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Essential oils: A novel source for food preservation

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Abstract
Essential oils are aromatic and volatile liquids extracted from plants. The chemicals in essential oils are secondary metabolites, which play an important role in plant defense as they often possess antimicrobial properties. The interest in essential oils and their application in food preservation has been amplified in recent years by an increasingly negative consumer perception of synthetic preservatives. Furthermore, food-borne diseases are a growing public health problem worldwide, calling for more effective preservation strategies. The antibacterial properties of essential oils and their constituents have been documented extensively. Pioneering work has also elucidated the mode of action of a few essential oil constituents, but detailed knowledge about most of the compounds' mode of action is still lacking. This knowledge is particularly important to predict their effect on different microorganisms, how they interact with food matrix components, and how they work in combination with other antimicrobial compounds. The article reviews the existing research work on the use of EOs as food preservatives as an alternative to synthetic preservatives and chemical additives in Mediterranean food products.

Keywords: Essential oils, novel, food preservation

Introduction

In view of the rapid increase in world population many countries experience perpetual food shortage resulting in chronic and often wide- spread hunger among significant number of people. Loss of food commodities due to storage pests is a major reason of food crisis particularly in tropical countries. According to FAO, food borne molds and their toxic metabolites render quantitative and qualitative losses of nearly 25% of agricultural food items throughout the world [1, 2]. Essential oils have been used since thousands of years in various cultures for medicinal and health purposes. Because of their antidepressant, stimulating, detoxifying, anti-bacterial, antiviral and calming properties. These are recently gaining popularity as a natural safe and cost-effective therapy for a number of health concerns [3]. Many essential oils have anti-inflammatory, anti-viral, anti-bacterial, antiseptic and anti-fungal properties that help to boost immune system and fight infections. The chemical found in essential oils, such as terpenes, esters, phenolics, ethers and ketones have the potential to fight foreign pathogens that can threaten health. Essential oils are considered to be secondary metabolites and important for plant defense as they often possess antimicrobial properties. The antibacterial properties of secondary metabolites were first evaluated using essential oil vapors by [4]. Although the food industry primarily uses essential oils as flavorings, they represent an interesting source of natural antimicrobials for food preservation. However, application of essential oils as food preservatives requires detailed knowledge about their properties, i.e., the minimum inhibitory concentration (MIC), the range of target organisms, the mode of action, and the effect of food matrix components on their antimicrobial properties. The purpose of this review is to provide an overview of current knowledge about the antimicrobial mode of action of essential oil constituents, and to identify research avenues that can facilitate implementation of essential oil constituents as natural food preservatives in foods.

Some synthetic organic formulations have been recommended to control storage losses of food items. However, in view of some serious drawbacks related to environmental issues, safety concerns, development of resistant races to pests and residual toxicities [5], there is a need for eco-friendly, biodegradable and safer alternatives to control bio deterioration and biodegradation of food items. At the same time, western society also appears to favour a trend of 'green consumerism' [6] desiring fewer synthetic food additives and products with a smaller impact on the environment. Recently, EOs of aromatic plants are the thrust area of interest of researchers throughout the world in view of their potential as natural source of antimicrobial and antioxidant compounds [7, 3]. Many essential oil products are on the 'Generally Recognized as Safe' (GRAS) list fully approved by the Food and Drug Administration (FDA) and Environment Protection Agency (EPA) in the USA for food and beverage consumption and their vapor activity as fumigant strengthens their application on food items with wide coverage [3].

Most studies concerning the antimicrobial mode of action of essential oil constituents have been performed on bacteria, while less is known about their action on yeast and molds. Gramnegative bacteria are generally less susceptible than Gram-positive bacteria [4]. The outer membrane of Gram negative bacteria contain hydrophilic lipopolysaccharides (LPS), which create a barrier toward macromolecules and hydrophobic compounds, providing Gram-negative bacteria with higher tolerance toward hydrophobic antimicrobial compounds like those found in essential oils [8]. Most essential oil constituents have several targets. It is therefore difficult to predict how susceptible a microorganism is and why the susceptibility varies from strain to strain. Predictions about the mode of action of crude essential oils require thorough investigations of their constituents' target site, their mode of action, and their interactions with the surrounding environment. In this context, the following is known about the mode of action of some selected essential oil constituents.

Terpenes

Terpenes are hydrocarbons produced from combination of several isoprene units (C_5H_8). Terpenes are synthesized in the cytoplasm of plant cells, and the synthesis proceeds via the mevalonic acid pathway starting from acetyl-CoA. Terpenes have a hydrocarbon backbone which can be rearranged into cyclic structures by cyclases, thus forming monocyclic or bicyclic structures [9]. The main terpenes are monoterpenes ($C_{10}H_{16}$) and sesquiterpene ($C_{15}H_{24}$), but longer chains such as diterpenes ($C_{20}H_{32}$), triterpenes ($C_{30}H_{40}$), etc., also exist. Examples of terpenes include p-cymene, limonene, terpinene, sabinene, and pinene. Terpenes do not represent a group of constituents with high inherent antimicrobial activity. For example, p-cymene, one of the major constituents in thyme, had no antimicrobial activity against several Gram-negative pathogens even at 85700µg/mL concentration [10]. In a large scale experiment the effect of α-pinene, β-pinene, p-cymene, β-myrcene, β-caryophyllene, limonene, and γ-terpinene against Escherichia coli, Staphylococcus aureus, and Bacillus cereus, and their antimicrobial activity were low or absent. p-Cymene and γ-terpinene were ineffective as fungicides against Saccharomyces cerevisiae [11, 12]. These *in vitro* tests indicate that terpenes are inefficient as antimicrobials when applied as single compounds.

P-Cymene

The carvacrol precursor p-cymene is a monoterpene that has a benzene ring without any functional groups on its side chains. Investigations on cell and vesicle systems confirm that p-cymene has no effect on the membrane permeability, but cause a decrease in the enthalpy and melting temperature of membranes [13] supporting the hypothesis that p-cymene acts as a substitutional impurity in the membrane

Terpenoids

Terpenoids are terpenes that undergo biochemical modifications via enzymes that add oxygen molecules and move or remove methyl groups [9]. Terpenoids can be subdivided into alcohols, esters, aldehydes, ketones, ethers, phenols, and epoxides. Examples of terpenoids are: thymol, carvacrol, linalool, linalyl acetate, citronellal, piperitone, menthol, and geraniol. The antimicrobial activity of most terpenoids is linked to their functional groups, and it has been shown that the hydroxyl group of phenolic terpenoids and the presence of delocalized electrons are important for antimicrobial activity. For example, the antimicrobial activity of the carvacrol derivatives carvacrol methyl ether and p-cymene were much lower than carvacrol [14, 15]. Exchanging the hydroxyl group of carvacrol with methyl ether affects its hydrophobicity, antimicrobial activity, and changes how the molecule interacts with the membrane [16].

Thymol

The mode of action of thymol, a phenolic monoterpenoids and one of the major constituents of thyme oil, has received much attention from researchers. Studies have shown that thymol interacts with cell membranes. The interaction affects membrane permeability, and this has been documented by loss of membrane potential, cellular uptake of ethidium bromide, and leakage of potassium ions, ATP, and carboxyfluorescein [17, 18]. Thymol also impaired the citrate metabolic pathway and affected many enzymes directly or indirectly involved in the synthesis of ATP [19]. Thymol's intracellular action indicates that it affects important energy-generating processes, which lower a cells ability to recover after exposure to thymol.

Carvacrol

Carvacrol is a phenolic monoterpenoids and a major constituent of oregano. Together with its closely related isomer thymol, it is one of the most extensively studied essential oil constituents. The antimicrobial effect of carvacrol is expected to be similar to that of thymol, causing structural and functional damages to the cell membrane [20]. The mechanism of antifungal activity of carvacrol resembles that of thymol, showing disruption of $Ca2^+$ and H^+ homeostasis, up- and down-regulation of gene transcription similar to $Ca2^{+-}$ stress and nutrient starvation [12]. disruption of membrane integrity and impairment of ergosterol biosynthesis in Candida strains [21].

Phenylpropenes

Phenylpropenes constitute a subfamily among the various groups of organic compounds called phenylpropanoids that are synthesized from the amino acid precursor phenylalanine in plants. Phenylpropanoids have their name from the six-carbon aromatic phenol group and the three-carbon propene tail of cinnamic acid, produced in the first step of phenylpropanoid biosynthesis. The phenylpropenes constitute a relatively small part of essential oils, and those that have been most thoroughly studied are eugenol, isoeugenol, vanillin, safrole, and cinnamaldehyde.

Eugenol

Eugenol is a major constituent in clove essential oil, and its antimicrobial activity is linked to its ability to permeabilize the cell membrane and interact with proteins. Eugenol's action on membranes occurs mainly by a non-specific permeabilization. Eugenol induced minor changes in the fatty acid profile of Pseudomonas fluorescens, E. coli, Brochotrix thermosphacta, S. enterica, and S. aureus, and cell damages to E. coli and B. thermosphacta cells [19]. The antifungal mode of action of eugenol needs further investigation, but it is known to depend on cell proliferation. Eugenol treatment altered cell membrane and cell wall structures of proliferating S. cerevisiae cells resulting in the release of cellular content [22].

Cinnamaldehyde

Aldehyde groups are reactive and have the ability to cross-link covalently with DNA and proteins through amine groups, thereby interfering with their normal function [23]. At sub-

lethal concentrations, cinnamaldehyde gains access to the periplasm and inhibits the activity of transmembrane ATPase. Sub-lethal concentrations of cinnamaldehyde did not affect the integrity of the outer membrane of E. coli, but it inhibited growth and bioluminescence of Photobacterium leiognathi, indicating that cinnamaldehyde does gain access to the periplasm and possibly also the cytoplasm [17].

Vanillin

The mode of action of the phenylpropene phenolic aldehyde vanillin is not well understood, but it has been proposed to function as a membrane active compound that might have intracellular targets. The proposed membrane and protein interactions of vanillin are based on one study. Vanillin inhibited respiration of E. coli and Listeria innocua cells, and disrupt the potassium and pH homeostasis of Lactobacillus plantarum cells [24]. Propidium iodide staining demonstrated that treatment with vanillin disrupted membrane integrity of only a sub-population of cells and it was proposed that although vanillin primarily is a membrane active compound, it may also have intracellular target sites [25].

Perspectives and limitations in application of essential oils in food

A range of essential oil components have been accepted by the European Commission for their intended use as flavorings in food products. The registered flavorings are, e.g., linalool, thymol, eugenol, carvone, cinnamaldehyde, vanillin, carvacrol, citral, and limonene, all of which are considered to present no risk to the health of the consumer. The United States Food and Drug Administration (FDA) also classifies these substances as generally recognized as safe (GRAS). The crude essential oils classified as GRAS by FDA include amongst others clove, oregano, thyme, nutmeg, basil, mustard, and cinnamon. There are regulatory limitations on the accepted daily intake of essential oils or essential oil components, so before they can be used in food products, a daily intake survey should be available for evaluation by FDA.

The intense aroma of essential oils, even low concentrations, can cause negative organoleptic effects exceeding the threshold acceptable to consumers [26]. Having to increase the concentration of essential oils to compensate for their interactions with food matrix components is therefore highly unfortunate and limits their application to spicy foods where the acceptable sensory threshold is relatively high. Different strategies can be used to circumvent this problem.

Conclusion

The essential oils effectiveness is attributed to the presence of phenolic natural compounds and they are an important and healthy alternative to synthetic preservatives and chemical additives. The FDA treats antimicrobial agents of natural origin as GRAS type products, including plant products and their essential oils. The improvement in food safety is due to the inhibition of pathogenic microbial growth and reduction of biogenic amines, mainly in meat and meat and dairy products, as a consequence of the inhibited growth of spoilage microorganisms. The extension of food products' shelf-life results from enzymatic reduction, mainly due to their antioxidant activity. However, it must be taken into account that essential oils have an intense taste and smell, which can modify the taste and aroma of food products. Therefore, studies should focus on the minimum necessary essential oil amount, which still maintains antimicrobial activity without changing the organoleptic characteristics of food products. Besides, the combined use of different essential oils, in lower concentrations, may satisfy the safety of food products not depreciating their sensory characteristics.

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