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Chemical composition and antimicrobial activity of essential oils from Cuminum cyminum L. collected in different areas of Morocco

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ABSTRACT

Essential oils from 8 Cuminum cyminum local populations collected in Morocco were investigated for their chemical composition and antimicrobial activity. The chemical composition, investigated by gas chromato-graphic technique, revealed a common fingerprint in all Moroccan samples: twenty-five compounds were identified with γ-terpinen-7-al being the major component in all samples studied. The antimicrobial activity of cumin essential oils were tested against 10 bacterial strains, belonging to 8 different species, and 6 yeast strains, belonging to 4 species. Lactic acid bacteria showed a good resistance to all essential oil tested while overall the cumin essential oils showed a strong antifungal activity that affected both maximum specific growth rate and lag time.

1. Introduction

Cuminum cyminum (C. cyminum) is a small, herbaceous, annual plant belonging to Umbelliferae. Its seeds are one of the most popular spices, regularly being used as a flavouring agent in the cuisines of several different cultures. Cumin plants are encountered in Asia, North Africa, Europe and America and are also cultivated in Arabia, India, China and in the countries bordering the Mediterranean Sea (Thippeswamy and Naidu, 2005). China is the biggest producer of C. cyminum and an im-portant exporter (Li and Jiang, 2004). All cumin varieties are used in traditional and veterinary medicines as stimulants, astringents and as carminatives for indigestion, flatulence and diarrhoea.

The steam distillation of C. cyminum gives an essential oil (EO) which is recognized as an interesting source of antibacterial, antifungal and antioxidants components which might be used as potent agents in food preservation and for therapeutic or nutraceutical industries (Hajlaoui et al., 2010; Khosravi et al., 2011). Several reports have fo-cused on the antimicrobial efficacy of C. cyminum EO (from now, CEO) against diverse species of bacteria, fungi and yeast, both pathogens and non-pathogens (Kivanç, Akgül, & Doğan, 1991; Mekawey, Mokhtar, & Farrag, 2009; Naeini, Jalayer Naderi, & Shokri, 2014). However, studies into the practical efficacy of EO as a preservative for food systems or into the diverse biological activity and EO yield dependence on culti-vation site are lacking (Kedia, Prakash, Mishra, & Dubey, 2014).

The chemical profile of the EO from a particular plant species can show different chemotype variations linked to ecological and geo-graphical variability, age of plant, and the time of harvesting (Petretto et al., 2016). Such chemotype variations definitely affect the biological activity of the EO, and it has been demonstrated that the ripening stage also significantly affects several physical properties of the cumin seed (Bettaieb R et al., 2014). For instance, EOs extracted from European and Iranian C. cyminum seeds showed different antimicrobial activities (Akrami et al., 2015), with the CEO from Iran showing better activity, compared to the European EOs. Maximum and minimum EO yields (4.3% and 2.7%) were observed at the mature and immature stage respectively. CEO analysis reveals different compositions, although cumin aldehyde is by far the major (and characteristic) compound of cumin EO, with Baser et al. (1992), showing that this component is responsible for the pungent odor of the cumin seeds, and another study showing that cumin seeds may contain up to 30% cumin aldehyde (Borges and Pino, 1993).

In Morocco, cumin seeds are used to flavor soft dates and other

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foods (Meunie, 1982) and, due its great resistance to the drought, cumin is grown in arid climate regions such as Chichaoua, Rhamna, Haouz, Essaouira, Errachidia and Kelâa Sraghna (Elmaghraoui, 1986). Cumin is a warm climate plant, but it grows also at low temperatures and withstands temperatures as low as −7 °C (Elmaghraoui, 1986). While the ideal temperature for germination is 25 °C, it can begin at only 8 or 9 °C; the plant is cold-sensitive in spring.

To the best of our knowledge no earlier investigations have studied the cumin collected in different areas of Morocco. The aim of the pre-sent study is to investigate various C. cyminum local populations from Morocco, focusing the analysis on volatiles chemical composition and the antimicrobial activity of EOs. A multivariate approach was applied to the data in order to study any possible discrimination of the popu-lations related to the geographical area of collection; furthermore, the EOs isolated by steam-distillation of the eight populations were screened against 16 microorganisms with the aim to correlate the biological activity of EOs with the cultivation area of cumin.

2. Material and methods

2.1. Cumin samples and EO extraction

For this study a total of 8 populations (CEO1-8) of Cuminum cym-inum samples were examined. The samples were collected from three different geographical areas of Morocco (Fig. 1) during February-March

2013. CEO1 and CEO2 are from Errachidiya region, this area belongs to very dry semi-desert climate. Precipitation is low and distributed in a irregular way in the time and in the space. The majority of the territory receive less than 100 mm of rain a year. It is a pre-Saharan region.

CEO3, CEO4, CEO5 and CEO6 are from Rhamna region which has also a semi arid climate. The annual average pluviometry is about 300 mm. CEO7 and CEO8 were collected in Figuig region which has a semi arid climate characterized by low precipitation. The annual average pluviometry oscillates between 25 mm and 150 mm.

The EOs were extracted according to the European Pharmacopoeia protocol, briefly: a sample weighing about 20 g of powdered cumin seeds was subjected to hydro distillation for 1.5 h using a Clevenger type apparatus. Four extractions were carried out for each sample, the obtained EOs were collected separately, dried over anhydrous sodium sulfate (Na_2SO_4) and then stored at 4 °C in amber glass vials until analysis.

2.2. GC-FID analysis

The GC analysis of the EOs was carried out using an Agilent 4890 N instrument equipped with a FID and an HP-5 capillary column (30 m × 0.25 mm, film thickness 0.17 μm). The column temperature was held at 60 °C for 3 min, then increased to 210 °C at a rate of 4 °C/min and held at 210 °C for 15 min, then increased to 300 °C at a rate of 10 °C/min, and finally held at 300 °C for 15 min. Injector and detector
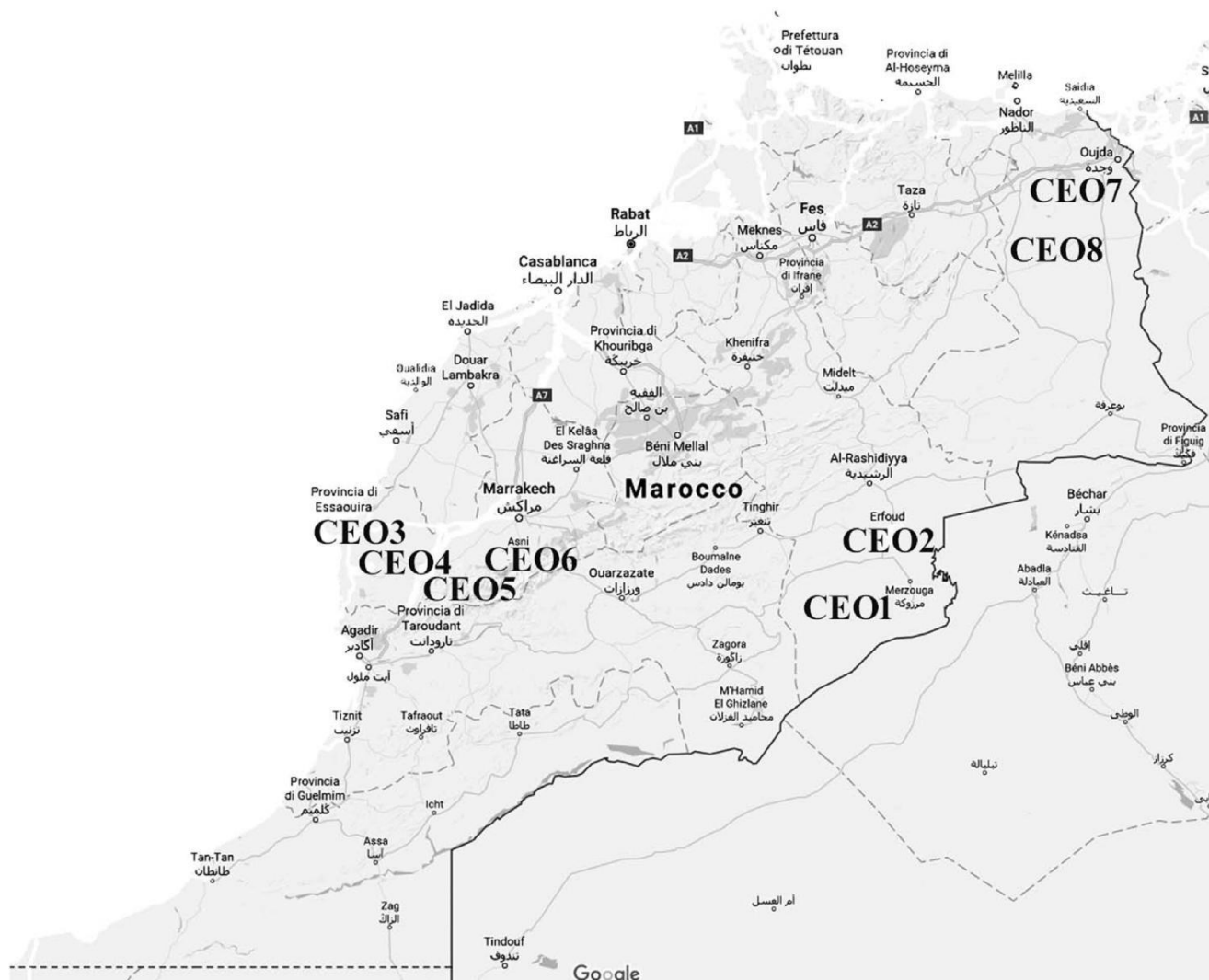

Fig. 1. Zone of harvest of the different Cumin in Morocco.

temperatures were 250 °C. Helium was used as carrier gas at a flow rate of 1 mL/min. The compound quantification in the EOs was carried out by the internal standard method, injecting 1 µL (split ratio 1:10) of a solution of EOs in hexane (dilution ratio 1:200). A calibration curve was constructed for each matching standard compound in the EOs. When standards were unavailable, quantification was performed with a cali-bration curve of a compound of the same classes of volatiles (mono-terpene hydrocarbons, oxygenated monoterpenes, sesquiterpene hy-drocarbons, oxygenates sesquiterpenes) occurring in the EOs; results were expressed as mg per mL of distilled EO.

2.3. GC-MS analysis

The GC-MS analysis was carried out injecting 1 µL (split ratio 1:10) of a solution of EOs in hexane (dilution ratio 1:200) into an Agilent 7890 GC equipped with a Gerstel MPS autosampler, coupled with an Agilent 7000 C MSD detector. The chromatographic separation was performed on a VF-Wax 60 m × 0.25 mm i.d., 0.5 µm film thickness column (Agilent), as well as on a HP-5MS capillary column (30 m × 0.25 mm, film thickness 0.17 µm), the following temperature program was used for the VF-Wax column: 40 °C hold for 4 min, then increased to 150 °C at a rate of 5.0 °C/min, held for 3 min then increased to 240 °C at a rate of 10 °C/min, and finally held for 12 min. For the HP-5MS column the following temperature program was used: 60 °C hold for 3 min, then increased to 210 °C at a rate of 4 °C/min, then held at 210 °C for 15 min, then increased to 300 °C at a rate of 10 °C/min, and finally held at 300 °C for 15 min. Helium was used as the carrier gas at a constant flow of 1 mL/min for both columns. The data was analysed using a MassHunter Workstation B.06.00 SP1, with identification of the individual components (Table S1) performed by comparison with the co-injected pure compounds and by matching the MS fragmentation patterns and retention indices with the built in libraries or literature data or commercial mass spectral libraries (NIST/EPA/NIH 2008; HP1607 purchased from Agilent Technologies).

2.4. Antimicrobial activity

The minimal inhibitory concentration (MIC) of Cumin Essential Oil (CEO) against 12 bacterial and 4 yeast species (see Table S1 for detailed information on strain used, culture condition and media used in this work) was performed according to literature procedures (Fancello et al., 2016). Briefly, EO stock solutions were first prepared with a concentration of 15 µL/mL. Stock solutions were then diluted, in 2× LAB susceptibility test medium (LSM) broth, cation adjusted Muller Hinton Agar (Oxoid, Basingstoke, England) and YEPD (Yeast Extract 2%, Peptone 1%, Dextrose 2%) for lactobacilli, pathogens and yeasts respectively, to give a series of final concentrations ranging from 0.015 to 7.5 µL/mL. Aliquots of 100 µL of diluted inoculation at desired cell concentration were added to each well in the 96-well micro-dilution plate already containing 100 µL of desired EOs dilutions. The plates were then incubated at 37 °C for 24 h. After incubation, MICs (µL/mL) values were determined as the lowest EO concentration that inhibited visible growth of the tested microorganism, which was indicated by absence of turbidity. DMSO alone (at 1% concentration) was used as negative control. Each test was performed in quadruplicate and the experiments were repeated twice. The influence of EO in the growth dynamics was performed in an automated microtiter dilution assay. Microtiter plates were prepared as above described using sub-MIC concentration (see Table S2 for detailed sub-MIC concentration tested) and were incubated at 37 °C for 48 h, in a plate reader (Spectro Nano Star, BMG LABTECH, Germany) with absorbance readings (OD600) taken every 30 min. SPECTRO star Nano MARS data analysis software integrated to the plate reader allowed an automated data recording.

2.5. Statistical analyses

Chemical analysis data were subjected to principal component analysis to evaluate any possible effects of the geographical origin of cumin on the chemical composition of its essential oil. A correlation analysis between the different chemical components of cumin essential oil was also performed using the Pearson correlation coefficient. The main discriminant chemical component between the 8 cumin oils stu-died were chosen according to the PCA and correlation analysis and were subjected to ANOVA analysis to investigate the effect of geo-graphical origin on their variation. When the effect was significant (P < 0.05), differences between means were separated by Tukey−Kramer multiple comparisons test. Data were statistically ana-lysed using SPSS software: Version 19.0.

Extensions in the lag time of growth of the studied microorganisms, when incubated with increasing concentration of the 8 cumin essential oils, were normalised by expressing them as percentage of the running time of the experiments according to Hayouni, Bouix, Abedrabba, Leveau, and Hamdi (2008).

The growth curves were fitted with the function of Baranyi, Roberts, and McClure (1993) to estimate the main growth parameters, namely, maximum specific growth rate (µ) and lag time (λ). Data were subjected to one-way ANOVA analysis to investigate the effect of different sub-MIC cumin EOs concentrations on main growth parameters using SPSS software (version 19.0).

3. Results

3.1. Chemical composition

The 1.5 h hydro distillation of the collected samples, in a Clevenger type apparatus, gave eight colorless EOs (4 of each sample) all with a pungent odor. The steam distillation yield varied from 2.9% of CEO2 to 3.7% of CEO4. The chemical characterization of the eight EOs was achieved by qualitative GC-MS analysis and quantitative internal standard method applied to GC-FID analysis. Twenty-five compounds were identified (Table 1) with γ-terpinen-7-al being the major compo-nent in all samples studied: its concentration ranging from 551 mg/mL of CEO1 to 227 mg/mL of CEO7. As shown in Table 1 a common fin-gerprint of the cumin EOs, from all the Moroccan areas, is found and is represented by six main components, namely β-pinene, p-cymene, γ-terpinene, cuminal, α-terpinen-7-al and γ-terpinen-7-al; between them, they cover over 95% of the total composition (based on the FID peak area normalization) in each sample.

Although the chemical composition of the EOs was relatively similar in all the studied areas, principal component analysis (PCA) and sta-tistical methods were applied to chromatographic data, with the aim of elaborating the relationship between C. cyminum populations and the compounds in the EO. PCA results are reported in Fig. 2: 62% of the total variance is explained by two first components; in the plane PC1-PC2 of the score plot in Fig. 2a are clustered the samples of cumin in 4 groups, populations D, F, G and H (CEO4, 6, 7 and 8) are separated each in a single group, whereas populations A, B, C and E (CEO1, 2, 3 and 5) are grouped in another cluster.

The results of correlation analysis are shown in Table S3. As ex-pected, monoterpene hydrocarbons were significantly positively cor-related to each other, and also with sesquiterpene hydrocarbons (Car-yophyllene, Farnesene-Z-β). Conversely, oxygenated monoterpenes, such as cumin-aldehyde showed a negative correlation with mono-terpene hydrocarbons, in particular with β-pinene, alpha α-pinene and phellandrene. γ-terpinen-7-al negatively correlated with sesquiterpene hydrocarbons.

ANOVA analysis was performed to highlight possible differences between the main chemical components as a function of the geo-graphical origin of the CEO tested. As we can see in Table S4, the proportion of the different components varied significantly with the

Table 1

Chemical composition of essential oils from Cuminum cyminum. RI: retention index, SD: standard deviation. Results are expressed as mean of four replicates.

	EO1	SD	EO2	SD	EO3	SD	EO4	SD	EO5	SD	EO6	SD	EO7	SD	EO8	SD	RI HP5	RI VF-WAX
α thujene	0.8	0.1	0.8	0.1	1.3	0.1	1.3	0.0	0.7	0.1	0.0	0.0	1.3	0.4	1.7	0.1	925.6	1038.9
α pinene	1.8	0.2	2.1	0.1	3.3	0.4	4.2	0.1	2.2	0.2	0.2	0.1	2.9	0.6	4.0	0.2	931.3	1035.4
sabinene	1.2	0.1	1.3	0.2	1.6	0.3	2.1	0.1	1.3	0.1	0.9	0.1	1.3	0.1	2.8	0.1	971.7	1138.7
β pinene	40.7	1.9	45.7	2.6	68.3	1.6	86.4	2.1	40.6	1.7	20.8	2.2	65.7	4.0	81.5	1.8	974.1	1126.9
myrcene	3.3	0.2	3.7	0.0	4.2	0.1	5.7	0.0	4.2	0.1	3.4	0.0	6.6	0.2	7.9	0.1	991.7	1176.3
α-phellandrene	3.7	0.0	4.0	0.0	4.2	0.1	7.6	0.0	2.6	0.1	1.9	0.0	3.2	0.1	6.2	0.0	1003.1	1184.4
α-terpinene	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.1	0.0	1.1	0.2	1.3	0.6	1015.4	1200.3
p-cymene	6.2	0.9	7.8	1.9	24.7	4.8	7.6	0.1	12.9	0.5	24.1	1.4	10.2	1.3	10.4	0.1	1023.2	1294.6
d3-carene	2.2	0.3	3.5	0.4	3.5	0.4	3.4	0.1	4.2	0.2	1.8	0.1	2.8	0.5	3.4	0.4	1026.9	1168.3
limonene	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	1028.0	1220.0
1,8-cineole	1.6	0.1	1.8	0.0	2.3	0.0	1.9	0.0	1.7	0.1	0.7	0.0	1.8	0.4	1.3	0.1	1028.7	1231.7
γ-terpinene	80.3	2.9	89.2	2.5	123.4	1.2	169.0	16.1	86.1	8.3	90.7	3.1	118.1	10.2	152.8	10.3	1058.9	1267.9
terpinolene	0.0	0.0	0.1	0.0	0.1	0.0	0.0	0.0	7.4	2.8	0.1	0.0	0.7	0.4	0.0	0.0	1087.6	1306.7
pinocarvone	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.4	0.0	1.3	0.9	0.0	0.0	0.0	0.0	1161.2	1552.2
terpinen−4-ol	1.1	0.1	0.0	0.0	1.6	0.4	0.0	0.0	1.2	0.3	2.4	0.1	2.9	0.2	0.9	0.6	1176.8	1634.0
α-terpineol	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1188.0	1725.0
cuminaldehyde	84.1	8.7	87.8	12.1	173.8	5.6	51.1	5.5	104.8	26.6	191.5	15.2	142.5	16.2	73.8	9.0	1240.5	1837.4
terpinen −7al-α	84.0	32.8	95.3	23.0	70.7	3.9	73.7	19.9	121.2	54.1	107.6	6.5	214.6	66.5	60.8	12.6	1284.1	1853.9
terpinen−7al-γ	550.6	25.9	531.5	29.1	369.4	9.5	468.2	76.7	524.3	131.9	411.4	58.6	226.9	31.8	446.4	48.6	1294.7	2146.0
daucene	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.9	0.1	0.6	0.1	1378.6	1526.7
cariophyllene	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.1	0.4	0.1	1417.7	
farnesene-(Z)-β	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.1	0.2	0.9	0.2	1458.5	1683.4
germacrene D	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.1	0.5	0.1	1469.6	
α-acoradiene	0.6	0.1	0.0	0.0	1.0	0.2	0.0	0.0	0.9	0.4	1.6	0.1	0.7	0.2	0.6	0.1	1473.6	1740.0
carotol	0.0	0.0	0.0	0.0	0.9	0.5	0.0	0.0	0.0	0.0	0.5	0.3	0.3	0.2	0.5	0.2	1597.1	

origin of CEO. A high variability was observed for β-pinene, myrcene, α-phellandrene, and cumin aldehyde widely, while the α-terpinen-7-al and γ-terpinen-7-al showed much less variability, even though the differences were significant.

3.2. Antimicrobial analysis

The antimicrobial activity of CEOs were tested against 10 bacterial strains, belonging to 8 different species, and 6 yeast strains, belonging to 4 species (See Table S1 for details). All CEOs were tested to de-termine the effect of the growth parameters in all strains using a con-centration range varying from 7.50 to 0.014 µL of EO/ mL of growth medium.

The MIC of yeast (Fig. 3) varied depending on the origin of the oil,

yeast species and strains tested. Overall, CEO2 and CEO6 showed the highest antifungal activity, whereas the CEO7 and CEO8 showed the lowest. These differences on antimicrobial activity can be attributed to the chemical composition of the oils tested. The CEO2 showed a strong antimicrobial activity against Candida albicans 3248, with the lowest value of MIC compared to others oils tested against yeast.

C. albicans 3993 and Saccharomyces cerevisiae EC1118 strains showed the highest MIC for all oils. In general, Lachancea thermotolerans J19 and Metschnikowia pulcherrima J20 were the most sensitive to CEOs, and the sensitiveness was oil dependent.

Lactic acid bacteria (LAB) showed a good resistance to all CEOs tested. Among LAB Lb plantarum 8014 ATCC was the most sensitive. For pathogens the antibacterial activity was also linked to the origin of the CEOs. CEO4, 7 and 8 inhibited the growth of L. monocytogenes DSMZ
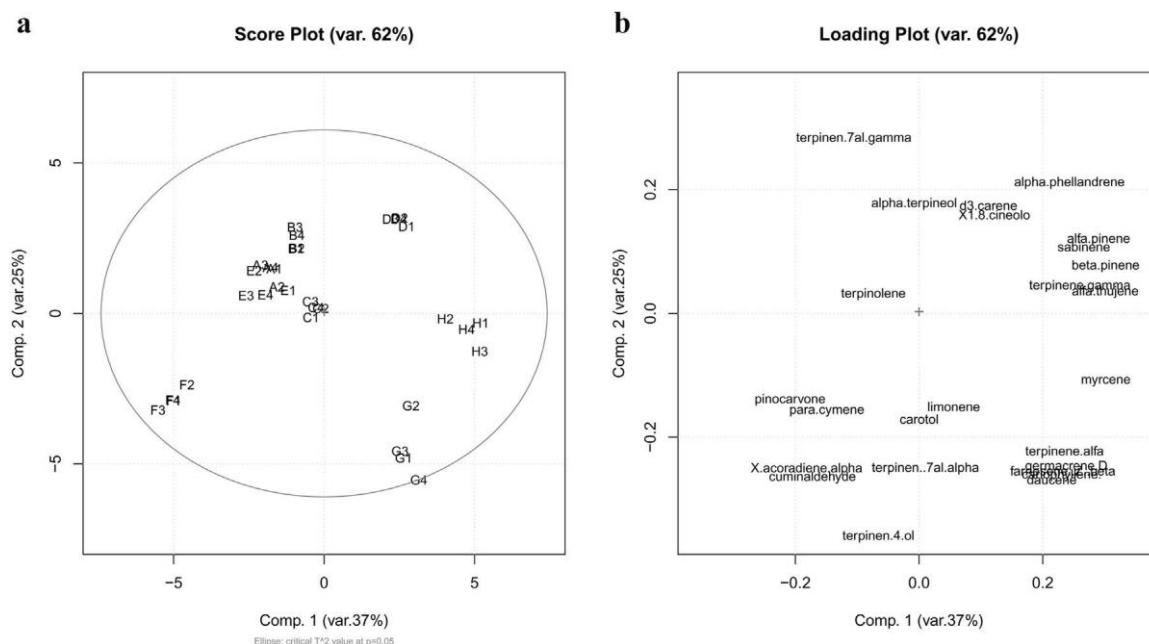

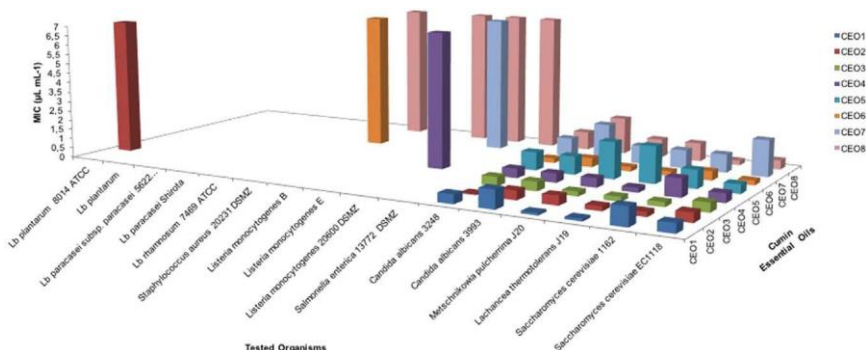Fig. 2. Principal component analysis (PCA) of C. cyminum populations and the chemical compounds.

Fig. 3. Minimum inhibitory concentrations (MIC) of the Cumin cyminum essential oils against the tested microorganisms.

20600, even at high concentration, while the other CEOs did not inhibit its growth. No differences were observed on maximum specific growth rate (μ_{max}) for Lactobacillus paracasei DSMZ 5622 and Lactobacillus rhamnosus ATCC 7469 (result not shown). Indeed, CEO1, 3, 4, 6 and 8 delayed the lag phases of Lb paracasei DSMZ 5622 (delay of 7.3; 11.7; 13.4; 11.1 and 6.8 h respectively, at maximum concentration tested) and CEO1 and 2 delayed the lag phase of Lb rhamnosus ATCC 7469 (of 10 and 3 h respectively) (Fig. S1). Generally, only for the high con-centrations tested (7.50 μL mL^{-1} and 3.75 μL mL^{-1}) was a delay on lag time on Lb paracasei DSMZ 5622 observed, with the exception of CEO3 and 8 (Fig. S1) which delayed the lag phase also at lower concentration (1.88 μL mL^{-1} and 0.94 μL mL^{-1}).

The growth rate of Lactobacillus paracasei SHIROTA was not affected by any essential oil tested except for CEO2 and 8 where a lag time delay was observed. The μ_{max} of L. plantarum was not affected by any CEO tested. Whereas the CEO2 inhibited the growth of L. plantarum 8014 ATCC, its growth parameters (growth rate and lag phase) were not af-fected by sub-MIC concentrations of CEO2. This oil did not inhibit the L. plantarum strains but extended the lag time by about 2 h.

The anti-listerial activity was also strain-dependent; in fact CEO8 inhibited the growth of L. monocytogenes E whereas the L. mono-cytogenes B was not inhibited by any CEOs. Likewise, CEO6 and 8 in-hibited the growth of S. aureus DSMZ 20231 while S. enterica DSMZ 13772 was inhibited only by CEO8.

As reported in the Figs. 3 and 4 and S2 it is evident that the relative extension of the lag phases (γ) was affected and the extent of delay is dose and oil dependent. Nevertheless, such correlation varied among species and strains. Hence, different CEOs affect differently the growth dynamic of the studied species.

The μ_{max} of L. monocytogenes B was significantly affected by CEO1 and CEO8 with respect to the control (p < 0.05, 0.42, 0.40 and 0.55 respectively), while all CEOs tested affected their lag time. At the highest concentration tested, the maximum extension of lag time with respect to the control (Fig. 4a) was observed for CEO1 (5 h), CEO8 (15 h) and CEO7 (17 h).

The μ_{max} of L. monocytogenes E was not affected by any CEO tested, whereas the lag time was delayed by all CEOs, with CEO6 and CEO7, at a concentration of 7.5 μL mL^{-1} delaying the lag phase of L. mono-cytogenes E, with respect to the control, by 12 and 6 h, respectively, and CEO8 extending the lag phase by 19 h at a concentration of 3.75 μL mL^{-1} (Fig. 4b).

The μ_{max} of L. monocytogenes DSMZ 20600 was not affected by any CEO, evenly at sub-MIC concentration. Conversely all CEOs tested ex-tended its lag time (Fig. 4c). At the maximum concentration tested, CEO1, 2, 3, delayed the lag time by about 4 h, CEO5 and CEO6 ex-tended the lag time by more than 10 h (namely 13.5 and 11 h respec-tively). At 3.5 μL mL^{-1} of CEO, only CEO5 appreciably extended the lag phase (about 9 h) and CEO4 and 8 extended the lag time by about 5 h, while CEO7 extended it by 8.62 h.

With regard to S. enterica DSMZ 13772, the μ_{max} was not affected by any CEO tested. The lag time was delayed with respect to control by

CEO1, 2, 5, 6 and 7 by 5.3 h, 5.8 h, 17.6 h, 13.2 h and 10.7 h respec-tively, at maximum concentration used (Fig. S2). At sub-MIC con-centration (MIC=7.50 μL mL^{-1}) CEO8 did not affect the growth parameters of S. enterica DSMZ 13772.

Interestingly, CEO1, 2 and 3 increased significantly the μ_{max} of Staphylococcus aureus DSMZ 20231 with respect to the control (p < 0.05, 0.75, 0.75, 0.89 h^{-1} vs 0.57), whereas the other CEO tested did not affect its μ_{max}.

All the oils tested delayed the lag time of Staphylococcus aureus DSMZ 20231 (Fig. 5). At the highest concentration, CEO5 extended the lag time by 15.2 h, CEO3 by 11.9 h, and CEO2 by 11.4 h. For CEO1 and 4 the delay with respect to the control was 9.4 and 9.9 h respectively, while for CEO6 and 7 there was a delay of 6.4 and 5.6 h respectively. Of CEO6 and 8, with a MIC of 7.50 μL mL^{-1}, only CEO8 showed a strong effect on lag time at the sub-MIC concentration, indeed at 3.5 μL mL^{-1} of CEO a delay respect to the control of 17.1 h was observed. CEO 3, 4 and 5 extended the lag time of 9, 6 and 11 h respectively (Fig. 5).

As noted before, the CEOs showed a strong antifungal activity that affected both maximum specific growth rate and lag time. The max-imum specific growth rate (μ^{max}) and lag time (λ) at sub-MIC con-centration tested (table S2) compared to μ^{max} and lag time of the con-trol are shown in Tables S5 and S6.

At sub-MIC concentration the CEO5 and CEO8 showed the highest reduction of μ^{max} of C. albicans 3248 whereas CEO2 and CEO6 were not significantly different from the control. Interestingly these last two had the lowest MIC while the first two had the highest, probably due to a different mechanism of action between different oils. Regarding C. al-bicans 3993, the CEO3, 7 and 8 significantly reduced the μ^{max}, whereas CEO2 and 5 had no significant effect on growth rate. Conversely all CEOs affected significantly the lag time (Fig. S3 and Table S6).

The CEO1, 3, 5, 6, 7 at sub-MIC concentration did not influence the μ^{max} of M. pulcherrima J20, likewise CEO1, 4, 6, 7 and 8 did not affect the growth rate of L. thermotolerans J19. For the M. pulcherrima J20 all oils except CEO8 showed a significant effect on lag time while CEO3, 4 and 8 extended the lag phase of L. thermotolerans J19 strains. Also for S. cerevisiae 1162 and EC1118 strains the CEOs work differently: the μ^{max} of S. cerevisiae 1162 was affected by all the CEOs except CEO7 and 8, whereas the μ^{max} of S. cerevisiae EC1118 was affected only by CEO1, 2 and 3. All CEOs significantly affected the lag time of S. cerevisiae strains (Table S6).

Overall there is a direct relation between CEO concentration and relative extension of the lag time as observed in Figs. S4–S6, even if such correlation varies from strain to strain. As rule, the relative ex-tension of lag time never exceeded 33% (~15 h).

4. Discussion

The variability in yield of plant hydro-distillation (HD) products is related to several variables, such as maturation (El-Sawi & Mohamed, 2002), genetic factors (Melito et al., 2013), environmental factors, and extraction method (Chatterjee et al., 2015). Since the maturation,
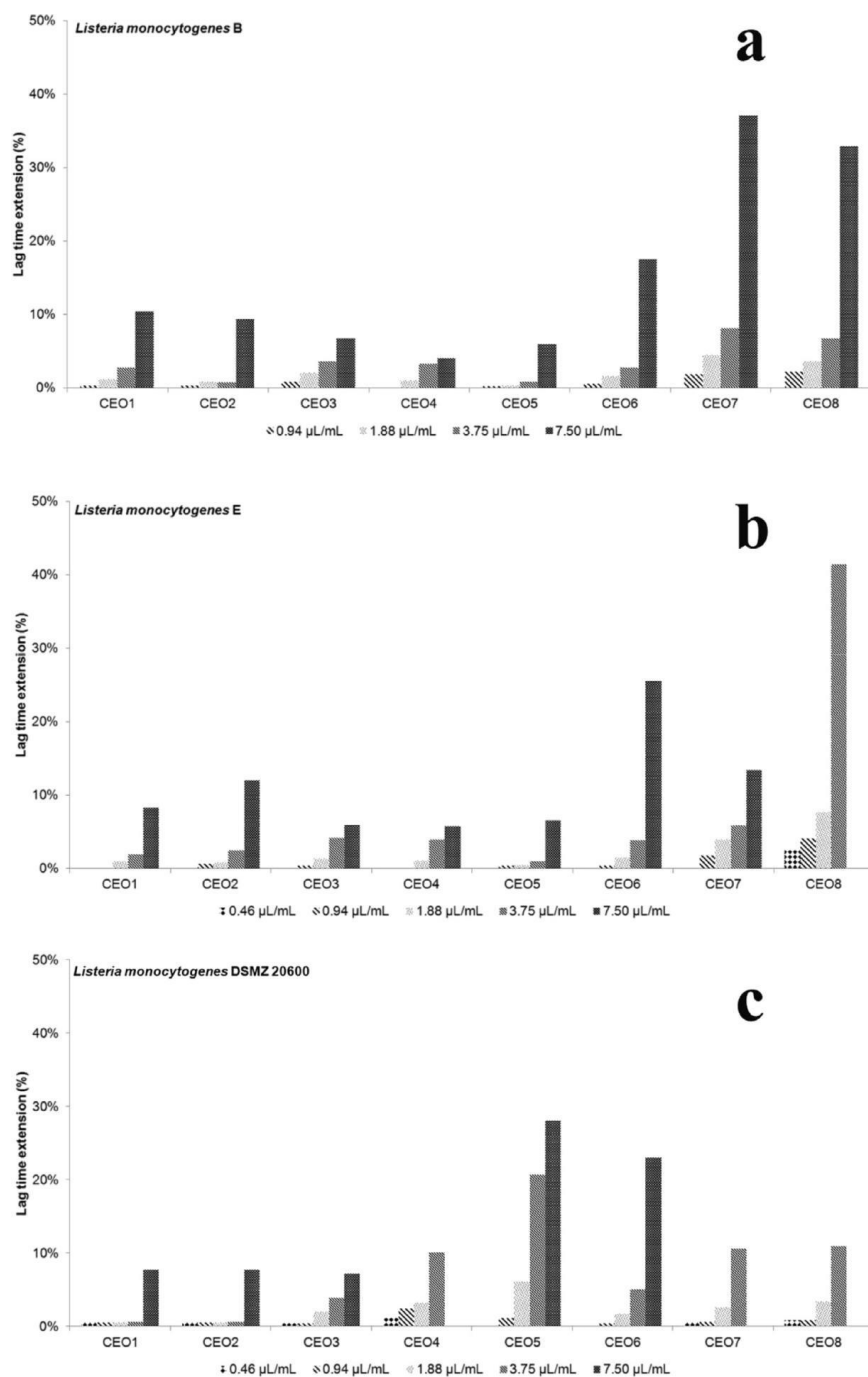
Fig. 4. The Lag time extension of three different strains of L. monocytogenes treated with the different cumin essential oils.

genetic and extraction method variables do not changes in our samples we would expect the moderate variation of yield in EO extraction we see in our samples could be related to the different environmental conditions of the growth sites. In the literature different yield values have been reported: Bettaieb et al. (2011) report a HD yield data for Indian cumin of 1.21% whereas Li and Jiang (2004) reported a HD yield of 3.8% for cumin from China.

Our results show that Moroccan cumin is characterized by high amount of γ-terpinen-7-al. γ-Terpinene has been demonstrated to be the precursor of corresponding aromatic derivatives such as p-cymene or thymol (Poulose & Croteau, 1978). The γ-terpinene/thymol transition is similar to the γ-terpinen-7-al/cuminal chemical transformation, there-fore in the same way it would be expected that terpinen 7al gamma functions as the precursor of the corresponding aldehyde cuminal which is often found to be the major compound in the cumin EO. The

conversion of γ-terpinen-7-al in cuminal could be related to several parameters such as climatic condition or time of collection.

The chemical composition of the EO extracted from cumin collected in different country show several differences in comparison with our results, confirming a variability according the grown area (Bettaieb et al., 2011). Cumin aldheyde is reported to be the main compound of the EO extracted from cumin collected in several regions (Beies, Azcan, Ozek, Kara & Baser, 2000; Bettaieb et al., 2010; El-Sawi & Mohamed, 2002; Jalali-Heravi, Zekavat & Sereshti, 2007). In our result cuminal was found in each case lower than its possible precursor γ-terpinen-7-al. Some authors (Bettaieb et al., 2011; Moghaddam & Pirbalouti, 2017; Moghaddam, Miran, Pirbalouti, Mehdizadeh, & Ghaderi, 2015; Viuda-Martos, Ruiz-Navajas, Fernández-López, & Pérez-Álvarez, 2008;) re-ported several chemo-types characterized by high content of γ-terpi-nene. Although it was not the major component, our study shown
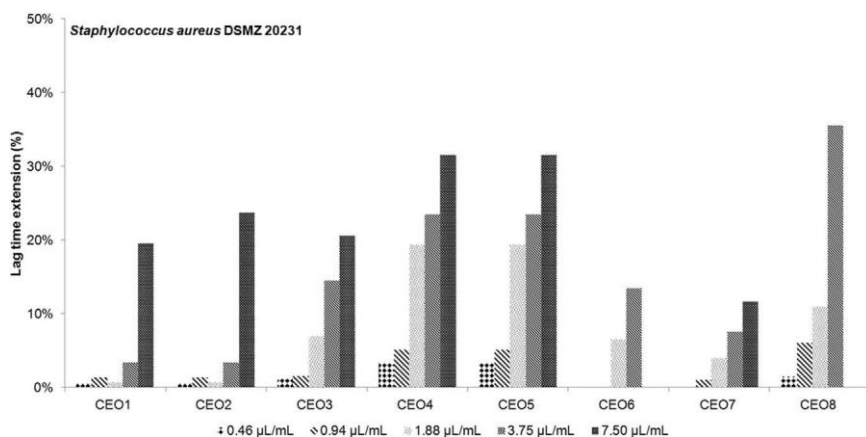
considerable amount of γ-terpinene particularly for CEO3, 4, 7, 8, in addition, γ-terpinene showed a negative correlation with cumin alde-hyde confirming previous results (Moghaddam & Pirbalouti, 2017).

PCA applied to the chromatographic data show some similarities between CEO1, 2, 3 and 5. As reported in material and method section, there is not any environmental factor which could be linked to this cluster. It should be then supposed that, besides to geographical grown area, the genetic factors play a key role in the variability/similarities of population chemo-types included in this cluster (Moghaddam & Pirbalouti, 2017).

The antibacterial activity of CEOs is much less impressive when compared to its antifungal activity. Overall CEOs show a good anti-microbial activity against Candida spp. strains tested whereas they slightly antagonize bacteria pathogens tested. To point out that CEOs not inhibited the LAB, which is one of the most important food related bacteria, owing to the essential role which perform in the production of fermented food.

The anti-bacterial and anti-fungal activity is known to vary with respect to cumin population, microbial species and strain. Several au-thors (Din, Sarfraz, & Shahid, 2015; Hajlaoui et al., 2010; Moghaddam et al., 2015; Pichersky, Noel, & Dudareva, 2006) found that the che-mical differences in the composition of EOs is directly related to dif-ferences in their biological properties, while Heywood (2002) observed that variation in morphological and phytochemical traits can be due to various soils and climatic conditions. In fact, the antibacterial activity of CEO is attributable to the high level of cumin aldehyde, a compound with known antimicrobial properties (Hyldgaard, Mygind, & Meyer, 2012; Saad, Muller, & Lobstein, 2013), and to α-pinene, the other main component of CEO, which inhibited the growth of bacteria. Limonene, sabinene, minor components of CEO, are known bactericides (Hyldgaard et al., 2012) and may contribute to the antimicrobial ac-tivity. So, as observed in our work, CEO components vary according to growth site, weather, extraction methods and storage conditions (Burt, 2004; Iacobellis, Lo Cantore, Capasso, and Senatore, 2005; Li and Jiang, 2004), and these variations influence the antimicrobial activity.

Among the 10 bacteria and 6 yeast species, we observed that the MIC and growth parameters varied depending on the origin of the oil, and, although the antibacterial action was assessed against a range of beneficial and pathogenic gram-positive and gram-negative bacterial strains, antimicrobial activity was always higher against yeast species. A strong antifungal activity in accord with our results was found by Hajlaoui et al. (2010) and Din et al. (2015). These authors found, also in accord with our results, that the MIC for yeast was lower than for the bacteria. Our results are also confirmed by Özcan and Erkmen (2001) who studied the antibacterial activities of nine Turkish plant species including C. cuminum, and found that this oil inhibited S. aureus and S. typhimurium at high concentration, compared to S. cerevisiae. Several authors also confirmed our data

(Chaudhary, Husain, & Ali, 2014; Minooeianhaghighi, Sepehrian, & Shokri, 2016; Wanner et al., 2010), having found that CEOs have a strong anti-candida activity. Aligiannis, Kalpoutzakis, Mitaku, and Chinou (2001) proposed a classification for plant material, based on the essential oil activities and MIC results as follows: strong inhibitors (MIC up to 0.5 mg/mL); moderate inhibitors (MIC between 0.6 an d 1.5 mg/ mL); weak inhibitors (MIC above 1.6 mg/mL). According to this clas-sification, the CEOs analysed here may be classified as strong inhibitors for yeast and weak inhibitors for bacteria.

As noted above, the antibacterial activity of the different CEOs was weaker compared to their action against yeast. This behaviour was observed for both food-related and pathogen bacteria. For pathogens, there are different studies with contrasting results. Gachkar et al. (2007) found that L. monocytogenes was very sensitivity to CEO. On the other hand, (Hyldgaard et al., 2012); Irkin and Korukluoglu (2009) found that CEO actively inhibited yeast and, to lesser extent, L. mono-cytogenes and other bacteria. Likewise a recent paper from Amrutha, Sundar, and Shetty (2017) found that CEO effectively reduced quorum regulated phenotypes in S. enterica such as bacterial swimming, swarming and biofilm formation along with reduction in exopoly-saccharide EPS production. Moreover, Sağdiç, Karahan, Özcan, and Özkan (2003) demonstrated that C. cyminum had no activity against S. aureus and S. enteritidis. Conversely, Raja et al. (2016) found that C. cyminum was the most effective against multi-drug resistant S. aureus and, finally, a recent work of Kakarla et al. (2017) showed that mul-tidrug-resistant strains like methicillin-resistant S. aureus growth was inhibited by CEO in a dosage-dependent manner, and cumin and its bioactive components inhibit the growth of bacterial cells with the LmrS multidrug efflux pump, suggesting that they be considered po-tential candidates for rational drug design.

CEOs tested in our work did not inhibit strains belonging to Lactobacillus genus, with exception of a strain of L. plantarum, even at high concentrations. Lactobacillus genus are food-grade bacteria, fun-damental for fermented food and probiotic production. Recently, Kozłowska, Ścibisz, Zaręba, and Ziarno (2015) found that cumin seeds extracts had no impact on the growth of the many tested LAB. In an-other work, Zamani-Zadeh, Soleimanian-Zad, Sheikh-Zeinoddin, and Hossein Goli (2014) used L. plantarum in combination with cumin as postharvest biocontrol agents against Botrytis spp. on strawberry fruit. On the other hand, Viuda-Martos et al. (2008), analyzing several spices against some bacteria commonly used in the food industry among which Lactobacillus spp, found that CEO showed a high antibacterial activity. These results are in accordance with two recent reviews where has been underlined the positive effect of cumin EOs on probiotic bacteria and their synergistic effects (Rasouli, Mahmoudi & Kazeminia, 2017; Shipradeep et al., 2012). Finally, the synergistic bactericidal ef-ficacy of the essential cumin in combination with other spices and/or compounds i.e. coriander or nisin as a potential source of safe and

effective natural antimicrobial and antioxidant agents in pharmaceu-tical and food industries has been recently observed (Bag & Chattopadhyay, 2015; Pajohi, Tajik, Farshid, & Hadian, 2011; Tavakoli, Mashak, Moradi, & Sodagari, 2015).

5. Conclusion

In conclusion, our work has shown that the chemical composition and antimicrobial activity of cumin essential oil is correlated and varies depending on climatic and edaphic condition of the growth site. The cumin essential oil analysed in this work showed a good antifungal activity but lacks antimicrobial activity against lactic acid bacteria, the most important food related bacteria; a slight antimicrobial activity against food borne pathogens was observed. Based on the findings of this work, and considering that EOs, and cumin is one of them, are becoming very important as natural food preservatives (Jessica Elizabeth, Gassara, Kouassi, Brar, & Belkacemi, 2017; Pandey, Kumar, Singh, Tripathi, & Bajpai, 2017), it will be very important in the future to evaluate the antimicrobial activity of any essential oil, taking into consideration its chemical composition and the growing site.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.fbio.2018.01.004.

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