

## Effects of spearmint and pennyroyal essential oils on the growth of *Staphylococcus aureus* inoculated in chocolate mousse during chilled storage and abused temperature

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### Summary

The chemical composition of *Mentha spicata* and *M. pulegium* essential oils, and their effect at various contents (2 ml·kg<sup>-1</sup> and 4 ml·kg<sup>-1</sup>) on the survival and growth of *Staphylococcus aureus* inoculated to chocolate mousse during chilled storage and abused temperature were investigated. Yields of essential oils of the air-dried aerial parts of *M. spicata* and *M. pulegium* were 0.9 % and 1.2 % (w/w, on dry weight basis), respectively. Carvone (53.3 %), D-limonene (16.9 %), eucalyptol (8.9 %) and terpinen-4-ol (2.7 %) were the highest represented components of spearmint essential oil, while pennyroyal essential oil contained mainly piperitenone (41.4 %), pulegone (27.1 %), *cis*-menthone (9.1 %) and piperitone (9 %). Incorporation of *M. spicata* or *M. pulegium* essential oils in chocolate mousse led to a significant ( $p < 0.05$ ) reduction of *Staph. aureus* growth during the entire storage time, as compared to control, indicating that these oils contain high levels of bioactive antimicrobials. Their efficiency was not also affected by abused temperature condition and/or essential oil contents in chocolate mousse, suggesting that these essential oils are potential candidates to be used as alternatives of synthetic preservatives in pastry products to control foodborne pathogens and improve product safety and quality.

### Keywords

spearmint; pennyroyal; essential oil; *Staphylococcus aureus*; chocolate mousse; abused temperature

Chocolate mousse is a famous classic French desserts and occupies an interesting portion of the food market covering sectors such as supermarkets, restaurants as well as institutional and catering businesses. However, non-conformity of standards of food hygiene may lead to an increased incidence of contamination with food-borne pathogens. The source of this contamination includes contaminated ingredients, poor control of storage times and temperature, cross-contamination during preparation, inadequate cleaning, poor food preparation practices and contamination by infected food handlers [1]. Important food-borne pathogens that have been responsible for the outbreaks of food poisoning often found in bakery

products basically include *Staphylococcus aureus*, *Bacillus cereus* and several serovars of *Escherichia coli*, including O157:H7 [1]. Among them, *Staph. aureus* is a major safety concern.

*Staph. aureus* has a great importance in the food industry due to its ability to grow and produce a range of extracellular protein toxins in food products. Staphylococcal food poisoning is characterized by rapid (in 2–6 h) onset of symptoms after the consumption of food containing relevant amounts of enterotoxin(s). Patients have symptoms such as watery diarrhea, violent vomiting, abdominal pain, headache and low blood pressure [1]. It is generally accepted that *Staph. aureus* contents of  $> 10^5$  CFU·g<sup>-1</sup> are needed for

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the production of sufficient amount of staphylococcal enterotoxin(s) to cause illness [2]. However, some authors estimate that staphylococcal enterotoxins were detected in food, although the count of *Staph. aureus* was  $< 10^5$  CFU·g<sup>-1</sup> [3]. For this reason, control of *Staph. aureus* in food products is a critical issue in public health policy.

Nowadays, consumers have shown increasing unease towards the use of synthetic antimicrobials in foods, due to concerns on their side effects such as carcinogenicity or teratogenicity. As a result, natural alternative antimicrobial agents such as essential oil compounds of aromatic plants and other plant extracts were used to inhibit the growth of serious foodborne pathogens [4]. Spearmint (*Mentha spicata*) and pennyroyal (*Mentha pulegium*) have been Generally Recognized As Safe (GRAS) [5]. Leaves, flowers and the stems of these plants are frequently used as additives to many foods, including chocolate mousse, to offer aroma and flavour [6]. Recent studies demonstrated in vitro antimicrobial activity of their essential oils against food-borne pathogens, including *Staph. aureus*, and this is valid also regarding their components [7, 8]. It has been postulated that *Escherichia coli* and *Pasteurella multocida* are more sensitive to the essential oils of botanicals than *Staph. aureus* and *Bacillus subtilis* [9].

*Staph. aureus* growth and the staphylococcal enterotoxin production in contaminated food products at temperature abuse, estimated by predictive microbiology, can be used as a tool for preventing food-borne diseases [10]. Given these considerations, the aim of the present work was to investigate (1) the chemical composition of *Mentha spicata* and *M. pulegium* essential oils, and (2) the survival and growth behaviour of *Staph. aureus* in chocolate mousse treated with spearmint and pennyroyal essential oils at contents of 2 ml·kg<sup>-1</sup> and 4 ml·kg<sup>-1</sup>, when stored at  $3 \pm 1$  °C or under temperature abuse conditions.

## MATERIALS AND METHODS

### Plant materials

*Mentha pulegium* and *M. spicata* were collected during the month of October 2019 in Laghouat region of Algeria. Cultivated *M. pulegium* and *M. spicata* were harvested in the regions of El Merdja and Bordj Senouci, province of Laghouat, Algeria, respectively. Identification of the plants was carried out by the botanists of the laboratory of Plant Ecology, Department of Agricultural Sciences, Amar Telidji University, Laghouat, Algeria. The aerial parts were shade-dried at room

temperature for 1 month and then stored in sealed paper bags until their use for analyses for a maximum of 1 month.

### Isolation of essential oils

According to the British Pharmacopoeia method [11], essential oils were extracted by hydrodistillation from the dried samples during approximately 4 h using a Clavenger-type apparatus (Schott Duran, Mainz, Germany). The essential oils were then stored in sealed glass vials at  $3 \pm 1$  °C until use for a maximum of 1 week.

### Analysis of essential oil

An Agilent 7000 Series Triple Quad gas chromatography-mass spectroscopy (GC-MS) instrument (Agilent Technologies, Santa Clara, California, USA), equipped with a HP-5ms column (30 m length  $\times$  0.25 mm inner diameter  $\times$  0.25  $\mu$ m film thickness, 5% phenyl methylpolysiloxane; Agilent Technologies) was used to determine the chemical composition of the obtained oils. The GC-MS analysis was carried out at following conditions: flow rate of carrier gas (helium, purity 99.95 %) 1 ml·min<sup>-1</sup>; splitless injection mode (flow 40 ml·min<sup>-1</sup>, ratio 20) ionization voltage 70 eV; mass spectra range 50–600 *m/z*; column oven temperature programmed from 50 °C to 240 °C (3 min) and injector temperature 250 °C. The injection volume of the diluted sample (1% *n*-hexane, v/v) was 1  $\mu$ l. Mass spectra from National Institute of Standards and Technology (NIST, Gaithersburg, Maryland, USA) and Wiley (Wiley and Sons, Hoboken, New Jersey, USA) libraries or those reported in the literature [12] were used to identify the oil components. From the peak area of the chromatogram, relative percentages of the oil constituents were calculated using the software provided by the manufacturer.

### Preparation of chocolate mousse samples

The chocolate mousse was manually prepared in the laboratory of the Department of Agricultural Sciences, Amar Telidji University, Laghouat, Algeria, following the homemade formulation previously described by HERME [13]. Chocolate butter cream sample recipe contained the following ingredients (w/w): chocolate bar 36 %, liquid cream 24 %, butter 4 %, egg (white and yolk) 33 % and sugar 3 %. All samples were prepared under aseptic conditions. The details are well described by HERME [13].

### Preparation of inocula

*Staph. aureus* ATCC 25923 was used as the test organism, having been stored at  $-80$  °C in brain

heart infusion (BHI) broth (Merck, Darmstadt, Germany) with 10% glycerol added as a cryoprotectant. Fresh culture was prepared by thawing it, transferring a loopful into BHI broth and then incubating it for 24 h at 35 °C. After that, 0.1 ml was spread onto BHI agar plates and incubated at 35 °C for 48 h. To obtain a working suspension at final content of  $10^8$  CFU·ml<sup>-1</sup>, 4–5 colonies were dispersed in 0.1% buffered peptone water (BPW; Difco, Sparks, Maryland, USA) and adjusting the suspension turbidity to a 0.5 McFarland standard at 600 nm using a UV/VIS spectrophotometer 6405 (Jenway, Stone, United Kingdom).

#### Treatments and inoculation of samples

For each essential oil treatment, 500 g of chocolate mousse, previously tested negative for *Staph. aureus*, was weighed in a sterile homogenization bag (SFB-0410; Spiral Biotech, Bethesda, Maryland, USA) under sterile conditions. Thereafter, the content of each bag was divided into 5 bags, each group containing 100 g of chocolate mousse. The control group was not treated by any essential oil, two groups were treated with 2 ml·kg<sup>-1</sup> of essential oils, and two other groups were treated with 4 ml·kg<sup>-1</sup> of essential oils. Prior to the experiment, the oil content of 2 ml·kg<sup>-1</sup> was chosen as an acceptability limit based on preliminary tests on flavour perception of chocolate mousse, while 4 ml·kg<sup>-1</sup> of essential oil was considered as maximum content of added oil. Then, the contents of each group were mixed by a stomacher for 2 min to attain uniform distribution of the added compounds. After treatment, all groups were inoculated with 1 ml of *Staph. aureus* suspension so that the final content was approximately  $10^6$  CFU·g<sup>-1</sup>. After inoculation, the contents of the bags were mixed by the stomacher for 2 min to ensure proper distribution of *Staph. aureus* cells.

#### Storage conditions

After inoculation and treatments, each group was put into a polystyrene box with a lid. One of each treated group was stored at  $3 \pm 1$  °C for 24 h, followed by 10 h at  $25 \pm 1$  °C and then at  $3 \pm 1$  °C until the end of storage (for 72 h, which is the expected shelf-life for cakes in confectioneries). This abused temperature condition was chosen to simulate a cold chain disruption during one or more stages in the storage of fresh pastry in manufacturing plant, during transport and during storage in confectioneries or in domestic refrigerators. Control and treated groups were stored at  $3 \pm 1$  °C for 72 h. Groups were tested for *Staph. aureus* counts after 0 h, 24 h, 48 h and 72 h of storage.

#### *Staphylococcus aureus* enumeration

*Staph. aureus* enumeration was performed on the chocolate mousse, with the aim to assess quantitatively the effect of *M. pulegium* and *M. spicata* essential oils at 2 ml·kg<sup>-1</sup> and 4 ml·kg<sup>-1</sup> on survival and growth of *Staph. aureus* in chocolate mousse under chilled storage and temperature abuse conditions. A 10 g sample was diluted 1:10 in a stomacher bag using 90 ml sterile 0.1% BPW and, subsequently, homogenized for 2 min. Serial dilutions of homogenized samples were made by adding 1 ml of diluted sample to 9 ml BPW. Sample dilutions (0.1 ml) were spread-plated on Baird-Parker agar base (Oxoid, Basingstoke, United Kingdom) supplemented with 5% (v/v) tellurite egg yolk emulsion (Difco). Plates were incubated for 48 h at 37 °C. On Baird-Parker agar, typical *Staph. aureus* colonies are shiny black with a distinctive clear zone in the surrounding agar after the incubation period. All experiments were performed in triplicate and the counts of *Staph. aureus* were converted to logarithms of colony-forming units.

#### Statistical analysis

Means and standard deviations of *Staph. aureus* counts were calculated from the data obtained from three repetitions for each tested group. One-way analysis of variance (ANOVA) and Duncan's multiple range tests were used for data analysis. All statistics were conducted using SigmaPlot for Windows, version 12.0 (Systat Software, San Jose, California, USA).

## RESULTS AND DISCUSSION

#### Chemical characterization of essential oils

Essential oil yields from the air-dried aerial parts of the representative samples of *M. spicata* and *M. pulegium* were 0.9 % and 1.2 % (w/w, on dry weight basis), respectively. In accordance with our results, the amount of essential oil obtained from *M. spicata* harvested in two other regions of Algeria was previously found to be 1.0 % [14] and 1.1 % [15]. However, ALEXOPOULOS *et al.* [7] and XYLIA *et al.* [16] showed a much higher rate of 2.3 % and 2.6 %, respectively. In the present study, the yield of essential oil from *M. pulegium* (1.2 %) was similar to other previous works, i.e. 1.5 % [17], and 1.1 % [18]. However, aerial parts of *M. pulegium* collected from Morocco were previously shown to yield 2.7 % [8] and 5.4 % essential oil [19].

Tab. 1 shows the main chemical components identified in essential oils of *M. spicata*

and *M. pulegium* as found by GC-MS analysis, together with the retention times of the compounds. Thirty-four compounds, corresponding to 99.9 % of total chemical compounds in the oil of *M. spicata*, were identified. Carvone (53.3 %), D-limonene (16.9 %), eucalyptol (8.9 %) and terpinen-4-ol (2.7 %) were the dominant components of spearmint essential oil (Fig. 1). It must be noted that this oil was mainly dominated by oxygenated monoterpenes and monoterpene hydrocarbons representing 70.2 % and 23.8 %, respectively. In the current study, the spearmint essential oil was characterized by the dominant presence of carvone (53.3 %), in agreement with our previous results [20] and with results of other authors [14], who found carvone level at 52.2 % and 49.5 % in Algerian Saharan atlas, respectively. On the other hand, BRAHMI et al. [15] found it at 48.5 % in Bejaia province (east northern region of Algeria). However, the amount of carvone obtained from *M. spicata* essential oil in the present study was lower compared to previous works (75.6 % [16] and 76.6 % [21]). Despite the moderate carvone level in our sample, it has been reported that the

biological activities of spearmint oil are preserved when carvone ratio is greater than 51 % while limonene is also present [22].

In *M. pulegium* essential oil, twenty-seven compounds were identified, accounting for 99.8 % of the total chemical compounds, only dominated by oxygenated monoterpenes (93.2 %; Tab. 1). This essential oil contained mainly piperitenone (41.4 %), pulegone (27.1 %), *cis*-menthone (9.1 %) and piperitone (9 %; Fig. 2). However, previous studies with the same plant harvested in Turkey, Uruguay, Iran, Bulgaria, Egypt, Spain, Portugal, Tunisia, Greece, Morocco, Iran, India and Algeria reported pulegone as the major component of the essential oil, but in different proportions [18]. Similar to our results, KOKKINI et al. [23] reported piperitenone (39.8 %) and pulegone (34.8 %) as the dominant constituents of *M. pulegium* essential oil of Island of Samothraki (near Chora, Greece), contrary to another study in which menthone (35.9 %) and pulegone (23.2 %) were reported as the major compounds, while the content of piperitenone was quite low (0.4 %) [24]. Nonetheless, there is a significant variability

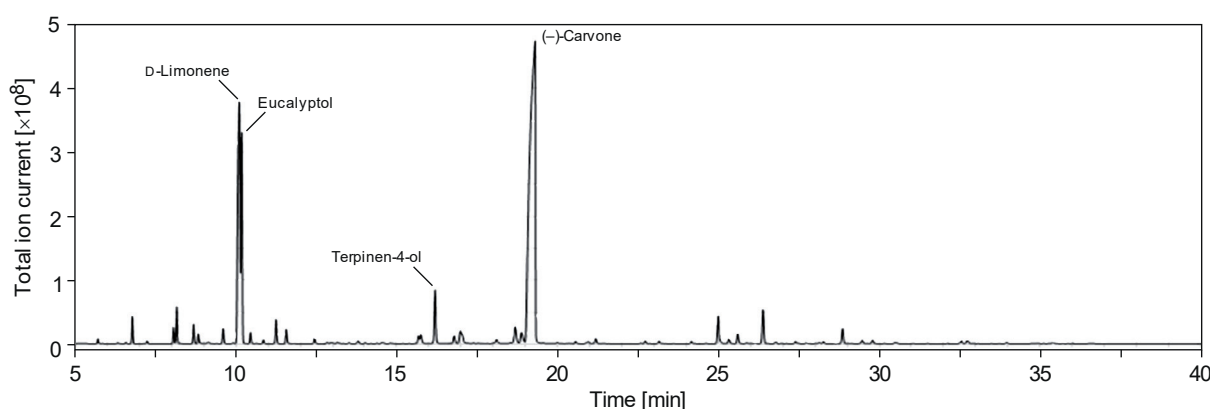


Fig. 1. Gas chromatography-mass spectrometry chromatogram of *Mentha spicata* essential oil.

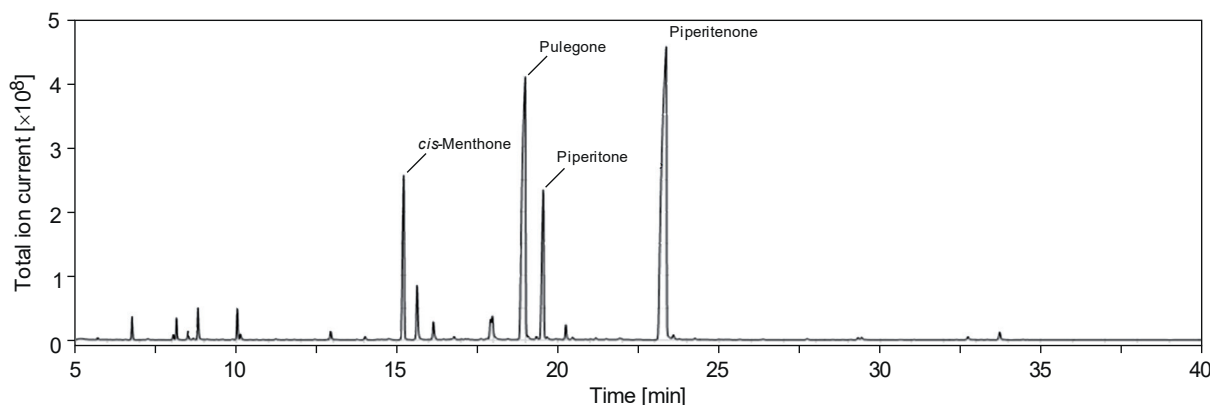


Fig. 2. Gas chromatography-mass spectrometry chromatogram of *Mentha pulegium* essential oil.

**Tab. 1.** Chemical constituents of the essential oils of *Mentha pulegium* and *Mentha spicata* characterized using gas chromatography-mass spectrometry.

No.	Components	Retention time [min]	Level [%]	
			<i>M. pulegium</i>	<i>M. spicata</i>
1	$\alpha$ -Pinene	6.77	0.7	0.9
2	Sabinene	8.05	0.2	0.6
3	$\beta$ -Pinene	8.15	0.8	1.4
4	3-Octanone	8.51	0.3	nd
5	$\beta$ -Myrcene	8.68	nd	0.7
6	3-Octanol	8.82	1.1	0.4
7	$\alpha$ -Terpinene	9.60	nd	0.6
8	D-Limonene	10.04	1.2	16.9
9	Eucalyptol	10.14	0.2	8.9
10	<i>cis</i> - $\beta$ -Ocimene	10.45	nd	0.4
11	<i>trans</i> - $\beta$ -Ocimene	10.85	nd	0.2
12	$\gamma$ -Terpinene	11.25	nd	1.0
13	<i>cis</i> -Sabinene hydrate	11.56	nd	0.6
14	Terpinolen	12.44	nd	0.3
15	Linalool	12.94	0.4	nd
16	3-Carene	13.80	nd	0.2
17	3-Octyl acetate	14.01	0.1	nd
18	<i>cis</i> -Menthone	15.21	9.1	nd
19	<i>trans</i> -Menthone	15.62	2.7	nd
20	endo-Borneol	15.67	nd	0.4
21	Terpineol	15.75	nd	0.5
22	Isopulegone	16.13	0.9	nd
23	Terpinen-4-ol	16.19	nd	2.7
24	$\alpha$ -Terpineol	16.78	0.1	0.5
25	1,6-Dihydrocarveol	16.98	nd	0.8
26	<i>cis</i> -Dihydrocarvone	17.04	nd	0.4
27	<i>cis</i> -Pulegone oxide	17.90	0.9	nd
28	8,9-Dehydrothymol	17.96	1.3	nd
29	<i>cis</i> -Carveol	18.10	nd	0.3
30	Carveol	18.68	nd	1.1

No.	Components	Retention time [min]	Level [%]	
			<i>M. pulegium</i>	<i>M. spicata</i>
31	Pulegone	18.98	27.1	0.8
32	D-Carvone	19.08	0.2	nd
33	(-)-Carvone	19.30	nd	53.3
34	Cyclohexanone, 3-methyl-	19.33	0.2	nd
35	Piperitone	19.54	9.0	nd
36	1,5,9,11-Tridecatetraene, 12-methyl-, (E,E)-	19.67	0.1	nd
37	Isopiperitenone	20.24	0.7	nd
38	Dihydroedulan	20.95	nd	0.2
39	Dihydroedulan II	21.18	nd	0.2
40	Carvacrol	21.93	0.2	nd
41	Piperitenone	23.36	41.4	nd
42	<i>trans</i> -Pulegone Oxide	23.58	0.2	nd
43	(-)- $\beta$ -Bourbonene	24.99	nd	1.6
44	$\beta$ -Elemen	25.32	nd	0.2
45	<i>cis</i> -Jasmone	25.59	nd	0.5
46	Caryophyllene	26.38	nd	1.8
47	Germacrene D	28.85	nd	0.8
48	Mintlactone	29.43	0.1	nd
49	Bicyclogermacrene	29.47	nd	0.2
50	Spathulenol	32.54	nd	0.2
51	Caryophyllene oxide	32.73	0.2	0.3
52	Humulene oxide II	33.72	0.4	nd

Total identified			99.8	99.9
Monoterpenes hydrocarbons			2.9	23.8
Oxygenated monoterpenes			93.2	70.2
Sesquiterpenes hydrocarbons			nd	4.6
Oxygenated sesquiterpenes			0.6	0.5
Others			3.1	0.8
Oil yield (w/w)			1.2	0.9

Retention time is given in elution order from the HP-5ms column. Level is based on peak area of the chromatogram. nd – not detected.



ity in the chemical composition of *M. spicata* and *M. pulegium* essential oils as compared with previously published data. Such variability may be attributed to various factors, mainly to chemotypes, climate, soil type, age of the leaves, fertility regime and methods of drying and distillation [18]. In fact, these factors affect and regulate the metabolism of essential oils via repression or activation of the enzyme genes responsible of synthesis of essential oils. This regulation is mediated by several epigenetic factors such as DNA methylation, remodelling of chromatin and histones modifications [19].

#### Effects of essential oils on *Staphylococcus aureus*

Tab. 2 shows the counts of *Staph. aureus* inoculated in chocolate mousse and treated with essential oils at 2 ml·kg<sup>-1</sup> and 4 ml·kg<sup>-1</sup> during chilled storage and abused temperature. For *M. spicata* essential oil treatment, the counts of *Staph. aureus* were significantly ( $p < 0.05$ ) reduced in treated groups during entire storage time, as compared with control. On the first day of storage, a reduction by 4 log CFU·g<sup>-1</sup> was observed in treated groups, while a small but significant increase was recorded after 48 h of storage in group 1 (4 ml·kg<sup>-1</sup> of this essential oil at 3 ± 1 °C) and group 4 (2 ml·kg<sup>-1</sup> of this essential oil at abused temperature), as compared with other treated groups. At the end of storage time, the counts of *Staph. aureus* remained stable and did not exceed 4 log CFU·g<sup>-1</sup> in all groups treated with *M. spicata* essential oil, except for group 4 treated with 2 ml·kg<sup>-1</sup> of essential oil under stimulated abuse temperature. The lowest counts ( $p < 0.05$ )

of *Staph. aureus* (3.53 log CFU·g<sup>-1</sup>) were recorded for group 2 treated with 4 ml·kg<sup>-1</sup> of *M. spicata* essential oil under stimulated abuse temperature. In general, it must be pointed out that *M. spicata* essential oil caused a significant reduction of *Staph. aureus* counts in chocolate mousse without remarkable differences between essential oil contents and/or storage conditions (Tab. 2). However, the use of *M. spicata* essential oils in food matrices is often limited due to flavour considerations. In agreement with our findings, SHAHBAZI and SHAVISI [25] studied the effects of *M. spicata* essential oil (0.1 % and 0.2 %) against *Staph. aureus* in fish soup at various storage conditions. They found that the groups treated with 0.2 % spearmint essential oil showed the best effects and delayed the growth of *Staph. aureus* during the storage time ( $p < 0.05$ ), and these effects were higher under refrigeration and abused temperature than at room temperature. In one study [16], the application of the spearmint essential oil at 0.1 % (v/v) significantly reduced the counts of *Staph. aureus* (by 0.3 log CFU·g<sup>-1</sup>) in endive (*Cichorium endivia* L.) at day 0, while its growth was observed during the next days. Several authors also indicated that *Staph. aureus* was the most sensitive bacterium to *M. spicata* essential oil and its growth was effectively inhibited in vitro [9, 26]. In contrast, BARDAWEEL et al. [14] and ALEXOPOULOS et al. [7] reported only moderate activities of spearmint oil against this bacterium.

Similarly, *M. pulegium* essential oil showed a significant reduction ( $p < 0.05$ ) of *Staph. aureus* counts in chocolate mousse during entire storage

**Tab. 2.** Effects of *Mentha pulegium* and *Mentha spicata* essential oils on counts of *Staphylococcus aureus* in chocolate mousse during storage.

Group	Essential oil	Content [ml·kg <sup>-1</sup> ]	Storage temperature	Storage time			
				0 h	24 h	48 h	72 h
				<i>Staphylococcus aureus</i> [log CFU·g <sup>-1</sup> ]			
Control	<i>Mentha pulegium</i>	0	3 ± 1 °C	6.00 ± 0.01 <sup>a</sup>	8.34 ± 0.06 <sup>a</sup>	7.96 ± 0.11 <sup>a</sup>	7.60 ± 0.45 <sup>a</sup>
1		4	3 ± 1 °C	6.00 ± 0.01 <sup>a</sup>	4.39 ± 0.01 <sup>b</sup>	3.96 ± 0.06 <sup>b</sup>	3.53 ± 0.39 <sup>bc</sup>
2		4	abused	6.00 ± 0.01 <sup>a</sup>	4.39 ± 0.01 <sup>b</sup>	4.11 ± 0.24 <sup>b</sup>	4.06 ± 0.03 <sup>b</sup>
3		2	3 ± 1 °C	6.00 ± 0.01 <sup>a</sup>	4.24 ± 0.02 <sup>c</sup>	3.84 ± 0.19 <sup>b</sup>	3.29 ± 0.05 <sup>c</sup>
4		2	abused	6.00 ± 0.01 <sup>a</sup>	4.26 ± 0.00 <sup>c</sup>	4.02 ± 0.04 <sup>b</sup>	3.75 ± 0.17 <sup>bc</sup>
Control	<i>Mentha spicata</i>	0	3 ± 1 °C	6.00 ± 0.02 <sup>a</sup>	8.24 ± 0.02 <sup>a</sup>	8.28 ± 0.03 <sup>a</sup>	8.36 ± 0.03 <sup>a</sup>
1		4	3 ± 1 °C	6.00 ± 0.02 <sup>a</sup>	3.88 ± 0.10 <sup>b</sup>	4.10 ± 0.02 <sup>b</sup>	3.94 ± 0.09 <sup>c</sup>
2		4	abused	6.00 ± 0.02 <sup>a</sup>	3.95 ± 0.13 <sup>b</sup>	3.92 ± 0.02 <sup>c</sup>	3.53 ± 0.02 <sup>d</sup>
3		2	3 ± 1 °C	6.00 ± 0.02 <sup>a</sup>	3.86 ± 0.11 <sup>b</sup>	3.94 ± 0.06 <sup>c</sup>	3.96 ± 0.03 <sup>c</sup>
4		2	abused	6.00 ± 0.02 <sup>a</sup>	3.80 ± 0.02 <sup>b</sup>	4.18 ± 0.04 <sup>b</sup>	4.19 ± 0.02 <sup>b</sup>

Values represent mean ± standard deviation ( $n = 3$ ). Different lowercase letters (a–c) in the same column indicate significant differences ( $p < 0.05$ ) between groups during storage time.

time, as compared with control (Tab. 2). After 24 h of storage, lower counts of *Staph. aureus* were determined in groups treated with 2 ml·kg<sup>-1</sup> of essential oil than in those treated with 4 ml·kg<sup>-1</sup> of essential oil (reduction by 4 log CFU·g<sup>-1</sup>). This reduction of *Staph. aureus* counts continued in all treated groups until the end of storage time, recording the lowest counts of *Staph. aureus* (3.29 log CFU·g<sup>-1</sup>) in group 3 treated with 2 ml·kg<sup>-1</sup> of *M. pulegium* essential oil under chilled storage. Noticeably, the addition of pennyroyal essential oil effectively reduced the growth of *Staph. aureus* in chocolate mousse during storage time, without remarkable differences between essential oil contents and/or storage conditions (Tab. 2). These results are difficult to discuss as only few reports on the effects of the *M. pulegium* essential oil application in food matrices are available. In one study [27], the addition of *M. pulegium* essential oil in Iranian white cheese inoculated with *Listeria monocytogenes* not only improved organoleptic properties of the cheese but also suppressed the counts of the of bacterium by 1 log CFU·g<sup>-1</sup>. Many active edible films incorporated with pennyroyal essential oil have been used to control food-borne pathogenic bacteria. For example, salep mucilage-based edible films containing various concentrations (0.05 %, 0.1 % and 0.15 %, v/v) of pennyroyal essential oil showed a powerful inhibitory effect against various bacteria including *Staph. aureus* [28]. SALARBASHI et al. [29] also reported that active packaging films incorporated with 2 % of *M. pulegium* essential oil effectively inhibited the growth of *Staph. aureus* and *B. cereus*. In addition, maize starch-based film incorporated with 1 %, 2 % and 3 % of *M. pulegium* essential oil effectively inhibited the growth of *Staph. aureus* and *E. coli* O157:H7, and this effect significantly increased at the highest oil concentration of 3 % (v/v) [30]. The antibacterial effect of *M. pulegium* essential oil was also observed in vitro against five bacterial strains including *Staph. aureus* [8]. However, BRAHMI et al. [18] and BOUKHEBTI et al. [31] recorded only weak inhibitory effects of *M. pulegium* essential oils against various *Staph. aureus* strains.

Generally, the antibacterial activity of essential oils can be attributed to high levels of oxygenated monoterpenes [8, 26], and the efficiency of studied essential oils may be explained by this observation. Most Gram positive bacteria are sensitive to essential oils due to the structure of their cell walls that consist of a thick peptidoglycan layer [16, 26]. The active compounds of essential oils can easily penetrate into the cell and disrupt the lipid structures of the bacterial membrane, damage its integrity, which leads to cell death. Moreover, the anti-

microbial action of essential oils was found to be dependent on many factors including the essential oil content in food matrix, the storage temperature and the food composition. Milk-based products like chocolate mousse containing liquid cream and eggs are excellent nutritive media for microbial growth, which necessitates not only suitable raw material during preparation but also storage under refrigeration conditions. Abused temperature or simulated cold chain disruption is used in experiments for the reason that *Staph. aureus* grows and produces toxins under inadequate temperatures usually above those used for refrigeration [32]. In this study, a significant reduction of *Staph. aureus* counts in chocolate mousse treated with essential oils was recorded without notable effects between refrigeration and abused temperature conditions. Similarly, SHAHBAZI and SHAVISI [25] reported that spearmint essential oil treatment significantly suppressed the counts of *Staph. aureus* in fish soup under both refrigeration and abused temperature, as compared with room temperature. The growth of *L. monocytogenes* was also stronger suppressed by clove essential oil at 25 °C than at 4 °C [33]. This phenomenon was explained on the basis of lower solubility of major antimicrobial components when exposed to refrigeration [34]. In addition, ALONSO-HERNANDO et al. [32] found that chemical compounds were most effective for decontamination of skinless chicken legs when stored under stimulated abuse temperature conditions. However, MOORE-NEIBEL et al. [35] concluded that storage temperature has a limited effect on the efficiency of oregano oil against *Salmonella* Newport suggesting that the antibacterial activity of essential oils need not be affected in the same way by incubation temperature in various food products.

## CONCLUSIONS

Incorporation of *M. spicata* or *M. pulegium* essential oils in chocolate mousse led to a significant reduction ( $p < 0.05$ ) in *Staph. aureus* counts during entire storage time, indicating that these essential oils contain high levels of bioactive antimicrobials. The efficiency of both essential oils was not affected by the abused temperature condition and/or essential oil contents in chocolate mousse. Our results suggest that they can be used as alternatives of synthetic preservatives in pastry products to control food-borne pathogens and improve product safety. Nevertheless, regulatory and toxicological investigations are required before using these antimicrobials as food additives.

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