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

Ignacio Gutiérrez-del-Río, Javier Fernández, Felipe Lombó*

Research Group BIONUC, Departamento de Biología Funcional, Área de Microbiología, University of Oviedo, Oviedo, Principality of Asturias, Spain; IUOPA (Instituto Universitario de Oncología del Principado de Asturias), IISPA (Instituto de Investigación Sanitaria del Principado de Asturias)

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Synthetic food additives generate a negative perception in consumers. Therefore, food manufacturers search for safer natural alternatives such as those involving phytochemicals and plant essential oils. These bioactive compounds have antimicrobial activities widely proven in in vitro tests. Foodborne diseases cause thousands of deaths and millions of infections every year, mainly due to pathogenic bacteria such as *Salmonella* spp., *Campylobacter* spp., *Escherichia coli*, *Bacillus cereus*, *Listeria monocytogenes* and *Staphylococcus aureus*. This review summarises industrially interesting antimicrobial bioactivities as well as their mechanisms of action for three main types of plant nutraceuticals, namely terpenoids (e.g. carnosic acid), polyphenols (e.g. quercetin) and thiols (e.g. 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 especially useful in food preservation as microbial growth inhibitors.

1. Antibacterial activities of plant nutraceuticals

A wide range of synthetic preservatives and antibacterial physical treatments are used to extend the shelf-life of food by inhibiting bacterial growth. Moreover, some foods require special protection against microbial spoilage during their preparation, storage and distribution in order to increase their shelf-life and organoleptic properties, avoiding microbial spoiling, which usually changes taste, odour, colour, and sensorial or textural properties [1]. The presence of specific micro-organisms, such as *Listeria monocytogenes*, *Escherichia coli* O157:H7, *Salmonella* spp., *Staphylococcus aureus*, *Bacillus cereus*, *Campylobacter* spp. and *Clostridium perfringens*, not only affects food quality but also constitutes a hazard for human health, causing food-borne diseases [2,3]. Foodborne diseases are an increasing public-health problem worldwide. For example, it is estimated that 31 pathogenic species are responsible for 9.4 million cases of foodborne diseases each year in the USA alone [4].

Food antimicrobials are chemical compounds that are naturally present in food or are directly added in order to inhibit the growth of pathogenic or spoilage micro-organisms with the aim of ensuring food safety and quality [5]. Approved food antimicrobials are classified as chemical preservatives, a category that also includes other types 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, sulphites, 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]. However, the use of some of these represents a nutritional or health threat for the consumer. For example, sulphites cause degradation of vitamin B1 (thiamine) in food, an essential nutrient [6].

Apart from chemical antimicrobials, food can undergo different physical processes, which are classified as thermal and non-thermal treatments. Thermal technologies are the most widely used preservation methods in the food industry owing to their high efficacy. However, the intensities needed to achieve high safety 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 and high hydrostatic pressure (HHP), are especially interesting as they do not affect food organoleptic properties. Nevertheless, under certain circumstances these techniques are not able to ensure food safety because some experiments have demonstrated that these physical treatments can lead only to sublethal damage to the bacterial cell wall [8–10].

Moreover, there is an increasing rejection among consumers of the use of synthetic additives as well as a demand for 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

* Corresponding author.
E-mail address: lombofelipe@uniovi.es (F. Lombó).

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antimicrobial must fulfil certain requirements, such as: (i) being active at low concentrations in its natural form; (ii) inexpensive; (iii) not generating sensorial changes in the product; (iv) inhibiting a wide range of spoilage and pathogenic micro-organisms; and (v) non-toxic [5].

2. Terpenoids and essential oils

Essential oils, also known as volatile odoriferous oils, are natural, volatile and complex liquids characterised by an intense smell and flavour that 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 areas, 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 deter certain predator animals. Their chemical constituents also play an important role as signal compounds and growth regulators (phytohormones) of plants [12]. Essential oils can be synthesised by all organs of a plant (e.g. buds, flowers, leaves, stems, seeds, fruits and wood) and can be stored in secretory cells, cavities, epidermal cells or glandular trichomes. They can be extracted from these plant organs in various ways; however, among 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 both of polar and non-polar components that can include 20–60 compounds at different concentrations. However, they are characterised by the presence of two or three main components found at relatively high concentrations (20–70%) in comparison with the others that are in trace amounts [13]. Components contained in essential oils can be divided into two groups, each with a different biosynthetic origin, although both are characterised by a low molecular weight: the main groups are terpenes (monoterpenes and sesquiterpenes) and terpenoids (monoterpenoids). Monoterpenes (C_{10}), which are made up by fusion of two isoprene units (C_5), are the most representative molecules in essential oils, achieving a percentage of up to 90%. Depending on the number of isoprene subunits, different terpene subfamilies are defined, such as hemi- (C_5), mono- (C_{10}), sesqui- (C_{15}), di- (C_{20}), sester- (C_{25}), tri- (C_{30}), tetra- (C_{40}) and polyterpenes $(C_5)_n$ with n (number of isoprene units) greater than 8 [12]. Other less abundant components in essential oils are aromatic and aliphatic compounds such as aldehydes, phenols and methoxy derivatives [14]. Medicinal plant parts such as roots, leaves, branches, stems, bark, flowers and fruits are commonly rich in terpenes such as carvacrol, citral (a natural mixture of geranial and neral), linalool, geraniol and many others [15].

Essential oils have been known since ancient times by their aroma as well as their antiseptic medicinal properties (i.e. bactericidal, fungicidal and virucidal). Essential oils perform a key function in plants defence, working as antibacterial, antifungal and antiviral agents. They even work as a defence 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 the flavour, their antimicrobial activities make them good candidates to replace chemical preservatives [16].

The antimicrobial activities of essential oils make them good candidates for use as natural additives in foods and food products as they can be added as bioactive components in packaging materials [1]. Currently, more than 3000 essential oils are known, with 300 of them having a commercial interest in food, pharmaceutical, sanitary or cosmetic industries. In fact, different terpene components of essential oils (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 flavourings for food products. These components have also been recognised by the US Food and Drug Administration (FDA) as GRAS (Generally Recognized as Safe) ingredients [14].

Owing to the wide range of constituents that make up essential oils, several cellular targets have been described for their antimicrobial activity (Fig. 1) and they are effective against a great variety of micro-organisms, including bacteria [17], viruses [18] and fungi [19] (Table 1). Terpenoids are active against a broad range of microorganisms, with carvacrol (a monoterpenoid phenol) (Fig. 2) being one of the most active components [14]. However, other common terpenes, such as *p*-cymene (Fig. 2), lack high antimicrobial activity, and many in vitro tests have shown that some terpenes are inefficient as antimicrobials when used as sole compounds [20]. The antimicrobial activity of terpenoids is related to their functional groups. Specifically, in phenolic terpenoids, hydroxyl groups as well as the presence of delocalised electrons perform an important function against micro-organisms [14]. In fact, if the carvacrol hydroxyl group is substituted by a methyl ether group, a change affecting its hydrophobicity, this also affects its antimicrobial activity because it modifies how this molecule interacts with the microbial cell membrane. The reason for this is that the carvacrol hydroxyl group has been proposed to function as a monovalent cation carrier across membranes, carrying H^+ into the cell cytoplasm and transporting K^+ back out [21].

As typical lipophilic substances, essential oils are able to cross the cell wall and the cytoplasmic membrane, having a different effect on Gram-positive and Gram-negative bacteria. The lipophilic ends of lipoteichoic acid in the Gram-positive cell wall facilitate penetration of hydrophobic compounds such as essential oils into these bacteria. However, Gram-negative bacteria show higher resistance to the action of essential oils, associated with the presence of the outer membrane. This higher resistance could be attributed to outer membrane proteins or to lipopolysaccharide (LPS), 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 phospholipid layers, increasing their permeability. Damage to these two structures is associated with ion leakage, reduction of membrane potential, proton pump collapse, ATP pool depletion and loss of macromolecules. All of these events lead to an impairment of essential processes in the cell and finally to cell lysis [22]. Essential oils can also coagulate the cytoplasm as well as causing direct damage to cellular lipids and proteins [23] (Fig. 1).

There are other antibacterial mechanisms for essential oils that are not yet completely understood, such as inhibition of specific bacterial essential 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 the FtsZ binding pocket and thus could be an important natural preservative [24].

Until now, owing 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 the doses used are above the lethal concentration. This cytotoxic activity is of vital importance, e.g. in the food industry to preserve fish or agricultural products [13].

Nevertheless, it is important to remember that the antimicrobial activity of essential oils is associated especially with synergistic interactions produced by their components. An excellent example is the synergistic interaction between carvacrol and *p*-cymene [25]. Carvacrol (a monoterpenoid) and *p*-cymene (its monoterpene precursor) are present in oregano and thyme, respectively, and have promising potential to be used as natural preservatives when

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