Semi-synthetic compounds as antimicrobial agents in food preservation

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In the last years, the number of papers describing new strategies to the synthesis and chemical modification of natural occurring compounds has exponentially increased. The synthesis of semi-synthetic compounds allows improving the biological activities presented by the natural, unmodified, molecules. A class of substances of natural source that has been the target of several research groups in pharmaceutical and food sciences is the essential oils. This complex mixture of volatile compounds presents antimicrobial activity by disrupting the microorganism's cell membrane, resulting in the inhibition of the electrons transport, affecting the protein translocation, the phosphorylation and other enzymatic activities, which causes the destruction of the cell membrane integrity, culminating with the microbial death. Semi-synthetic compounds can be used as an alternative to the synthetic antimicrobial agents largely used in the food industry. These compounds can be directly added in the food or incorporated in the food packaging, affording an active package, where the diffusion of the antimicrobial agent from the package to the food surface occurs in a controlled mode. Alternatively, the active molecules can be adsorbed to the covering or films used in food. The final goal in using semi-synthetic compounds is to extend the shelf life and/or to ensure the food safety. The use of natural occurring feedstock, such as essential oils and their constituents, in the synthesis of new semi-synthetic molecules is in agreement with the green chemistry. This is a new philosophy, which aims to minimize the collateral effects caused by the chemical activity to the environment. In this chapter, we will discuss the synthesis, antimicrobial properties, mechanism of action and the applicability of semi-synthetic antimicrobials in food conservation. We have analysed and critically discussed around 60 articles covering the last improvements on the field.

Keywords: green chemistry; food preservative; antimicrobial; semi-synthetic; natural compounds

1. Introduction

Foodborne diseases represent a worldwide public health problem, and annually affecting around 30% of people in developed countries [1]. Most of the foodborne diseases is caused by the food contamination by pathogenic microorganisms or by their toxins and aiming to control the microorganism proliferation, the food industry uses mainly synthetic antimicrobial agents [2]. However, the use of these products is limited, once they are associated to several undesired toxic effects, including carcinogenic and teratogenic ones. Besides, synthetic antimicrobial agents generally have a low degradation in the environment [3]. Consequently, there is an increasing demand for natural occurring products as an alternative to the synthetic food preservatives [4]. Besides, in the last two decades, the consumer's preference for minimally processed foods over processed ones has increased and it made the industry turn on the yellow light regarding the amount and identity of the additives added to the processed food [5,6].

1.1 Essential Oils

Many of the naturally occurring compounds present in plants have antimicrobial activity [7]. Most of these compounds are secondary metabolites, of which phenols and their derivatives substituted on the aromatic ring are the most representative [8]. Besides phenolic derivatives, quinones, saponins, flavonoids, tannins, coumarins, terpenoids and alkaloids are responsible for the antimicrobial activity of plants [9]. Alterations on the chemical composition of and in the structure of the natural compounds result in changing the antimicrobial activity [10]. Essential oils (EOs) are natural compounds whose the antimicrobial activities have been widely demonstrated [11-13].

EOs are aromatic oily liquid obtained from different parts of plants (leaves, bark, flowers, rhizomes, etc.) which are constituted by a mixture of compounds such as terpenes, alcohols, ketones, phenols, esters and aldehydes [14]. The antimicrobial activity of EOs is related to the presence of volatile bioactive phenolic compounds, such as carvacrol and eugenol and terpenoids like menthol and 1,8-cineol [15]. These compounds present antimicrobial activity against several pathogenic microorganisms of importance in foods, such as *Escherichia coli* O157:H7, *Salmonella* Typhimurium, *Listeria monocytogenes*, *Staphylococcus aureus*, *Bacillus cereus* and *Shigella dysenteriae*, at concentrations of 0.2 to 10 $\mu L.mL^{-1}$ [12].

The effectiveness of the antimicrobial action of an EO depends on the chemical structure and concentration of their active constituents as well as the number and identity of the microorganisms present in the sample. Besides, the antimicrobial effect is influenced by the pH, once the lower the pH, the greater the hydrophobicity of the EO, which facilitates its penetration in the plasmatic membrane of the microorganism cell, increasing its efficacy [12].

1.2 Extraction of EOs

EOs can be extracted from the plant by several physic methods, including dry distillation, expression or cold pressing (for citrus EOs), hydro-distillation and steam distillation, this being the most used method for commercial purposes [16,17]. These traditional methods to extraction of EOs however, can lead to loss of some of their components and to degradation of some unsaturated compounds by thermal effects or hydrolysis. Besides, methods that use organic solvents in the extraction of EOs can contaminate it with toxic residuals [18]. Another point to be considered is the influence of the extraction methods on the sensorial profile of the EO. The chemical profile of the EOs vary with the used method to obtaining it and this can influence their biological properties as, for example, its antimicrobial effect [12,19].

Taking in consideration the disadvantages of the traditional extraction methods, several research groups on natural compounds have studied ways to improve and optimize the existing extraction technics, aiming to develop greener procedures to access these valuable products. The development of new methodologies that are economic, sustainable, efficient and environmentally friendly are pivotal nowadays, considering the large amount of toxic wastes generated and also the high demand for energy in the chemical industry. Some examples of these "new technologies" include the use of alternative solvents obtained from natural source or supercritical carbon dioxide and even solvent-free protocols using microwaves and ultrasound as non-classical energy sources. By using these new approaches, EOs with excellent quality and safe can be obtained [20-23].

1.3 Chemical Composition of EOs

As mentioned in the Section 1.1, EOs are a complex mixture of volatile organic compounds, which are produced in the secondary metabolism of aromatic plants. Two photosyntheses pathways can be involved in the biosynthesis of the components of the EOs: the multiplication isopentenyl pyrophosphate C_5 to its adducts and the shikimic acid biosynthesis, which is the responsible for the formation of the aromatic components of EOs. Consequently, the constituents of EO can be classified in two groups: hydrocarbons (mono, sesqui and diterpenes) and oxygenated (phenols, esters, ketones, aldehydes, alcohols, etc.) [17].

Gas chromatography coupled to mass spectrometry (GC-MS) data have shown that an EO can contain dozens or even hundreds of components at different concentrations. However, the aromatic properties of some EOs are determined by only few of their components, even at low concentrations or trace amounts [24-27]. There is a general understanding that the phenolic compounds are the main responsible for the antimicrobial activity of the EO. However, the minority components are also protagonist in the whole EO's activity and possible there is a synergic effect between their minor and major components [12,28].

2. Synthesis of New Semi-Synthetic Antimicrobial Agents from EOs

Currently, most of the starting materials used in organic synthesis are petroleum-based, i.e., the chemical industry deals mainly with non-renewable, toxic, inflammable feedstock, which are novice to the human health and the environment. As a response to the worldwide concern about the depletion of the non-renewable natural resources, many research groups on organic synthesis have faced the problem searching for natural, bio-based alternatives to those obtained from petroleum. In this sense, EOs and their constituents have been explored regarding their potential as starting material to bioactive chemicals [29,17]. The use of natural compounds in organic synthesis meets several of the Green Chemistry Principles, and more specifically Principles # 5, use of safer solvents and auxiliaries, and #7, use of renewable raw material (biomass) in substitution to the non-renewable ones (petroleum derivatives) [30].

The volatile components of the EO (e.g. linalool, eugenol, limonene, etc.) are essentially terpenic and phenolic derivatives with low molecular weight (< 350 g mol^{-1}). Due the presence of unsaturated bonds, functional groups (carbonyl, alcohol, epoxide, and aromatic) and asymmetrical centers, these are very interesting starting materials to functional group modifications [17].

EOs and their components are known for presenting several biological activities, such as antibacterial [31], antioxidant [17], insecticidal [32], antiviral [33], antifungal [34], among others, which out them as potential natural food preservative candidates [35]. However, due their volatility, EOs and most of their constituents alter the food sensorial characteristics, such as taste and smell, and may exceed its acceptable aroma threshold [36-37].

Combined, these aspects of EOs and their components have inspired several research groups around the world to modify the chemical structure of the major components of EOs aiming to improve their biological activity while reducing their volatility, aiming thus to obtain semi-synthetic antimicrobial compounds which could be employed in food preservative.

Chakravorty et al. [38] described the synthesis and evaluated the antimicrobial activity of natural occurring (*Z*)- and (*E*)-hex-3-en-1-yl nonanoate **1a** and **1b** and of two semi-synthetic analogues, *n*-hexanyl nonanoate **1c** and (*Z*)-hex-2-en-1-yl nonanoate **1d** (Figure 1). The (*Z*)- isomer **1a** is the major component (16 %) of the EO of *Heteropyxis natalensis* leaves, a deciduous tree endemic to South Africa. The authors observed a good antimicrobial activity for all the tested esters with the natural (*E*)-hex-3-en-1-yl nonanoate **1b** being slightly more active for most of tested microbes.

Noteworthy, the chemically modified (*Z*)-hex-2-en-1-yl nonanoate **1d** was the more active against *Enterococcus facealis* ATCC 29212 (MIC= 1.06 *vs.* 1.25 mg mL^{-1} for ciprofloxacin), while the saturated ester **1c** was a potent inhibitor of *Pseudomonas aeruginosa* (MIC= 0.49 *vs.* 0.25 mg mL^{-1} for ciprofloxacin).

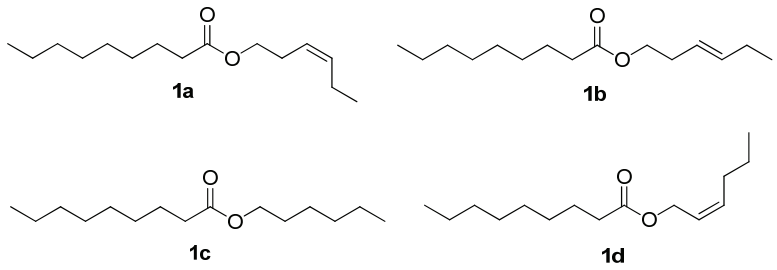
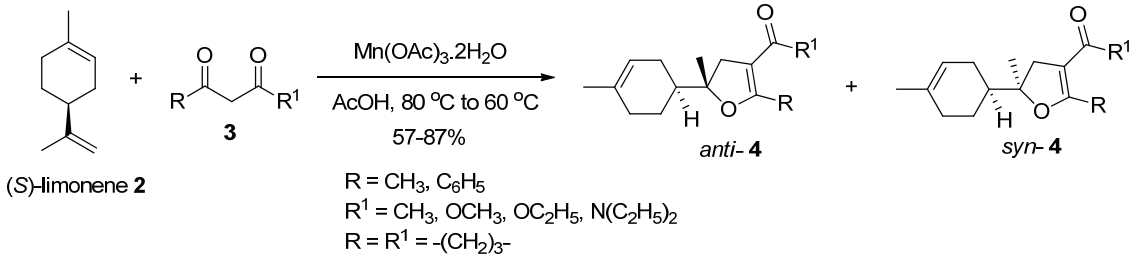


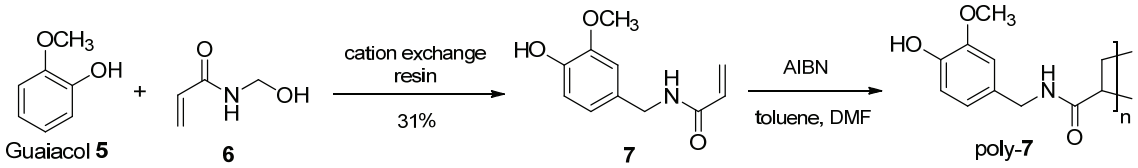
Fig. 1 Natural (**1a-b**) and semi-synthetic (**1c-d**) compounds from *Heteropyxis natalensis* EO.

Findik et al. [39] synthesized several 4,5-dihydrofuranes derivative from limonene **4** by the $Mn(OAc)_3$ mediated radical cyclization of 1,3-dicarbonyl compounds with *S*-(-)-limonene **2** in the presence of acetic acid (Scheme 1). The authors decided to explore the effect of the chemical modification in limonene in its already described antimicrobial activity. The antimicrobial activity of the mixture of *anti* and *syn*-tetrahydrofurans **4** was evaluated by the disc-diffusion technique against 12 different human pathogen microorganisms (two yeasts, five Gram-negative and five Gram-positive bacteria). All the tested compounds presented good antimicrobial activity which compound derivative from cyclohexane-1,3-dione (**3**, R=R^1 = -$(CH_2)_3$-) being the more active, comparable to the control [Sulbactam (30 μg) + cefoperazona (75 μg)].



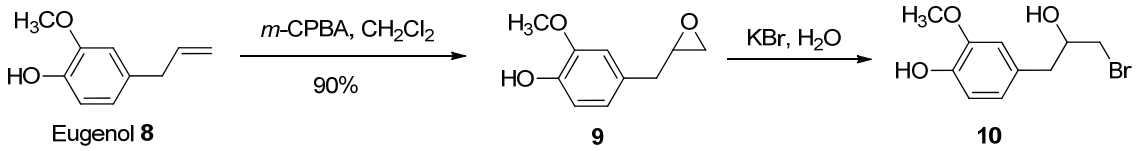
Scheme 1

Liu et al. [40] described the synthesis of new guaiacol-based polymers (poly-**7**) and tested their anti-biofilm activity against *Bacillus subtilis*. Guaiacol **5** (or 2-methoxy phenol) is present in a sort of EOs and it was used to prepare a new acrylamide-type monomer [*N*-(4-hydroxy-3-methoxy-benzyl)-acrylamide] **7**, which was polymerized by a radical reaction in the presence of AIBN. To prepare *N*-(4-hydroxy-3-methoxy-benzyl)-acrylamide **7** the authors used the Friedel-Crafts reaction between guaiacol **5** and *N*-hydroxymethylacrylamide **6** in EtOH and in the presence of a cationic exchange resin (Scheme 2). The polymer could not be tested directly against the bacteria due the low water-solubility; however, anti-adhesion test showed a high activity of poly-**7** against bio-adhesion of *B. subtilis*, with a strong anti-biofilm activity. The authors suggested that poly-**7** could be potentially used in the preparation of antibacterial materials.



Scheme 2

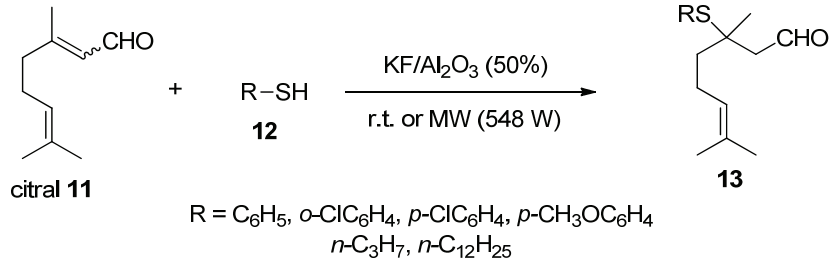
Eugenol **8** is the major component of *Eugenia caryophyllata* (clove) plant EO and the responsible for most of their biological activities, including antibacterial one. Eyambe et al. [41] recently studied the synthesis and the antimicrobial activity of two derivatives of eugenol, namely epoxy-eugenol **9** and 3-bromo-2-hydroxy eugenol **10** (Scheme 3). The antimicrobial activity of **9** and **10** against *S. aureus* ATCC 25923 was evaluated and compared to that of eugenol **8** and the doxycline (the control). The authors observed that epoxy-eugenol **9** was more active than the precursor eugenol 8, while the bromo derivative **10** presented the same activity than eugenol. Values of MIC and MBC for compound **9** were of 57 and 115 μg mL^{-1} *vs.* 1.2 and 2.4 μg mL^{-1} for the control and 115 and 230 μg mL^{-1} for eugenol.



Scheme 3

Organic compounds with at least one C-Ch bond (Ch = sulfur, selenium or tellurium) are known as organochalcogen. In recent years, this class of compounds has attracted the attention of organic synthetic chemists and biochemists due to their synthetic versatility and also their interesting biological properties, including antioxidant activity [42-43].

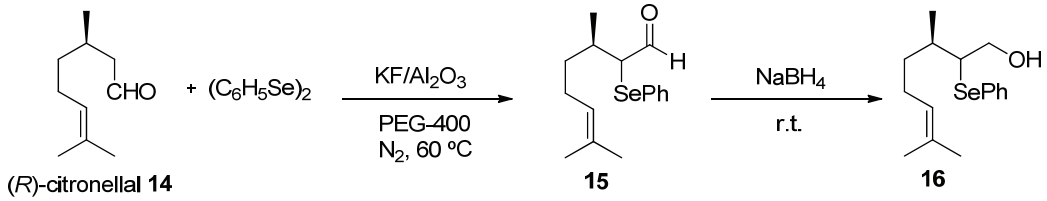
The strategy of combining the biological properties of natural compounds with those of organochalcogen ones was explored by Lenardão et al. [29] in the synthesis of a new class of compounds derivative from citronellal. By reacting citral **11**, the main component of *Cymbopogon citratus* (lemon grass) EO with a series of thiol **12** in the presence of the solid supported base KF/Al_2O_3 , the authors prepared the Michael adducts 3-organylthio citronellal **13** in good yields (Scheme 4). Besides starting from a natural compound, the authors used a green procedure to prepare the desired product, in the absence of solvent and under irradiation with microwaves to accelerate the reaction. These features turned the synthesis even greener than those previously discussed in this section. In a preliminary screening for the antimicrobial activity, the new semi-synthetic compounds **13** shown better activity than the precursor citral **11** and the unsubstituted (*R*)-citronellal **14** [29].



Scheme 4

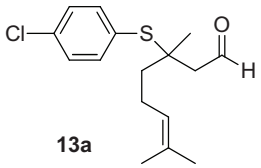
Victoria et al. [44] described in 2012 a study on the antimicrobial activity of α-phenylseleno citronellal **15** e 2-phenylseleno citronellol **16**, which were easily prepared from the natural terpenoid (*R*)-citronellal **14**, the major constituent of the EO of *Cymbopogon nardus* or citronella (Scheme 5). The synthesis was achieved by using PEG-400 in the presence of KF/Al_2O_3 [45]. This clean procedure, similarly to depicted in Scheme 4 for the 3-organylthio-citronellal **13**, allowed the synthesis of functionalized semi-synthetic organochalcogen compound in good yield and in a simple way. The system solvent/solid base could be reused for up four successive reactions with good performance, increasing the greenness of the reaction.

It was observed that the insertion of a phenylselenium group in the molecule of citronellal increased its antimicrobial activity, with good results observed for the Se-citronellal **15** against three economically important pathogenic food-occurring bacteria (*L. monocytogenes, S. aureus* e *S.* Typhimurium).



Scheme 5

More recently, Goldbeck et al. [46] evaluated the antibacterial activity of 3-(*p*-chlorophenyl)thio citronellal **13a** (Figure 2), obtained according the Scheme 4, using citral **11** and *p*-chlorobenzenethiol **12a** (R= *p*-Cl-C_6H_4) as the starting materials [29]. The possible action mechanism and cell targets were investigated and the authors observed that compound 13a is a potent antimicrobial agent, being more effective against Gram-positive bacteria. *S.* Typhimurium, *S. aureus*, *S. dysenteriae*, *L. monocytogenes*, *E. coli* and *P. fluorescens* were subjected to the action of citral **11**, (*R*)-citronellal **14** and 3-(*p*-chlorophenyl)thio citronellal **13a**. It was observed that cell death was faster when the bacterial cells were treated with the semi-synthetic compound 3-(*p*-chlorophenyl)thio citronellal. Tests *in vitro* showed no acute toxicity. SEM images showed cell damage with the formation of pores in the cell wall and membrane, which are possibly the cellular targets of the compound **13a**.



13a

Fig. 2 3-(*p*-Chlorophenyl)thio citronellal **13a**.

3. Mechanism of Action of Semi-synthetic Compounds in Bacteria Cells

Despite many studies describing the potential of using semi-synthetic compounds as food preservative, their use *in vivo* is not allowed. This restriction occurs mainly to the safety rules required by the USA- Food and Drug Administration (FDA). Among the difficulties in using these compounds are the limited number of studies on their action mechanism and cellular target combined with the fact that not all compounds bioactive *in vitro* present the same antibacterial activity when added to the food [46].

With the aim to change this picture, recently some studies have been conducted to elucidate both the action mode and the target of these compounds in bacterial cells. One of the strategies to access the action mode of a new bio-preservative semi-synthetic molecule is through the analysis by similarity on the molecular weight, density or volatility with antibiotics used in the bacteria control. Thus, by analogy with the biochemical and genetic mechanisms that are triggered when the bacterial cells under study come in contact with an antibiotic of known action, it is possible to predict the mechanism of the novel semi-synthetic compound [47-48]. By monitoring metabolic pathways as well as characterizing and quantifying genes and proteins expressed during and after the exposition to the new molecule, it is possible to predict the local of action and the target of the compound.

There are some studies, which report that, the initial passage of the semi-synthetic compounds to the microbial cell interior seems to be by a passive diffusion of the active molecule through the cell wall of Gram-positive or the external membrane of the Gram-negative bacteria [49]. These are typically lipophilic compounds and are capable of crossing the cytoplasmic bacterial cell membrane increasing its permeability by damaging the structure of the diverse layers of polysaccharides, fatty acids and phospholipids [50].

The current information on the action mechanism of organochalcogen compounds is that Gram-positive bacteria are more sensitive to them than Gram-negative ones. This finding corroborates the evidence that, possibly, the cell wall and the cytoplasmic membrane are the main target of these new bio-preservative agents [12,51-54]. The action of these semi-synthetic compounds can promote the disruption of the peptidoglycan layer, altering the cytoplasmic membrane, changing its permeability and releasing cellular constituents. Besides, the membrane dysfunction can influence in the electron transportation, the nutrients absorption and in the synthesis of nucleic acid [55-56].

The transmission electron microscopy (TEM) and the scanning electron microscopy (SEM) are important tools to visualizing any damage in the cell wall and in the cytoplasmic membrane. In this case, it is possible clearly observe the formation of pores in the cell wall with extravasation of intracellular material [57]. Other relatively simple way to access damage in the cytoplasmic membrane is by measuring the crystal violet uptake; the greater the uptake, the greater the membrane damage [57-58].

It is important to highlight that despite the addition of an organochalcogen moiety in the natural molecule does not alter the action mechanism, the inhibitory activity increases. A possible explanation to an increase in the bioactivity could be the interaction between the organochalcogen molecule and sulfur-containing amino acids present in the cell wall and in the cytoplasmic membrane of the bacteria, resulting in the formation of Ch-S (Ch= chalcogen) bonds and destabilizing the cell wall and the cytoplasmic membrane [46].

Additionally, other possible cellular targets have been investigated, such as damage to membrane proteins, cytoplasm coagulation and depletion of the driving force; however, there are no information enough on these interactions [57].

4. Semi-synthetic compounds as Food Preservatives

Studies on the use of semi-synthetic compounds as food preservative are rare. However, in view of the higher bioactivity showed by the chemically modified compounds compared to their parent ones, there are reasons to believe that they could be a viable alternative to the synthetic preservatives. In this sense, our group have been working in the last years to collect more information on the synthesis and the action mechanism of these new compounds as an additive in food.

The use of EOs in food has already been explored. It was observed that the presence of intrinsic factors in the food, such as water activity, fat content, the presence of proteins and enzymes and pH contribute to decrease the EOs efficacy [59]. Besides, the incorporation of the EO in food is limited, once sometimes are necessary high doses to achieve antimicrobial effect, which might impair the sensorial acceptance of the product [60]. Another critical point is the hard dispersion of the EO in aqueous products [61]. To circumvent these limitations some studies have shown the possibility

to incorporate EOs in packages [62-63], which allows a controlled releasing of the antimicrobial agent during the food storage, i.e. the formation of a bioactive package [64]. Another alternative to facilitate the use of EOs as a food preservative is by means of their encapsulation. Recently, Pan et al. showed that encapsulated thymol is more effective than free thymol in inhibiting pathogenic microorganisms [65].

EOs can also be used in combination with other methods of preservation, which can contributes to increase its antimicrobial activity. In this sense, Friedly et al. observed that the amount of citrus EOs necessary to inhibit *Listeria* was diminished tenfold when the EO was used combined with organic acids [59]. A similar increase in the antimicrobial activity of citrus EO was observed by Espina et al. against *E. coli* and *L. monocytogenes* [66]. In this case, the authors performed a simultaneous mild heating (54 ^{o}C for 10 min).

5. Conclusions

The utilization of compounds of natural source, like EOs or their semi-synthetic derivatives, is a potential alternative to the synthetic preservatives, widely used by the food industry. Despite a number of studies have shown the antimicrobial activity of EOs, it is due especially to their phenolic compounds, which are volatile. The chemical modification of the EOs' constituents is an efficient tool to enhance their bioactivity and potential use as food preservatives. The cell wall and cytoplasmic membrane are among the targets of EOs and their semi-synthetic derivatives, being the Gram-positive bacteria more sensitive than the Gram-negative ones. Even if the cellular target and action mechanisms of EOs and the semi-synthetic compounds are close related, the latter are generally more active. The use of EOs and their semi-synthetic derivatives as greener food preservatives has a promising future, especially in the composition of bioactive packages.

Acknowledgements The authors are grateful to FAPERGS/CNPq (PRONEM 11/2026-4) and CAPES for financial support.

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