Journal homepage: www.fia.usv.ro/fiajournal
Journal of Faculty of Food Engineering,
Ştefan cel Mare University of Suceava, Romania
Volume XIX, Issue 2- 2020, pag. 98 - 115

FOOD AND ENVIRONMENT SAFETY

IN VITRO GROWTH INHIBITION OF PATHOGENIC AND FOOD SPOILAGE YEASTS AND FUNGI BY PEPPERMINT (*MENTHA PIPERITA*) ESSENTIAL OIL AND SURVIVAL OF *SACCHAROMYCES CEREVISIAE* IN FRUIT JUICES

Sarah KEHILI [1], Mohamed Nadjib BOUKHATEM [2,3]*, Hussein EL-ZAEDDI [4], Dahbia KELLOU [5], Amina-Bouchra BENELMOUFFOK [5], Mohamed Amine FERHAT [1], Angel A. CARBONELL-BARRACHINA [4], William N. SETZER [6,7]

[1] *Laboratoire de Recherche sur les Produits Bioactifs et Valorisation de la Biomasse, Département de Chimie, Ecole Normale Supérieure, Kouba, Alger, Algeria.*
[2] *Département de Biologie et Physiologie Cellulaire, Faculté des Sciences de la Nature et de la Vie, Université – Saad Dahlab – Blida 1, Blida, Algeria. Mail: mn.boukhatem@yahoo.fr*
[3] *Laboratoire Ethnobotanique et Substances Naturelles, Ecole Normale Superieure, Kouba, Alger, Algeria.*
[4] *Research Group « Food Quality and Safety », Department of Agro-Food Technology, Escuela Politécnica Superior de Orihuela, Unviersidad Miguel Hernández de Elche, Carretera de Beniel, km 3.2, 03312-Orihuela, Alicante, Spain.*
[5] *Laboratoire de Mycologie, Institut Pasteur d'Algérie, Route du petit Staouéli, Dely-Brahim, Alger, Algeria.*
[6] *Department of Chemistry, University of Alabama in Huntsville, Huntsville, AL 35899, USA.*
[7] *Aromatic Plant Research Center, 230 N 1200 E, Suite 100, Lehi, UT 84043, USA.*
*Corresponding author
Received 26th March 2020, accepted 28th June 2020

Abstract: *In the present research, the antifungal and antioxidant activities of Mentha piperita essential oil (MPEO) were investigated, and its potential as a natural food preservative in Orangina juices was evaluated. The major component was menthol (54.47%). The percentage inhibitions of MPEO were dose dependent with IC_{50} values of 2.53±1.77 mg/mL in DPPH test and 8.24±1.16 mg/mL in metal complexing ability. The microbial inhibition of MPEO was assessed against different food spoiling strains. The MPEO strongly inhibited the growth of Rhodotorula sp. and Saccharomyces cerevisiae with a diameter of the inhibitory zone (DIZ) ranging from 17–85 mm at the lower dose (20 µL), and from 35-85 mm at the higher quantity (60 µL). The minimum inhibitory concentration varied from 0.0078 to 0.5% (v/v) for yeasts. In addition, the anti-yeast effectiveness of MPEO alone and in association with moderate heat treatment was investigated in Orangina juices. The juices treated with association of MPEO at different doses (1, 2 and 6 µL/mL) and medium heat treatment (80 °C for 2 min) improved the reduction of Saccharomyces cerevisiae viability cells. Present data confirmed the superior performance of integrated thermal treatment over individual use of peppermint oil for Orangina juices preservation.*

Keywords: *Mentha piperita essential oil, Natural food preservative, Antimicrobial activity, Saccharomyces cerevisiae, Menthol, Antioxidant activity, Orangina juices.*

1. Introduction

Abbreviation List
BHA = Butylated Hydroxyanisole
BHT = Butylate Hydroxytoluene
CFU = Colony-Forming Unit
DIZ = Diameter of Inhibitory Zone
DPPH = 1,1-Diphenyl-2-Picrylhydrazyl
EOs = Essential Oils
MIC = Minimum Inhibitory Concentration
MPEO = *Mentha piperita* essential oil
GC-MS = Gas Chromatography-Mass Spectrometry
Hex = Hexamidine
IC_{50} = Median Inhibitory Concentration

NIST = National Institute of Standards & Technology
Rt = Retention time
RI = Retention index
SDA = Sabouraud-chloramphenicol Dextrose Agar

Orange juice is healthful and energizing because of its vitamin C (ascorbic acid) quantity, sweet, acidic taste, pleasing color, scented and nutritious. Though, even after a few minutes of extraction, the juice starts decaying and its taste, flavor and color get off. This is due to substantial bacterial and fungal charging and enzymatic activity, which spoils the organoleptic and nutritive properties of juice, making it unhealthy for consumers. The main reasons of alteration must be related to the development and growth of pathogens (bacteria, yeast, and fungi), physical and chemical reactions, structural modifications and packing conditions [1,2].
Consequently, some processing methods, quickly after the removal of fruit juices, are required to maintain the freshness of juices. For example, freezing, purification, sanitization, and adding of chemical additives are some examples of the current practices applied to accomplish microbiological and chemical stabilities and to control the safety and quality of food and fruit juices [3]. Some alternative techniques such as modified CO_2 atmosphere packaging, ozone treatment, organic acids, irradiation, thermal processing, steam or hot water have been demonstrated to be active for shelf life prolongation in new or processed foods. Currently, there is a growing tendency of consuming packed fruit juices as they can be drunk at desire and are simple to bring. Nevertheless, fruit juices are processed before packing, and several synthetic and chemical additives are added in order to control their safety and quality. Some synthetic preservatives generally uesed in juice are sorbic and benzoic acids and their derivatives, formic acid, formaldehyde, salicylic acid and SO_2 [4]. In addition, the disputation over the safety of some synthetic and chemical additives has encouraged and prompted the search for their natural alternative compounds. The growing claim for natural preservative molecules has linked in their extended utility. The numerous chemical disinfectants and preservatives are mostly not accepted by users because of their side effects and harmful as well. Therefore, natural sanitizers such as vinegar, lemon juice, phytochemicals and essential oils (EOs) extracted from medicinal herbs, spices and aromatic plants, not only give flavor and aroma to foods but they also have the benefit of being safe, healthy and natural preservatives [5,6].
Natural food preservatives with high antibacterial, antifungal and antioxidant actions that extend the shelf life of juices are appreciated. Most medicinal herbs and aromatic plants synthesize and produce antimicrobial biomolecules. These plant-based antimicrobials can be suggested and used as natural food preservatives in fruit juices [7,8].
Peppermint (*Mentha piperita*) is a member of Lamiaceae family that spreads mostly in the temperate and Mediterranean areas of the globe such as Algeria. It is considered a rich source of EOs, which is commonly used in food production, cosmetic, pharmaceutical and nutraceutical industries. The famous and usually used peppermint is a cultivated natural hybrid of *Mentha spicata* (spearmint) and *Mentha aquatica* (water mint) [9,10]. Besides it is used in cosmetics, herbal tea preparations, food industry, and sweets. Peppermint was reported as a medicinal and aromatic herb, with an EO having several pharmaceuticals and food uses. The therapeutic and medical

uses of *Mentha piperita* essential oil (MPEO) comprise anti-inflammatory, antispasmodic, wound-healing, antidiabetic, analgesic and antiemetic applications. In some provinces of the North Africa countries, MPEO is used for the treatment of infections, fever, vomiting, nausea, common cold, bronchitis and stimulation of appetite. Most recently, MPEO has gained enlarged scientific importance, principally as an antioxidant, analgesic and antibacterial bioactive natural agent [11,12].

However, EOs present considerable activity when used in fruit juice matrix, but quantities necessary (33–100 times of *in vitro* concentrations) are very great [13], and such doses generally exceed the sensory and organoleptic satisfactory ranks. The association of a minor heat processing with different doses of volatile oils can be considered as an important approach to decrease or inhibit bacterial and fungal growth in various food products, eliminating the issues of sensorial impact on juices. Consequently, the association of EOs with medium heat treatment can be investigated for discovering new active food preservation practices.

As per our knowledge, there is no report on the preservation of Orangina fruit juices by MPEO which has remarkable anti-inflammatory and analgesic properties [11]. Therefore, an effort has been made to improve the shelf life of Orangina fruit juices with natural phytochemical food additives extracted from peppermint plants. In the current investigation, the effect of MPEO against different food spoiling fungal and yeast species was studied using different *in vitro* assays (disc diffusion and disc volatilization methods, agar dilution test) as well in Orangina fruit juices. Also, to decrease the dose of MPEO in the Orangina juices, the integrated influence of the MPEO with medium thermal treatment (80 °C for 2 min) was also investigated. The chemical composition profile of MPEO was done by gas chromatography.

2. Material and methods

2.1. Material

2.1.1. Extraction of *Mentha piperita* volatile oil

The MPEO used in our research was a commercial sample produced by steam distillation from the aerial part of the plant in industrial conditions (a stainless steel alembic). The MPEO was obtained from Extral-Bio Company (Blida, Algeria).

2.1.2. Food-spoilage microorganisms

The *in vitro* microbial inhibitory action of MPEO was assessed against several mycelial fungi and yeast strains: six *Candida* strains comprising *Candida glabrata*, *C. albicans* and *C. tropicalis*; two *Saccharomyces cerevisiae*; two food spoiling *Aspergillus* strains including *A. flavus* and *A. niger* and one *Fusarium* sp. strain. Such isolates were obtained from food matrix in the laboratory of food quality and microbiology (Laboratoire d'Hygiène, Blida, Algeria) and from the mycology laboratory (Institute Pasteur of Algeria, Algiers, Algeria). These microbial species were identified by standard microbiology assays and stored in sabouraud-chloramphenicol dextrose agar (SDA) for yeast and fungi.

2.1.3. Chemicals and reagents

The following drugs and chemicals were used: dimethyl sulfoxide (DMSO), tween 80, gallic acid, butylated hydroxyanisole (BHA), L-ascorbic acid (vitamin C), FerroZine™ iron reagent, 1,1-diphenyl-2-

picrylhydrazyl (DPPH), iron (II) chloride ($FeCl_2$) were obtained from Sigma Aldrich (St. Louis, MO, USA). The antiseptic solution of Isomedine® 0.1% (hexamidine dermal solution, Isopharma, Algiers, Algeria) was used in order to control the sensitivity of tested isolated microorganism strains.

2.2. Methods

2.2.1. Determination of chemical composition of peppermint volatile oil

Analysis and identification of the volatile compounds of *Mentha piperita* EO were done using a Shimadzu GC-17A gas chromatograph apparatus combined with a Shimadzu QP-5050A mass spectrometer detector (Shimadzu Corporation, Kyoto, Japan). The GC-MS system has a TRACSIL Meta.X5 (95% dimethylpolysiloxane, and 5% diphenylpolysiloxane) column (60 m×0.25 mm, 0.25 μm film thickness; Teknokroma, Barcelona, Spain). The chromatography analyses were done using helium as the carrier gas at a column flow rate of 0.3 mL/min and a total flow of 3.9 mL/min in a split ratio of 1:200 and the following temperature program: (a) 45 °C for 6 min; (b) increase of 3 °C/min from 45 °C to 210 °C and hold for 4 min; (c) increase of 25 °C/min from 210 °C to 290 °C and hold for 4 min. The temperatures of the detector and injector were 290 °C and 300 °C, respectively. All chemical compounds of MPEO were detected and identified using two different analytical techniques: (1) comparison of experimental retention indexes (RI) with those of the literature and standards; and, (2) mass spectra (NIST05 spectral library collection available). Only fully identified chemical compounds detected in MPEO are reported in the current research.

2.2.2. Determination of *in vitro* antioxidant Activity

2.2.2.1. Evaluation of DPPH radical scavenging technique

DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay was evaluated and determined according to Wang et al. [14] with some slight modifications, based on the capability of MPEO chemical compounds to neutralize DPPH radical. The conversion of the DPPH radical into a yellow color reduced form (DPPH/H+) is detected immediately. Briefly, a series of dilutions of MPEO were done in a pure ethanol solvent. In parallel, the negative control was prepared comprising all reagents except the MPEO. After 25 min incubation period at room temperature in a dark cupboard, the absorbance at 520 nm (maximum absorbance of DPPH) was measured and recorded using a spectrophotometer. A blank reading was taken using a covert containing solution without the MPEO, and the absorbance was measured. The DPPH free radical scavenging action of selected MPEO concentration was then calculated as percentage inhibition in accordance to the below formula:

$$\text{DPPH radical scavenging Inhibition \%} = \left(\frac{\text{Abs Blank} - \text{Abs Sample}}{\text{Abs Blank}}\right) * 100$$

where Abs Sample is the optical density or absorbance of DPPH free radical with the tested sample and Abs Blank is the optical density of DPPH free radical without MPEO.

The *in vitro* antioxidant property of the MPEO was calculated and expressed as IC_{50} (Median inhibitory concentration), defined as the dose of the MPEO necessary to make a 50% reduction or inhibition in initial DPPH solution. Ascorbic acid (vitamin C) and BHA were used as

positive standards. All these analyses were carried out in triplicate.

2.2.2.2. Evaluation of metal complexing activity

The complexation of ferrous ions by the MPEO and standards was evaluated and estimated following to the method of Ye et al. [15] with slight modifications. MPEO samples were added and mixed to a solution of 2 mM $FeCl_2$ (0.05 mL). The reaction was started by adding a quantity of 5 mM ferrozine (0.2 mL) and the combination was shaken strongly and left standing at laboratory temperature for 12 min in a dark cupboard. After the solution mixture had reached equilibrium, the optic density was then measured and recorded by an apparatus of spectrophotometer at 562 nm. All tests and assays were done in triplicate. The percentage inhibition of ferrozine–Fe^{2+} complex creation was calculated following this equation:

$$\%\ \text{inhibition} = \left(\frac{A0 - A1}{A0}\right) * 100$$

where A0 was the optical density of the negative control and A1 was the optical density in the presence of the MPEO or standards. The control contains only $FeCl_2$ and ferrozine complex formation molecules.

Gallic acid, BHA and vitamin C (ascorbic acid) were used as positive standards. The dose of inhibition of the tested samples was expressed and reported as the percentage of concentration required to do 50% inhibition (IC_{50}).

2.2.3. *In vitro* antifungal activity of peppermint volatile oil

The *in vitro* fungal inhibitory action of MPEO against several filamentous fungi and yeast strains was done using different methods: disc diffusion, disc volatilization and agar macrodilution assays.

2.2.3.1. Agar disc diffusion test

Agar disc diffusion test was employed for the evaluation and determination of the antifungal property of MPEO [12]. The fungal inoculum of each strain was prepared with fresh cultures by suspending the microorganisms in sterile saline (0.9% NaCl). Filter paper discs (diameter of 9 mm, Schleicher and Schull, Dassel, Germany) were saturated with 3 different quantities (20, 40, and 60 µL) of peppermint EO per disc and positioned on the inoculated plates (SDA for fungi and yeast). After maintaining at laboratory temperature for 40 min, the plates were incubated under aerobic conditions for 72 h (yeast) and 5 days (fungi). The fungal inhibitory potential was estimated by calculating the diameter of the inhibitory zone (DIZ) in millimeters (including disc diameter of 9 mm). Antiseptic solution of Hexamidine was used as a positive control in order to control the sensitivity of tested isolated microorganisms.

2.2.3.2. Vapor diffusion test

Because EOs extracted from aromatic plants are volatile, methods and techniques that test the fungal inhibitory effect of such agents in their vapor phase have been done in this research. A standard experimental setup as published by Tyagi et al. [12] was followed with some modifications. The fungal inhibitory potential of MPEO in vapor phase was assessed using the disc volatilization assay at three different amounts (20, 40, and 60 µL per disc). In brief, SDA was inoculated over the solidified medium surface with 100 µL of suspension of the mycelial or yeast strains under study. A paper disc was placed on the inside surface of the upper lid and a suitable volume of MPEO was placed on selected paper disc. Then, the plate was immediately inverted on top of the lid and closed with parafilm to avoid the runoff of MPEO vapor. Plates were incubated under

aerobic conditions for 72h (yeast) and for 5 days (fungi). The effectiveness of the *in vitro* fungal inhibitory action of MPEO was calculated and reported by measuring the DIZ (in millimeters) above the disc.

2.2.3.3. Determination of the fungal minimum inhibitory concentration (MIC)

The agar macro dilution assay was performed as recommended by Tyagi et al. [12] with some modifications. All experiments were made in SDA medium added with Tween 80 (final dose of 0.5% v/v). Filamentous fungi and yeast strains were cultured for 24h and geometric dilutions ranging from 2% to 0.007% (v/v) of MPEO were prepared in a culture medium plate, including one growth control (SDA + Tween 80). Petri dishes were incubated under aerobic conditions for 48-72h. The inhibitory effect of fungal growth was detected by the absence of the colonies on the SDA medium. The MIC values were estimated and expressed as the lowest dose of peppermint EO stopping visible growth of fungal species.

2.2.4. Anti-yeast effect of MPEO in Orangina juices

2.2.4.1. Inoculation of Orangina juices with *Saccharomyces cerevisiae*

Orangina juices were purchased from a local company (Djgaguen, Blida, Algeria). Orangina is a lightly carbonated beverage made from carbonated water, orange juice and other citrus juice from concentrate 12%. Orangina is sweetened with sugar and natural flavors are added. This beverage contains also synthetic additives such as citric acid (SIN330), benzoate sodium (SIN211) and potassium sorbate (SIN202) as preservatives, and ascorbic acid (SIN300) as an antioxidant ingredient. The yeast suspension of *Saccharomyces cerevisiae* was added and then mixed with Orangina juices and the inoculated juices were transferred in 100 mL sterilized glass vials.

2.2.4.2. Anti-yeast effect of peppermint EO alone

Tween 80 solution (0.5%) of MPEO was added and then mixed in the inoculated Orangina juices at several concentrations (1, 2 and 6 µL/mL). Orangina juices sample inoculated with *Saccharomyces cerevisiae* alone was considered as a control group. Then, the treated vials were stored at laboratory temperature up to 9 days and juice samples were drawn on 0, 2^{nd}, 3^{rd}, 6^{th}, and 8^{th} day. All treated juice samples were successively diluted in isosaline solution (0.9% NaCl) and plated on SDA medium. All petri dishes were incubated for 72 h at 25 °C to observe and count the growth and number of yeast colonies that appeared in all plates. The observations were recorded as the number of colonies present in 10 mL of Orangina juice samples (CFU/mL).

2.2.4.3. Anti-yeast effect of peppermint EO in association with medium thermal treatment

A set of inoculated Orangina juices vials added with three different concentrations of peppermint EO was exposed to a moderate heat treatment (80 °C) for a short time (2 min). Each juice was treated in triplicate. Then, the treated vials were deposited at laboratory temperature up to 9 days and Orangina juice samples were drawn on 0, 2^{nd}, 3^{rd}, 6^{th}, and 8^{th} day. All treated Orangina juices were consecutively diluted in isosaline solution and plated on SDA medium. All plates were incubated for 72 h at 25 °C to detect and count the number of yeast colonies that appeared in all plates. The results were calculated and recorded as the number of colonies present in 10 mL of juice sample (CFU/mL).

The effectiveness of the medium thermal treatment (80 °C for 2 min) in association with different doses of peppermint EO was measured and expressed by the variation in log CFU of the inoculated yeast strains with time (up to 8 days).

2.2.5. Statistical Analyses

All the analyses were performed in triplicates to report the results as mean with standard deviation and subjected to one-way analysis of variance (ANOVA) followed by HSD Tukey's post hoc multiple comparison tests to establish whether the differences in experimental results for different samples were significant ($p<0.05$) or not ($p>0.05$). The statistical analysis was done using XLstat 2014 software (Addinsoft, Paris, France).

3. Results and discussion

3.1. Chemical composition of peppermint volatile oil

In the current investigation, the EO from the aerial parts of peppermint (*Mentha piperita* L.) a medicinal and aromatic herb grown in Algeria and commonly used in phytomedicine, was extracted using steam distillation method. The determination of the chemical composition profile of MPEO was made by GC-MS, and quantitative and qualitative compositions are reported in Table 1 and Figure 1.

Table 1.
Chemical composition of the essential oil obtained from peppermint (*Mentha piperita* L.) using a steam distillation method.

Peak No.	Rt† (min)	Compound	RI, Exp.	RI, Lit.	Difference	Area (%)
1	9.017	*cis*-3-Hexen-1-ol	845	849	4	0.01
2	9.716	1-Hexanol	860	858	-2	0.01
3	12.624	Thujene	918	923	5	0.04
4	13.051	*α*-Pinene	925	921	-4	0.61
5	15.526	Sabinene	964	963	-1	0.4
6	15.815	*β*-Pinene	969	964	-5	0.79
7	16.133	1-Octen-3-ol	974	978	4	0.1
8	16.674	ß-Myrcene	982	983	1	0.07
9	17.28	3-Octanol	992	992	0	0.22
10	18.491	*α*-Terpinene	1010	1018	8	0.15
11	19.034	*p*-Cymene	1017	1023	6	0.28
12	19.413	Limonene	1023	1027	4	1.44
13	19.637	Eucalyptol	1026	1030	4	4.9
14	20.65	*trans*-ß-Ocimene	1040	1043	3	0.05
15	21.455	*γ*-Terpinene	1051	1053	3	0.27
16	22.352	*cis*-Sabinenehydrate	1063	1068	5	0.87
17	24.613	Linalool	1095	1098	3	0.29
18	25.171	Amyl isovalerate	1102	1108	6	0.09
19	27.617	Sabinol	1135	1142	7	0.02
20	28.155	Isopulegol	1142	1146	4	0.09
21	28.877	Menthone	1152	1155	3	16.75

22	29.275	Menthofuran	1157	1164	7	3.91
23	29.399	Isomenthone	1159	1164	5	2.53
24	30.974	Menthol	1180	1185	5	54.47
25	34.664	Pulegone	1231	1237	6	1.2
26	35.773	Piperitone	1246	1250	4	0.46
27	38.472	Menthyl acetate	1283	1287	4	6.93
28	47.139	*trans*-Caryophyllene	1408	1411	3	1.75
29	51.138	Germacrene D	1469	1467	-2	1.02
30	52.078	ß-Elemene	1483	1484	1	0.28
				Oxygenated Monoterpenes		91.64
				Monoterpene Hydrocarbons		5.31
				Sesquiterpene Hydrocarbons		3.05

† Rt = Retention time; Exp. = Experimental; Lit. = Literature; ‡ All compounds were identified using Retention Indexes (RI) and mass spectra.

The main compound identified was menthol (54.47), followed by menthone (16.75%), menthyl acetate (6.93%) and 1.8-cineole (eucalyptol) (4.9%). Other chemical compounds were detected but less than 4%. Also, MPEO showed a high content of oxygenated monoterpene (93.26%) and low amounts of monoterpene hydrocarbons (2.69%) and sesquiterpene hydrocarbons (3.69%). Thus, Algerian MPEO extracted by steam distillation may be categorized as a "menthol/menthone chemotype".

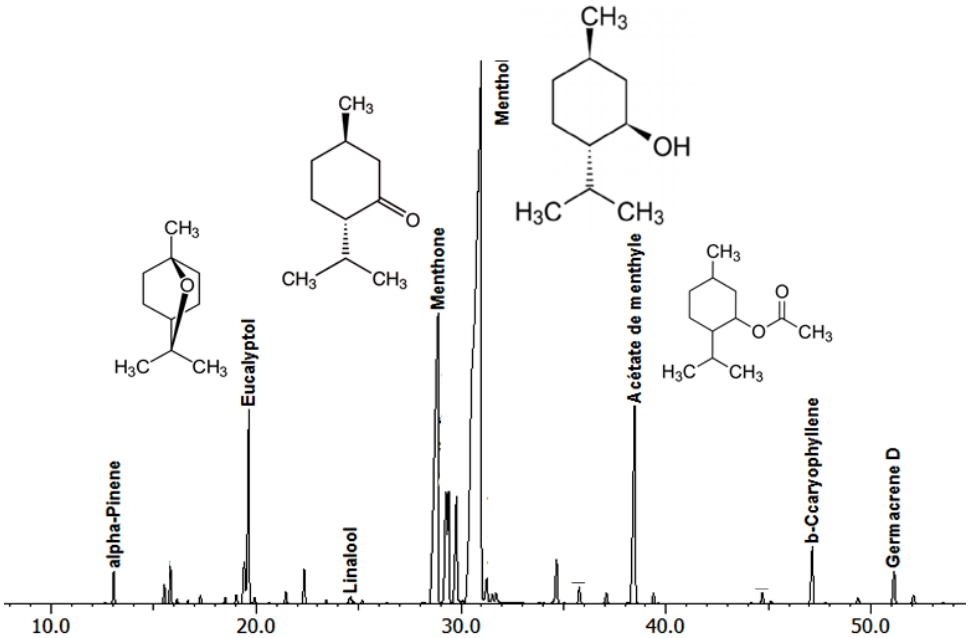


Fig. 1. The chemical profile of peppermint (*Mentha piperita* L.) essential oil determined by GC-MS.

Several research groups have analyzed aroma profiles for the various peppermint, which can vary and fluctuate significantly depending on several factors [9,10,12]. Experimental findings are in agreement with other published papers, wherein menthone and menthol were the main abundant compounds in EO extracted from

peppermint [16,17]. According to Derwich et al. [18], menthone (29%), menthol (5.58%) and menthyl acetate (3.3%) were the major components in peppermint EO from Morocco. In Turkey, the study of Kizil et al. [19] found that (+)-menthol (38%), (-)-menthol (35.6%) and neo-menthol (6.7%) were the major compounds of MPEO.

Remarkably, the MPEO under examination of this research seems to be richer in some important constituents such as menthone, menthol, eucalyptol and oxygenated monoterpenes. A review of the literature accessible on this area shows that several papers have previously been published on MPEO chemical characterization [20]; however, there are no research articles on the chemical profile of MPEO from Algerian Mitidja area.

It has been revealed that the EO distillated from the peppermint leaves grown in Unicamp (Brazil) is characterized by the dominance of a monoterpenic alcohol (linalool) with a rate of 51%, followed by carvone (23.42%) [21]. In another investigation, the dominant compounds of MPEO from Iran were α-terpinene (19.7%) and pipertitinone oxide (19.3%) [22]. Several papers have reported that the chemical composition of these MPEOs differs in accordance to the countries, or the regions in the same state. These variations seem to depend on several reasons such as climate changes, external environment and other factors such as the method and the period of extraction, collected parts of the plant, irrigation techniques, and phenological transformations [23,24].

3.2. *In vitro* antioxidant activity of peppermint EO

The *in vitro* antioxidant effect of MPEO harvested from Algeria was investigated using DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging and ferrous ion complexation assays. The median inhibitory concentrations (IC_{50}) were calculated and values are presented in Table 2.

Table 2.

***In vitro* antioxidant activity of peppermint (*Mentha piperita* L.) essential oil.**

Sample	DPPH radical scavenging IC_{50} (mg/mL)	Complexing power IC_{50} (mg/mL)
Mentha piperita essential oil	2.53±1.77	8.24±1.16
Positive Control (BHA)	0.32 ± 0.28	31.22 ± 15.87
Positive Control (vitamin C)	0.01 ± 0.01	14.19 ± 6.27
Positive Control (Gallic Acid)	—	31.92 ± 24.18

BHA: Butylhydroxyanisol; IC_{50} = Median inhibitory concentration 50%; Values are given as mean ± SD (n = 3).

The decrease ability of DPPH free radical was assessed by the reduction in its optical density at 520 nm induced by antioxidant compounds. In the DPPH assay, the IC_{50} value for the MPEO was 2.53±1.77 mg/mL (Table 2), indicating a moderate electron transfer capacity for the EO when compared to the standards of BHA and ascorbic acid, that presented IC_{50} values of 0.32±0.28 and 0.01±0.01 mg/mL, respectively. Scavenging activity of vitamin C and BHA, recognized as powerful antioxidant standards, were comparatively more active than that of peppermint EO.

The ferrous complexing capacity test was used to evaluate and confirm the capacity of antioxidant molecules to disrupt the formation of the complexes or to prevent interaction between metal and lipids.

Because of the importance of metal complexation as one of the antioxidant mechanisms, the aptitude of the MPEO to compete with ferrozine for iron ions in free solution was evaluated, and the corresponding IC_{50} values are presented in Table 2. Unlike the DPPH assay, the iron complexing ability of MPEO is more pronounced with an IC_{50} value of 8.24±1.16 mg/mL, followed by ascorbic acid (14.19±6.27 mg/mL). Current findings showed that MPEO has an excellent ferrous ion complexation action.

Results from current study agreed with those of previous reports in which the *in vitro* antioxidant power of the MPEO was assessed and linked to the major oxygenated monoterpenes including menthol, menthone, menthyl acetate and 1,8-cineole [25,26]. Other MPEO minor chemical compounds that contain molecules in the active methylene group, such as terpinolene, α- and γ-terpinene, were also listed and recognized for their powerful antioxidant action, which is equivalent to the positive standard (vitamin E or α-tocopherol) [27]. Because the *in vitro* antioxidant ability of the whole aroma is the consequence of the interaction of all minor and major compounds, it is difficult to attribute the EOs antioxidant power to a single molecule, as other MPEO components can contribute showing synergistic, additive or antagonistic effects [28].

3.3. *In vitro* anti-yeast and antifungal activity of peppermint volatile oil

3.3.1. Agar disc diffusion test

The *in vitro* antifungal effect of peppermint EO was assessed using three different quantities. The resultant diameters of inhibition zones (DIZ) are presented in Table 3. In the current investigation, the inhibitory action of peppermint EO was done against 13 isolates of filamentous fungi and yeast species using the agar diffusion method. As can be seen in Table 3, MPEO showed various degrees of *in vitro* anti-yeast antifungal actions depending on the microorganism strains tested. It is essential to state that in comparison to the positive standard (antiseptic solution of Hexamidine), MPEO showed a potent fungal inhibitory action against *Rhodotorula* sp. with a total inhibition zone. The MPEO strongly inhibited the growth of *Saccharomyces cerevisiae*, *Candida tropicalis* (Ct1) and *C. albicans* (Ca2) with DIZ ranging from 13-25 mm at the lower volume of MPEO (20 µL/mL), and from 35-81 mm at the higher amount (60 µL/mL). Among the filamentous fungi, *Aspergillus flavus* (Af2) was the most susceptible strains; the application of 60 µL of MPEO resulted in a DIZ of 36 mm. The DIZ extended with increasing MPEO volume. Using 60 µL of MPEO, highest DIZ was shown by *Rhodotorula* sp. (85 mm), *Candida albicans* (Ca2) (81 mm) and *C. tropicalis* (Ct2) (42 mm), in comparison to the positive standard (antiseptic solution of Hexamidine).

Agreeing to the literature, several authors and scientists [29,30] state that the percentage of chemical constituents and the dominant compounds detected in different EOs determined the bacterial and fungal inhibitory effect *in vitro*. The researchers go on to explain the maximum antifungal property [29,31] is caused by chemical elements containing hetero atoms such as oxygen. Interestingly, oxygenated monoterpenes and sesquiterpenes were clearly reported as powerful antifungal agents in the chemical composition of different volatile oils, comprising MPEO [32]. Furthermore, menthone, menthol and menthyl acetate were shown to display significant fungal and bacterial inhibitory effect against a wide range of pathogens

and food spoiling microorganisms [33]. Nevertheless, as EOs have multiple chemical constituents their *in vitro* fungal inhibitory effect is rather due to synergistic, additive or antagonistic actions of the pure compounds. Current research has shown that the volatile oil obtained from the fresh aerial parts of peppermint plant has the potential to be an antifungal agent with a superior activity against a wide variety of food spoilage yeast when compared to synthetic drugs.

Table 3.
***In vitro* susceptibility of fungal strains to MPEO through liquid and vapor phases in comparison with an antiseptic solution**

	Diameter of Inhibition Zone (DIZ, mm) †								
	Disc diffusion test			Vapor diffusion test			Positive control ‡		
	Quantity of MPEO (µL/disc)								
Yeast strains	**20**	**40**	**60**	**20**	**40**	**60**	**20**	**40**	**60**
Candida albicans (Ca1)	15.6	23.3	28.3	10.3	27.6	35.0	-	-	-
Candida albicans (Ca2)	13.0	19.5	81.0	16.0	31.0	11.0	15.0	15.0	18.0
Candida glabrata (Cg1)	12.6	14.6	18.0	20.3	25.0	31.3	-	-	22.0
Candida glabrata (Cg2)	13.5	19.0	21.5	-	17.0	63.0	-	-	-
Candida tropicalis (Ct1)	23.6	31.3	35.0	22.3	24.0	30.3	-	-	-
Candida tropicalis (Ct2)	17.0	32.0	42.0	3.5	13.0	71.0	-	-	-
Saccharomyces cerevisiae	25.0	30.0	40.0	11.0	19.0	39.0	-	-	-
Rhodotorula sp.	85.0	85.0	85.0	85.0	85.0	85.0	38.0	38.0	40.0
Filamentous fungi									
Aspergillus niger (An1)	12.6	15.0	18.6	-	-	-	21.6	28.3	31.3
Aspergillus niger (An2)	-	12.5	16.0	-	-	-	13.0	17.0	21.0
Aspergillus flavus (Af1)	10.6	18.0	36.0	-	-	-	-	-	-
Aspergillus flavus (Af2)	-	-	14.5	-	-	-	-	-	-
Fusarium sp.	10.0	11.6	12.3	-	-	-	19.3	20.3	25.6

† Diameter of inhibition zone includes the disc diameter of 9 mm. ‡ Antiseptic solution (Hexamidine 0.1%) used as a positive control for fungal strains. MPEO: *Mentha piperita* essential oil; (-) no inhibitory activity.

3.3.2. Disc volatilization technique

The antifungal inhibitory effect of peppermint EO was also evaluated in the vapor phase using the disc volatilization technique (Table 3). As observed in the agar disc diffusion method, the DIZ due to the vapors increased with increasing volumes of the peppermint EO. Current data revealed that for the tested fungal strains (*Candida albicans* (Ca1), *C. glabrata* (Cg1 and Cg2) and *C. tropicalis* (Ct2), the DIZ resulting from exposure to peppermint EO vapors was higher than that resulting from a similar volume of MPEO in the liquid phase with 60 µL of MPEO per disc. This may be correlated with the difference in the chemical composition of the two phases (liquid and vapor), with the vapor phase being richer in the more volatile chemical elements [12,34]. For example, *Rhodotorula* sp. and *Candida tropicalis* were the most susceptible yeast species to MPEO vapors since a total inhibition zones were generated using 60 µL. Nevertheless, no fungal inhibitory activity was noticed in the case of filamentous fungi such as *Aspergillus niger*, *A. flavus* and *Fusarium* sp.

In view of these data, it is surprising that regardless of the publication of several

original research articles on the antibacterial and antifungal effects of peppermint EO using the agar disc diffusion assay, the *in vitro* antimicrobial property of the vapor phase of MPEO has been largely ignored. Both the liquid oil and oil vapor have been established to have some antifungal activity. The efficacy of MPEO as an antifungal agent against 17 fungal and micromycetal food poisoning, animal, plant, and human pathogens was reported and published by Sokovic et al. [35]. Peppermint EO presented strong *in vitro* antibacterial and antifungal activities, higher than bifonazole (commercial fungicide used as a positive control) but lower than that of pure major chemical compound (menthol).

In addition, the application of the vapor phase has the supplementary advantages of simplicity of use and avoiding the need for direct interaction with the MPEO. A minor dose of peppermint EO is essential to accomplish the same level of fungal inhibition. The current findings are in conformity with our previous publications on this point [36,37].

3.3.3. Minimum inhibitory concentrations of peppermint EO

The MIC of MPEO was determined against different food spoiling fungi and yeast species using the agar macrodilution method. The MIC values are shown in Table 4.

Table 4.
Determination of minimum inhibitory concentrations (MIC) using the agar dilution method

Fungal strains	MIC (% v/v)
Candida albicans (Ca1)	0.125
Candida albicans (Ca2)	0.25
Candida glabrata (Cg1)	0.25
Candida glabrata (Cg2)	0.5
Candida tropicalis (Ct1)	0.125
Candida tropicalis (Ct2)	0.5
Saccharomyces cerevisiae	0.5
Rhodotorula sp.	0.007
Aspergillus niger (An1)	1
Aspergillus flavus (Af2)	0.5
Fusarium sp.	0.5

The MPEO exhibited dose-dependent inhibition of the yeast and fungal growth, and the MIC varied from 0.125% to 0.5% for *Candida* spp., and from 0.5% to 1% for filamentous fungi. The lowest MIC (0.007%) was shown by *Rhodotorula* sp., followed by *C. albicans* and *C. tropicalis* (0.125%).

The difference in the *in vitro* fungal inhibitory effect could be linked to different microbial species and also to the chemical profile of the MPEO used. The quantity of major antimicrobial compounds (oxygenated monoterpenes such as menthol and menthone) in MPEO used is higher (61%). The oxygenated monoterpenes can raise the permeability and penetrability of the fungal cell membrane, leading to leakage of the cell substances [9,20]. Fungal cell membrane alteration, loss of cytoplasmic constituents and inhibition of respiratory activity due to some oxygenated terpenes (menthol, menthone and menthyl acetate) have been previously reported and published [38]. The existence of oxygenated monoterpenes as major chemical elements could be the

cause for greater *in vitro* anti-yeast effect of MPEO.

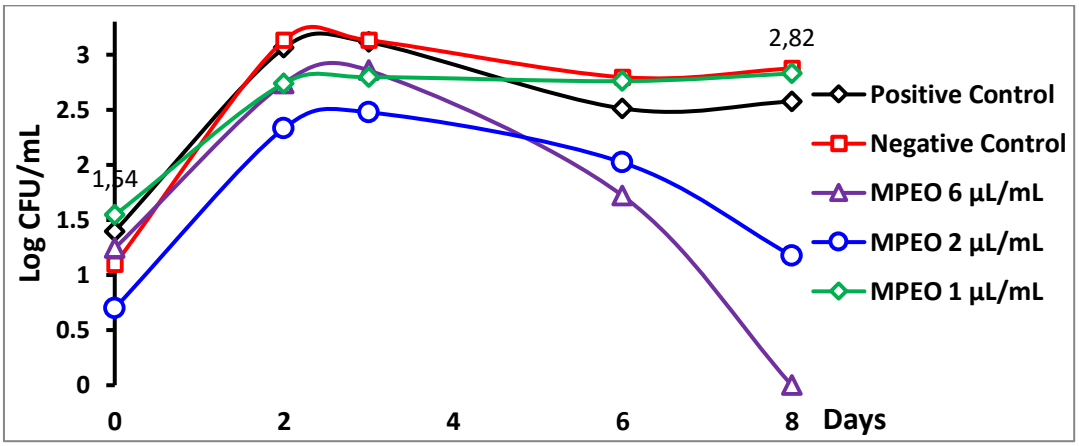
Furthermore, it has been reported that menthol was the principal inhibitory compound of peppermint volatile oil against the fungus *Trametes versicolor* [39]. This result is consistent with the fact that EO extracted from peppermint grown in Brazil, containing high quantities of oxygenated monoterpenes such as linalool (51%), but no menthol, had only a medium fungal inhibitory activity with MIC value of 0.6 mg/mL against the strain of *Candida albicans* [40].

The different doses at which MPEO exerted significant *in vitro* anti-yeast action indicate that there may be possibilities and opportunities for its use as safe and natural food preservative, where a reduction in food spoilage fungi and yeast is necessary. Therefore, additional experiments and assays were done to confirm and validate the efficacy of peppermint EO in association with other food preservation technique in Orangina fruit juices.

3.4. Orangina juices' preservation using peppermint EO alone or in association with moderate thermal treatment

3.4.1. Anti-yeast effect of peppermint EO at different concentrations

As peppermint oil was able to inhibit *in vitro* the growth of several food spoilage yeasts and fungi, it's potential as a safe food additive in Orangina juices was also studied and reported (Figure 2). The decrease in yeast viability of the yeast *Saccharomyces cerevisiae* cells due to peppermint EO application in time-dependent ways (i.e., 0, 2, 3, 6 and 8 days) and dose-dependent (1, 2 and 6 µL/mL) manner was described and demonstrated.



Positive control or juice with synthetic antimicrobial additives (sodium benzoate and potassium sorbate)); MPEO: *Mentha piperita* essential oil; CFU: Colony-Forming Unit**)**

Fig. 2. Variation in viability of yeast strain (*Saccharomyces cerevisiae*) in Orangina juices during storage after peppermint EO treatment at various doses (1, 2 and 6 µL/mL).

As illustrated in Figure 2, a complete growth inhibition of the yeast specie (*Saccharomyces cerevisiae*) was observed and recorded in Orangina juices at only higher concentration (6 µL/mL) and only on the 8th day. The viable count of yeast cells in Orangina juices increased with the decreasing of peppermint EO dose used. However, the quantity of 1 µL/mL of MPEO did not show a significant reduction

in the final number of yeast cells (1.54 log CFU/mL) at the beginning of the experiment as compared to the end of the assay (2.82 log CFU/mL).

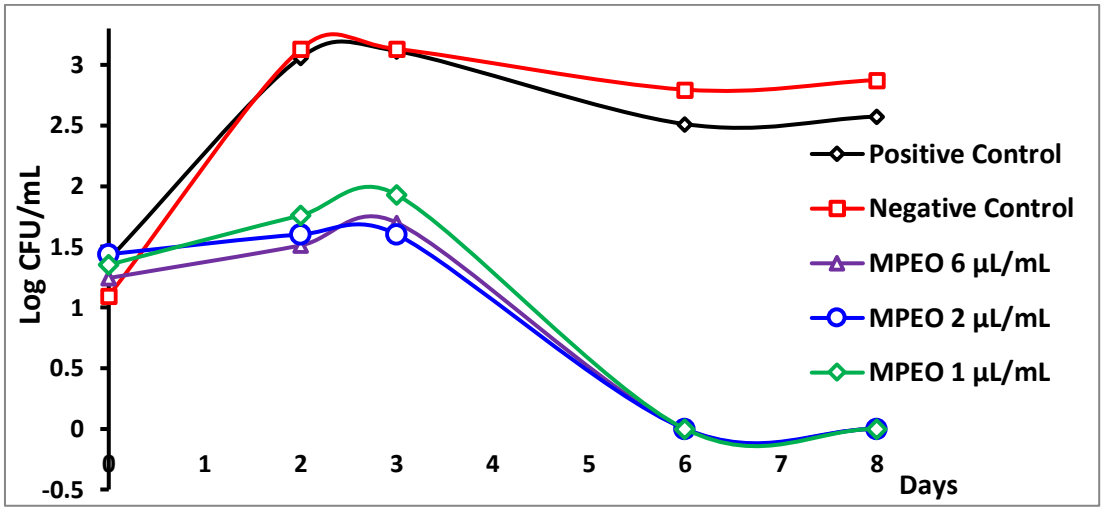
Due to the increasing data about the injurious and dangerous effects of synthetic or chemical food additives, there is an incessant and nonstop pressure from consumers and scientific societies to avoid or diminish the quantity of theses chemical preservatives and ingredients [41] and also to deliver minimally processed food products, without compromising food quality, safety and organoleptic properties. Consequently, substitute sources of acceptable and effective natural food preservatives extracted from natural and safe products need to be discovered and investigated, such as EOs. For example, rosemary EO has been applied not only as a condiment, but also for its effective antifungal and antioxidant properties. Indeed, carnosic acid, one of its major components, is not only an antiseptic and antiviral compound but it also has got a higher antioxidant potential than the powerful food additive antioxidants such as vitamin C, butylate hydroxytoluene (BHT) and BHA [42].

Current data suggested the efficacy of peppermint EO to decrease the yeast load of *S. cerevisiae* in Orangina fruit juices but only at a high concentration. However, this aspect can intensely disturb the physical and chemical properties and sensorial or organoleptic characteristics of the Orangina juices. To overcome these concerns, numerous approaches have been studied and investigated for the improvement of antifungal and antibacterial effects of volatile oils in food matrix or systems. To further diminish the necessary MPEO dose for controlling *Saccharomyces cerevisiae* load in Orangina fruit juices, the association between peppermint EO and medium thermal treatment was also studied.

3.4.2. Anti-yeast effect of peppermint EO in association with medium thermal treatment

The cell viable counts of food-spoiling yeast strain (*Saccharomyces cerevisiae*) after exposure to the integrated effect of peppermint EO at three different doses (1, 2 and 6 µL/mL) together with medium thermal treatment (80 °C/2 min) in Orangina juices was investigated and noted at different days (i.e. after 0, 2, 3, 6 and 8 days) (Figure 3). It is surprising to reveal that in the Orangina juices exposed to moderate thermal treatment and MPEO at the doses of 1, 2 and 6 µL/mL, total growth inhibition of the yeast *S. cerevisiae* was detected after 6 days. Additionally, no fungal growth was noticed up to 8 days of storage. Hence, the association of medium thermal treatment with MPEO decreased the oil dose requirement to exactly 1/5 of the MIC level. The association of moderate thermal treatment with MPEO can offer improved Orangina juices preservative.

In the current research, the MPEO dose required was decreased by the association with an additional food preservation technique (moderate thermal treatment). This association can be considered as a better method to preserve and control Orangina fruit juices from yeast-spoiling contamination without an important influence on the sensory or organoleptic characteristics of the drink. The integration of moderate thermal treatment with MPEO suggests a very valuable synergy, whereby an increase in temperatures during juice storage could improve the vapor phase concentration of chemical volatiles, thereby improving the fungal inhibitory actions for better and superior food preservation.



Positive control or juice with synthetic antimicrobial additives (sodium benzoate and potassium sorbate)); LAEO: *Mentha piperita* essential oil; CFU: Colony-Forming Unit**)**

Fig. 3. Anti-yeast effect of peppermint EO in association with medium thermal treatment.

The synergistic influence of medicinal herbs, aromatic spices and EOs on other food preservation matrix, such as mild heat processing, has been also evaluated and reported in the past. Essia Ngang et al. [43] investigated how to decrease the thermal influence during fruit juice extraction. They showed and proved that pasteurizing pineapple fruit juice at the temperature of 60 °C in presence of coriander EO, dropped the time needed for a 97% reduction of Gram-positive bacteria such as *Listeria monocytogenes* compared to juice samples without volatile oils. Another study demonstrated that mint, eucalyptus and lemongrass EOs functioned in a synergy manner with mild heat treatment to decrease and inhibit totally the yeast growth of *S. cerevisiae* in mixed juices prepared using different fruits [12].
The combination of peppermint EO with a moderate thermal treatment has never been previously investigated or published for controlling Orangina juice yeast spoilage. The current study could be considered as the first report on the potential application or use of peppermint EO as a natural Orangina juice preservative in an association with a moderate thermal treatment.

4. Conclusion

In the current investigation, the significant fungal inhibitory effect of the peppermint volatile oil against several food spoiling fungi and yeast has been assessed using different techniques, *in vitro* and in a real Orangina juice matrix in association with a moderate thermal treatment. The fungal inhibitory action of peppermint oil in Orangina juice supports its use in food preservation, because this EO is reported as safe. These data could be considered as a significant platform for the innovation and improvement of active natural food preservatives.

5. Acknowledgments

The authors would like to thank the Laboratory of Water Microbiology (Algérienne des Eaux, Chiffa, Blida, Algeria) and the Laboratory of Food Microbiology (Laboratoire d'Hygiène de Blida, Blida, Algeria)", especially Mr. Djamel TEFFAHI and Mr. Abdenacer

HMIDA for their help and making the facilities available for carrying out this research.

6. Conflict of interest statement

The authors have declared that there is no conflict of interest.

7. Sources of funding

This study did not receive any specific grant from funding agencies in the commercial, public, or not for-profit sectors.

8. References

[1]. TOURNAS VH., HEERES J., BURGESS L., Moulds and yeasts in fruit salads and fruit juices, *Food Microbiology*, 23: 684-688, (2016). https://doi.org/10.1016/j.fm.2006.01.003
[2]. AHMED T., DAS KK., UDDIN MA., The Microbiological Quality of Commercial Fruit Juices-Current perspectives, *Bangladesh Journal of Microbiology*, 35: 128-133, (2018). https://doi.org/10.3329/bjm.v35i2.42643
[3]. RUPASINGHE HV., YU LJ., Emerging preservation methods for fruit juices and beverages, *Food Additive*, 65-82, (2012).
[4]. OLADIPO IC., ADELEKE DT., ADEBIYI AO., The effect of pH and chemical preservatives on the growth of bacterial isolates from some Nigerian packaged fruit juices, *Pakistan Journal of Biological Sciences*, 13: 16, (2010). https://doi.org/10.3923/pjbs.2010.16.21
[5]. KAPOOR IPS., SINGH B., SINGH S., SINGH G., Essential oil and oleoresins of black pepper as natural food preservatives for orange juice, *Journal of Food Processing and Preservation*, 38: 146-152, (2014). https://doi.org/10.1111/j.1745-4549.2012.00756.x
[6]. EKANEM JO., EKANEM OO., The effect of natural and artificial preservatives and storage temperature on the pH and microbial load of freshly produced apple (*Malus domestica*) juice, *Agro-Science*, 18: 16-21, (2019). https://doi.org/10.4314/as.v18i1.3
[7]. PANDEY AK., KUMAR P., SINGH P., TRIPATHI NN., BAJPAI VK., Essential oils: Sources of antimicrobials and food preservatives, *Frontiers in Microbiology*, 7: 2161, (2017). https://doi.org/10.3389/fmicb.2016.02161
[8]. GRANATA G., STRACQUADANIO S., LEONARDI M., NAPOLI E., CONSOLI GML., CAFISO V., GERACI C., Essential oils encapsulated in polymer-based nanocapsules as potential candidates for application in food preservation, *Food Chemistry*, 269: 286-292, (2018). https://doi.org/10.1016/j.foodchem.2018.06.140
[9]. DESAM NR., AL-RAJAB AJ., SHARMA M., MYLABATHULA MM., GOWKANAPALLI RR., ALBRATTY M., Chemical constituents, *in vitro* antibacterial and antifungal activity of *Mentha Piperita* L. (peppermint) essential oils, *Journal of King Saud University-Science*, 31: 528-533, (2019). https://doi.org/10.1016/j.jksus.2017.07.013
[10]. BENZAID C., TICHATI L., DJERIBI R., ROUABHIA M., Evaluation of the chemical composition, the antioxidant and antimicrobial activities of *Mentha piperita* essential oil against microbial growth and biofilm formation, *Journal of Essential Oil Bearing Plants*, 22: 335-346, (2019). https://doi.org/10.1080/0972060X.2019.1622456
[11]. BALAKRISHNAN A., Therapeutic uses of peppermint-a review. *Journal of Pharmaceutical Sciences and Research*, 7: 474, (2015).
[12]. 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*, 18: 201, (2018). https://doi.org/10.1186/s12906-018-2274-x
[13]. TYAGI AK., GOTTARDI D., MALIK A., GUERZONI ME., Chemical composition, *in vitro* anti-yeast activity and fruit juice preservation potential of lemon grass oil, *LWT-Food Science and Technology*, 57: 731-737, (2014). https://doi.org/10.1016/j.lwt.2014.02.004
[14]. WANG W., WU N., ZU YG., FU YJ., Antioxidative activity of *Rosmarinus officinalis* L. essential oil compared to its main components, *Food Chemistry*, 108: 1019-1022, (2008). https://doi.org/10.1016/j.foodchem.2007.11.046
[15]. YE CL., DAI DH., HU WL., Antimicrobial and antioxidant activities of the essential oil from onion (*Allium cepa* L.), *Food control*, 30: 48-53, (2013). https://doi.org/10.1016/j.foodcont.2012.07.033
[16]. OH JY., PARK MA., KIM YC., Peppermint oil promotes hair growth without toxic signs. *Toxicological Research*, 30: 297-304, (2014). https://doi.org/10.5487/TR.2014.30.4.297

[17]. MCKAY DL., BLUMBERG J B., A review of the bioactivity and potential health benefits of peppermint tea (*Mentha piperita* L.), *Phytotherapy Research*, 20: 619-633, (2006). https://doi.org/10.1002/ptr.1900
[18]. DERWICH E., CHABIR R., SENHAJI O., *In-vitro* antioxidant activity and GC/MS studies on the leaves of *Mentha piperita* (Lamiaceae) from Morocco, *International Journal of Pharmaceutical Sciences and Drug Research*, 3: 130-136, (2011).
[19]. KIZIL S., HASIMI N., TOLAN V., KILINC E., YUKSEL U., Mineral content essential oil components and biological activity of two *Mentha* species (*M. piperita* L., *M. spicata* L.), *Turkish Journal of Field Crops*, 15: 148-153, (2010).
[20]. FITSIOU E., MITROPOULOU G., SPYRIDOPOULOU K., TIPTIRI-KOURPETI A., VAMVAKIAS M., BARDOUKI H., PAPPA A., Phytochemical profile and evaluation of the biological activities of essential oils derived from the Greek aromatic plant species *Ocimum basilicum*, *Mentha spicata*, *Pimpinella anisum* and *Fortunella margarita*, *Molecules*, 21: 1069, (2016). https://doi.org/10.3390/molecules21081069
[21]. SARTORATTO A., MACHADO A.LM., DELARMELINA C., FIGUEIRA GM., DUARTE MCT., REHDER VLG., Composition and antimicrobial activity of essential oils from aromatic plants used in Brazil, *Brazilian Journal of Microbiology*, 35: 275-280, (2004). https://doi.org/10.1590/S1517-83822004000300001
[22]. YADEGARINIA D., GACHKAR L., REZAEI MB., TAGHIZADEH M., ASTANCH SA., RASOOLI I., Biochemical activities of Iranian *Mentha piperita* L. and *Myrtus communis* L. essential oils, *Phytochemistry*, 67: 1249-1255, (2006). https://doi.org/10.1016/j.phytochem.2006.04.025
[23]. ALLALI H., CHIKHI I., DIB MEA., MUSELLI A., FEKIH N., MELIANI N., COSTA J., Antioxidant activity and chemical analysis of *Mentha spicata* cultivated from west northern region of Algeria by headspace solid phase micro-extraction and hydro-distillation, *Natural Products*, 9: 258-63, (2013).
[24]. LAGGOUNE S., ÖZTÜRK M., EROL E., DURU ME., ABAZA I., KABOUCHE A., KABOUCHE Z., Chemical composition, antioxidant and antibacterial activities of the essential oil of *Mentha spicata* L. from Algeria, *Journal of Materials and Environmental Sciences*, 7: 4205-4213, (2016). https://doi.org/10.1016/j.foodchem.2007.03.059
[25]. YANG SA., JEON SK., LEE EJ., SHIM CH., LEE IS., Comparative study of the chemical composition and antioxidant activity of six essential oils and their components, *Natural Product Research*, 24: 140-151, (2010). https://doi.org/10.1080/14786410802496598
[26]. ROZZA AL., DE FARIA FM., BRITO ARS., PELLIZZON CH., The gastroprotective effect of menthol: involvement of anti-apoptotic, antioxidant and anti-inflammatory activities, *PloS one*, 9, (2014). https://doi.org/10.1371/journal.pone.0086686
[27]. RUBERTO G., BARATTA MT., Antioxidant activity of selected essential oil components in two lipid model systems, *Food Chemistry*, 69: 167-174, (2000). https://doi.org/10.1016/S0308-8146(99)00247-2
[28]. ANTHONY KP., DEOLU-SOBOGUN SA., SALEH MA., Comprehensive assessment of antioxidant activity of essential oils, *Journal of Food Sciences*, 77: C839-C843, (2012). https://doi.org/10.1111/j.1750-3841.2012.02795.x
[29]. DORMAN HJD., DEANS SG., Antimicrobial agents from plants: antibacterial activity of plant volatile oils, *Journal of Applied Microbiology*, 88: 308-316, (2000). https://doi.org/10.1046/j.1365-2672.2000.00969.x
[30]. REDDY DN., AL-RAJAB AJ., Chemical composition, antibacterial and antifungal activities of Ruta graveolens L. volatile oils, *Cogent Chemistry*, 2: 1220055, (2016). https://doi.org/10.1080/23312009.2016.1220055
[31]. LAMBERT RJW., SKANDAMIS PN., COOTE PJ., NYCHAS GJ., A study of the minimum inhibitory concentration and mode of action of oregano essential oil, thymol and carvacrol, *Journal of Applied Microbiology*, 91: 453-462, (2001). https://doi.org/10.1046/j.1365-2672.2001.01428.x
[32]. KNOBLOCH K., PAULI A., IBERL B., WEIGAND H., WEIS N., Antibacterial and antifungal properties of essential oil components, *Journal of Essential Oil Research*, 1: 119-128, (1989). https://doi.org/10.1080/10412905.1989.9697767
[33]. BARATTA MT., DORMAN HD., DEANS SG., FIGUEIREDO AC., BARROSO JG., RUBERTO G., Antimicrobial and antioxidant properties of some commercial essential oils, *Flavour and Fragrance Journal*, 13: 235-244, (1998). https://doi.org/10.1002/(SICI)1099-1026(1998070)13:4<235::AID-FFJ733>3.0.CO;2-T
[34]. GOÑI P., LÓPEZ P., SÁNCHEZ C., GÓMEZ-LUS R., BECERRIL R., NERÍN C., Antimicrobial activity in the vapour phase of a combination of cinnamon and clove essential

oils, *Food Chemistry*, 116: 982-989, (2009). https://doi.org/10.1016/j.foodchem.2009.03.058
[35]. SOKOVIĆ MD., VUKOJEVIĆ J., MARIN PD., BRKIĆ DD., VAJS V., VAN GRIENSVEN LJ., Chemical composition of essential oils of *Thymus* and *Mentha* species and their antifungal activities, *Molecules*, 14: 238-249, (2009). https://doi.org/10.3390/molecules14010238
[36]. EDRIS AE., FARRAG ES., Antifungal activity of peppermint and sweet basil essential oils and their major aroma constituents on some plant pathogenic fungi from the vapor phase, *Food/Nahrung*, 47: 117-121, (2003). ttps://doi.org/10.1002/food.200390021
[37]. BOUKHATEM MN., FERHAT MA., KAMELI A., KEBIR HT., Lemon grass (*Cymbopogon citratus*) essential oil as a potent anti-inflammatory and antifungal drugs, *Libyan Journal of Medicine*, 9: 25431, (2014). https://doi.org/10.3402/ljm.v9.25431
[38]. SAMBER N., VARMA A., MANZOOR N., Evaluation of *Mentha piperita* essential oil and its major constituents for antifungal activity in *Candida* spp, *Evaluation*, 3, (2014).
[39]. MATAN N., WORAPRAYOTE W., SAENGKRAJANG W., SIRISOMBAT N., MATAN N., Durability of rubberwood (*Hevea brasiliensis*) treated with peppermint oil, eucalyptus oil, and their main components, *International Biodeterioration & Biodegradation*, 63: 621-625, (2009). https://doi.org/10.1016/j.ibiod.2008.12.008
[40]. DUARTE MCT., FIGUEIRA GM., SARTORATTO A., REHDER VLG., DELARMELINA C., Anti-*Candida* activity of Brazilian medicinal plants, *Journal of Ethnopharmacology*, 97: 305-311, (2005). https://doi.org/10.1016/j.jep.2004.11.016
[41]. CALO JR., CRANDALL PG., O'BRYAN CA., RICKE SC., Essential oils as antimicrobials in food systems–A review, *Food Control*, 54: 111-119, (2015). https://doi.org/10.1016/j.foodcont.2014.12.040
[42]. DE LA TORRE TORRES JE., GASSARA F., KOUASSI AP., BRAR SK., BELKACEMI K., Spice use in food: properties and benefits, *Critical Reviews in Food Science and Nutrition*, 57: 1078-1088, (2017). https://doi.org/10.1080/10408398.2013.858235
[43]. ESSIA NGANG JJ., NYEGUE MA., NDOYE FC., TCHUENCHIEU KAMGAIN AD., SADO KAMDEM SL., LANCIOTTI R., ETOA FX., Characterization of Mexican coriander (*Eryngium foetidum*) essential oil and its inactivation of *Listeria monocytogenes in vitro* and during mild thermal pasteurization of pineapple juice, *Journal of Food Protection*, 77: 435-443, (2014). https://doi.org/10.4315/0362-028X.JFP-13-323