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Antioxidant activity of *Mentha longifolia* aromatic oils and their application in extending beef burger shelf life

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ABSTRACT

Background: Lipid oxidation is a major factor affecting the chemical stability and shelf life of processed meat products during frozen storage. Natural plant oils rich in bioactive compounds have gained attention as effective alternatives to *Mentha longifolia* antioxidants. These compounds can reduce oxidative deterioration and improve product quality.

Objective: This study aimed to evaluate the effect of different concentrations of ***Mentha longifolia*** oil on the chemical properties and oxidative stability of processed burger patties during frozen storage.

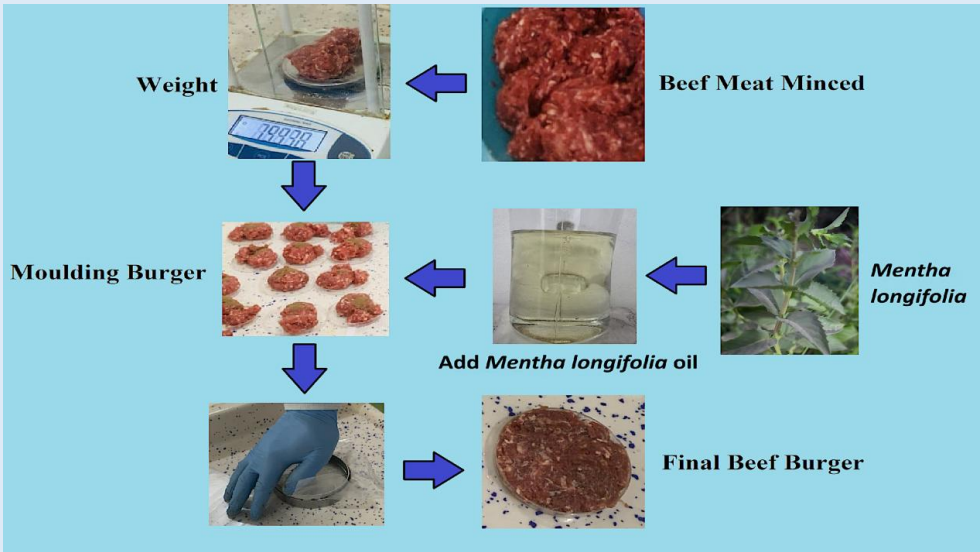
Materials and Methods: Crude *Mentha longifolia* oil was extracted using a Clevenger apparatus. Burger samples were divided into four treatments: control (T1) without oil addition, and T2, T3, and T4, each supplemented with 1, 2, and 3 g of *Mentha longifolia* oil, respectively. Chemical analyses were conducted before freezing and during 60 days of frozen storage at −18 °C, with testing performed twice per month. Fatty acid composition and flavonoid profiles were determined using GC–MS and HPLC, while oxidative stability was evaluated by measuring free fatty acids (FFA), peroxide value (PV), and thiobarbituric acid (TBA).

Results: *Mentha longifolia* oil exhibited a high content of essential fatty acids and 38 identified flavonoid compounds, dominated by caffeic acid, apigenin, quercetin, and 4-hydroxy derivatives. No significant differences ($P \geq 0.05$) were

observed among treatments before freezing. During frozen storage, oxidative indices increased in all samples; however, oil-treated burgers showed significantly lower FFA, PV, and TBA values, with treatments T3 and T4 recording the lowest values compared to the control.

Conclusion: The results show that Mentha longifolia oil improved the oxidative stability of burger patties during frozen storage. Patties containing 2 or 3 g of the oil showed markedly lower FFA, PV, and TBA values, with performance comparable to the control, suggesting reduced lipid oxidation. These findings support the potential of Mentha longifolia oil as a natural antioxidant for extending the shelf life of meat products.

Keywords: antioxidant activity; natural antioxidants; flavonoids; *Mentha longifolia* aromatic oils; beef burger; shelf-life



Graphical Abstract: Antioxidant activity of *Mentha longifolia* aromatic oils and their application in extending beef burger shelf life

INTRODUCTION

Chemical therapies are commonly used in disease treatment. Nevertheless, concerns regarding adverse side impacts, the development of resistant bacterial strains, economic burdens, and other related factors have brought medicinal plants back to the forefront of scientific research [1]. The antimicrobial and insecticidal activities of medicinal plants are largely attributed to essential oils derived from secondary metabolism [2-3], which are volatile compounds found in leaves and flowers. They contribute to the characteristic aromatic profiles of plants, which hold significant nutritional, industrial, and commercial value and enable differentiation among plant species. They are used as flavourings in various foods, spices, and beverages. They are also considered natural active ingredients used in the pharmaceutical industry [4,5]. The *Mentha longifolia* is a plant belonging to the Lamiaceae family. It is an evergreen, fragrant shrub growing in several regions [6-7]. The *Mentha longifolia* plant has been used since ancient times to add flavor to cheeses and as an herbal medicine to treat alopecia, inflammatory skin diseases,

coughs, bronchitis, skin infections, and digestive system disorders. It is also antimicrobial, anti-gas, an antiseptic, and antioxidant [8-10]. Meat products constitute an important and essential component of the human diet, owing to their high nutritional value and their provision of essential amino acids necessary for growth, as well as vitamins, minerals, carbohydrates, and fats. It is a food that encourages microbial growth, making it perishable. The need to control microbial growth in meat has led humans to develop preservation methods that extend its usability for consumption.

The food industry faces significant issues due to the rancidity and spoilage of fats. Questions have emerged regarding the quality and safety of processed foods and regulated food ingredients. The production environment and storage conditions, along with thawing procedures, can lead to a higher level of microorganisms in meat products. Maintaining meat product quality requires producers and consumers to focus on minimizing microbial changes and chemical which affect shelf life and nutritional value. Artificial preservatives help extend meat storage duration by slowing down qualitative alterations. Meat storage duration increases when storage temperature and humidity in cold stores get regulated along with ionizing radiation, antibiotics and natural preservatives stop microorganisms from growing [17-18] or chemical. The artificial food preservatives BHA and BHT together with antimicrobials like nitrite and benzoic acid carry potential health hazards because of their toxic properties.

Research efforts have mainly focused on using antimicrobials, natural antioxidants, and their extracts through direct incorporation into foods and plants [19-18]. Food preservatives include meat product spices that use active ingredients to block bacterial and mold growth. Research shows that these ingredients possess antioxidant properties [20-22]. The preservation of processed meat products involves using natural antioxidants to enhance their sensory qualities and prevent microbial spoilage by protecting meat quality through oxidation inhibition and bacterial enzyme activity inhibition. Natural medicinal herbs are increasingly popular as natural food additives because they provide active compounds that replace industrial chemical additives to prevent food spoilage. Active ingredients of ginger solutions [25], sea dew [24], watercress, galbanum powder [23], and cinnamon extract [22] improve meat quality and prolong the shelf life when used in meat products.

Mentha longifolia oil is wild mint has Antioxidants derived from *Mentha longifolia* oil help prevent spontaneous oxidation of frozen meat production while extending their shelf life and inhibit microbial growth. *Mentha longifolia* oil contains phenolic compounds, contributing to its aromatic features and characteristic pungent taste [11]. *Mentha longifolia* oil is characterized by its high oil content, which reduces the adverse effects of excessive protein intake and improves the quality of meat products. This research aimed to demonstrate how the inclusion of *Mentha longifolia* oil which contains essential fatty acids, tocopherols and flavonoids used as natural antioxidants and antimicrobials affects the technological process of food manufacturing specifically in making meat products like burgers.

METHODS AND MATERIALS

Sample Preparation: We bought beef from butcher shops located in Ramadi for this research. Scientists transferred the meat to a refrigerated corrugated box before grinding it twice to create a homogeneous sample. Researchers performed chemical analysis on the beef samples used in this research. The burger mix preparation followed the 2000 Iraqi specification guidelines. The recipe for burger mix follows the ingredient list presented in Table 1.

Table 1. Standard burger mix.

Sample/ g	**Percentage %**
1 - 1.5	Salt
2	Spices (Cubeb pepper, Coriander, Paprika, Garlic, Ginger, Black pepper)
10	Fat (belly fat)
0.5	Ground garlic and onion
80	Meat
3	*Mentha longifolia* oil
3	Filler (flour, breadcrumbs)

Table 2 lists the control treatment together with three *Mentha longifolia* oil treatments where each 100 g of the burger mixture contained oil.

Table 2. Empirical treatments combined control mixture without adding spices.

T1	A standard mix without adding ***Mentha longifolia*** oil
T2	A standard mix without adding ***Mentha longifolia*** oil
T3	A standard mix containing 2% added ***Mentha longifolia*** oil
T4	A standard mix containing 3% added ***Mentha longifolia*** oil

Bacteriological analysis was performed on the transaction products before being individually sealed in polyethylene bags with a heat-sealing method and information written before being stored in a freezer at -18°C until testing was done after 60 days of freezing.

Extracting *Mentha longifolia* Oil: The *Mentha longifolia* oil was taken from Anbar Governorate, specifically in the Al-Buaitha area, dried, and then ground into a fine powder using an electric grinder. Then, the material was sterilized in an electric oven at 105 °C for 5 min to suppress enzymatic activity. The powder was stored under sterile conditions in tightly sealed glass containers until use.

Estimation of Phenolic Compounds: Charcoal-decolorized plant extract was filtered through a Buchner funnel, and the filtrate was concentrated by rotary evaporation under vacuum and then solubilized in 1 ml HPLC-grade methanol. They filtered the extract through a 2.5-micron filter before storing it at 4 degrees Celsius for future analysis. The characteristics of several flavonoid compounds were analyzed by HPLC using a Phenomenex C-18 column (50 x 2.0 mm I.D., 3 µm particle size). A linear gradient mobile phase was employed, consisting of solution B (acetonitrile: methanol:0.1% formic acid; 1:3:6, v/v/v) and A (0.1% formic acid). Elution was carried out from (0- 100) % solution B within 10 min. UV detection was set at 290 nm, and the flow rate was maintained at 1.5 mL/min [12].

Identification of Bioactive Compounds in Essential Oils: Profiling of the lively principles of essential oils studied was made through gas chromatography equipment coupled to mass spectrometry, type JAPAN, SHIMADZU, Ultra QP2010 MS-GC, using a 50 µm film capillary column. The oven began at a temperature of 40°C before reaching its final temperature of 280°C while holding the temperature program at (120-210ºC) at 8°C/min, which was then maintained for 45 minutes. Gas chromatography analysis required a sample injection volume of 1 microliter, and both the injection zone and detector temperatures were set to 280°C. The gas chromatography system used helium as the carrier gas, maintained consistently at 1.96 kPa pressure. A constant carrier gas flow rate of 71.1 mL/min was applied [18].

Chemical Tests

Thiobarbituric acid (TBA) determination: This was achieved by dissolving 0.2883g of TBA reagent in a 90% glacial acetic acid solution and the use of gentle use a water bath by heating to increase the rate of dissolution and the contents of the flask were then made up to 100ml. The burger sample weighing 10g was combined with 47.5ml of distilled water and 4 N hydrochloric acid (HCl) until reaching pH 1.5. The volumetric flask contents were moved to a 500 ml distillation flask after reaching 100ml volume and the addition of a boiling stone. The distillation apparatus was connected and heated using an electric heater until 50 ml of distilled liquid was collected within 10 minutes. 5 ml of the distilled liquid was taken and mixed with 5 ml of TBA reagent in a glass test tube with a tight stopper. Meanwhile, the blank solution was prepared by mixing 5 mL of distilled water with 5 mL of the reagent. The tubes were sealed, shaken, and incubated in a water bath for 35 min. Following the TBA procedure, the tubes were then cooled for 10 min, and the absorbance was measured using a spectrophotometer at 538 nm. No. mg of malondialdehyde/kg meat according to the following equation:

Value = TBA7.8 × sample absorbance

Free Fatty Acid (FFA) Estimation: The percentage of free fatty acids was estimated using the method [12]. 25 ml of chloroform and 50 g of burger sample (homogenized with a Hamilton Beach apparatus for two minutes) were taken. The mixture was filtered through filter paper, 25 ml of ethanol was added, and the mixture was sieved using 0.1 N sodium hydroxide in the presence of phenolphthalein indicator until the equivalence point was reached (appearance of a pink color). Free fatty acid content (%) was calculated as oleic acid using the following equation:

$$FFA\ (\%) = \frac{V \times N \times 28.2}{weight\ of\ sample}$$

Peroxide Value (PV) Estimation: The peroxide value was estimated according to the method of Pearson (1972) [15] by mixing 25 ml of chloroform with 50 g of Berger and sorting it well, then filtering it and taking 25 ml of the filtrate and adding to its 37 ml of glacial acetic acid and 1 ml of saturated potassium iodide, freshly prepared, using a 1% starch solution as an indicator and sifting it using sodium thiosulfate. The peroxide value (PV) was determined using the following equation:

$$PV = \frac{(V - Vb) \times N \times 1000}{weight\ of\ sample\ \ (gm)}$$

RESULTS AND DISCUSSION

The current study aimed to produce an innovative burger with a long shelf life, enriched with (Mentha longifolia), which contains natural antioxidants to improve its sensory properties and prevent microbial spoilage. Mentha longifolia oil contains phenolic compounds that contribute to its aromatic properties and distinctive pungent flavor. It has a high content of essential fatty acids and 38 flavonoid compounds, most notably caffeic acid, apigenin, quercetin, and 4-hydroxy derivatives. It also protects meat quality by preventing oxidation and inhibiting bacterial enzyme activity. Mentha longifolia oil acts as a natural food additive, providing effective compounds that replace synthetic chemical additives. This improves quality and extends shelf life when used in burger products, offering sustainable solutions for enhancing the quality of processed meats.

Figure 1 and Table 3 illustrate, through HPLC analysis, the total phenolic compounds identified in *Mentha longifolia* oil, revealing the presence of 38 compounds. The maximum peak areas were recorded for apigenin (4862.397), caffeic acid (230.154), quercetin (181.058), and 4-hydroxy compounds (174.521). The studied phenolic compounds demonstrated antioxidant activity and the ability to scavenge free radicals, as the DPPH radical is used to evaluate antioxidant activity. The role of antioxidants in reducing DPPH is due to their

ability to donate a hydrogen atom. DPPH is considered a free radical capable of receiving an electron and a hydrogen atom to form a stable molecule or increase the number of hydroxyl groups in the compound. The interaction mechanism relates to the chemical structure relationship between the DPPH radical and antioxidant compounds [27, 28]. The standard between antioxidants and S.O.R. is crucial for intracellular activities, given that the mitochondrial respiratory chain is a major source of S.O.R. in eukaryotic cells. As weak acids with antioxidant properties, flavonoids exert antimicrobial effects partly by reducing the respiratory rate. At elevated concentrations, specific flavonoids, including quercetin and dihydromycetin, can inhibit mitochondrial respiration and ATP synthesis in fungal cells, resulting in cell death [29]. The biological effectiveness of phenolic compounds is mainly attributed to the presence of hydroxyl (OH^-) groups on the polar aromatic phenolic ring. According to kind of compound, one or more hydroxyl groups are available to interact and bind with the active sites of coenzymes in the organism through hydrogen bonding [25]. Phenolic compounds impetuous proteins because of their ability to form Hydrogen bonds between phenolic hydroxyl groups and proteins in the body, disrupting the action of enzymes essential for the organism [26]. In general, phenolic compounds with antioxidant activity are phenolic acids and flavonoids. Different classes of flavonoids differ in the level of oxidation and saturation in the C-ring. In contrast, individual compounds of the same class differ in the substitution pattern of rings A and B. Variations in structural features and substitution patterns leverage the stability of the phenoxyl radical, characteristics of flavonoids [22], and the antioxidant. Everal studies suggest to antioxidant activity of phenolic compounds arises from synergistic interactions among different phenolic constituents rather than being ascribed to a single compound. The HPLC Figure 1. chromatography used for the separation, identification of total phenolic compounds in *Mentha longifolia* oil is shown.

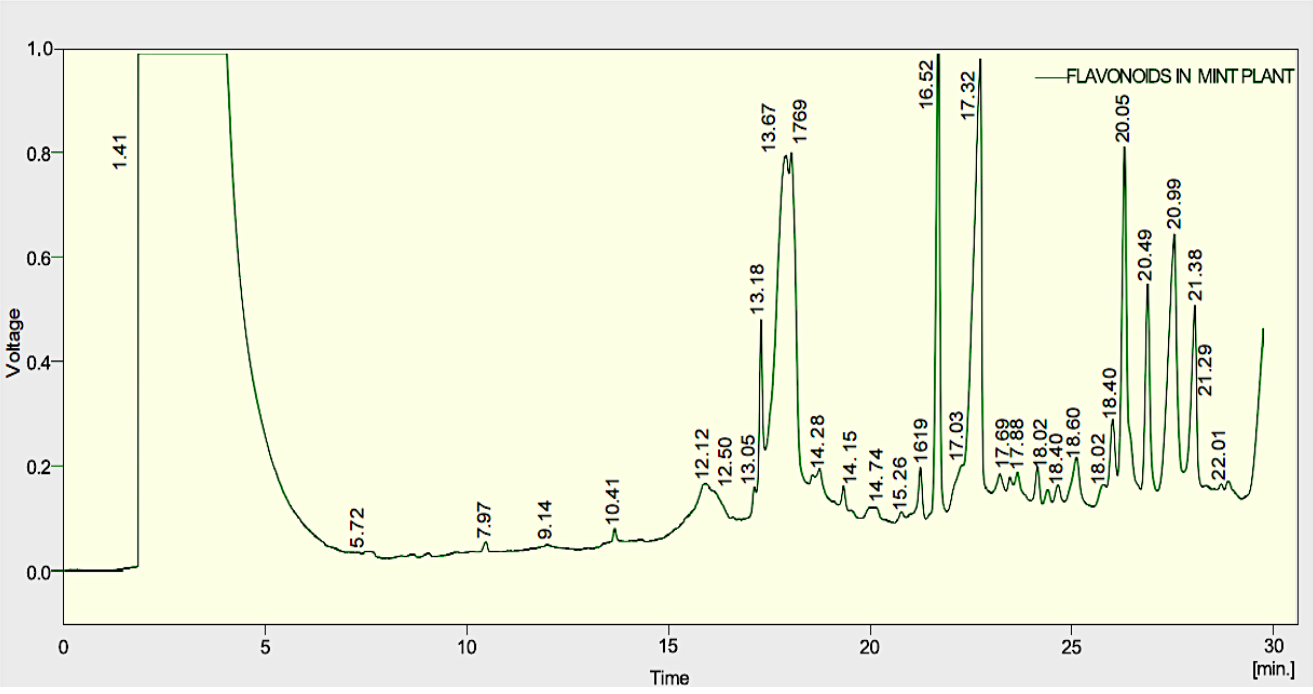


Figure 1. The chemical compounds identified by GC–MS and their percentage in *Mentha longifolia* oil are presented. The analyzed sample (FLAVONOIDS) was obtained from mint plant extract, injected at a volume of 0.001 mL, with no internal standard or dilution applied [29].

Table 3. Result of Uncal – Flavonoids in Mint Plant [29].

	Reten. Time [min]	Area [%]	Height [%]	Area [mV.s]	Height [mV]	W 05 [min]	Compound Name
1	0.180	0.0	0.0	18.627	1.246	0.27	
2	1.407	0.0	11.5	126505.711	985.361	1.99	
3	5.720	0.0	0.1	98.122	7.077	0.21	
4	7.973	0.0	0.2	230.154	19.900	0.09	CAFFEIC ACID
5	9.140	0.0	0.1	174.521	6.988	0.15	4-HYDROXY
6	10.410	0.0	0.3	181.058	23.884	0.06	QUERCETIN
7	12.123	0.0	1.1	3255.040	93.387	0.56	
8	12.500	0.0	0.4	416.353	35.884	0.21	
9	13.050	0.0	0.8	449.336	71.500	0.08	
10	13.177	0.0	4.5	1979.471	389.178	0.08	
11	13.657	0.0	8.1	11256.844	696.091	0.25	
12	13.750	0.0	8.1	7100.636	700.765	0.16	
13	14.150	0.0	0.9	551.905	76.929	0.14	
14	14.283	0.0	1.0	1224.552	86.910	0.21	
15	14.740	0.0	0.5	223.796	45.330	0.07	
16	15.263	0.0	0.3	419.837	24.785	0.26	
17	15.833	0.0	0.2	137.452	20.646	0.11	
18	16.187	0.0	1.2	704.840	102.115	0.08	
19	16.517	0.0	10.4	4862.397	893.222	0.08	APIGENIN
20	17.030	0.0	1.3	1390.769	109.733	0.23	
21	17.323	0.0	10.2	10279.699	875.805	0.18	
22	17.693	0.0	0.9	920.114	79.672	0.25	
23	17.883	0.0	0.8	633.541	72.499	0.18	
24	18.023	0.0	0.9	843.671	79.605	0.17	
25	18.403	0.0	1.0	644.027	86.885	0.10	
26	18.597	0.0	0.5	330.549	43.513	0.13	
27	18.793	0.0	0.6	460.485	50.888	0.14	
28	19.140	0.0	1.2	1231.416	101.161	0.17	
29	19.823	0.0	2.0	1709.015	169.263	0.12	
30	19.963	0.0	1.7	297.241	147.041	0.03	
31	20.050	0.0	8.0	3869.588	689.480	0.10	
32	20.120	0.0	1.9	1167.111	165.558	0.10	
33	20.487	0.0	4.9	2829.073	423.523	0.10	
34	20.990	0.0	6.0	6006.143	514.794	0.18	
35	21.297	0.0	2.1	1105.514	182.752	0.06	
36	21.377	0.0	4.4	2079.467	376.422	0.11	
37	21.427	0.0	1.4	496.769	119.374	0.03	
38	22.013	0.0	0.4	815.736	35.961	0.48	
Total	8,605.127	0.0	590.492	196,900.58	99.9	7.92	

Estimation of fatty acids in *Mentha longifolia* oil: Figure 2. represented the chromatographic results of the separated chemical compounds by MS-GC of *Mentha longifolia* oil. The number of chemical compounds is [30] *Mentha longifolia*. Analysis of the Figure 2. reveals D-Carvone as the dominant chemical compound in the essential oil with a concentration of 23.52%, which parallels the finding of [18] at 23.5% and exceeds the percentage reported by [19] at 22.76%. The recorded percentages for l-Menthone, D-Limonene, and Eucalyptol were 0.19%, 9.68%, and 6.13% respectively which aligns with the findings of the values that were 17.44%, 7.65%, and 5.26%.

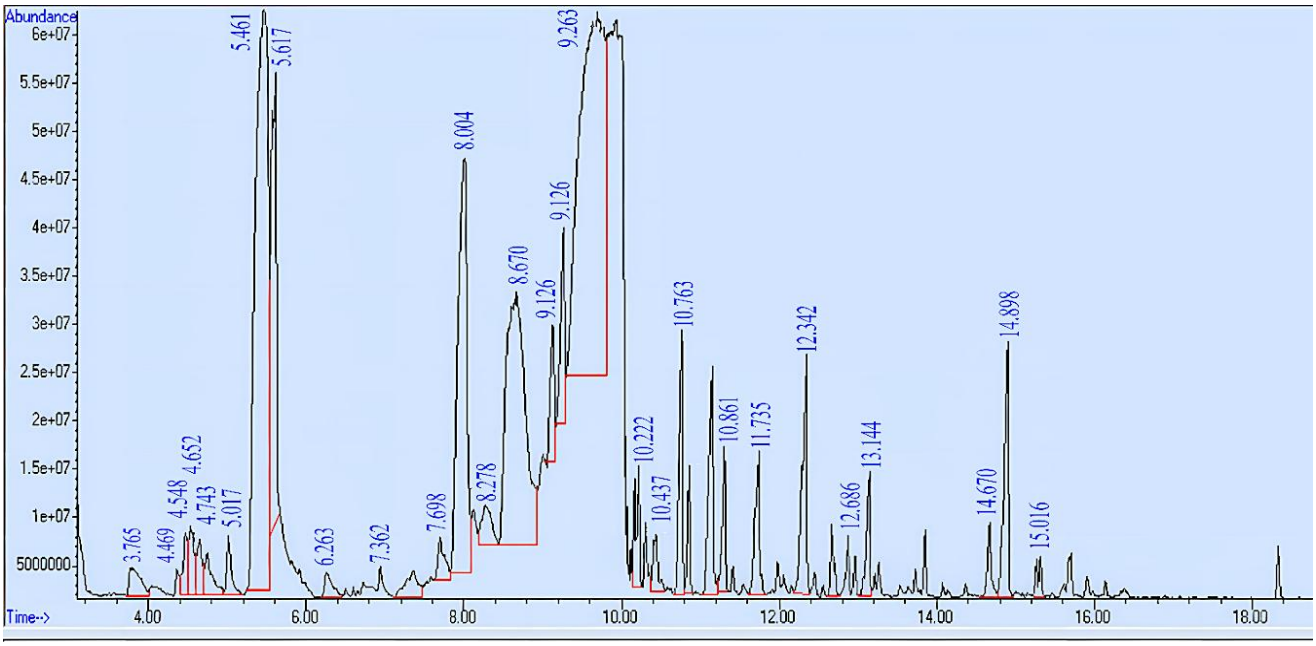


Figure 2. The chemical compounds identified by GC–MS and their percentage in *Mentha longifolia* oil are presented [29].

Table 4. The GC–MS utilized to identify the chemical compounds in *Mentha longifolia* oil and determine their proportions.

Pk#	RT	Area%	Library/ID	Ref#	CAS#	Qual	Compound Name
1	3.765	0.80	C:\GCMS\firmware\NIST11.L	15854	007785-70-8	94	{1R}-2,6,6-Trimethylbicyclo[3.1.1]hept-2-ene (α-Pinene)
2	4.469	0.68	C:\GCMS\firmware\NIST11.L	15891	003387-41-5	91	Bicyclo[3.1.0]hexane, 4-methylene-1-(1-methylethyl)-
3	4.548	0.95	C:\GCMS\firmware\NIST11.L	15695	000127-91-3	53	β-Pinene
4	4.652	0.74	C:\GCMS\firmware\NIST11.L	15694	000127-91-3	80	β-Pinene
5	4.743	0.81	C:\GCMS\firmware\NIST11.L	15694	000127-91-3	90	β-Pinene
6	5.017	0.68	C:\GCMS\firmware\NIST11.L	13704	018720-66-6	45	3-Heptanol, 6-methyl-
7	5.461	19.03	C:\GCMS\firmware\NIST11.L	15682	005989-27-5	99	D-Limonene
8	5.617	6.13	C:\GCMS\firmware\NIST11.L	26625	000470-82-6	94	Eucalyptol
9	6.263	0.50	C:\GCMS\firmware\NIST11.L	26992	015537-55-0	60	Bicyclo[3.1.0]hexan-2-ol, 2-methyl-5-(1-methylethyl)-
10	7.352	0.83	C:\GCMS\firmware\NIST11.L	25376	018881-04-4	22	Bicyclo[3.1.1]hept-3-en-2-ol, 4,6,6-trimethyl-

Pk#	RT	Area%	Library/ID	Ref#	CAS#	Qual	Compound Name
11	7.698	0.72	C:\GCMS\firmware\NIST11.L	26618	014073-97-3	83	l-Menthone
12	8.004	9.68	C:\GCMS\firmware\NIST11.L	26618	014073-97-3	98	l-Menthone
13	8.278	1.01	C:\GCMS\firmware\NIST11.L	5994	029253-64-3	25	1,3-Hexadiene, 2,5-dimethyl-
14	8.670	12.47	C:\GCMS\firmware\NIST11.L	25335	005948-04-9	99	Cyclohexanone, 2-methyl-5-(1-methylethenyl)-
15	9.126	1.38	C:\GCMS\firmware\NIST11.L	23675	002244-16-8	52	D-Carvone
16	9.263	1.94	C:\GCMS\firmware\NIST11.L	25066	000089-82-7	97	Pulegone
17	9.694	23.52	C:\GCMS\firmware\NIST11.L	23675	002244-16-8	70	D-Carvone
18	10.222	1.25	C:\GCMS\firmware\NIST11.L	57973	000076-49-3	98	Bornyl acetate
19	10.437	0.74	C:\GCMS\firmware\NIST11.L	58019	057287-13-5	43	(-)-8-p-Menthen-2-yl, acetate, trans
20	10.763	2.18	C:\GCMS\firmware\NIST11.L	58019	057287-13-5	93	(-)-8-p-Menthen-2-yl, acetate, trans
21	10.861	0.82	C:\GCMS\firmware\NIST11.L	56288	001205-42-1	99	2-Cyclohexen-1-ol, 2-methyl-5-(1-methylethenyl)-, acetate, cis-
22	11.155	2.51	C:\GCMS\firmware\NIST11.L	23893	000491-09-8	70	2-Cyclohexen-1-one, 3-methyl-6-(1-methylethylidene)-
23	11.305	1.09	C:\GCMS\firmware\NIST11.L	56156	001134-95-8	95	trans-Carveyl acetate
24	11.735	1.55	C:\GCMS\firmware\NIST11.L	64360	005208-59-3	91	(-)-β-Bourbonene
25	12.342	2.55	C:\GCMS\firmware\NIST11.L	64275	000087-44-5	83	Caryophyllene
26	12.668	0.60	C:\GCMS\firmware\NIST11.L	114224	000134-28-1	37	5-Azulenemethanol, 1,2,3,4,5,6,7,8-octahydro-α,α,3,8-tetramethyl-, acetate
27	13.144	1.05	C:\GCMS\firmware\NIST11.L	20830	105977-13-7	49	(Z)-4-Chloro-2,3-dimethyl-1,3-hexadiene
28	14.670	0.72	C:\GCMS\firmware\NIST11.L	48616	1000241-27-7	52	6-Cyano-5-methoxyquinoline
29	14.898	2.63	C:\GCMS\firmware\NIST11.L	77539	001139-30-6	91	Caryophyllene oxide
30	15.316	0.45	C:\GCMS\firmware\NIST11.L	64515	000514-51-2	47	4,7-Methanoazulene, 1,2,3,4,5,6,7,8-octahydro-1,4,9,9-tetramethyl-

Pk: Peak number in the chromatogram; RT: Retention time in minutes; Area%: The percentage of area under the peak, which indicates the relative amount of the compound in the sample; Library/ID: The library used for identification (in this case, the NIST11 library); Ref: The reference number of the compound in the library; CAS: The CAS (Chemical Abstracts Service) number of the compound; Qual: The quality of the match between the sample spectrum and the library spectrum (the higher the ratio, the better the match); Compound Name: The name of the identified chemical compound; Thiobarbituric acid (TBA) determination

Table 4. shows the TBA values for the burger treatments with 1, 2, and 3% added *Mentha longifolia* oil compared to those without *Mentha longifolia* oil. At an indication level of $P \leq 0.05$, no considerable variations were observed among treatments in TBA values prior to freezing, with a mean value of 0.30 mg malondialdehyde/kg fat. After freezing for 15 days, the treatments recorded an increase in TBA values, Showing statistically significant variations at the probability degree of $P \leq 0.05$. The highest value was 1.03 mg malondialdehyde/kg fat in the control treatment, and the lowest was 0.358 mg malondialdehyde/kg fat in handling of the T4. The treatments of the TBA range were these 2 values, with the TBA value decreasing with the raise in the proportion of *Mentha longifolia* oil included to the treatments. This demonstrates the role of starchy oil as an antioxidant in the fat-reducing burger mix under study. These results are like those reached by [13], as the TBA value increased slightly in minced meat with ginger added (1 g/100 g meat), from 0.75 mg malondialdehyde/kg fat before freezing to 0.91 mg malondialdehyde/kg fat after 60 days of freezing. The

Table 4. also shows an increase in TBA values after freezing the treatments for 30 days. The results explain indication variations at the significance degree (P ≤ 0.05). The control treatment recorded the highest value of 1.18 mg malondialdehyde/kg fat. The values gradually decreased, reaching a TBA value in treatment T4 of 0.366 mg malondialdehyde/kg fat. The values of the remaining treatments fell between these two values. The Table 4. also shows an increase in TBA values after freezing the treatments for 45 days. The results showed significant differences at the probability level (P ≤ 0.05). The control treatment recorded the highest value of 1.95 mg malondialdehyde/kg fat. The values gradually decreased, reaching a TBA value in treatment T4 of 0.563 mg malondialdehyde/kg fat. The values of the remaining treatments fell between these two values.

Table 5. Influence of *Mentha longifolia* oil on thiobarbituric acid content of beef burgers.

Treatment (mg)	Days				
	1	15	30	45	60
T1	0.30	1.033	1.18	1.954	2.23
T2	0.30	0.491	0.530	1.066	1.63
T3	0.30	0.413	0.397	0.967	1.18
T4	0.30	0.358	0.366	0.563	0.73
LSD Value	0.00 NS	0.637 *	0.541 *	0.683 *	0.763 *
* (P≤0.05) NS: not significant,					

After freezing the treatments for 60 days, the results showed significant differences in probability (P ≤ 0.05). The control treatment recorded the highest value of 2.23 mg malondialdehyde/kg fat. The values gradually decreased, with the TBA value in treatment T4 reaching 0.73 mg malondialdehyde/kg fat. These results were consistent with [14], who found that adding ginger oil (1 g/100 g) to minced beef reduced the TBA value from 2.63 mg malondialdehyde/kg fat in the control treatment to 1.12 mg malondialdehyde/kg fat when frozen for 100 days. noted that adding *Mentha longifolia* oil to the treatments was essential in inhibiting fat oxidation and preventing rancidity, as the TBA value decreased with an increasing proportion of added *Mentha longifolia* oil [16]. This delay in fat oxidation in burgers, especially in the treatments with a higher percentage of *Mentha longifolia* oil, results from the presence of active flavonoid compounds, which play a role in inhibiting the oxidation process by binding to free radicals.

Peroxide Value (PV): Table 6. shows the peroxide values for the treatments to which *Mentha longifolia* oil was added. The results show no significant differences at the probability degree (P ≤ 0.05) between the treatments before freezing. The values ranged between 0.92 and 0.93 mEq/kg fat.

After freezing for 15 days, significant differences appeared at the probability level (P ≤ 0.05) between the treatments. All treatments recorded increased PV values inversely comparative to the percentage of *Mentha longifolia* oil added. The highest value was 1.17 mEq/kg fat in the control treatment, and the lowest was 0.46 mEq/kg in the T4 treatment. The remaining treatments of values were 2 values. The PV values were decreased compared with the control treatment, along with the *Mentha longifolia* oil was added to the treatments to increase the percentage, is attributed to the *Mentha longifolia* oil containing active flavonoid compounds, which are substances that contribute to the production of *Mentha longifolia* oil Antioxidant [10].

After freezing for 45 days, significant differences appeared at the probability level ($P < 0.05$) between treatments. All treatments recorded increased PV values inversely proportional to the percentage of added *Mentha longifolia* oil. The highest value was 4.05 mEq/kg fat in the control treatment. The lowest value was 1.03 mEq/kg fat in the T4 treatment, while the remaining treatments exhibited intermediate values between these two extremes.

PV values increased for all treatments upon freezing for 60 days, and notable variations appeared at the significance degree ($P < 0.05$) between treatments. The control treatment recorded the highest PV value of 7.11 mEq/kg fat, while the lowest was 1.76 mEq/kg fat in the T4 treatment. The PV values of the other therapies ranged between these values.

Table 6. Influence of *Mentha longifolia* oil on the peroxide number of beef burgers.

Treatment(mEq/kg)	Days					LSD Value
	1	15	30	45	60	
T1	0.93	1.17	1.65	4.05	7.11	0.962 *
T2	0.93	1.12	1.43	2.09	3.45	0.739 *
T3	0.93	1.06	1.20	1.47	1.94	0.577 *
T4	0.92	0.98	1.15	1.16	1.76	0.526 *
LSD Value	0.00 NS	0.466 *	0.479 *	1.033 *	1.57 *	---
* (P≤0.05) NS: not significant,						

Estimation of Free Fatty Acids (FFA): Table 7. shows the free fatty acid values for the burger treatments with added *Mentha longifolia* oil. At a prominence degree of $P \leq 0.05$, the findings demonstrated no significant differences between treatments before freezing, and the values ranged between 0.79 and 0.80 milliequivalents per 100g of meat.

After freezing for 15 days, FFA values increased across all treatments, and significant differences appeared between them at the probability degree ($P \leq 0.05$). The highest value was for the control treatment, reaching 1.53 milliequivalents per 100g of meat, while treatments T4 and T3 observed the minimum values, reaching 0.98 and 0.88 milliequivalents per 100g of meat. The FFA values for the remaining treatments ranged between these two values. After freezing for 30 days, FFA values increased for all treatments, and significant differences were found between them at the significance degree ($P \leq 0.05$). The peak value was for the control treatment, reaching 1.95 mEq/100g of meat, while the T4 treatment recorded the lowest value, reaching 0.96 mEq/100g of meat. The FFA values for the remaining treatments ranged between these two values. It is noted that the FFA values of the control treatments increased above the permissible limits set by the Iraqi standard (1987), which stipulated that the FFA value should not exceed 1.5 mEq/kg for frozen or fresh beef. This indicates the onset of fat decomposition in the burger. After freezing for 45 days, FFA values increased for all treatments, and significant differences appeared between them at the probability level ($P \leq 0.05$). The highest value was for the control treatment, which reached 3.03 mEq/kg meat, while treatment T4 recorded the lowest value, which reached 1.11 mEq/100g meat. The FFA values for the remaining treatments ranged between these two values. It is noted that there was an increase in the FFA value for the treatment above the permissible limits specified by the Iraqi specification (1987), which selected the FFA value not to exceed 1.5 mEq/kg for frozen or fresh beef. This indicates the occurrence of fat decomposition in the burger [30-31].

When frozen for 60 days, there was a further increase in FFA values, and notable variations appeared at the significance degree [P ≤ 0.05] between treatments. The control treatment recorded the peak value, reaching 5.11 mEq/100g of meat, higher than the permissible limits mentioned above. Treatment T4 remained within the allowable limits, reaching 1.33 mEq/100g of meat. The FFA values for the remaining treatments fell between those mentioned [32]. The Figure 2. shows that the increase in FFA values is negatively comparative to the percentage of wheat germ added to the treatments. This is indicated by [33], indicating that the flavonoid compounds in *Mentha longifolia* oil play an important role as natural antioxidants in frozen-stored meat [34].

Table 7. Beef burgers affected by adding *Mentha longifolia* oil degree of free fatty acids

Treatment(mEq/100g)	Days					LSD Value
	1	15	30	45	60	
T1	0.80	1.53	1.95	3.03	5.11	1.64 *
T2	0.80	1.21	1.17	2.09	2.53	0.843 *
T3	0.80	0.98	1.15	1.51	2.61	0.835 *
T4	0.79	0.88	0.96	1.11	1.33	0.719 NS
LSD Value	0.00 NS	0.414 *	0.509 *	0.388 *	0.724 *	---
* (P≤0.05) NS: not significant,						

CONCLUSION

Natural, inexpensive antioxidants can help slow lipid oxidation and maintain quality in frozen meat products.Mentha longifolia essential oil is a safe, plant-derived source of bioactive compounds that can enhance the preservation of meats containing oxidizable fats by delaying rancidity and quality deterioration during frozen storage. Incorporating Mentha longifolia oil may reduce oxidative changes and support better overall stability of beef burgers, which can help extend shelf life while decreasing reliance on synthetic preservatives. To reduce oxidation in foods, antioxidants should be prioritized from natural sources with documented efficacy and acceptable sensory impact. Future work should optimize extraction and dosing, evaluate performance in different packaging and storage conditions, and confirm consumer acceptance, safety, and cost-effectiveness to support wider use of Mentha longifolia oil as a natural preservative in meat and other fat-rich food systems.

Recommendations: Further studies are recommended to optimize the extraction methods of *Mentha longifolia* aromatic oils to enhance their antioxidant efficiency. Evaluating their application under different storage and packaging conditions, as well as assessing their safety, sensory acceptance, and economic feasibility, is essential to support their wider use as natural preservatives in meat products and other functional food system.

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