Preservative effect of *Juniperus phoenicea* essential oil and ethanolic extract against *Escherichia coli* and *Staphylococcus aureus* in soft fresh cheese during storage

LAID DJOKHDEM – ABDELKADER BENSID – ABDERRAHMANE HOUICHER – TAHA MOSSADAK HAMDI – FATIH ÖZOGUL

Summary

This study investigated the antibacterial activity of the *Juniperus phoenicea* essential oil water emulsion and ethanolic extract solution against *Escherichia coli* and *Staphylococcus aureus* inoculated on soft fresh cheese stored under refrigeration and abused temperatures to simulate incidental post-processing contamination. The gas chromatography-mass spectrometry analysis of essential oil showed that α-pinene was the major component (57.8 %). Polyphenols were highly abundant in the ethanolic extract with an average of 131.8 g gallic acid equivalent per kilogram of dry weight. The results showed that the growth of inoculated *E. coli* and *Staph. aureus* significantly decreased in treated samples during 5-day storage time when compared to the controls ($p < 0.05$). However, treated samples with plant essential oil emulsion generally showed greater reductions in the growth of these food-borne pathogens (0.4 log CFU·g^{-1} to 2.6 log CFU·g^{-1}) than the samples treated with ethanolic extract solution (0 log CFU·g^{-1} to 0.8 log CFU·g^{-1}). Furthermore, the antibacterial effectiveness of these treatments was not influenced by the simulated temperature abuse conditions. These findings extend knowledge about the behaviour of natural antimicrobials under non-ideal storage situations, which very often occur in the cheese supply chain.

Keywords

Juniperus phoenicea; ethanolic extract; essential oil; *Escherichia coli*; *Staphylococcus aureus*; soft fresh cheese; preservative effect

The white soft cheese, known under the name of Jben, is one of the most popular dairy products in Algeria. It is obtained by enzymatic coagulation of raw or pasteurized cows' milk with rennet and with or without starter cultures [1]. Its specific characteristics including low salt content (1.4–1.6 %), pH values above 5.0, high water activity and intrinsic nutritional ingredients, introduce a potential risk of infection from cheese-borne bacterial species such as *Listeria monocytogenes*, *Salmonella enterica, Staphylococcus aureus* or enteropathogenic *Escherichia coli* [2]. Unsuitable storage, unhygienic practices and poor sanitation may cause proliferation of pathogenic microorganisms that contaminate the surface of the product. Indeed, cheese products are involved in serious diseases of humans such as staphylococcal intoxication. The onset of its symptoms is quite rapid, usually 1–6 h after ingestion of food containing the enterotoxin [3]. Pathogenic *E. coli* strains can also cause very severe forms of food poisoning by cheese products. These pathogenic strains acquired virulence factors conferring the ability to cause gastrointestinal diseases, including enteritis and colitis, or extraintestinal manifestations outside of the gastrointestinal tract [3]. *Staph. aureus* and *E. coli* O157:H7 are two important food-borne pathogens of major concern for

Laid Djokhdem, Taha Mossadak Hamdi, Laboratory of Food Hygiene and Quality Insurance System (HASAQ), Higher National Veterinary School, Rue Issad Abbes, Oued Smar, 16000 Algiers, Algeria.
Abdelkader Bensid, Department of Agronomy, Faculty of Natural Sciences and Life, Ziane Achour University, BP 3117, 17000 Djelfa, Algeria.
Abderrahmane Houicher, Department of Agricultural Sciences, Faculty of Science, Amar Telidji University, BP 37 G, 03000 Laghouat, Algeria.
Fatih Özogul, Department of Fishing and Fish Processing Technology, Faculty of Fisheries, Cukurova University, Balcali, 01330 Adana, Turkey,

Correspondence author:
Abderrahmane Houicher, e-mail: a.houicher@yahoo.fr; a.houicher@lagh-univ.dz

the dairy industry. The occurrence of these pathogens in cheese products was verified by several studies [4, 5]. It is known that *E. coli* survives in cheese under refrigerated storage and grows in a large range of temperatures between 10 °C and 45 °C even in the presence of lactic acid bacteria if added as a starter. In addition, it was reported that *E. coli* O157:H7 grows from a primary number of about 10^5 CFU·g^{-1} in fresh cheese to a final level of 10^7 CFU·g^{-1}, during cottage cheese production [6, 7]. In addition, *Staph. aureus* is one of the most common pathogenic or toxinogenic bacteria related to cheese products, typically causing human poisoning through the ingestion of the toxin released to foods. This microorganism is destroyed by heat treatment, whereas its toxin is heat stable [8]. It has been postulated that *Staph. aureus* contents of $> 10^5$ CFU·g^{-1} are needed for the production of sufficient amounts of staphylococcal enterotoxin to cause illness [9]. The microbial contamination of cheese products is responsible for substantial economic losses for manufacturers and for health risk of transmitting potentially pathogenic microorganisms to consumers.

Over the past few years, the interest in replacement of synthetic additives with natural substances as bio-preservatives, such as extracts and essential oils, which are effective and non-toxic, is increasing [10]. Various studies focused on application of herbal extracts and essential oils, including antimicrobial phenolic compounds, in cheese products [2, 5, 7, 11, 12]. *Juniperus phoenicea* is a medicinal and aromatic plant, belonging to Cupressaceae family. It grows wild over the whole Mediterranean region [13]. The inhibitory effects of its extracts (essential oil, alcoholic and water extracts) against a wide range of food-borne pathogens, particularly *Staph. aureus* and *E. coli*, were reported in growth media, foods and food-based broth [14–17].

Given these considerations, the objectives of this study were to (1) analyse the chemical composition of essential oil of *J. phoenicea*, (2) characterize total polyphenols of its ethanolic extract, and (3) study the survival and/or growth behaviour of inoculated *Staph. aureus* and *E. coli* in soft white cheese treated with *J. phoenicea* essential oil and ethanolic extract, when stored at 4 ± 1 °C or under temperature abuse conditions.

MATERIALS AND METHODS

Bacterial strains and preparation of inocula

Staph. aureus ATCC 25923 and *E. coli* ATCC 25922 strains obtained from the culture collection of Regional Veterinary Laboratory of Laghouat (Algeria) were maintained at –80 °C in brain heart infusion (BHI) broth (Merck, Darmstadt, Germany) with 10% glycerol added as a cryoprotectant. Prior to use, each strain was cultured directly in BHI broth and incubated at 35 °C for 24 h. After incubation, 0.1 ml was spread onto BHI agar plates and left at 35 °C for 48 h to obtain cells in the stationary phase of growth. Subsequently, well isolated colonies were dispersed in 0.1% buffered peptone water (BPW; Difco, Sparks, Maryland, USA) and optical density of the culture was adjusted at 600 nm to be equivalent to McFarland standard of 0.5 using a UV/VIS spectrophotometer Libra S6 (Biochrom, Cambridge, United Kingdom), so the cell concentration was approximately 10^7 CFU·ml^{-1}.

Plant material

The aerial parts (stem and leaves) of *Juniperus phoenicea* were collected during winter (2021) from Ain Ousmane Mountain around the town of Oued Morra (Aflou, province of Laghouat) in north-central part of Algeria (34°12'07.0°N and 2°1925.9°E). The identity of the plant was confirmed by the Department of Agricultural Sciences, Faculty of Science, Laghouat University (Laghouat, Algeria). Plant samples were hand-cleaned from impurities, dried in shade at room temperature (< 30 °C) and ground to a homogenous powder using a blender. Then they were stored in sealed paper bags until extractions for a maximum of 1 month.

Preparation of plant extract

The air-dried and finely ground sample was subjected to ethanolic extraction according to Bensid et al. [18]. One kilogram of powdered sample was extracted with 5 000 ml of absolute ethanol (99.94%) at room temperature for 24 h. The resultant extract was filtered through Whatman Filter paper No. 1 (GE Healthcare, Maidstone, United Kingdom). After filtration, activated carbon (Merck) was added to filtrate (1 : 5, w/w; 10 g of activated carbon per 50 g of plant material) and immediately was removed from the filtrate using Whatman No. 1 filter paper. The resultant filtrate was concentrated under reduced pressure at 50 °C using a rotary evaporator Laborota 4000 (Heidolph, Schwabach, Germany) until a semisolid residue was obtained. The extract was weighed, treated by UV-light (30 W, 50 cm irradiation distance) for 30 min to reduce the naturally existing microflora and kept at 4 °C in the dark until utilization for a maximum of 1 month.

Determination of total phenolic compounds

For the determination of total concentration of phenolic compound in *J. phoenicea* extract, Folin-Ciocalteu colorimetric method described by SINGLETON et al. [19] and slightly modified by HOUICHER et al. [20] was used. A volume of 50 μl of sample, or blank, was pipetted into separate tubes and, to each, 1.85 ml of ultrapure water was added. Then, the diluted aqueous solution of each solution was mixed with 200 μl of Folin-Ciocalteu reagent and allowed to stand at room temperature (approximately 25 °C). After 5 min, 900 μl of 20 % Na_2CO_3 solution was added, the mixture was shaken intermittently and kept in the dark for 2 h at room temperature (approximately 25 °C). The absorbance of the mixture was determined at 765 nm with a spectrophotometer (Lambda 25; Perkin Elmer, Waltham, Massachusetts, USA). The total phenolic content was calculated from the calibration curve using gallic acid as a standard (0 mg·l^{-1} to 500 mg·l^{-1})

$$y = 0.0019x + 0.0802; R^2 = 0.9982 \quad (1)$$

The results were expressed as grams of gallic acid equivalent (GAE) per kilogram of dry weight (DW) extract. All determinations were performed in triplicate.

Extraction and analysis of essential oil

In order to extract the essential oil from this plant, 150 g of ground material from a dry sample was completely immersed in distilled water (1 500 ml) and subjected to hydrodistillation for 4 h, using a Clevenger-type apparatus according to the European Pharmacopoeia method [21]. The essential oil yield was calculated as percentage per air-dried weight. The obtained essential oil was stored in a dark glass bottle at 4 °C until the gas chromatography coupled to mass spectrometry analysis for a maximum of 1 week.

The chemical composition of *J. phoenicea* essential oil was analysed by gas chromatography-mass spectrometry using a Clarus 500 instrument (Perkin Elmer), equipped with SGE capillary column (60 m × 0.25 mm inside diameter; BPX5, 0.25 μm film thickness, Perkin Elmer) at the following conditions: oven temperature programmed from 60 °C to 250 °C (holding for 10 min), temperature ramp 4 °C·min^{-1}, and injector temperature at 240 °C. The carrier gas was helium (purity 99.95 %) and the flow rate was 1.5 ml·min^{-1}. A sample was diluted in *n*-hexane (1%, v/v) and the injection volume was 1 μl. A splitless injection mode was used with ionization energy of 70 eV, mass spectra range was m/z 35–425. The relative percentage values of the oil constituents were obtained by semi-quantification by peak area integration from peaks using the software provided by the manufacturer.

Emulsions preparation

Essential oil of *J. phoenicea* was used in the form of emulsion for immersing application. The essential oil emulsion was prepared by adding 5 ml of oil to 1 000 ml of distilled water, in which 0.3 ml of Tween 20 (Merck) was added to ensure miscibility of the essential oil in water. The solution was emulsified with a mechanical homogenizer (150 Handheld homogenizer, Thermo Fisher Scientific, Waltham, Massachusetts, USA) at 583 Hz for 2 min until obtaining a homogenous mixture. The emulsion was used immediately after preparation for immersion of the tested cheese.

Manufacture of soft white cheese

The soft fresh cheese (Jben) was produced by a traditional method with fresh and whole cows' milk. This milk was pasteurized at 65 °C for 30 min and cooled immediately to 4 °C. The pasteurized milk was slowly heated to 45 °C, which was followed by addition of stock solution of rennet based on chymosin (CHY MAX, 2235 International Milk Clotting Units per gram; Chr. Hansen, Hørsholm, Denmark). After 12 h from adding rennet, the resulting coagulum was allowed to drain for 24 h until the appropriate texture of Jben was obtained. Finally, the cheese samples were packaged in sterile polyethylene bags and stored at refrigeration (4 °C).

Preparation of cheese samples and storage conditions

Samples of 800 g of white soft cheese that had been placed in sterile containers were aseptically cut into uniform cubes (of approximately 10 g) for microbiological analysis. Each cheese cube was treated by dipping in 1 000 ml of 0.5 % plant extract solution (5 g of ethanolic extract dissolved in 1 000 ml distilled water) or 1 000 ml of 0.5% plant essential oil emulsion for 4 min at 25 °C, and allowed to dry at room temperature (approximately 25 °C). exactly for 5 min. The contents of plant extract and essential oil yielding best appearance and less presence of odour and colour in cheese were chosen. The cheese samples were then inoculated separately by spreading 0.1 ml of inoculum (*Staph. aureus* or *E. coli*, 10^7 CFU·ml^{-1}) across the surface of the samples so that the final content in the cheese sample was approximately 10^5 CFU·g^{-1}.

Samples of cheese were randomly divided into six separate groups: two controls were not treated

by plant extract or essential oil but inoculated separately with bacteria. Two groups were treated by plant extract at 0.5 % and inoculated separately with *Staph. aureus* and *E. coli*. Two other groups of samples were treated by essential oil emulsion at 0.5 % and also inoculated separately with *Staph. aureus* and *E. coli*. After treatments and inoculation, cheese samples were packed separately in sterile polystyrene foam boxes with lids. All groups of the tested cheese samples were stored at 4 ± 1 °C for 24 h, followed by 12 h at 25 ± 1 °C and then at 4 ± 1 °C until the end of storage (for 120 h, which is the expected shelf-life for white soft cheese). This abused temperature condition was chosen to simulate a cold chain disruption during one or more stages in the storage of white soft cheese in the manufacturing plant, during transport and during storage in retail stores or in domestic refrigerators. Groups were tested for *Staph. aureus* and *E. coli* counts after 0 h, 24 h, 36 h, 72 h, 96 h, and 120 h of storage.

Bacteriological analyses of cheese samples

Ten grams of each cheese sample were aseptically mixed with 90 ml of 0.1% sterile BPW (Difco) in a sterile Stomacher bag. The mixtures were blended for 2 min using Stomacher 400 Labblender (Seward, London, United Kingdom). Ten-fold serial dilutions of these homogenates were prepared using 0.1% sterile BPW. Two sterile Petri dishes were prepared from each dilution by spreading 0.1 ml onto the surface of eosin methylene blue (EMB) agar (Condalab, Madrid, Spain) for *E. coli* and Baird–Parker agar supplemented with egg yolk and tellurite (Oxoid, Basingstoke, United Kingdom) for *Staph. aureus*. The plates were then incubated under aerobic conditions at 37 °C for 24 h. Species identification was done based on colony morphology on EMB agar (blue-black with a metallic green sheen) and Baird-Parker agar supplemented with egg yolk and tellurite (shiny black with a distinctive clear zone in the surrounding agar). All samples were assayed in triplicate. Bacterial counts were expressed as logarithms of the number of colony-forming units per gram of cheese.

Statistical analysis

The data were presented as mean ± standard deviation (*SD*). SigmaPlot 12.0 Systat Software (Jandel Scientific Software, San Jose, California, USA) was used to perform statistical analysis of the results. One-way analysis of variance (ANOVA) was used according to the Duncan multiple comparison tests to determine significant differences among treatments, at a 5% significance level.

RESULTS AND DISCUSSION

Extraction yield and total phenolics content

We found that the yield of the crude extract from *J. phoenicea* leafy twigs, conducted by absolute ethanol (99.94 %), reached 5 % (w/w). The extraction yield obtained in the present study was much lower than that reported by Menaceur et al. [22] and Ennajar et al. [23], who recorded 32% and 35.4% ethanolic extract from *J. phoenicea* leaves, respectively. In other works, Amalich et al. [24] and Dane et al. [25] found 16.6% and 21% methanolic extract from *J. phoenicea* leaves and leafy twigs, respectively. In general, the percentage of extraction yield is dependent mainly on the extraction procedure, particularly the polarity of the extracted compounds, the temperature used for the extraction, as well as the ratio of solvent to sample at extraction [25].

The total phenolics content was found to be 131.8 ± 27.9 $g \cdot kg^{-1}$ DW (expressed as GAE) in *J. phoenicea* extract. The results of the present study correlated with the findings of other researchers. For example, Ennajar et al. [23] recorded 169 ± 2 $g \cdot kg^{-1}$ DW of total phenolics content of *J. phoenicea* leaves from Tunisia. However, the phenolics content obtained in the current study was higher than that reported by Bakchiche et al. [26], who recorded 70.31 ± 5.64 $g \cdot kg^{-1}$. The high content of phenolic compounds in our extract can be attributed to the low temperature applied (50 °C) in the rotary evaporator to prepare our extract. The temperature used for the extraction is selected to ensure higher retention of total phenolic compounds since the rate of polyphenols degradation increases as temperature and time increase [27]. Such variation in the content of phenolic compounds can be also attributed to the diversity of geographic and climatic factors (temperature, precipitation, soil, sunlight, etc.), genetic factors, the degree of maturation of the plant and the storage [24].

Chemical characterization of essential oil

The essential oil was obtained by steam-distillation from the leafy twigs of *J. phoenicea* with an average yield of 0.9 % (w/w) based on air-dried weight of sample. The comparison with the average essential oil yields coming from other regions of Algeria revealed a little difference. The essential oil yields obtained from leafy twigs of this species, collected from Menâa, Elhadjaz, T'kout, Boutaleb and Boussâda regions, all in Algeria, were 0.7 %, 0.9 %, 0.9 %, 0.8 % and 0.7 %, respectively [13]. The essential oils yield of the plant species tested was lower than that obtained by

HARMOUZI et al. [28], which was 1.7 ± 0.3 % for the same plant from the Tounfit region, Morocco. However, it was higher than 0.5 % as found by AIT-OUAZZOU et al. [15] using extraction from aerial parts of *J. phoenicea* collected from the Taourirt region, Morocco. In general, this important variation in plant essential oil yields depends on the plant organ, geographical location and extraction conditions [28].

The gas chromatography-mass spectrometry analysis of the *J. phoenicea* essential oil led to identification of 48 compounds (Tab. 1), which constituted 98.4 % of the total oil. The essential oil was predominantly composed of monoterpene hydrocarbons (68.1 %), with α-pinene as the major constituent (57.8 %). δ-Cadinene (5.5 %), τ-cadinol (3.7 %), limonene (3.5 %), δ-3-carene (3.1 %) and α-terpinyl acetate (2.9 %) were the further most important constituents of the *J. phoenicea* essential oil (Fig. 1). Our results are in agreement with those of RAMDANI et al. [13] and HARMOUZI et al. [28], when α-pinene was the main constituent of essential oil obtained from *J. phoenicea* aerial parts. A similar result was also found by AIT-OUAZZOU et al. [15], when α-pinene was found to be the major component of leafy twigs of *J. phoenicea* essential oil, although at a lower content (24.9 %) compared to our study (57.8 %). This important variation in chemical composition could be due to genetic and environmental factors (climatic, seasonal, geographical and geological) [23].

Preservative effect of the applied treatments

The antimicrobial effectiveness of *J. phoenicea* essential oil and extract on the growth of *Staph. aureus* and *E. coli* in soft white cheese during refrigerated storage and abused temperature conditions is shown in Tab. 2. The bacterial population counts in the control group inoculated with *E. coli* increased to its maximum count at 24 h (from

Tab. 1. Chemical constituents of the essential oil of *Juniperus phoenicea* analysed using gas chromatography-mass spectrometry.

No.	Component	*RT* [min]	Relative percentage [%]
1	α-Pinene	12.53	57.8
2	Camphene	12.85	0.5
3	Sabinene	13.04	0.1
4	β-Pinene	14.02	0.8
5	β-Myrcene	14.81	1.3
6	δ-3-Carene	15.46	3.1
7	Cymene	16.10	0.4
8	Limonene	16.35	3.5
9	γ-Terpinene	17.42	0.4
10	*cis*-Linalool oxide	18.00	0.1
11	Terpinolene	18.43	0.2
12	Linalool	19.16	0.5
13	α-Campholenal	19.91	0.2
14	*trans*-Pinocarveol	20.44	0.2
15	Camphor	20.55	0.2
16	Isopinocamphone	21.03	0.1
17	*trans-p*-menth-2-ene-1,8-diol	21.52	0.3
18	Terpinen-4-ol	21.73	0.1
19	α-Terpineol	22.29	0.8
20	Verbenone	22.65	0.2
21	Fenchyl acetate	22.92	0.2
22	Pulegone	23.56	0.5
23	Linalyl acetate	24.00	0.5
24	Isopulegyl acetate	24.59	0.5
25	Bornyl acetate	24.96	0.1
26	Thymol	25.55	0.6
27	2,4-Decadien-1-ol	26.11	0.3
28	δ-Elemene	26.36	0.3
29	α-Terpinyl acetate	26.77	2.9
30	β-Bourbonene	27.66	0.1
31	β-Elemene	27.85	0.4
32	trans-Caryophyllene	28.63	1.6
33	Thujopsene	28.93	1.5
34	α-Humulene	29.56	0.7
35	Germacrene-D	30.23	1.3
36	β-Selinene	30.50	1.4
37	Acenaphthene	30.68	1.2
38	δ-Cadinene	31.23	5.5
39	α-Calacorene	31.71	0.3
40	Guaiol	31.95	0.7
41	Junenol	32.14	1.0
42	Citronellyl valerate	32.53	0.2
43	τ-Cadinol	33.84	3.7
44	α-Cadinol	34.45	1.1
45	Cadalene	34.78	0.1
46	Juniper camphor	35.40	0.3
47	13-epi-manoyl oxide	41.46	0.5
48	Totarol	47.62	0.1
Total identified			98.4
Monoterpene hydrocarbons			68.1
Oxygenated monoterpenes			8.0
Sesquiterpene hydrocarbons			13.2
Oxygenated sesquiterpenes			7.0
Others			2.1
Oil yield (w/w)			0.9

RT – retention time (given in elution order from SGE column).

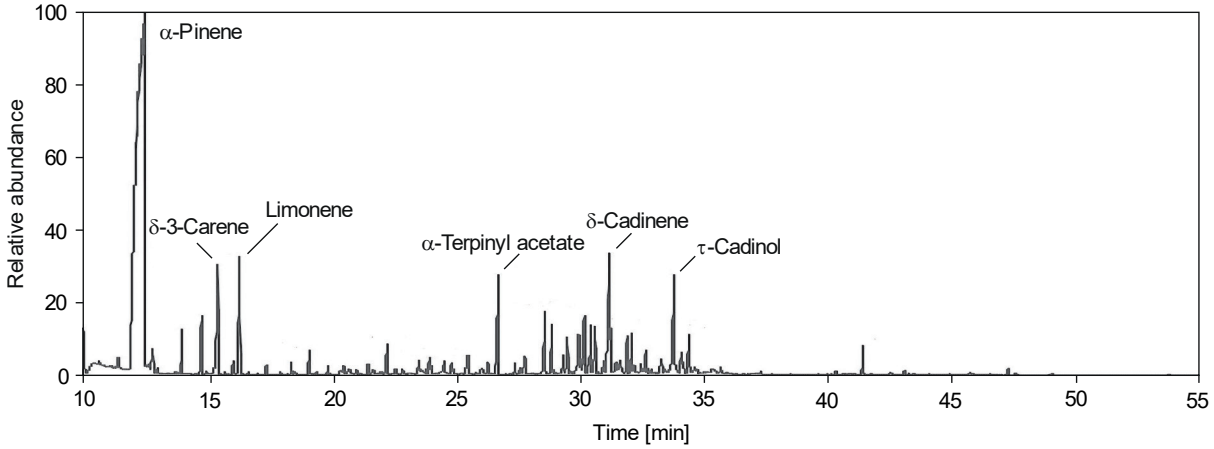

Fig. 1. Gas chromatography-mass spectrometry chromatogram of *Juniperus phoenicea* essential oil.

5.88 log CFU·g^{-1} to 7.54 log CFU·g^{-1}) and were then stable thereafter, while *Staph. aureus* counts increased slowly with the duration of storage (from 5.86 log CFU·g^{-1} to 6.83 log CFU·g^{-1}). The growth of *E. coli* and *Staph. aureus* was significantly reduced in all experimental cheese samples immersed in essential oil emulsion during the storage period when compared to controls ($p < 0.05$). Additionally, the treatment with the plant extract solution showed significantly lower counts than the controls, except for *E. coli* at 96 h and *Staph. aureus* at 24 h and 72 h of storage. After the application of the plant extract solution, a reduction by approximately 0.5 log CFU·g^{-1} in the *E. coli* numbers was observed until 72 h of storage, while the treatment with essential oil emulsion caused a significant reduction (> 1 log CFU·g^{-1}) especially during later periods of storage. On the other hand, a reduction of > 2 log CFU·g^{-1} was observed for *Staph. aureus* counts in cheese samples treated with essential oil emulsion over the storage of 120 h. However, there was not enough reduction in the treated group with plant extract solution during storage. The antimicrobial potential of plant extract and essential oil, in this study, was predominantly related to the contained bioactive substances, which may combine to perform their antimicrobial action. Our findings are in accordance with previous studies for single strains or cocktail of strains of *Staph. aureus* and *E. coli* in other types of cheese [2, 12, 29].

Regarding *J. phoenicea* essential oil, it was reported that its main active component is α-pinene, which was previously found to be an antimicrobial agent effective against *E. coli* and *Staph. aureus* [30]. Monoterpene hydrocarbons were also detected at high levels in the studied oil. These compounds have low water solubility and limited hydrogen-bonding capacity that may limit their activity in water emulsions. In addition, several authors [23, 25, 31] reported that various extracts of *J. phoenicea* are rich sources of numerous phenolic acids, flavonoid aglycones and flavonoid glycosides that possess significant antimicrobial pro-

Tab. 2. Effects of *Juniperus phoenicea* essential oil and ethanolic extract on the growth of *Escherichia coli* and *Staphylococcus aureus* in soft fresh cheese during storage.

Microorganism	Group	Count of microorganisms at the storage conditions [log CFU·g^{-1}]					
		0 h/4 °C	24 h/4 °C	36 h/25 °C	72 h/4 °C	96 h/4 °C	120 h/4 °C
Escherichia coli	Control 1	5.88 ± 0.08^{a}	7.54 ± 0.05^{a}	7.29 ± 0.16^{a}	7.52 ± 0.04^{a}	7.43 ± 0.06^{a}	7.50 ± 0.06^{a}
	Extract	5.88 ± 0.08^{a}	7.14 ± 0.19^{b}	6.48 ± 0.03^{b}	7.14 ± 0.02^{b}	7.45 ± 0.02^{a}	7.35 ± 0.02^{b}
	Essential oil	5.88 ± 0.08^{a}	7.16 ± 0.12^{b}	6.40 ± 0.02^{b}	6.34 ± 0.25^{c}	6.27 ± 0.21^{b}	6.46 ± 0.02^{c}
Staphylococcus aureus	Control 2	5.86 ± 0.10^{a}	5.93 ± 0.06^{a}	6.61 ± 0.22^{a}	6.55 ± 0.05^{a}	6.75 ± 0.26^{a}	6.83 ± 0.06^{a}
	Extract	5.86 ± 0.10^{a}	6.07 ± 0.18^{a}	6.33 ± 0.01^{b}	6.54 ± 0.01^{a}	6.25 ± 0.05^{b}	6.22 ± 0.03^{b}
	Essential oil	5.86 ± 0.10^{a}	4.46 ± 0.04^{b}	4.39 ± 0.06^{c}	4.25 ± 0.13^{b}	4.11 ± 0.08^{c}	4.17 ± 0.37^{c}

The values are mean ± standard deviation ($n = 3$). Different lowercase letters in superscript in the same column indicate significant differences ($p < 0.05$) between groups during storage.

perties against a wide range of bacteria and fungi. However, crude extracts generally contain not only bioactive compounds but also sugar, which may decrease their effectiveness against some bacteria. Previous in vitro studies also showed the antimicrobial efficacy of the *J. phoenicea* extract and essential oil against these pathogens [15, 16, 31, 32]. Various bioactive agents were suggested to influence the synthesis of DNA and RNA, interfere with membrane-integrated enzymes, cause denaturation of proteins, disrupt cytoplasmic membrane or alter the permeability of microbial cell, permitting macromolecules loss from the cell, which may eventually lead to bacterial cell death [2].

In the present study, *Staph. aureus* displayed higher susceptibility to *J. phoenicea* essential oil and extract compared to *E. coli*. The higher resistance of *E. coli* may be due to the complexity of its outer membrane, which provides a hydrophilic surface able to act as a barrier to the partition of plant essential oil and extract constituents [7]. Phenolic compounds, which are present in the plant essential oils and extracts, generally show antimicrobial activity against Gram-positive bacteria. Their effect depends on the amount of the compound present as, at low concentrations, they can interfere with enzymes involved in the production of energy and, at higher concentrations, they can denature proteins [2].

The results presented here also show that the antimicrobial activity against these pathogens determined in cheese immersed in plant extract solution was significantly lower than that in the samples immersed in plant essential oil emulsion. This could be explained by the possible affinity existing between the essential oil components (nonpolar) and the system used (soft fresh cheese), which contains approximately 26.9 % lipids [33]. This characteristic facilitates the migration of the essential oil to the surface of the product and allows for a better dissolution and/or dispersion in the food [34]. Similar behaviour was observed for mint (*Mentha spicata*) essential oil used for coating lor cheese (the traditional whey cheese of Turkey) stored at 4 °C for 15 days [35].

In our study, the antimicrobial effectiveness of plant essential oil and extract was not influenced by the simulated temperature abuse conditions from 24 h to 36 h. The use of this type of immersion may be the most effective method when a mild disturbance in the cold chain occurred. Similarly, the efficiency of *M. spicata* and *M. pulegium* essential oils on the growth of *Staph. aureus* was not affected by abused temperature conditions in chocolate mousse [9], indicating that storage temperature has a limited effect on the antibacterial activity of plant extracts in such experiments. However, the diffusibility of antimicrobial compounds is related to the storage temperature, storage time and acidity of food, which may also influence their effectiveness [11, 12, 35].

CONCLUSION

In this study, it was found that the essential oil used was rich in monoterpenes, with α-pinene as the major compound in leaves and berries. Moreover, total polyphenols were present at high levels with an average of 131.8 $g \cdot kg^{-1}$ DW (expressed as GAE) of extract. Furthermore, it can be concluded that the *J. phoenicea* essential oil and extract used had an antimicrobial effect against *E. coli* and *Staph. aureus* during refrigerated storage but also when a disruption in the cold chain took place. This activity was higher in the cheese samples immersed in essential oil emulsion than in the samples immersed in ethanolic extract solution. Although the tested strains were not eliminated and inhibited completely, plant essential oil and extract were able to effectively restrict proliferation of these food-borne pathogens in soft fresh cheese. The delay in growth of the pathogenic bacteria could particularly be useful in terms of food safety. These results demonstrated the potential advantages of using *J. phoenicea* essential oil and extract as a natural antimicrobial in cheese products.

Acknowledgements

The authors would like to thank Professor Mohamed Yousfi, Dr. Mohamed Harrat and Dr. Tayeb Berramdane (Laboratory of Fundamental Sciences, Amar Telidji University, Laghouat, Algeria) for their help in obtaining plant extracts and co-operation.

REFERENCES

1. Zantar, S. – Yedri, F. – Mrabet, R. – Laglaoui, A. – Bakkali, M. – Zerrouk, M. H.: Effect of *Thymus vulgaris* and *Origanum compactum* essential oils on the shelf life of fresh goat cheese. Journal of Essential Oil Research, *26*, 2014, pp. 76–84. DOI: 10.1080/10412905.2013.871673.
2. Tayel, A. A. – Hussein, H. – Sorour, N. M. – El-Tras, W. F.: Foodborne pathogens prevention and sensory attributes enhancement in processed cheese via flavoring with plant extracts. Journal of Food Science, 80, 2015, pp. M2886–M2891. DOI: 10.1111/1750-3841.13138.
3. Ayana, B. – Turhan K. N.: Use of antimicrobial methylcellulose films to control *Staphylococcus*

aureus during storage of Kasar cheese. Packaging Technology and Science, *22*, 2009, pp. 461–469. DOI: 10.1002/pts.870.

4. Ehsani, A. – Mahmoudi, R.: Effects of *Mentha longifolia* L. essential oil and *Lactobacillus casei* on the organoleptic properties and on the growth of *Staphylococcus aureus* and *Listeria monocytogenes* during manufacturing, ripening and storage of Iranian white-brined cheese. International Journal of Dairy Technology, *66*, 2013, pp. 70–76. DOI: 10.1111/j.1471-0307.2012.00865.x.
5. Langroodi, A. M. – Mehdizadeh, T. – Majidi, L. – Neyriz-Naghadehi, M.: *Lactobacillus acidophilus* and *Anethum graveolens* essential oil in Iranian cheese against *Escherichia coli* O157:H7. Flavour and Fragrance Journal, *36*, 2021, pp. 190–196. DOI: 10.1002/ffj.3629.
6. Mehdizadeh, T. – Narimani, R. – Langroodi, A. M. – Moghaddas Kia, E. – Neyriz-Naghadehi, M.: Antimicrobial effects of *Zataria multiflora* essential oil and *Lactobacillus acidophilus* on *Escherichia coli* O157 stability in the Iranian probiotic white-brined cheese. Journal of Food Safety, *38*, 2018, article e12476. DOI: 10.1111/jfs.12476.
7. Diniz-Silva, H. T. – Brandao, L. R. – de Sousa Galvao, M. – Madruga, M. S. – Maciel, J. F. – Leite de Souza, E. – Magnani, M.: Survival of *Lactobacillus acidophilus* LA-5 and *Escherichia coli* O157:H7 in Minas frescal cheese made with oregano and rosemary essential oils. Food Microbiology, *86*, 2020, article 103348. DOI: 10.1016/j.fm.2019.103348.
8. De Souza, G. T. – De Carvalho, R. J. – De Sousa, J. P. – Tavares, J. F. – Schaffner, D. – De Souza, E. L. – Magnani, M.: Effects of the essential oil from *Origanum vulgare* L. on survival of pathogenic bacteria and starter lactic acid bacteria in semihard cheese broth and slurry. Journal of Food Protection, *79*, 2016, pp. 246–252. DOI: 10.4315/0362-028X.JFP-15-172.
9. Houicher, A. – Bensid, A. – Oudenani, B. – Rahmania, F. – Özogul, F.: Effects of spearmint and pennyroyal essential oils on the growth of *Staphylococcus aureus* inoculated in chocolate mousse during chilled storage and abused temperature. Journal of Food and Nutrition Research, *59*, 2020, pp. 332–340. ISSN: 1336-8672. <https://www.vup.sk/download.php?bulID=2082>
10. Amatiste, S. – Sagrafoli, D. – Giacinti, G. – Rosa, G. – Carfora, V. – Marri, N. – Tammaro, A. – Bovi, E. – Rosati, R.: Antimicrobial activity of essential oils against *Staphylococcus aureus* in fresh sheep cheese. Italian Journal of Food Safety, *28*, 2014, pp. 148–150. DOI: 10.4081/ijfs.2014.1696.
11. Lin, L. – Gu, Y. – Cui, H.: Moringa oil/chitosan nanoparticles embedded gelatin nanofibers for food packaging against *Listeria monocytogenes* and *Staphylococcus aureus* on cheese. Food Packaging and Shelf Life, *19*, 2019, pp. 86–93. DOI: 10.1016/j.fpsl.2018.12.005.
12. Kavas, N. – Kavas, G. – Saygili, D.: Use of ginger essential oil-fortified edible coatings in Kashar cheese and its effects on *Escherichia coli* O157:H7 and *Staphylococcus aureus*. CyTA - Journal of Food, *14*, 2015, pp. 317–323. DOI: 10.1080/19476337.2015.1109001.
13. Ramdani, M. – Lograda, T. – Silini, H. – Zeraib, A. – Chalard, P. – Figueredo, G. – Bouchaala, M. – Zerrar, S.: Antibacterial activity of essential oils of *Juniperus phoenicea* from Eastern Algeria. Journal of Applied Pharmaceutical Science, *3*, 2013, pp. 22–28. DOI: 10.7324/JAPS.2013.31105.
14. Espina, L. – García-Gonzalo, D. – Laglaoui, A. – Mackey, B. M. – Pagán, R.: Synergistic combinations of high hydrostatic pressure and essential oils or their constituents and their use in preservation of fruit juices. International Journal of Food Microbiology, *161*, 2013, pp. 23–30. DOI: 10.1016/j.ijfoodmicro.2012.11.015.
15. Ait-Ouazzou, A. – Lorán, S. – Arakrak, A. – Laglaoui, A. – Rota, C. – Herrera, A. – Pagán, R. – Conchello, P.: Evaluation of the chemical composition and antimicrobial activity of *Mentha pulegium, Juniperus phoenicea* and *Cyperus longus* essential oils from Morocco. Food Research International, *45*, 2012, pp. 313–319. DOI: 10.1016/j.foodres.2011.09.004.
16. Angioni, A. – Barra, A. – Russo, M. T. – Coroneo, V. – Dessi, S. – Cabras, P.: Chemical composition of the essential oils of *Juniperus* from ripe and unripe berries and leaves and their antimicrobial activity. Journal of Agricultural and Food Chemistry, *51*, 2003, pp. 3073–3078. DOI: 10.1021/jf026203j.
17. Oztürk, M. – Tümen, I. – Ugur, A. – Aydogmus-Öztürk, F. – Topcu, G.: Evaluation of fruit extracts of six Turkish *Juniperus* species for their antioxidant, anticholinesterase and antimicrobial activities. Journal of the Science of Food Agriculture, *91*, 2011, pp. 867–876. DOI: 10.1002/jsfa.4258.
18. Bensid, A. – Yilmaz, U. – Bendeddouche, B. – Özogul F.: Effect of the icing with thyme, oregano and clove extracts on quality parameters of gutted and beheaded anchovy (*Engraulis encrasicholus*) during chilled storage. Food Chemistry, *145*, 2014, pp. 681–686. DOI: 10.1016/j.foodchem.2013.08.106.
19. Singleton, V. L. – Orthofer, R. – Lamuela-Raventos, R. M.: Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin–Ciocalteu reagent. Methods in Enzymology, *299*, 1999, pp. 152–178. DOI: 10.1016/S0076-6879(99)99017-1.
20. Houicher, A. – Kuley, E. – Bendeddouche, B. – Özogul, F.: Effect of *Mentha spicata* L. and *Artemisia campestris* extracts on the shelf-life and quality of vacuum packed refrigerated sardine (*Sardina pilchardus*) fillets. Journal of Food Protection, *76*, 2013, pp. 1719–1725. DOI: 10.4315/0362-028X.JFP-13-118.
21. British Pharmacopoeia 1988. London : Her Majesty's Stationary Office, 1990. ISBN: 0113212704.
22. Menaceur, F. – Benchabane, A. – Hazzit, M. – Baaliouamer, A.: Chemical composition and antioxidant activity of Algerian *Juniperus phoenicea* L. extracts. Journal of Biologically Active Products from Nature, *3*, 2013, pp. 87–96. DOI: 10.1080/22311866.2013.782754.

23. Ennajar, M. – Bouajila, J. – Lebrihi, A. – Mathieu, F. – Abderraba, M. – Raies, A. –Romdhane, M.: Chemical composition and antimicrobial and antioxidant activities of essential oils and various extracts of *Juniperus phoenicea* L. (Cupressacees). Journal of Food Science, *74*, 2009, pp. M364–M371. DOI: 10.1111/j.1750-3841.2009.01277.x.
24. Amalich, S. – Fadili, K. – Fahim, M. – El Hilali, F. – Zair, T.: Polyphenols content and antioxidant power of fruits and leaves of *Juniperus phoenicea* L. from Tounfite (Morocco). Morrocan Journal of Chemistry, *4*, 2016, pp. 177–186. DOI: 10.48317/IMIST.PRSM/morjchem-v4i1.4013.
25. Dane, Y. – Mouhouche, F. – Canela-Garayoa, R. – Delpino-Rius, A.: Phytochemical analysis of methanolic extracts of *Artemisia absinthium* L. 1753 (Asteraceae), *Juniperus phoenicea* L., and *Tetraclinis articulata* (Vahl) Mast, 1892 (Cupressaceae) and evaluation of their biological activity for stored grain protection. Arabian Journal for Science and Engineering, *41*, 2016, pp. 2147–2158. DOI: 10.1007/s13369-015-1977-2.
26. Bakchiche, B. – Gherib, A. – Smail, A. – Custódia, G. M. – Graca, M.: Antioxidant activities of eight Algerian plant extracts and two essential oils. Industrial Crops and Products, *46*, 2013, pp. 85–96. DOI: 10.1016/j.indcrop.2013.01.020.
27. Mba, O. – Kwofie, E. – Ngadi, M.: Kinetic modelling of polyphenol degradation during common beans soaking and cooking. Heliyon, *5*, 2019, article e01613. DOI: 10.1016/j.heliyon.2019.e01613.
28. Harmouzi, A. – Boughdad, A. – El Ammari, Y. – Chaouch, A.: Chemical composition and toxicity of Moroccan *Tetraclinis articulata* and *Juniperus phoenicea* essential oils against *Aphis citricola* Goot, 1912 (Homoptera, Aphididae). Research on Chemical Intermediates, *42*, 2016, pp. 7185–7197. DOI: 10.1007/s11164-016-2528-5.
29. Shan, B. – Cai, Y. Z. – Brooks, J. D. – Corke, H.: Potential application of spice and herb extracts as natural preservatives in cheese. Journal of Medicinal Food, *14*, 2011, pp. 284–290. DOI: 10.1089/jmf.2010.0009.
30. Zavareh, S. T. – Ardestani, F.: Antibacterial effects of chitosan coating containing *Mentha aquatica* L. essence against *Escherichia coli, Staphylococcus aureus* and *Listeria monocytogenes* in Iranian white cheese. International Journal of Dairy Technology, *73*, 2020, pp. 585–593. DOI: 10.1111/1471-0307.12678.
31. Elmhdwi, M. F. – Attitalla, I. H. – Khan, B. – Menaa, F.: Evaluation of antibacterial activity and antioxidant potential of different extracts from the leaves of *Juniperus phoenicea*. Journal of Plant Pathology and Microbiology, *6*, 2015, pp. 1–8. DOI: 10.4172/2157-7471.1000300. 32. Ghouti, D. – Rached, W. – Abdallah, M. – Pires, T. C. S. P. – Calhelha, R. C. – Alves, M. J. – Abderrahmane, L. H. – Barros, L. – Ferreira, I. C. F. R.: Phenolic profile and in vitro bioactive potential of Saharan *Juniperus phoenicea* L. and *Cotula cinerea* (Del) growing in Algeria. Food and Function, *9*, 2018, pp. 4664– 4672. DOI: 10.1039/c8fo01392f.
33. O'Callaghan, Y. C. – O'Connor, T. P. – O'Brien, N. M.: Nutritional aspects of cheese. In: Fox, P. F. – Guinee, T. P. – Cogan, T. M. – McSweeney, P. L. H. (Eds.): Fundamentals of cheese science. 2nd edition. New York : Springer, 2017, pp. 715–730. DOI: 10.1007/978-1-4899-7681-9.
34. Dannenberg, G. D. – Funck, G. D. – Cruxen, C. E. – Marques, J. D. – Silva, W. –Fiorentini, Â. M.: Essential oil from pink pepper as an antimicrobial component in cellulose acetate film: Potential for application as active packaging for sliced cheese. LWT - Food Science and Technology, *81*, 2017, pp. 314–318. DOI: 10.1016/j.lwt.2017.04.002.
35. 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, *12*, 2014, pp. 40–45. ISSN: 1459–0255.

Received 2 August 2021; 1st revised 15 October 2021; 2nd revised 15 November 2021; accepted 15 December 2021; published online 22 February 2022.