Falcó, I.; Verdeguer Sancho, MM.; Aznar, R.; Sánchez, G.; Randazzo, W. (14-0). Sanitizing food contact surfaces by the use of essential oils. Innovative Food Science and Emerging Techonologies. Innovative Food Science & Emerging Technologies. 51:220-228. https://doi.org/10.1016/j.ifset.2018.02.013

Sanitizing food contact surfaces by the use of essential oils

I. Falcó[1,2], M. Verdeguer[3], R. Aznar[1,2], G. Sánchez[1,2], W. Randazzo[1,2 *]

[1]*Department of Preservation and Food Safety Technologies, (IATA-CSIC). Av. Agustín Escardino 7. 46980 Paterna. Valencia, Spain;*

[2]*Departament of Microbiology and Ecology, University of Valencia. Av. Dr. Moliner, 50. 46100 Burjassot. Valencia. Spain;*

[3]*Instituto Agroforestal Mediterráneo, Universitat Politecnica de València, Camino de Vera, s/n 46022, Valencia, Spain.*

[*]Corresponding author: Walter Randazzo.

Carrer del Catedràtic Agustín Escardino Benlloch, 7, 46980 Paterna, Valencia. Spain.

Tel.: + 34 96 3900022; Fax: + 34 96 3939301; E-mail: walran@uv.es

ABSTRACT

Chemical sanitizers continue to be widely used by the food industry to disinfect food contact surfaces. However, as some chemical disinfectants have been reported to produce unhealthy by-products, alternative and natural compounds need to be investigated. To this end, nine essential oils (EOs) were screened to develop a natural sanitizing solution (SAN) for disinfecting food contact surfaces. Once extracted, their antimicrobial activity and chemical composition were determined. An exploratory multivariate approach was used to investigate the relationships between the chemical and microbiological data sets. Among the tested EOs, *Thymbra capitata* EO, containing up to 93.31% oxygenated monoterpenes (mainly carvacrol), showed the strongest antimicrobial activity and thus was assayed as a potential SAN for food contact surfaces. To this end, a SAN consisting of 1% *T. capitata* EO was first validated according to the AOAC standard, which showed about an 8 log reduction for *Escherichia coli* and *Salmonella enterica* after 30 and 60 seconds of contact time, respectively. Then, the SAN was evaluated at various concentrations, cleanliness conditions, and contact times on stainless steel, glass, and polypropylene surfaces for sanitizing purposes. The results showed that the SAN containing 2.5% of *T. capitata* EO applied for 10 min, reduced the levels of *E. coli* by more than 3 log and *S. enterica* by 1 log under clean working conditions on the three tested surfaces. These findings indicate that EOs can be used as natural disinfectants to decontaminate food contact surfaces, thus lowering the risk of the indirect transfer of bacterial pathogens to food or persons.

Keywords: Essential oils; Natural sanitizers; Foodborne pathogens; Food contact surfaces; Food safety.

1. Introduction

Microbial safety of food products is a key concern of consumers, the food industry, and regulatory bodies. Thus, different guidelines have been proposed to limit and control the occurrence of pathogens in food products (Codex alimentarius, 2007), and they agree that these risks can be reduced through safe food preparation, consumption, and storage practices by increasing hygienic measures along the entire food chain. On top of that, diarrheal diseases caused by bacteria are one of the most common illnesses resulting from the consumption of contaminated food (World Health Organization, 2014).

In the European Union, *Salmonella* and Shiga toxin-producing *Escherichia coli* have been identified as the first and seventh most common causes of foodborne illness outbreaks, respectively (EFSA, 2016). Moreover, *Salmonella* and *E. coli* are considered safety/hygiene indicators because their presence in food and water is due to fecal contamination and\or inadequate hygiene practices (Ceuppens et al., 2015).

The role that contaminated surfaces play in spreading pathogenic bacteria such as *Salmonella* and *E. coli* to foods is already well established in food processing, catering, and domestic environments such as chopping boards, knives, processing machines, tanks, and vats that can act as reservoirs and/or vehicles of pathogens. Food contact surfaces and equipment are commonly made by different materials such as stainless steel, glass and polypropylene plastic that can divergently play in harboring pathogens (Chia, Goulter, McMeekin, Dykes, & Fegan, 2009; Duffy, O'Callaghan, McAuley, Fegan, & Craven, 2009).

In the food industry, to reduce the spread of bacteria through contaminated surfaces, chemicals are routinely used to sanitize and disinfect food contact surfaces (Phillips, 2016; Simões, Simões, & Vieira, 2010). However, some of these chemicals (e.g. chlorine compounds, peroxide and peroxyacid mixtures, carboxylic acids, quaternary ammonium compounds, acid anionic, and iodine compounds) may generate the formation of by-products (e.g. trihalomethanes, haloacetic acids, and other potentially carcinogenic compounds), or contribute to the development of antibiotic resistance

in bacteria (e.g. triclosan) (Coroneo et al., 2017; Davidson & Harrison, 2002; Doyle, 2006; Halden, 2014; Marques et al., 2007; Xue et al., 2017). Alternative antimicrobial compounds would, therefore, be beneficial, especially for the development of natural sanitizers. In recent years, because of increased consumer awareness and concern regarding synthetic chemical additives or sanitizers, foods and food-contact surfaces treated with EOs or their main active compounds have become very popular since they are safer for humans and environmentally-friendly (S. Burt, 2004). Moreover, many of them show antimicrobial, antifungal, and virucidal properties, and thus represent potential 'natural' alternatives to chemical preservatives in the food industry (S. Burt, 2004; da Cruz Cabral, Fernández Pinto, & Patriarca, 2013; Sánchez & Aznar, 2015).

The selection and standardization of EOs is a critical task because many factors (e.g. plant material, ecological conditions, and extraction method) affect their chemical composition and, consequently, their biological and antimicrobial properties (S. Burt, 2004; Settanni et al., 2014).

Some EOs such as *Citrus* spp. (Fisher & Phillips, 2008), cinnamon (Van Haute, Raes, Devlieghere, & Sampers, 2017), oregano, and thyme (Yemiş & Candoğan, 2017) have been used as natural antimicrobials in food application, while uncommon, plant-derived EOs have received limited attention. So far, some well characterized EOs or their main active compounds have been directly applied as flavoring agents in food, used in washing solutions for vegetables, or incorporated in packaging materials to control foodborne pathogens (Irkin & Esmer, 2015). Furthermore, the application of well-characterized EOs to sanitize food contact surfaces has also been investigated (Giaouris et al., 2014; Rhoades et al., 2013; Valeriano et al., 2012)

Thus, this study aims to (i) collect, extract, and chemically characterize EOs from little-known plants; (ii) screen their antimicrobial activity against the common foodborne pathogens *S. enterica* and *E. coli*; and (iii) develop a natural EO-based sanitizer and evaluate its antibacterial activity on stainless steel, glass, and polypropylene surfaces.

2. Materials and methods

2.1. Plant material and extraction of EOs and aqueous extracts

Aerial parts (leaves and/or sprigs) from *Eriocephalus africanus* L. (EO1), *Artemisia absinthium* L. (EO2), *Santolina chamaecyparissus* L. (EO3), *Mentha longifolia* (L) L. (EO4), *Thymbra capitata* (L.) Cav. (EO6), *Citrus limon* (L.) Osbeck (EO7), *Citrus reticulata Blanco* (EO8) and *Eucalyptus camaldulensis Dehnh* (EO9) were collected in different areas of Spain to obtain their EOs (Table 1S). *Pelargonium odoratissimum* (L.) L'Hér. EO (EO5) was purchased from Titolchimica (Italy).

After collection, fresh plant material was immediately subjected to hydro-distillation for 3 h using a Clevenger-type apparatus (European Pharmacopoeia Commission, 2004; ISO 9235:2013; Zuzarte & Salgueiro, 2015). In particular, raw material was weighted (150-200 grams, depending of the volume occupied) and transferred to 2 l round flasks in which 1 litter of distilled water was added. Heat was applied by heating mantles (Selecta, Spain) and the process was maintained at least for 3 h, until no more oil was obtained. The oil was collected carefully and anhydrous sodium sulfate was used to remove residual water. EOs were stored at 4°C in air-tight sealed glass vials covered with aluminum foil until use. The yield (v/w) was calculated as volume of oil (ml) obtained from 100 g of plant material (Table 1S).

2.2. Chemical characterization of EOs

The quantification of the samples was performed by gas chromatography (GC) using a Clarus 500GC Perkin–Elmer apparatus equipped with a flame ionization detector (FID), and capillary column ZB-5 (30 m × 0.25 mm i.d. × 0.25 μm film thickness). The injection volume was 1 μl. The GC oven temperature was set at 60ºC for 5 min, with 3ºC increases per min to 180ºC, then 20ºC increases per min to 280ºC which was maintained for 10 min. Helium was the carrier gas (1.2 ml/min). Injector and detector temperatures were set at 250°C. The percentage composition of the EO was computed from GC peak areas without correction factors by means of the software Total Chrom 6.2 (Perkin-Elmer Inc., Wellesley, PA, USA).

For the identification of the compounds, gas chromatography coupled to mass spectrometry (GC-MS) was used (Adams, 2007) using a Clarus 500 GC-MS from Perkin-Elmer Inc., equipped with

the same column, carrier and operating conditions as described above for GC analysis. Ionization source temperature was set at 200°C and 70 eV electron impact mode was employed. MS spectra were obtained by means of total ion scan (TIC) mode (mass range m/z 45-500 uma). The total ion chromatograms and mass spectra were processed with the Turbomass 5.4 software (Perkin-Elmer Inc.). Retention indexes were determined by injection of C8–C25 n-alkanes standard (Supelco) under the same conditions. The EO components were identified by comparison of their mass spectra with those of computer library NIST MS Search 2.0 and available data in the literature. Identification of the following compounds was confirmed by comparison of their experimental RI with those of authentic reference standards (Sigma-Aldrich): α-pinene, β-pinene, camphene, myrcene, limonene, camphor, terpinolene, β-thujone, borneol, terpinen-4-ol, bornyl acetate, geraniol and linalool.

2.3. Screening for antimicrobial activity and minimum inhibitory concentration determination

The reference strains *E. coli* O157:H7 CECT 5947 (non-toxigenic) and *S. enterica subsp. enterica* CECT 4138 supplied by the Spanish Type Culture Collection (CECT) were used to test the antibacterial activity of nine EOs. Firstly, paper disc diffusion assay (PDDA) was used as rapid screening method (Balouiri, Sadiki, & Ibnsouda, 2016; Settanni et al., 2014). Briefly, bacterial cells were grown overnight at 37°C on tryptic soy broth (TSB), the concentration adjusted to 7 log CFU/ml and seed on tryptic soy agar (TSA) using a cotton swab. Once dried, sterile paper discs (Sigma-Aldrich) were placed on the plate surface. Each disk was soaked with 10 µl of each undiluted EO. Sterile water and streptomycin (10%, w/v) were used as negative and positive control, respectively. Each test was performed in duplicate and the experiments were repeated twice. Additionally, the minimum inhibitory concentration (MIC) was determined. For that, bacterial cultures of ca. 6 log CFU/ml were exposed to increasing EO concentrations (0, 0.025, 0.05, 0.1, 0.5 and 1%) and incubated overnight at 37°C. Growth inhibition was evaluated after 4 and 24h of incubation by plate count on TSA.

2.4. Evaluation of the EO-based sanitizer following AOAC 960.09 and EN 13697:2015 standards

Based on preliminary antimicrobial assays (PDDA and MIC), a sanitizer solution (SAN) was prepared using EO6 and ethanol mixed in a ratio 1:1. SAN was freshly prepared immediately before each assay. Initially, the SAN was evaluated following the AOAC 960.09 standard method "Germicidal and detergent sanitizing action of disinfectants". Briefly, 9.90 ml solution of 2 % SAN prepared in synthetic hard water of 400 ppm $CaCO_3$ (AOAC 960.09) was inoculated with 0.1 ml of bacterial inoculum, resulting in a final concentration of ca. 8 log CFU/ml, and incubated for 30 and 60 seconds at room temperature (RT). Then, serial dilutions were performed using peptone water (PW) as neutralizer (previously validated according to the method) and colony forming units (CFU) enumerated on TSA after 24 h at 37ºC.

2.5. Surface disinfection tests

Surface disinfection tests were performed using the EN 13697:2015 standard "Chemical disinfectants and antiseptics. Quantitative non-porous surface test for the evaluation of bactericidal and/or fungicidal activity of chemical disinfectants used in food, industrial, domestic and institutional areas. Test method and requirements without mechanical action". The bactericidal activity of SAN was evaluated on stainless steel, glass and polypropylene discs. Discs (2 x 2 cm) were sterilized with 70% (V/V) of isopropanol for 15 min before each assay. Briefly, *E. coli* and *S. enterica* suspensions were diluted (ratio 1:1) with 0.3 and 3 g/l bovine serum albumin (BSA) to mimic clean and dirty working conditions (as in EN 13697:2015). Then 50 µl of resulting inocula (ca. 6 log CFU/ml) were spotted into sterile discs and dried at RT for 15 min. Afterward, 100 µl of 0.5, 1 and 5% SAN prepared on hard water as diluent according to EN 13697:2015, were spotted on the inoculated discs, followed by incubation at RT for 1, 5 and 10 min. Then, the effect of the SAN was stopped by transferring the discs into a flask with 10 ml of peptone water as neutralizer and 5 g of glass beads. After 1 min in a shaker at 240 rpm (VWR, The Netherlands), bacterial cells were enumerated as described above. Positive controls were performed using discs treated with hard water contained the same ethanol concentration as applied for SAN.

2.6. *Statistical and explorative multivariate analyses*

Data obtained from chemical characterization and antimicrobial activities of EOs were analyzed using an explorative multivariate analysis, including a hierarchical cluster analysis (HCA) and a principal component analysis (PCA). Firstly, HCA was carried out for grouping EOs samples measured by Euclidean distances; whereas cluster aggregation was based on the single linkage method (Todeschini, 1998). The input matrix used for HCA consisted of chemical compounds and MIC for both *E. coli* and *S. enterica.* The PCA explored the input matrix based on the 9 EOs introduced as cases and the normalized average data of 178 chemical compounds grouped according to their chemical classes and MIC for both *E. coli* and *S. enterica* considered as explanatory variables, preliminary evaluated by using the Barlett's sphericity test (Alfonzo et al., 2017; Bautista Gallego et al., 2011). Eigenvalues were calculated and score and loading plots including both EOs samples and GC-MS constituents were generated (Torregiani et al., 2017). The analysis of variance (ANOVA), followed by a pairwise comparison with the post-hoc Tukey's test, was applied to identify significant differences for SAN efficacies (Figures 3 and 4) with a statistical significance attributed to p values <0.05. All statistical data processing and graphic constructions were performed using STATISTICA software version 7 (StatSoft Inc., Tulsa, OK, USA).

3. Results and discussion

3.1. *Extraction and GC/GC-MS characterization of EOs*

The EOs' extraction yields are reported in Table 1S and ranged between 0.22 for EO2 and 3.00% (v/w) for EO6. Similar extraction yields have already been reported for *E. africanus* (0.43% v/w), *E. camaldulensis* (0.71% v/w) and *T. capitata* (2.1-5.6% v/w) (Bounatirou et al., 2007; Verdeguer, Blázquez, & Boira, 2009). The main chemical compounds constituting more than 10% of the total composition determined by GC/GC-MS for each of the nine EOs are reported in Table 1 whereas the complete composition is reported in Table 2S. A high percentage of compounds were identified for all EOs (92.69 - 99.20%), and they are grouped into different chemical classes as monoterpene hydrocarbons (MH, ranging from 2.05 to 64.47%), oxygenated monoterpenes (OM, from 28.82 to

93.3%), sesquiterpene hydrocarbons (SH, from 0 to 5.05%), oxygenated sesquiterpenes (OS, from 0 to 21.82%) and esters (EST, from 0 to 0.83%).

EO1 was mainly characterized by artemisia ketone (57.54%) among MH and intermedeol (10.54%) among OS. For EO2, the OMs epoxy-ocimene <(E)-> (34.01%) and cis-chrysanthenyl acetate (28.35%) were the most abundant among a total of 28 compounds. The OM camphor (31.43%), 1,8-cineole (11.74%), terpinen-4-ol (8.64%), and the OS β-copaen-4-α-ol (10.11%) were the main compounds in EO3. EO4 was mainly characterized by the OM α-terpineol acetate (32.59%), pulegone (14.15%), carvone acetate (10.29%), and isomenthone (9.16%). Citronellol (20.40%), α-terpineol (12.60%) and geraniol (12.30%) were the main compounds of the EO5. EO6 showed 23 different compounds (99.31%), with a slight amount of MH (3.51%) and a high percentage of OM (93.06%). Among OM, it is worth noting that carvacrol contributed to a significant percentage of the EO composition (91.56%), while only 0.03% of thymol was detected. This high carvacrol level distinguishes this species from others of the *Thymus* genre (e.g. *Thymus vulgaris)*, which are characterized by high levels of thymol, another OM showing antimicrobial activity (S. Burt, 2004). EO7 showed limonene (30.14%) and β-pinene (17.28%), both MHs, together with geranial (11.91%), an OM, as its main compounds. EO8 was characterized by sabinene (34.41%) and linalool (21.27%). EO9 exhibited a total of 40 compounds; p-cymene (28.34%), cryptone (14.12%), and spathulenol (17.99%) were the most abundant.

Comparing the EOs' chemical compositions, the types of compounds and their concentrations showed wide variability due to the botanical diversity of the plant material used for EO extraction. Thus, plant material deeply influences the final EO constituents, their relative concentrations (S. Burt, 2004; Chang, Chen, & Chang, 2001), and, finally, the EO antibacterial activities.

3.2. *Antimicrobial activity of EOs*

The antimicrobial activity of the nine EOs against *E. coli* and *S. enterica* is shown in Table 2. Both PDDA, and MIC determinations identified EO6 as the most effective; it had the widest inhibitory

haloes (2.75 and 2.47 cm for *E. coli* and *S. enterica*, respectively) and inhibited the growth of both tested strains at the lowest concentration (MIC of 0.05% v/v). Considering its chemical composition, it could be inferred that carvacrol (comprising 91.56% of the 99.31% identified compounds) was directly responsible for the antimicrobial effect. This finding is not surprising since the antimicrobial activity of carvacrol has already been reported against several foodborne pathogens (Friedman, 2014; Nostro & Papalia, 2012), and resistant isolates (Memar, Raei, Alizadeh, Aghdam, & Kafil, 2017). In addition, similar MIC values (0.025-0.03%) have been reported for pure carvacrol against *S. Typhimurium* (Kamlesh et al., 2013) and *S. enterica* (Engel, Heckler, Tondo, Daroit, & da Silva Malheiros, 2017).

In line with these results, the poor antibacterial activity of *E. africanus* (EO1), *A. absinthium* (EO2), and *M. longifolia* (EO4) have already been reported (Anwar, Alkharfy, Najeeb-ur-Rehman, Adam, & Gilani, 2017; Mkaddem et al., 2009; Riahi et al., 2015; Salie, Eagles, & Leng, 1996). EO3, extracted from *S. chamaecyparissus*, showed an MIC of 0.5%, a higher value with respect to the 0.0001% v/w reported for *E. coli* by Bel Hadj Salah-Fatnassi et al. (2017). In contrast, EO5, extracted from *P. Odoratissimum*, showed MIC values of 1% for both strains, indicating only moderate activity, while poor antimicrobial activity has been previously reported (Andrade, Cardoso, Batista, Freire, & Nelson, 2011; Lis-Balchin & Roth, 2000).

Compared to previous research, poor antibacterial activity (MIC≥0.5%, v/v) was found for *Citrus* EOs (EO7 and EO8) (Fisher & Phillips, 2008; Randazzo, Jiménez-Belenguer, et al., 2016; Settanni et al., 2014). These discrepancies can be explained by several factors, such as intrinsic factors of the plants (e.g. genotype and, part of the plant harvested, such as leaves vs peel), harvest time, geographical and ecological conditions, extraction method, and the method for antimicrobial determination, including the types of bacterial strains tested (S. Burt, 2004; Randazzo, Jiménez-Belenguer, et al., 2016). In addition, the structural characteristics of the EOs' active compounds (i.e. aliphatic ring, hydroxyl group) may change depending on the extraction procedure applied and/or

storage time, resulting in a different level of antimicrobial activity, such as that reported for carvacrol (Veldhuizen, Tjeerdsma-Van Bokhoven, Zweijtzer, Burt, & Haagsman, 2006).

In case of EO9, a MIC of 0.5% v/v was recorded against *E. coli* according to Nasir, Tafess, and Abate (2015), while Sliti et al. (2015) reported higher values (1.5% v/v for *E. coli* and 1.0% for *S. enteritidis*).

3.3. Explorative multivariate analysis of chemical composition and antibacterial activities

Since HCA gathers cases according to their overall similarity and PCA plots cases and variables together to provide information on their correlation, the two methods are complementary in their ability to present and discuss chemical and microbiological results (Alfonzo et al., 2017; Bendiabdellah et al., 2014; Randazzo, Guarcello, et al., 2016).

HCA mainly classified the EOs into two mega-clusters at around 95% of their mutual dissimilarity (Fig. 1); EO6 was clustered separately from the remaining EOs. In this last group, the EOs shared 66% of dissimilarity with EO1 and only 54% among themselves. In general, the high linkage distance among the cases (>46%) reflects the high complexity of the EOs's chemical composition and antimicrobial activity, which were used as variables for the HCA analysis.

Regarding PCA, EO1 and EO2 were not included in the analysis due to their negligible antimicrobial activity (lowest PDDA values). Four Factors displayed eigenvalues higher than 1, explaining 95.32% of the total variance (Table 3S). In particular, the scatterplots represent the relationship between the three main Factors and EOs (score plot, Fig. 2A), and, between the three main Factors and variables (loading plot, Fig. 2B), accounting for 82.59% of the total variance. Factor 1 represents 33.69% of the total variance and it is positively correlated with OM and negatively correlated with MH, OS and MIC (Fig. 2B and Table 4S). Factor 3 (22.64%) is positively correlated with OM, OS, and MIC variables for both *E. coli* and *S. enterica*; it is the Factor most correlated to the EOs' antimicrobial traits. Similarly, MH, EST, and OTH correlated

negatively with Factor 3. Interestingly, EO6 showed the highest correlation value with Factor 3 (associated with antimicrobial traits, Tab. 5S).

In summary, the discrimination of EOs based on the scatterplots highlighted differences among the samples that resulted in widely spaced points (Fig. 2A). The PCA indicated a high correlation among antimicrobial traits (MIC) and oxygenated compounds, like OM and OS as previously reported for *Citrus* EOs (Randazzo, Jiménez-Belenguer, et al., 2016; Settanni et al., 2014).

3.4. Evaluation of the antibacterial activity of the EO-based sanitizer

According to the antibacterial results, EO6 was chosen to be prepared into a SAN solution to be evaluated as food contact surface sanitizer according to official methods. The SAN's efficacy was tested according to AOAC 960.09 and is reported in Table 3. In this case, the SAN containing 1% of EO6 was highly effective, inhibiting aproximately 8 log CFU/ml of *E. coli* and *S. enterica* after 30 and 60 seconds of contact time, respectively. According to method validation, a 99.999% (5 log CFU/ml) reduction was achieved for both strains within 30 seconds. Consequently, the developed SAN passed the validation recommended by the AOAC method.

Studies evaluating EOs for bacterial inhibition within food service environments remain somewhat limited (Phillips, 2016; Simões et al., 2010). Therefore, this SAN was further evaluated at various concentrations, cleanness conditions, contact times, and on different material surfaces commonly employed in food industries (Figure 3, Figure 4 and Table 6S).

As expected, the SAN's inhibitions were higher when tested at higher percentages (0.5<1<5 %) and for longer contact time (1<5<10 min) as reported by Messager, Hammer, Carson, and Riley (2005) for tea tree oil. The SAN was also tested on simulated clean and dirty surfaces (by preparing bacterial inocula in 0.3 and 3.0 g/l BSA, respectively, as in ISO 13697:2015). Figures 3 and 4 show titers of recovered *E. coli* and *S. enterica* on stainless steel, glass and polypropylene surfaces before, and after 1, 5, and 10 min treatment with a 5% SAN solution.

Titers of control samples were 5.75 ± 0.14 and 5.63 ± 0.25 log CFU/ml for *E. coli* and *S. enterica*, respectively. On clean stainless steel, the 5% SAN solution reduced *E. coli* counts by 1.38, 2.72,

and 3.60 1og after 1, 5, and 10 min of exposure, respectively, while for *S. enterica* reductions of 0.32, 0.50 and 1.13 log were recorded. On clean glass, 0.77, 1.99 and 3.01 log reductions were recorded for *E. coli* treated with the 5% SAN solution after 1, 5, and 10 min, respectively, and *S. enterica* was reduced by 0.33, 0.43, and 1.13 log. On clean polypropylene, 5% SAN reduced 0.94, 2.59 and 3.46 log *E. coli* and 0.23, 0.43 and 1.03 log *S. enterica* after 1, 5, and 10 min, respectively. Statistically significant inhibitions were reported for *S. enterica* after 10 min of contact with the 5% SAN solution under clean working conditions for the three material tested, with reductions of 1.03-1.13 log CFU/ml. Higher reductions have been reported by other authors when extending the time of contact. For instance, reductions of 3.71 to 7.41 log CFU/cm^2 were reported for *Salmonella* spp. biofilms on polypropylene treated for 1 h with 312 µg/ml (0.03%) of carvacrol (Amaral et al., 2015). Similarly, approximately 6 log CFU/cm^2 reductions were achieved for *Salmonella* spp attached on stainless steel after 10 min contact with 0.03% carvacrol (Engel et al., 2017).

Generally, Gram-negative bacteria are more resistant than Gram-positive bacteria to EOs (Nazzaro, Fratianni, De Martino, Coppola, & De Feo, 2013), and, among Gram-negative bacteria, *E. coli* usually reported as more sensitive than *Salmonella* spp. (Semeniuc, Pop, & Rotar, 2017). The SAN's limited activity against *Salmonella* could be explained by the EO's effect on some outer membrane proteins involved in the formation of an efflux system (e.g. TolC), that may be up-regulated by the EO and constitute a final mechanism of resistance, as observed for thymol (Baucheron, Mouline, Praud, Chaslus-Dancla, & Cloeckaert, 2005).

In general, the results showed more effectiveness on clean surfaces than on dirty ones, and significant differences ($p < 0.05$) were recorded among the different surface materials (Table 2S). Regarding the latter, the higher disinfectant efficacy of sanitizers on smooth (i.e. steel) rather than rough (i.e. plastic) surfaces has been previously reported (Lin, Sheu, Hsu, & Tsai, 2010).

On all clean surfaces tested, the 5% SAN solution was able to reduce *E. coli* counts by more than 3 log CFU/ml compared to the control (99.9%). In dirty conditions, the 5% SAN solution achieved lower reductions (2.65 log CFU/ml on plastic). The presence of organic matter also reduced the

effectiveness of chemical sanitizers, such as sodium hypochlorite (Kich et al., 2004; Souza & Daniel, 2005) or sodium dichloroisocyanurate (NaDCC) (Williams, Denyer, Hosein, Hill, & Maillard, 2009), because the higher amount of proteins in dirty conditions may protect bacteria cells from the disinfectant action, as previously reported (Hammer, Carson, and Riley (1999) and Messager et al. (2005)).

The 5% SAN solution was effective against both bacterial strains when applied for 10 min (Figures 3 and 4). These different inhibitions between the two bacteria could depend on the species and strain tested, since various authors have reported heterogeneous antibacterial effects depending on the bacterial species (S. Burt, 2004; Fisher & Phillips, 2008) and strain (Settanni et al., 2014). For all the experiments, the ethanol used as a control did not show any significant inhibitory effect.

T. capitata EO demonstrated antimicrobial properties to certain extent, therefore, SAN improvement should be evaluated for example by the addition of stabilizers (S. A. Burt, Vlielander, Haagsman, & Veldhuizen, 2005).

Conclusions

Considering the increasing resistance of bacteria to chemical compounds and sanitizers, searching for natural antibacterial products is becoming a priority.

This study demonstrated the antimicrobial activity of *T. capitata* EO, and, for the first time, its potential use as a natural sanitizing product.

The EO-based sanitizer was developed by applying official methods (AOAC 960.09 and ISO 13697:2015) and testing different concentrations (0.25, 0.5 and 2.5%), cleanness conditions (clean and dirt), contact times (1, 5 and 10 minutes), and on stainless steel, glass and polypropylene surfaces commonly employed in food industries. Finally, a natural sanitizer containing 2.5% of *T. capitata* EO was effective against *E. coli* (> 3 log redution in all three clean material tested), but had limited effect on *S. enteridis* when evaluated on different food contact surfaces, suggesting an

interesting potential of its application in real conditions even further improvements are needed to widen its efficacy against a wider range of bacterial pathogens.

Acknowledgments

This work was supported by the Spanish Ministry of Economy and Competitiveness (MINECO) (RYC-2012-09950) and the Spanish National Institute for Agriculture and Food Research and Technology (INIA) co-financed by the European Social Fund (Project RTA2014-00024-C03). GS was supported by the "Ramon y Cajal" Young Investigator.

References

Adams, R. P. (2007). *Identification of Essential Oil Components by Gas Chromatography/Mass Spectrometry*. Illinois, USA.: Allured Publishing Corporation.

Alfonzo, A., Randazzo, W., Barbera, M., Sannino, C., Corona, O., Settanni, L., . . . Francesca, N. (2017). Effect of Salt Concentration and Extremely Halophilic Archaea on the Safety and Quality Characteristics of Traditional Salted Anchovies. *Journal of Aquatic Food Product Technology*, 1-18. doi:10.1080/10498850.2016.1251521

Amaral, V. C. S., Santos, P. R., da Silva, A. F., dos Santos, A. R., Machinski, M., & Mikcha, J. M. G. (2015). Effect of carvacrol and thymol on Salmonella spp. biofilms on polypropylene. *International Journal of Food Science and Technology, 50*(12), 2639-2643. doi:10.1111/ijfs.12934

Andrade, M. A., Cardoso, M. G., Batista, L. R., Freire, J. M., & Nelson, D. L. (2011). Antimicrobial activity and chemical composition of essential oil of Pelargonium odoratissimum. *Revista Brasileira de Farmacognosia, 21*(1), 47-52.

Anwar, F., Alkharfy, K. M., Najeeb-ur-Rehman, Adam, E. H. K., & Gilani, A.-u.-H. (2017). Chemo-geographical Variations in the Composition of Volatiles and the Biological Attributes of Mentha longifolia (L.) Essential Oils from Saudi Arabia. *INTERNATIONAL JOURNAL OF PHARMACOLOGY, 13*(5), 408-424.

Balouiri, M., Sadiki, M., & Ibnsouda, S. K. (2016). Methods for in vitro evaluating antimicrobial activity: A review. *Journal of Pharmaceutical Analysis, 6*(2), 71-79. doi:https://doi.org/10.1016/j.jpha.2015.11.005

Baucheron, S., Mouline, C., Praud, K., Chaslus-Dancla, E., & Cloeckaert, A. (2005). TolC but not AcrB is essential for multidrug-resistant Salmonella enterica serotype Typhimurium colonization of chicks. *Journal of Antimicrobial Chemotherapy, 55*(5), 707-712. doi:10.1093/jac/dki091

Bautista Gallego, J., Arroyo López, F. N., Romero Gil, V., Rodríguez Gómez, F., García García, P., & Garrido Fernández, A. (2011). Chloride salt mixtures affect Gordal cv. green Spanish-style table olive fermentation. *Food Microbiology, 28*(7), 1316-1325. doi:10.1016/j.fm.2011.06.002

Bel Hadj Salah-Fatnassi, K., Hassayoun, F., Cheraif, I., Khan, S., Jannet, H. B., Hammami, M., . . . Harzallah-Skhiri, F. (2017). Chemical composition, antibacterial and antifungal activities of flowerhead and root essential oils of Santolina chamaecyparissus L., growing wild in Tunisia. *Saudi Journal of Biological Sciences, 24*(4), 875-882. doi:10.1016/j.sjbs.2016.03.005

Bendiabdellah, A., Dib, M. E. A., Djabou, N., Hassani, F., Paolini, J., Tabti, B., . . . Muselli, A. (2014). Daucus carota ssp. hispanicus Gouan. essential oils: Chemical variability and fungitoxic activity. *Journal of Essential Oil Research, 26*(6), 427-440. doi:10.1080/10412905.2014.956189

Bounatirou, S., Smiti, S., Miguel, M. G., Faleiro, L., Rejeb, M. N., Neffati, M., . . . Pedro, L. G. (2007). Chemical composition, antioxidant and antibacterial activities of the essential oils isolated from Tunisian Thymus capitatus Hoff. et Link. *Food Chemistry, 105*(1), 146-155. doi:10.1016/j.foodchem.2007.03.059

Burt, S. (2004). Essential oils: their antibacterial properties and potential applications in foods—a review. *International journal of food microbiology, 94*(3), 223-253.

Burt, S. A., Vlielander, R., Haagsman, H. P., & Veldhuizen, E. J. A. (2005). Increase in activity of essential oil components carvacrol and thymol against Escherichia coli O157:H7 by addition of food stabilizers. *Journal of Food Protection, 68*(5), 919-926.

Ceuppens, S., Johannessen, G. S., Allende, A., Tondo, E. C., El-Tahan, F., Sampers, I., . . . Uyttendaele, M. (2015). Risk factors for Salmonella, shiga toxin-producing Escherichia coli and Campylobacter occurrence in primary production of leafy greens and strawberries. *International Journal of Environmental Research and Public Health, 12*(8), 9809-9831. doi:10.3390/ijerph120809809

Chang, S. T., Chen, P. F., & Chang, S. C. (2001). Antibacterial activity of leaf essential oils and their constituents from Cinnamomum osmophloeum. *Journal of Ethnopharmacology, 77*(1), 123-127. doi:10.1016/S0378-8741(01)00273-2

Chia, T. W. R., Goulter, R. M., McMeekin, T., Dykes, G. A., & Fegan, N. (2009). Attachment of different Salmonella serovars to materials commonly used in a poultry processing plant. *Food Microbiology, 26*(8), 853-859. doi:10.1016/j.fm.2009.05.012

Codex alimentarius. (2007). *Guidelines on the application of general principles of food hygiene to the control of Listeria monocytogenes in foods*. (CAC/GL 61 - 2007).

Coroneo, V., Carraro, V., Marras, B., Marrucci, A., Succa, S., Meloni, B., . . . Schintu, M. (2017). Presence of Trihalomethanes in ready-to-eat vegetables disinfected with chlorine. *Food Additives and Contaminants - Part A Chemistry, Analysis, Control, Exposure and Risk Assessment*, 1-7. doi:10.1080/19440049.2017.1382723

da Cruz Cabral, L., Fernández Pinto, V., & Patriarca, A. (2013). Application of plant derived compounds to control fungal spoilage and mycotoxin production in foods. *International journal of food microbiology, 166*(1), 1-14. doi:10.1016/j.ijfoodmicro.2013.05.026

Davidson, P. M., & Harrison, M. A. (2002). Resistance and adaptation to food antimicrobials, sanitizers, and other process controls. *Food Technology, 56*(11), 69-78.

Doyle, M. P. (2006). Dealing with antimicrobial resistance. *Food Technology, 60*(8), 22-29.

Duffy, L. L., O'Callaghan, D., McAuley, C. M., Fegan, N., & Craven, H. M. (2009). Virulence properties of Escherichia coli isolated from Australian dairy powder factory environments. *International Dairy Journal, 19*(3), 178-179. doi:10.1016/j.idairyj.2008.09.002

EFSA. (2016). The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2015. *EFSA Journal, 14*(12), 4634. doi:10.2903/j.efsa.2015.4329

EN 13697:2015. Chemical disinfectants and antiseptics. Quantitative non-porous surface test for the evaluation of bactericidal and/or fungicidal activity of chemical disinfectants used in food, industrial, domestic and institutional areas. Test method and requirements without mechanical action (phase 2, step 2). In.

Engel, J. B., Heckler, C., Tondo, E. C., Daroit, D. J., & da Silva Malheiros, P. (2017). Antimicrobial activity of free and liposome-encapsulated thymol and carvacrol against Salmonella and Staphylococcus aureus adhered to stainless steel. *International journal of food microbiology, 252*(Supplement C), 18-23. doi:https://doi.org/10.1016/j.ijfoodmicro.2017.04.003

European Pharmacopoeia Commission. (2004). *European Pharmacopoeia*: Council of Europe.

Fisher, K., & Phillips, C. (2008). Potential antimicrobial uses of essential oils in food: is citrus the answer? *Trends in food science & technology, 19*(3), 156-164.

Friedman, M. (2014). Chemistry and multibeneficial bioactivities of carvacrol (4-isopropyl-2-methylphenol), a component of essential oils produced by aromatic plants and spices. *Journal of Agricultural and Food Chemistry, 62*(31), 7652-7670. doi:10.1021/jf5023862

Giaouris, E., Heir, E., Hébraud, M., Chorianopoulos, N., Langsrud, S., Møretrø, T., . . . Nychas, G.-J. (2014). Attachment and biofilm formation by foodborne bacteria in meat processing environments: Causes, implications, role of bacterial interactions and control by alternative novel methods. *Meat Science, 97*(3), 298-309. doi:https://doi.org/10.1016/j.meatsci.2013.05.023

Halden, R. U. (2014). On the Need and Speed of Regulating Triclosan and Triclocarban in the United States. *Environmental Science & Technology, 48*(7), 3603-3611. doi:10.1021/es500495p

Hammer, K., Carson, C., & Riley, T. (1999). Influence of organic matter, cations and surfactants on the antimicrobial activity of Melaleuca alternifolia (tea tree) oil in vitro. *Journal of Applied Microbiology, 86*(3), 446-452.

Irkin, R., & Esmer, O. K. (2015). Novel food packaging systems with natural antimicrobial agents. *J Food Sci Technol, 52*(10), 6095-6111. doi:10.1007/s13197-015-1780-9

ISO 9235:2013. ISO 9235:2013 . Aromatic natural raw materials - Vocabulary. In. Geneva, Switzerland.

Kamlesh, S., Ademola, O., Ramakrishna, N., Wes, S., Juan, S., Benjy, M., & Hartford, B. (2013). Inhibition and Inactivation of Salmonella Typhimurium Biofilms from Polystyrene and Stainless Steel Surfaces by Essential Oils and Phenolic Constituent Carvacrol. *Journal of Food Protection, 76*(2), 205-212. doi:10.4315/0362-028x.jfp-12-196

Kich, J., Borowsky, L., Silva, V., Ramenzoni, M., Triques, N., Kooler, F., & Cardoso, M. (2004). Evaluation of the antibacterial activity of six commercial disinfectants against Salmonella typhimurium strains isolated from swine. *Acta Sci Vet, 32*, 33-39.

Lin, C.-M., Sheu, S.-R., Hsu, S.-C., & Tsai, Y.-H. (2010). Determination of bactericidal efficacy of essential oil extracted from orange peel on the food contact surfaces. *Food Control, 21*(12), 1710-1715.

Lis-Balchin, M., & Roth, G. (2000). Composition of the essential oils of Pelargonium odoratissimum, P. exstipulatum, and P.× fragrans (Geraniaceae) and their bioactivity. *Flavour and Fragrance Journal, 15*(6), 391-394.

Marques, S. C., Rezende, J. D. G. O. S., Alves, L. A. D. F., Silva, B. C., Alves, E., De Abreu, L. R., & Piccoli, R. H. (2007). Formation of biofilms by Staphylococcus aureus on stainless steel and glass

surfaces and its resistance to some selected chemical sanitizers. *Brazilian Journal of Microbiology, 38*(3), 538-543.

Memar, M. Y., Raei, P., Alizadeh, N., Aghdam, M. A., & Kafil, H. S. (2017). Carvacrol and thymol: Strong antimicrobial agents against resistant isolates. *Reviews in Medical Microbiology, 28*(2), 63-68. doi:10.1097/MRM.0000000000000100

Messager, S., Hammer, K., Carson, C., & Riley, T. (2005). Assessment of the antibacterial activity of tea tree oil using the European EN 1276 and EN 12054 standard suspension tests. *Journal of Hospital Infection, 59*(2), 113-125.

Mkaddem, M., Bouajila, J., Ennajar, M., Lebrihi, A., Mathieu, F., & Romdhane, M. (2009). Chemical composition and antimicrobial and antioxidant activities of mentha (longifolia L. and viridis) essential oils. *Journal of Food Science, 74*(7), M358-M363. doi:10.1111/j.1750-3841.2009.01272.x

Nasir, M., Tafess, K., & Abate, D. (2015). Antimicrobial potential of the Ethiopian Thymus schimperi essential oil in comparison with others against certain fungal and bacterial species. *BMC Complementary and Alternative Medicine, 15*(1). doi:10.1186/s12906-015-0784-3

Nazzaro, F., Fratianni, F., De Martino, L., Coppola, R., & De Feo, V. (2013). Effect of Essential Oils on Pathogenic Bacteria. *Pharmaceuticals, 6*(12), 1451-1474. doi:10.3390/ph6121451

Nostro, A., & Papalia, T. (2012). Antimicrobial activity of carvacrol: Current progress and future prospectives. *Recent Patents on Anti-Infective Drug Discovery, 7*(1), 28-35. doi:10.2174/157489112799829684

Phillips, C. A. (2016). Bacterial biofilms in food processing environments: a review of recent developments in chemical and biological control. *International Journal of Food Science & Technology, 51*(8), 1731-1743. doi:10.1111/ijfs.13159

Randazzo, W., Guarcello, R., Francesca, N., Germanà, M. A., Erten, H., Moschetti, G., & Settanni, L. (2016). Development of new non-dairy beverages from Mediterranean fruit juices fermented with water kefir microorganisms. *Food Microbiology, 54*, 40-51. doi:10.1016/j.fm.2015.10.018

Randazzo, W., Jiménez-Belenguer, A., Settanni, L., Perdones, A., Moschetti, M., Palazzolo, E., . . . Moschetti, G. (2016). Antilisterial effect of citrus essential oils and their performance in edible film formulations. *Food Control, 59*, 750-758.

Rhoades, J., Gialagkolidou, K., Gogou, M., Mavridou, O., Blatsiotis, N., Ritzoulis, C., & Likotrafiti, E. (2013). Oregano essential oil as an antimicrobial additive to detergent for hand washing and food contact surface cleaning. *Journal of Applied Microbiology, 115*(4), 987-994. doi:10.1111/jam.12302

Riahi, L., Ghazghazi, H., Ayari, B., Aouadhi, C., Klay, I., Chograni, H., . . . Zoghlami, N. (2015). Effect of environmental conditions on chemical polymorphism and biological activities among Artemisia absinthium L. essential oil provenances grown in Tunisia. *Industrial Crops and Products, 66*(1), 96-102. doi:10.1016/j.indcrop.2014.12.036

Salie, F., Eagles, P. F. K., & Leng, H. M. J. (1996). Preliminary antimicrobial screening of four South African Asteraceae species. *Journal of Ethnopharmacology, 52*(1), 27-33. doi:10.1016/0378-8741(96)01381-5

Sánchez, G., & Aznar, R. (2015). Evaluation of Natural Compounds of Plant Origin for Inactivation of Enteric Viruses. *Food and Environmental Virology, 7*(2), 183-187. doi:10.1007/s12560-015-9181-9

Semeniuc, C. A., Pop, C. R., & Rotar, A. M. (2017). Antibacterial activity and interactions of plant essential oil combinations against Gram-positive and Gram-negative bacteria. *Journal of Food and Drug Analysis, 25*(2), 403-408. doi:https://doi.org/10.1016/j.jfda.2016.06.002

Settanni, L., Randazzo, W., Palazzolo, E., Moschetti, M., Aleo, A., Guarrasi, V., . . . Moschetti, G. (2014). Seasonal variations of antimicrobial activity and chemical composition of essential oils extracted from three Citrus limon L. Burm. cultivars. *Natural product research, 28*(6), 383-391.

Simões, M., Simões, L. C., & Vieira, M. J. (2010). A review of current and emergent biofilm control strategies. *LWT - Food Science and Technology, 43*(4), 573-583. doi:https://doi.org/10.1016/j.lwt.2009.12.008

Sliti, S., Ayadi, S., Kachouri, F., Khouja, M. A., Abderrabba, M., & Bouzouita, N. (2015). Leaf essential oils chemical composition, antibacterial and antioxidant activities of eucalyptus camaldulensis and E. rudis from korbous (Tunisia). *Journal of Materials and Environmental Science, 6*(2), 531-537.

Souza, J. B. d., & Daniel, L. A. (2005). Comparison between sodium hipoclorite and peracetic acid for E. coli, coliphages and C. perfringens inactivation of high organic matter concentration water. *Engenharia Sanitaria e Ambiental, 10*(2), 111-117.

Todeschini, R. (1998). Introduzione alla chemiometria. *EdiSES, Napoli*, 321.

Torregiani, E., Lorier, S., Sagratini, G., Maggi, F., Vittori, S., & Caprioli, G. (2017). Comparative Analysis of the Volatile Profile of 20 Commercial Samples of Truffles, Truffle Sauces, and Truffle-Flavored Oils by Using HS-SPME-GC-MS. *Food Analytical Methods, 10*(6), 1857-1869. doi:10.1007/s12161-016-0749-2

Valeriano, C., de Oliveira, T. L. C., de Carvalho, S. M., Cardoso, M. D. G., Alves, E., & Piccoli, R. H. (2012). The sanitizing action of essential oil-based solutions against Salmonella enterica serotype Enteritidis S64 biofilm formation on AISI 304 stainless steel. *Food Control, 25*(2), 673-677. doi:10.1016/j.foodcont.2011.12.015

Van Haute, S., Raes, K., Devlieghere, F., & Sampers, I. (2017). Combined use of cinnamon essential oil and MAP/vacuum packaging to increase the microbial and sensorial shelf life of lean pork and salmon. *Food Packaging and Shelf Life, 12*, 51-58. doi:10.1016/j.fpsl.2017.02.004

Veldhuizen, E. J. A., Tjeerdsma-Van Bokhoven, J. L. M., Zweijtzer, C., Burt, S. A., & Haagsman, H. P. (2006). Structural requirements for the antimicrobial activity of carvacrol. *Journal of Agricultural and Food Chemistry, 54*(5), 1874-1879. doi:10.1021/jf052564y

Verdeguer, M., Blázquez, M. A., & Boira, H. (2009). Phytotoxic effects of Lantana camara, Eucalyptus camaldulensis and Eriocephalus africanus essential oils in weeds of Mediterranean summer crops. *Biochemical Systematics and Ecology, 37*(4), 362-369.

Williams, G. J., Denyer, S. P., Hosein, I. K., Hill, D. W., & Maillard, J. Y. (2009). Use of sodium dichloroisocyanurate for floor disinfection. *Journal of Hospital Infection, 72*(3), 279-281. doi:http://dx.doi.org/10.1016/j.jhin.2009.02.017

World Health Organization, W. (2014). *Advancing food safety initiatives: strategic plan for food safety including foodborne zoonoses 2013-2022*.

Xue, R., Shi, H., Ma, Y., Yang, J., Hua, B., Inniss, E. C., . . . Eichholz, T. (2017). Evaluation of thirteen haloacetic acids and ten trihalomethanes formation by peracetic acid and chlorine drinking water disinfection. *Chemosphere, 189*, 349-356. doi:10.1016/j.chemosphere.2017.09.059

Yemiş, G. P., & Candoğan, K. (2017). Antibacterial activity of soy edible coatings incorporated with thyme and oregano essential oils on beef against pathogenic bacteria. *Food Science and Biotechnology, 26*(4), 1113-1121. doi:10.1007/s10068-017-0136-9

Zuzarte, M., & Salgueiro, L. (2015). Essential oils chemistry. In *Bioactive Essential Oils and Cancer* (pp. 19-61).

Table 1. Main chemical compounds (>10%) characterizing extracted EOs by GC and GC–MS analysis.

Compound[a,b]	Class compound	IK	EO1	EO2	EO3	EO4	EO5	EO6	EO7	EO8	EO9
Sabinene	MH	980		0.34	0.42	2.11	t		3.13	34.41	0.14
β-Pinene	MH	982	1.47	0.66	0.46	0.53	0.58	0.03	17.28	2.2	1.12
p-Cymene	MH	1027	0.73	0.50	3.40	0.10		1.69		0.17	28.34
Limonene	MH	1033		0.89		1.13		0.05	30.14	3.69	t
1.8-Cineole	OM	1037	0.07		11.74	4.72	1.34				6.99
Artemisia ketone	OM	1065	57.54		3.15						
Linalool	OM	1107		4.87		0.35	4.19	0.64	1.20	21.27	0.28
Epoxy-ocimene <(E)->	OM	1140		34.01							
Camphor	OM	1149		7.96	31.43		0.87				
Cryptone	OM	1192									14.12
α-Terpineol	OM	1195		t		3.09	12.60	0.02	0.34	0.77	1.01
Citronellol	OM	1237					20.40				
Pulegone	OM	1245				14.15					
Geraniol	OM	1250					12.30		0.65		
cis-Chrysanthenyl acetate	OM	1267		28.35							
Geranial	OM	1269		t			1.20		11.91	0.02	
Carvacrol	OM	1317						91.56			
α-Terpineol acetate	OM	1353				32.59					
β-Copaen-4-α-ol	OS	1580			10.11						
Carvone acetate	OM	1574				10.29					
Spathulenol	OS	1580	1.32								17.99
Intermedeol	OS	1667	10.54	0.07							
Monoterpene hydrocarbons. (MH)			5.98	4.56	11.17	6.41	2.05	3.25	59.11	64.47	34.66
Oxygenated monoterpenes. (OM)			66.03	80.16	66.16	87.44	75.1	93.31	34.51	28.82	38.61
Sesquiterpene hydrocarbons. (SH)			0.6	5.05	2.9	3.56		2.17	3.15	1.66	0.83
Oxygenated sesquiterpenes. (OS)			21.35	0.85	14.31	0.30	1.52	0.53		4.25	21.82

Esters. (EST)	0.04		0.38	0.83					
Others. (OTH)		2.07	0.38	0.66	17.25	0.05	0.42		
Total identified (%)	94.00	92.69	95.30	99.19	95.92	99.31	97.19	99.20	95.92

[a]Compounds listed in order of elution in the ZB-5 column.

[b]The complete list of identified compounds is in Table 6S.

t. traces (<0.02%); IK. Kovats retention index relative to C_8–C_{25} n-alkanes on the ZB-5 column.

Table 2. Inhibitory activity of EOs tested by paper disc diffusion assay (PDDA) and minimum inhibitory concentration (MIC)

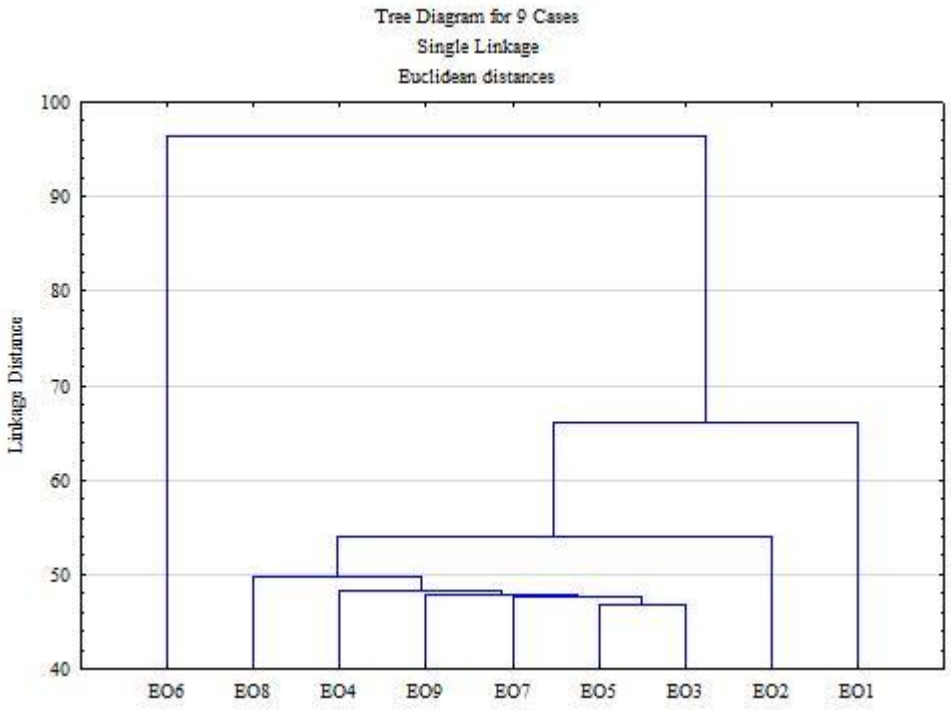
	Escherichia coli		***Salmonella enterica***	
	PDDA (cm)	**MIC** (%)	**PDDA** (cm)	**MIC** (%)
EO1	1.00±0.00	nd	1.00±0.00	nd
EO2	1.10±0.00	nd	1.10±0.00	nd
EO3	1.43±0.19	0.5	1.60±0.18	1
EO4	1.58±0.10	1	1.33±0.10	1
EO5	1.88±0.15	1	1.88±0.12	1
EO6	2.75±0.35	0.05	2.47±0.28	0.05
EO7	1.45±0.06	0.5	1.38±0.05	>1
EO8	1.75±0.10	0.5	2.23±0.26	0.5
EO9	1.83±0.13	0.5	1.50±0.00	0.5

nd. not determined. The results are expressed in cm and represent the mean value of the inhibition haloes of four determinations (carried out in duplicate and repeated twice) ± standard deviation.

Table 3. Evaluation of 1% natural sanitizing solution (SAN) against *Escherichia coli* and *Salmonella enterica* after 30 and 60 seconds of contact time according to AOAC 960.09 standard method.

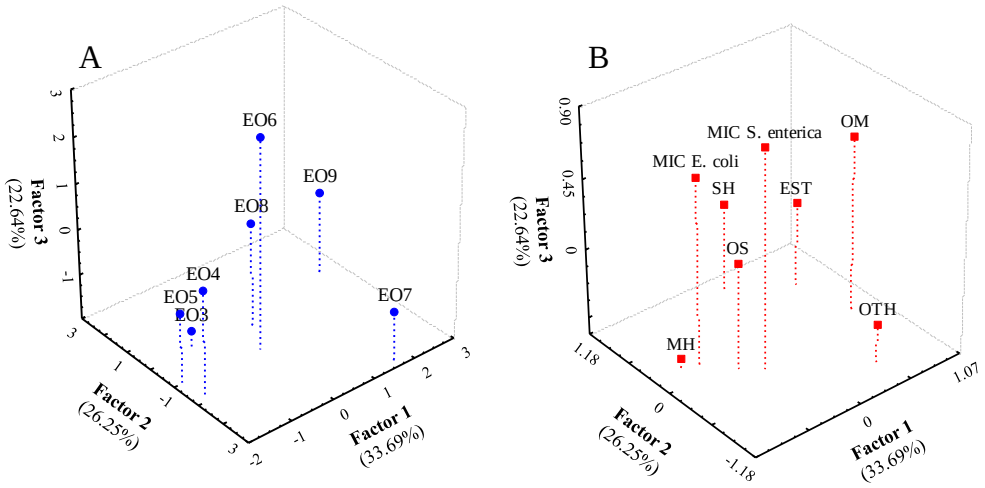
	Escherichia coli				***Salmonella enterica***			
	30” Plate counts (log CFU/ml)	30” Reduction	60” Plate counts (log CFU/ml)	60” Reduction	30” Plate counts (log CFU/ml)	30” Reduction	60” Plate counts (log CFU/ml)	60” Reduction
Untreated	8.28±0.56	-	8.28±0.56	-	7.83±0.16	-	7.83±0.16	-
Ethanol 1%	8.00±0.13	0.28	8.05±0.25	0.23	7.72±0.03	0.11	7.65±0.03	0.18
SAN 1%	0	8.28	0	8.28	2.32±0.01	5.51	0	7.83

Figure 1. Hierarchical clustering analysis of the nine essential oils according to their chemical and antimicrobial characterizations.



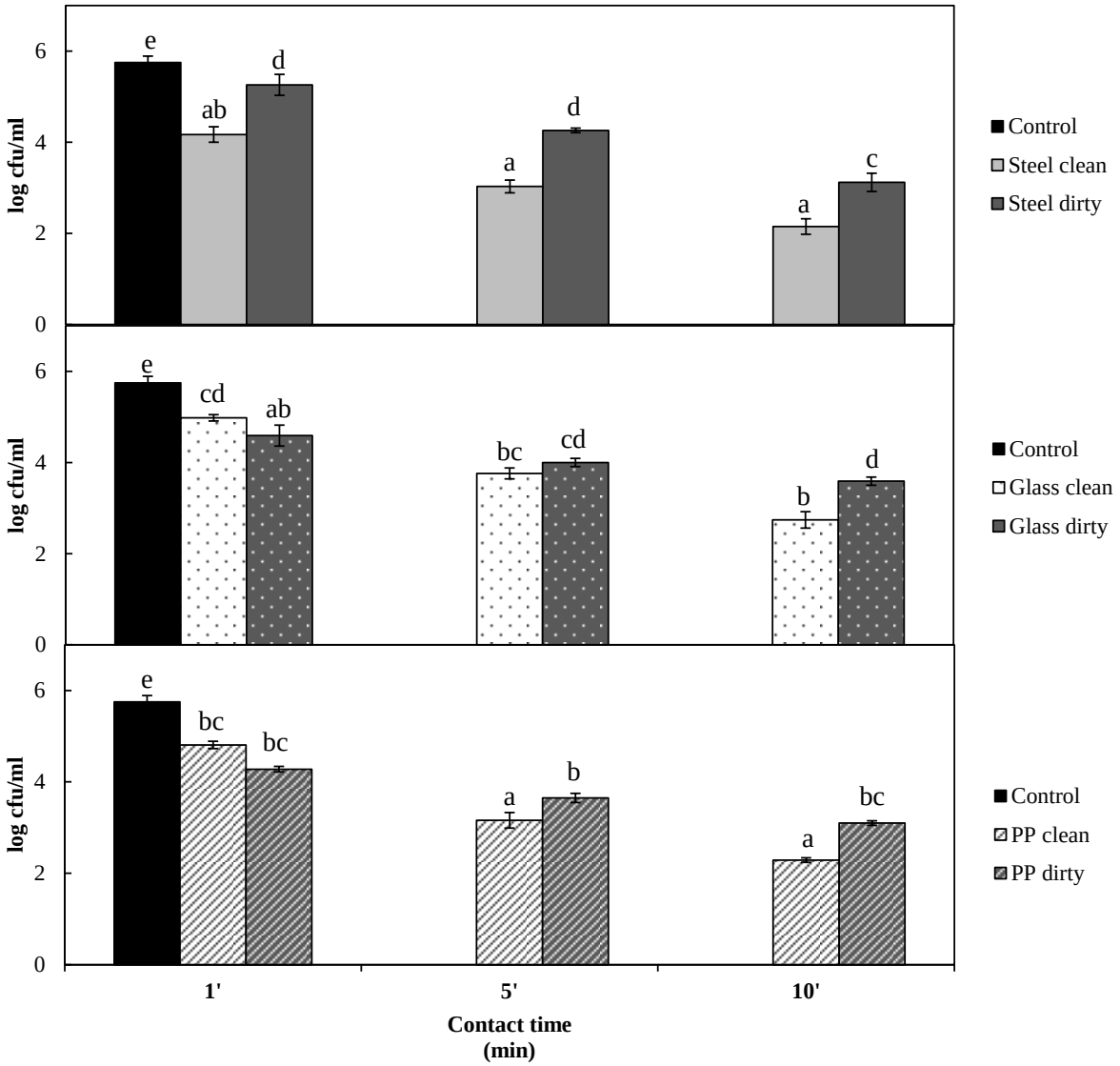
Abbreviations: EO1. *Eriocephalus africanus*; EO2. *Artemisia absinthium*; EO3. *Santolina chamaecyparissus*; EO4. *Mentha longifolia*; EO5. *Pelargonium odoratissimum*; EO6. *Thymbra capitata*; EO7. *Citrus limon*; EO8. *Citrus reticulata*; EO9. *Eucalyptus camaldulensis*.

Figure 2. Principal component analysis based on chemical compositions and antimicrobial activity of essential oils. Scatterplots show relationship between Factors and essential oils samples (score plot. A). and variables (loading plot. B).



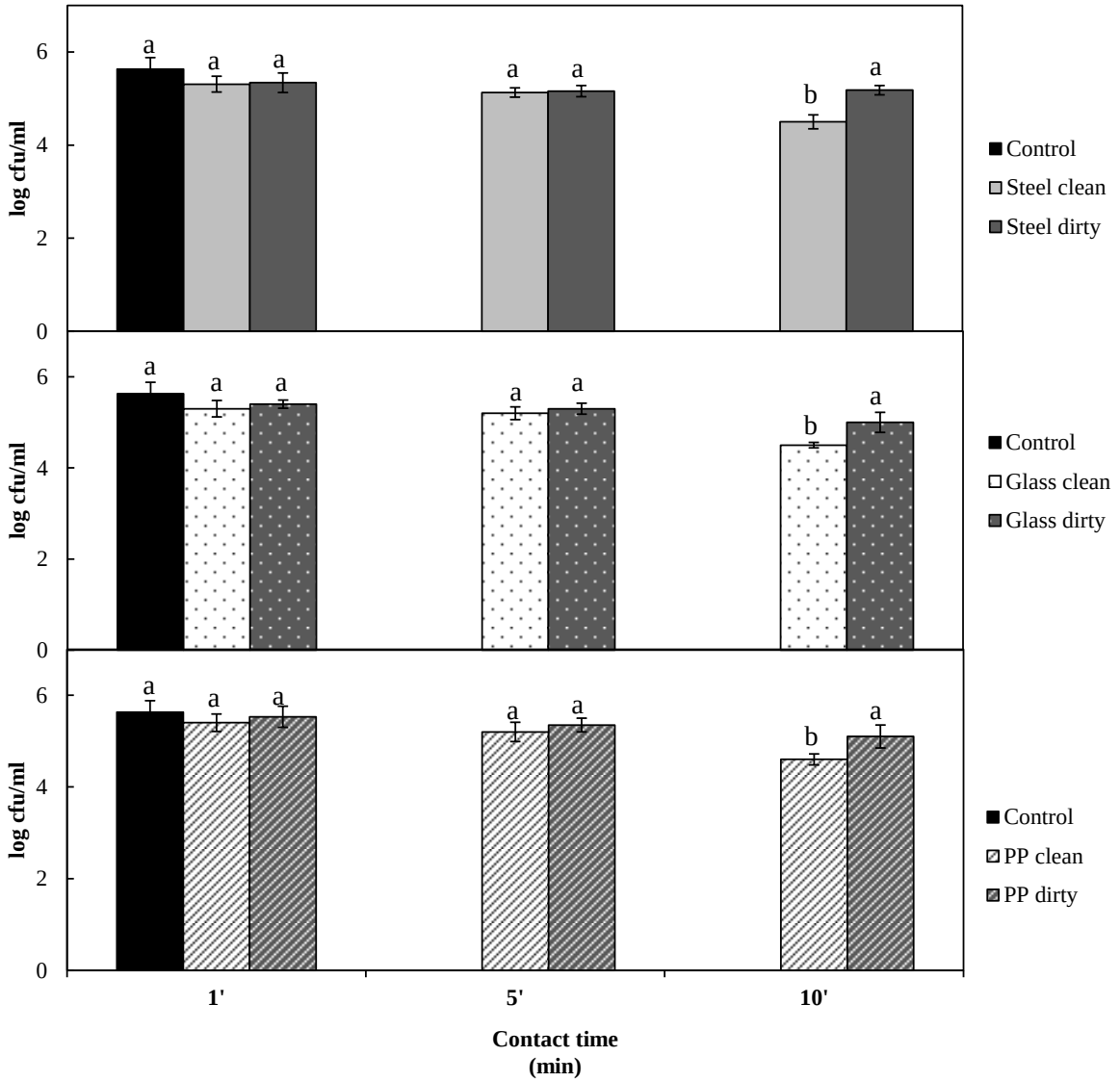
Abbreviations: EO1. *Eriocephalus africanus*; EO2. *Artemisia absinthium*; EO3. *Santolina chamaecyparissus*; EO4. *Mentha longifolia*; EO5. *Pelargonium odoratissimum*; EO6. *Thymbra capitata*; EO7. *Citrus limon*; EO8. *Citrus reticulata*; EO9. *Eucalyptus camaldulensis*. MIC E. coli and S. enterica, minimum inhibitory concentration for *E. coli* and *S. enterica*. respectively; MH. monoterpene hydrocarbons; OM. oxygenated monoterpenes; SH. sesquiterpene hydrocarbons; OS. oxygenated sesquiterpenes; EST. esters; OTH. others.

Figure 3. Bactericidal activity of 5% of a natural sanitizing solution (SAN) against *Escherichia coli* on different food contact surfaces (stainless steel, glass and polypropylene, PP, discs) cleanness conditions and contact times according to EN 13697:2015.



Error bars indicate standard errors of the means. For each contact time. samples with different letters are statistically different according to the analysis of variance followed by Tukey's test ($p \leq 0.05$).

Figure 4. Bactericidal activity of 5% of a natural sanitizing solution (SAN) against *Salmonella enterica* on different food contact surfaces (stainless steel, glass and polypropylene discs) cleanness conditions and contact times. according to EN 13697:2015.



Error bars indicate standard errors of the means. For each contact time. samples with different letters are statistically different according to the analysis of variance followed by Tukey's test ($p \leq 0.05$).

Research Highlights:

- Nine essential oils (EO) were screened for antibacterial activity and chemical composition
- *Thymbra capitata* EO shows high antimicrobial activity
- *T. capitata* EO-based natural sanitizing solution (SAN) was validated according to AOAC 960.09 standard
- SAN was effective against *Escherichia coli* for food contact surfaces disinfection according to EN 13697:2015
- SAN represents a natural sanitizing solution for cleaning steel, glass and plastic food contact surfaces