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Isolation and identification of the active compounds found in aromatic oils extracted from *Mentha longifolia* and their use in the extension shelf life of frozen beef patties

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ABSTRACT

Background: Wild mint is a perennial herb from the mint family, native to Europe. It is considered beneficial to the human body, as it has long been used to treat many diseases and problems that affect humans. For thousands of years, medicinal herbs and plants have been used to treat various types of diseases and to improve health care for individuals.

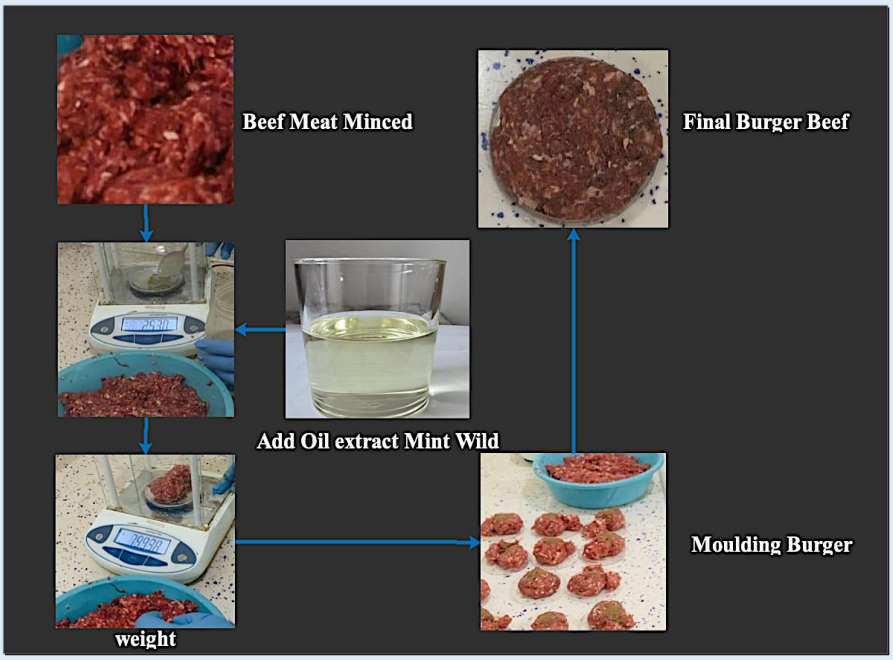
Objective: The study aimed to demonstrate the effect of using Mentha longifolia, as it is a substance rich in essential fatty acids, tocopherols, and flavonoids, as effective natural antioxidants and antimicrobials, and its inclusion in food manufacturing, such as the manufacture of meat products such as burgers.

Materials and Methods: Our main goal was to use Mentha longifolia, extracted from the Stachys plant using a Clevenger apparatus, and a standardized burger mix was prepared from it. The mixture was divided into four treatments, with Mentha longifolia (T2, T3, and T4) added at 1, 2, and 3% oil, respectively, in addition to the control treatment, T1, without any addition. Microbial tests were conducted on burger patty samples for all treatments before freezing. The treatments were stored at -18°C for 60 days, and tests were performed twice a month.

Results: The results of estimating fatty acids using GC-MS technology confirmed that Mentha longifolia contained reasonable amounts of some essential fatty acids. Examining flavonoid content using HPLC showed that Mentha longifolia contains 38 compounds, the most important of which are Caffeic Acid, 4-Hydroxy, Quercetin, and Apigenin. The logarithm of the total numbers of aerobic bacteria, refrigeration bacteria, and staphylococci reached 5.56-5.69, 1.47-

1.60, and 4.39-4.44 / g, freezing for 60 days led to a decrease in these values, and the severity of the decline was directly proportional to the percentage of adding Mentha longifolia. Treatments T3 and T4 recorded the lowest values compared to the control treatment. Statistical analysis revealed that the results were not significant at P ≤ 0.05.

Keywords: burgers, Wild mint, Mentha longifolia, Antimicrobials from plant sources, Storing meat in the freezer, antioxidant.



INTRODUCTION

Wild mint is a perennial herb from the mint family, native to Europe. It is considered beneficial to the human body, as it has long been used to treat many diseases and problems that affect humans [1]. For thousands of years, medicinal herbs and plants have been used to treat various types of diseases and to improve health care for individuals [2]. Many studies have proven that Mentha longifolia have multiple benefits, especially in medicine, industry, and cosmetics [1,2]. The wild mint plant grows naturally in the eastern Mediterranean region, including Palestine. It has a pleasant minty scent has been used in folk medicine to treat many diseases (4). Chemical treatments have been used to treat many diseases. Still, their harmful side effects and the emergence of resistant bacterial strains, in addition to their economic cost and other factors, have led to medicinal plants being placed again before the eyes of scientists and researchers [1]. Medicinal plants have antibacterial, antiviral, antifungal, and insecticidal properties due to the essential oils produced by the secondary metabolism of plants. [2] Essential oils are stachys substances found in flowers and plant leaves. They are responsible for the aromatic smell in these plants, which is essential nutritionally, industrially, and commercially, distinguishing them from each other. They are responsible for the aromatic smell in these plants, which is essential nutritionally, industrially, and commercially, distinguishing them from each other. They are used as spices, flavorings, and food flavorings in various foods and drinks and are considered

natural active substances, so they are used in the pharmaceutical industry [3,19,20,21. The Mentha longifolia plant has been used since ancient times to add flavor to dairy products, in addition to being a herbal medicine for treating alopecia, skin infections, bronchitis, coughs, inflammatory skin diseases, and digestive system disorders. It is also an antiseptic, anti-gas, antimicrobial, and antioxidant [4,5]. Meat and meat products are an essential source of human nutrition due to their high nutritional value in providing the body with a good combination of essential amino acids necessary for growth, in addition to fats, carbohydrates, vitamins, and salts. They encourage microbial growth, which makes them perishable food. For this reason, humans have tried to find ways to preserve them. Meat should be kept for the most extended period to be suitable for human consumption [6]. Mentha longifolia is characterized by its good content of fatty acids that reduce the negative impact of processed meat and play an essential role in improving the quality of meat products.

Rancidity and spoilage of fats are among the most critical problems facing the food industry. The safety and quality of foods, mainly processed foods and specific and permitted food additives, have raised several questions [2]. Poor manufacturing and storage conditions and the thawing process are among the factors that increase the number of microorganisms in meat and meat products [5,7,17,19]. The concern of producers and consumers lies in preserving the quality of meat products by limiting chemical and microbial changes, thus keeping their nutritional value and extending their shelf life. Meat and meat products can be preserved by adding artificial preservatives that delay or prevent qualitative changes and extend the storage period. The storage period of meat can also be extended by controlling several factors, including temperature and humidity during cold storage, or by using ionizing radiation, antibiotics, and chemical or natural preservatives to inhibit the growth of microorganisms [8,16]. The use of artificial preservatives in foods, such as BHA (Butylated hydroxyanisole) and (BHT) (Butylated hydroxytoluene), and antimicrobials, such as nitrite and benzoic acid, may pose a potential risk due to their toxicity. Therefore, much research has been directed toward using natural antioxidants and antimicrobials, such as plants and their extracts, by adding them directly to foods [9,13,14,15].

The development of the qualitative and sensory characteristics of processed meat products and the inhibition of microbial spoilage is achieved by using natural antimicrobial substances to preserve meat quality, prevent and reduce microbial spoilage, and inhibit the activity of enzymes that play a role in the life processes of bacterial cells. There has been a trend towards using natural medicinal herbs, an essential source for obtaining active compounds used as natural food additives instead of industrial chemical additives to prevent food spoilage. Cinnamon extract [6], galbanum powder [7], watercress, sea dew [8], and ginger solutions [3] have been added to meat and meat products because they contain active natural substances that improve meat qualities and extend their storage period.

Mentha longifoliais a by-product of the Stachys plant (wild mint). It is a source of antimicrobial agents that prevent microbial spoilage of frozen meat products and extend their shelf life, as it contains phenolic compounds responsible for their aromatic properties and distinctive pungent taste [10,11,12].

METHODS AND MATERIALS

Sample preparation: The beef was purchased from butcher shops in Ramadi city. The meat was transported to the laboratory in a refrigerated cork box, which was minced twice for homogeneity. A chemical analysis of the beef used in the study was conducted. The burger mix was manufactured according to the Iraqi specification (2000). This mix consists of the materials shown in Table 1.

Table 1. Standard burger mix.

Sample	Percentage %
3	Mentha longifolia
80	Meat
10	Fat (belly fat)
3	Filler (flour, breadcrumbs)
2	Spices
1 - 1.5	Salt
0.5	Ground garlic and onion

Table 2. Experimental treatments for the standard mix with and without spices.

T1	Standard mix without adding Mentha longifolia
T2	Standard mixture with 1% added Mentha longifolia
T3	Standard mixture with 2% added Mentha longifolia
T4	Standard mixture with 3% added Mentha longifolia

Mentha longifolia was added to every 100 grams of burger mix in three treatments, in addition to the control treatment, as shown in Table 2.

Bacteriological tests were conducted on the transactions, and then they were packed in polyethylene bags, each individually, and sealed well using a heat sealing method. The information was written on them, and they were stored in the freezer at a temperature of -18°C to conduct tests after freezing them for (60) days.

Extracting Mentha longifolia: The Mentha longifolia was obtained by drying the stachys from farms located in the Al-Buaitha area of Anbar Governorate, grinding it in an electric grinder to a fine powder, and sterilizing it in an electric oven at 105°C for 5 minutes to inhibit enzyme activity. The powder was stored in a sterile, tightly sealed glass container until ready to use.

Estimation of phenolic compounds: The plant extract was treated with charcoal to remove pigments, then filtered using a Buechner funnel, concentrated under vacuum using a rotary evaporator and dissolved in 1 ml of HPLC-grade methanol. The mixture was filtered through a 2.5 µm filter and stored at 4°C until analysis. The quality and quantity of some flavonoid compounds were characterized by HPLC using a Phenomenex c-18 column (50x2.0mm I.D., particle size 3 µm). The mobile phase was a linear gradient of solution (A) 0.1% formic acid and solution (B) acetonitrile: methanol: 0.1% formic acid (v/v/v) (1:3:6). Separation was carried out using a linear gradient elution mode from (0-100% solution B) over 10 minutes, and detection was carried out using a UV detector at a wavelength of 290 nm. The flow rate was 1.5 ml/min [18,27,23].

Identification of active compounds in essential oils under study: The active compounds in the essential oils under study were identified using a gas chromatography device connected to a mass spectrometer, type JAPAN, SHIMADZU, Ultra QP2010 MS-GC. A 50 µm film capillary column was used for separation. The initial oven temperature was 40°C, and the final temperature was 280°C. To maintain the program temperature, it was held

for 1 minute at 120°C, and the temperature increased at 8°C/min until it reached 210°C. Then, it was held for 45 minutes at 210°C. The sample volume required for injection was one microliter, the injection zone temperature was 280°C, and the detector temperature was 280°C. The carrier gas was helium at a constant pressure of 1.96 kPa. The flow rate of the carrier gas in the column was 71.1 ml/min—stoffel minute (18).

Bacteriological tests for burger products

Serial dilution: The method given in A.O.A.C (2005) was followed for bacteriological tests. 25g of beef burger patties were weighed and mixed with 225ml of Nutrient Broth medium using an electric mixer for 2 minutes at 230 rpm. This first dilution was calculated as 10-1. The remaining decimal dilutions were completed using P.W. Peptone Water (P.W) dilution solution. 9ml of P.W. was placed in each tube, and 1ml of the first dilution was added, making the dilution -210 and so on up to -510. These dilutions were used for bacteriological determination. The method given in APHA (1992) was followed for all bacteriological tests.

Total plate count: Plate Count Agar was used as a culture medium. 1 ml of each dilution prepared in 3-5-1 was transferred to each Petri dish using a sterile pipette. The medium was then cooled to 45°C and poured onto the plates. The plates were gently swirled to homogenize and distribute evenly and then allowed to solidify. The plates were inverted and incubated at 37°C for 24 hours. The number of bacterial colonies growing on the plates was counted.

Coliform bacteria: MacConkey agar was used to estimate the number of coliform bacteria. The medium was poured into plates and allowed to solidify. 0.1 ml of the dilution was applied and spread evenly over the surface. Another layer of medium was poured over the dilution to provide anaerobic conditions. The plates were allowed to solidify, then inverted and incubated at 37°C for 24 hours. Colonies growing on the medium were counted to estimate the number of coliform bacteria.

Staphylococcus bacteria: Manitol Salt Agar was used to estimate the number of Staphylococcus bacteria. The medium was poured into plates and allowed to solidify. 0.1 ml of the appropriate dilution was added and spread well. The plates were inverted and incubated at 37°C for 48 hours. The number of growing colonies was then counted.

Psychrophilic bacteria: Nutrient agar was used by pouring it into plates, allowing it to solidify, then adding 0.1 ml of the appropriate dilution and spreading it evenly over the surface of the agar. The plates were inverted and incubated at 5°C for 5-7 days, and the colonies growing on the medium were counted.

Statistical Analysis: The Statistical Packages of Social Sciences -SPSS (2019) program was used to detect the effect of difference Treatments and Period in study parameters. Least significant difference-LSD was used to significant compare between means in this study [31].

RESULTS AND DISCUSSION

Table 3 and Figure 1 show the total phenolic compounds present in the Mentha longifolia, which were detected by high-performance liquid chromatography (HPLC). The identification revealed the presence of 38 compounds, with the highest percentage of isolated compounds being Caffeic Acid, 4-Hydroxy, Quercetin, and Apigenin, with an area of 230.154, 174.521, 181.058, 4862.397). The studied phenolic compounds showed a positive effect on microorganisms. The mitochondrial respiratory chain is a significant source of S.O.R. species in most eukaryotic cells, and the balance between antimicrobials and S.O.R. is essential for cellular activities. Flavonoids are weak acids with antimicrobial properties [6]. Reducing respiration rate is one of the crucial antimicrobial mechanisms of flavonoids. Some flavonoids, such as Quercetin and dihydromycetin, at high concentrations can reduce the rate of mitochondrial respiration and ATP synthesis in fungal cells, leading to cell death [29,24,28]. The effectiveness of phenolic compounds is attributed to the presence of the hydroxyl group (OH) in the polar

phenolic aromatic ring, as it has one or more groups (depending on the type of compound) that can bind and interact via hydrogen bonds with the active groups of coenzymes in the organism's body [25]. Phenolic compounds precipitate proteins due to their ability to form hydrogen bonds between phenolic hydroxyl groups and body proteins, thus disrupting the work of enzymes essential to the organism's body [26]. Phenolic compounds with antimicrobial activity are phenolic compounds and flavonoids [22,30]. Some researchers have suggested that the antimicrobial activity of phenolic compounds is expressed as a result of synergy between different types of phenolic compounds and cannot be explicitly attributed to a single component

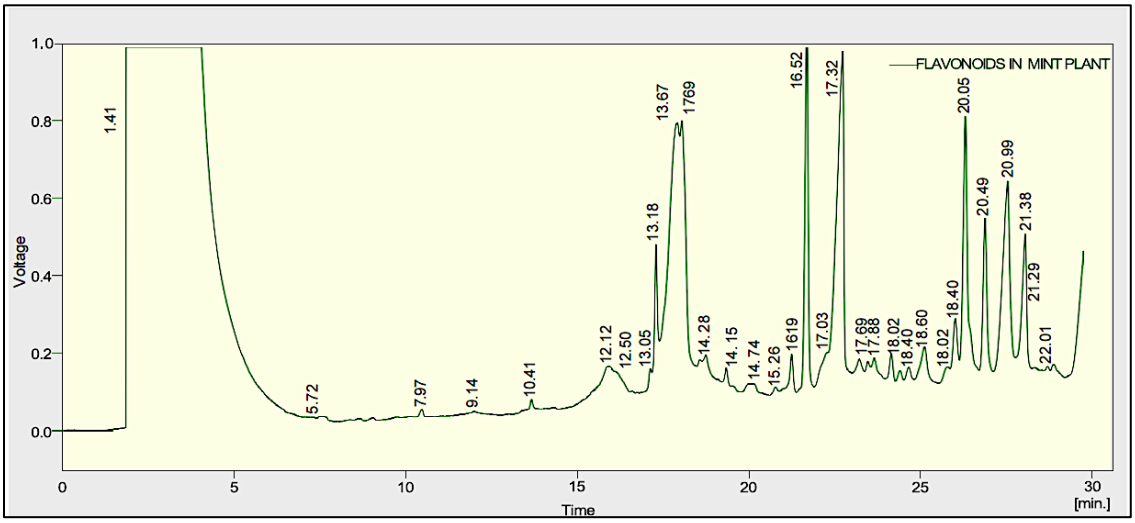


Figure 1. HPLC chromatography for separating and identifying total phenols in Mentha longifolia.

Table 3. Shows the HPLC chromatography for separating and identifying total phenols in Mentha longifolia.

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W 05 [min]	Compound Name
1	0.180	18.627	1.246	0.0	0.0	0.27	-
2	1.407	126505.711	985.361	0.0	11.5	1.99	-
3	5.720	98.122	7.077	0.0	0.1	0.21	-
4	7.973	230.154	19.900	0.0	0.2	0.09	CAFFEIC ACID
5	9.140	174.521	6.988	0.0	0.1	0.15	4-HYDROXY
6	10.410	181.058	23.884	0.0	0.3	0.06	QUERCETIN
7	12.123	3255.040	93.387	0.0	1.1	0.56	-
8	12.500	416.353	35.884	0.0	0.4	0.21	-
9	13.050	449.336	71.500	0.0	0.8	0.08	-
10	13.177	1979.471	389.178	0.0	4.5	0.08	-
11	13.657	11256.844	696.091	0.0	8.1	0.25	-
12	13.750	7100.636	700.765	0.0	8.1	0.16	-
13	14.150	551.905	76.929	0.0	0.9	0.14	-
14	14.283	1224.552	86.910	0.0	1.0	0.21	-
15	14.740	223.796	45.330	0.0	0.5	0.07	-
16	15.263	419.837	24.785	0.0	0.3	0.26	-
17	15.833	137.452	20.646	0.0	0.2	0.11	-
18	16.187	704.840	102.115	0.0	1.2	0.08	-
19	16.517	4862.397	893.222	0.0	10.4	0.08	APIGENIN
20	17.030	1390.769	109.733	0.0	1.3	0.23	-
21	17.323	10279.699	875.805	0.0	10.2	0.18	-
22	17.693	920.114	79.672	0.0	0.9	0.25	-

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W 05 [min]	Compound Name
23	17.883	633.541	72.499	0.0	0.8	0.18	-
24	18.023	843.671	79.605	0.0	0.9	0.17	-
25	18.403	644.027	86.885	0.0	1.0	0.10	-
26	18.597	330.549	43.513	0.0	0.5	0.13	-
27	18.793	460.485	50.888	0.0	0.6	0.14	-
28	19.140	1231.416	101.161	0.0	1.2	0.17	-
29	19.823	1709.015	169.263	0.0	2.0	0.12	-
30	19.963	297.241	147.041	0.0	1.7	0.03	-
31	20.050	3869.588	689.480	0.0	8.0	0.10	-
32	20.120	1167.111	165.558	0.0	1.9	0.10	-
33	20.487	2829.073	423.523	0.0	4.9	0.10	-
34	20.990	6006.143	514.794	0.0	6.0	0.18	-
35	21.297	1105.514	182.752	0.0	2.1	0.06	-
36	21.377	2079.467	376.422	0.0	4.4	0.11	-
37	21.427	496.769	119.374	0.0	1.4	0.03	-
38	22.013	815.736	35.961	0.0	0.4	0.48	-
Total	590.492	196,900.58	8,605.127	0.0	99.9	7.92	-

Estimation of fatty acids in Mentha longifolia: The results in Figure 2 show the chemical compounds separated by MS-GC for Mentha longifolia, which numbered 30. From these results, it is clear that the compound Methanoazulene was present in the highest concentration in the essential oil, reaching 15.3%, which is close to the percentage obtained by (20, 21, 22), which was 15.5%, and higher than the percentage obtained by (13.76%). We also note that the percentages of each of Caryophyllene oxide, 6-Cyano-5-methoxyquinoline, and 4-Chloro-2,3-Dimethyl-1,3-hexadiene were 89.14%, 14.58%, and 13.14%, respectively, which is close to what was obtained by (10), which was 14.44%, 13.65%, and 12.26%, respectively.

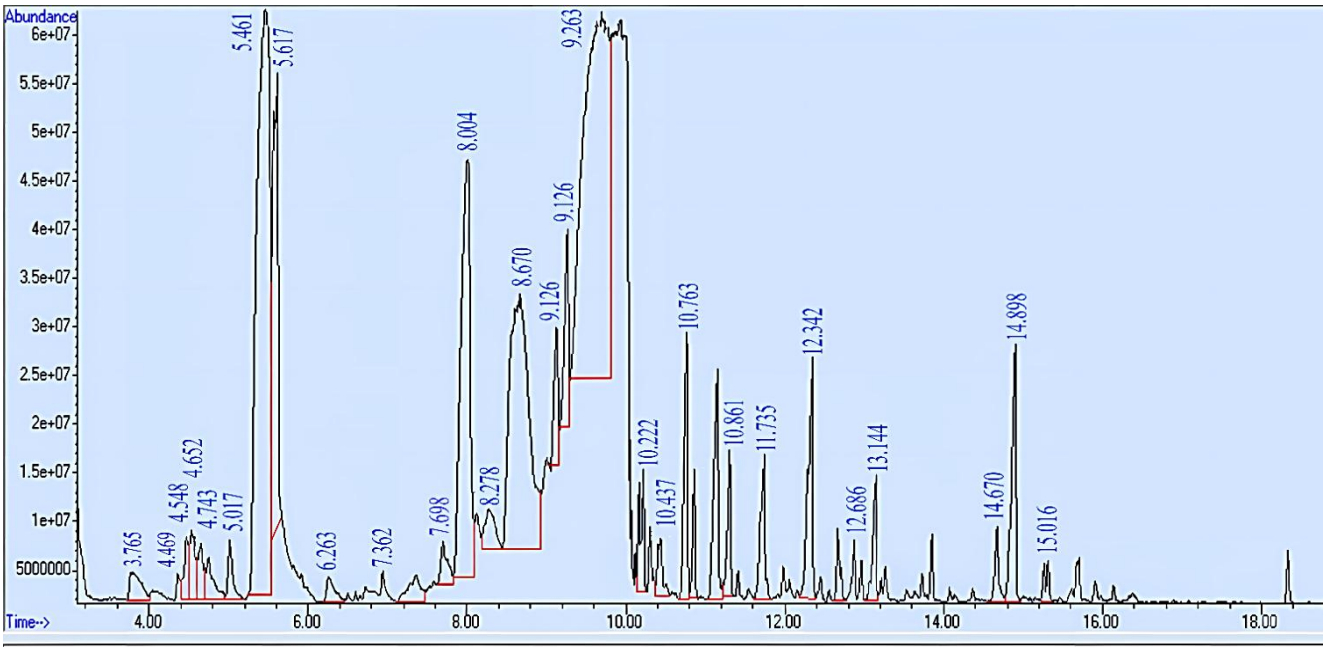


Figure 2. Shows the names of the chemical compounds separated by MS-GC technology and their proportions in Mentha longifolia.

Bacteriological Tests for Burger Processes

Total Aerobic Bacteria Counts: Table 4 shows the logarithm of the total aerobic bacteria counted when starchy oil was added to beef burgers at ratios of (0, 1, 2, and 3) %. Before freezing, the logarithm of the total aerobic bacteria counts reached (5.69, 5.60, 5.57, and 5.56) / g. After 15 days, the logarithm of the total aerobic bacteria counts was (5.66, 5.58, 5.56, and 5.50) / g, respectively. The logarithm of the total aerobic bacteria counts continued to decline after 30 days, reaching (5.63, 5.57, 5.54, and 5.42) / g, respectively. The logarithm values of the total aerobic bacterial counts decreased for all treatments after 45 days of freezing to reach (5.62, 5.55, 5.05, and 4.65) / g, respectively. After freezing for 60 days, the logarithm values of the total aerobic bacterial counts were (5.61, 5.53, 4.74, and 4.24) / g when adding (0, 1, 2, and 3) % of Mentha longifolia, respectively. These results were similar to those of several researchers using natural antimicrobial agents (11, 18). (16, 2) indicated a decrease in aerobic bacteria from 5.80/g of meat to 1.7/g of meat when adding natural antioxidants to ground beef at a concentration of 1g/kg of meat upon freezing. The apparent decrease in bacterial counts before freezing, which is directly proportional to the increase in the percentage of Mentha longifolia added to the processed burger treatments, indicates the inhibitory role of Mentha longifolia on the growth of aerobic microorganisms due to the presence of a high percentage of phenolic compounds that inhibit the growth of aerobic bacteria. This is consistent with what was found by (1, 13, 2, 14). The total aerobic bacterial counts were close to the acceptable limits set by the Iraqi specification (2000) and the American specification (1992) APHA, which stipulated that the total aerobic bacterial counts in meat should not exceed the established specification of 1 x 10^2 CFU/gm of beef.

Table 4. The effect of adding Mentha longifolia to beef burgers on the total number of aerobic organisms.

Treatment (CFU/gm)	Freezing Period (days)					
	1	15	30	45	60	L. S. D.
T1	5.69	5.66	5.63	5.62	5.61	0.372 NS
T2	5.60	5.58	5.57	5.55	5.53	0.297 NS
T3	5.57	5.56	5.23	5.05	4.74	0.588 *
T4	5.56	5.50	5.21	4.65	4.24	0.602 *
L. S. D.	0.287 NS	0.205 NS	0.397 *	0.711 *	0.684 *	--
* (P≤0.05) , NS: Non-Significant.						

Coliform bacteria Counts: Table 5 shows the coliform counts in burger treatments containing added Mentha longifolia. Bacterial counts gradually decreased with increasing levels of Mentha longifolia added to the treatments before freezing, from 2.43×10^3 CFU/gm of meat in the control treatment (T1) to 0.78×10^3 CFU/gm of meat in treatment (T4). The bacterial counts in the remaining treatments were between these two values. This apparent decrease in coliform counts between the control treatment and treatment (T4) indicates that Mentha longifolia has an inhibitory effect against the growth of coliform bacteria in processed burgers. It is noted that treatments (T1-T3) had bacterial counts higher than the permissible limits specified in the American Standard for Food Safety (APHA) (1992), which determined that the total number of coliform bacteria for acceptable meat quality should not exceed (1×10^3) CFU/gm of meat. In the remaining treatments, bacterial counts were below the permissible limits.

These results were somewhat consistent with (2), which indicated a decrease in the number of coliform bacteria in minced beef treated with Mentha longifolia

(1g/100g) from 3×10^4 CFU/gm of meat to 2×10^3 CFU/gm of beef. After freezing the treatments for 15 days, the increase in coliform bacteria counts was slight in the control treatment, reaching 2.41×10^3 CFU/gm of meat. However, the bacterial counts in the remaining treatments decreased compared to the pre-freezing period, reaching 2.04×10^3 CFU/gm of meat in treatment T4. However, the bacterial counts in treatment T4 remained within acceptable limits. This indicates the continued inhibitory effect of Mentha longifolia during freezing.

Table 5. Estimation of the number of coliform bacteria in the treatment of the burger with added Mentha longifolia.

Treatment (CFU/gm)	Freezing Period (days)					
	0	15	30	45	60	L. S. D.
T1	2.43	2.41	2.40	2.39	2.38	0.194 NS
T2	2.39	2.24	2.12	2.06	1.88	0.375 *
T3	2.38	2.13	2.05	1.85	1.34	0.522 *
T4	2.37	2.04	1.57	1.13	0.78	0.629 *
L. S. D.	0.283 NS	0.361 *	0.528 *	0.673 *	0.626 *	--
* (P≤0.05) , NS: Non-Significant.						

These results differ from those reported by (3), who indicated an increase in the number of coliform bacteria in frozen minced beef treated with Mentha longifolia (1g/100g) from 2×10^3 CFU/gm of meat before freezing to 2.55×10^3 CFU/gm of meat after freezing for 60 days. An increase in coliform bacteria was also observed after 60 days of freezing, reaching a maximum value of 2.38×10^3 CFU/gm of meat in the control treatment (T1). Meanwhile, the increase in bacterial counts in the remaining treatments was slight and inversely proportional to the percentage of added Mentha longifolia. The lowest value was 0.78×10^3 CFU/gm of meat in treatment (T4), while bacterial counts in treatments (T3-T4) remained within acceptable limits.

Psychrophilic bacteria counts: Table 6 shows the logarithm of the live counts of psychrophilic bacteria when Mentha longifolia was added to beef burgers at ratios of (0, 1, 2, and 3) g. The logarithm of the live counts of psychrophilic bacteria reached (1.60, 1.60, 1.47, and 1.47) / g, respectively, before freezing. After 15 days, the logarithm values of the live counts of psychrophilic bacteria were (1.57, 1.57, 1.46, and 1.43) / g, respectively. The logarithm values of the live counts of psychrophilic bacteria continued to decrease after 30 days, reaching (1.57, 1.54, 1.41, and 1.37) / g, respectively. The logarithm values of live cryobiotic bacteria decreased for all treatments after 45 days of freezing to reach (1.56, 1.54, 1.39, and 1.27)/g, respectively. Upon freezing for 60 days, the logarithm values of live cryobiotic bacteria were (1.54, 1.51, 1.32, and 1.17)/g at 0, 1, 2, and 3% of Mentha longifolia, respectively. The apparent decrease in bacterial counts before freezing, which is directly proportional to the increased percentage of Mentha longifolia added to the processed burger treatments, indicates the inhibitory role of Mentha longifolia on the growth of cryobiotic microorganisms due to the high rate of flavonoid compounds that inhibit the growth of aerobic bacteria. This is consistent with the findings of (1, 25, 26, and 27). The number of cold-loving bacteria was close to the acceptable limits specified by the Iraqi standard (2000) and the American standard (APHA) (1992), which specified that the number of cold-loving bacteria in meat should not exceed 1.8 x 101 mg/g of meat (30).

Table 6. Effect of adding Mentha longifolia to beef burgers on the level of cold-loving organisms.

Treatment (CFU/gm)	Freezing Period (days)					
	0	15	30	45	60	L. S. D.
T1	1.6	1.57	1.57	1.56	1.54	0.188 NS
T2	1.6	1.57	1.55	1.54	1.51	0.174 NS
T3	1.47	1.46	1.43	1.39	1.32	0.202 NS
T4	1.47	1.43	1.37	1.27	1.17	0.315 *
L. S. D.	0.217 NS	0.182 NS	0.215 NS	0.285 *	0.325 *	--
* (P≤0.05) , NS: Non-Significant.						

Staphylococcus counts: Table 7 shows the logarithm of the live counts of Staphylococcus bacteria when Mentha longifolia was added to beef burgers at ratios of (0, 1, 2, and 3) %. The logarithm of the live counts was (4.43, 4.39, 4.38, and 4.37)/g, respectively, in all treatments manufactured before freezing. After 15 days of freezing, the logarithm of the live counts was (4.41, 4.36, 4.23, and 4.12)/g, respectively. The logarithm values of the live counts of staphylococci continued to decline after 30 days, reaching 4.40, 4.24, 3.84, and 3.47/g, respectively. The logarithm values of the live counts of staphylococci for all treatments decreased after 45 days of freezing to 4.39, 4.16, 3.46, and 3.14/g, respectively. Upon freezing for 60 days, the logarithm values of the live counts of staphylococci were 4.38, 4.07, 3.13, and 2.67/g, respectively. This apparent decrease in the live numbers of staphylococci after freezing is inversely proportional to the increase in the percentage of Mentha longifolia added to the manufactured burger treatments, and this decrease is clear evidence that Mentha longifolia contains flavonoid compounds that have a high inhibitory effect against the growth of staphylococci in frozen-stored burgers, thus extending the storage period. This is what was found by (15, 2, 20). The number of staphylococci was close to the acceptable limits set by the Iraqi specification (2000) and the American specification APHA (1992), which specified that the number of staphylococci should not exceed 2×10^2 CFU/gm of meat in frozen meat (30).

Table 7. Effect of adding Mentha longifolia to beef burgers on the level of Staphylococcus.

Treatment (CFU/gm)	Freezing Period (days)					
	0	15	30	45	60	L. S. D.
T1	4.43	4.41	4.40	4.39	4.38	0.161 NS
T2	4.39	4.36	4.24	4.16	4.07	0.348 NS
T3	4.38	4.24	3.84	3.46	3.13	0.522 *
T4	4.37	4.12	3.47	3.14	2.67	0.610 *
L. S. D.	0.143 NS	0.387 NS	0.594 *	0.517 *	0.692 *	--
* (P≤0.05), NS: Non-Significant.						

Recommendations: Study using Mentha longifolia as a natural preservative to replace synthetic additives in the preservation of frozen beef, buffalo, poultry, and fish by adding it directly to these meats. Study the use of Mentha longifolia to inhibit the growth of microorganisms and hinder their production of toxins in food products and fresh and processed meats.

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