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Antioxidant activity of the essential oil of *citrus limon* before and after its encapsulation in amorphous SiO_2

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ABSTRACT

In recent years, the consumer demand for natural ingredients has resulted in sustained growth in the global market of essential oils. Indeed, essential oils are considered today as a product "trend" to the general public, which in particular raises considerable appeal from the agro-food sector. However, this application sector has to face many disadvantages related to the variability of their composition, and their volatility and storage instability.

The objective of this work was to extract the essential oil of *Citrus limon*, determine its chemical composition, evaluate its antioxidant activity and see the effect of its encapsulation on such activity. The cold pressing of the lemon peel yielded 1.24±0.07% of the essential oil. This oil was characterized by a composition rich in limonene (67.1%) followed by α-pinene (11.0%) and α-terpinene (8.0%).The antioxidant activity of the oil expressed by the antiradical power DPPH and by the reducing power CUPRAC gave rise to results comparable to that of the standards. The encapsulation of this oil in silica did not modify its antioxidant activity. The thermogravimetric analysis showed a weak thermal estabilization of the encapsulated oil compared to the free oil. FTIR-ATR showed that the encapsulation did not alter the composition of the oil.

Introduction

Essential oils are liquids concentrated in molecules derived from plant metabolism (terpenoid and aromatic molecules). They are very popular in perfumery and cosmetics, as well as in the food and pharmaceutical industries [1]. The market dynamics of essential oils is supported by an ever increasing demand for natural ingredients. This pushes manufacturers in the cosmetics and perfume sector to further integrate essential oils in their formulations. The substitution of a synthetic product with an essential oil increases the added value of a given formulation [2].

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However, the industrial use of essential oils in food, cosmetics and perfumery has to face many problems dealing with their composition variability and storage instability. Currently, it is recognized that temperature, light and oxygen availability have a major impact on preserving the integrity of essential oils [3]. Another limitation to the use of essential oils is their volatile nature [1]. Certain techniques make possible to reduce the effect of these problems which condition the industrial use of essential oils. Encapsulation in porous solids such as porous silicas [4], zeolites [5] and MOFs [6] is a commonly used technique. Encapsulation can make possible to immobilize the most volatile compounds of the essential oil, to stabilize them and to protect them (against light, oxygen and temperature) as well as to modulate their release by prolonging the kinetic profile. A complementary issue relates to the fact that encapsulation can facilitate the industrial processing of the final solid product [7].

The objective of this work is initially to extract the essential oil from *Citrus limon* (Eureka variety) by cold pressing and to characterize it by GC/MS. In a second step, the essential oil is encapsulated in amorphous porous silica and then characterized by thermogravimetric analysis (TGA), scanning electron microscopy (SEM) and attenuated total reflection (ATR) spectroscopy. Finally, the antioxidant activity and the chelating power of the extracted essential oil before and after encapsulation are determined.

Materials and methods

Process of extraction of essential oil

The essential oil of *Citrus limon* (Eureka) was manually extracted from its peel by cold pressing. The oil was carried down to the decantation vessel in a stream of water, the emulsion being collected and then separated by centrifugation. The essential oil was dried over anhydrous sodium sulfate and stored in glass vials covered by aluminum foil at 4 °C. The extraction yield was calculated according to the weight of the plant material before extraction (expressed in wt% of the fresh vegetable material).

Gas chromatography-mass spectrometry (GC/MS)

The essential oil was analyzed by gas chromatography coupled to mass spectrometry (Hewlett Packard 5973A), using a nonpolar column (HP5 MS) (30 m × 0.25 mm, 0.25 µm film thickness). The oven temperature was at 60 °C and was held for 8 min, then was heated to 250 °C and maintained for 10 min. Helium gas was used as carrier at a constant flow rate of 0.7 mL/min. Injector and MS transfer line temperatures were set at 250 °C and 280 °C, respectively. The temperature of electronic impact at 70 eV source was 230 °C. Samples (1 µL) were injected at 250 °C and the split ratio was1:20. The identification of the components was made by determination of their retention indices (I) relative to those of a homologous series of n-alkanes (C_8–C_{28}) (Sigma-Aldrich and Fluka chemicals) and by matching their recorded mass spectra with those stored in the spectrometer database (NIST MS Library v. 2.0) and the bibliography [8]. Component relative percentages were calculated based on GC peak areas.

Encapsulation of the extracted essential oil

A sample was prepared by mixing 1 mL of the essential oil with 500 mg of silica, the whole is agitated until a homogeneous mixture. Commercial amorphous porous silica IBERSIL® A-400, with a BET specific surface area of 168 m^2/g, a pore volume of 0.62 cm^3/g and an average pore size of about 14 nm [7], was generously provided by the IQE S.A. company. This silica was industrially prepared by precipitation and corresponds to food and animal additives E-551 and E-551a, respectively.

Determination of encapsulation efficiency

The percentage of encapsulation efficiency was determined according to the protocol described by Keawchaoon and Yoksan [9].Where 10 mg of essential oil-silicate was dispersed in 4 mL ofHCl solution (2 M), then incubated at 95 °C for 30 min. After cooling, 2 mL of ethanol was added to the mixture. The whole was centrifuged at 9000 rpm for 5 min at room temperature. The supernatant was recovered and the encapsulated essential oil content was measured in a UV–visible at 275 nm. The amount of essential oil was calculated by the calibration curve of the free essential oil in methanol.

The percentage of encapsulation efficiency was calculated by the following equation:

$$\text{Encapsulation efficiency (\%)} = (\text{amount of oil encapsulated} \,/\, \text{initial amount of oil}) \times 100$$

Characterization

Thermal analysis of the samples was carried out by thermogravimetric analysis (TGA) using Mettler Toledo TGA/SDTA 851^e equipment. Samples (10 mg) placed in 70 µL alumina pans were heated in nitrogen flow up to 400 °C at a heating rate of 10 °C/min. The different samples of silica and encapsulated silica, were characterized by scanning electron microscopy

(SEM, FEI Inspect F50) with a voltage of 5–15 kV, the specimens were previously coated with a thin film of platinum under vacuum conditions.

Free essential oil, silica and encapsulated oil were analyzed by Attenuated Total Reflection Fourier-transform infrared spectroscopy (ATR-FTIR, Bruker Vertex 70) in the range 600–4000 cm^{-1} with a Golden Gate diamond ATR accessory.

Evaluation of the antioxidant activity of the essential oil before and after encapsulation

The antioxidant power is estimated by the DPPH (2,2′-diphenyle-1-picryl hydrazyl, Sigma Aldrich) test, CUPRAC (Cupric ion-Reducing Antioxidant Capacity) reducing power and the chelating power test.

Scavenging activity on DPPH

The DPPH solution was prepared according to the protocol described by Mansouri et al. [10], by the dissolution of 2.4 mg of DPPH in 100 mL of methanol. 25 µL of each of the methanolic solutions of the essential oil tested or the reference antioxidant (α-tocopherol) was added to 975 µL of the methanolic solution of DPPH. The mixture was left at room temperature in the dark for 30 min and the discoloration with respect to the negative control containing only the DPPH solution was measured at 517 nm. The antiradical activity was estimated according to the following equation [11]:

$$\text{Antiradical activity } (\%) = [(A_0 - A_t)/A_0] \times 100$$

Where A_t and A_0 are the absorbance values of the tested and blank samples, respectively. The percentage of inhibition was plotted after 30 min against concentration, and the equation for the line was used to obtain the EC_{50}value (effective concentration to reduce 50% of radical).

Cupric ion reducing (CUPRAC) method

The test consists in following the decrease of the absorbance of the Cu (II) neocuproine (Nc), complex ([Nc_2–Cu^{2+}]) reduced by the presence of an antioxidant. According to Apak et al., [12], a solution was prepared by adding 1 mL of $CuCl_2$(Sigma-Aldrich) solution (10 mM), 1 mL of Nc (Sigma-Aldrich) solution(7.5 mM) and 1 mL of ammonia acetate buffer solution (1 M, pH = 7) in a test tube where 0.5 mL of different concentrations of essential oil (or Trolox, Sigma-Aldrich, a vitamin E analogue, as a positive control) was added. The volume of this mixture was adjusted to 4.1 mL with distilled water. The absorbance against the blank was read at 450 nm after 30 min of incubation at room temperature and in the dark.

The reducing power represented in percentage was calculated by the following equation:

$$\text{Reducing power } (\%) = [(A_0 - A_t / A_0)] \times 100$$

Where A_t and A_0 are the absorbance values of the tested and blank samples, respectively. The equation for the line was used to obtain the EC_{50} value.

Metal chelating activity

The chelating capacity was determined according to the method of Lee et al. [13]. The method is based on the inhibition of Fe (II)-ferrozine complex formation after sample processing with Fe^{2+} ions.100 µL sample or standard EDTA (ethylene diamine tetraacetic acid, Sigma-Aldrich) was added to 50 µL of iron chloride ($FeCl_2$,Sigma-Aldrich, 2 mM). After vigorous stirring for 5 min, 100 µL of ferrozine (5 mM) was added, followed by 2.75 mL of distilled water. The mixture was left standing for 10 min at room temperature, thus allowing the complexation of the residual iron and the formation of the Fe (II)-ferrozine complex. A negative control (without sample) was prepared under the same conditions and the absorbance of the complex was measured at 562 nm. The chelating activity was expressed as a percentage according to the following equation:

$$\text{Chelating activity } (\%) = \text{CA}(\%) = [(A_0 - A_t)/A_0] \times 100$$

Where A_t and A_0 are the absorbance values of the tested and blank samples, respectively. The equation for the line was used to obtain the EC_{50} value.

All the assays were carried out in triplicate. The results were expressed as average values with their corresponding standard deviations (SD). The differences between the oils (free and encapsulated) and the standards were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's honestly significant difference ($p<0.05$).This treatment was carried out using XLSTAT 2009 software.

Results and discussion

Chemical composition of the essential oil

The average yield in essential oil is 1.24± 0.07%. This oil is yellowish and have an aromatic odor characteristic of lemon. The yield cited by Himed et al. [14] of the Lisbon variety extracted by cold pressing was 0.81% ± 0.09%. The two yields represent a significant difference ($p<0.05$) in agreement with the fact, that the variety may affect the yield.

Table 1
Chemical composition of the essential oil of *Citrus limon* of the Eureka variety.

No.	Compound	I	%
Monoterpene			**88.96**
1	α-Thujene	931.196	0.33
2	α- Pinene	938.610	11.01
3	β-Pinene	981.766	1.60
4	β-Myrcene	995.024	0.72
5	Limonene	1045.348	67.08
6	α-Terpinene	1067.373	8.03
7	α-Terpinolene	1108.546	0.19
Oxygented monoterpene			**6.19**
8	Linalool	1109.571	0.43
9	cis-Limonene oxide	1138.187	0.79
10	trans-Limonene oxide	1143.310	0.70
11	cis-Citronellol	1148.022	0.49
12	trans-Citronellol	1149.254	0.73
13	Citronellal	1153.643	0.18
14	trans-Carveol	1231.182	0.24
15	cis-Carveol	1244.105	0.11
16	Geranial	1279.198	2.52
Sesquiterpenes			**1.21**
17	β-Elemene	1373.211	0.11
18	Caryophyllene	1391.324	0.26
19	α-Humulene	1450.000	0.68
20	Germacrene	1477.154	0.16
Oxygenated sesquiterpenes			**2.07**
21	Caryophyllene oxide	1584.603	1.13
22	γ-Cadinol	1615.546	0.67
23	α-Cadinol	1640.325	0.27
Other oxygenated compounds			**0.27**
24	Neryl acetate	1361.966	0.11
25	Citronellyl acetate	2552.941	0.16
Total			**98.7**

I: Retention indices relative to C_8–C_{28} n-alkanes calculated on non-polar HP5MS capillary column. Percentages calculated by GC/MS on non-polar HP5MS capillary column.

Table 1 provides both qualitative and quantitative analyses for the *Citrus limon* essential oil volatile profiles.

A total of 25 compounds, which represented 98.7% of the oil, were identified. The lemon oil is generally monoterpenoid in nature. As shown in Table 1, the essential oil contains mainly monoterpene hydrocarbons (88.96%), oxygenated monoterpenoids (6.19%), followed by oxygenated sesquiterpenes (1.21%) and sesquiterpene hydrocarbons (2.07%). The dominant compounds of the essential oil are limonene (67.08%) followed by α-pinene (11.01%) and α-terpinene (8.03%).

Determination of encapsulation efficiency

The percentage of encapsulation efficiency was calculated by the formula described in the part of the materials and methods section using the calibration curve of the essential oil of Fig. 1.This curve allowed us to determine the amount of encapsulated oil.

The calculated encapsulation efficiency is 93.98%; this result means that there was an efficient adsorption of the essential oil in the silicate used.

Thermogravimetric analysis (TGA)

Thermogravimetry was used for the evaluation of the thermal stability of the free and encapsulated essential oil. Fig. 2 shows that the loss of weight occurred mainly in two stages. From room temperature to ca. 100 °C, due to water (probably present in the silica powder) the loss of weight was 10%. And from ca. 100 °C to ca. 170 °C, where the loss of weight reached the value of approximately 63% (approximately 50% of essential oil). It is worthy mention a certain resistance of the silica to release the oil in agreement with the slight thermal stabilization of the guest observed in Fig. 2. This is in line with the previous publication by Paseta et al. [7] dealing with the encapsulation of several essential oils in amorphous mesoporous silica, zeolites and MOFs. In that work only the microporous materials (zeolite and MOFs) gave rise to an appreciable thermal stabilization upon thermogravimetry analysis.

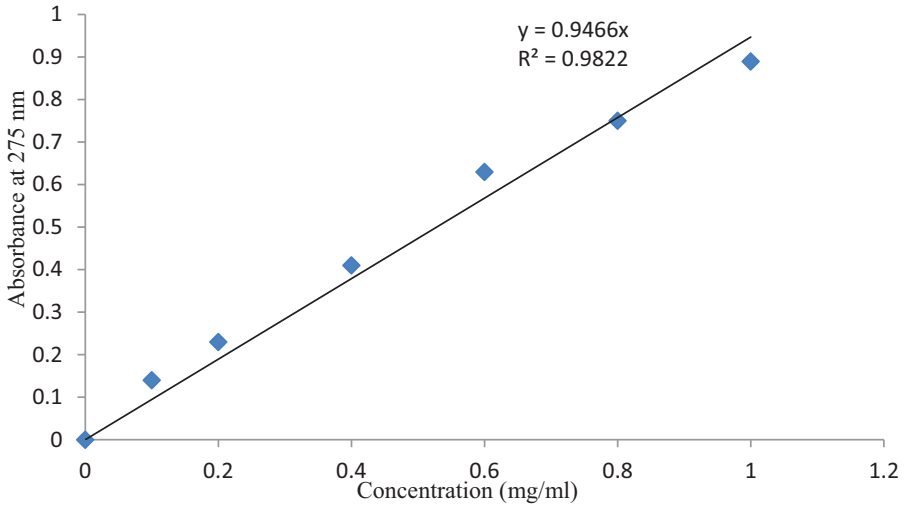

Fig. 1. Calibration curve of the essential oil in methanol.

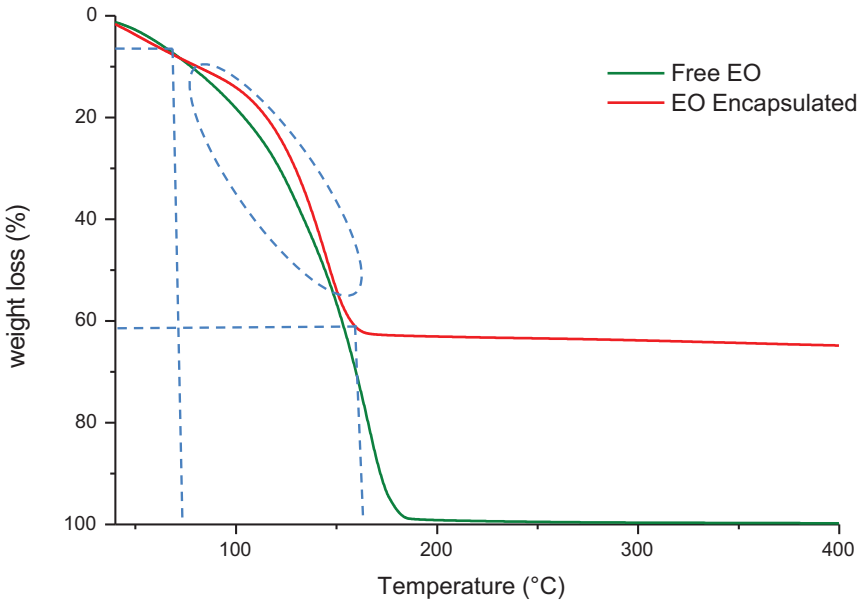

Fig. 2. TGA curves of free and encapsulated essential oil.

Scanning electron microscope (SEM)

The SEM observations of the silicate samples and the silicate encapsulated oil are shown in Fig. 3 (a and b). The electron beam scanning on the surface of the silicate samples and the essential oil capsules yielded similar images, which means that the encapsulated essential oil did not importantly affect the structure of silicates.

Attenuated total reflection (ATR)-Fourier-transform infrared spectroscopy (FTIR)

The results of the analysis of free essential oil, encapsulated and silica by ATR-FTIR are shown in Fig. 4. The peaks of the encapsulated oil are the result of the addition effect between the peaks of the free oil and those of the amorphous silica, which confirms the previous results of the analysis of the samples by SEM and suggests that there is not a strong host-guest chemical interaction between the essential oil and the silica.

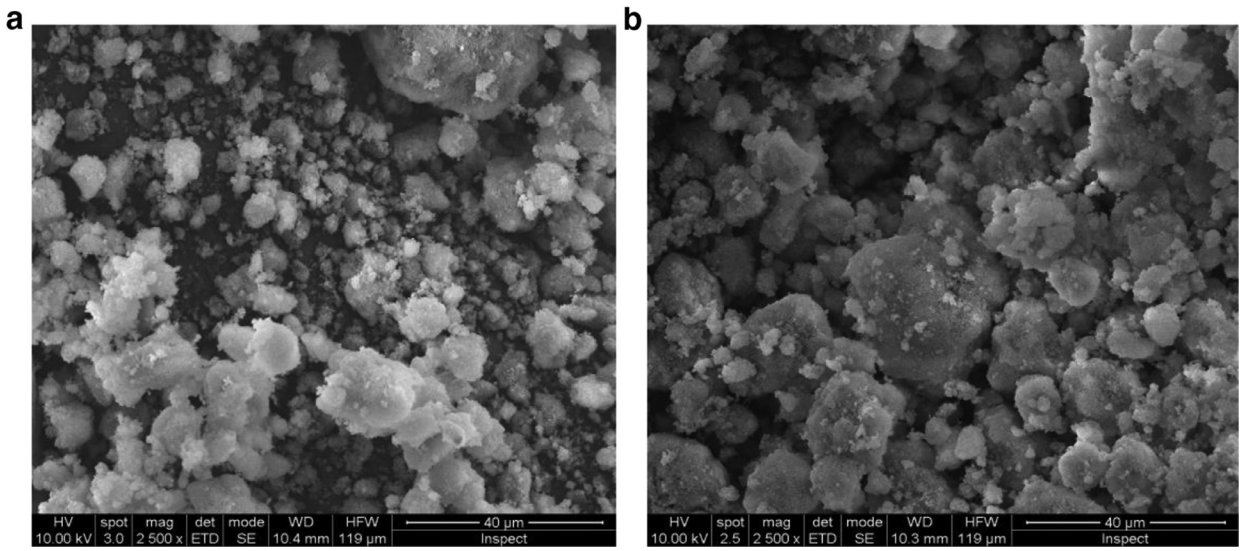

Fig. 3. SEM images of: (a) as provided silicates; (b) lemon essential oil capsules.

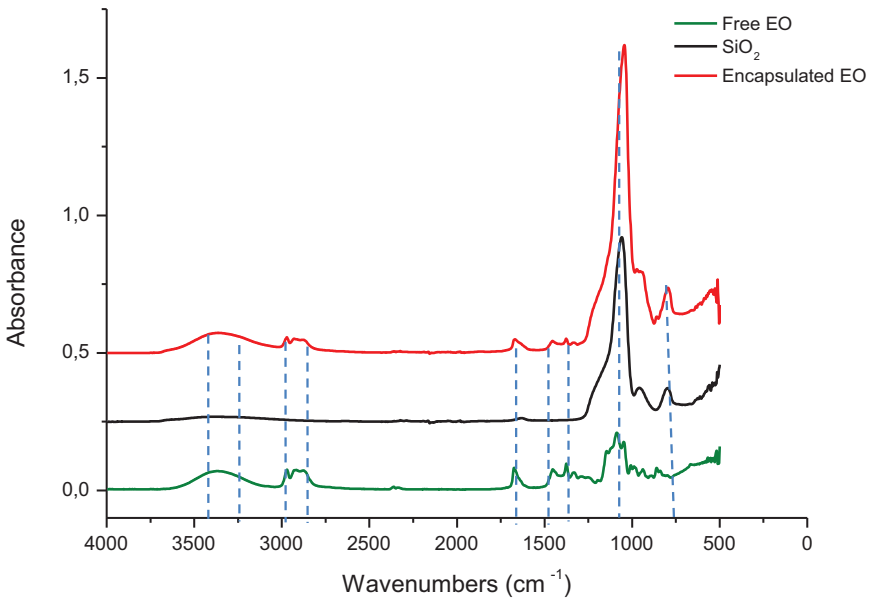

Fig. 4. ATR-FTIR spectra of the free essential oil, encapsulated and SiO_2.

Antioxidant activity

The antioxidant activity of the free and encapsulated essential oil was determined by using three complementary techniques: the DPPH, CUPRAC and iron chelation tests. The results of the antiradical activity determined by the DPPH test of free essential oil and encapsulated essential oil tested are shown in Fig. 5. It can be inferred from this figure that the antiradical activity of the oils tested was comparable to that of the standard (α-tocopherol). In addition, the encapsulated essential oil presented an antiradical activity comparable to that of the pure oil. This allows to conclude that the encapsulation process did not alter the antioxidant properties of the essential oil, even though such encapsulation brought to the resulting oil-silica hybrid important properties such as enhancement of compatibility and process ability to produce adequate formulations of industrial interest [7].

The results of the percentage of reducing power are shown in Fig. 6. These results, show that the reducing power of the essential oils tested (free and encapsulated) is important compared to the positive control (Trolox).

The results of the percentage of chelating power are shown in Fig. 7. This figure depicts that the chelating power of the essential oils (free and encapsulated) is lower than that of the standard (EDTA).

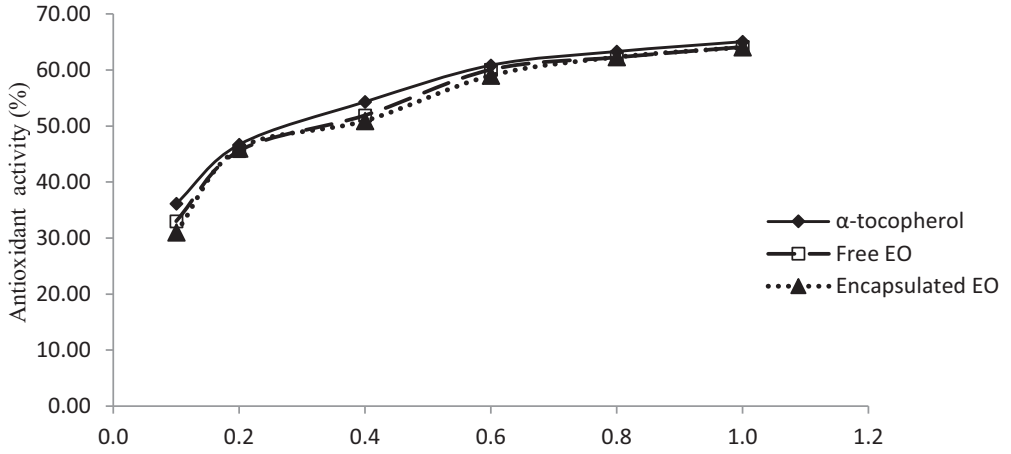

Fig. 5. Antiradical activity of free and encapsulated essential oil.

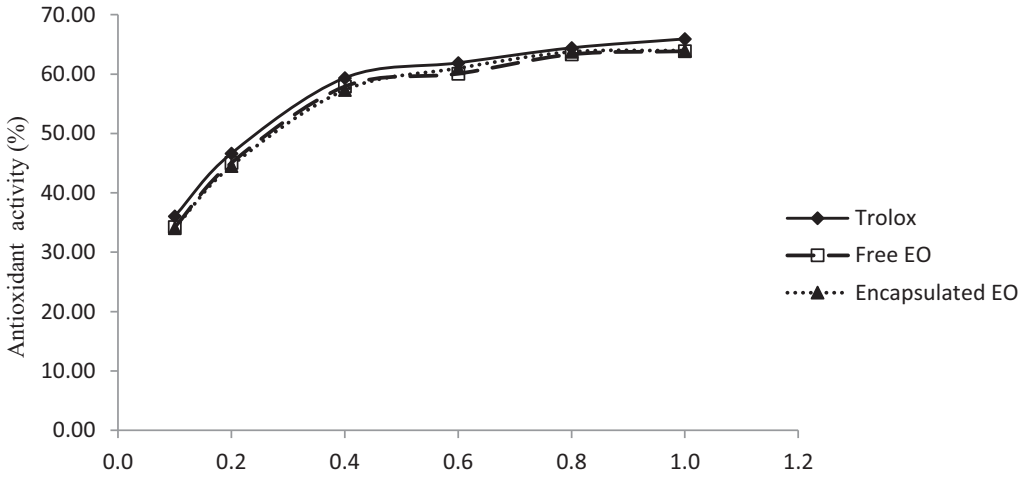

Fig. 6. Reducing power of free and encapsulated essential oil.

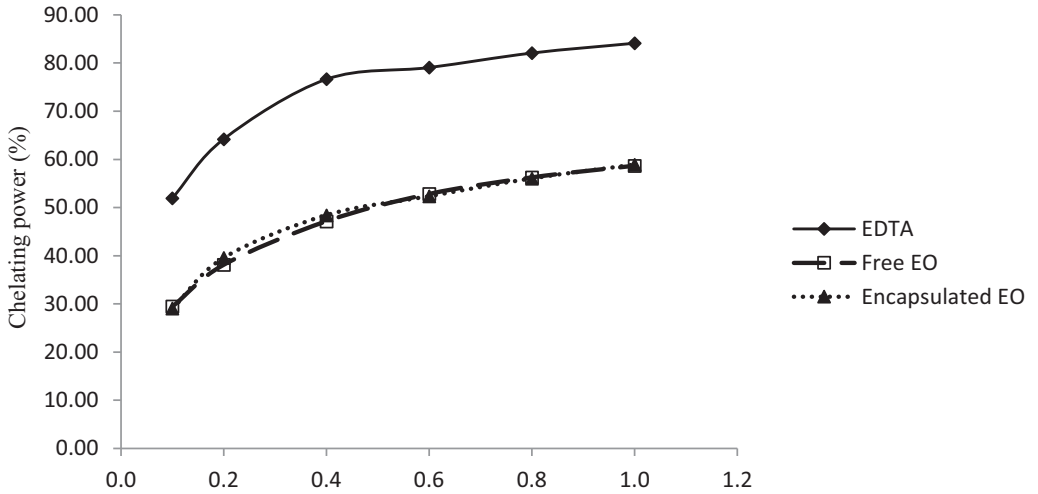

Fig. 7. Chelating power of free and encapsulated essential oil.

Table 2
EC_{50} results of free and encapsulated essential oils.

Tests	EC_{50} (mg/mL)	
	Free EO	Encapsulated EO
DPPH test	0.66±0.03 (a)	0.67 ± 0.04 (a)
CUPRAC test	0.55±0.04 (a)	0.55 ± 0.02 (a)
iron chelation test	0.73±0.02 (a)	0.74 ± 0.01 (a)

The same letter means that there is no significant difference ($p<0.05$) between free and encapsulated essential oil.

The antioxidant activity of the oil extracted from lemon peel was evidenced, from the results combined of the different tests above described compared to the performances of the different standards. This activity is related to the chemical composition of this oil (whose main principles are limonene, α-pinene and α-terpinene, see Table 1). Studies on the relationship between the chemical composition of essential oils and their biological activity are abundant. It has been established in numerous bibliography that the activity of an essential oil is related to the major compounds and the possible synergistic effects estabilished between them [15,16,17 and 18].

Wei and Shibamoto [19] noted significant antioxidant activity of essential oils rich in monoterpenes (limonene and α-pinene). In general, the essential oils rich in oxygenated compound exhibit a very important antiradical activity [20].Gauthier et al. [21] revealed a positive correlation between antioxidant activity and α-pinene level. Similarly, Mimica-Dukicet al. [22] reported that monoterpenes (limonene) and sesquiterpenes (caryophyllyene) are responsible for the neutralization of the DPPH radical. Mohammedi and Atik [23] reported the antioxidant activity of essential oils with oxygenated sesquiterpenes. Moreover, Kelen and Tepe [24] have detected that monoterpenes (limonene, α-pinene and β-pinene) individually tested did not have a significant antioxidant activity compared to the same constituents when tested together. This suggests there is a synergistic effect between such compounds. Ruberto et al. [25] reported that α-terpinene was responsible for the antioxidant activity detected for an essential oil in which that component had content greater than 8%.

Finally, to compare the antioxidant activity of the free essential oil with the encapsulated essential oil, the EC_{50} results for each are summarized test in Table 2. The comparison results between the EC_{50} of the free and encapsulated essential oil showed no significant difference ($p<0.05$). This means that the encapsulation of the essential oil in the amorphous porous silica did not change the antioxidant activity thereof.

Conclusion

In conclusion, the antioxidant activity of the oil extracted from lemon peel was evidenced by means of three different antioxidant activity tests. The main principles of this essential oil are limonene (67.1%), α-pinene (11.0%) and α-terpinene (8.0%).The encapsulation of the essential oil in amorphous porous silica no significant difference ($p<0.05$) in terms of same antioxidant activity as compared to the activity of the free oil. The characterizations carried out by SEM and FTIR-ATR showed that there was no relevant interaction between the essential oil and silica and each component preserved is individual properties in the oil-silica hybrid produced. Only the TGA revealed some minor thermal stabilization of the additive upon encapsulation.

The results achieved suggest that the encapsulation the essential oil in silica may produce a material ready to be used as component of active packages or even as a food preservative since silica is a common food additive.

Declaration of Competing Interest

The authors report no conflicts of interest.

CRediT authorship contribution statement

Louiza Himed: Funding acquisition, Writing - original draft. **Salah Merniz:** Funding acquisition, Writing - original draft. **Rebeca Monteagudo-Olivan:** Funding acquisition, Writing - original draft. **Malika Barkat:** Funding acquisition, Writing - original draft. **Joaquín Coronas:** Funding acquisition, Writing - original draft.

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References

[1] Y. Li, A.-S. Fabiano-Tixier, F. Chemat, Essential oils: from conventional to green extraction, in: Essential Oils as Reagents in Green Chemistry, 2014, pp. 9–21.

[2] A. Kerdudo, A. Dingas, X. Fernandez, C. Faure, Encapsulation of rutin and naringenin in multilamellar vesicles for optimum antioxidant activity, Food Chem. 159 (2014) 12–19.
[3] C. Turek, F.C. Stintzing, Stability of essential oils: a review, Compr. Rev. Food Sci. Food Saf. 12 (1) (2013) 40–53.
[4] N. Liédana, E. Marín, C. Téllez, J. Coronas, One-step encapsulation of caffeine in SBA-15 type and non-ordered silicas, Chem. Eng. J. 223 (2013) 714–721.
[5] E. Pérez, L. Martín, C. Rubio, J.S. Urieta, E. Piera, M.A. Caballero, C. Téllez, J. Coronas, Encapsulation of a-tocopheryl acetate into zeolite y for textile application, ind, Eng. Chem. Res. 49 (2010) 8495–8500.
[6] P. Horcajada, C. Serre, M. Vallet-Regi, M. Sebban, F. Taulelle, G. Ferey, Metalorganic frameworks as efficient materials for drug delivery, Angew. Chem. Int. Ed. 45 (2006) 5974–5978.
[7] L. Paseta, E. Simón-Gaudó, F. Gracia-Gorría, J. Coronas, Encapsulation of essential oils in porous silica and MOFs for trichloroisocyanuric acid tablets used for water treatment in swimming pools, Chem. Eng. J. 292 (2016) 28–34.
[8] R.P. Adams, Identification of Essential Oil Components by Gas chromatography/quadrupole Mass Spectroscopy, Allured Publishing Corporation, Illinois, 2001.
[9] L. Keawchaoon, R. &Yoksan, Preparation characterization and in vitro release study of carvacrol-loaded chitosan nanoparticles, Colloids Surf. B: Biointerface 84 (2011) 163–171.
[10] A. Mansouri, G. Embarek, E. Kokkalou, P. Kefalas, Phenolic profile and antioxidant activity of the algerian ripe date palm fruit (*Phoenix dactylifera*), Food Chem. 89 (2005) 411–420.
[11] C. Sanchez-Moreno, Methods used to evaluate the free radical scavenging activity in foods and biological systems, Int. J. Food Sci. Technol. 8 (2002) 121–137.
[12] R. Apak, K. Güçlü, M. Ozyürek, S.E. Karademir, Novel total antioxidant capacity index for dietary polyphenols and vitamins c and E, using their cupric ion reducing capability in the presence of neocuproine: cuprac method, J. Agric. Food Chem. 52 (26) (2004) 7970–7981.
[13] J. Lee, N. Koo, D.B. Min, Reactive oxygen species, aging and antioxidative nutraceuticals, Compr. Rev. Food Sci. Food Saf. 1 (2006) 21–33.
[14] L. Himed, S. Merniz, M. Barkat, Evaluation of the chemical composition and antioxidant activity of *citrus limon* essential oil and its application in margarine preservation, Alger. J. Nat. Product. 4 (2016) 316–322.
[15] D.J.Y. Saint Laumer, E. Frérot, A. Herrmann, Controlled release of perfumery alcohols by neighboring-group participation. comparison of the rate constants for the alkaline hydrolysis of 2-acyl-, 2-(hydroxymethyl)-, and 2-carbamoylbenzoates, Helv. Chim. Acta 86 (2003) 2871–2899.
[16] D. Kalemba, A. Kunicka, Antibacterial and antifungal properties of essential oils, Curr. Med. Chem. 10 (2003) 813–829.
[17] K.R. Oussou, in: Etude Chimique Et Activitébiologiques Des Huilesessentielles De Sept Plantesaromatiques De La Pharmacopéeivoirienne, Doctorat de l'Université de Cocody-Abidjan, 2009, p. 241. p.
[18] K.R. Oussou, S. Youlou, C. Kanko, B. Tue Bi, C. Kanko, J.B. Boti, C. Ahibo, J. Casanova, Etude chimique bio-guidée de l'huile essentielle de *ocimumgratissimum*(lamiaceae), Eur. J. Sci. Res. 1 (2010) 50–59.
[19] A. Wei, T. Shibamoto, Antioxidant activities and volatile constituents of essential oils, J. Agric. Food Chem. 54 (2007) 1737–1742.
[20] L. Benov, N. Georgeiv, The antioxidant activity of flavonoids isolated from, Coryl. Phy. Res. 8 (1994) 92–94.
[21] R. Gauthier, M. Gourai, J. Bellakhdar, A propos de l'huile essentielle de myrtuscommunis L. var. italicarécolté au maroc. I. rendements et compositions durant un cycle vegetative annuel, Rev. Mar. Pharm. 4 (1988) 97–116.
[22] N. Mimica-Dukic, B. Bozin, M. Sokovic, N. Simin, Antimicrobial andantioxidant activities of melissa officinalis L. (Lamiaceae) essential oil, J. Agric. Food Chem. 54 (2004) 5313–5322.
[23] Z. Mohammedi, F. Atik, Impact of solvent extraction type on total polyphenols content and biological activity from tamarixaphylla (L.) karst, Int. J. Pharma Bio. Sci. 2 (2011) 609–615.
[24] M. Kelen, B. Tepe, Chemical composition, antioxidant and antimicrobial properties of the essential oils of three salvia species from turkish flora, Biores. Technol. 99 (2008) 4096–4104.
[25] G. Ruberto, M.T. Baratta, Antioxidant activity of selected essential oil components in two lipid model systems, Food Chem. 69 (2000) 167–174.