

# Plant nutraceuticals as antimicrobial agents in food preservation: terpenoids, polyphenols and thiols

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## Abstract

Synthetic food additives generate a negative perception in consumers. Therefore, food manufacturers search for safer natural alternatives as those involving phytochemicals and plant essential oils. These bioactives have antimicrobial activities widely proved in *in vitro* tests. Foodborne diseases cause thousands of deaths and millions of infections every year, mainly due to pathogenic bacteria as *Salmonella* spp., *Campylobacter* spp., *Escherichia coli*, *Bacillus cereus*, *Listeria monocytogenes* or *Staphylococcus aureus*. This review summarizes industrially interesting antimicrobial bioactivities, as well as their mechanisms of action, for three main types of plant nutraceuticals, terpenoids (as carnosic acid), polyphenols (as quercetin) and thiols (as allicin), which are important constituents of plant essential oils with a broad range of antimicrobial effects. These phytochemicals are widely distributed in fruits and vegetables and are really useful in food preservation as they inhibit microbial growth.

**Keywords:** antibacterial, terpenoid, polyphenol, thiol, essential oil.

## Antibacterial activities of plant nutraceuticals

A wide range of synthetic preservatives and antibacterial physical treatments are used to extend food shelf life by inhibiting bacterial growth. Also, some foods require special protection against microbial spoilage during their preparation, storage or distribution in order to increase their shelf life and organoleptic properties, avoiding microbial spoiling, which usually changes taste, odor, color and sensory or texture food properties [1]. Presence of specific microorganisms such as *Listeria monocytogenes*, *Escherichia coli* O157:H7, *Salmonella* spp., *Staphylococcus aureus*, *Bacillus cereus*, *Campylobacter* spp. or *Clostridium perfringens* does not only affect

food quality, as they constitute a hazard for human health, causing food-borne diseases [2,3]. Actually, food-borne diseases are an increasing worldwide problem in public health. As an example, it is estimated that 31 pathogen species are responsible for 9.4 million cases of food-borne diseases each year only in the USA [4].

Food antimicrobials are chemical compounds that are naturally present in food or are directly added in order to inhibit microbial growth of pathogenic or spoilage microorganisms, with the aim of ensuring food safety and quality [5]. Approved food antimicrobials are classified as chemical preservatives, a category that also includes other type of agents such as antioxidant compounds, whose objective is to delay food spoilage [5]. Various synthetic antimicrobials, including several organic acids and salts (sodium benzoates and propionates, potassium sorbates, sorbic acid, sulfites, chlorides, nitrites, triclosan, nisin, natamycin, potassium lactate, ascorbic acid, citric acid, tartaric acid, etc.) have been approved by regulatory agencies and are used as food preservatives [3]. The use of some of these, however, represents nutritional or health threats for the consumer. For example, sulfites cause degradation of vitamin B1 (thiamine) in food, an essential nutrient [6].

Apart from chemical antimicrobials, food can go through different physical processes which are classified as thermal and non-thermal treatments. Thermal technologies are the most widely used preservation methods in food industry due to their high efficacy. However, the intensities needed to achieve high security levels generate undesirable changes in the sensorial and nutritional properties of some foods [7]. On the other hand, non-thermal technologies for food preservation, such as pulsed electric fields (PEFs) and high hydrostatic pressure (HHP), are really interesting since they do not affect food organoleptic properties. Nevertheless, under certain circumstances, these techniques are not able to ensure food safety, since some experiments have demonstrated that these physical treatments can lead only to sub-lethal damages in the bacterial cell walls [8][9,10].

Moreover, there is an increasing rejection among consumers on the use of synthetic additives, as well as a demand for a better food quality, free of artificial preservatives, but maintaining its long shelf life. For all these reasons, research has focused on finding natural alternatives to traditional solutions [11]. A good natural antimicrobial has to fulfil some requirements such as: (a) to be active in low concentrations in its natural form, (b) to be inexpensive, (c) not to generate sensorial changes in the product, (b) to inhibit a wide range of spoilage and pathogenic microorganisms, and (e) not to be toxic [5].

### **Terpenoids and essential oils**

Essential oils, also known as volatile odoriferous oils, are natural, volatile and complex liquids characterized by an intense smell and flavor which varies depending on the type of constituents that form the oil. They are generated by aromatic plants as secondary metabolites, especially by plants generally located in warm areas such as tropical and Mediterranean ones, where they represent an important part of the traditional pharmacopoeia. Many plants produce these volatile oils in order to attract specific insects for pollination or to expel certain predator animals. Its chemical constituents play also an important role as signal compounds and

growth regulators (phytohormones) of plants [12]. Essential oils can be synthesized by all the organs of a given plant (e.g. buds, flowers, leaves, stems, seeds, fruits and wood), and can be stored in secretory cells, cavities, epidermic cells or glandular trichomes. They can be extracted from these plant organs in various ways, but among all the extraction methods, steam distillation, first developed in the Middle Age by Arab chemists, is the most widely used, especially for commercial scale production [1,13].

Essential oils are complex mixtures of both polar and non-polar components that can even include 20-60 compounds in quite different concentrations. However, they are characterized by the presence of two or three main components that are found in relatively high concentrations (20-70 %) in comparison with other ones that are in trace amounts [13]. Components contained in essential oils can be divided into two groups, each with a different biosynthetic origin, but both of them are characterized by a low molecular weight: the main groups are terpenes (monoterpenes and sesquiterpenes) and terpenoids (monoterpenoids). In fact, monoterpenes (C<sub>10</sub>), which are made up by the fusion of two isoprene units (C<sub>5</sub>), are the most representative molecules in essential oils, achieving a percentage of up to 90 %. Depending on the number of isoprene subunits, different terpenes subfamilies are defined as hemi- (C<sub>5</sub>), mono- (C<sub>10</sub>), sesqui- (C<sub>15</sub>), di- (C<sub>20</sub>), sester- (C<sub>25</sub>), tri- (C<sub>30</sub>), tetra- (C<sub>40</sub>) and polyterpenes (C<sub>5</sub>)<sub>n</sub> with n higher than 8 units [12]. Other less abundant components in essential oils are aromatic and aliphatic compounds as aldehydes, phenols and methoxy derivatives [14]. Medicinal plant parts as roots, leaves, branches, stems, barks, flowers and fruits are commonly rich in terpenes like carvacrol, citral (a natural mixture of geranial and neral), linalool, geraniol and many others [15].

Essential oils are known since ancient times by their aroma, as well as by their antiseptic medicinal properties (i.e. bactericide, fungicide and virucide). In fact, essential oils carry out a key function in plants defense, working as antibacterial, antifungal and antiviral agents. They even work as a defense against herbivores, as they reduce their appetite for plants containing these compounds [13]. Despite the fact that essential oils have originally been added to food in order to change or improve flavors, their antimicrobial activities make them good candidates to replace chemical preservatives [16].

Antimicrobial activities of essential oils make them good candidates to be used as natural additives in foods and food products, as they can be added as bioactive components in packaging materials [1]. Currently, more than 3,000 essential oils are known, with 300 of them having a commercial interest in food, pharmaceutical, sanitary or cosmetic industries. In fact, different essential oils terpene components (e.g. linalool, thymol, carvone, carvacrol, citral and limonene) from a total number of 30,000 described molecules, have been accepted by the European Commission as flavorings for food products. These components have also been recognized by the FDA (Food and Drug Administration, USA) as GRAS ingredients (Generally Recognized as Safe) [14].

Due to the wide range of constituents that make up essential oils, several cellular targets have been described for antimicrobial activity (Fig. 1), and they are effective against a great variety of microorganisms, including bacteria [17], virus [18] or fungi [19] (Table 1). Terpenoids are active against a broad spectrum of microorganisms, with carvacrol (a monoterpenoid phenol) (Fig. 2) as one of the most active components [14]. However, other common terpenes, such as *p*-cymene (Fig. 2), lack a high antimicrobial activity, and many *in*

*vitro* tests have shown that some terpenes are inefficient as antimicrobials, when used as sole compounds [20]. Antimicrobial activity of terpenoids is related to their functional groups. Specifically, in phenolic terpenoids, hydroxyl groups as well as the presence of delocalized electrons carry out an important function against microorganisms [14]. In fact, if the carvacrol hydroxyl group is substituted by a methyl ether group, a change that affects its hydrophobicity, this affects also its antimicrobial activity, because this modifies how this molecule interacts with the microbial cell membrane. The reason for this is that carvacrol hydroxyl group has been proposed to function as a monovalent cations carrier across membranes, carrying H<sup>+</sup> into the cell cytoplasm and transporting K<sup>+</sup> back out [21].

As typical lipophilic substances, essential oils are able to cross the cell wall and the cytoplasmic membrane, with a different effect on Gram-positive and Gram-negative bacteria. Lipophilic ends of lipoteichoic acids in Gram-positive cell walls facilitate penetration of hydrophobic compounds such as essential oils in these bacteria. However, Gram-negative bacteria show higher resistance to the action of essential oils, associated to the presence of the outer membrane. This higher resistance could be attributed to the outer membrane proteins or to its lipopolysaccharides, which may limit the diffusion rate of these hydrophobic compounds [1].

Essential oils are also able to disrupt cell wall and cytoplasmic membrane structures by affecting the conformation of their different polysaccharides (Fig. 1), fatty acids and phospholipids layers, increasing their permeability. Damage to these two structures is associated with ions leakage, reduction of membrane potential, proton pumps collapse, ATP pool depletion and loss of macromolecules. All these events lead to an impairment of essential processes in the cell and finally to its lysis [22]. Essential oils can also coagulate the cytoplasm as well as cause direct damage to cellular lipids and proteins [23] (Fig. 1).

There are other antibacterial mechanisms for essential oils that are not still completely understood, as inhibition of bacterial essential specific enzymes. A clear example of this is FtsZ protein (from "Filamenting Temperature Sensitive strain Z"), which is a promising target because of its key role in bacterial division. For example, the sesquiterpene germacrene D (Fig. 2) interacts with FtsZ binding pocket, so it could be an important natural preservative [24].

Until now, due to the fact that essential oils are able to affect diverse cell targets at the same time, particular resistances or bacterial adaptations have hardly ever been described, provided that used doses were higher than lethal concentration. This cytotoxic activity is of vital importance for example in food industry, in order to preserve fish or agricultural products [13].

Anyway, it is important to remind that the antimicrobial activity of essential oils is associated especially to synergistic interactions produced by their components. An excellent example is the synergistic interaction between carvacrol and *p*-cymene [25]. Carvacrol (a monoterpene) and *p*-cymene (its monoterpene precursor) are present in oregano and thyme respectively, and they have a promising potential to be used as natural preservatives when both are used together [23]. Carvacrol has antimicrobial activity against a wide range of bacteria, while *p*-cymene barely inhibits their microbial growth, but it is able to improve the antimicrobial

properties of carvacrol by a synergistic process (Table 1). This process has been well described in carrot juice, where *Vibrio cholerae* growth was inhibited when both terpenes were added at the same time to the spiked carrot juice. However, this bacterial inhibition was observed at a lesser extent when each compound was present separately in the juice [26]. Several studies have demonstrated that  $p$ -cymene acts as a substitutional impurity in the bacterial membrane, partially affecting the membrane potential of intact cells. This situation facilitates the carvacrol activity, and therefore, less concentrations of each component are needed [21].

Due to this growing interest in natural additives, a great variety of essential oils have been used in food industry, especially together with other traditional preservatives or techniques under the concept of “hurdle technology” as a new barrier to ensure that all pathogens in a given food are eliminated. Therefore, these essential oils can be used as an efficient alternative to conventional additives as a green technology strategy [1]. In fact, it has been thoroughly demonstrated that it is possible to reduce food-borne pathogens and to extend shelf life when multiple antimicrobial compounds are incorporated. This can be achieved in minimally processed foods by combining natural antimicrobials with mild heat treatments [5]. Also, food quality can be improved when thermal and non-thermal techniques are applied in combination with essential oils [27]. For example, the use of *Laurus nobilis* and *Myrtus communis* essential oils as food additives (both of them rich in monoterpenes) reaches better rates of bacteria inactivation and less adverse effects in organoleptic properties when these essential oils are used in combination with mild heat and HHP treatments, because then lower doses are required [11]. These good results can be explained because there is again a synergistic effect between HPP or heat and essential oils, since physical treatments generate a sub-lethal damage in the bacterial membrane, which facilitates entrance in the cell of essential oils [28].

Essential oil from Rosemary (*Rosmarinus officinalis*) mainly consists of monoterpenes such as  $\alpha$ -pinene,  $\beta$ -pinene, myrcene, borneol, camphor and verbenone (Fig. 2), and the main components carnosic acid (Fig. 2), rosmarinic acid (a polyphenol, Fig. 3) and carnosol (Fig. 2), which have a powerful antimicrobial activity by also disrupting bacterial membrane integrity [29]. Different studies have demonstrated their success against Gram-positive (*S. aureus* and *B. subtilis*) and Gram-negative (*E. coli* and *Klebsiella pneumoniae*) bacteria [30]. After its ingestion by animals, rosemary terpenoids, as carnosic acid, are stored in lamb muscle at sufficient levels to exert antimicrobial effects in meat [31]. For example, feeding lambs with 200 mg/kg of rosemary dry extract, a reduction in bacterial spoilage was observed, as rosemary polyphenols affected bacterial membranes, inhibiting their multiplication rates [32].

Oregano essential oil (rich in thymol and carvacrol) showed a greater antimicrobial activity against Gram-positive bacteria (*S. aureus*) than against Gram-negative ones (*E. coli* and *Pseudomonas aeruginosa*) [33,34]. Addition of *Satureja horvatii* essential oil, rich in  $p$ -cymene (33.14 %) and thymol (26.11 %), to pork meat inhibited *L. monocytogenes* growth. In this case, it was also noticed an improvement in flavor and odor of treated food, after 4 days of storage, in comparison with controls [2].

Fruit juice processing involves a thermal step to inactivate vegetative forms of pathogenic and spoilage microorganisms. However, it is well known that heat leads to a loss of vitamins and to flavor changes. When a

combination of carvacrol and *p*-cymene was added to juices, lower doses of both of them and heat were required to become efficient against *E. coli* O157:H7 [35].

An increasing demand of organic food is raising the risk of foodborne diseases outbreaks due to the consumption of contaminated products. In the USA, organic food production is regulated by the USDA National Organic Program, and chemical antimicrobials approved for post-harvest treatments of organic products are very limited. Therefore, it is necessary to develop new GRAS additives such as essential oils, which are awakening interest for their use in “ready-to-eat” vegetables, such as packaged salads [36]. In this sense, some polymeric films containing oregano essential oil (carvacrol) and citral (from citrus fruits odor glands) showed a drastic reduction in spoilage microbiota and inhibited the growth of some pathogens in spiked salads (*E. coli*, *Salmonella enterica* and *Listeria* spp.), with a greater effect in Gram-negative cells. In addition, salads in containers with essential oils had better sensorial qualities [37]. The combined activity of carvacrol and nisine (a bacteriocin commonly used in food industry) was able to eliminate *L. monocytogenes* in ready-to-eat carrots in an effective way, decreasing the necessary dose for each hurdle [38].

In other studies, citron oil was effective against *Salmonella* spp., *E. coli* and *L. monocytogenes* in fruit-based salads [39]; oregano essential oil (carvacrol) was effective against *C. jejuni*, *E. coli* O157:H7, *L. monocytogenes* and *S. enterica* in apple juice [40]; and packaging films which incorporated carvacrol were able to reduce *Salmonella* populations in spiked bagged leaves [36].

Despite all these activities and the fact that most essential oils possess GRAS status, their use as food preservatives is very limited due to flavoring considerations, as their intense aroma could modify the typical flavor or odor of certain foods [1]. Therefore, the use of these natural additives in food industry is very limited due to the high concentrations needed to achieve an optimal antimicrobial activity, which negatively affects the foodstuffs organoleptic properties [41]. Nevertheless, when low concentrations of essential oils are added to packaging films (25 %), it does not generate a detriment in food acceptability [1]. Combined treatments would also allow to generate safe foodstuff by decreasing the concentration of each hurdle at the same time [5].

However, essential oils present many other problems when they are used as food preservatives. In certain foods, they are impaired by interactions with components contained in the food matrix such as fat and proteins [42], and for this reason it is difficult to extrapolate results obtained in *in vitro* tests. Therefore, under industrial real conditions, a lower antimicrobial activity of these compounds is expected, being necessary to increase their concentration due to these interactions, but this fact will most probably alter some food organoleptic properties [43]. A real solution to this problem could be active packaging, in which the antimicrobial volatile agents are incorporated into a carrier material that acts as a vehicle for slow release of these bioactives towards the surface of the food product, where microbial contamination is supposed to be higher [44]. This method is more effective than directly applying the antimicrobial compound all over the surface of the product via a spray solution, because it reduces the organoleptic impact [45]. Another way to reduce the concentration of essential oils without compromising their antimicrobial activity would be using synergistic strategies with other compounds

[46]. Finally, in some cases, replacement of essential oils for its main chemical components could provide a similar activity, avoiding certain compounds that could alter food flavor [47].

An innovative method to intensify the efficacy of natural preservatives could be the encapsulation of the compound into food-grade materials (nano-emulsions). Nano-encapsulation has various objectives, including (a) stabilization of the compound against undesirable reactions with matrix components as fat, (b) stabilization of volatile compounds such as essential oils, (c) retardation of compound delivery rate in order to increase the exposure of this component to food and therefore its shelf life, and (d) protection against physical treatments that could damage it [48].

The antimicrobial effect of polyphenols has been also demonstrated in animal models for infections. For example, in an mice model for *L. monocytogenes* infection, lutein was able to reduce mortality and the bacterial damages to spleen and liver, due to inhibition of hemolysis associated to the virulence factor listeriolysin O. Lutein prevents the oligomerization of this toxin, therefore blocking *L. monocytogenes* transfer from vacuoles to cytoplasm of infected cells in these organs [49].

### **Polyphenols**

Polyphenolic compounds are the second biggest family of plant nutraceuticals, after terpenoids. These phytochemicals are a group of secondary metabolites sharing a common chemical structure characterized by the presence of at least one aromatic ring, tailored with one or more hydroxyl groups [50]. This family contains more than 10,000 compounds described to date in vascular plants, several hundreds of which are found in edible plants [51]. These phenolic compounds can be found in a broad spectrum of food and beverages from plant origin such as fruits, vegetables, coffee, tea, beer, wine or chocolate [52].

Polyphenols are usually classified in subfamilies, depending on their chemical structure, the number of phenolic rings and the structural elements that link these rings: (a) phenolic acids (as gallic acid), (b) flavonoids (as quercetin), (c) stilbenes (as resveratrol), (d) lignans (as secoisolariciresinol), (e) coumarins (as coumarin), and (f) tannin polymers (as proanthocyanidins) (Fig. 3) [53].

Flavonoids (from the latin word *flavus*, yellow) represent about 60 % of dietary polyphenols and are ubiquitously distributed in plants [54]. All flavonoids also share a generic chemical structure composed of 15 carbon atoms (C6-C3-C6) distributed in two aromatic rings (A and B rings) linked by a bridge of three carbons which is part of a heterocyclic pyrane ring (C ring), forming a characteristic phenyl-benzopyrone structure. This basic skeleton can bear a lot of different substituents, such as hydroxyl groups in C4', C5 and C7 positions, or even sugars (Fig. 3) [50].

Flavonoids can be classified in several subgroups according to their hydroxylation pattern and the substituents that are linked to the C ring: (a) anthocyanidins (as malvidin and delphinidin), (b) flavan-3-ols (as catechins), (c) flavones (as apigenin and luteolin), (d) flavanones (as naringenin and eriodictyol), (e) flavonols (as quercetin and kaempferol), and (f) isoflavonoids (as genistein and daidzein) (Fig. 3). Individual compounds within each family differ on the pattern of substituents in A and B rings [53].

Some common spices are rich in polyphenols with antibacterial activity. Cinnamon contains for example 3.6% epicatechins, 23.2% proanthocyanidans and 64.1% cinnamaldehydes (Fig. 3). This plant extract is able to inhibit the growth of food pathogens as *B. cereus* at concentrations of 625 µg/ml, with a minimum bactericidal concentration of 2,500 µg/ml [55,56]. Another spice, rosemary, is also a rich source of antibacterial polyphenols with potential applications in meat industry, as its introduction in the fed (as a rosemary extract, 0.6 mg/kg) during last stage in lamb fattening was able to inhibit lipid oxidation and rancidity in chilled packed lamb cuts [57].

Olive oil has also a high polyphenolic content, especially in hydroxytyrosol (Fig. 3). Aqueous extract from olive pulp (a by-product after processing olives for oil extraction) contains 6% polyphenols and has interest as food preservative. Its major constituents are hydroxytyrosol (50-70%), oleuropein (5-10%) and tyrosol (0.3%) (Fig. 3) [58]. Hydroxytyrosol shows toxicity to human intestinal and respiratory tract pathogens as *S. aureus*, *Salmonella* spp., *Vibrio parahaemolyticus*, *Moraxella catarrhalis* and *Haemophilus influenza*, with minimum inhibitory concentrations between 0.24-7.85 µg/ml [59].

Epicatechin gallate (ECG) and epigallocatechin gallate (EGCG, Fig. 3) are two flavonoids present in green tea (*Camellia sinensis*) with the capacity to sensitize multidrug resistant *S. aureus* (MRSA) strains to β-lactam antibiotics, reducing the oxacillin MIC values from 512 to 4 µg/mL [60] (Table 1).

Red grapes juice is rich in diverse polyphenols as resveratrol, ellagic acid, quercetin, myricetin, catechin and epicatechin (Fig. 3). This juice is able to inactivate *in vitro* high populations of *C. sakazakii*, allowing its use as food preservative for baby foods [61]. Quercetin, one of the main polyphenols in wine, shows inhibitory effect on *E. coli* DNA gyrase. This flavonoid also increases the membrane permeability and reduces the membrane potential, avoiding ATP synthesis in this intestinal bacterium. Quercetin inhibitory activity on the growth of this enterobacteria is observed already at 25 µg/mL, allowing its use as food preservative [62].

In animal models, flavonoids as the common quercetin have shown antimicrobial activity against *Salmonella enterica* infection. In mice, this polyphenol was able to prevent intestinal infection and to reduce bacterial titers in livers, preventing liver damage and enhancing animal survival rates [63]. All these examples show how important polyphenolic compounds are with respect to bacterial growth inhibition, alone or together with other phytochemicals, as a promising alternative to chemical synthesis preservatives.

## Thiols

Organosulfur compounds are defined as molecules that contain one or more carbon-sulfur bonds. *Allium* and *Brassica* plant genera contain high concentration of these compounds, specifically thiosulfonates (between 1.1-3 % in garlic, *Allium sativum*). A wide range of microorganisms have been shown to be sensitive to crushed garlic. The compound responsible for this antibacterial capacity is the L-Cys sulfoxide alliin (Fig. 4), a volatile compound, hydrophobic, which gives the characteristic smell to these vegetables. Damage to garlic cells during storage or cooking causes activation of the vacuolar enzyme alliinase, which converts two molecules of alliin



into allicin (Fig. 4), which renders diverse oxidation derivatives as diallyl disulfide and diallyl trisulfide (Fig. 4) [64][64,65].

The allicin is effective as antimicrobial against Gram-positive (*Bacillus* spp., *Streptococcus* spp., *Staphylococcus aureus* MRSA) as well as against Gram-negative bacteria (*E. coli*, *S. typhimurium*, *P. syringae*, *V. cholerae*, *H. pylori*; *Shigella* spp.), with inhibitory concentrations ranging from 1.72  $\mu\text{M}$  (*E. coli*) to 80  $\mu\text{M}$  (*V. cholerae*, *S. typhimurium*, *Bacillus* spp., *L. monocytogenes*, *Mycobacterium tuberculosis*) [64] (Table 1). This bioactivity is due to the reactivity of allicin with thiol-containing microbial enzymes, including interference during protein synthesis elongation steps [64,66]. Particularly, allicin reacts with protein L-cysteines to form an S-thiolation product called S-allylmercaptocystein (Fig. 4). This reaction inhibits many bacterial enzymes in the microorganism, therefore reducing its virulence, and acting as bacteriostatic or bactericide compound. In this way, 0.2 mM allicin causes a rapid inhibition of *S. typhimurium* RNA synthesis (primary target) and also a partial inhibition of DNA synthesis [67].

## Conclusion

Current industrial food processes have available a broad array of techniques and chemical synthesis additives for their use in food preservation. These additives are used mainly to diminish microbial contamination (as pathogenic and spoilage bacterial species) and to prolong shelf live. However, some of these antimicrobial additives may alter the nutritional properties of a given food, as in the case of sulfites, which destroy vitamin B1, or the addition of nitrates to meat, which renders their microbial conversion to nitrites and the subsequent formation of the carcinogens nitrosamines. These type of scientific data, together with an increasing consumer perception on the need for safer and more natural food processing techniques and additives has caused that during last years, increasing efforts at scientific and industrial levels are devoted to trigger the use of plant metabolites as terpenoids, polyphenols and thiols, among others, as food additives. The antibacterial activities for some of these plant nutraceuticals has been tested *in vitro*, in the stored food matrix, and in some cases in animal models for infections, shedding natural light in the complex field of food additives and preservatives.

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## Conflict of Interests

Authors declare absence of conflict of interest.

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## Figure legends

**Figure 1:** Targets of essential oils in bacterial cells, showing different mechanisms for antimicrobial activities: degradation, damage or leakage at cell wall, cytoplasm proteins coagulation or inhibition, and depletion of proton force.

**Figure 2:** Chemical structures of antibacterial terpenoids. A: carvacrol, B: citral, C: linalool, D: geraniol, E: thymol, F: carvone, G: limonene, H: p-cymene, I: germacrene D, J:  $\alpha$ -pinene, K:  $\beta$ -pinene, L: myrcene, M: borneol, N: camphor, O: verbenone, P: carnosic acid, Q: carnosol.

**Figure 3:** Chemical structures of antibacterial polyphenols. A: gallic acid, B: quercetin, C: resveratrol, D: secoisolariciresinol, E: coumarin, F: malvidin, G: delphinidin, H: apigenin, I: luteolin, J: naringenin, K: eriodictyol, L: kaempferol, M: genistein, N: daidzein, O: cinnamaldehyde, P: rosmarinic acid, Q: hydroxytyrosol, R: oleuropein, S: epicatechin gallate, T: epigallocatechin gallate, U: myricetin, V: ellagic acid, W: catechin.

**Figure 4:** Chemical structures of antibacterial thiols. A: alliin, B: allicin, C: diallyl disulfide, D: diallyl trisulfide, E: S-allylmercaptocystein.



## Tables

**Table 1:** Summary of antibacterial bioactivities described for terpenoids, polyphenols and thiols.

Compound	Bioactivity	References
Carvacrol	Antibacterial (alteration of membrane proton transport)	Hyltdgaard et al., 2012
Citral	Antibacterial	Ndoti-Nembe et al., 2015
Thymol	Antibacterial	Aguirre et al., 2013
Carvone	Antibacterial	Raut et al., 2013
Limonene	Antibacterial (membrane biosynthesis inhibition)	Di Pasqua et al., 2006
p-Cymene	Antibacterial (alteration of membrane proton transport)	Ultee et al., 2002
Germacrene D	Antibacterial (cell division inhibition)	Šarac et al., 2014
$\alpha$ -Pinene, $\beta$ -Pinene	Antibacterial (membrane alteration)	Knobloch et al., 1989
Myrcene	Antibacterial	Santoyo et al., 2005
Borneol	Antibacterial	Santoyo et al., 2005
Camphor	Antibacterial	Santoyo et al., 2005
Verbenone	Antibacterial	Santoyo et al., 2005
Carnosic Acid	Antibacterial	Santoyo et al., 2005
Carnosol	Antibacterial (membrane alteration)	Knobloch et al., 1989
Gallic Acid	Antibacterial	Pagnussatt et al., 2014
Quercetin	Antibacterial (inhibition of DNA gyrase, membrane potential disruption)	Rodríguez Vaquero et Manca de Nadra, 2008
Resveratrol	Antibacterial	Lee et Lee; 2015
Kaempferol	Antibacterial	García et al., 2013

Cinnamaldehyde	Antibacterial	Ooi et al., 2006
Rosmarinic Acid	Antibacterial (membrane potential disruption)	Knobloch et al., 1989
Hydroxytyrosol	Antibacterial	Bisignano et al., 1999
Epicatechin Gallate, Epigallocatechin Gallate	Antibacterial	Stapleton et al., 2004
Ellagic Acid	Antibacterial	Kim et al, 2010
Myricetin	Antibacterial	Kim et al, 2010
Allicin	Antibacterial (inactivation of thiol-containing enzymes)	Amagase et al., 2001 Borlinghaus et al. 2014
Diallyl Disulfide, Diallyl Trisulfide	Antibacterial	Amagase et al., 2001 Borlinghaus et al. 2014