Essential Oils from three species of *Mentha* harvested in Sardinia: Chemical Characterization and evaluation of their biological activity

Running title: Mint essential oil as functional food ingredient

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ABSTRACT

The chemical composition, antimicrobial activity and antioxidant activity of *Mentha (M.) requienii*, *M. pulegium* L. and *M. aquatica* (L.) Huds from Sardinia, Italy were tested. The chemical composition was determined by Gas Chromatography-Mass Spectrometry (GC-MS) and GC-Flame Ionization Detector (GC-FID) analysis. The antiradical activity was assessed by DPPH and ABTS assay and minimal inhibition concentration was determined by microdilution

broth analysis. The major components found in *M. requienii* EO were pulegone, menthone and limonene; similarly the major components of *M. pulegium* EO were pulegone and iso-menthone. By contrast, the major components of the EO from *M. aquatica* are terpinyl acetate and eucalyptol. The antioxidant activity of *M. aquatica* EO was higher than that of *M. requienii* and *M. pulegium*. The EO from *M. requienii* had good antifungal and antioxidant activity. In the concentration range tested, the mint EOs did not show any antibacterial activity against *Staphylococcus aureus* 20231 DSMZ*, Salmonella enterica* subsp. *bongori* serovar 66:z41:-13772 DSMZ and four strains of *Listeria monocytogenes.* Lactic acid bacteria strains were not inhibited by the EOs tested, with the exception of *Lactobacillus brevis* which showed slight sensitivity to *M. pulegium* and *M. requenii* EOs. Yeast strains were in general sensitive to the different *Mentha* EOs tested and was species and strain dependent; generally, the EOs of *M. pulegium* and *M. requenii* were found to be more active than that of *M. aquatica.*

Keywords: Mint oil, probiotics, antioxidant activity, composition, functional food

INTRODUCTION

The global demand trend for functional food is increasing with consumers frequently request foods with nutraceutical features, which in turn has stimulated fundamental and applied research on functional food. Generally, a functional food contains various factors that ensure or enhance

health, such as plant bioactive compounds, antioxidants, dietary fiber, probiotic, prebiotics, functional starches and bioactive peptides. Among functional foods, probiotic and prebiotic containing food are the most popular. Alongside the increasing demand for functional food, the spread of antimicrobial resistance has assumed a global dimension and this has stimulated the search for new alternatives, such as the use of probiotics and essential oils (EOs), to replace antibiotic use [1] and synthetic-derived antimicrobial agents EOs have different functional characteristics such as antioxidant, antidepressant-like, anti-angiogenic and anti-diabetic activity [2-4]. Together with these features, some EOs have shown strong antimicrobial activity that can be exploited against food pathogens. There are few studies on the effect of EOs on probiotic strains [5] and, considering that they are both are used in combination for functional products, it is important to investigate the effect of EOs on probiotics [5-7].

Mint is the common name for more than twenty perennial species belonging to the genus *Mentha* (*Mentha sp*. *Lamiaceae*, family *Lamiaceaeare*) . Among those included in the genus mentha, are several economically important plants, sources of raw material for flavour and fragrance. Worldwide, the most important mint species are spearmint (*Mentha spicata,),* peppermint *(M. piperita)* and Japanese mint *(M. arvensis).*

The hydro-distillation of mint aerial parts provides EO, the chemical composition of which varies with species. *M. arvensis* EO has a high proportion of isomenthone and menthol, *M. spicata* has an EO rich in limonene, carvone and caryophyllene [8] while *M. piperita* produces an EO rich in menthol and menthyl acetate [9].The study of the antimicrobial activity of mint EO has shown contrasting results, though this difference is mainly due to the differences in chemical

composition and, to a lesser extent, the experimental protocol utilized. It has been shown that the EOs extracted from different mint species exhibited antimicrobial activity against several foodborne pathogens and spoilage microorganisms [10]. At higher concentration of mint EO (1% v/v) a bactericidal effect was observed whereas at lower concentration (0.1 % v/v) there is a dose-dependent decrease of bacterial growth rate [11]. The antimicrobial activities of mint EOs were correlated with their major lipophilic constituents such as menthol, isomenthone, pulegone and piperitone, which caused irreversible damage to the bacterial membrane [12-13].

Much attention has also been paid to the antioxidant capacity of EOs. Among terpene compounds, the most active molecules have found to be thymol and carvacrol, which exhibit antioxidant properties comparable to those of -tocopherol [14]. The antioxidant capacity of an EO is often difficult to predict due to synergistic or antagonistic effects of the complex mixtures of molecules that are present in the EOs [15]. Overall, mint EO has shown to have good antioxidant capacity [16].

Mentha requienii Bentham (***M. requienii***) is an endemic Sardinian-Corsican species described for the first time by Valsecchi [17]. The same authors described ***M. requienii*** as a paleoendemism due to its morphological isolation and because it has several differences as compared with other species of the genus [17]. Despite the great number of studies focused on EOs extracted from ***Mentha*** spp., little is known about the EO extracted from ***M. requienii*** other than that it has a pulegone rich EO that exhibits considerable activity, mainly against moulds and ***Aspergillus fumigatus*** [18]. Herein we present the results of our in-depth study on ***M. requienii*** EO in terms of chemical composition, antioxidant and antimicrobial activity. We pay particular attention to

the effect of the EO on probiotic, food borne, bacteria pathogens and yeast and make comparisons with the EOs extracted from two other species well represented in Sardinia, namely *M. pulegium* and *M. aquatica*.

MATERIAL AND METHODS

Plant material and extraction of EOs

Four samples of the aerial parts of wild *M. requienii* Bentham were collected from different plants growing in the same area, in June 2016 from the Bruncu Spina, NU, Sardinia, Italy. Four samples of the aerial parts of wild *M. aquatica* were collected from different plants growing in the same area, in July 2016 from the Laconi, OR, Sardinia, Italy. Four samples of the aerial parts of wild *M. pulegium* were collected from different plants growing in the same area, in June 2016 from La Maddalena Island, OT, Sardinia, Italy. A sample weighing 50g of each *Mentha* spp. leaves was subjected to hydro-distillation using a Clevenger type apparatus for 2 h, according to the European Pharmacopoeia protocol (2002). The extraction was carried out in duplicate for each sample, and the obtained EOs were collected separately, dried over anhydrous sodium sulfate (Na_2SO_4) and then stored under a nitrogen atmosphere at 4ºC in amber glass vials until analyzed**.**

Chemicals and reagent

Unless stated otherwise, all chemicals and reagents were supplied by Sigma (Dorset, UK). The following terpenoid compound commercial reference standards were used: α-pinene, α-

phellandrene, β-pinene, myrcene, p-cymene, limonene, 1-8-cineole, γ-terpinene, linalool, menthone, borneol, α-terpineol, bornyl acetate and terpinyl acetate.

Gas Chromatography-Mass Spectrometry (GC-MS) analysis

The GC-MS analysis was carried out using an Agilent 7890 GC equipped with a Gerstel MPS autosampler, coupled with an Agilent 7000C MSD detector. The chromatographic separation was performed on a HP-5MS capillary column (30 m × 0.25 mm, film thickness 0.17 m); the following temperature program was used: 60 ºC hold for 3 min, then increased to 210 ºC at a rate of 4 ºC/min, then held at 210 ºC for 15 minutes, then increased to 300 ºC at a rate of 10 ºC/min, and finally held at 300 ºC for 15 minutes. Helium was used as the carrier gas at a constant flow of 1mL/min. The data was analyzed using Mass Hunter Workstation B.06.00 SP1, with identification of the individual components (Table 1) 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).

Gas Chromatography-Flame Ionization Detector (GC-FID) analysis

The GC analysis of the an EO solution in hexane (dilution ratio 1:200 v/v) was carried out using an Agilent 4890N instrument equipped with a FID and an HP-5 capillary column (30 m × 0.25 mm, film thickness 0.17 m). The column temperature program was the same described for the HP5 column in GC-MS section. The compound quantification in the EOs was carried out by the internal standard method, according to previous literature methods [19]. A calibration curve was

constructed for each matching standard compound in the EOs. When standards were unavailable, quantification was performed with a calibration curve of a compound of the same classes of volatiles (monoterpene hydrocarbons, oxygenated monoterpenes, sesquiterpene hydrocarbons, oxygenates sesquiterpenes) occurring in the EOs; results are expressed as mg per mL of distilled EO. (Table 1)

Antiradical activity

The radical scavenging activity assay was performed according to literature methods [19]. Different concentrations of EOs, from 0.125 mg and up to a maximum of 5 mg were added to solution of DPPH (100 μM in ethyl acetate) to reach a final volume of 1 mL. Mixtures were shaken and incubated at 25 °C in the dark. The reduction of the DPPH radical was determined by measuring the absorbance of the solution at 517 nm using a 1 cm quartz cuvette on an Ultrospec 4300 pro UV–vis spectrophotometer, equipped with a temperature controller set to 25 °C, at pre-chosen time intervals of 60, 180 and 300 min, whereupon a constant value was reached. A Trolox calibration curve in the range 0.25-7.5 μg/mL was used as positive reference. The scavenging activity of the DPPH radical was calculated as follows:

$$\% \text{ scavenging of DPPH radical} = [(A_{blank} - A_{sample})/A_{blank}] \times 100$$

where A_{blank} is the absorbance of the control reaction (containing all reagents except the test compound), and A_{sample} is the absorbance in presence of EOs. Experiments were carried out in triplicate, and results are expressed as mean ± SD.

Antioxidant Capacity Assay (ABTS).

The ABTS free radical-scavenging activity of each sample was determined according to literature methods [19]. This involves the direct production of the blue/green ABTS·+ chromophore through the reaction between ABTS and potassium persulfate. Addition of antioxidants to the preformed radical cation reduces it to ABTS, to an extent and on a timescale dependant on the antioxidant activity, the concentration of the antioxidant, and the duration of the reaction. ABTS was dissolved in water to give a final concentration of 7 mM. $ABTS^{\cdot+}$ was produced by the reaction of ABTS stock solution with 2.45 mM potassium persulfate (final concentration) and allowing the mixture to stand in the dark at room temperature for 12–16 h before use. The $ABTS^{\cdot+}$ solution was diluted with ethanol to an absorbance of 0.7 (±0.02) at 734 nm. In order to measure the antioxidant activity of the EOs, 100 µL of EOs solution at various concentrations (0.1, 0.2, 0.5, 1.0, 2.0 mg/mL) were added to 900 µL of diluted $ABTS^{\cdot+}$ and the absorbance recorded at time zero and after 50 min whereupon the absorbance was stable. Each concentration was analysed in triplicate. The decoloration percentage at 734 nm was calculated for each point; the antioxidant capacity of the test compound was expressed in percent inhibition (%), and the IC_{50} value was calculated from regression analysis and is expressed as the mean ± SD.

Antimicrobial activity

The minimal inhibitory concentration (MIC) of the *M. pulegium, M. requenii* and *M. aquatica* EOs against 13 bacterial and 5 yeast species (see table 2 for detailed information on strain used, culture condition and media used in this work), were tested according to previous literature

methods [20]. Briefly, EO stock solutions were first prepared with a concentration of 30 µL/mL. Stock solutions were then diluted, in 2× Lactic Acid Bacteria (LAB) susceptibility test medium (LSM, is a mixture of 90% of ISO-Sensitest broth (Oxoid, Basingstoke, England) and 10% of MRS broth (Oxoid, Basingstoke, England), cation adjusted Muller Hinton broth (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 0.059 to 15 µL/mL. Aliquots of 100 µL of diluted inoculation solutions at desired cell concentration were added to each well in the 96-well micro-dilution plate already containing 100 µL of appropriate EOs dilutions. The plates were then incubated at 37°C for 48 h for lactobacilli and 24 h for yeast (*Saccharomyces cerevisiae* EC1118 was incubated at 30°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 (4 technical replicate for each microbial strain tested) and the experiments were repeated twice.

Principal component analysis procedure

The multivariate analysis of *Mentha* samples was carried out subjecting the chromatographic data (four biological replicates) to principal component analysis (PCA). The relative percent (by internal normalization of FID chromatogram) of components in the EOs of the *Mentha* were used to define a m × n matrix, where m are the samples and n are the compounds in the EOs. The data were centered and autoscaled before the PCA. All PCA analyses were performed with R-based chemometric software designed by Chemometric group of Chemical Italian Society.

RESULTS AND DISCUSSION

The chemical composition of EOs obtained from *M. aquatica, M. pulegium* L. and *M. requienii* Bentham are reported in the table 1. A total of 24 compounds were identified or tentatively identified by GC/MS analysis with a quantification of components being performed by an internal standard method applied to GC/FID analysis. EO from *M. requienii* is characterized by a high amount of pulegone (over 500 mg per mL of EO with menthone and limonene (66 and 13 mg/mL respectively) well represented. The chemical composition of EO from *M. requienii* is in line with the same EO extracted from plants collected in a different geographical area: Chessa et al. [18] reported a similar fingerprint in which EO composition was as also dominated by pulegone.

The chemical composition of *M. requienii* EO shows several similarities with EO extracted from Sardinian *M. pulegium*. Both EOs are dominated by pulegone and, with exception of some minor compounds, the chemical compositions show qualitative and quantitative similarities (Table 1). There is considerable variation in reported chemical composition of EO from *M. pulegium*: Mahbubi and Haghi [21] reported that EO of *M. pulegium* from Iran was characterized by high amounts of piperitone and piperitenone (together, they accounted for 77% of the whole EO), while Teixeira et al. [22] showed that EO extracted from *M. pulegium* collected in Portugal is characterized by high amount of menthone (36% of the whole). Conversely, our data on the EO from Sardinian *M. pulegium* are in agreement with results on EOs extracted from samples collected in India, Algeria and Uruguay [23-25]. These differences in chemical composition might be linked to the different geographical area of grown and/or to the climatic conditions.

M. aquatica EO is different from *M. pulegium* and *M. requienii* EOs, and is characterized by a high amount of terpinyl acetate (560 mg/mL) together with eucalyptol (86 mg/mL). The GC/FID analysis of the EO also shows a non negligible amount of borneol and mentha-1(7),8-dien-2-ol (15.3 and 19.2 mg/mL respectively). The EO of *M. aquatica* from Iran and former Yugoslavian states [8-9] show fingerprints quite different from our results. Those EOs were characterized by medium/high amount of eucalyptol (18-14%), menthofuran (15-17%) and caryopyllene (12-16%). Agostini et al. [26] studied the chemical characterization of EO extracted from *M. aquatica* collected in Brazil and reported that EO chemical composition was dominated by menthofuran (53%), with a number of other minor compounds.

We subjected our chromatographic data on EO chemical composition to a PCA multivariate analysis to allows us to graphically represent the system in fewer dimensions [27]. Figure 1a shows the score plot and Figure 1b the loadings plot. The first and second principal component represent the 66% of the total variance and by looking the score plot (figure 1a) it is clear that the information contained in the variables is able to discriminate the three variety of *Mentha*. In the loading plot (figure 1b) are distributed the list of detected volatiles (the variables) according to their contribution to each principal component.

To evaluate the antioxidant capacity of *Mentha* EOs we used the DPPH assay, a direct method based on the scavenging of the radical, and the ABTS assay, based on the inhibition of cation radical by antioxidants. The antioxidant activity was calculated as IC_{50}, the amount of EO that neutralizes 50% of the radical. IC_{50} is used because the determination of the absolute value for the antioxidant capacity of EO depends on many variables, including degradation during the

analysis, matrix interference, and may give a misleading value. As shown in table 3, the antioxidant activity of *M. aquatica* EO was higher compared with *M. requienii* and *M. pulegium*, at time zero and also at 30 min of DPPH assay, the time when the reaction was stable. ABTS results were in line with DPPH data but gave the best results, as shown in table 3. The superiority of the ABTS assay over DPPH can be explained because ABTS is usable over a wider range of pH, and it can measure the antioxidant activity of both water-soluble and lipid-soluble antioxidants because it can be solubilized in both aqueous and in organic media, whereas DPPH can only be dissolved in alcoholic media [28-29].

The similar antioxidant activity of *M. pulegium* and *M. requienii* EOs is probably linked to their similar chemical profiles, in which pulegone, menthone and limonene were well represented. On the other hand, the higher antioxidant activity registered by *M. acquatica* EOs could be explained by the high amount of terpinyl acetate and eucalyptol present. This suggestion is supported by the literature data in which terpenes like terpinyl acetate or eucalyptol, are described as good antioxidant compounds [14, 30-32]. In any event, the antioxidant activity of EOs has frequently found to be higher, though sometimes lower, than that registered for their individual components [15]. This indicates the existence of synergetic or antagonistic effects due to the complex multi-component composition of the oils [31-33].

The *in vitro* antimicrobial activity of *M. pulegium, M. requenii* and *M. aquatica* aerial parts EOs was assayed against 13 bacterial and 5 yeast species. Table 4 shows the MIC of the different strains tested. The EO of *M. pulegium* and *M. requenii* has been found to be more active compared with the *M. aquatica* EO [8, 34]. *M. aquatica* EO showed the lowest antimicrobial

activity, with only *Candida kruseii* J8 inhibited by this EO (MIC, 3.75 µL/mL). The strain members of LAB species, which include some of the most important bacteria in food fermentations, along with all bacteria pathogens, showed the highest antimicrobial resistance against *Mentha* EOs. These findings are in accordance with the results obtained by several authors [5, 8, 20] who found that the members of LAB groups showed low sensitivity to EOs extracted from different plant species.

Among LAB, only *Lactobacillus brevis,* a bacterium common in beer and fruit juices spoilage contaminants, showed a slight sensitivity to *M. pulegium* and *M. requienii* [36-37], suggestingthe use of EOs could be an alternative to the use of chemicals against these microorganisms. Our results on the antimicrobial activity against pathogens differ from the results obtained by Abdelli et al. [38] with *M. pulegium*. These authors found for *S. aureus* and for *E. feacalis* a MIC of 1.25 and 10 µL mL^{-1}, lower than the high concentration value (15 µL) found in this work. These differences could be due to the variation of chemical composition of EOs of *M. pulegium* harvested in different regions or to the sensitivity of strains tested. Differences in the antimicrobial activity have also been observed for *M. aquatica* EO, contrasting with our results: Dhifi et al. [34] and Golestan et al. [8] found an antimicrobial activity against *S. aureus*. It has been suggested that the biological activity of water mint (*M. aquatica*) EO correlates with its richness in oxygenated monoterpenes like menthofurane; the absence of this compound in the EO of *M. aquatica* analyzed in our study might explain the absence of antimicrobial activity.

The yeast strains analyzed were more sensitive than bacteria to the *Mentha* EOs. The sensitivity to the *Mentha* EOs was species and strain dependent, as can be seen for the different behavior of

the two strains of *C. albicans*, with respect to the effect of *M. pulegium* and *M. requenii.* These results are in agreement with Bona et al. [39], who it was found that mint has a more efficient anti-*Candida* action than clotrimazole. Finally, *M. aquatica* did not show any inhibitory effect against the Candida *spp.* yeast, with the exception of *C. krusei,* as already noted [34].

Our results for *M. pulegium* EO are in agreement with those obtained by Brahmi et al. [24] on *M. pulegium* cultivated in Algeria. Conversely, opposite effects of *M. pulegium* EO were observed by other authors [22, 40]. As suggested earlier, such differences could be due to different plant origins causing different EO chemical compositions. Indeed, in comparison with our results, both Teixeira et al. [22] and Hajlaoui [40] founded an EO of *M. pulegium* with lower concentration of pulegone and higher concentration of menthone and isomenthone.

CONCLUSION

M. requienii, a poorly studied variety of *mentha* endemic to Sardinia, was subjected to steam distillation for the extraction of the EO. A comparison with the EOs of two other mint species, namely *M. pulegium* and *M. aquatica*, collected in Sardinia, was carried out and the chemical composition of the EOs studied by qualitative GC-MS analysis and quantitative GC-FID analysis. The results confirm that the *M. requenii* has a pulegone rich EO and that it shows several similarities with EO extracted from Sardinian *M. pulegium,* indeed both EOs are dominated by pulegone. Conversely the EO from *M. aquatica* collected in Sardinia is characterized by high quantities of terpinyl acetate. The antioxidant activity of the EOs was evaluated by DPPH and ABTS methods: overall the antioxidant activity of *M. aquatica* EO is

higher than those of *M. pulegium* and *M. requienii*. Mint EOs did not show, in the concentration range tested, any antibacterial activity against *Staphylococcus aureus* 20231 DSMZ*, Salmonella enterica* 13772 DSMZ and the four strains of *Listeria monocytogenes* tested. LAB strains were not inhibited, with the exception of *Lactobacillus brevis,* which showed slight sensitivity to *M. pulegium* and *M. requenii* EOs. Yeast strains were in general more sensitive than bacteria to the different *mint* EOs tested. The mint EOs tested showed good antifungal and antioxidant activity and could be potential alternatives to chemical additives in pharmaceutical and food industries.

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Figure 1 Caption: Principal component analysis of the components of essential oils from *Mentha aquatica, Mentha pulegium* and *Mentha requienii*

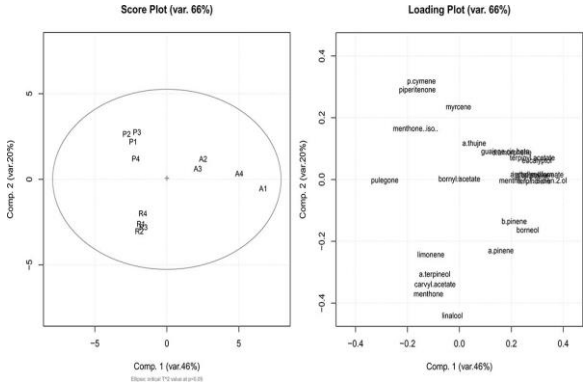

Table 1 chemical composition of essential oils from mentha species. compounds are listed according crescent elution time in an HP5 column. results are expressed as mg per mL of essential oil. RI: retention index on an HP5 column, ID: identification method, STD: comparison of MS fragmentation pattern, retention index and retention time pure standard with those of target analyte

	aquatic	pulegium	requienii		
	mg/mL	mg/mL	mg/mL	RI	ID
α-thujene	5.8	3.7	3.0	929	MS, RI
α-pinene	5.7	0.4	1.4	943	MS,RI,STD
α-phellandrene	8.9	0.6	0.4	969	MS,RI,STD
β-pinene	9.1	2.5	3.5	971	MS,RI,STD
myrcene	3.0	0.4	0.5	989	MS,RI,STD
p-cymene	nd	0.5	nd	1022	MS,RI,STD
limonene	nd	5.2	12.6	1025	MS,RI,STD
eucalyptol	86.4	0.8	nd	1030	MS,RI,STD

γ-terpinene	1.1	nd	nd	1057	MS,RI,STD
terpinolene	2.5	nd	nd	1086	MS, RI
linalool	3.1	nd	6.4	1100	MS,RI,STD
menthone	nd	10.1	66.5	1153	MS,RI,STD
menthone	nd	34.8	4.9	1162	MS, RI
borneol	15.3	nd	3.7	1164	MS,RI,STD
mentha-1(7),8-dien-2-ol	19.2	nd	nd	1191	MS, RI
α-terpineol	nd	nd	1.8	1193	MS,RI,STD
isobornyl formate	4.8	nd	nd	1239	MS, RI
pulegone	nd	668.7	559.2	1246	MS, RI
bornyl acetate	nd	nd	nd	1274	MS,RI,STD

piperitenone	nd	9.2	1.3	1343	MS, RI
terpinyl acetate	560.2	nd	0.9	1360	MS,RI,STD
carvyl acetate	nd	nd	0.9	1363	MS, RI
guaiene cis-β	1.0	nd	nd	1496	MS, RI
δ-amorphene	1.6	nd	nd	1517	MS, RI

Table 2. List of microorganisms, medium and culture condition used in this work for testing the antimicrobial activity of mint essential oils.

Tested Organisms	**Source**	**Medium**	**Temperature and Time of incubation**
Bacteria			
Lb brevis 20054	DSMZ	MRS	37°C x 24 h
Lb plantarum 8014	ATCC	MRS	"
Lb plantarum ICE5	DAFES	MRS	"
Lb paracasei subsp. *paracasei* 5622	DSMZ	MRS	"
Lb rhamnosus 7469	ATCC	MRS	"
E. faecalis 20478 DSMZ	DSMZ	MRS	"
E.faecium 20477 DSMZ	DSMZ	MRS	"
Staphylococcus aureus 20231 DSMZ	DSMZ	BHI	"

Listeria monocytogenes B	DAFES	BHI	“
Listeria monocytogenes E	DAFES	BHI	“
Listeria monocytogenes C	DAFES	BHI	“
Listeria monocytogenes 20600	DSMZ	BHI	“
Salmonella enterica subsp. bongori serovar 66:z41:- 13772 DSMZ	DSMZ	BHI	“
Yeast			
Candida albicans 3248	DAFES	YPD	37°CX24 h
Candida albicans 3993	DAFES	YPD	“
Candida bracarensis J6	CBS	YPD	“
Candida kruseii J8	CBS	YPD	“

Saccharomyces cerevisiae EC1118	Commercial strains	YPD	30°CX24 h

MRS: de Man, Rogosa and Sharpe; BHI: Brain Earth Infusion; ATCC, American Type Culture Collection; DSMZ, Deutsche SammLung von Mikroorganismen und Zellkulturen, German Collection of Microorganism of Cell Cultures; CBS, Centraalbureau voor Schimmelcultures Fungal Biodiversity Centre, Utrecht, The Netherlands; DAFES, Collection of Microorganisms of Dipartimento di Agraria of the University of Sassari, Section of Food and Environmental Science.

Table 3. Scavenging of 50% of DPPH and ABTS radical by Trolox and EOs of *Mentha requienii* Bentham, *Mentha pulegium* and *Mentha acquatica* at different time points.

	DPPH IC_{50}		**ABTS IC_{50}**	
	0 min	**30 min**	**0 min**	**50 min**
Trolox (µg/mL)	7.93	5.26	3.41	3.23
***Mentha requienii* Bentham** (mg/mL)	70.07	22.54	10.77	0.31
(mg/mL) ***Mentha pulegium***	182.90	19.82	24.55	0.43
Mentha acquatica (mg/mL)	45.63	16.02	7.38	0.28

Table 4. Minimum inhibitory concentrations (MIC, µL/mL) of the *Mentha* spp. essential oils against the tested microorganisms.

Tested Organisms			
Bacteria	*M. pulegium*	*M. requeni*	*M.acquatica*
Lb brevis 20054 DSMZ	3.75	7.5	>15
Lb plantarum 8014 ATCC	>15	>15	>15
Lb plantarum ICE5	>15	>15	>15
Lb paracasei subsp. *paracasei* 5622 DSMZ	>15	>15	>15
Lb rhamnosus 7469 ATCC	>15	>15	>15
Staphylococcus aureus 20231 DSMZ	>15	>15	>15
E. faecalis 20478 DSMZ	>15	>15	>15
E.faecium 20477 DSMZ	>15	>15	>15
Listeria monocytogenes B	>15	>15	>15
Listeria monocytogenes E	>15	>15	>15

Listeria monocytogenes C	>15	>15	>15
Listeria monocytogenes 20600 DSMZ	>15	>15	>15
Salmonella enterica subsp. bongori 13772 DSMZ	>15	>15	>15
Yeast			
Candida albicans 3248	0.94	0.94	>15
Candida albicans 3993	3.75	3.75	>15
Candida kruseii J8	0.94	3.75	3.75
Candida bracarensis J6	3,75	7.50	>15
Saccharomyces cerevisiae EC1118	0.94	3.75	>15