

Original Research

Efficacy of Warming Essential Oil Emulsions in Extending the Shelf Life of Vacuum-Packed Chicken Legs

Zohra Madani^{1,2}, Talal Lahreche³, Kheira Gasmi^{1,2,*}, Abdelkader Bensid³,
Rym N Benamara^{1,4}

¹Faculty of Sciences of Nature and Life, University of Mustapha Stambouli, 29000 Mascara, Algeria

²Research Laboratory on Biological Systems and Geomatics (LRSBG), University Mustapha Stambouli, 29000 Mascara, Algeria

³Faculty of Sciences of Nature and Life, Ziane Achour University of Djelfa, 17000 Djelfa, Algeria

⁴Laboratory of Microbiology Applied to Agri-food, Biomedical and Environment (LAMAABE), Faculty of SNV/STU, University of Tlemcen, 13000 Tlemcen, Algeria

*Correspondence: kheira.gasmi@univ-mascara.dz (Kheira Gasmi)

Academic Editor: Corinna Kehrenberg

Submitted: 25 January 2025 Revised: 12 March 2025 Accepted: 21 March 2025 Published: 11 April 2025

Abstract

Background: This study evaluates the impact of dipping chicken legs in 0.4% *Mentha spicata* and *Rosmarinus officinalis* essential oil (EO) emulsions, warmed to the same temperature and for the same duration as the soft scalding process (53 ± 1 °C for 3 min), on the EO's antimicrobial activity against spoilage bacteria to extend its shelf life. Samples were stored at 4 ± 1 °C under air packaging (AP) and vacuum packaging (VP). **Methods:** Microbial analysis of *Enterobacteriaceae*, lactic acid bacteria (LAB), *Pseudomonas* spp., psychrotrophic bacteria, and H_2S -producing bacteria, pH determination, and sensory evaluations of raw and cooked chicken legs were performed on days 0, 3, 6, 9, 12, and 16 for samples stored under AP and continued on days 20, 24, and 28 for samples stored under VP. **Results:** Gas chromatography-mass spectrometry (GC-MS) analysis revealed that carvone 25.36% and eucalyptol 52.09% were the main components in *M. spicata* and *R. officinalis* EO, respectively. Warming the EO emulsions increased their antibacterial effect. In addition, vacuum packaging and dipping chicken legs in a warmed emulsion of *R. officinalis* or *M. spicata* EO extended the shelf life by 15 and 19 days, respectively. However, under air, dipping chicken legs in both warmed EO emulsions extended the shelf life by 10 days. Furthermore, the sensory shelf lives of raw and cooked chicken legs under air and vacuum generally matched those determined by microbial analysis. *M. spicata* EO performed better under all conditions. **Conclusion:** The use of warmed EO emulsions is considered an inexpensive and effective method of preserving raw and cooked chicken meat. However, the most effective combination for extending the shelf life of chicken legs is warmed *M. spicata* EO emulsion and vacuum packaging.

Keywords: chicken legs; shelf life; soft scalding; spoilage bacteria; warmed emulsion

1. Introduction

Meat and meat products have a relatively short shelf life due to their high water activity (aw) and nutrient richness, which create a favorable environment for microbial growth [1]. Producers face a challenge in establishing an appropriate shelf life for meat and poultry products. Shelf life, on the other hand, can be defined as the time it takes for a product to remain suitable for consumption while meeting safety, nutritional, regulatory, and sensory standards [2,3]. Meat spoilage is defined as an alteration of physicochemical properties that exceeds the acceptable limit and is caused by microbial activity or metabolic processes [4].

Microbial growth is one of the major causes of quality deterioration in meat, so the level of microbial growth is closely related to sensory acceptability and serves as a spoilage indicator [5]. According to Fletcher *et al.* [6] (2018), meat is considered spoiled when undesirable changes in its sensory properties are detected. It is estimated that more than 20% of the meat produced worldwide is lost or wasted, primarily due to microbial spoilage. How-

ever, in the United States, it is estimated that 2 to 4% of processed meat is lost due to spoilage, with a total value of \$83,127 million [7]. Another study found that in Canada, red meat accounted for 39.73% of total waste, while poultry waste was estimated to be around 42.74% [8]. Thus, poultry spoilage is an economic burden for both producers and consumers. The development of alternative strategies to extend the shelf life of meat products is a major focus of meat processing research.

Various combinations of modified atmosphere, vacuum packaging (VP), and essential oils (EO) have been used to control spoilage bacteria and increase the shelf life of meat and poultry products [3]. To extend the shelf life of chicken meat, previous research has used EO as emulsions at room temperature in conjunction with vacuum packaging, but in this study, a third combination is added: warming the EO emulsions to the same temperature used in the soft scalding process. This is the process of immersing the carcass in warm water at 53 °C for 3 minutes to effectively remove feathers [9]. It is commonly used because it causes little damage to the bird's outer layer of skin.



The EO used in this study were derived from *Rosmarinus officinalis* and *Mentha spicata*, two aromatic plants grown worldwide for their unique aroma and commercial value. As a result, microbiological analysis, sensory evaluation of raw and cooked chicken legs under air and vacuum packaging, and the effect of warming the EO emulsions on the antimicrobial activity of the EO were used to determine the best combination treatment to extend the shelf life of chicken meat.

2. Materials and Methods

2.1 Essential Oil Extraction (GC-MS) Analysis and Emulsion Preparation

Fresh leaves of *Rosmarinus officinalis* and *Mentha spicata* were collected from Blida-Djelfa, Algeria (April-May 2023), botanically identified, dried in the dark, and subjected to essential oil extraction following the European Pharmacopoeia method [10]. The extracted essential oils were stored in airtight glass bottles at 4 °C. Their composition was analyzed at PTAPC-CRPC (Laghouat, Algeria) using the Gas chromatography-mass spectrometry (GC-MS) QP2020 (Shimadzu Corporation, Kyoto, Japan) with a fused Rxi®-5ms capillary column. The analysis involved split-mode injection (1:10), a temperature program from 50 °C to 310 °C, helium as the carrier gas, and electron ionization at 70 eV. EO components were identified by comparing their retention indices and mass spectra to the National Institute of Standards and Technology (NIST) and Wiley libraries, as well as data from the literature [11].

R. officinalis and *M. spicata* EO emulsions were created by combining 0.4 mL of each EO with 100 mL of sterile distilled water and 0.2 mL of tween 80 (MERCK, Darmstadt, Germany). The solutions were emulsified separately with a mechanical homogenizer (850 Homogenizer, Thermo Fisher Scientific, Waltham, MA, USA) for 4 minutes until the mixture became completely uniform. These emulsions, each containing 10 liters, were immediately used to immerse the chicken legs. The concentrations of EO were determined based on preliminary testing to ensure any negative sensory impacts on chicken meat while maintaining its desirable flavor.

2.2 Sample Preparation and Storage Conditions

One hundred eighty chicken legs were purchased from a local retail market and delivered to the laboratory in a refrigerated container. Chicken legs were divided into 2 batches according to storage conditions. Samples from the first batch were randomly divided into five groups (control group, group dipped in 0.4% *M. spicata* EO emulsion at room temperature MNA (*M. spicata* EO, Not warmed, Air-packed) or warmed to 53 ± 1 °C MWA (*M. spicata* EO, Warmed, Air-packed); group dipped in 0.4% *R. officinalis* EO emulsion at room temperature RNA (*R. officinalis* EO, Not warmed, Air-packed) or warmed to 53 ± 1 °C RWA (*R. officinalis* EO, Warmed, Air-packed). The dipping period

of the treated samples is 3 minutes. After treatment, the chicken legs were drained for 10 min at room temperature, placed in sterile polystyrene trays (two legs per tray) and wrapped with an oxygen-permeable film for air packaging (AP).

The same preparation method was used for the second batch of samples, except that they were vacuum packed using a vacuum packaging machine (Aukey 75 kpa, Hamburg, Germany) instead of air packed, resulting in the following groups: MNV, MWV, RNV, and RWV. The thickness of the film was 90 µm. All trays were stored at 4 ± 1 °C. Samples were tested for sensory and microbiological properties. For the first batch, microbial analyses targeted *Pseudomonas* spp., *Enterobacteriaceae*, psychrotrophic bacteria, and *H₂S*-producing bacteria at 0, 3, 6, 9, 12, and 16 days. For the second batch, analyses focused on *Enterobacteriaceae*, lactic acid bacteria (LAB), and *H₂S*-producing bacteria at 0, 3, 6, 9, 12, 16, 20, 24, and 28 days. All analyses were conducted in duplicate, based on the shelf life of each group of chicken legs.

2.3 Microbiological Analysis and PH Determination

Microbial analyses were performed as described by Alessandrini *et al.* [12] (2022). Raw chicken meat (10 g) of each sample was aseptically mixed with 90 mL of peptone water (0.1% w/v) (CONDALAB, Madrid, Spain) in a sterile stomacher bag and homogenized using a stomacher at room temperature. Decimal dilutions were performed using the same diluents, and 0.1 mL of each appropriate dilution was poured into plates of selective medium: *Pseudomonas* spp. growth at 25 °C for 48 hours on Cetrimid, Fucidin, and Cephaloridin (CFC) agar (MERCK, Darmstadt, Germany). Psychrotrophic bacteria were investigated on plate count agar after incubation at 7 °C for 10 days. For *H₂S*-producing bacteria were determined on peptone iron agar (MERCK, Darmstadt, Germany) supplemented after autoclaving L-cysteine [13,14]. These formed black colonies as a result of the formation of ferrous sulfide.

To determine LAB count and *Enterobacteriaceae*, 1 mL of the appropriate dilution was plated on Man, Rogosa, Sharpe (MRS) Agar (MERCK, Darmstadt, Germany) and Violet Red Bile Glucose (VRBG) Agar (CONDALAB, Madrid, Spain), respectively, and overlaid with 5–10 mL of the same molten medium. The plates were incubated for 24 h at 37 °C for *Enterobacteriaceae* and stored under anaerobic conditions for 3 days at 25 °C for LAB. Microbiological data are expressed as \log_{10} CFU·g⁻¹. The PH of the homogenate from each sample was determined using a PH meter (OHAUS, ST3100, Parsippany, NJ, USA).

2.4 Sensory Analysis

A panel of 6–7 members of Food Hygiene and Quality, Zian Achour University, Algeria, competent in meat evaluation, was invited to evaluate raw and cooked chicken legs. Each group of samples was labeled at random with

Table 1. Chemical composition of *Rosmarinus officinalis* and *Mentha spicata* analyzed using GC-MS.

No.	Compounds	<i>Rosmarinus officinalis</i>		<i>Mentha spicata</i>	
		R. time	Area%	R. time	Area%
1	α -Pinene	8.826	10.31	8.830	1.16
2	Camphene	9.389	3.25	9.387	tr
3	Sabinene	-	-	10.427	1.10
4	β -Pinene	10.512	2.62	10.555	1.81
5	Myrcene	11.143	1.07	11.178	1.36
6	3-Octanol	-	-	11.416	0.94
7	δ -3-Carene	11.945	0.14	-	-
8	α -Terpinene	12.229	0.43	12.266	0.62
9	o-Cymene	12.565	1.77	12.600	tr
10	Limonene	-	-	13.103	23.93
11	Eucalyptol	12.896	52.09	-	-
12	(Z)- β -Ocimene	-	-	13.272	0.64
13	γ -Terpinene	14.105	0.46	14.167	0.90
14	Sabinene hydrate	-	-	14.554	1.55
15	Linalool	15.968	0.76	16.032	tr
16	Camphor	17.985	11.71	-	-
17	Borneol	18.991	4.26	19.125	1.31
18	Terpinen-4-ol	19.536	0.85	19.645	1.58
19	α -Terpineol	20.165	3.96	20.270	0.32
20	Trans-Dihydrocarvone	-	-	20.576	4.20
21	Pulegone	-	-	22.452	0.95
22	Carvone	22.594	tr	23.168	25.36
23	Trans-Carvone oxide	-	-	23.569	2.05
24	Carvacrol	24.812	0.64	25.073	tr
25	Dihydrocarvyl acetate	-	-	26.554	0.58
26	β -Bourbonene	-	-	29.080	4.00
27	β -Elemene	-	-	29.339	1.69
28	(Z) Jasmone	-	-	29.569	0.77
29	Carvacrylacetate	30.350	3.60	30.571	5.09
30	α -Humulene	31.750	0.47	31.861	0.43
31	Cis-Muurola-4(15),5-diene	-	-	32.257	1.12
32	Germacrene D	-	-	33.082	3.96
33	Bicyclogermacrene	-	-	33.648	1.28
34	Trans- β -Guaiene	-	-	33.982	0.48
35	δ -Cadinene	-	-	34.663	0.76
36	Spathulenol	-	-	36.791	0.51
37	Caryophyllene oxide	-	-	37.003	0.51
38	epi- α -Muurolol	-	-	39.185	0.86
	Total		98.39		91.82

R. time, Retention time; tr, Trace; (-), Not detected.

a three-digit code number and presented in a randomized order. Sensory analysis of raw chicken meat was carried out according to Kreyenschmidt *et al.* [15] (2010), with a slight modification. Odor (O), color (C) and texture (T) were evaluated using a scoring system ranging from 1 to 4, with 1 corresponding to the highest quality score and 2.5 corresponding to the cut-off score for unacceptability. The weighted Sensory Index (SI) was calculated using the following equation:

$$SI = \frac{2 * O + 1 * C + 1 * T}{4}$$

Sensory evaluation of cooked chicken meat was assessed according to Patsias *et al.* [16] (2006). Chicken legs from each group were cooked in a microwave oven (Condor CMW-M2307B, Bordj Bou Arréridj, Algeria) at 700 W for 6 min. An acceptability scale ranging from 5 to 1 was used to assess acceptability as a composite of odor, texture and taste, with 5 corresponding to the most-liked sample and 1 to the least-liked chicken sample. A score of 3 was considered the lower limit of acceptability.

Table 2. Effect of dipping a warmed or not warmed emulsion of *M. spicata* and *R. officinalis* essential oils on the growth of spoilage bacteria in a chicken leg under air at 4 ± 1 °C.

		D0	D3	D6	D9	D12	D16
<i>Enterobacteriaceae</i>	Control CA	3.44 ± 0.15 a	5.24 ± 0.06 a	6.20 ± 0.03 a	Na	Na	Na
	MNA	3.28 ± 0.35 a	5.00 ± 0.03 b	5.70 ± 0.01 b	5.89 ± 0.01 b	6.28 ± 0.11 a	Na
	MWA	3.21 ± 0.16 a	4.08 ± 0.15 d	4.80 ± 0.09 d	5.19 ± 0.20 c	5.31 ± 0.09 c	5.99 ± 0.09 b
	RNA	3.34 ± 0.15 a	5.04 ± 0.03 b	5.47 ± 0.04 c	6.09 ± 0.02 a	Na	Na
	RWA	3.31 ± 0.35 a	4.52 ± 0.18 c	5.31 ± 0.04 c	5.45 ± 0.21 b, c	5.66 ± 0.17 b	6.18 ± 0.06 a
<i>Psychrotrophic bacteria</i>	CA	4.62 ± 0.01 a	5.54 ± 0.09 a	7.13 ± 0.00 a	Na	Na	Na
	MNA	4.50 ± 0.06 a	5.29 ± 0.02 b	5.63 ± 0.01 b	6.32 ± 0.00 b	6.98 ± 0.07 a	Na
	MWA	4.51 ± 0.05 a	4.91 ± 0.08 c	5.17 ± 0.00 c	5.82 ± 0.07 c	6.56 ± 0.05 b	7.12 ± 0.00 b
	RNA	4.52 ± 0.01 a	5.36 ± 0.04 b	5.71 ± 0.03 b	6.91 ± 0.02 a	Na	Na
	RWA	4.51 ± 0.06 a	4.82 ± 0.08 c	4.94 ± 0.05 d	5.61 ± 0.02 d	6.40 ± 0.01 b	7.48 ± 0.02 a
<i>Pseudomonas</i> spp.	CA	4.21 ± 0.05 a	5.58 ± 0.00 a	6.38 ± 0.00 a	Na	Na	Na
	MNA	4.17 ± 0.05 a	5.54 ± 0.02 a	5.46 ± 0.00 b	6.02 ± 0.03 b	6.91 ± 0.05 a	Na
	MWA	4.11 ± 0.05 b	4.34 ± 0.15 b	4.57 ± 0.07 d	4.96 ± 0.07 d	5.21 ± 0.09 c	6.59 ± 0.02 b
	RNA	4.15 ± 0.05 a	5.58 ± 0.01 a	5.49 ± 0.01 b	6.31 ± 0.07 a	Na	Na
	RWA	4.11 ± 0.05 a	4.39 ± 0.30 b	4.96 ± 0.01 c	5.47 ± 0.04 c	6.73 ± 0.06 b	7.12 ± 0.00 a
<i>H₂S</i> -producing bacteria	CA	3.13 ± 0.12 a	5.58 ± 0.00 a	6.21 ± 0.04 a	Na	Na	Na
	MNA	3.03 ± 0.12 a	5.43 ± 0.05 b	5.47 ± 0.03 b	5.90 ± 0.06 b	6.73 ± 0.06 a	Na
	MWA	3.08 ± 0.12 a	4.44 ± 0.00 c	4.57 ± 0.07 d	4.96 ± 0.07 d	5.97 ± 0.04 c	6.59 ± 0.02 b
	RNA	3.03 ± 0.12 a	5.47 ± 0.00 a, b	5.52 ± 0.02 b	6.31 ± 0.07 a	Na	Na
	RWA	3.11 ± 0.12 a	4.56 ± 0.12 c	4.96 ± 0.01 c	5.47 ± 0.03 c	6.08 ± 0.02 b	7.12 ± 0.00 a

Values are expressed as the mean ± standard deviation (n = 2).

D, day; C, Control; A, air; M, *M. spicata* EO; R, *R. officinalis* EO; W, warmed emulsion to 53 ± 1 °C; N, at room temperature = not warmed. Na, Not analyzed; the sample had a spoiled appearance with an off-odor.

a, b, c, d indicate significant differences ($p < 0.05$) between the different dipping treatments at the same sampling time.

2.5 Statistical Analyses

All microbiological experiments were performed in duplicate for each sample. Before analyses, data were checked for normality and homogeneity of variance using the Shapiro-Wilk and Levene tests, respectively. Analysis of variance (ANOVA) followed by Duncan's post-hoc multiple interval test was performed to evaluate the significant differences ($p < 0.05$) and determine the most effective treatment for each storage condition. All analyses were performed using SPSS 19.0 for Windows (SPSS Inc. Chicago, IL, USA).

3. Results and Discussion

3.1 Volatile Composition of EO

The chemical compositions of the EO compounds were determined using GC-MS analysis. Table 1 only shows compounds with a retention rate of more than 0.3%. It can be noted that *M. spicata* and *R. officinalis* had 35 and 18 compounds, respectively, that account for 91.82 and 98.39% of their total EO. The main constituents detected in rosemary essential oil were eucalyptol (52.09%), camphor (11.71%), α -pinene (10.31%), borneol (4.26%), and α -terpineol (3.96%). According to González-Minero *et al.* [17] (2020), Moroccan and Tunisian *R. officinalis* essential oils contain a high level of 1.8 cine-

ole (eucalyptol) ranging from 38 to 55%, whereas Spanish rosemary essential oils contain 16 to 20% 1.8 cine-ole. The main compounds in spearmint essential oil (EO) were carvone (25.36%), limonene (23.93%), carvacrylacetate (5.09%), Trans-Dihydrocarvone (4.20%), and β -Bourbonene (4.00%). Similar to another study, the essential oil of *M. spicata* from Laghouat, Algeria, contained a high concentration of carvone (49.5%) and limonene (16.1%) [18].

These bioactive compounds, found in essential oils, exhibit various biological activities, such as antimicrobial effects against spoilage and pathogenic bacteria, antioxidant properties that delay lipid oxidation, and consequently, preservative effects, especially in the case of eucalyptol, borneol, camphor, and carvone [19].

3.2 Microbiological Changes

Assessments of microbial growth under air (Table 2) and vacuum packaging (Table 3) were carried out by measuring *Enterobacteriaceae*, LAB, *Pseudomonas* spp., psychrotrophic bacteria, and *H₂S*-producing bacteria. The growth of spoilage bacteria causes chicken meat deterioration and mainly contributes to a reduction in its shelf life, especially in the control group. At the beginning of storage, the initial count of *Enterobacteriaceae*, LAB, and *H₂S*-pr-

Table 3. Effect of dipping a warmed or not warmed emulsion of *M. spicata* and *R. officinalis* essential oil on the growth of spoilage bacteria in a chicken leg under vacuum at 4 ± 1 °C.

		D0	D3	D6	D9	D12	D16	D20	D24	D28
<i>Enterobacteriaceae</i>	CV	3.58 ± 0.16 a	5.04 ± 0.06 a	5.21 ± 0.06 a	6.44 ± 0.03 a	Na	Na	Na	Na	Na
	MNV	3.43 ± 0.16 a	4.81 ± 0.06 b	4.90 ± 0.06 b	5.91 ± 0.06 b	6.01 ± 0.08 a	6.38 ± 0.08 b	Na	Na	Na
	MWV	3.45 ± 0.16 a	4.48 ± 0.14 c	4.37 ± 0.19 c	5.29 ± 0.17 c	5.49 ± 0.11 c	5.71 ± 0.09 d	5.82 ± 0.06 b	5.97 ± 0.09 a	6.27 ± 0.05 a
	RNV	3.48 ± 0.16 a	4.93 ± 0.06 a, b	5.02 ± 0.08 a	5.89 ± 0.03 b	5.95 ± 0.09 a, b	6.48 ± 0.01 a	Na	Na	Na
	RWV	3.46 ± 0.16 a	4.41 ± 0.24 c	4.47 ± 0.32 c	5.49 ± 0.12 c	5.78 ± 0.09 b	5.97 ± 0.06 c	6.01 ± 0.04 a	6.41 ± 0.04 a	Na
LAB	CV	3.48 ± 0.01 a	5.29 ± 0.02 a	5.75 ± 0.02 a	6.33 ± 0.05 a	Na	Na	Na	Na	Na
	MNV	3.43 ± 0.16 a	5.24 ± 0.03 a	5.43 ± 0.09 b	5.89 ± 0.04 b	6.03 ± 0.04 a	6.39 ± 0.11 a	Na	Na	Na
	MWV	3.44 ± 0.16 a	4.91 ± 0.03 c	5.11 ± 0.05 c	5.40 ± 0.02 c	5.47 ± 0.02 c	5.36 ± 0.01 c	5.66 ± 0.08 b	5.91 ± 0.03 b	6.12 ± 0.04 a
	RNV	3.43 ± 0.01 a	5.06 ± 0.02 b	5.49 ± 0.01 b	5.54 ± 0.04 c	5.85 ± 0.05 b	6.21 ± 0.05 a	Na	Na	Na
	RWV	3.43 ± 0.01 a	4.77 ± 0.00 d	5.11 ± 0.20 c	5.58 ± 0.14 c	5.39 ± 0.00 d	5.97 ± 0.06 b	6.09 ± 0.11 a	6.33 ± 0.03 a	Na
<i>H₂S</i> -producing bacteria	CV	3.13 ± 0.12 a	5.14 ± 0.04 a	5.15 ± 0.06 a	6.24 ± 0.03 a	Na	Na	Na	Na	Na
	MNV	3.03 ± 0.01 a	4.90 ± 0.06 a, b	4.99 ± 0.02 a, b	5.90 ± 0.03 b	6.01 ± 0.08 a	6.28 ± 0.11 a, b	Na	Na	Na
	MWV	3.10 ± 0.12 a	4.37 ± 0.19 c	4.44 ± 0.07 c	5.19 ± 0.02 d	5.82 ± 0.03 b	5.91 ± 0.04 c	5.95 ± 0.08 b	6.10 ± 0.00 b	6.71 ± 0.09 a
	RNV	3.13 ± 0.01 a	4.81 ± 0.01 b	4.91 ± 0.07 b	5.93 ± 0.03 b	6.09 ± 0.07 a	6.48 ± 0.01 a	Na	Na	Na
	RWV	3.01 ± 0.12 a	4.37 ± 0.19 c	4.48 ± 0.14 c	5.49 ± 0.12 c	5.80 ± 0.05 b	6.01 ± 0.08 b	6.08 ± 0.02 a	6.91 ± 0.04 a	Na

Values are expressed as the mean ± standard deviation (n = 2).

D, day; C, Control; V, vacuum; M, *M. spicata* EO; R, *R. officinalis* EO; W, warmed emulsion to 53 ± 1 °C; N, at room temperature = not warmed. Na, Not analyzed; the sample had a spoiled appearance with an off-odor.

a, b, c, d indicate significant differences ($p < 0.05$) between the different dipping treatments at the same sampling time.

Table 4. Change in the pH value during storage under vacuum packaging or air.

		D0	D3	D6	D9	D12	D16	D20	D24	D28
Under vacuum	CV	7.02 ± 0.01 a	6.96 ± 0.03 b	7.04 ± 0.02 a, b	7.01 ± 0.03 a, b	7.08 ± 0.02 c	7.32 ± 0.00 a	Na	Na	Na
	MNV	6.96 ± 0.01 b	7.00 ± 0.01 a, b	7.01 ± 0.02 b	6.96 ± 0.01 c	7.08 ± 0.01 b	7.24 ± 0.01 b	7.31 ± 0.00 a	Na	Na
	MWV	6.97 ± 0.00 b	6.99 ± 0.01 a, b	7.10 ± 0.02 a	6.95 ± 0.00 c	7.13 ± 0.01 a, b	7.16 ± 0.04 c	7.26 ± 0.02 b	7.30 ± 0.01 a	7.32 ± 0.01 a
	RNV	7.03 ± 0.01 a	7.01 ± 0.00 a	7.00 ± 0.03 b	7.03 ± 0.05 a	7.17 ± 0.02 a	7.32 ± 0.01 a	7.32 ± 0.01 a	Na	Na
	RWV	6.96 ± 0.00 a	7.02 ± 0.01 a	7.09 ± 0.04 a	6.99 ± 0.01 b	7.09 ± 0.04 b	7.24 ± 0.02 b	7.27 ± 0.01 b	7.32 ± 0.01 a	Na
Under air	CA	7.03 ± 0.01 a	7.02 ± 0.00 b	6.94 ± 0.01 c	7.13 ± 0.06 a	Na	Na	Na	Na	Na
	MNA	6.97 ± 0.00 b	6.99 ± 0.01 c	7.11 ± 0.01 a	7.03 ± 0.11 a	7.31 ± 0.02 a	Na	Na	Na	Na
	MWA	6.97 ± 0.00 b	7.02 ± 0.00 b	6.95 ± 0.01 b, c	6.98 ± 0.01 b	7.23 ± 0.04 b	7.33 ± 0.01 a	Na	Na	Na
	RNA	6.97 ± 0.01 b	7.06 ± 0.02 a	7.11 ± 0.01 a	7.02 ± 0.01 a	7.32 ± 0.01 a	Na	Na	Na	Na
	RWA	6.98 ± 0.01 b	7.06 ± 0.01 a	7.01 ± 0.06 b	7.02 ± 0.01 a	7.30 ± 0.00 a	7.33 ± 0.00 a	Na	Na	Na

Values are expressed as the mean ± standard deviation (n = 2).

D, day; C, Control; V, vacuum; A, air; M, *M. spicata* EO; R, *R. officinalis* EO; W, warmed emulsion to 53 ± 1 °C; N, at room temperature = not warmed. Na, Not analyzed; the sample had a spoiled appearance with an off-odor.

a, b, c indicate significant differences ($p < 0.05$) between the different dipping treatments at the same sampling time.

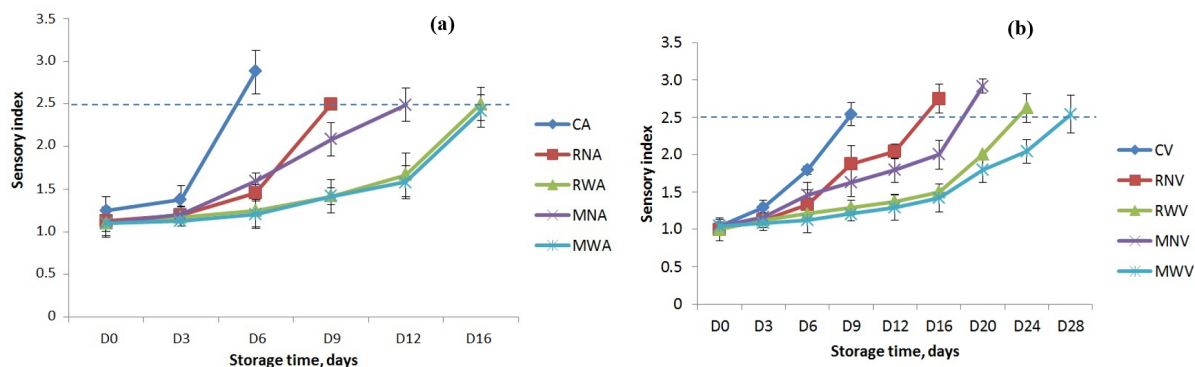


Fig. 1. Sensory assessment of raw chicken legs with different dipping treatments stored at 4 ± 1 °C under (a) air and (b) vacuum packaging. C, control; A, air; V, vacuum; M, *M. spicata* EO; R, *R. officinalis* EO; W, warmed emulsion to 53 ± 1 °C; N, at room temperature = not warmed. Standard deviations are indicated by bars. A score of 2.5 is the cut-off score for unacceptability.

oducing bacteria were approximately $3 \log_{10}$ CFU·g⁻¹, indicating the acceptable microbial quality of chicken meat [20]. However, the initial counts of *Pseudomonas* spp. and psychrotrophic bacteria were approximately $4 \log_{10}$ CFU·g⁻¹. During chilled storage at 4 ± 1 °C, the microbial counts in all groups of chicken legs increased, reaching levels of $6\text{--}7 \log_{10}$ CFU·g⁻¹ at the end of the storage period, depending on the chicken legs group.

At each sampling day, all microbial counts of treated chicken legs were significantly lower than those of control groups throughout the entire storage period ($p < 0.05$). Under air packaging AP, *Enterobacteriaceae*, *Pseudomonas* spp. and H_2S -producing bacteria levels in the control group under air (CA) exceeded the acceptable limit of $6 \log_{10}$ CFU·g⁻¹ by day 6. In contrast, the limit was reached on day 9 for RNA, day 12 for MNA, and day 16 for both the RWA and MWA groups. However, psychrotrophic bacteria in these same groups and on the same days mentioned reached $7 \log_{10}$ CFU·g⁻¹.

Most of the psychrotrophic bacteria found on the carcass immediately after processing are not of intestinal origin but originate from the feathers and feet of the live bird, the processing plant water supply, cooling tanks and processing equipment [21]. However, under vacuum packaging, spoilage bacteria in the control group under vacuum (CV) exceeded the acceptable limit by day 9, indicating rapid microbial proliferation in the absence of antimicrobial intervention. In contrast, treated chicken leg groups experienced delayed bacterial spoilage, with RNV and MNV thresholds reached on day 16, RWV on day 24, and MWV on day 28. These variations highlight the antimicrobial properties of *M. Spicata* and *R. officinalis* essential oils. The longer shelf life observed in MWV (19 days) and RWV (15 days) indicates that the combination of VP and warmed EO emulsions, particularly spearmint, had a strong antimicrobial effect. Additionally, it can be noted that VP itself plays a crucial role by creating an anaerobic environment that suppresses the growth of aerobic spoilage bacteria and limits lipid oxidation [22].

H_2S -producing bacteria play an important role in fish and meat spoilage. Bonilla *et al.* [14] (2007) reported that the end of the shelf life of raw cod fillets was defined as the point at which the H_2S -producing bacteria count reached approximately $7 \log_{10}$ CFU·g⁻¹. However, these bacteria are primarily represented by *Shewanella putrefaciens*, which produces off-odors resembling ammonia [21]. However, the main microorganism responsible for the deterioration of fresh meat is *Pseudomonas*. On the other hand, LAB is the most common spoilage microorganism in vacuum-packed meats [23].

Table 4 shows pH changes in chilled chicken legs under AP and VP conditions. The initial pH values for all chicken sample groups ranged from 6.96 to 7.03.

During AP storage, pH values increased almost to the end of the shelf life, reaching 7.13 for the control group and ranging from 7.31 to 7.33 for the treated groups. The pH values in VP meats initially decreased slightly due to the activity of LAB, which characterizes the natural microflora. These bacteria produce organic acids as part of their metabolism, resulting in a slight pH decrease. pH values rose to 7.30 in the control group and from 7.31 to 7.32 at the end of shelf life. Warmed EO emulsions typically resulted in lower pH values ($p < 0.05$) than those treated with the EO emulsions. The production of ammonia and volatile amines by spoilage bacteria that metabolize proteins and other meat components contributes to an increase in pH values [24].

The spoilage threshold for chicken legs was determined using microbial counts and pH changes. Specifically, spoilage was considered to have occurred when *Enterobacteriaceae*, LAB, H_2S -producing bacteria, *Pseudomonas* spp., and psychrotrophic bacteria exceeded 6 to $7 \log_{10}$ CFU·g⁻¹, which are associated with significant deterioration in meat quality. Furthermore, an increase in pH above 7.3 in most cases indicated advanced protein degradation and increased bacterial metabolic activity, which contributed to undesirable sensory changes, such as the development of off-odors. These combined indicators provide

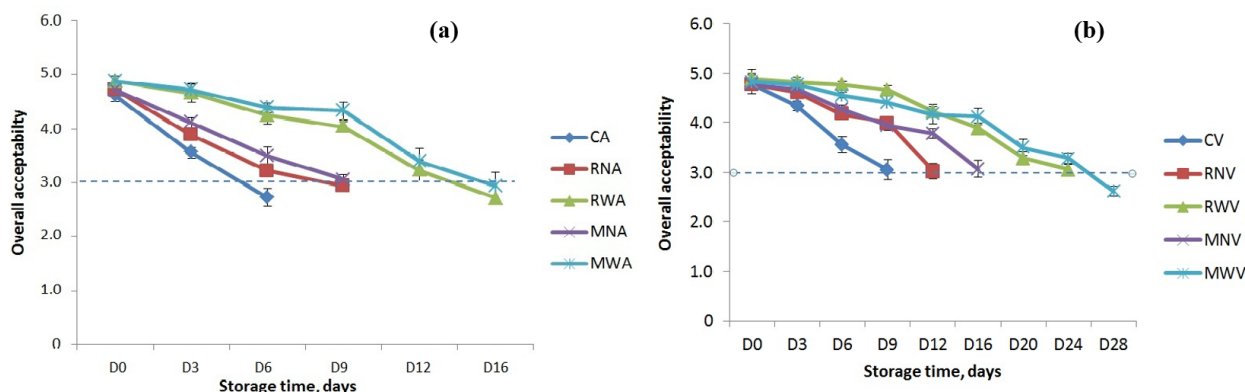


Fig. 2. Change in overall acceptability score of cooked chicken legs with different dipping treatments stored at 4 ± 1 °C under (a) air and (b) vacuum packaging. C, control; A, air; V, vacuum; M, *M. spicata* EO; R, *R. officinalis* EO; W, warmed emulsion to 53 ± 1 °C; N, at room temperature = not warmed. Standard deviations are indicated by bars. A score of 3 is the lower limit of acceptability.

reliable criteria for determining the end of chicken meat's shelf life under various storage conditions.

3.3 Results of Sensory Analysis of Chicken Meat

Fig. 1a,b show the sensory index quality of raw chicken legs stored at 4 ± 1 °C under air and vacuum conditions, with and without dipping treatment. The control groups (CV and CA) had lower quality parameters (color, odor, and texture) compared to the treated groups ($p < 0.05$). Using a sensory score of 2.5 as the unacceptability cut-off score, the control group's shelf life did not exceed 6 days under AP. In contrast, the limit was reached on day 9 for RNA, day 12 for MNA, and day 16 for both the RWA and MWA groups. In contrast, under VP, the observed shelf life was 9 days for CV, 12–16 days for RNV, 16–20 days for MNV, 24 days for RWV, and 28 days for the MWV group. As a result, the sensory-determined shelf lives of both treated and control groups of raw chicken legs under AP and VP generally agreed with those determined by microbial analysis. Exceptions were the RNV and MNV groups, which differed by about two days.

According to Russell *et al.* [21] (1995), eviscerated fresh carcasses have an average shelf life of 6 to 8 days when stored at 4.4 °C. Dipping chicken legs in a 0.4% *M. spicata* or *R. officinalis* EO emulsion warmed to 53 ± 1 °C extended shelf life by 10 days compared to the control group CA. However, under VP, warmed emulsions of *R. officinalis* and *M. spicata* EO increased shelf life by 15 and 19 days, respectively, compared to the control group CV. According to a 2020 Kanatt study [25], packaging films increase the shelf life of food products while also ensuring their safety by extending the lag phase and reducing bacterial growth. The sensory evaluation results (odor, texture, and taste attributes) of cooked chicken legs stored under AP and VP are shown as overall acceptability scores in Fig. 2 for both AP (Fig. 2a) and VP (Fig. 2b). The overall acceptability score of cooked chicken decreased with storage time in all groups.

Significant differences ($p < 0.05$) were observed between the control and treated groups under both AP and VP conditions. Thus, panelists strongly preferred cooked chicken legs treated with various EO emulsions. This finding could be attributed to the bioactive phenolic compounds found in *M. spicata* and *R. officinalis* EO. Using a sensory score of 3 as the acceptability threshold, the control group was no longer acceptable after 6 days (2.68). However, this limit was reached on day 9 for both RNA and MNA, 12–16 days for RWA, and 16 days for MWA. While under VP, the acceptability limit was 9 days for the control group, 12 days for RNV, 16 days for MNV, 24 days for RWV, and 24–28 days for MWV groups. There were no significant differences ($p > 0.05$) between RN and MN or RW and MW under AP and VP conditions. At the rejection points, the general acceptability scores of cooked chicken legs were largely consistent with the raw samples from each dipping treatment.

In this study, both EO emulsions demonstrated antimicrobial activity under all conditions. This effect is due to *R. officinalis* EO's high concentration of oxygenated monoterpenes, particularly eucalyptol and camphor, which demonstrated antibacterial properties by increasing membrane permeability and disrupting bacterial metabolism [26].

A previous study found that adding rosemary essential oil (0.2% v/w) to vacuum-packed precooked chicken meat reduced spoilage bacteria but only extended the shelf life by 7 days [27]. Under various conditions, spearmint EO outperforms rosemary EO in terms of antimicrobial activity despite the latter's high eucalyptol content (52.09%). Similarly, in food science, Burt [19] (2004) reviewed and ranked the antibacterial effects of essential oils, with mint outperforming rosemary. This finding could be explained by the stability of *M. spicata* EO, which retained its activity at 80 °C and was effective for up to 24 months when stored at room temperature [28].

Furthermore, its bioactive compounds, particularly carvone, are now important in the agri-food chain due to

their inhibitory effect on various microorganisms that cause food spoilage; however, the mechanism of action is not fully understood. Some authors have reported that it works by influencing the metabolic energy status of the microbial cell [28]. Additionally, carvone exhibits antimicrobial activity by disrupting bacterial cell membranes and interfering with key enzymatic functions, leading to impaired cellular homeostasis [19]. Another hypothesis to explain the higher activity of *M. spicata* EO compared to *R. officinalis* used in this study to immerse chicken legs is that spearmint's antibacterial activity is strongly related to food characteristics, which increase in low-fat products [28]; chicken meat is known for its low-fat content.

Additionally, *M. spicata* EO and sodium chloride have a synergistic effect. When salt is added to chicken meat, the antimicrobial activity of this essential oil may increase dramatically. The current findings are consistent with previous research, which found that adding 0.5% *M. spicata* to raw minced camel meat stored at 4 °C extended the shelf life to 12 days while decreasing total mesophilic, psychrotrophic bacteria, *Pseudomonas* spp., and *Enterobacteriaceae* counts by about 1–2 log CFU/g in treated samples compared to the control [29]. In another study, authors found that edible films enriched with 2% *M. spicata* EO used for cheese coating improved shelf life and had antimicrobial activity against pathogenic bacteria [30]. However, another study used a lower concentration of *M. spicata* EO than ours and found the same results in extending the shelf life of vacuum-packed chicken thighs by 16 days, but only with 0.2% spearmint EO [31].

In this study, the chicken legs were immersed in EO emulsions warmed to the same temperature and duration as soft scalding, which involves immersing the carcass in warm water at 53 °C for 3 minutes to effectively remove feathers. This aids in the release of feather quills from feather follicles located within the skin. As a result, warmed EO emulsions perform better than room-temperature emulsions. This finding could be explained by the penetration of EO emulsions into the skin's dilated pores, which host a variety of bacteria. Chicken legs stored in air or vacuum were found to be better preserved with warmed EO emulsions of *M. spicata* and *R. officinalis* at 0.4% while maintaining acceptable sensory and microbial quality during chilled storage. As expected, the ideal treatment for preserving raw and cooked chicken legs was MWV.

4. Conclusion

Dipping chicken meat in warmed emulsions of *Mentha spicata* and *Rosmarinus officinalis* essential oils (0.4%) was found to be an effective strategy for increasing shelf life under both aerobic and vacuum packaging conditions. Warming the EO emulsions significantly increased their antibacterial activity. Notably, chicken legs immersed in warmed emulsions of *R. officinalis* or *M. spicata* and vacuum-packed had a shelf life of 15 and 19 days longer,

respectively, than the control group. This finding demonstrates the ability of this natural preservation method to inhibit spoilage bacteria while preserving the sensory properties of the meat. Furthermore, the shelf lives determined by sensory tests were generally consistent with those discovered through microbial analysis.

In addition to their antimicrobial effects, the potential antioxidant properties of warmed essential oils necessitate further research to comprehensively elucidate their function in meat preservation. Moreover, subsequent investigations should concentrate on enhancing the utilization of essential oils in meat products, especially regarding potential interactions between the bioactive compounds of essential oils and meat constituents that may affect their effectiveness and sensory attributes. Evaluating consumer acceptability and regulatory considerations of essential oil-based treatments will be vital for their effective integration into the food industry.

Availability of Data and Materials

The datasets generated and/or analyzed during the current study are available in the formats specified on the journal's website. Additional data may be available from the corresponding author upon reasonable request.

Author Contributions

ZM and TL designed and performed the study. KG contributed to the interpretation of results and critically revised the manuscript. AB and RNB provided help and analyzed the data. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

Not applicable.

Acknowledgment

We thank the Microbiology Station team of the Laboratory of Quality Control and Repression of Frauds, Djelfa, Algeria, for their support.

Funding

This research received no external funding.

Conflict of Interest

The authors declare no conflict of interest.

References

- [1] Zhou GH, Xu XL, Liu Y. Preservation technologies for fresh meat - a review. *Meat Science*. 2010; 86: 119–128. <https://doi.org/10.1016/j.meatsci.2010.04.033>.
- [2] Soro AB, Noore S, Hannon S, Whyte P, Bolton DJ, O'Donnell C, Tiwari BK. Current sustainable solutions for extending the

- shelf life of meat and marine products in the packaging process. *Food Packaging and Shelf Life*. 2021; 29: 100722. <https://doi.org/10.1016/j.foodpack.2021.100722>.
- [3] Nethra PV, Sunooj KV, Aaliya B, Navaf M, Akhila PP, Sudheesh C, *et al.* Critical factors affecting the shelf life of packaged fresh red meat—A review. *Measurement: Food*. 2023; 10: 100086. <https://doi.org/10.1016/j.meaf.2023.100086>.
 - [4] Bekhit AEDA, Holman BW, Giteru SG, Hopkins DL. Total volatile basic nitrogen (TVB-N) and its role in meat spoilage: A review. *Trends in Food Science & Technology*. 2021; 109: 280–302. <https://doi.org/10.1016/j.tifs.2021.01.006>.
 - [5] Corradini MG. Shelf Life of Food Products: From Open Labeling to Real-Time Measurements. *Annual Review of Food Science and Technology*. 2018; 9: 251–269. <https://doi.org/10.1146/annurev-food-030117-012433>.
 - [6] Fletcher B, Mullane K, Platts P, Todd E, Power A, Roberts J, *et al.* Advances in meat spoilage detection: A short focus on rapid methods and technologies. *CyTA-Journal of Food*. 2018; 16: 1037–1044. <https://doi.org/10.1080/19476337.2018.1525432>.
 - [7] Karwowska M, Łaba S, Szczepański K. Food loss and waste in meat sector- why the consumption stage generates the most losses? *Sustainability*. 2021; 13: 6227. <https://doi.org/10.3390/su13116227>.
 - [8] Abdulla M, Martin RC, Gooch M, Jovel E. The importance of quantifying food waste in Canada. *Journal of Agriculture, Food Systems, and Community Development*. 2013; 3: 137–151. <https://doi.org/10.5304/jafscd.2013.032.018>.
 - [9] Irshad A, Arun TS. Scalding and its significance in livestock slaughter and wholesome meat production. *International Journal of Livestock Research*. 2013; 3: 45–53.
 - [10] European Pharmacopoeia 6.0. Determination of essential oils in herbal drug. 2008. Available at: <http://www.uspbpep.com/ep60/essential%20oils%202098e.pdf> (Accessed: 20 March 2025).
 - [11] Adams RP. Identification of essential oil components by gas chromatography/mass spectrometry (pp. 804). 4th edn. Allured Publishing Co. Carol Stream: Illinois. 2007.
 - [12] Alessandrini L, Caprioli G, Faiella F, Fiorini D, Galli R, Huang X, *et al.* A shelf-life study for the evaluation of a new biopackaging to preserve the quality of organic chicken meat. *Food Chemistry*. 2022; 371: 131134. <https://doi.org/10.1016/j.foodchem.2021.131134>.
 - [13] Malle P, Valle M, Demarque P, Eb P, Tailliez R. Detection and enumeration of H2S+ bacteria: Application to *Shewanella putrefaciens* (CIP). *Journal of Rapid Methods & Automation in Microbiology*. 1998; 6: 93–102. <https://doi.org/10.1111/j.1745-4581.1998.tb00190.x>.
 - [14] Bonilla AC, Sveinsdottir K, Martinsdottir E. Development of Quality Index Method (QIM) scheme for fresh cod (*Gadus morhua*) fillets and application in shelf life study. *Food Control*. 2007; 18: 352–358. <https://doi.org/10.1016/j.foodcont.2005.10.019>.
 - [15] Kreyenschmidt J, Hübner A, Beierle E, Chonsch L, Scherer A, Petersen B. Determination of the shelf life of sliced cooked ham based on the growth of lactic acid bacteria in different steps of the chain. *Journal of Applied Microbiology*. 2010; 108: 510–520. <https://doi.org/10.1111/j.1365-2672.2009.04451.x>.
 - [16] Patsias A, Chouliara I, Badeka A, Savvaidis IN, Kontominas MG. Shelf-life of a chilled precooked chicken product stored in air and under modified atmospheres: microbiological, chemical, sensory attributes. *Food Microbiology*. 2006; 23: 423–429. <https://doi.org/10.1016/j.fm.2005.08.004>.
 - [17] González-Minero FJ, Bravo-Díaz L, Ayala-Gómez A. *Rosmarinus officinalis* L. (Rosemary) An ancient plant with uses in personal healthcare and cosmetics. *Cosmetics*. 2020; 7: 77. <https://doi.org/10.3390/cosmetics7040077>.
 - [18] Bardaweel SK, Bakchiche B, ALSalamat HA, Rezzoug M, Gherib A, Flamini G. Chemical composition, antioxidant, antimicrobial and Antiproliferative activities of essential oil of *Mentha spicata* L. (Lamiaceae) from Algerian Saharan atlas. *BMC Complementary and Alternative Medicine*. 2018; 18: 201. <https://doi.org/10.1186/s12906-018-2274-x>.
 - [19] Burt S. Essential oils: their antibacterial properties and potential applications in foods—a review. *International Journal of Food Microbiology*. 2004; 94: 223–253. <https://doi.org/10.1016/j.ijfoodmicro.2004.03.022>.
 - [20] Projahn M, Pacholewicz E, Becker E, Correia-Carreira G, Bandick N, Kaesbohrer A. Reviewing Interventions against Enterobacteriaceae in Broiler Processing: Using Old Techniques for Meeting the New Challenges of ESBL *E. coli*? *BioMed Research International*. 2018; 2018: 7309346. <https://doi.org/10.1155/2018/7309346>.
 - [21] Russell SM, Fletcher DL, Cox NA. Spoilage bacteria of fresh broiler chicken carcasses. *Poultry Science*. 1995; 74: 2041–2047. <https://doi.org/10.3382/ps.0742041>.
 - [22] Hernández-Macedo ML, Barancelli GV, Contreras-Castillo CJ. Microbial deterioration of vacuum-packaged chilled beef cuts and techniques for microbiota detection and characterization: a review. *Brazilian Journal of Microbiology*. 2011; 42: 1–11. <https://doi.org/10.1590/S1517-83822011000100001>.
 - [23] Jay JM, Loessner MJ, Golden DA. Fresh meats and poultry. *Modern Food Microbiology* (pp. 63–99). Springer New York: NY. 2005.
 - [24] Kaban G. Changes in the composition of volatile compounds and in microbiological and physicochemical parameters during pastirma processing. *Meat Science*. 2009; 82: 17–23. <https://doi.org/10.1016/j.meatsci.2008.11.017>.
 - [25] Kanatt SR. Development of active/intelligent food packaging film containing *Amaranthus* leaf extract for shelf life extension of chicken/fish during chilled storage. *Food Packaging and Shelf Life*. 2020; 24: 100506. <https://doi.org/10.1016/j.fpsl.2020.100506>.
 - [26] Sharifi-Rad J, Sureda A, Tenore GC, Daglia M, Sharifi-Rad M, Valussi M, *et al.* Biological Activities of Essential Oils: From Plant Chemoecology to Traditional Healing Systems. *Molecules*. 2017; 22: 70. <https://doi.org/10.3390/molecules22010070>.
 - [27] Ntzimani AG, Giatrakou VI, Savvaidis IN. Combined natural antimicrobial treatments EDTA, lysozyme, rosemary and oregano oil on semi cooked coated chicken meat stored in vacuum packages at 4°C: Microbiological and sensory evaluation. *Innovative Food Science & Emerging Technologies*. 2010; 11: 187–196. <https://doi.org/10.1016/j.ifset.2009.09.004>.
 - [28] Morcia C, Tumino G, Ghizzoni R, Terzi V. *Carvone* (*Mentha spicata* L.) oils. Essential oils in food preservation, flavor and safety. Academic Press: New York. 2016.
 - [29] Shahbazi Y, Karami N, Shavisi N. Effect of *Mentha spicata* essential oil on chemical, microbial, and sensory properties of minced camel meat during refrigerated storage. *Journal of Food Safety*. 2018; 38: e12375. <https://doi.org/10.1111/jfs.12375>.
 - [30] Kavas G, Kavas N. The effects of mint (*Mentha spicata*) essential oil fortified edible films on the physical, chemical and microbiological characteristics of lor cheese. *Journal of Food, Agriculture and Environment*. 2014; 12: 40–45.
 - [31] Kačániová M, Petrová J, Terentjeva M, Mellen M, Kántor A, Kluz M, *et al.* Microbiological quality of chicken thighs after vacuum packaging, EDTA, *Coriandriaetheroleum* and *Mentha spicata aetheroleum*. *Scientific Papers Animal Science and Biotechnologies*. 2015; 48: 143–143.