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Review Article

Applications of Essential Oils in Food Preservation

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Abstract

Many food products are perishable by nature and require protection from spoilage during their preparation, storage and distribution to give them desired shelf-life. Because food products are now often sold in areas of the world far distant from their production sites, the need for extended safe shelf-life for these products has also expanded. Currently, there is a strong debate about the safety aspects of chemical preservatives since they are considered responsible for many carcinogenic and teratogenic attributes as well as residual toxicity. For these reasons, consumers tend to be suspicious of chemical additives and thus the demand for natural and socially more acceptable preservatives has been intensified. One such possibility is the use of essential oils (EOs) as antibacterial additives. In the production of food it is crucial that proper measures are taken to ensure the safety and stability of the product during its whole shelf-life. In particular, modern consumer trends and food legislation have made the successful attainment of this objective much more of a challenge to the food industry. EOs comprise a large number of components and it is likely that their mode of action involves several targets in the bacterial cell. It is most likely that their antibacterial activity is not attributable to one specific mechanism but that there are several targets in the cell. The potency of naturally occurring antimicrobial agents or extracts from plants, ranges of microbial susceptibility and factors influencing antimicrobial action and their antioxidative properties, aimed at food preservation, are reviewed in this article. Methods employed for estimation of inhibitory activity, mode of action and synergistic and antagonistic effects are evaluated. Hence, it is recommended that more safety studies be carried out before EOs are more widely used or at greater concentrations in foods that at present. There is therefore scope for new methods of making food safe which have a natural or 'green' image.

Key words: Antimicrobials; Essential oils; Food; Safety; Toxicity

Introduction

Food preservation is a continuous fight against microorganisms spoiling the food or making it unsafe. Several food preservation systems such as heating, refrigeration and addition of antimicrobial compounds can be used to reduce the risk of outbreaks of food poisoning; however, these techniques frequently have associated adverse changes in organoleptic characteristics and loss of nutrients. Within the disposable arsenal of preservation techniques, the food industry investigates more and more the replacement of traditional food preservation techniques by new preservation techniques due to the increased consumer demand for tasty, nutritious, natural and easy-to-handle food products. The most common classical preservative agents are the weak organic acids, for example acetic, lactic, benzoic and sorbic acid. These molecules inhibit the outgrowth of both bacterial and fungal cells and sorbic acid is also reported to inhibit the germination and outgrowth of bacterial spores. In the production of food it is crucial that proper measures are taken to ensure the

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safety and stability of the product during its whole shelf-life. In particular, modern consumer trends and food legislation have made the successful attainment of this objective much more of a challenge to the food industry. An increasing number of consumers prefer minimally processed foods, prepared without chemical preservatives. Many of these ready-to-eat and novel food types represent new food systems with respect to health risks and spoilage association. Against this background, and relying on improved understanding and knowledge of the complexity of microbial interactions, recent approaches are increasingly directed towards possibilities offered by biological preservation. Throughout the development of both Western and Eastern civilization, plants, plant parts, and derived oils and extracts have functioned as sources of food and medicine, symbolic articles in religious and social ceremonies, and remedies to modify behavior. Taste and aroma not only determine what we eat but often allow us to evaluate the quality of food and, in some cases, identify unwanted contaminants. Originally added to change or improve taste, spices and herbs can also enhance shelf-life because of their antimicrobial nature. Some of these same substances are also known to contribute to the self-defense of plants against infectious organisms. There is therefore scope for new methods of making food safe which have a natural or 'green' image. One such possibility is the use of essential oils (EOs) as antibacterial additives. The perception that these products are "natural" and have a long history of use has, in part, mitigated the public's need to know whether these products work or are safe under conditions of intended use. Actually, however, essential oils and their components are gaining increasing interest because of their relatively safe status, their wide acceptance by consumers, and their exploitation for potential multi-purpose functional use [1]. Besides antibacterial properties [2-4], essential oils or their components have been shown to exhibit antiviral [5], antimycotics [6], antioxidative [7,8], antitoxigenic [9-11], and antiparasitic [12,13] properties. It is therefore scientifically sound to evaluate the impact of essential oils on food and food products safety. This review presents the current understanding of the mode of action of these compounds and their possible applications in food protection.

Antimicrobial activities of essential oils compositions

The antibacterial activity of EOs is influenced by the degree to which oxygen is available. This could be due to the fact that when little oxygen is present, fewer oxidative changes can take place in the EOs and/or that cells obtaining energy via anaerobic metabolism are more sensitive to the toxic action of EOs [14]. The use of vacuum packing in combination with oregano EO may have a synergistic effect on the inhibition of *Listeria monocytogenes* and spoilage flora on beef fillets; 0.8% v/w oregano EO achieved a 2–3 log initial reduction in the microbial flora but was found to be even more effective in samples packed under vacuum in low-permeability film when compared to aerobically stored samples and samples packaged under vacuum in highly permeable film [15]. It is well established that bacterial biofilms exhibit more resistance to antimicrobial treatments than the individual cells grown in suspension [16,17]. It was postulated that individual components of EOs exhibit different degrees of activity against gram-positives and gram-negatives [18] and it is known that the chemical composition of EOs from a particular plant species can vary according to the geographical origin and harvesting period (*vide supra*). It is therefore possible that variation in composition between batches of EOs is sufficient to cause variability in the degree of susceptibility of Gram-negative and Gram-positive bacteria. Little information is available on interaction among constituents in essential oils and the effects they have on antimicrobial activity. The study of the sensory characteristics of the supplemented broths suggested that low concentration of cinnamaldehyde enhanced the taste of carrot broth, and that it did not have any adverse effect on the taste and smell of carrot broth at concentrations less than 6 µl 100 ml^{-1} [19].

In vitro antimicrobial assay methods

It appears that no standardized test has been developed for evaluating the antibacterial activity of possible preservatives against food-related microorganisms, although the need for such has been indicated. The NCCLS method for antibacterial susceptibility testing, which is principally aimed at the testing of antibiotics has been modified for testing Eos [20]. Researchers adapt experimental methods to better represent possible future applications in their particular field. The minimum inhibitory concentration (MIC) is cited by most researchers as a measure of the antibacterial performance of EOs. The definition of the MIC differs between publications and this is another obstacle to comparison between studies. In some cases, the minimum bactericidal concentration (MBC) or the bacteriostatic concentration is stated, both terms agreeing closely with the MIC. Screening of EOs for antibacterial activity is often done by the disk diffusion method, in which a paper disk soaked with EO is laid on top of an inoculated agar plate. This is generally used as a preliminary check for antibacterial activity prior to more detailed studies. Factors such as the volume of EO placed on the paper disks, the thickness of the agar layer and whether a solvent is used vary considerably between studies. This means that this method is useful for selection between EOs but comparison of published data is not feasible. In order to make bacterial growth easier to visualize, triphenyltetrazolium chloride may be added to the growth medium [2,21]. The strength of the antibacterial activity can be determined by dilution of EO in agar or broth. Despite these variations, the MICs of EOs determined by agar dilution generally appear to be in approximately the same order of magnitude [22]. In broth dilution studies a number of different techniques exist for determining the endpoint, the most used methods are that of optical density (OD) measurement and the enumeration of colonies by viable count. The former method has the advantage of being automated; the latter is labor intensive. A very useful development has been the adoption of microwelltitre plates containing broth to which 0.15% (w/v) agar is added to suspend partially soluble antimicrobials in the colloidal agar matrix [23]. This method continues to be used [24] and as originally described the broths contained resazurin dye as a redox indicator to give a visual signal reflecting bacteria growth. A prerequisite is that each test strain must be calibrated for its ability to reduce the resazurin dye before tests are conducted since rates may vary. The optimum test cell concentration is about one log lower than the level necessary to reduce resazurin to a pink/purple color. Microwelltitre plates are attractive for this type of study because only small reaction volumes (≤300 μl/test) are needed, replicate tests are easily prepared using multi-channel pipettors, and multi-well plates lend themselves to adoption of protocols where two or more antimicrobials can be used simultaneously in gradients to examine reactants for interactive (synergistic or antagonistic) effects. A patented color indicator based on resazurin has been used to determine the MICs for methanolic extracts of plant materials [25] and EOs [26]. The rapidity of a bactericidal effect or the duration of a bacteriostatic effect can be determined by time-kill analysis (survival curve plot) whereby the number of viable cells remaining in broth after the addition of EO is plotted against time. The most frequently used methods for this are measurement of OD and viable count after plating out onto agar. Decimal reduction value (D value has been employed to find out microbial elimination time by others [7,8]. The hydrophobicity of phenolics limits the value of agar disc/diffusion tests for estimating antimicrobial potency accurately. Since essential oils are characterized as being volatile, methods that test the antimicrobial activity of such agents in their vapor phase have been outlined [27], and modified [28]. There is clearly still a need for the adoption of standardized protocols for the evaluation of natural antimicrobials during *in vitro* tests to avoid the generation of contradictory results. However, it is unlikely that a single standard method will have general appeal. This is because studies often have different purposes and objectives, and frequently employ different experimental designs. Perhaps the greatest source of variation in study results arises from the use of unstandardized natural antimicrobials of different potency and composition.

Antioxidative characteristics of essential oils

Many experiments have indicated that free radicals are necessary to support life, though they are also dangerous in biological tissues. Lipid peroxidation is a complex process occurring in aerobic cells and reflects the interaction between molecular oxygen and polyunsaturated fatty acids. Radicals are known to take part in lipid peroxidation, which causes food deterioration, aging organisms and cancer promotion [29]. Antioxidants act as radical-scavengers, and inhibit lipid peroxidation and other free radical-mediated processes: therefore, they are able to protect the human body from several diseases attributed to the reactions of radicals [30]. Use of synthetic antioxidants to prevent free radical damage has been reported to involve toxic side effects [31], making attractive the search for antioxidant and scavenger natural compounds. The most widely used synthetic antioxidants in food (butylated hydroxytoluene BHT, butylated hydroxyanisole BHA, propyl galate PG and tertiary butyl hydroquinone TBHQ) have been suspected to cause or promote negative health effects [32]. The spoilage and poisoning of foods by oxidation and/or microorganisms is still a problem that is not yet overcome despite of the range of robust preservation techniques available. The screening of plant extracts and natural products for antioxidative activity has revealed the potential of higher plants as a source of new. Free radicals provoked by various environmental chemicals as well as endogenous metabolism are involved in a number of diseases like tumors, inflammation, shock, atherosclerosis, diabetes, infertility, gastric mucosal injury, and ischemia due to the oxidative damage to DNA, lipids, and proteins and which can result in failure of cellular functions [33]. Consumption of antioxidants from plant materials that inhibit free radical formation or accelerate their elimination has been associated with a lowered incidence of these diseases as a consequence of alleviating the oxidative stress of free radicals [34]. Hence, there is a growing interest in studies of natural additives as potential antioxidants. Many sources of antioxidants of plant origin have been studied in recent years. Essential oil compounds, such as carvacrol and thymol, will both prevent the microbial and chemical deterioration when added to food [26,35-37].

Applications of essential oils in food

A reaction between carvacrol, a phenolic component of various EOs, and proteins has been put forward as a limiting factor in the antibacterial activity against *Bacillus cereus* in milk [38]. Protein content has also been put forward as a factor inhibiting the action of clove oil on *Salmonella enteritidis* in diluted low-fat cheese [39]. Carbohydrates in foods do not appear to protect bacteria from the action of EOs as much as fat and protein do [40]. A high water and/or salt level facilitates the action of EOs [41].

A high fat content appears to markedly reduce the action of EOs in meat products. It is generally supposed that the high levels of fat and/or protein in foodstuffs protect the bacteria from the action of the EO in some way. For example, if the EO dissolves in the lipid phase of the food there will be relatively less available to act on bacteria present in the aqueous phase [42]. Another suggestion is that the lower water content of food compared to laboratory media may hamper the progress of antibacterial agents to the target site in the bacterial cell [39]. In addition to fat and protein, the pH of food systems is an important factor affecting the activity of oils. At low pH, the hydrophobicity of some essential oils (for example, thyme oil and the phenolic oleuropein) increases and while they may tend to partition in the lipid phase of the food, they can also dissolve more easily in the lipid phase of the bacterial membrane and have enhanced antimicrobial action.

The antimicrobial activity of EOs in vegetable dishes is benefited by a decrease in storage temperature and/or a decrease in the pH of the food [41]. Vegetables generally have a low fat content, which may contribute to the successful results obtained with EOs.

Synergism and antagonism

The simultaneous application of nisin (0.15 μg/ml) and carvacrol or thymol (0.3 mmol/l or 45 μg/ml) caused a larger decline in viable counts for strains of *B. cereus* than was observed when the antimicrobials were individually applied. The maximum reduction of viability was achieved in cells that had experienced prior exposure to mild heat treatment at 45°C (5 min for exponentially growing cells and 40 min for stationary phase cells) [43]. Essential oils have been used in combination with other antibacterial agents and with a variety of treatments such as mild heat [44], hydrostatic pressure [45], sodium citrate and monolaurin [46,47]. Yamazaki *et al.* [47] investigated plant-derived essential oil components in combination with nisin and diglycerol fatty acid esters for their antibacterial activity against *Listeria monocytogenes*. Antagonistic effects of salt were found with carvacrol and *p*-cymene against *B. cereus* in rice: carvacrol and *p*-cymene worked synergistically, but this effect was reduced when salt was added (1.25 g/l rice) [48].

Damage to microbial cells and safety concerns

Some EOs and their components have been known to cause allergic contact dermatitis in people who use them frequently. Preventive measures may be needed to ensure the well-being of workers if these substances were to be used on a larger scale [49, 50]. Some oils used in the fields of medicine, paramedicine and aromatherapy have been shown to exhibit spasmolytic or spasmogenic properties, although these are difficult to associate with a particular component [51]. Therefore data on the percent range or upper limit of concentration of congeneric groups in the EO, target constituents monitored in an ongoing quality control program, and the amount of trace unidentified constituents that stipulate the composition of the essential oil become key specifications linking the product distributed in the marketplace to the chemically-based safety evaluation.

The chemical structure of the individual EO components affects their precise mode of action and antibacterial activity [18]. EOs are mixtures of molecules often characterized by a poor solubility in water and by a high hydrophobicity. In addition, the different components of an EO can have antagonistic, synergistic or additive effects on microbial cells. Ultee and co workers [52] suggested that carvacrol exerts its activities by interacting with the cytoplasmic membrane via its own hydroxyl group, thus changing the permeability of membrane for protons and potassium ions.

Considering the large number of different groups of chemical compounds present in EOs, it is most likely that their antibacterial activity is not attributable to one specific mechanism but that there are several targets in the cell [41,53]. Currently, there is a strong debate about the safety aspects of chemical preservatives since they are considered responsible for many carcinogenic and teratogenic attributes as well as residual toxicity. For these reasons, consumers tend to be suspicious of chemical additives and thus the demand for natural and socially more acceptable preservatives has been intensified [54]. The evaluation of the safety of essential oils that have a documented history of use in foods starts with the presumption that they are safe based on their long history of use over a wide range of human exposures without known adverse effects. The close relationship of flavor complexes to food itself has made it difficult to evaluate the safety and regulate the use of essential oils. For essential oils, there is a requirement to specify the chemical constituents and their range of concentrations for the oil to be evaluated for Generally Recognized as Safe (GRAS). However, the chemical description represents the chemical composition of material considered for GRAS. It is not a required specification, since different batches of the commercial oil will not contain all listed constituents in the reference concentration ranges. Instead different batches will be required to exhibit upper concentration limits for congeneric group that comprises the essential oil.

Mutagenicity was evaluated by the *Salmonella*/microsome assay (TA100, TA98, TA97a and TA1535 tester strains), without and with addition of an extrinsic metabolic activation system (rat liver S9 fraction induced by Aroclor 1254). The mutagenic activity was initially screened using *Salmonella typhimurium* strains TA98 and TA100, with or without S9 metabolic activation. No mutagenicity was found in the oil to the both strains either with or without S9 mixture whereas significant mutagenic activity was induced by carvacrol generally in the absent of metabolic activity. Many of the individual constituents of the essential oils are themselves used as flavoring substances and pose no toxicological threat [55]. Besides, chronic studies have also been performed on over 30 major chemical constituents including methyl chavicol and cinnamldehyde and results have not revealed any safety concern [55].

Hence, it is recommended that more safety studies be carried out before EOs are more widely used or at greater concentrations in foods that at present.

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