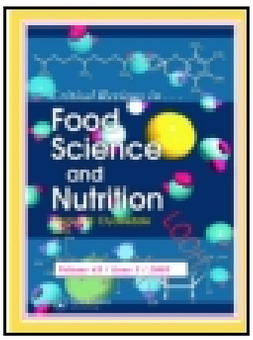

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REVIEW

Effectiveness and mechanisms of essential oils for biofilm control on food-contact surfaces: An updated review

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ABSTRACT

Microbial biofilms represent a constant source of contamination in the food industry, being also a real threat for human health. In fact, most of biofilm-producing bacteria are becoming resistant to sanitizers, thus arousing the interest in natural alternatives to prevent biofilm formation on foods and food-contact surfaces. In particular, studies on biofilm control by essential oils (EOs) application are increasing, being EOs characterized by unique mixtures of compounds able to impair the mechanisms of biofilm development. This review reports the anti-biofilm properties of EOs in bacterial biofilm control (inhibition, removal and prevention of biofilm dispersion) on food-contact surfaces. The relationship between EOs effect and composition, concentration, involved bacteria, and surfaces is discussed, and the possible sites of action are also elucidated. The findings prove the high biofilm controlling capability of EOs through the regulation of genes and proteins implicated in motility, Quorum Sensing and exopolysaccharides (EPS) matrix. Moreover, incorporation in nanosized delivery systems, formulation of blends and combination of EOs with other strategies can increase their anti-biofilm activity. This review provides an overview of the current knowledge of the EOs effectiveness in controlling bacterial biofilm on food-contact surfaces, providing valuable information for improving EOs use as sanitizers in food industries.

KEYWORDS

Biofilm; essential oil; food-contact surface; biofilm control; biofilm inhibition; mechanism of action

Introduction

The interest in microbial adhesion and biofilm formation on food and food-contact surfaces has considerably increased since the late 1990s (Kumar and Anand 1998; Shi and Zhu 2009) and is undoubtedly of current relevance. In fact, living in a biofilm confers different advantages to bacteria, such as interaction and cooperation, nutrient and enzyme entrapment, heterogeneous habitat and tolerance to antimicrobial agents (Flemming et al. 2016). Biofilm formation is a cyclical and dynamic process that comprises different steps, starting from a reversible attachment of planktonic cells to a surface, followed by an irreversible attachment with the production of EPS that include proteins, polysaccharides, lipids and DNA. This primary biofilm structure matures over time and provides mechanical and chemical stability to the cells. However, detachment and dispersion of bacterial cells from the biofilm matrix into the environment can occur, thus becoming a new source of contamination (Coughlan et al. 2016). Biofilm formation is affected by three main factors: the characteristics of the involved bacteria (i.e. cell surface appendages, strain, metabolic activity, surface charge), the attachment surface (material, roughness, wettability, hydrophobicity/hydrophilicity, etc.) and the surrounding environment (temperature, nutrient availability, pH, etc.) (Van Houdt and Michiels 2010; García-Gonzalo and Pagán 2015).

Biofilms are usually made up of a single or multiple species, forming the so-called multi-species biofilms, which show also higher resistance to antimicrobial agents and could involve spoiling and pathogenic bacteria (Galiè et al. 2018). In biofilms, sessile bacterial cells are different from their planktonic form (free floating cells), as they present an altered phenotype (García-Gonzalo and Pagán 2015) and different metabolic characteristics (Davey and O'toole 2000).

Bacteria in biofilms colonize different industrial environments, compromising food safety and quality. Moreover, due to the increased tolerance to common sanitizers, the study of new agents able to inhibit/prevent biofilm formation and eradicate/remove already established biofilms is of particular importance for the food industry. Furthermore, in response to consumer demands for natural products, alternatives to the use of chemicals are required. Among the environmental-friendly strategies, essential oils (EOs) have great potential for food industry application (Rossi, Chaves-López et al. 2018; Rossi et al. 2019); these oily systems contain concentrated secondary metabolites produced by aromatic plants and exert varying antimicrobial activity depending on the chemical composition. In detail, essential oils interact with different cellular constituents, also affecting several metabolic and physiological processes, including the respiratory process (Cui et al. 2019), crucial for the cell

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vitality (Paparella et al. 2008). Although some bacteria showed increased EOs tolerance after repeated exposure (Becerril, Nerín, and Gómez-Lus 2012), having simultaneously multiple targets in microbial cells, they behave as effective antimicrobials, without stimulating the development of resistance phenomena and thus preventing the emergence of drug-resistant bacteria (Yap et al. 2014). Moreover, they are biodegradable and they are applied in very low quantities, without toxic side effects (Cui, Zhang et al. 2018).

The literature suggests a promising use of EOs in sanitizing formulations to control microbial biofilms in food industries, and EOs based solutions applied to sanitize surfaces appear to be easily removed with washing procedures without residual odor problems (de Oliveira et al. 2012).

Therefore, the aim of this review is to gather and discuss published data on EOs anti-biofilm properties, considering the involved microorganisms, the adhesion surface, the EO and its composition. In this paper we analyze both microbial biofilm inhibition and prevention, as well as current understanding of the possible mechanisms of action. Furthermore, some approaches to improve EOs activity such as the use of natural carriers, blends and combined strategies are discussed. Finally, the activity of hydrolates in biofilm control is also considered.

Biofilms in the food industries

In the food industry environment, biofilm-associated bacteria present on food processing surfaces and utensils such as pipelines, tables, filters, conveyor belts, blades, etc., may acquire protection against physical (desiccation, radiations), mechanical (cleaning procedures) and chemical (disinfectants and sanitizing agents) treatments. This aspect is of crucial importance for food manufacturing, as bacterial biofilms are a threat for human and animal health, representing a continuous source of contamination (Coughlan et al. 2016). In fact, pathogenic bacteria and/or toxins can contaminate food products when they come in contact with contaminated surfaces or equipment, leading to foodborne illnesses cases or outbreaks. In detail, *Campylobacter, Salmonella*, shiga-toxin producing *Escherichia coli*, *Yersinia enterocolitica* and *Listeria monocytogenes* were responsible of about 356.000 confirmed human cases of infection or intoxication in Europe during the year 2018, and all these pathogens are known to produce biofilm (EFSA, 2019). Also the economical aspect has to be considered, since food products contamination with spoilage microorganisms leads to shelf life reduction, with significant economic losses. In addition, material corrosion, reduced heat transfer, mechanical blockage, and altered permeability of filtration membranes could occur in presence of biofilms (Tang et al. 2009; García-Gonzalo and Pagán 2015; Galiè et al. 2018). For the above mentioned aspects, the study of strategies to control biofilms in the food industry is worth to receive extreme attention, taking into consideration three main approaches: i) prevention/inhibition of biofilm formation ii) removal/eradication of existing biofilm, and iii) prevention of biofilm dispersion through the control of environmental factors and quorum sensing.

Prevention is clearly the preferred and the most successful plan, which is based on accurate and properly executed cleaning and disinfection of all the equipment. In this context, the choice of the appropriate detergents, disinfectants, frequency of cleaning and disinfection is of crucial importance. Food-contact surfaces materials (stainless steel, plastic, etc.) have to be carefully chosen too, to guarantee high resistance to corrosion and avoid scratches and cracks (Coughlan et al. 2016). Moreover, the equipment design should avoid corners, laminar flow and static product (Van Houdt and Michiels 2010).

Although prevention is deemed to be the best way to control biofilm formation, it is also important to study and improve methods to remove the pre-formed biofilms. Usually the combination of chemical and physical methods is the most adopted solution. Chlorine-based sanitizers, hydrogen peroxide, peracetic acid, quaternary ammonium compounds are some of the widely used chemicals, as well as brushing, scrubbing, foam formation, water jets, pulsed electric fields are some of the commonly employed physical methods (Kumar and Anand 1998; Galiè et al. 2018).

Unfortunately, most biofilm-forming microorganisms are becoming resistant to the aforementioned antimicrobial agents, urging the need of new anti-biofilm strategies. Furthermore, the growing consumers negative impression of conventional synthetic chemicals and the problems related to their toxic residues motivates the increasing interest in natural and green alternatives. These may include the use of i) enzymes, to disrupt the EPS matrix and facilitate the action of disinfectants, ii) bacteriophages, that diffuse in the biofilm and perform their antibacterial effect, iii) bacteriocins and bacteriocins-producing bacteria with their competitive exclusion action, and iv) quorum sensing inhibitors, since this cell-to-cell communication mechanism regulates the expression of genes involved in biofilm formation (Coughlan et al. 2016; Galiè et al. 2018). Moreover, nanotechnology in drug delivery systems supports and enhances biofilm inhibition by improving the stability and slow-release of essential oils, thanks to the high surface area to volume ratio of nanomaterials. Among these new biocontrol strategies, EOs are of relevant importance, having shown very high efficiency in controlling biofilm formation and eradication both in food products and food-contact surfaces.

Essential oils in biofilm control

Essential oils are natural, complex mixtures of organic, volatile and lipophilic compounds, characterized by a very intense odor (Paparella et al. 2016). They are generally obtained from aromatic plants by steam distillation, hydrodistillation or cold-pressing method, although alternative extraction techniques have been employed, such as solvents, supercritical fluids, and microwaves extraction (Schillaci et al. 2013; Friedman 2017). Different parts of the plant, i.e. bark, roots, leaves, flowers, buds, seeds, or even the whole plant, are employed to produce EOs. Their composition depends on different factors such as climate, genetics, soil, culture methods, age, part of the plant used, geographical

sources, and extraction techniques. The interaction among these factors gives a unique mixture of molecules, also named phytocomplex, which globally defines the properties and characteristics of each EO. Usually, terpenes, terpenoids, alcohols, esters, phenols are the main chemical compounds (Bazargani and Rohloff 2016).

The use of EOs has very ancient origins and nowadays is widespread in cosmetics, pharmaceuticals, agriculture, food and sanitary products (Schillaci et al. 2013). In food manufacturing, they were originally applied as flavoring agents, while now they are principally considered for their antimicrobial properties. In fact, EOs have cytotoxic effect, mainly due to the impairment of microbial cytoplasmic membranes and enzymatic systems (Vetas et al. 2017).

In recent times, the interest in the anti-biofilm activity of essential oils has been increasing, because their different chemical components can be exploited as antimicrobials not only against planktonic cells but also against the sessile counterpart. In fact, EOs can interfere with the mechanisms involved in biofilm formation, thus enabling to impair and control this process. The effect exerted depends both on the chemical characteristics and on the target microorganism. It is worthy of mention that both hydrophilic and hydrophobic compounds of essential oils can contribute to anti-biofilm activity. However, according to Shahabi et al. (2017), the most hydrophilic EOs compounds show a higher anti-biofilm activity. Specifically, the hydrophilic moieties of the oils penetrate through the biofilm EPS matrix, while the hydrophobic ones enable to permeate the lipid components of the bacterial membranes (Desai et al. 2012; Kostoglou, Protopappas, and Giaouris 2020; Wang et al. 2020).

Literature regarding biofilms mainly refers to biofilms formed on food-contact surfaces, where food processing conditions are simulated by using food-based broths and adhesion surfaces, such as plastic or steel materials. In fact, in situ studies on food matrices are limited and specifically concern bacterial adhesion and biofilm formation on fresh produce. Although the use of essential oils as anti-adhesive or anti-biofilm on vegetable surfaces are limited (Cui, Ma, and Lin 2016a; Rossi et al. 2019), several studies describe the possibility of using EOs as alternative sanitizers for fresh produce washing (Singh et al. 2002; Zhang et al. 2014; Dunn et al. 2019; Pellegrini et al. 2020).

The EOs anti-biofilm activity can depend also on the biofilm-forming microorganism. In fact, the bacteria sensitivity to EOs is species- and strain- dependent. Selected recent data concerning the wide-spectrum anti-biofilm activity of OEs (biofilm inhibition and removal from different surfaces commonly used in the food industry), published since 2010, are reported in Tables 1 and 2.

Inhibition of biofilm formation by EOs

EOs rich in monoterpenes or phenylpropanoids demonstrated high efficacy in bacterial biofilm prevention. In particular, these two classes of compounds are more active in the early stages of biofilm formation, when planktonic cells are still involved, and decrease their effectiveness with the increase of sessile population (Zygadlo et al. 2017).

To investigate the effect of EOs in disrupting the initial cell attachment and hindering biofilm maturation, the principal method used is to keep EOs in contact with the inoculum during the entire incubation period. Then, biofilm biomass, viability and EPS measurements can be assessed by different procedures that rely on microbiological and molecular methods, or on physical or chemical properties of the biofilm. In addition, microscopy techniques (e.g. Confocal Laser Scanning Microscopy and Scanning Electron Microscopy) are important to describe biofilm spatial organization, heterogeneity and links with the community functions (Azeredo et al. 2017). On the other hand, two classical methods for in vitro biofilm detection are commonly used: i) crystal violet assay (OD measurement) and ii) agar plating technique, although the first is the most exploited method, thanks to its simple but reliable quantification of total biomass. As evidenced in Table 1, the great part of the studies concerning the EO capacity to inhibit/prevent biofilm formation is carried out on polystyrene material, while some studies (dos Santos Rodrigues et al. 2017) reported also the use of stainless steel (SS) with good inhibition rates, in some cases more pronounced than on plastic surfaces (i.e for *Listeria* spp. as reported by Jadhav et al. (2013)).

As depicted in Table 1, *S. aureus* is the most investigated microorganism for biofilm inhibition by means of EOs, as it is persistent in food products. Despite the capability to form biofilm and colonize different kind of surfaces (Paparella et al. 2018), *Syzygium aromaticum* (clove) and *Cinnamomum zeylanicum* (cinnamon) EOs at 0.106 mg/mL (Budri et al. 2015) showed a significant biofilm inhibition on SS (63.6% and 45.3%, respectively). In spite of more studied pathogens, few data are available for *Vibrio* spp. biofilm, nevertheless it was inhibited by *Ocimum basilicum* EOs, at 50% of the minimal bactericidal concentration, and in less extent by *Petroselinum crispum* EO (Snoussi et al. 2016).

The biofilm-forming capacity of *Pseudomonas* spp., including *P. fluorescens,* was investigated considering its role in food discoloration and food spoilage, in general (Rossi, Serio et al. 2018), with *Thymus vulgare* EO ($< 20\,\mu L/mL$) showing a strong biofilm inhibition on SS (Myszka et al. 2016).

By comparing the activity of each essential oil to its major components, several studies generally witnessed a greater anti-biofilm effect of EOs with respect to their major components (Kerekes et al. 2013; Piovezan et al. 2014; Budri et al. 2015; Kim et al. 2016; Myszka et al. 2016), suggesting that minor compounds may have a synergistic effect with major bioactive compounds (Zhang, Gan et al. 2020). In fact, as shown in Table 3 which reports the comparison of the anti-biofilm effectiveness of some oils and their major components, most EOs (*Cinnamomum zeylanicum, Citrus lemon, Pimenta officinalis* and *Syzygium aromaticum*) exert greater activity than single compounds (cinnamaldehyde, limonene, eugenol, and eugenol).

Furthermore, although Budri et al. (2015) registered higher MIC values for the EOs than the individual compounds, by using the same concentrations, better performances in contrasting the biofilm were highlighted for the EOs. These findings are in contrast with the study of Kerekes et al. (2013) where single components (alpha-

Table 1. Overview of essential oils effect on biofilm formation of food spoiling and pathogenic microorganisms.

Name of plant source and extraction method	Plant part used	Major compounds	Type of surface	Tested microorganism	MIC	EO concentration (contact time)	Effect on biofilm formation	Reference
Achillea millefolium Commercial EO	–	Caryophyllene (15.9%), 1,8-cineole (12.0%), bornyl acetate (9.9%), 1-terpinen-4-ol (8.2%), beta-pinene (8.1%), camphor (6.6%), linalool (5.5%), sabinene (5.0%)	PS	*Listeria innocua*	3.13%	1.56% (24 h)	≈ 80% inhibition	Jadhav et al. (2013)
			PS	*Listeria monocytogenes*	3.13%	1.56% (24 h)	≈ 80% inhibition	Jadhav et al. (2013)
			SS	*L. innocua*	3.13%	1.56% (24 h)	≈ 4.5 Log reduction	Jadhav et al. (2013)
			SS	*L. monocytogenes*	3.13%	1.56% (24 h)	≈ 3 Log reduction	Jadhav et al. (2013)
			HDPE	*L. innocua*	3.13%	1.56% (24 h)	≈ 2.5 Log reduction	Jadhav et al. (2013)
			HDPE	*L. monocytogenes*	3.13%	1.56% (24 h)	≈ 2.2 Log reduction	Jadhav et al. (2013)
Calamintha nepeta L. Savi subsp. *glandulosa* (Req.) Ball Steam distillation	Fresh aerial parts	–	PS	*Pseudomonas aeruginosa*	>25 mg/mL	3.125 mg/mL (18 h)	≈ 0-60% inhibition	Artini et al. (2018)
			PS	*P. aeruginosa*	>25 mg/mL	3.125 mg/mL (18 h)	Increase	Artini et al. (2018)
Cinnamomum zeylanicum Hydrodistillation	Barks	E-cinnamaldehyde (94.9%)	SS	*Salmonella* Saintpaul	312 μg/mL	156 μg/mL (48 h)	≈ 2 Log CFU/cm^2 reduction	Piovezan et al. (2014)
Cinnamomum zeylanicum Steam distillation	–	Cinnamaldehyde (86.5%), benzaldehyde (4.2%), cineole (1.7%), cinnamic acid (1.5%), eugenol (0.1%)	PS	*Staphylococcus aureus*	0.243 mg/mL	0.106 mg/mL (48 h)	74.7% inhibition	Budri et al. (2015)
			SS	*S. aureus*	0.243 mg/mL	0.106 mg/mL (48 h)	45.3% inhibition	Budri et al. (2015)
Cinnamon Commercial EO	–	Cinnamonaldehyde, eugenol	PS	*Acinetobacter* spec.	0.063-0.016%	0.016% (24 h)	Complete inhibition	Szczepanski and Lipski (2014)
Citrus lemon Commercial EO	–	–	P	*Escherichia coli*	4 μL/mL	2 μL/mL (24 h)	≈ 77% inhibition	Kerekes et al. (2013)
Citrus medica L. var. *sarcodactylis Swingle* Steam distillation	Peel	D-limonene (44.87 %), α-pinene (3.36 %), β-myrcene (2.73 %), β-ocimene (2.61 %), carveol (2.32 %), camphene (1.88 %), α-vetivone (1.84 %), β-pinene (1.83 %), 2,4,6-octatriene (1.78 %), limonene oxide (1.73 %), α-bisabolene epoxide (1.42 %), caryophyllene oxide (1.33 %), α-cadinol (1.17 %), alloaromadendrene oxide (1.15 %)	PS	*L. monocytogenes*	4%	2% (24 h)	52% inhibition	Gao et al. (2020)
Coriandrum sativum L. Hydrodistillation	Seeds	Linalool (56.79%), γ-terpinene (9.80%), geranyl acetate (7.75%), α-pinene (7.67%), menthol (3.24%), octanol (3.02%), *p*-cymene (3%)	P	*E. coli*	1.6 μL/mL	1.6 μL/mL (8 h)	72.3% inhibition	Bazargani and Rohloff (2016)
			P	*S. aureus*	0.8 μL/mL	0.8 μL/mL (8 h)	100% inhibition	Bazargani and Rohloff (2016)
Echinops ritro L. Hydrodistillation	Aerial parts	Machilol (8.99%), nerolidol (7.64%), α-terpineol (4.14%), (-)-β-cadinene (4.14%), α-pinocarvone (3.93%)	P	*E. coli*	5 mg/mL	5 mg/mL (24 h)	50% inhibition	Jiang et al. (2017)
			P	*S. aureus*	0.21 mg/mL	0.21 mg/mL (24 h)	50% inhibition	Jiang et al. (2017)
			P	*Salmonella* Enteritidis	0.45 mg/mL	0.45 mg/mL (24 h)	50% inhibition	Jiang et al. (2017)
Foeniculum vulgare Miller Steam distillation	Fresh aerial parts	–	PS	*P. aeruginosa*	>25 mg/mL	3.125 mg/mL (18 h)	≈ 20-80% inhibition	Artini et al. (2018)
Juniperus communis Commercial EO	–	–	P	*E. coli*	2 μL/mL	1 μL/mL (24 h)	≈ 37% inhibition	Kerekes et al. (2013)
Mentha piperita -	–	Menthol (36.87%), menthone (16.44%), neoisomenthol (11.33%), isomenthone (10.47), menthyl acetate (7.47%), 2-isopropyl-5-methylcyclohexanol (2.74%), piperitone (2.17%), limonene (0.53%)	P	*P. aeruginosa*	3.2%	0.37-3% (24 h)	42.7-84% inhibition	Husain et al. (2015)
Mentha x piperita L. Hydrodistillation	Leaves	Menthol (33.19%), menthone (23.69%), 1,8-cineole (7.18%), menthyl acetate (5.04%), isomenthone (4.11%)	P	*E. coli*	6.3 μL/mL	6.3 μL/mL (8 h)	98.4% inhibition	Bazargani and Rohloff (2016)
			P	*S. aureus*	3.1 μL/mL	3.1 μL/mL (8 h)	74.7% inhibition	Bazargani and Rohloff (2016)
Mentha spicata L. Hydrodistillation	Aerial parts	Carvone (40.8% ± 1.23%), limonene (20.8% ± 1.12%), 1,8-cineole, (17.0% ± 0.60%), β-pinene (2.2% ± 0.25%), *cis*-dihydrocarvone (1.9% ± 0.49%), dihydrocarveol (1.7% ± 0.31%)	PS	*Vibrio vulnificus*	0.023-0.047 mg/mL	0.092 mg/mL	40% inhibition	Snoussi et al. (2015)
Murraya koenigii Supercritical fluid CO_2	–	Alpha logipinen (31.77%), sativen (6.55%), spathulenol (5.85%), disulfide,bis(1-methypropyl) (5.17%), valencene (4.36%), naphthalene (3.58%)	P	*P. aeruginosa*	2.5%	0.3% (48 h)	80% inhibition	Ganesh and Vittal (2015)
Ocimum basilicum Hydrodistillation	–	Linalool (42.1%), (*E*)-methyl cinnamate (16.9%), 1,8-cineole (7.6%)	PS	*Vibrio alginolyticus*	0.019-0.039 mg/mL	>5 mg/mL (24 h)	50% inhibition	Snoussi et al. (2016)
			PS	*Vibrio parahaemolyticus*	0.019-0.039 mg/mL	>5 mg/mL (24 h)	50% inhibition	Snoussi et al. (2016)
			PS	*Vibrio cholerae*	0.019-0.039 mg/mL	> 2.5 mg/mL (24 h)	50% inhibition	Snoussi et al. (2016)
Oregano Commercial EO	–	Carvacrol, *p*-cymol	PS	*Sphingomonas* spec.	0.031-0.016%	0.0005% (24 h)	50% inhibition	Szczepanski and Lipski (2014)
Origanum majorana Commercial EO	–	–	P	*Bacillus cereus*	0.25 μL/mL	0.125 μL/mL (24 h)	≈ 41% inhibition	Kerekes et al. (2013)
			P	*Pseudomonas putida*	2 μL/mL	1 μL/mL (24 h)	≈ 94% inhibition	Kerekes et al. (2013)
	Flowers		PS	*Pseudomonas fluorescens*	0.63-40 μg/mL	0.63 μg/mL (48 h)		

Origanum vulgare L. Commercial EO		Carvacrol (69.1%), *o*-cymene (5.9%), thymol (3.7%), terpinene (3.7%), caryophyllene (2.9%), *β*-pinene (1.6%)					38.54%-87.41% inhibition	Rossi, Chaves-López et al. (2018)
Origanum vulgare L. Commercial EO	–	Carvacrol (67%), thymol (16.22%), *p*-cymene (3.88%), linalool (2.73%), α-pinene (1.17%), myrcene (1.02%)	SS	*S. aureus*	5 μL/mL on planktonic, 10 μL/mL on sessile cells	2.5 μL/mL (360 h)	2.5 Log CFU/cm^2 reduction	dos Santos Rodrigues et al. (2017)
			SS	*S. aureus*	5 μL/mL on planktonic, 10 μL/mL on sessile cells	0.62 μL/mL (288 h)	1.5 Log CFU/cm^2 increase	dos Santos Rodrigues et al. (2017)
Peppermint Commercial EO	–	Menthol (40.39%), menthone (18.90%), (+)-isomenthone (9.06%), (+)-isomenthol (5.66%)	PS	*S. aureus*	0.5 mg/mL	2 mg/mL (24 h)	≈ 6 Log CFU/mL reduction	Kang et al. (2019)
Petroselinum crispum Hydrodistillation	Aerial parts	1,3,8-*p*-menthatriene (24.2%), *β*-phellandrene (22.8%), apiole (13.2%), myristicin (12.6%), terpinolene (10.3%)	PS	*V. parahaemolyticus*	0.011-0.044 mg/mL	> 5.62 mg/mL (24 h)	62.5% inhibition	Snoussi et al. (2016)
			PS	*V. vulnificus*	0.011-0.044 mg/mL	> 2.81 mg/mL (24 h)	52% inhibition	Snoussi et al. (2016)
Pimenta officinalis Commercial EO	Berry	Eugenol (71.10%), methyleugenol (13.67%), *β*-caryophyllene (7.83%)	PS	*E. coli*	–	0.005% (24 h)	≈ 90% inhibition	Kim et al. (2016)
Pimenta racemosa Commercial EO	–	Eugenol (61.99%), chavicol (16.48%), myrcene (7.93%)	PS	*E. coli*	–	0.005% (24 h)	≈ 79% inhibition	Kim et al. (2016)
Pimpinella anisum L. Hydrodistillation	Seeds	(E)-anethole (86.77%), estragole (3.92%), carvone (3.83%)	P	*E. coli*	12.5 μL/mL	12.5 μL/mL (8 h)	56.2% inhibition	Bazargani and Rohloff (2016)
			P	*S. aureus*	3.1 μL/mL	3.1 μL/mL (8 h)	90.3% inhibition	Bazargani and Rohloff (2016)
Rhanterium suaveolens Hydrodistillation	Aerial parts	Perillaldehyde (45.79%), caryophyllene oxide (24.82%), *β*-cadinol (5.61%), *β*-caryophyllene (5.17%), 8-cedren-13-ol (4.98%), *β*-pinene (3.21%), α-irone (1.62%)	P	*Staphylococcus epidermidis*	20 μg/mL	20 μg/mL (48 h)	50.3% inhibition	Chemsa et al. (2016)
			P	*Bacillus subtilis*	40 μg/mL	40 μg/mL (48 h)	45.55% inhibition	Chemsa et al. (2016)
			P	*B. cereus*	10 μg/mL	10 μg/mL (48 h)	30.35% inhibition	Chemsa et al. (2016)
			P	*Streptococcus mutans*	40 μg/mL	40 μg/mL (48 h)	24.04% inhibition	Chemsa et al. (2016)
Ridolfia segetum Moris Steam distillation	Fresh aerial parts	–	PS	*P. aeruginosa*	–	3.125 mg/mL (18 h)	≈ 0-60% inhibition	Artini et al. (2018)
Salvia sclarea Commercial EO	–	–	P	*E. coli*	2 μL/mL	1 μL/mL (24 h)	≈ 37% inhibition	Kerekes et al. (2013)
Satureja hortensis L. Hydrodistillation	Fresh leaves	Thymol (41.28%), γ-terpinene (37.63%), *p*-cymene (12.2%), α-terpinene (3.52%)	PS	*S. aureus*	0.125 μL/mL	0.0625 μL/mL (24 h)	≈ 64% inhibition	Sharifi et al. (2018)
Syzygium aromaticum Steam distillation	–	Eugenol (90.2%), eugenol acetate (6.5%), *β*-caryophyllene (1.3%)	PS	*S. aureus*	0.392 mg/mL	0.106 mg/mL (48 h)	69.4% inhibition	Budri et al. (2015)
			SS	*S. aureus*	0.392 mg/mL	0.106 mg/mL (48 h)	63.5% inhibition	Budri et al. (2015)
Syzygium aromaticum Commercial EO	–	Eugenol (77.08%), *β*-caryophyllene (9.10%)	PS	*E. coli*	–	0.005% (v/v) (24 h)	≈ 78% inhibition	Kim et al. (2016)
Syzygium aromaticum -	–	–	PS	*P. aeruginosa*	3.2% (v/v)	1.6% (v/v)	65% inhibition	Husain et al. (2013)
			PS	*Aeromonas hydrophila*	0.8% (v/v)	0.05-0.4% (v/v)	35-66% inhibition of biofilm foarming ability	Husain et al. (2013)
Thyme Commercial EO	–	Thymol, *p*-cymol	PS	*Sphingomonas* spec.	0.031-0.016%	0.001% and 0.004% (24 h)	50% and complete inhibition	Szczepanski and Lipski (2014)
			PS	*Acinetobacter* spec.	0.031-0.016%	0.008% (24 h)	> 50% inhibition	Szczepanski and Lipski (2014)
Thymus daenensis L. Hydrodistillation	Fresh leaves	Carvacrol (40.69%), γ-terpinene (30.28%), α-terpinene (5.52%)	PS	*S. aureus*	0.0625 μL/mL	0.03 μL/mL (24 h)	≈ 94% inhibition	Sharifi et al. (2018)
Thymus vulgaris Hydrodistillation	Dried leaves	Thymol (55.42%), carvacrol (6.84%), *p*-cymene (5.33%), *trans*-caryophyllene (3.89%), linalol (3.08%), caryophyllene oxide (3.08%), γ-terpinene (2.98%)	SS	*P. fluorescens*	20 μL/mL	< 20 μL/mL	5 dominant adhesion degrees reduction	Myszka et al. (2016)

SS: stainless steel, P: plastic, PS: polystyrene, HDPE: high density polyethylene, -: information not specified.

Table 2. Overview of essential oils effect against pre-formed biofilm of food spoiling and pathogenic microorganisms.

Name of plant source and extraction method	Plant part used	Major compounds	Type of surface	Tested microorganism	MIC	EO concentration (contact time)	Effect on pre-formed biofilm	Reference
Achillea millefolium Commercial EO	–	Caryophyllene (15.9%), 1,8-cineole (12.0%), bornyl acetate (9.9%), 1-terpinen-4-ol (8.2%), beta-pinene (8.1%), camphor (6.6%), linalool (5.5%), sabinene (5.0%)	PS	*Listeria innocua*	3.13%	3.13% (1 h)	28.7% reduction	Jadhav et al. (2013)
			PS	*Listeria monocytogenes*	3.13%	3.13% (1 h)	52.2% reduction	Jadhav et al. (2013)
Allium cepa Commercial EO	–	Trisulfide dipropyl (35.46%), dipropyl disulfide (31.11%), tetrasulfide, dipropyl (17.65%), trisulfide, mehtyl propyl (6.69%), disulfide, mehtyl propyl (5.11%)	PS	*L. monocytogenes*	0.025 mg/mL	0.025 mg/mL (1 h)	53% inhibition	Somrani et al. (2020)
Allium sativum Commercial EO	–	Diallyl trisulfide (25.13%), Diallyl disulfide (22.74%), Diallyl sulfide (20.89%), tetrasulfide, di-2-propenyl (13.54%), trisulfide, methyl 2-propenyl (2.94%), sulfide allyl mehtyl (1.41%), allyl methyl disulfide (1.12%)	PS	*L. monocytogenes*	0.1 mg/mL	0.1 mg/mL (1 h)	68% inhibition	Somrani et al. (2020)
Cinnamomum cassia Commercial EO	–	E-cinnamaldehyde 76.54%, Z)-2-mehtoxy cinnamaldehyde 10.30%, acetic acid, cinnamyl eeter 4.21, coumarin 2.59%	PS	*L. monocytogenes*	0.1 mg/mL	0.1 mg/mL (1 h)	61% inhibition	Somrani et al. (2020)
Cinnamomum cassia Commercial EO	Barks	Cinnamaldehyde 81%	SS	*Escherichia coli*	–	0.73% (16 min)	4.86 ± 0.00 Log CFU/cm^2 reduction	de Oliveira et al. (2012)
			SS	*L. monocytogenes*	–	1.00% (20 min)	2.54 ± 0.35 Log CFU/cm^2 reduction	de Oliveiraet al. (2012)
Cinnamomum cassia Commercial EO	Leaves	(*E*)-cinnamaldehyde 82.66%, (*E*)-*o*-methoxy cinnamaldehyde 10.12%	SS	*Staphylococcus aureus*	1.25%	2.5% (90 min)	≈ 4 Log CFU/cm^2 reduction (60%)	Campana et al. (2017)
Cinnamomum zeylanicum Hydrodistillation	Barks	*E*-cinnamaldehyde (94.9%)	SS	*Salmonella* Saintpaul	312 μg/mL	312 μg/mL (1 h)	≈ 0.6 Log CFU/cm^2 reduction	Piovezan et al. (2014)
Citrus limonia Hydrodistillation	Fresh peels	Limonene (33.67%), *ρ*-cimene (14.16%), carvone (9.50%), ciclohexanodiol (7.67%)	PS	*E. coli*	–	1% (15 min)	2.99 Log CFU/cm^2 reduction	Millezi et al. (2012)
			PS	*S. aureus*	–	1% (15 min)	2.49 Log CFU/cm^2 reduction	Millezi et al. (2012)
Citrus Changshan-huyou Y.B. Steam distillation	Peel	–	PS	*L. monocytogenes*	4%	4% (24 h)	67.3% inhibition	Guo et al. (2019)
Citrus medica L. var. *sarcodactylis Swingle* Steam distillation	Peel	D-limonene (44.87 %), α-pinene (3.36 %), *β*-myrcene (2.73 %), *β*-ocimene (2.61 %), carveol (2.32 %), camphene (1.88 %), α-vetivone (1.84 %), *β*-pinene (1.83 %), 2,4,6-octatriene (1.78 %), limonene oxide (1.73 %), α-bisabolene epoxide (1.42 %), caryophyllene oxide (1.33 %), α-cadinol (1.17 %), alloaromadendrene oxide (1.15 %)	PS	*L. monocytogenes*	4%	4% (24 h)	67.3% inhibition	Gao et al. (2020)
Cymbopogon citratus Hydrodistillation	Leaves	Geranial (42.26%), neral (32.23%), *β*-myrcene (12.69%)	SS	*L. monocytogenes*	0.4%	4% (1 h)	≈ 71% reduction	Reis-Teixeira et al. (2019)
			GL	*L. monocytogenes*	0.4%	4% (1 h)	≈ 38% reduction	Reis-Teixeira et al. (2019)
Cymbopogon nardus Commercial EO	Herbs	–	SS	*S. aureus*	0.5%	0.10-8% (30 min)	1-3.5 Log CFU/cm^2 reduction	Vázquez-Sánchez, Cabo, and Rodríguez-Herrera (2015)
Cymbopogon nardus Hydrodistillation	Fresh leaves	Citronellal (30.48%), geraniol (17.12%), citronellol (14.32%), elemol (6.11%)	PS	*E. coli*	–	1% (15 min)	3.64 Log CFU/cm^2 reduction	Millezi et al. (2012)
			PS	*S. aureus*	–	1% (15 min)	2.51 Log CFU/cm^2 reduction	Millezi et al. (2012)
Dodartia orientalis L. Hydrodistillation	–	Linalool (32.25%), α-terpineol (13.83%), 3,7-Dimethyl-2,6-octadien-1-ol (9.21%)	P	*S. aureus*	0.5 μL/mL	0.5 μL/mL (24 h)	≈ 84% reduction	Wang et al. (2017)
			P	*E. coli*	1 μL/mL	1 μL/mL (24 h)	≈ 87% reduction	Wang et al. (2017)
			P	*Salmonella* Enteritidis	2 μL/mL	2 μL/mL (24 h)	≈ 85% reduction	Wang et al. (2017)
Lippia sidoides Hydrodistillation	Leaves and branches	Thymol (53.54%), *p*-cymene (13.29%), *β*-caryophyllene (7.16%), γ-terpinene (6.75%)	PS	*L. monocytogenes*	–	0.5% (30 min)	4 Log CFU/cm^2 reduction	Vázquez-Sánchez, Galvão, Ambrosio, et al. (2018)
			SS	*L. monocytogenes*	–	1% (30 min)	4 Log CFU/cm^2 reduction	Vázquez-Sánchez, Galvão, Ambrosio et al. (2018)
			PS	*E. coli*	–	0.5% (30 min)	4 Log CFU/cm^2 reduction	Vázquez-Sánchez et al. (2019)
			SS	*E. coli*	–	2% (30 min)	4 Log CFU/cm^2 reduction	Vázquez-Sánchez et al. (2019)
			PS	*S. aureus*	–	1.25% (30 min)	4 Log CFU/cm^2 reduction	Vázquez-Sánchez, Galvão, Mazine, et al. (2018)
			SS	*S. aureus*	–	2.25% (30 min)	4 Log CFU/cm^2 reduction	Vázquez-Sánchez, Galvão, Mazine, et al. (2018)
Mentha spicata L. Hydrodistillation	–	Carvone (40.8% ± 1.23%), limonene (20.8% ± 1.12%), 1,8-cineole, (17.0% ± 0.60%), *β*-pinene (2.2% ± 0.25%), *cis*-dihydrocarvone (1.9% ± 0.49%), dihydrocarveol (1.7% ± 0.31%)	PS	*Vibrio alginolyticus*	0.023-0.047 mg/mL	0.092 mg/mL (24 h)	> 50% reduction	Snoussi et al. (2015)
			PS	*Vibrio cholerae*	0.023 mg/mL	0.092 mg/mL (24 h)	> 50% reduction	Snoussi et al. (2015)
	–	*D*-carvone (59.39%), limonene (20.76%)	PS	*S. aureus*	1.25%	5-15% (6 min)	0.8-1.2 Log CFU/cm^2 reduction	Vetas et al. (2017)

Mentha spicata Commercial EO								
Ocimum basilicum Hydrodistillation	–	Linalool (42.1%), (*E*)-methyl cinnamate (16.9%), 1,8-cineole (7.6%)	PS	*V. cholerae*	0.019-0.039 mg/mL	0.152 mg/mL (24 h)	50% reduction	Snoussi et al. (2016)
			PS	*Vibrio parahaemolyticus*	0.019-0.039 mg/mL	5 mg/mL (24 h)	50% reduction	Snoussi et al. (2016)
			PS	*Vibrio vulnificus*	0.019-0.039 mg/mL	> 5 mg/mL (24 h)	64.8% reduction	Snoussi et al. (2016)
Oregano Commercial EO	–	–	PS	*L. monocytogenes*	–	0.05% (24 h)	3.9 Log CFU/well reduction of 1 day old biofilm	Desai et al. (2012)
			PS	*L. monocytogenes*	–	0.1% (24 h)	Complete inactivation of 1-day-old biofilm (7.4 Log CFU/well)	Desai et al. (2012)
			SS	*L. monocytogenes*	–	0.1% (24 h)	3.7 Log CFU reduction of 4-day-old biofilm	Desai et al. (2012)
			SS	*L. monocytogenes*	–	0.25% (4 h)	2-3 Log CFU reduction of 4-day-old biofilm	Desai et al. (2012)
			SS	*L. monocytogenes*	–	0.25% (24 h)	Complete inactivation of 4-day-old biofilm (7 Log CFU/coupon)	Desai et al. (2012)
Origanum vulgare L. Steam distillation	–	Carvacrol (67%), thymol (16.22%), *p*-cymene (3.88%), linalool (2.73%), α-pinene (1.17%), myrcene (1.02%)	SS	*S. aureus*	–	10 μL/mL (10 min)	≥ 2 Log CFU/cm^2 reduction	dos Santos Rodrigues et al. (2018)
			SS	*S. aureus*	–	10 μL/mL (15 min)	≈ 6 ± 0.2 Log CFU/cm^2 reduction	dos Santos Rodrigues et al. (2018)
Petroselinum crispum Hydrodistillation	Aerial parts	1,3,8-*p*-menthatriene (24.2%), β-phellandrene (22.8%), apiole (13.2%), myristicin (12.6%), terpinolene (10.3%)	PS	*V. alginolyticus*	0.011-0.044 mg/mL	> 11.25 mg/mL (24 h)	55% reduction	Snoussi et al. (2016)
			PS	*V. parahaemolyticus*	0.011-0.044 mg/mL	> 5.62 mg/mL (24 h)	56% reduction	Snoussi et al. (2016)
Pimenta pseudochariophyllus Hydrodistillation	Leaves and branches	Chavibetol (33.57%), 1,8-cineole (11.25%), eugenol (8.89%), β-pinene (8.08%), *p*-cymene (7.73%), α-pinene (7.10%)	PS	*L. monocytogenes*	–	2.75% (30 min)	4 Log CFU/cm^2 reduction	Vázquez-Sánchez, Galvão, Ambrosio, et al. (2018)
			SS	*L. monocytogenes*	–	3% (30 min)	4 Log CFU/cm^2 reduction	Vázquez-Sánchez, Galvão, Ambrosio, et al. (2018)
			PS	*E. coli*	–	2.75% (30 min)	4 Log CFU/cm^2 reduction	Vázquez-Sánchez et al. (2019)
			SS	*E. coli*	–	3% (30 min)	4 Log CFU/cm^2 reduction	Vázquez-Sánchez et al. (2019)
			PS	*S. aureus*	–	1.75% (30 min)	4 log CFU/cm^2 reduction	Vázquez-Sánchez, Galvão, Mazine, et al. (2018)
			SS	*S. aureus*	–	3% (30 min)	4 Log CFU/cm^2 reduction	Vázquez-Sánchez, Galvão, Mazine, et al. (2018)
Pogostemon patchouli Commercial EO	Leaves	–	SS	*S. aureus*	0.5%	0.10-8% (30 min)	1-4.2 Log CFU/cm^2 reduction	Vázquez-Sánchez, Cabo, and Rodríguez-Herrera (2015)
Salvia officinalis Commercial EO	–	Eucalyptol (43.06%), camphor (17.45%)	PS	*S. aureus*	1.25%	5-15% (6 min)	1.9-3 Log CFU/cm^2 reduction	Vetas et al. (2017)
Salvia officinalis Commercial EO	–	*Cis*-thujone (23.90%), camphor (19.22%), 1.8-cineole (10.62%), α-humulene (7.52%), (*E*)-caryophyllene (6.34%), *trans*-thujone (6.22%), camphene (5.68%), α-pinene (5.08%), borneol (4.31%)	SS	*S. aureus*	5%	5% (90 min)	≈ 3.5 Log CFU/cm^2 reduction (45%)	Campana et al. (2017)
Satureja hortensis Hydrodistillation	Fresh leaves	Thymol (41.28%), γ-terpinene (37.63%), *p*-cymene (12.2%), α-terpinene (3.52%)	PS	*S. aureus*	0.125 μL/mL	0.0625 μL/mL (24 h)	≈ 38% reduction	Sharifi et al. (2018)
Tea tree Commercial EO	–	Terpinen (28.3%), gamma terpinen (18.9%), alpha terpine (9.7%), beta fenchol (8.5%)	SS	*E. coli*	0.08%	0.08 and 0.1% (2 h)	1.8 and 2.8 Log CFU/cm^2 reduction	Sadekuzzaman et al. (2018)
			SS	*L. monocytogenes*	0.09%	0.09 and 0.1% (2 h)	2.0 and 3.2 Log CFU/cm^2 reduction	Sadekuzzaman et al. (2018)
			SS	*S.* Enteritidis	0.07%	0.07 and 0.1% (2 h)	2.3 and 3.1 Log CFU/cm^2 reduction	Sadekuzzaman et al. (2018)
			SS	*Salmonella* Typhimurium	0.07%	0.07 and 0.1% (2 h)	2.3 and 3.1 Log CFU/cm^2 reduction	Sadekuzzaman et al. (2018)
Thyme Commercial EO	–	Cymene (28.5%), thymol (17.8), alpha pinene (10.6%), carvacrol (9.2%)	SS	*E. coli*	0.01%	0.01 and 0.1% (2 h)	1.4 and 3.0 Log CFU/cm^2 reduction	Sadekuzzaman et al. (2018)
			SS	*L. monocytogenes*	0.06%	0.06 and 0.1% (2 h)	1.5 and 3.3 Log CFU/cm^2 reduction	Sadekuzzaman et al. (2018)
			SS	*S.* Enteritidis	0.03%	0.03 and 0.1% (2 h)	1.8 and 3.3 Log CFU/cm^2 reduction	Sadekuzzaman et al. (2018)
			SS	*S.* Typhimurium	0.03%	0.03 and 0.1% (2 h)	1.8 and 3.3 Log CFU/cm^2 reduction	Sadekuzzaman et al. (2018)
Thyme Commercial EO	–	–	PS	*S.* Typhimurium	0.025%	0.05% (1 h)	≈ 7 Log CFU/well reduction	Soni et al. (2013)
Oregano Commercial EO	–	–	PS	*S.* Typhimurium	0.025%	0.05% (1 h)	≈ 7 Log CFU/well reduction	Soni et al. (2013)
Thymus daenensis L. Hydrodistillation	Fresh leaves	Carvacol (40.69%), γ-terpinene (30.28%), α-terpinene (5.52%)	PS	*S. aureus*	0.0625%	0.03 μL/mL (24 h)	≈ 30% reduction	Sharifi et al. (2018)
	Plant with flowers	–	SS	*S. aureus*	–	0.10-8% (30 min)	1-4.3 Log CFU/cm^2 reduction	

(*continued*)

Table 2. Continued.

Name of plant source and extraction method	Plant part used	Major compounds	Type of surface	Tested microorganism	MIC	EO concentration (contact time)	Effect on pre-formed biofilm	Reference
Thymus vulgaris Commercial EO								Vázquez-Sánchez, Cabo, and Rodríguez-Herrera (2015)
Thymus vulgaris Hydrodistillation	Leaves and branches	Thymol (31.74%), *p*-cymene (24.37%), γ-terpinene (9.45%), carvacrol (4.84%), linalool (3.45%)	PS	*L. monocytogenes*	–	1.5% (30 min)	4 Log CFU/cm^2 reduction	Vázquez-Sánchez, Galvão, Ambrosio, et al. (2018)
			SS	*L. monocytogenes*	–	2.5% (30 min)	4 Log CFU/cm^2 reduction	Vázquez-Sánchez, Galvão, Ambrosio, et al. (2018)
			PS	*E. coli*	–	1% (30 min)	4 Log CFU/cm^2 reduction	Vázquez-Sánchez et al. (2019)
			SS	*E. coli*	–	1% (30 min)	4 Log CFU/cm^2 reduction	Vázquez-Sánchez et al. (2019)
			PS	*S. aureus*	–	1.25% (30 min)	4 Log CFU/cm^2 reduction	Vázquez-Sánchez, Galvão, Mazine, et al. (2018)
			SS	*S. aureus*	–	2.25% (30 min)	4 Log CFU/cm^2 reduction	Vázquez-Sánchez, Galvão, Mazine, et al. (2018)
White thyme oil Commercial EO	–	–	PS	*L. monocytogenes*	–	0.05% (24 h)	4.8 Log CFU/well reduction of 1 day-old biofilm	Desai et al. (2012)
			PS	*L. monocytogenes*	–	0.1% (24 h)	Complete inactivation of 1-day-old biofilm (7.4 Log CFU/well)	Desai et al. (2012)
			SS	*L. monocytogenes*	–	0.1% (24 h)	1.5 Log CFU reduction of 4-day-old biofilm	Desai et al. (2012)
			SS	*L. monocytogenes*	–	0.25% (4 h)	2-3 Log CFU reduction of 4-day-old biofilm	Desai et al. (2012)
			SS	*L. monocytogenes*	–	0.25% (24 h)	Complete inactivation of 4-day-old biofilm (7 Log CFU/coupon)	Desai et al. (2012)
Zataria multiflora Hydrodistillation	–	Thymol (44.5%), γ-terpinene (16.12%), *p*-cymene (9.13%), β-mycrene (6.51%), α-thujene (3.71%), carvacrol (2.4%)	PS	*L. monocytogenes*	312.5 ppm	0.3% (24 h)	Complete elimination of 1-day-old biofilm	Tajik et al. (2015)
			SS	*L. monocytogenes*	312.5 ppm	0.3% (10 min)	Complete elimination of 6-day-old biofilm (6 Log CFU/cm^2)	Tajik et al. (2015)
Zingiber officinale Hydrodistillation	Leaves	α-zingiberene (13.56%), geranial (10.23%), α-curcumene (10.06%), neral (7.37%), β-sesquiphellandrene (7.04%)	SS	*L. monocytogenes*	0.4%	0.8% (1 h)	≈ 9% reduction	Reis-Teixeira et al. (2019)
			GL	*L. monocytogenes*	0.4%	0.8% (1 h)	≈ 15% reduction	Reis-Teixeira et al. (2019)

SS: stainless steel, P: plastic, PS: polystyrene, GL:glass; -: information not specified.

pinene, terpinen-4-ol and linalool) showed better biofilm inhibitory effect than parent EOs (*Juniperus communis, Origanum majorana* and *Salvia sclarea*), at leasts against *B. cereus* and *E. coli*.

In assessing biofilm inhibition, the choice of the EOs concentration is of crucial importance to obtain the desired result, as using too low dosages can have the opposite effect. In fact, an increasing number of sessile cells was observed in mature biofilm of *S. aureus* after exposure to low concentration of *Origanum vulgare* EO (dos Santos Rodrigues et al. 2017). According to the authors, the inductive effect of the oil occurs in presence of sub-MICs of phenolic compounds, as a response to stressful conditions, enhancing the biofilm formation capability of the microorganism.

However, several studies reported in Table 1 described an anti-biofilm effect of the EO at sub-MIC concentration. The fact highlights that the capability of the oils to inhibit the biofilms is not only due to the antimicrobial effect but also linked to other mechanisms, as reported below.

Removal of mature pre-formed biofilm by EO

The removal of established biofilms implies dealing with microbial cells entrapped inside a complex structure that acts as a protective barrier against anti-biofilm agents. Therefore, to retain its efficacy at this late stage of biofilm formation, it is important that the EO penetrates the EPS matrix and reaches the surface-attached cells.

Several EOs were analyzed, and Table 2 reports studies regarding their effect on the eradication of established biofilms.

As mentioned before, the effectiveness of an EO should be evaluated considering different aspects. In the case of biofilms formed on food-contact surfaces, the kind of material on which the biofilm is developed is of major importance. SS and plastic (P), usually polystyrene (PS), are the most studied materials, being the most common materials in the food industries. In this regard, different studies (Vázquez-Sánchez, Galvão, Vázquez-Sánchez, Galvão, Mazine, et al. 2018; Vázquez-Sánchez et al. 2019) highlighted that PS-related biofilms resulted more susceptible to EOs treatments compared to SS ones, since a reduction was generally reached at lower EOs concentrations than those needed for SS coupons. The enhanced efficacy of different EOs on PS material could be related to the hydrophobicity of the surface. In fact, PS and SS possess hydrophobic and hydrophilic physical and chemical characteristics, respectively (Di Ciccio et al. 2015). Probably, depending on their chemical composition, EOs are more attracted by hydrophobic surface and consequently exert higher interaction with bacterial biofilms, whereas the presence of more hydrophilic compounds (e.g. thymol and carvacrol) could facilitate the penetration of the EPS matrix. Although other authors (Desai et al. 2012) observed the greater resistance of biofilm on SS than on PS, independently on the applied EO, it has to be underlined that biofilms of different ages have probably different resistance to sanitizers and antimicrobials and therefore results are difficult to be compared. Moreover, EOs could exhibit different affinity for different surfaces, thus suggesting the need to choose the EO to be applied not only on the basis of the biofilm-forming microorganism, but also on the surface where the biofilm has been constructed.

As demonstrated for *Salmonella* spp., one of the principal causes of foodborne illnesses, thanks to its ability to form biofilm and persist in food establishments, both thyme and oregano EO were strongly effective in removing *Salmonella* Typhimurium pre-formed biofilms on PS (Soni et al. 2013). The efficacy is ascribable to the main component carvacrol, which passes through the EPS matrix due to its relative hydrophilicity and intrinsic antibacterial characteristics (Nostro et al. 2007). In fact, as showed in Table 3, 0.05% concentrations of oregano EO, thyme EO and carvacrol exerted the same effect, inactivating about 7 Log CFU of the 1-day-old *Salmonella* biofilms (Soni et al. 2013).

In general, the decrease of biofilm population is strain-, concentration- and exposure time- dependent, with increasing concentration and exposure time generally resulting in greater biofilm eradication performances (Campana et al. 2017). Another important feature to consider was highlighted by Millezi et al. (2012), who investigated differences in susceptibility of mono- and dual-species biofilm to EOs treatments. *Staphylococcus aureus* and *Escherichia coli* were considered, while *Cymbopogon nardus* and *Citrus limonia* were the chosen EOs. The number of sessile cells was higher in single-species than in dual-species biofilms, and also the effect of EOs was different depending on whether it is a mono or multi-species biofilm. In fact, *S. aureus* showed a slightly higher resistance in multi-species biofilm with respect to the single-species, while *E. coli* demonstrated a greater sensitivity to EOs treatments in dual-species biofilm. On the contrary, de Oliveira et al. (2012) found that enteropathogenic *E. coli* sessile cells, both in single and dual-species biofilm with *L. monocytogenes*, were 100% reduced by *Cinnamomum zeylanicum* EO. As regards *L. monocytogenes*, a greater sensitivity was observed in presence of *E. coli*, where a 100% reduction of sessile population compared to 45% decrease in the case of mono-species biofilm was revealed.

Therefore, it is essential to study multi-species biofilms, since they are those most commonly found in nature and in the food industry, and the biofilm can show different behavior and characteristics depending on the species involved.

Prevention of biofilm dispersion by EO

Biofilm dispersion, which is the last step in the biofilm formation process, is no less critical than the previous ones. At this stage, planktonic cells detach from biofilm and dissolve in the neighboring spaces, determining the beginning of a new biofilm and becoming a new source for food contamination. Therefore, the control of this phenomenon is of great importance. According to Zhao et al. (2017), biofilm dispersion is influenced by environmental and bacteria-related factors, such as quorum sensing (QS), EPS release, enzyme systems alteration, etc. Since it is known that EOs act on these systems (Khan et al. 2009; Myszka et al. 2016; Hu,

Table 3. EOs and EOs-related major compounds effect on biofilms of food spoiling and pathogenic microorganisms.

Tested microorganism	EO/single compound	MIC	Concentration and contact time	Effect on biofilm	Reference
Biofilm Formation					
S. aureus	*Cimbopogon nardus* EO (Geraniol 33.88%)	0.5 mg/mL	0.5-4 mg/mL, 24 h	95-100% inhibition	Pontes et al. (2019)
	Geraniol	0.25 mg/mL	0.25-4 mg/mL, 24 h	95.0-99.9% inhibition	
S. Saintpaul	*Cinnamomum zeylanicum* EO (E-cinnamaldehyde 94.9%)	312 μg/mL	156 μg/mL, 48 h	≈ 2 Log CFU/cm^2 reduction	Piovezan et al. (2014)
	E-cinnamaldehyde	312 μg/mL	156 μg/mL, 48 h	≈ 0.7 Log CFU/cm^2 reduction	
S. aureus	*Cinnamomum zeylanicum* EO (Cinnamaldehyde 86.5%)	0.243 mg/mL	0.106 mg/mL, 48 h	74.7% inhibition	Budri et al. (2015)
	Cinnamaldehyde	0.199 mg/mL	0.106 mg/mL, 48 h	69.4% inhibition	
S. aureus	*Cinnamomum zeylanicum* EO (Cinnamaldehyde 86.5%)	0.243 mg/mL	0.106 mg/mL, 48 h	45.3% inhibition	Budri et al. (2015)
	Cinnamaldehyde	0.199 mg/mL	0.106 mg/mL, 48 h	44.9% inhibition	
E. coli	*Citrus lemon* EO	4 μL/mL	2 μL/mL, 24 h	≈ 77% inhibition	Kerekes et al. (2013)
	Limonene	6 μL/mL	3 μL/mL, 24 h	≈ 60% inhibition	
E. coli	*Juniperus communis* EO	2 μL/mL	1 μL/mL, 24 h	≈37.0% inhibition	Kerekes et al. (2013)
	Alpha-pinene	1 μL/mL	0.5 μL/mL, 24 h	≈54.0% inhibition	
B. cereus	*Origanum majorana* EO	0.25 μL/mL	0.125 μL/mL, 24 h	≈ 41% inhibition	Kerekes et al. (2013)
	Terpinen-4-ol	6 μL/mL	3 μL/mL, 24 h	≈ 66% inhibition	
S. aureus	*Origanum vulgare* L. EO (Carvacrol 67%)	5 μL/mL on planktonic, 10 μL/mL on sessile cells	0.62 μL/mL-2.5 μL/mL, 288 h-360 h	1.5-2.5 Log CFU/cm^2 reduction	dos Santos Rodrigues et al. (2017)
	Carvacrol	2.5 μL/mL on planktonic, 10 μL/mL on sessile cells	0.62-1.25 μL/mL, 72 h	1 Log CFU/cm^2 reduction	
E. coli	*Pimenta officinalis* EO (Eugenol 71.1%)	–	0.005%, 24 h	≈ 90% inhibition	Kim et al. (2016)
	Eugenol	–	0.005%, 24 h	87% inhibition	
E. coli	*Salvia sclarea* EO	2 μL/mL	1.0 μL/mL, 24 h	≈ 37% inhibition	Kerekes et al. (2013)
	Linalool	3 μL/mL	1.5 μL/mL, 24 h	≈ 70% inhibition	
S. aureus	*Syzygium aromaticum* EO (Eugenol 90.2%)	0.392 mg/mL	0.106 mg/mL, 48 h	69.4% inhibition	Budri et al. (2015)
	Eugenol	0.237 mg/mL	0.106 mg/mL, 48 h	52.8% inhibition	
S. aureus	*Syzygium aromaticum* EO (Eugenol 90.2%)	0.392 mg/mL	0.106 mg/mL, 48 h	63.5% inhibition	Budri et al. (2015)
	Eugenol	0.237 mg/mL	0.106 mg/mL, 48 h	19.2% inhibition	
P. fluorescens	*Thymus vulgaris* EO (Thymol 55.42%) (Carvacrol 6.84%)	20 μL/mL	< 20 μL/mL	5 dominant adhesion degrees reduction	Myszka et al. (2016)
	Thymol	4 μL/mL	< 4.0 μL/mL	1 dominant adhesion degrees reduction	
	Carvacrol	2 μL/mL	< 2.0 μL/mL	1-2 dominant adhesion degrees reduction	
Formed Biofilm					
S. Saintpaul	*Cinnamomum zeylanicum* EO (E-cinnamaldehyde 94.9%)	312 μg/mL	312 μg/mL, 1 h	≈ 0.6 Log CFU/cm^2 reduction	Piovezan et al. (2014)
	E-cinnamaldehyde	312 μg/mL	312 μg/mL, 1 h	≈ 1 Log CFU/cm^2 reduction	
E. coli	*Cinnamomum cassia* EO (Cinnamaldehyde 81%)	–	0.73%, 16 min	4.86 ± 0.00 Log CFU/cm^2 reduction	de Oliveira et al. (2012)
	Cinnamaldehyde 81%	–	0.60%, 16 min	4.86 ± 0.00 Log CFU/cm^2 reduction	
L. monocytogenes	*Cinnamomum cassia* EO (Cinnamaldehyde 81%)	–	1.00%, 20 min	2.54 ± 0.35 Log CFU/cm^2 reduction	de Oliveira et al. (2012)
	Cinnamaldehyde 81%	–	0.80%, 20 min	2.79 ± 0.50 Log CFU/cm^2 reduction	
S. aureus	*Origanum vulgare* L. EO (Carvacrol 67%)	–	10 μL/mL, 10 min	≥ 2 Log CFU/cm^2 reduction	dos Santos Rodrigues et al. (2018)
	Carvacrol	–	5 μL/mL, 10 min	≥ 2.4 Log CFU/cm^2 reduction	
S. aureus	*Origanum vulgare* L. EO (Carvacrol 67%)	–	10 μL/mL, 15 min	≈ 6 ± 0.2 Log CFU/cm^2 reduction	dos Santos Rodrigues et al. (2018)
	Carvacrol	–	2.5 μL/mL, 15 min	≥ 3.0 Log CFU/cm^2 reduction	
S. Typhimurium	Thyme EO	0.025%	0.05%, 1 h	≈ 7 Log CFU/well reduction	Soni et al. (2013)
	Carvacrol	0.025%	0.05%, 1 h	≈ 7 Log CFU/well reduction	

-: information not specified.

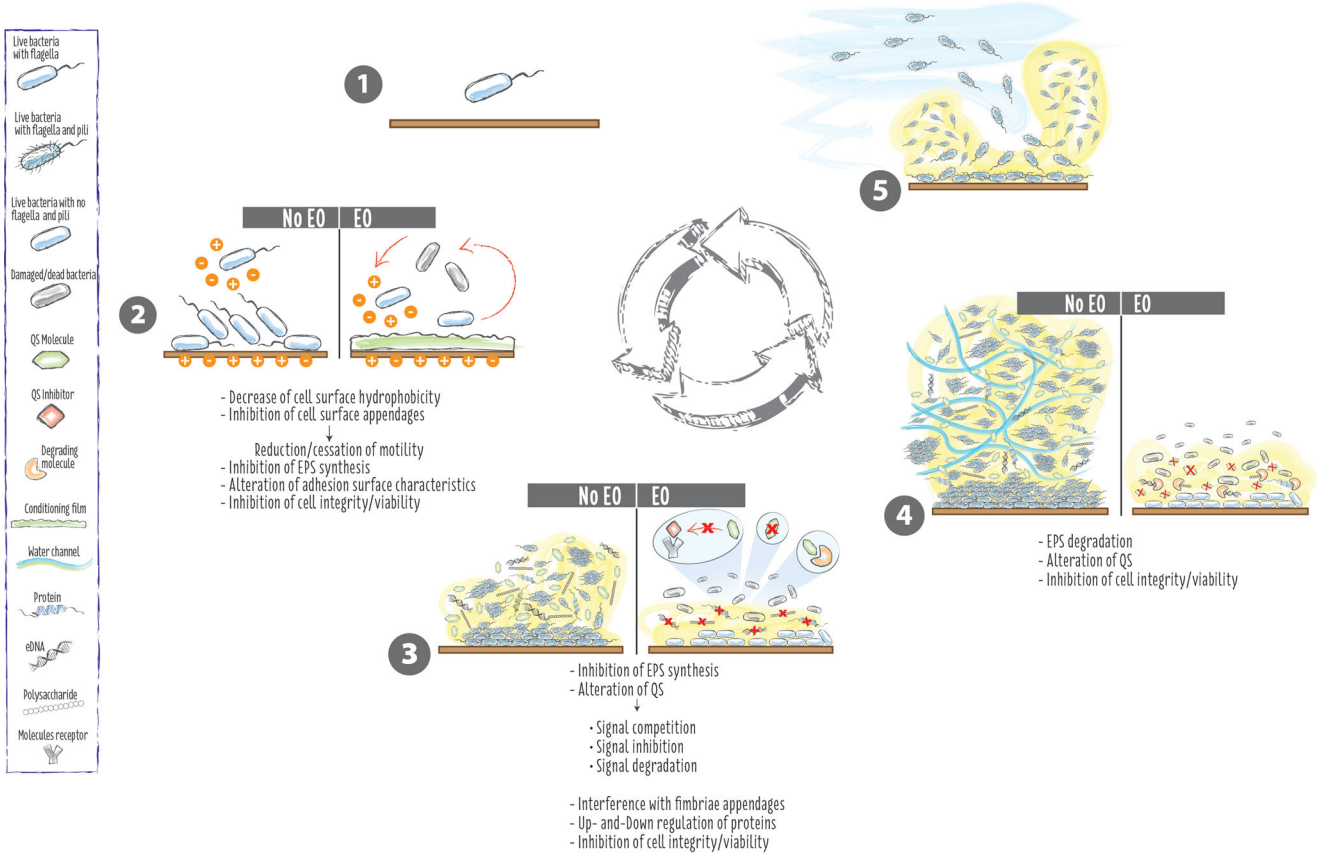

Figure 1. Representation of biofilm formation process in five steps with particular focus on the mechanisms of anti-biofilm action of essential oils: planktonic cell landing to a surface (1), bacterial cell adhesion (2), microcolonies formation (3), biofilm maturation (4), dissemination of cells that revert back to a planktonic state (5).

Zhou, and Wei 2018) it can be assumed that they could represent an interesting alternative strategy in preventing biofilm dispersion, even though no specific studies on this topic have been published.

The mechanisms of anti-biofilm action of essential oils

Indeed, during the whole evolution of the biofilm formation process, EOs exert antimicrobial activity on planktonic and sessile cells; most of the studies on the mechanisms of action of EOs (Serio et al. 2010; Zhang et al. 2016) reported that they can increase membrane permeability, disturbe cell membrane integrity, then inhibiting microbial growth. Although the interest in biofilm is growing, the reports describing the anti-biofilm mechanisms of action of EOs are limited and the phenomenon is not completely understood. However, some hypotheses have been proposed, and are here illustrated. Overall, the potential controlling mechanism of EOs is mainly due to the action on the multiple-stages of biofilm formation (Figure 1). In fact, during the life cycle of biofilms (adhesion, microcolonies formation, and maturation) the anti-biofilm effects are principally related to the inhibition of EPS matrix, the suppression of cell adhesion and the QS system alteration.

Adhesion

At the beginning of biofilm formation, when cell adhesion occurs because of cell hydrophobicity, flagella and motility, EOs probably act by interfering with the physiological cell characteristics.

Cell surface hydrophobicity is provided by several factors such as outer membrane proteins, lipopolysaccharides, S-layer proteins, lipoteichoic acids, and bacterial appendages. Among these latter, the fimbriae contain a high number of hydrophobic amino acids that probably help to overcome the initial electrostatic repulsion barrier between bacterial cell and the attachment surface (Lahesaare et al. 2016). About cell hydrophobicity, Wang et al. (2017) observed a decrease in different pathogens (*S. aureus*, *E. coli*, and *S.* Enteritidis), after exposure to *Dodartia orientalis* EO. The study revealed that the oil was capable of reducing the adhesive capacity of the strains, belonging to its anti-biofilm mechanisms. Likewise, selected monoterpenes of EOs caused changes in cell surface hydrophobicity of *E. coli* and *S. aureus* (Lopez-Romero et al. 2015).

As concerns motility, the swimming capability of individual cells allows the upstream movement of bacteria, with the consequent deposition of cells on areas not available for non-motile cells (de Kerchove and Elimelech 2008), while the rapid migration of swarming cells on solid surfaces is correlated with cell growth, thus resulting in a rapid colonization and adhesion on available areas (Vicario, Dardanelli,

and Giordano 2015). Moreover, motility is correlated with the cells release from mature biofilm.

To explain the previously assessed anti-biofilm capacity of *Origanum vulgare* EO, Rossi, Chaves-López et al. (2018) evaluated the effects of the EO on microbial motility of 10 blue pigmenting *P. fluorescens*. In this study, a significant reduction of swimming and swarming motility was observed for some of the tested strains after treatment with sub-MIC concentration of *Origanum vulgare* EO (5 µL/mL). This effect could be linked to the inhibition of cell surface appendages synthesis by microorganisms as a survival tactic, to store energy for other cell functions. Moreover, Myszka et al. (2016) reported the suppression of *P. fluorescens* motility and the reduction of mRNA level of flagella gene, after treatment with *Thymus vulgare* EO. The authors stated that the expression of this gene significantly inhibited the biofilm formation on stainless steel surfaces. Furthermore, Khan et al. (2009) and Kim et al. (2015) observed the inhibition of *Pseudomonas aeruginosa* swarming motility by the use of clove and cinnamon bark EOs, respectively. Also carvacrol inhibited the flagellin synthesis, causing a reduction or cessation of *E. coli* O157:H7 motility depending on compound concentration (Burt et al. 2007); in addition, carvacrol, eugenol, thymol and *trans*-cynnamaldehyde inhibited the expression of *fliC*, *fimA*, *lpfA* genes that encode for appendages related to migration (Baskaran et al. 2016).

EOs can also interfere with the EPS secreted by bacteria during the initial stages of biofilm formation, where the secretion enhances cell-to surface-adhesion and allows to pass from reversible to irreversible attachment (Zhao et al. 2017). Nevertheless, EPS secretion goes on during the growth, proliferation and maturation of biofilm. Hu, Zhou, and Wei (2018) highlighted an attenuation of *P. aeruginosa* exopolysaccharides production by clove oil, while a major component of cinnamon bark EO (*trans*-cinnamaldehyde) showed inhibitory activity on the synthesis of *C. sakazakii* exopolysaccharide (Amalaradjou and Venkitanarayanan 2011). Moreover, ten EOs components influenced the motility and inhibited the EPS production of *Erwinia carotovora* and *P. fluorescens* (Zhang et al. 2018). Recently, in addition to the inhibition of EPS secretion, Cui et al. (2020) found that EOs can regulate also the expression of multiple genes involved in the complex process of biofilm formation. In fact, they reported that cardamom EO affects the formation of methicillin-resistant *S. aureus* biofilms by up-regulating (*sigB* gene) or down-regulating (*sarA* gene) the expression of biofilm-forming genes. On the other hand, with respect to *L. monocytogenes*, clove EO was found to affect the formation of biofilm by regulating the transcription levels of genes in the Agr system, and of genes *sigB* and *prfA* (Zhang, Li, et al. 2020).

Probably, also an alteration of adhesion surface characteristics determined by EOs could eventually interfere with cells surface colonization. In fact, analyses on the development of EO component-based polymer films revealed a reduction of surface hydrophobicity in presence of oil components, thereby reducing bacteria adhesion (Nostro et al. 2013).

Microcolonies formation

The QS system, due to its involvement in biofilm development, is an attractive target for novel anti-biofilm strategies. In this respect, some EOs exert anti-QS activity (Husain et al. 2015; Venkadesaperumal et al. 2016; Poli et al. 2018; Reichling 2020) and were recently classified for their Quorum Quenching activity, together with other molecules that degradate or inactivate QS signaling molecules (Coughlan et al. 2016). This bacterial communication system mainly occurs in microcolonies formation stage and during biofilm maturation. Sub-MIC concentrations of thyme EO limited of 90% the production of the *P. fluorescens* signaling molecule N-acyl-homoserine lactone (AHL) (Myszka et al. 2016), whereas cinnamon bark oil inhibited the production of *P. aeruginosa* QS molecules (Kim et al. 2015). Also *cis-cis-p*-menthenolide extracted and isolated from *Mentha suaveolens* ssp. *insularis* acted as a quencher of the QS signaling pathway and altered the biofilm matrix of *Chromobacterium violaceum,* without affecting bacterial density (Poli et al. 2018). Therefore, the anti-QS action of EOs might be associated to i) the binding/interference of oil components to the signal receptor (Rathinam, Vijay Kumar, and Viswanathan 2017) ii) the biosynthesis inhibition of signaling molecules (Kumar et al. 2015), and iii) the degradation of signaling molecules (Bai A and Vittal 2014).

Another possible explanation for EOs anti-biofilm activity is their interference with fimbriae appendages involved in twitching motility and in microcolonies formation (Talagrand-Reboul, Jumas-Bilak, and Lamy 2017), as demonstrated by Kim et al. (2015) for *E. coli* O157:H7. During microcolonies formation, when the intensity of the cell chemical signal crosses a threshold, the attached cells also produce additional polymers, whose chemical characteristics can also influence the EOs anti-biofilm action and effect. In fact, some microorganisms produce cellulose as major polysaccharide while other produce alginate or polysaccharide intercellular adhesin as main component of their biofilms (Branda et al. 2005).

Moreover, EOs chemical components appear to up- or down-regulate the proteins involved in biofilm biosynthesis. For example, *trans*-cynnamaldehyde was able to inhibit the expression of two ribosomal proteins and the conjugal transfer nickase/helicase TraI involved in protein synthesis. Moreover, it down-regulated protective proteins like peroxiredoxin, essential in bacterial survival, and serine hydroxymethyltransferase which is implicated in biofilm matrix component production (Vasconcelos, Croda, and Simionatto 2018).

Maturation and dissemination

The mature biofilm with a three-dimensional structure, made of cells embedded in an extracellular matrix with water channels that facilitate the exchange of compounds, is generally more resistant than planktonic cells to anti-biofilm agents. Nevertheless, some active components of EOs can penetrate through the polysaccharide membrane of biofilm matrices thanks to their relative hydrophilicity and strong

antimicrobial activity. Essential oils primarily destabilize the biofilm structure, and once diffused, they damage and detach the sessile cells from the surfaces (dos Santos Rodrigues et al. 2017). Moreover, Millezi et al. (2013) observed that the treatment with *Thymus vulgaris* and *Cymbopogon citratus* EOs caused a breakdown of the EPS and the dispersion of cells of *Aeromonas hydrophila* biofilm on SS surfaces.

EOs could also affect the end of biofilm evolution when detachment and dispersion take place. In fact, beside environmental factors, also changes in bacterial systems (enzyme degradation, release of EPSs and protein, QS systems) potentially affected by EOs, can be involved in detachment.

Strategies to increase the anti-biofilm activity of EOs

Considering the high price and their difficult compatibility with industry applications, the reduction of EOs doses, without loosing an adequate anti-biofilm effect, could be achieved by an improvement of EOs activity or by combining multiple hurdles. In particular, experimental design could help to optimize the parameters of disinfection.

The possible strategies to increase the anti-biofilm effect of EOs can be pursued by the i) improvement of EOs stability by means of natural carriers, ii) formulation of blends containing different EOs, and iii) combination of EOs with common sanitizers or innovative strategies.

Improvement of EOs stability by natural delivery systems

Encapsulation of EOs in delivery systems can improve EOs stability and support a controlled and sustainable release, therefore extending their effectiveness during time. There are different types of delivery systems for EOs, but the application of nanotechnology is considered one of the most promising technologies. The increased surface-to-volume ratio of these nano-sized delivery systems improves their reactivity and provides an efficient absorption through cells, controlled release and targeting of bioactive compounds at the site of action (Reichling 2020). The solubility and thermal stability of encapsulated bioactive compounds can also be enhanced, and they can be protected against chemical, enzymatic, and physical instability (Sedaghat Doost et al. 2020).

Generally, the delivery systems are divided into three groups that are lipid, polymeric, and inorganic carrier systems. Among the lipid-based nanocarriers, we can mention nanoemulsion, liposomes, solid lipid nanoparticles, and nanostructured lipid carriers (Montes, Villaseñor, and Ríos 2019; Yousefi, Ehsani, and Jafari 2019) Emulsions have been extensively reported in scientific literature as delivery systems of EOs (Paparella et al. 2008; Acevedo-Fani, Soliva-Fortuny, and Martín-Belloso 2017; Grande-Tovar et al. 2018), and particularly EO-based nanoemulsions exhibit promising anti-biofilm activity (Prakash et al. 2018). A nanoemulsion is a dispersion of an immiscible liquid, dispersed in another liquid in the form of nanosized droplets (20-200 nm), where surfactants are used to reduce interfacial tension, thereby stabilizing the emulsion. Different approaches have been proposed for EOs nanoemulsions production, based on two classifications including top-down and bottom-up methods (Sedaghat Doost et al. 2020). Recently, Lou et al. (2017) evaluated the anti-biofilm potential of *Citrus medica* EO against *S. aureus* and demonstrated that the effect of nanoemulsion was more pronounced than the pure essential oil. Likewise, the nanoemulsion of *Zataria multiflora* EO decreased the pre-formed biofilm of *L. monocytogenes* and *S.* Typhimurium (Shahabi et al. 2017); in particular, the conversion of EO to nanoemulsion enhanced anti-biofilm properties, while the low efficacy of the pure EO was attributed to a phase separation. Also da Silva Gündel et al. (2018) observed a strong increase of the anti-biofilm activity of *Cymbopogon flexuosus* in nanoemulsion and a greater reduction in biofilm formation of *S. aureus* and *P. aeruginosa* with respect to the free EO. In contrast, no significant differences were observed between *Thymus daenensis* EO and its nanoemulsion against *Acinetobacter baumannii* biofilm (Moghimi et al. 2018).

Liposomes are simple microscopic vesicles, composed of one or several lipid bilayers entrapping a small volume of the aqueous phase. As a result of this structure, liposomes can incorporate either hydrophilic, hydrophobic, or amphiphilic molecules (de Matos, Lucca, and Koester 2019). In fact, the versatility of liposomes as carriers also for the delivery of the EOs compounds is well reported in literature (Trifan et al. 2020). Cui, Li, Li, Vittayapadung, et al. (2016) found that liposomes boost cinnamon EO anti-biofilm activity against *S. aureus* and hypothesized that the liposome-encapsulated EO could be slowly released, thus increasing bacterial cell damage and biofilm removal. The liposomes were also used to encapsulate proteinase K and *Thymus vulgaris* EO with the aim of improving chemical stability and obtaining a controlled release of these compounds (Cui, Ma, and Lin 2016b). In detail, the liposomes exhibited a longer duration of action and a higher anti-biofilm activity than free proteinase/EO against *E. coli* O157:H7 biofilms. The same EO (*T. vulgaris*) was studied by Perez et al. (2019), who formulated the EO into lipid nanovesicles, prepared from polar archaeolipids. The authors reached the same anti-biofilm effect at a lower concentration than EO emulsion, probably because of its low size and high structural stability.

With respect to the delivery systems based on nanostructured polymers, several morphologies including polymeric NPs, nanogels, micelles, nanocapsules, and vesicles are used (Venditti 2019). Biopolymers such as polysaccharides and proteins are commonly used to produce food grade delivery nanoparticles (Flamminii et al. 2020) using different preparation methods, including milling, ion gelation, coacervation, or supercritical fluid technology. The association of EOs with polymeric NPs displays many advantages and has been recently applied to active packaging formulations. In a study of Cui, Bai et al. (2018), the stability of clove EO was enhanced by loading it into chitosan NPs that were

incorporated within gelatin nanofibers and tested against *E. coli* O157:H7 biofilms. The nanofibres exhibited good stability, biofilm eradication effect in vitro, and satisfactory anti-biofilm activity on cucumber. Cui et al. (2017) also reported the use of chitosan nanoparticles (30-40%) to co-encapsulate D-amino acids (D-AAs) and nutmeg EO, and electrospun nanofibres to immobilize them in a packaging material. This nanofilm was used on soya bean products to significantly reduce attached bacteria and biofilm formation, with persistent resistance to biofilm formation.

Among the inorganic delivery system, inorganic nanoparticles are important as potential carriers for antimicrobials. They have an inorganic core (gold, silica, silver, iron oxide etc.), while their shells can be composed of organic polymers or metals (Ahmad et al. 2021). However, considering the limitations on synthesis of metal-based NPs with conventional methods (slow processes, high cost, environmental burden), plant-mediated synthesis could represent an interesting alternative. In fact, EOs may be associated with inorganic carriers for NPs green biosynthesis, where EOs biomolecules can act as reducing agents of metal ions in solution (Maciel et al. 2019). In a study on the potential of *Nigella sativa* EO coated gold NPs, Manju et al. (2016) observed the inhibition of *S. aureus* biofilm formation by decreasing the microbial hydrophobicity index. The authors hypothesized that the inhibition was due to the impairment of exopolysaccharides synthesis by metallic nanoparticles. More recently, Obeizi et al. (2020) investigated the anti-biofilm activity of ZnO NPs prepared by green synthesis route using *Eucalyptus globulus* EO against *S. aureus* and *P. aeruginosa*. The biosynthesized NPs exhibited significant biofilm inhibition rate against both microorganisms (85-97% with 100 µg/mL NPs), demonstrating a great potential for application in different fields.

Considering the above statements, the use of nanotechnology for EO-based formulations could represent a strategy for a successful biofilm control in food manufacturing.

Formulation of blends containing different EOs

EOs combination has been proposed as a strategy to potentiate the anti-biofilm effect of single EOs. In fact, different molecular structures of the active compounds of single EOs could promote varying mechanisms of action, resulting in a greater anti-biofilm activity. In this regard, the study of the EO chemical profile becomes important to increase the antimicrobial activity. The effectiveness of EOs in single application or in combination against the biofilms of *L. monocytogenes*, *S. aureus* and *E. coli*, has been studied (Vázquez-Sánchez, Galvão, Ambrosio, et al. 2018; Vázquez-Sánchez, Galvão, Mazine et al. 2018; Vázquez-Sánchez et al. 2019). The efficacy of binary combinations of *Lippia sidoides* and *Thymus vulgaris* EOs to completely eradicate 24-h-old *S. aureus* biofilms, using lower doses than those required by a single treatment, was reported. From the different investigations it is clear that the effectiveness of the blends depends not only on EOs synergism but also on contact time. Also Campana et al. (2017) observed the anti-biofilm effectiveness of *Cinnamomum cassia* and *Salvia officinalis* blend against *S. aureus* biofilm and desiccated ones up to 98% in 90 min. EO-based emulsions containing *Cymbopogon citratus* and *C. nardus* worked in synergism against *L. monocytogenes* biofilms (de Oliveira et al. 2010), reaching 100% of reduction in 240-h-old biofilm after 60 min of treatment. Furthermore, the application of *Lippia sidoides* and *Thymus vulgaris* in blends allowed a reduction of the concentrations needed to control *E. coli* biofilms (Vázquez-Sánchez et al. 2019). Moreover, by combining different EOs with distinct mechanisms of action, the activity spectrum of the blend can be enlarged (Lang et al. 2016).

Combination of EOs with common sanitizers or innovative strategies

Many reports showed enhanced biofilm removal through the use of EOs combined with antibiotics (Duarte et al. 2012; Coelho and Pereira 2013; Vitanza et al. 2019) and in general, the effect of EOs combined with innovative strategies or common sanitizers in foods is increasingly investigated. The development of EOs-based sanitizers for applications in the food industry could be an efficient and environmental-friendly strategy to reduce the dose of disinfectants and the risk of resistant strains. Recently, peracetic acid in combination with *L. sidoides* EO enhanced the efficacy of single treatments against *L. monocytogenes*, *S. aureus* and *E. coli* biofilms (Vázquez-Sánchez, Galvão, Ambrosio et al. 2018; Vázquez-Sánchez, Galvão, Mazine et al. 2018; Vázquez-Sánchez et al. 2019), probably thanks to the possibility to reach different cell targets or to modify the tolerance of bacteria to common sanitizers. The employment of sanitizing solution (1% NaOH and 0.5% Tween 80) containing *Mentha piperita* and *Cymbopogon citratus* EOs showed powerful sanitizing action against biofilm formation by *Salmonella enterica* biofilm on SS (Valeriano et al. 2012).

The combination of natural preservatives from plant and microorganism sources has been proposed by Iseppi et al. (2020). The authors investigated the effect of *Thymus vulgaris* EO, *Salvia officinalis* EO and bacteriocin bacLP17 alone and in combination against the mature biofilm formed by *L. monocytogenes*. The synergistic association between bacteriocin/EO led to the best anti-biofilm effect, compared to both control and the single use of the EOs.

Among the emerging green, non-thermal technology, Cui, Ma, and Lin (2016a) demonstrated a synergistic effect of clove essential oil combined with cold nitrogen plasma on the eradication of *E. coli* O157:H7 biofilms on lettuce. Regarding the application on food-contact surfaces, a reduction in *S. aureus* biofilm was observed after plasma treatment combined with *Helichrysum italicum* on containers and plastic surfaces (Cui, Li, Li, and Lin 2016), with a better biofilm dispersion in combined treatments. In addition, a greater effect was detected on the biofilms formed on plastic surface, probably due to the hydrophobic nature of this material.

Light-based antimicrobial technology is one of the innovative non-thermal antimicrobial strategies, including

the application of ultraviolet irradiation. The study of Silva-Espinoza et al. (2020), who investigated the combination of clove EO with ultraviolet light against *Salmonella* Thyphimurium biofilms, demonstrated a synergistic effect of the two combined treatments with a complete bacterial reduction (6.8 Log/cm^2) on biofilms.

In the light of the considerations shown up to this point, the synergy among treatments can be considered a promising strategy to improve the effect of EOs on bacterial biofilm.

Anti-biofilm action of hydrolates

Hydrolates or hydrosols are the co-products of essential oil extraction, and specifically the residual water of aromatic plants hydrodistillation, containing a small quantity of EO and secondary metabolites (D'Amato et al. 2018). The main and secondary components, which can coincide with those of their EOs, confer antimicrobial activity to hydrolates. Although their antimicrobial activity has been reported by several researchers (Ghavidel et al. 2018; Hay et al. 2018; Di Vito et al. 2019), the information regarding the anti-biofilm effects are still limited. In 2008, bactericidal effect of *Satureja thymbra* EO and its hydrosol was evaluated and compared to acid-base sanitizers (Chorianopoulos et al. 2008). These agents were tested against useful technological (*Staphylococcus simulans* and *Lactobacillus fermentum*), spoiling (*Pseudomonas putida*) and pathogenic bacteria (*Salmonella enterica* and *Listeria monocytogenes*), either as mono-species or as mixed-culture biofilms on SS. Interestingly, both EO (1% v/v) and its hydrosol (100%) were the most effective treatments against biofilms, as the activity of the acid-base disinfectants was lower than the natural sanitizers. Surprisingly, *S. thymbra* hydrolate revealed a strong action on bacterial biofilm, in most cases reducing sessile cells at levels below the detection limit as observed for the EO. The authors linked its efficacy to the bioactive compounds of *S. thymbra* EO (carvacrol and thymol), probably dissolved in the hydrosol during steam distillation. More recently, Karampoula et al. (2016) observed a complete eradication of *Salmonella enterica* serotype Typhimurium biofilms with *Thymbra capitata* hydrosol at 100% (v/v) concentration, displaying a total cell permeabilization throughout the biofilm in few minutes. The strong efficacy of *T. capitata* hydrosol was attributed to the high content of carvacrol, well known for its antimicrobial activity. The activity of hydrosols as natural washing sanitizers has been proved also in reducing pathogenic microorganisms on fresh-cut fruits and vegetables (Ozturk et al. 2016; Tornuk et al. 2011).

In contrast, recent studies highlighted low effectiveness of hydrolates with respect to EOs. In particular, Khalaf and Zahra (2020), in a study on the use of *Eucalyptus camaldulensis* EO and its hydrosol against biofilms of some Gram negative and positive bacteria, showed that the EO was highly effective against bacterial biofilms in comparison with the hydrosol. In addition, with respect to the antimicrobial efficacy, Garzoli et al. (2020) investigated the effect of nanoemulsion formulations of *Lavandula x intermedia* EO and its hydrolate against *Escherichia coli* and *Bacillus cereus*. The authors found no effect of pure hydrolate against the two bacterial strains compared to the crude EO form that exhibited bactericidal or bacteriostatic activity. However, hydrosol formulated in nanoemulsion became active against both microorganisms, with MIC values of 0.75 (v/v%) for *E. coli* and 0.06 (v/v%) for *B. cereus*. Therefore, if this effectiveness will also be confirmed on microbial biofilms, nanoemulsion as a delivery system could be an effective formulation strategy to increase the activity of both EOs and hydrolates in an anti-biofilm application.

Hydrosols may provide an alternative in biofilm control on food and food-contact surfaces because, as aqueous solutions, they could be easily applied and removed from surfaces, do not have a strong sensory impact, and are normally less expensive than EOs. Furthermore, hydrolates could be used in combination with EOs or other sanitizing agents to explicate synergistic or additive effect. However, the hydrosol application has been not much considered until now and further researches are needed to establish the potential of these promising natural compounds.

Conclusions, challenges and future perspectives

In the last years, studies on green strategies to reduce the microbial growth have been intensified, revealing the potential role of the essential oils as new anti-biofilm agents against food spoilage and pathogenic bacteria. The anti-biofilm effect of EOs is the results of combination of different factors including essential oil composition (interaction among all components) and biofilm characteristics (involved bacteria, stage of formation). Moreover, microbial susceptibility against EOs is species- and strain- dependent, and strictly related to the employed concentration.

In summary, this review presents evidence that EOs may be considered an environmentally friendly approach to control biofilms in the food industry, targeting both spoilage and pathogenic microorganisms.

The present study discloses important information on EOs effectiveness in controlling bacterial biofilms on food-contact surfaces such as plastic and SS materials, and highlights how pre-formed and mature biofilms are more resistant to EOs compared to planktonic cells and biofilms in the early stages of formation. Consequently, inhibition of biofilm formation can be considered the first strategy to control biofilms in the food industry. On the other side, the removal of an already formed biofilm is certainly more important but yet more difficult to achieve. Moreover, the kind of surface on which the biofilm is developed is an important factor in assessing the anti-biofilm effect of an EO.

The mechanisms by which EOs inhibit and remove microbial biofilms are diverse and not completely clear. EOs presumably act by damaging planktonic and sessile cells, interfering with cell physiological characteristics, inhibiting QS mechanisms, and interacting with the EPS matrix. Even though we have garnered information that allows a better understanding of the EOs anti-biofilm mechanisms, several

efforts are still required. In fact, further studies are needed to open up a range of perspectives for EOs use as anti-biofilm agents in food industry, as given below:

1. studies on the EOs mechanisms of action to increase efficiency and effectiveness of the treatments;
2. potential resistance to EOs, although the large number of antimicrobial components in the phytocomplex represents a hurdle to bacterial cell adaptation;
3. toxicological issues to ensure EOs application in food manufacturing, in particular concerning EOs safety, allergenic potential, and mutagenic capability;
4. possible synergism among different EOs and combination of different hurdles, to favor applications at lower EOs doses with interesting economical and technological implications;
5. preparation of scientific dossiers to allow the registration of selected EOs as biocides for the surfaces treatment in the food industry, also collecting data on EOs stability to quantify the residual activity and maximize the effectiveness in working conditions;
6. finally, also studies on the EOs anti-biofilm potential in future distribution conditions are extremely important, considering the search for plastic-free alternatives for packaging and food contact materials, where the incorporation of EOs could prevent biofilm formation by spoilage and pathogenic microorganisms.

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No potential conflict of interest was reported by the authors.

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