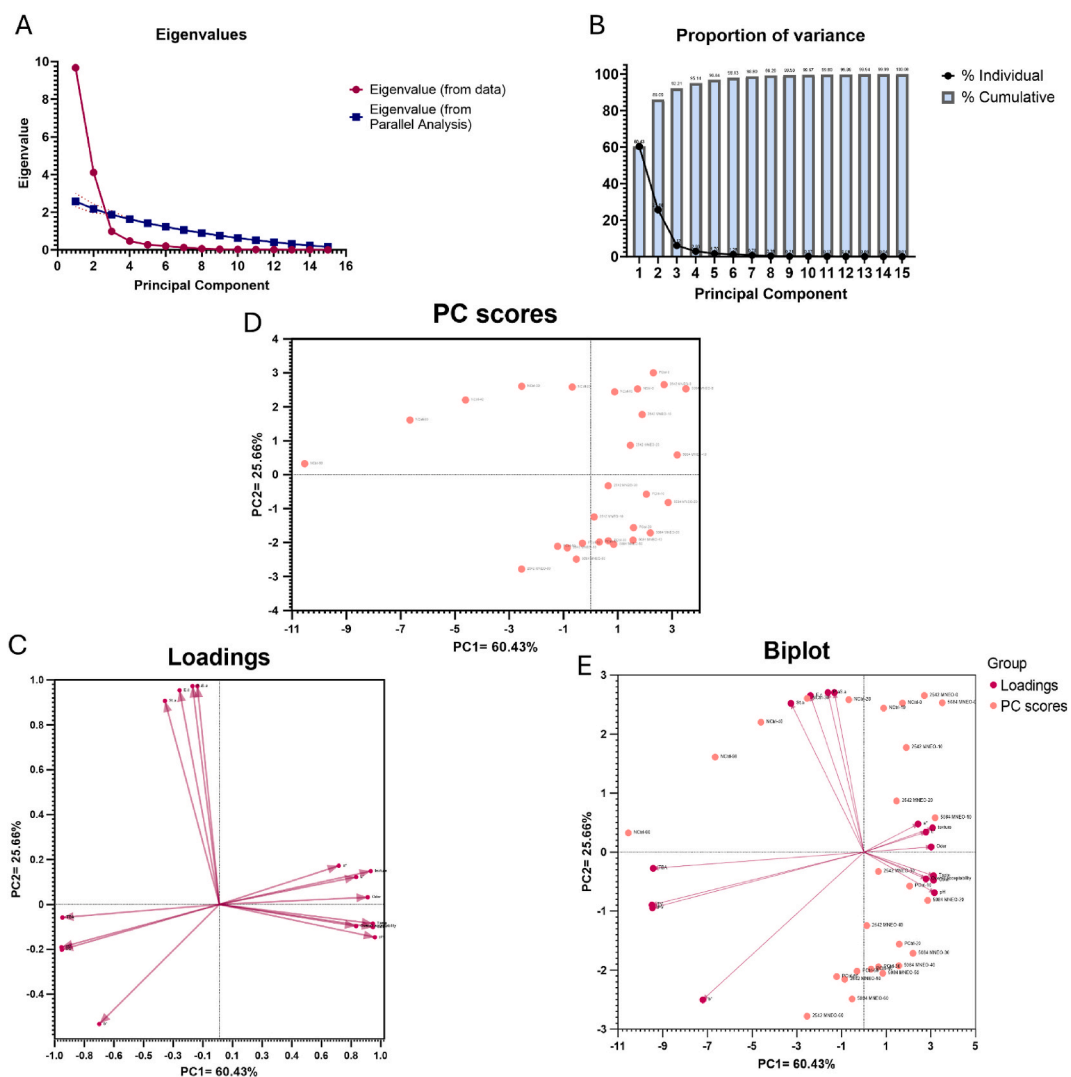


Fig. 4. Principal component analysis of investigated variables in mayonnaise samples. (A) Scree plot (based variable eigenvalues), (B) Proportion of variance, (C) Loading plot, (D) PC scores plot, and (E) Biplot. St.a, *S. aureus*; E.c, *E. coli*; P.a, *P. aeruginosa*; S.a, *S. enteritidis*; TBA, Thiobarbituric acid; PV, Peroxide value; TV, TOTOX value.

Table 4

Principal component analysis (PCA) of investigated variables in mayonnaise samples under various treatments.

Variable	PC1	PC2	Eigenvectors (PC1)	Eigenvectors (PC2)	Contribution of variables (PC1)	Contribution of variables (PC2)
<i>S. aureus</i>	-0.331	0.907	-0.106	0.448	0.011	0.201
<i>E. coli</i>	-0.242	0.954	-0.078	0.471	0.006	0.222
<i>P. aeruginosa</i>	-0.163	0.972	-0.052	0.480	0.003	0.230
<i>S. enteritidis</i>	-0.134	0.972	-0.043	0.480	0.002	0.230
pH	0.944	-0.147	-0.303	-0.072	0.092	0.005
TBA	-0.953	-0.059	-0.307	-0.029	0.094	0.001
PV	-0.957	-0.202	-0.308	-0.100	0.095	0.010
TV	-0.959	-0.191	-0.308	-0.094	0.095	0.009
L*	0.830	0.122	0.267	0.060	0.071	0.004
a*	0.724	0.172	0.233	0.085	0.054	0.007
b*	-0.729	-0.533	-0.235	-0.263	0.055	0.069
Odor	0.900	0.032	0.289	0.016	0.084	0.004
Color	0.932	-0.101	0.300	-0.050	0.090	0.003
Taste	0.931	-0.085	0.300	-0.042	0.090	0.002
Texture	0.918	0.148	0.295	0.073	0.087	0.005
Overall acceptability	0.829	-0.097	0.267	-0.047	0.071	0.002
Eigenvalue	9.668	4.106	-	-	-	-
Proportion of variance (%)	60.43	25.66	-	-	-	-
Cumulative proportion of variance (%)	60.43	86.09 %	-	-	-	-

suppressor effect versus pathogenic bacteria, effectively reducing or preventing the generation of oxidation mediators, as well as promoting the color (L*, a*) and sensorial (odor, color, taste, texture, and overall acceptability) characteristics of mayonnaise specimens at 0–50 days of storage (Fig. 4E).

Fig. 5 illustrates the correlation coefficients among the microbial, chemical, and sensory features of the mayonnaise specimens. Most of the connections demonstrated a positive correlation and were statistically meaningful ($P < 0.05$). The plot indicates a strong and positive correlation, along with a synergistic effect, between the development trends of the bacterial agents examined ($P < 0.05$). The increase in microbial populations resulted in a decline in the sensorial features, attributed to the negative and statistically significant relationship ($P < 0.05$) identified between these variables. The increase in microbial counts resulted in a reduction of pH level, L* index, and color scores, highlighting the adverse relationship between these factors. In a similar manner, the overall acceptability decreased in relation to the increase in microbial scores. The hue of the mayonnaise samples emerged as the primary sensory attribute influencing their overall acceptability. A positive and statistically significant relationship ($P < 0.05$) was identified between color and overall acceptability.

4. Conclusion

The bio-preservation agent *Malva neglecta* essential oil (MNEO) effectively prolongs the freshness of mayonnaise samples by inhibiting chemical and microbiological alterations. The preservation of the mayonnaise sample's quality was enhanced by increasing the concentration of MNEO. Our findings indicate that utilizing 5084 ppm MNEO provided the most effective protection against chemical alterations, inhibition of microbiological growth, and preservation of sensory attributes throughout refrigerated storage. Thus, employing MNEO at 5084 ppm is recommended to enhance mayonnaise shelf life, meeting consumer demand for natural and

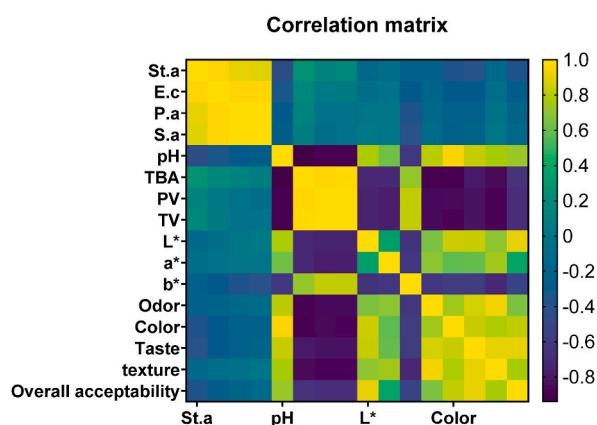


Fig. 5. Heatmap visualization of the correlation matrix of microbial, chemical, and sensory characteristics of the mayonnaise samples.

safe food preservatives.

Ethics statement

The Ethics Committee of Shahid Beheshti University of Medical Sciences approved this study (Number: IR.SBMU.RETECH.REC.1402.770). All ethical considerations have been observed during this research. All participants gave informed written consent, voluntarily participated in this study, and received no compensation.

CRedit authorship contribution statement

Amin Abbasi: Writing – review & editing, Visualization, Validation, Methodology, Formal analysis, Data curation, Conceptualization. **Hedayat Hosseini:** Writing – review & editing, Writing – original draft, Data curation. **Hadi Pourjafar:** Writing – original draft, Validation, Methodology, Formal analysis. **Samaneh Moradi:** Writing – original draft, Validation, Methodology, Data curation. **Saeedeh Shojaee-Aliabadi:** Writing – review & editing, Writing – original draft, Resources, Project administration, Funding acquisition.

Data availability

Data will be made available on request.

Declaration of competing interest

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

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