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ANTIMICROBIAL AND ANTIOXIDANT ACTIVITIES OF *CITRUS LIMON* PEEL ESSENTIAL OILS AND THEIR APPLICATION AS A NATURAL PRESERVATIVE IN FRESH CREAM: EFFECTS ON OXIDATIVE AND SENSORY PROPERTIES

– Research paper –

Farida KEHAL [1,2,] *, Loucif CHEMACHE[2], Louiza HIMED [1], Malika BARKAT [1]

[1] Laboratoire de recherche Biotechnologie et Qualité des Aliments (BIOQUAL), Institut de la Nutrition, de l'Alimentation et des Technologies Agro-Alimentaires (INATAA), Université Frères Mentouri Constantine 1, Route de Ain-El-Bey 25000, Constantine, Algérie.
[2] Laboratoire de Nutrition et Technologie Alimentaire (LNTA), Institut de la Nutrition, de l'Alimentation et des Technologies Agro-Alimentaires (INATAA), Université Frères Mentouri Constantine 1, Route de Ain-El-Bey 25000, Constantine, Algérie.

Abstract: The aim of this study is to enhance the use of *Citrus limon* peel by utilizing its essential oil as a natural aroma and preservative in fresh cream. The essential oil was extracted through hydrodistillation and analyzed using gas chromatography-mass spectrometry (GC-MS). The antioxidant and antimicrobial activities of the essential oil were assessed at varying concentrations prior to its incorporation into fresh cream. Subsequently, the antioxidant and sensory stabilities of the fresh cream were evaluated. The essential oils were characterized by a dominance of monoterpenes with a high limonene content (61%), oxygenated monoterpenes, sesquiterpenes and oxygenated sesquiterpenes. The essential oils effectively reduced the DPPH° radical, which was confirmed by the β-carotene bleaching assay. *Debaryomyces sp.* and *Rhodotorula sp.* showed sensitivity to the essential oils and have MICs of 0.25% and 0.5% respectively. However, *Zygosaccharomyces sp1* and *Zygosaccharomyces sp2* were resistant. Their MICs were 1 and > 4%, respectively. The TBARS test showed that fresh creams with *Citrus limon* essential oils were resistant to forced oxidation. Incorporation into fresh cream at 0.125 and 0.25% did not alter the flavor of the product, which did not differ from the control. From all results; we can conclude that lemon essential oil can be used as an antioxidant and aromatic agent in fresh cream.

Keywords: *Citrus limon*, essential oil, GC-MS, fresh cream, natural preservative.

INTRODUCTION

Fresh cream is a dairy product that contains several nutrients, such as proteins, carbohydrates, fat-soluble vitamins, and most notably, fat (Jeantet et al., 2016). Due to its high water content, fresh cream is a perishable product and is susceptible to various types of microbial and physicochemical alterations. Any alteration in the hygienic quality of fresh cream poses a health risk to consumers. These alterations are generally invisible and result from the development of pathogenic microorganisms, which can cause food poisoning of varying degrees of severity. In addition, any alteration in the market value of fresh cream leads to changes in its textural, rheological, and sensorial properties.

Although such alterations are not dangerous to consumers, they render the product unmarketable (Chémat et al., 2019). The most noticeable consequence of such alterations is the emergence of unpleasant odors, which often leads to consumers rejecting the product (Choe and Min, 2009; Falleh et al., 2020).

There has been a growing focus on the use of natural preservatives to reduce the risks associated with synthetic preservatives. Essential oils, phenolic extracts, vitamin C, vitamin E, and citric acid are among the most commonly used natural compounds for preserving food. Essential oils are frequently used in the food industry to enhance the taste, flavor, and color of foods (Aprotosoaie et al., 2010). Furthermore, essential oils have distinct

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* Corresponding author.
E-Mail address: kehal.farida@umc.edu.dz, khlfarida@yahoo.fr

chemical composition profiles that enable them to be utilized as natural agents for extending the shelf life of food (Holley et al., 2005; Adelakun et al., 2016). The use of essential oils in food preservation necessitates a thorough understanding of their properties, such as the susceptibility of the relevant microorganisms, their specific mode of action, their antimicrobial and antioxidant power, and the effect of the food matrix composition on their antimicrobial and antioxidant properties (Adelakun et al., 2016). It has been reported in the literature that essential oils derived from *Citrus* fruits, particularly *Citrus limon*, exhibit antioxidant (Djenane, 2015; Himed et al., 2019; Taktak et al., 2021) and antimicrobial activities (Caccioni et al., 1998; Hammer et al., 1999; Moreira et al., 2005; Taktak et al., 2021), which are particularly intriguing and can be advantageous and promising when incorporated into food to limit the risk of alterations caused by microorganisms or lipid oxidation.

Previous research has shown that the essential oils of *Citrus limon* can prevent both microbiological and physicochemical spoilage when applied to a range of products, including sardines (Djenane, 2015), margarine (Himed et al., 2016), and ice cream (Tomar and Akarca, 2019). Based on these findings, the present study was carried out to investigate the potential use of *Citrus limon* peel essential oil as a natural preservative and flavoring agent in a high-fat dairy product, namely fresh cream. Thus, this study aims to explore the effects of the incorporation of essential oils of *Citrus limon* at varying concentrations (0, 0.125, 0.25, and 0.5%) on the antioxidant properties and the ability to prevent lipid oxidation to improve the preservation of fresh cream without altering its sensory properties.

MATERIALS AND METHODS

Essential oils extraction

Lemon peel (*Citrus limon*), *Eureka* variety, was used to extract essential oils through the hydrodistillation method using the Clevenger apparatus. To perform the extraction, 100 grams of lemon peel were added to a 2 L double-necked Erlenmeyer flask that was filled with distilled water up to two-thirds of its capacity. The mixture was then boiled for 2h, and the resulting vapor was condensed with a refrigerant. The condensate was collected in a separating funnel, where the two immiscible phases, i.e., the aqueous phase and the organic phase (which constituted the essential oil), were separated. The organic phase was then dried with anhydrous sodium sulfate, which eliminated all traces of water (Chanthaphon et al., 2008).

GC/MS Analysis

The essential oil that was extracted underwent analysis using the gas chromatography-mass spectrometry (GC/MS) technique, utilizing an Agilent-Technologies Inc. Hewlett-Packard - MSD 5973 A instrument, equipped with an apolar capillary column DB-5. The column has a diameter of 0.25 mm, a length of 30 m, and a film thickness of 0.25 µm. A split mode with a ratio of 1:20 was used for the injection of 0.2 µL of each sample, and helium was used as the carrier gas. The analysis was conducted using programmed temperature conditions, starting at 60 °C for 8 min, followed by a ramp from 60 to 280 °C over 110 min, and a final temperature of 280 °C for 15 min. The injection was performed at 250 °C, the interface temperature was 280 °C, the equilibration time was 0.5 min, and the flow rate was 3 mL/min with a pressure of 10^{-7} mbar. Electron impact (EI) mode with at least 70 eV was utilized for the mass spectrometric detection. Identification of the components was accomplished by comparing the experimentally obtained retention indices (Kovats indices) and ion fragments with those reported by Adams (2017). To prepare the solution of n-alkanes ranging from C8 to C26, 0.1 g of each alkane was mixed in 20 mL of pentane.

Determination of antioxidant activity

β-carotene bleaching test

The β-carotene bleaching test was performed according the method reported by Mayachiew and Devahastin (2008). β-carotene (2 mg) was dissolved in 20 mL of chloroform, and 3mL of the obtained solution was introduced into a flask containing 40 mg linoleic acid and 400 mg Tween 20. After evaporation of chloroform in the rotavapor (Buchi R-210) at 50 °C for 5 min, 100 mL of oxygen-saturated distilled water were added. From the new solution, 3 mL were mixed with 120 µL of the solution of the essential oil in ethanol at a concentration of 0.004 g/mL. The essential oil was replaced by α-tocopherol and ethanol in the positive and negative controls, respectively. The experiment involved placing test tubes in a 50°C water bath (Memmert). The oxidation of the β-carotene emulsion was then monitored for 120 min at 20 min intervals using spectrophotometry (Shimadzy UV 1800), at 470 nm. The antioxidant activity was then calculated using the equation 1:

$$AA(\%) = \left(1 - \frac{AE0 - AEt}{AC0 - ACt}\right) * 100 \quad (1)$$

AA (%): antioxidant activity, AE0: absorbance of the emulsion with essential oil or vitamin E measured at t=0, AC0: absorbance of the negative control at t=0, AEt: absorbance with essential oil or vitamin E at t=120 min, and ACt: absorbance of the negative control at t=120 min.

Antiradical activity by DPPH assay

To determine the free radical scavenging activity, the method outlined by Dung et al. (2008) and Nikhat et al. (2009) was followed. In test tubes, 2.9 mL of each essential oil dilution (16, 8, 4, 2, 1, and 0.5 µg/mL) was mixed with 100 µL of a 0.004% (w/v) DPPH° ethyl solution. Following shaking, the tubes were kept in the absence of light at ambient temperature for 30 minutes, and the absorption was measured at a wavelength of 517 nm. The essential oil dilutions were replaced in the negative control by 100 µL of ethanol, and in the positive control by α-tocopherol dilutions (16, 8, 4, 2, 1, and 0.5 g/mL). DPPH° free radical inhibition was expressed in percentages (PR%) and calculated according to the equation 2:

$$PR(\%) = \frac{AC - AE}{AC} * 100 \quad (2)$$

where PR: power of reduction in percentage, AE: absorbance of DPPH° solution with essential oil or vitamin E, and AC: absorbance of DPPH° solution with essential oil and vitamin E. The antioxidant activity of the essential oil or vitamin E was expressed by the Effective Concentration (EC 50), which was calculated graphically from three separate tests, where the abscissa represents the concentration of the test compound and the ordinate the reduction power in percent.

Antimicrobial activity

Two bacteria were tested: *Pseudomonas aeruginosa* and *Escherichia coli*, which were provided to us by the public hospital of El-Milia, Jijel. These two bacteria are Gram-negative and pathogenic, and they most often contaminate fresh cream. Four strains of yeast were also tested: *Zygosaccharomyces* sp1, *Debaryomyces* sp., *Rhodotorula* sp., and *Zygosaccharomyces* sp2. These strains were isolated and identified from contaminated fresh cream. The identification was accomplished through the study of cultural and morphological characteristics, as well as the study of sugar fermentation. Bacteria were plated in Petri dishes containing Hektoen medium and incubated for 24 h. Several isolated and identical colonies were selected from a culture that was 18 h old and placed into 5 mL of sterile physiological water containing 0.9% NaCl. The transmittance was taken on a spectrophotometer (Shimadzy UV1800) at 620 nm.

Aromatogram method

A sterile cellulose disk, 6 mm in diameter and impregnated with essential oil, was placed on the surface of an agar in a petri dish and inoculated with the microorganism. After incubation, the results were expressed by measuring the diameter (mm) of the clear zone around the disc, called the inhibition zone. According to Hussain et al. (2010), 20 mL of agar media (Muller-Hinton agar for bacteria and Sabouraud agar for yeast) in super cooling were poured into Petri dishes. After solidification of the medium, 100 µL of the microbial suspension was spread on the surface. Under aseptic conditions, sterile discs soaked with 5µL of *Citrus limon* essential oil are deposited on the agar. The incubation was completed at 25°C for 48 h for yeast and 37 °C for 24 h for bacteria.

Agar dilution method

Antimicrobial activity was also evaluated by the agar dilution method described by Hammer et al. (1999). A volume of 20 mL of Muller Hinton Agar melted and added with 0.5% (v/v) tween 20 were put into test tubes by aseptically adding 200, 400, 800, and 1600 µL of the essential oil to obtain the concentrations of 1, 2, 4, and 8%. Subsequently, the same amount of Sabouraud with 0.5% (v/v) tween 20 was put into test tubes with the addition of 25, 50, 100, 200, 400, and 800 µL to obtain the essential oil concentrations of 0.125, 0.25, 0.5, 1, 2, and 4%. The culture media with the different dilutions of the essential oil and tween 20 were poured into petri dishes. After the agar had solidified, the plates were placed at 35 °C for 30 min. The dishes poured with Mueller-Hinton medium were divided into two parts, in which the two bacterial strains were put as 1 µL deposits containing 107 germs/ mL

The dishes poured with Sabouraud media were divided into four parts, where the four strains of yeast were placed at the same concentration as the bacteria. Afterwards, the petri dishes were incubated at 37ºC for 24 h for the bacteria and at 28ºC for 48 h for the yeast. The minimum inhibitory concentration (MIC) represents the lowest concentration of essential oil that inhibits the visible growth of a given strain of bacteria or yeast after the incubation (Memmert HPP 110) period at 37 and 28°C.

Fresh creams preparation

The fresh cream used in this study has a fat and moisture content of 30 and 15%, respectively. The incorporation of the essential oil into the fresh cream was performed under aseptic conditions after the pasteurization step. The essential oil was sterilized by passing it through a 0.22 µm sterile filter (MILLIPORE MILLEX®-GS) before it was added to the fresh cream. The essential oil was incorporated into the cream at rates of 0.125%, 0.25%, and 0.5%. They were determined by the biological activity tests and preliminary sensory analysis. Samples of raw milk, cream before pasteurization, creams, and elaborated fresh creams were collected, and the analyses were made every two days from the first day of opening until the day of their alteration.

Determination of pH and titratable acidity

The determination of pH was conducted with a pH meter at a temperature of 20 to 25°C. The acidity of raw milk and fresh cream was also determined by measuring lactic acid with sodium hydroxide at 0.11 mol/L (N/9). The phenolphthalein, used as a colored indicator, indicates the limit of neutralization by changing color (to a pale pink). This acidity is expressed in degrees Dornic (°D), where 1 °D represents 0.1 g of lactic acid in one liter of milk (ISO/TS 22113, 2012).

Oxidative stability assessment by Schaal oven test

The oxidative stability of fresh cream was assessed by the Schaal oven test, which consists of heating 50 g of fresh cream in open capsules in air at a temperature of 63°C until the appearance of oxidation odors (8 days), which are evaluated at regular intervals by the thiobarbituric acid reactive substances (TBARS) determination (Bandyopadhyay et al., 2008). A 20 mL of trichloroacetic acid (20%) at pH 3.5 was added to 20 g of fresh cream. The mixture was centrifuged (Hettich EBA 20) at 4000 rpm for 15 min. A volume of 2mL of the thiobarbituric acid (TBA) solution (0.02 mol/L) was added to 2mL of the obtained supernatant, which is the extracted malondialdehyde (MDA), with stirring for 5 sec. Thereafter, the mixture was transferred to a water bath set at 95°C for 10 min to promote the TBA and MDA reaction. After cooling the mixture, butan-1-ol (5 mL) was added and stirring for 5 min. The upper butan-1-ol phase obtained after centrifugation at 1500 rpm for 10 min was used for absorbance measurement at 532 nm (Djenane et al., 2012). The amount of thiobarbituric acid reactive substances was calculated using the equation 3 (Draper and Hadley, 1990):

$$\text{MDA mg equi/Kg} = (A * V_{TCA} * 2 * M * 10^{-1})/(1{,}66 * m) \quad (3)$$

where V_{TCA}: volume of trichloroacetic acid (mL), m: weight of the test sample (g), and M: molecular weight of MDA (72 g/mol).

Sensory analysis

The organoleptic analyses were carried out to evaluate, in a general way, the degree of appreciation of the fresh creams, added with the essential oil of lemon, by carrying out sensory profiles. The panel of tasters was composed of 40 male and female members. The tasters evaluated coded samples of each product by indicating their level of appreciation. The hedonic scale employed a verbal nine-point scale to rate the stimuli, which was then translated into numerical scores. Specifically, the ratings ranged from 1 (indicating an extreme dislike) to 5 (indicating a neutral response) to 9 (indicating an extreme liking). The fresh creams were presented simultaneously in identically coded containers. The tasters were asked to complete the questionnaires based on the attributes describing the texture, color, smell, taste, and aroma of the products.

Statistical analysis

The analyses were carried out in triplicate, and the data are expressed as mean ± standard deviation. The results were analyzed by one-way analysis of variance and the student test at a significance level of 0.05. The relationships between the essential oil incorporation rate and the physicochemical and sensory characteristics of fresh creams were evaluated by Pearson's correlation analysis using Minitab 16 (Minitab Inc., State College, PA, USA).

RESULTS AND DISCUSSION

Chemical composition

The chemical composition of the extracted essential oils is reported in Table 1 and Figure 1. A total of 30 compounds were identified and divided into monoterpenes (78.98%), oxygenated monoterpenes (11.04%), sesquiterpenes (3.76%), oxygenated sesquiterpenes (1.52%), and other oxygenated compounds (2.51%).

Limonene was the main component identified in the essential oil, and it represents 61.62% of the total identified compounds. Other monoterpenes were also identified, and according to their dominance, we can mention α-pinene (9.65%), γ-terpinene (3.84%), β-pinene (1.47%), β-myrcene (1.35%), cis-α-bergamotene (0.80%), and other compounds at different concentrations. In addition, other oxygenated monoterpenes were identified at varying concentrations; the most dominant were α-citral (4.22%), cis-citral (2.39%), linalyl propionate (1.07%), trans-citronellol (0.78%) and cis-litronellol (0.60%). In addition, sesquiterpenes and oxygenated sesquiterpenes were also identified. These results showed that the analyzed essential oil contains β-elemene (2.22%), γ-cadinene (1.21%), myristicin (0.81%) and caryophyllene oxide (0.71%). In the literature, Hosni et al. (2010) reported that in Tunisian *Citrus*, the monoterpenes ranged between 97.6 and 99.3%, and they represented about 80.03% according to Mushtaq et al. (2006). Several other studies have reported that limonene is the major compound identified in *Citrus* essential oils. The concentration of limonene obtained in the present study (61.62%) is significantly higher than those reported by Mushtaq et al. (2006) and Djenane (2015), which are 53.61% and 51.40%, respectively. According to previous studies, the number and percentage of compounds identified in essential oils extracted from *Citrus* differ from one study to another. As most constituents of essential oils are volatile and unstable, it is possible that these fluctuations can be due to genetic factors, extraction methods (El Aboubi et al., 2023), and storage conditions. According to Bakkali et al. (2008), the chemical composition of *Citrus* essential oils differs even from one variety to another. These authors have identified 25 to 60 compounds, of which limonene is the major compound. Indeed, the physicochemical and biological properties of these essential oils are strongly related to the content of this compound.

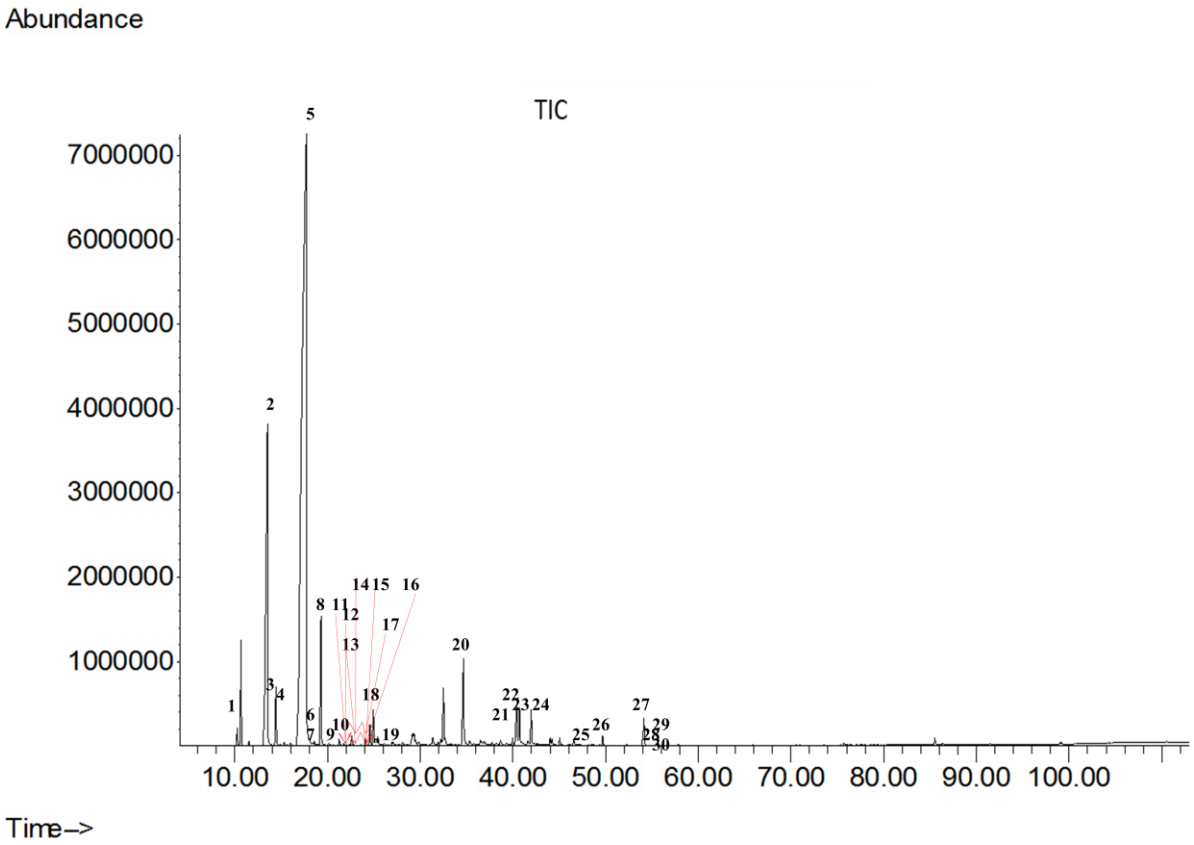

Figure 1. Total ion chromatograms (TIC) for GC-MS analyses of *Citrus limon* peel essential oils

Table 1. Chemical composition of extracted *Citrus limon* peel essential oils

N°	**Components**	**Class**	**IR**	**Content (%)**
1	α-thujene	Monoterpenes	931.196	0.24
2	α- pinene	Monoterpenes	938.610	9.65
3	β-pinene	Monoterpenes	981.766	1.47
4	β-myrcene	Monoterpenes	995.024	1.35
5	Limonene	Monoterpenes	1045.348	61.26
6	β-ocimene	Monoterpenes	1056.486	0.13
7	α-terpinolene	Monoterpenes	1108.546	0.19
8	γ-terpinene	Monoterpenes	1066.498	3.84
9	linalool	Oxygenatedmonoterpenes	1109.571	0.39
10	cis -limoneneoxide	Oxygenatedmonoterpenes	1138.187	0.13
11	camphor	Oxygenatedmonoterpenes	1147.971	0.26
12	citronellal	Oxygenatedmonoterpenes	1150.334	0.25
13	α-terpineol	Oxygenatedmonoterpenes	1192.000	0.37
14	trans-carveol	Oxygenatedmonoterpenes	1231.182	0.20
15	cis-Citronellol	Oxygenatedmonoterpenes	1148.022	0.61
16	trans-citronellol	Oxygenatedmonoterpenes	1149.254	0.78
17	cis-carveol	Oxygenatedmonoterpenes	1244.105	0.20
18	cis-citral	Oxygenatedmonoterpenes	1248.850	2.39
19	geranial	Oxygenatedmonoterpenes	1279.198	0.17
20	α-citral	Oxygenatedmonoterpenes	1248.850	4.22
21	linalylpropionate	Oxygenatedmonoterpenes	1199.414	1.07
22	neryl acetate	Otheroxygenatedcompounds	1361.966	1.40
23	piperitenone oxide	Otheroxygenatedcompounds	1363.013	1.11
24	β-elemene	Sesquiterpenes	1373.211	2.22
25	trans-caryophyllene	Sesquiterpenes	1391.324	0.11
26	Cis-α-bergamotene	Monoterpenes	1135.896	0.85
27	γ-cadinene	Sesquiterpenes	1501.000	1.21
28	myristicin	Oxygenatedsesquiterpenes	1531.537	0.81
29	germacrene	Sesquiterpenes	1477.154	0.22
30	caryophylleneoxide	Oxygenatedsesquiterpenes	1584.603	0.71
Monoterpenes				78.98
Oxygenated monoterpenes				11.04
Sesquiterpenes				3.76
Oxygenated sesquiterpenes				1.52
Other oxygenated compounds				2.51
Total (%)				**97.81**

IR: Retention Index

Antioxidant activity

The results showed that the inhibition of coupled oxidation of linoleic acid and β-carotene was significant ($p < 0.05$) at a concentration of 4000 µg/mL of lemon essential oil, with an antioxidant activity of 33.57%, which was significantly higher than that of vitamin E (23.38%) (Table 2). It was also found that lemon EO has a higher DPPH° radical scavenging power than vitamin E. For EC50, the essential oil showed a significantly low EC50 (5.5 µg/mL) compared with that of vitamin E (12.90 µg/mL). This result can be confirmed by the β-carotene bleaching test, which showed that the antioxidant activity of lemon EO was significantly higher than that of vitamin E, and are 33.57 and 23.38%, respectively. The antioxidant activity of *C. limon* EO is probably related to the major components, which are mainly monoterpenes, including limonene, β-pinene, and γ-terpinene (Ben Hsouna et al., 2017; Taktak et al., 2021). According to Tang et al. (2001), the antioxidant power of β-pinene and limonene can reduce the normal rate of chemical oxidation reactions by scavenging the hydroxyl radical. According to Liyana-Pathriana and Shahidi (2006), an antioxidant that inhibits or delays β-carotene bleaching is a free radical scavenger as well as a primary antioxidant.

Antimicrobial activity

The results of the antimicrobial activity obtained by measuring the diameter of inhibition of the growth of microorganisms are reported in Table 2. Both bacterial strains (*P. aeruginosa* and *E. coli*) showed resistance against lemon essential oil, with a slight sensitivity of *E. coli* and an inhibition zone diameter of 8.35 mm. *Zygosaccharomyces sp1* and *Zygosaccharomyces sp2* strains were resistant against lemon essential oil, with mean inhibition zone diameters of 7.25 and 8.75 mm for S1 and S7, respectively. However, this essential oil was able to slightly inhibit the growth of *Debaryomyces sp*. and *Rhodotorula sp*., which showed average inhibition zone diameters of 13.5 and 10.25 mm for *Debaryomyces sp.* and *Rhodotorula sp*., respectively. The obtained results showed that the essential oil has a very high MIC against the strains studied. Bacteria were more resistant than yeasts, and the MICs were >8% for *P. aeruginosa* and 4% for *E. coli*. The yeast strains *Debaryomyces sp.* and *Rhodotorula sp*. were inhibited at low concentrations of 0.25 and 0.5%, respectively. However, Z*ygosaccharomyces sp1* and *Zygosaccharomyces sp2* were inhibited at higher concentrations, which are 1 and > 4%, respectively.

Both methods used in this study showed that the tested bacterial strains (*P. aeruginosa*and *E. coli*) were resistant to lemon essential oil, which can be explained by the Gram-negative nature of these two bacteria. Gram-negative bacteria have a layer of peptidoglycan enclosed between the plasma membrane and an outer layer of lipopolysaccharides and proteins. This structure can prevent the penetration of the essential oil and protect the peptidoglycan layer against the essential oil. The outer membrane of the lipopolysaccharides of Gram-negative bacteria forms a barrier to the permeability of hydrophobic substances, which prevent the growth of bacteria upon entry (Chao et al., 2000). Furthermore, the high resistance observed with *P. aeruginosa* may be due to its particular outer membrane and its ability to metabolize several organic compounds (Chao et al., 2000; Ferhat et al., 2010). Bacteria of the genus *Pseudomonas* use terpenes as a source of carbon and energy (Boontawan et Stuckey, 2006); they convert limonene to perillyl alcohol, perillyl acid, α-terpineol, or limonene-6,8-diol (Malekey, 2007). Likewise, the hydrophobic components of essential oils can increase cell membrane permeability, causing changes in the contents of bacterial and fungal cells (Cox et al., 2000; Burt, 2004; Cristani et al., 2007). Adegoke et al. (2000) showed that α-terpinene, and limonene affect the permeability of the cytoplasmic membrane of *Candida tropicalis* by causing the loss of cytoplasmic components of the cell. In yeast, essential oils may potentially weaken the enzymatic processes responsible for energy production and structural compound synthesis (Conner and Beuchat, 1984).

Table 2. Antioxidant and antimicrobial activities of *Citrus limon* peel essential oils

	Antioxidant activity		Antimicrobial activity			
	DPPH (EC 50) (μg/mL)	β-carotene (%)	Strains	Aromatogram method		Agar dilution method
				Diameter (mm)	Interpretation	MIC (%)
Essential oils	5.5 ± 0.17[a]	33.57 ± 4.9[a]	*Pseudomonas aeruginosa*	6.00 ± 0.00	No inhibited	> 8
			Escherichia coli	8.35 ± 0.25	No inhibited	4
			Zygosaccharomyces sp1	7.25 ± 0.95	No inhibited	1
Vitamin E	12.90 ± 2.77[b]	23.38 ± 0.3[b]	*Debaryomyces sp.*	13.50 ± 1.73	Inhibited	0.25
			Rhodotorula sp.	10.25 ± 1.5	Inhibited	0.5
			Zygosaccharomyces sp2	8.75 ± 2.5	No inhibited	> 4

Data in columns with the identical letter are not significantly different ($p < 0.05$). MIC: Minimum Inhibitory Concentration, EC50: Effective Concentration

In the same connection, Uribe et al. (1985); Leigh-de Rapper & van Vuuren (2020) reported that the major constituents of lemon essential oil (β-pinene and D-limonene) could inhibit respiratory activity in intact yeast cells and in isolated mitochondria. In several studies, the antimicrobial activity of *C. limon* essential oil is linked to the existence of volatile components like limonene and linalool (Alma et al., 2004; Tepe et al., 2006). This activity can be determined by the effect of a single component or by the synergistic or antagonistic effect of various components (Deba et al., 2008). Veldhuizen et al. (2006) attributed this activity to phenolic compounds, whose amphipathicity may explain their interactions with membrane constituents and thus the antimicrobial activity. According to Knobloch et al. (1989), bacteria are more sensitive than yeasts to *Citrus* EO. Indeed, terpenols are more effective against bacteria than fungi. In this study, yeasts were found to be more sensitive than bacteria. This can be due to the high concentration of terpene hydrocarbons (limonene and β-pinene) in the extracted essential oil (Brahmi et al., 2021).

pH and titratable acidity

The pH of the fresh creams elaborated has registered a significant evolution through three phases (Table 3). In the first phase, no significant variation was noted after skimming ($p > 0.05$), while a net decrease was shown after pasteurization. The recorded pH ranged between 5.57 and 6.5, depending on the rate of essential oil incorporation. This decrease in pH is most probably due to possible peptization, which results in the unwinding and dissociation of protein chains under heat treatment while releasing H^+ ions (Jeantet et al., 2016). Even after opening the package, the decrease in pH continued at a significantly faster rate due to possible fermentation caused by microbial contamination. Finally, a stabilization of the pH can be observed beyond the second day, and no significant variations ($p > 0.05$) in pH were recorded after 4, 6, and 8 days following the opening of the containers, and all values were close to pH 4.5. This was confirmed with all the incorporation rates of the essential oil in the fresh cream (0, 0.125, 0.25, and 0.5%). According to Juillard et al. (1987), bacterial fermentation can exhibit the self-inhibition phenomenon by keeping the pH constant at an ultimate value. This acidification of creams provides biological protection against microorganisms that can deteriorate the product (Jeantet et al., 2016).

Furthermore, the results showed that skimming did not influence acidity and that pasteurization could slightly increase the developed acidity to a value of 19°D in the developed fresh creams (Table 3). The opening of the package can explain the significant increase in acidity up to 53.33, 52.00, 51.33, and 50.00 °D for fresh creams at 0, 0.125, 0.25, and 5% of *Citrus limon* peel essential oil, respectively, after two days. Up to eight days, the results showed that the acidity remained stable without any significant variation and ranged between 50 and 53°D. These results can attest to the positive effect of the incorporation of the essential oil of *Citrus limon* in the fresh cream. It can reduce the acidity through a possible inhibition of the development of lactic bacteria that ferment lactose into lactic acid.

Table 3. Changes in the pH and titratable acidity of fresh creams over time

EO incorporation rate (%)	Raw milk	FCBP	Fresh creams after opening the package: Day 0	Day 2	Day 4	Day 6	Day 8
pH							
0	6.6 ± 0.24[a]	6.5 ± 0.42[a]	5.57 ± 0.19[a]	4.41 ± 0.37[b]	4.42 ± 0.10[b]	4.62 ± 0.22[b]	4.57 ± 0.17[b]
0.125			5.57 ± 0.23[a]	4.45 ± 0.41[b]	4.45 ± 0.34[b]	4.55 ± 0.11[b]	4.65 ± 0.14[b]
0.25			5.64 ± 0.12[a]	4.50 ± 0.26[b]	4.46 ± 0.27[b]	4.56 ± 0.07[b]	4.49 ± 0.09[b]
0.5			5.71 ± 0.31[a]	4.50 ± 0.20[b]	4.44 ± 0.18[b]	4.34 ± 0.21[b]	4.51 ± 0.12[b]
Titratable acidity (°D)							
0	18.33 ± 0.57[a]	18.33 ± 0.46 [a]	20.00 ± 0.60[a]	53.33 ± 0.57[b]	53.33 ± 0.57[b]	52.91 ± 0.57[b]	53.62 ± 1.23[b]
0.125			19.00 ± 0.57[a]	52.00 ± 2.00[b]	52.00 ± 1.80[b]	52.60 ± 2.34[b]	52.45 ± 2.76[b]
0.25			18.66 ± 0.71[a]	51.33 ± 2.51[b]	51.23 ± 1.31[b]	51.66 ± 2.11[b]	52.00 ± 3.00[b]
0.5			19.00 ± 0.97[a]	50.00 ± 1.27[b]	50.58 ± 1.00[b]	49.94 ± 2.08[b]	50.73 ± 2.64[b]

There is no significant difference ($p < 0.05$) between values in rows with the same letter. FCBP: Fresh Cream Before Pasteurization, OE: Essential Oil

Oxidative stability

The variation of MDA content in fresh creams containing the essential oil was monitored after opening the package, and the results are shown in Figure 2. An increase in MDA content was recorded in all products for 8 days, and it was highly evident in the first 6 days. It can also be noted that fresh cream with HE is more resistant to forced oxidation and contains low levels of MDA. It appears that the MDA content increases strangely only in the first 4 days in the fresh creams incorporated with the EO, in contrast to the control, where the increase continues until the 6th day after opening the package. After the 4th day, the MDA levels remained unchanged until the 8th day, and the levels recorded were around 6.22, 3.89, 3.64, and 3.18 mg MDA/Kg at 0, 0.125, 0.25, and 0.5% of *Citrus limon* EO. The control fresh cream showed a high sensitivity to oxidation, which can be explained by its high content of unsaturated fatty acids. Dairy lipids contain about 20-30% oleic acid, 1-3% linoleic acid, and 0.5-2% linolenic acid (Huppertz et al., 20099). The presence of lemon peel essential oil in other fresh creams can prevent the forced peroxidation of these fatty acids caused by heat. Falleh et al. (2020) stated that essential oils have direct and indirect modes of action. EOs contain monoterpenes, aldehydes, ketones, alcohols, ethers, and phenolic compounds that play a crucial role in preventing oxidation. Indeed, these compounds can be hydrogen atom donors to release radicals and convert them into more stable molecules (Kehal et al. 2021).

Sensory properties

Figure 3 shows the sensory profiles of the four fresh creams prepared with different concentrations of lemon essential oil (A: 0% control, B: 0.125%, C: 0.25%, and D: 0.5%). Sensory attributes describing lemon aroma and smell were more intense in all fresh creams containing lemon essential oil. However, no remarkable differences were noted for the other attributes. The analysis of variance (ANOVA) on the ratings of sensory characteristics showed that for the characteristics of texture and color, no significant difference (p>0.05) was shown between all of the fresh creams. Nevertheless, significant differences (p<0.05) were noted for lemon odor and aroma. From these results, it can be concluded that the incorporation of EO into the fresh cream changed the aroma and smell without affecting the texture or color. The acceptability of these fresh creams with lemon EO was not affected since there was no significant difference (p> 0.05) in the classification of the four fresh creams. The 0.5% EO incorporation rate resulted in a product with an overly pronounced odor and taste of lemon, but was appreciated by some tasters, and its overall appreciation was not influenced.

The results of the analysis of the correlation between the incorporation rate of lemon essential oil and the sensory properties of fresh creams are grouped in Figure 4. It was shown that the incorporation rate of EO was significantly correlated with the aroma of raw milk and yellow color. This correlation was negative with raw milk aroma (r = -0.998) and positive with yellow color (r = 0.956).

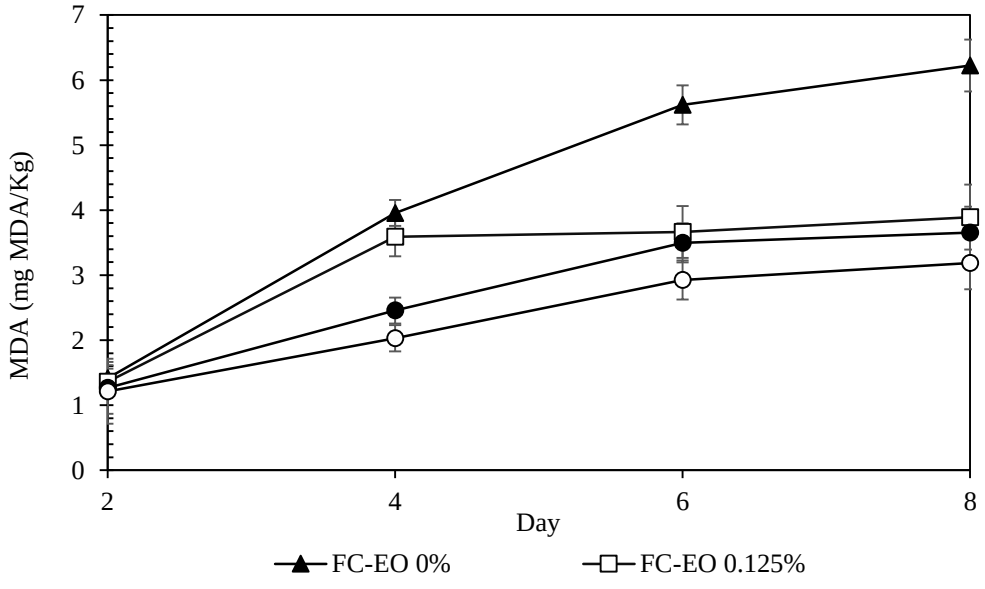

Figure 2. Evolution of the malondialdehyde (MDA) content in fresh creams as a function of storage time at a temperature of 63°C.FC-EO (%): Fresh Cream with Essential Oil (incorporation rate)

There was also a negative correlation between the rate of EO incorporation and yeast odor (r = - 0.938), fresh cream odor (r = - 0.934), and fresh cream aroma (r = - 0.898). Furthermore, positive correlations were also found between EO incorporation rate and creamy texture (r = 0.940), green herb odor (r = 0.818), lemon odor (r = 0.883), and lemon aroma (r = 0.897). In addition to the sensory properties, a negative correlation (r = - 0.824) between the rate of incorporation of essential oil and the MDA content was revealed. This result indicates that each increase in the rate of incorporation of EO leads to a decrease in the content of MDA, which is one of the major products of lipid oxidation, indicating that the presence of EO promotes the oxidation stability of fresh cream.

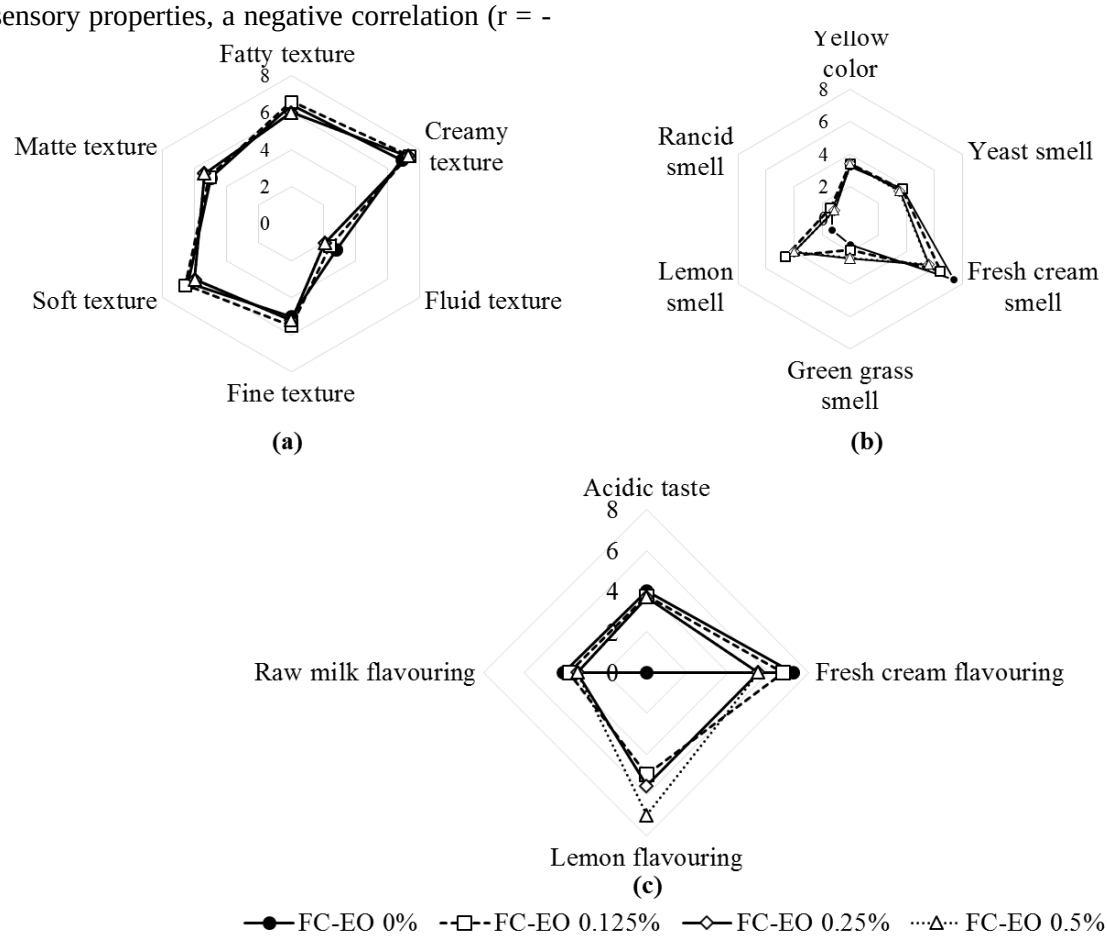

Figure 3. Sensory profiles of four fresh creams prepared with different concentrations of *Citrus limon* peel essential oils. (a) texture, (b) smell and color, and (c) taste and flavouring. FC-EO (%): Fresh Cream with Essential Oil (incorporation rate).

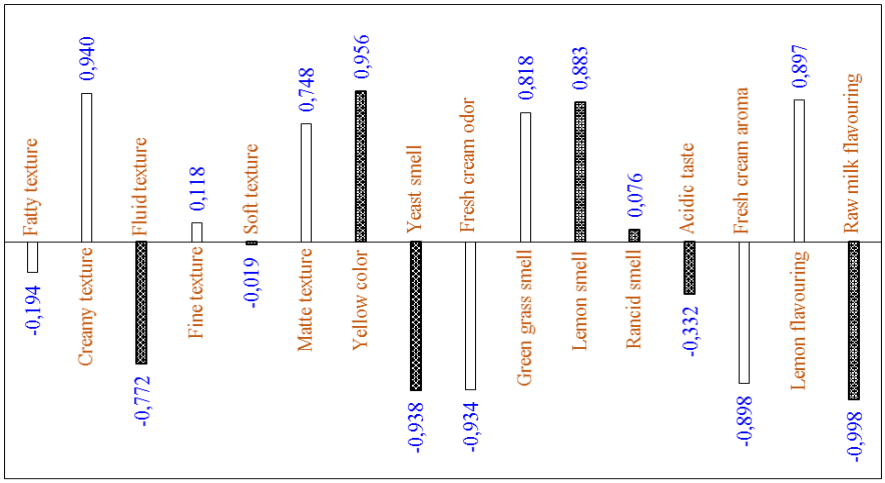

Figure 4. Correlation coefficients (r) between the incorporation rate of *Citrus limon* essential oil and the sensory attributes of fresh cream

CONCLUSION

The analysis of the essential oil extracted from *Citrus limon* peel using GC-MS allowed the identification of 30 compounds with a predominance of monoterpenes, mainly limonene (61%), followed by oxygenated monoterpenes, oxygenated sesquiterpenes, and finally sesquiterpenes. The antioxidant activity of lemon essential oil was assessed in vitro using DPPH° reduction and β-carotene bleaching methods, indicating that this essential oil has antioxidant power, suggesting that its use in fresh cream may be a possible wayto prevent lipid oxidation. The antimicrobial test of the essential oil revealed that both bacteria tested exhibited resistance against it, leading to extremely high MICs. The yeast strains tested, *Debaryomyces sp.* and *Rhodotorula sp*., showed a MICs of 0.25 and 0.5%, respectively, which indicated that they were sensitive to the impact of the essential oil. As for the other strains, such as *Zygosaccharomyces sp1* and *Zygosaccharomyces sp2*, it was found that they are resistant, and their MICs were 1 and > 4%, respectively.

The elaboration of fresh creams with the incorporation of the lemon essential oil was carried out by elaborating three fresh creams with incorporation rates of 0.125, 0.25, and 0.5%, and the characteristics of the fresh creams were in conformity with the standards. According to the Schaal test, the evaluation of oxidative stability showed that fresh creams containing lemon essential oil exhibited greater resistance to forced oxidation compared to the control and that the fresh cream with 0.5% EO showed better resistance to oxidation. As determined by sensory analysis, incorporating essential oil into fresh cream at concentrations of 0.125% and 0.25% did not lead to any significant difference in flavor. It was shown that the rate of incorporation in EO is negatively correlated with the aroma of raw milk and positively correlated with the yellow color. From all these results, we can conclude that it can be used as an antioxidant and aromatic agent in fresh cream.

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REFERENCES

1. Adams, R.P. (2017). Identification of essential oil components by gas chromatography/mass spectroscopy. 4th ed. Allured Publishing Corporation, Carol Stream, 806 p.
2. Adegoke, G. O., Iwahashi, H., Komatsu, Y., Obuchi, K., & Iwahashi, Y. (2000). Inhibition of food spoilage yeasts and aflatoxigenic moulds by monoterpenes of the spice Aframomum danielli. *Flavour and Fragrance Journal*, *15*(3), 147-150. https://doi.org/10.1002/1099-1026(200005/06)15:3<147::AID-FFJ883>3.0.CO;2-0
3. Adelakun, O. E., Oyelade, O. J., & Olanipekun, B. F. (2016). Use of essential oils in food preservation. In *Essential oils in food preservation, flavor and safety* (pp. 71-84). Academic Press. https://doi.org/10.1016/B978-0-12-416641-7.00007-9
4. Alma, M. H., Nitz, S., Kollmannsberger, H., Digrak, M., Efe, F. T., & Yilmaz, N. (2004). Chemical composition and antimicrobial activity of the essential oils from the gum of Turkish pistachio (Pistacia vera L.). *Journal of agricultural and food chemistry*, *52*(12), 3911-3914. https://doi.org/10.1021/jf040014e
5. Aprotosoaie, A. C., Spac, A., Hancianu, M., Miron, A., Tanasescu, V. F., Dorneanu, V., & Stanescu, U. (2010). The chemical profile of essential oils obtained from fennel fruits (*Foeniculum vulgare Mill.). Farmacia*, *58*(1), 46-53.
6. Arctander, S. (1994). Perfume and flavor chemicals (aroma chemicals) Carol Stream. *IL: Allured Publishing Company*.
7. Bakkali, F., Averbeck, S., Averbeck, D., & Idaomar, M. (2008). Biological effects of essential oils–a review. *Food and chemical toxicology*, *46*(2), 446-475. https://doi.org/10.1016/j.fct.2007.09.106

8. Bandyopadhyay, M., Chakraborty, R., &Raychaudhuri, U. (2008). Antioxidant activity of natural plant sources in dairy dessert (Sandesh) under thermal treatment. *LWT-Food Science and Technology*, *41*(5), 816-825. https://doi.org/10.1016/j.lwt.2007.06.001
9. Ben Hsouna, A., Ben Halima, N., Smaoui, S., & Hamdi, N. (2017). Citrus lemon essential oil: Chemical composition, antioxidant and antimicrobial activities with its preservative effect against Listeria monocytogenes inoculated in minced beef meat. Lipids in health and disease, 16, 1-11. DOI 10.1186/s12944-017-0487-5
10. Boontawan, A., & Stuckey, D. C. (2006). A membrane bioreactor for the biotransformation of α-pinene oxide to isonovalal by Pseudomonas fluorescens NCIMB 11671. *Applied microbiology and biotechnology*, *69*(6), 643-649. https://doi.org/10.1007/s00253-005-0025-7
11. Brahmi, F., Mokhtari, O., Legssyer, B., Hamdani, I., Asehraou, A., Hasnaoui, I., Asehraou, A. & Tahani, A. (2021). Chemical and biological characterization of essential oils extracted from citrus fruits peels. *Materials Today: Proceedings*, *45*, 7794-7799. https://doi.org/10.1016/j.matpr.2021.03.587
12. Burt, S. (2004). Essential oils: their antibacterial properties and potential applications in foods—a review. *International journal of food microbiology*, *94*(3), 223-253. https://doi.org/10.1016/j.ijfoodmicro.2004.03.022
13. Caccioni, D. R., Guizzardi, M., Biondi, D. M., Renda, A., &Ruberto, G. (1998). Relationship between volatile components of *Citrus* fruit essential oils and antimicrobial action on *Penicillium digitatum* and *Penicillium italicum*. *International journal of food microbiology*, *43*(1-2), 73-79. https://doi.org/10.1016/S0168-1605(98)00099-3
14. Campêlo, L. M. L., de Almeida, A. A. C., de Freitas, R. L. M., Cerqueira, G. S., de Sousa, G. F., Saldanha, G. B., Feitosa, C.M. & de Freitas, R. M. (2011). Antioxidant and antinociceptive effects of *Citrus limon* essential oil in mice. *Journal of Biomedicine and Biotechnology*, 1-8.
15. Chanthaphon, S., Chanthachum, S., & Hongpattarakere, T. (2008). Antimicrobial activities of essential oils and crude extracts from tropical *Citrus* spp. against food-related microorganisms. *Songklanakarin Journal of Science & Technology*, *30*. 125-131
16. Chao, S. C., Young, D. G., & Oberg, C. J. (2000). Screening for inhibitory activity of essential oils on selected bacteria, fungi and viruses. *Journal of essential oil research*, *12*(5), 639-649. https://doi.org/10.1080/10412905.2000.9712177
17. Chemat, F., Abert-Vian, M., Fabiano-Tixier, A. S., Strube, J., Uhlenbrock, L., Gunjevic, V., & Cravotto, G. (2019). Green extraction of natural products. Origins, current status, and future challenges. *TrAC Trends in Analytical Chemistry*, *118*, 248-263. https://doi.org/10.1016/j.trac.2019.05.037
18. Choe, E., & Min, D. B. (2009). Mechanisms of antioxidants in the oxidation of foods. *Comprehensive reviews in food science and food safety*, *8*(4), 345-358. https://doi.org/10.1111/j.1541-4337.2009.00085.x
19. Collomb, M., &Spahni, M. (1996). Revue des méthodes de dosage des produits d'oxydation des lipides, principalement des lipides des produits laitiers. *Schweizerische Milchwirtschaftliche Forschung*, *25*(1), 3-24.
20. Conner, D. E., & Beuchat, L. R. (1984). Effects of essential oils from plants on growth of food spoilage yeasts. *Journal of food science*, *49*(2), 429-434. https://doi.org/10.1111/j.1365-2621.1984.tb12437.x
21. Cox, S. D., Mann, C. M., Markham, J. L., Bell, H. C., Gustafson, J. E., Warmington, J. R., & Wyllie, S. G. (2000). The mode of antimicrobial action of the essential oil of *Melaleuca alternifolia* (tea tree oil). *Journal of applied microbiology*, *88*(1), 170-175. https://doi.org/10.1046/j.1365-2672.2000.00943.x
22. Cristani, M., D'Arrigo, M., Mandalari, G., Castelli, F., Sarpietro, M. G., Micieli, D., Venuti, V., Bisignano, G., Saija, A., & Trombetta, D. (2007). Interaction of four monoterpenes contained in essential oils with model membranes: implications for their antibacterial activity. *Journal of agricultural and food chemistry*, *55*(15), 6300-6308. https://doi.org/10.1021/jf070094x
23. Deba, F., Xuan, T. D., Yasuda, M., & Tawata, S. (2008). Chemical composition and antioxidant, antibacterial and antifungal activities of the essential oils from *Bidens pilosa* Linn. var. *Radiata*. *Food control*, *19*(4), 346-352. https://doi.org/10.1016/j.foodcont.2007.04.011
24. Djenane, D., Yangueela, J., Gomez, D., & Roncales, P. (2012). Perspectives on the use of essential oils as antimicrobials against *Campylobacter jejuni* CECT 7572 in retail chicken meats packaged in microaerobic atmosphere. *Journal of Food Safety*, *32*(1), 37-47. https://doi.org/10.1111/j.1745-4565.2011.00342.x

25. Djenane, D. (2015). Chemical profile, antibacterial and antioxidant activity of Algerian *Citrus* essential oils and their application in *Sardina pilchardus*. Foods, 4(2), 208-228.https://doi.org/10.3390/foods4020208
26. Draper, H. H., & Hadley, M. (1990). [43] Malondialdehyde determination as index of lipid Peroxidation. In *Methods in enzymology* (Vol. 186, pp. 421-431). Academic press. https://doi.org/10.1016/0076-6879(90)86135-I
27. Dung, N. T., Kim, J. M., & Kang, S. C. (2008). Chemical composition, antimicrobial and antioxidant activities of the essential oil and the ethanol extract of *Cleistocalyx operculatus* (Roxb.) Merr and Perry buds. *Food and chemical Toxicology*, *46*(12), 3632-3639. https://doi.org/10.1016/j.fct.2008.09.013
28. Eberhard, T., Robert, A., & Annelise, L. (2005). Plantes aromatiques, épice aromates, condiments et huiles essentielles. *Tec et Doc. Lavoisier. Paris France*.
29. El Aboubi, M., Hdech, D. B., Bikri, S., Benayad, A., El Magri, A., Aboussaleh, Y., & Aouane, E. M. (2023). Chemical composition of essential oils of *Citrus limon* peel from three Moroccan regions and their antioxidant, anti-inflammatory, antidiabetic and dermatoprotective properties. Journal of Herbmed Pharmacology, 12(1), 118-127. doi: 10.34172/jhp.2023.11
30. Falleh, H., Jemaa, M. B., Saada, M., & Ksouri, R. (2020). Essential oils: A promising eco-friendly food preservative. *Food Chemistry*, *330*, 127268.https://doi.org/10.1016/j.foodchem.2020.127268
31. Ferdeş, M., & Ungureanu, C. (2012). Antimicrobial activity of essential oils against four food-borne fungal strains. *University Politehnica of Bucharest Scientific Bulletin*, *74*(2), 87-98
32. Ferhat, M.A., Meklati B.Y., Chemat F. (2010). *Citrus d'Algérie : les huiles essentielles et leurs procédés d'extractions* .Ed. Office des publications universitaires, Alger. 157 p.
33. Hammer, K. A., Carson, C. F., & Riley, T. V. (1999). Antimicrobial activity of essential oils and other plant extracts. *Journal of applied microbiology*, *86*(6), 985-990. https://doi.org/10.1046/j.1365-2672.1999.00780.x
34. Himed, L., Merniz, S., & Barkat, M. (2016). Evaluation of the chemical composition and antioxidant activity of *Citrus limon* essential oil and its application in margarine preservation. *J. Nat. Product*, *4*, 316-322.
35. Himed, L., Merniz, S., Monteagudo-Olivan, R., Barkat, M., & Coronas, J. (2019). Antioxidant activity of the essential oil of *Citrus limon* before and after its encapsulation in amorphous SiO2. Scientific African, 6, e00181. https://doi.org/10.1016/j.sciaf.2019.e00181
36. Holley, R. A., & Patel, D. (2005). Improvement in shelf-life and safety of perishable foods by plant essential oils and smoke antimicrobials. *Food microbiology*, *22*(4), 273-292. https://doi.org/10.1016/j.fm.2004.08.006
37. Huang, C., Pu, H., Zhang, Z., & Song, J. (2010). Evaluation of antioxidant and antitumour activities of lemon essential oil. *Journal of Medicinal Plants Research*, *4*(18), 1910-1915.
38. Huppertz, T., Kelly, A. L., & Fox, P. F. (2009). Milk lipids—Composition origin and properties. *Dairy fats and relatedproducts*, *1*.
39. Hussain, A. I., Anwar, F., Chatha, S. A. S., Jabbar, A., Mahboob, S., & Nigam, P. S. (2010). *Rosmarinus officinalis* essential oil: antiproliferative, antioxidant and antibacterial activities. *Brazilian Journal of Microbiology*, *41*, 1070-1078. https://doi.org/10.1590/S1517-83822010000400027
40. Inouye, S., Takizawa, T., & Yamaguchi, H. (2001). Antibacterial activity of essential oils and their major constituents against respiratory tract pathogens by gaseous contact. *Journal of antimicrobia lchemotherapy*, *47*(5), 565-573.
41. ISO (2012). Milk and milk products — Determination of the titratable acidity of milk fat (Vol. Method ISO/TS 22113).Geneve: International Organization for Standardization.
42. Jeantet, R., Croguennec, T., Schuck, P., & Brule, G. (Eds.). (2016). *Handbook of food science and technology 3: Food biochemistry and technology* (Vol. 3). John Wiley & Sons.
43. Journal officiel de la république algerienne N°35/1998, Arrêté interministériel du 27 Mai 1998. Critères microbiologiques relatifs à certaines denrées alimentaires.
44. Juillard, V., Spinnler, H. E., Desmazeaud, M. J., & Boquien, C. Y. (1987). Phénomènes de coopération et d'inhibition entre les bactéries lactiques utilisées en industrie laitière. *Le lait*, *67*(2), 149-172. https://doi.org/10.1051/lait:1987210
45. Kehal, F., Chemache, L., Chaalal, M., Benbraham, M., Capanoglu, E. & Barkat, M. (2021). A Comparative analysis of different varietal of fresh and dried figs by in vitro bioaccessibility of phenolic compounds and antioxidant activities. *Acta Universitatis Cibiniensis. Series E: Food Technology*, *25* (1), 15-30. https://doi.org/10.2478/aucft-2021-0002

46. Knobloch, K., Pauli, A., Iberl, B., Weigand, H., & Weis, N. (1989). Antibacterial and antifungal properties of essential oil components. *Journal of essential oil research*, *1*(3), 119-128. https://doi.org/10.1080/10412905.1989.9697767
47. Leigh-de Rapper, S., & van Vuuren, S. F. (2020). Odoriferous therapy: A review identifying essential oils against pathogens of the respiratory tract. *Chemistry & Biodiversity*, *17*(6), e2000062. https://doi.org/10.1002/cbdv.202000062
48. Liyana-Pathirana, C. M., & Shahidi, F. (2006). Antioxidant properties of commercial soft and hard winter wheats (*Triticum aestivum* L.) and their milling fractions. *Journal of the Science of Food and Agriculture*, *86*(3), 477-485. https://doi.org/10.1002/jsfa.2374
49. Mayachiew, P., &Devahastin, S. (2008). Antimicrobial and antioxidant activities of Indian gooseberry and galangal extracts. *LWT-Food Science and Technology*, *41*(7), 1153-1159. https://doi.org/10.1016/j.lwt.2007.07.019
50. Moreira, M. R., Ponce, A. G., Del Valle, C. E., & Roura, S. I. (2005). Inhibitory parameters of essential oils to reduce a food borne pathogen. *LWT-Food Science and Technology*, *38*(5), 565-570. https://doi.org/10.1016/j.lwt.2004.07.012
51. Nikhat, F., Satynarayana, D., & Subhramanyam, E. V. S. (2009). Isolation, charectrisation and screening of antioxidant activity of the roots of Syzygiumcuminii (L) Skeel. *Asian Journal of Research in Chemistry*, *2*(2), 218-221.
52. Roy, S. D., Bania, R., Chakraborty, J., Goswami, R., Laila, R., & Ahmed, S. A. (2012). Pharmacognostic, phytochemical, physicochemical property and antimicrobial activity studies of lemon peel oil. *Journal of Natural Product and Plant Resources*, *2*, 431-435.
53. Rusenova, N., & Parvanov, P. (2009). Antimicrobial activities of twelve essential oils against microorganisms of veterinary importance. *Trakia Journal of sciences*, *7*(1). 37-43
54. Taktak, O., Ben Ameur, R., Ben Youssef, S., Pieters, L., Foubert, K., & Allouche, N. (2021). Chemical Composition and Biological Activities of Essential Oils and Organic Extracts from Fresh and Sun-Dried *Citrus limon* Peels. *Chemistry Africa*, *4*(1), 51-62. https://doi.org/10.1007/s42250-020-00212-w
55. Tang, S., Sheehan, D., Buckley, D. J., Morrissey, P. A., & Kerry, J. P. (2001). Anti-oxidant activity of added tea catechins on lipid oxidation of raw minced red meat, poultry and fish muscle. *International journal of food science & technology*, *36*(6), 685-692. https://doi.org/10.1046/j.1365-2621.2001.00497.x
56. Tepe, B., Akpulat, H. A., Sokmen, M., Daferera, D., Yumrutas, O., Aydin, E., & Sokmen, A. (2006). Screening of the antioxidative and antimicrobial properties of the essential oils of *Pimpinella anisetum* and *Pimpinella flabellifolia* from Turkey. *Food Chemistry*, *97*(4), 719-724. https://doi.org/10.1016/j.foodchem.2005.05.045
57. Tomar, O., & Akarca, G. (2019). Effects of ice-cream produced with lemon, Mandarin, and orange peel essential oils on some physicochemical, microbiological and sensorial properties. *Kocatepe Veteriner Dergisi*, *12*(1), 62–70. https://doi.org/10.30607/kvj.499415
58. Tserennadmid, R., Takó, M., Galgóczy, L., Papp, T., Pesti, M., Vágvölgyi, C., Almássy, K., & Krisch, J. (2011). Anti yeast activities of some essential oils in growth medium, fruit juices and milk. *International journal of food microbiology*, *144*(3), 480-486.
59. Uribe, S. A. L. V. A. D. O. R., Ramirez, J. O. R. G. E., & Peña, A. N. T. O. N. I. O. (1985). Effects of beta-pinene on yeast membrane functions. *Journal of bacteriology*, *161*(3), 1195-1200. https://doi.org/10.1128/jb.161.3.1195-1200.1985
60. Veldhuizen, E. J., Tjeerdsma-van Bokhoven, J. L., Zweijtzer, C., Burt, S. A., & Haagsman, H. P. (2006). Structural requirements for the antimicrobial activity of carvacrol. *Journal of agricultural and Food Chemistry*, *54*(5), 1874-1879. https://doi.org/10.1021/jf052564y