Use of essential oils in food preservation

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New alternatives for food conservation and preservation are now emerging, as many studies have shown that the use of synthetic preservatives and chemical additives is leading to intoxication, cancer and other degenerative diseases. This has led to a growing consumer concern and the desire to consume healthier products containing natural preservatives and additives instead of synthetic ones. This generates the need to look for conservation alternatives that cover the same antimicrobial properties and compatibility with food. In this search, new antimicrobial agents of natural origin, as is the case of essential oils (EOs) obtained from aromatic and medicinal plants, have been found. EOs have antimicrobial activity against a wide range of microorganisms and antioxidant activity, this is generally attributed to phenolic compounds owned by EOs. Many studies *in vitro* have defined EOs as effective antimicrobial and antioxidant compounds, but they are not much used in industry. Mediterranean dietary food products (meat and meat products, cheeses and fruits) are highly appreciated by consumers; their preservation with EOs would represent an added value. The present study 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 food safety; antibacterial; antifungal; phenolic compounds; minimum inhibitory concentration

1. Introduction

The main cause of food spoilage is the occurrence of different types of microorganisms (bacteria, yeasts and moulds).

This problem economically affects manufacturers, distributors and consumers. It is estimated that more than 20% of all food produced in the world is spoiled by microorganisms (1). The control of physical, chemical and particularly microbiological factors is essential for food preservation (1, 2).

In recent years, consumers prefer food of easy preparation and good quality, safe, natural and low processed, but with longer shelf-life. With food preservation technologies, more long-lasting products are obtained, maintaining their initial nutritional and sensory characteristics (1, 3-5).

For many years, synthetic preservatives have been used in the food industry, the most used being antimicrobial preservatives, but at present there are studies indicating that the consumption of chemical additives can lead to allergies, intoxications, cancer and other degenerative diseases (1, 6). For this reason, they are depreciated by consumers, which motivates the need to look for other alternatives. This search has uncovered new antimicrobial agents of natural origin, as substitutes of those traditionally used (7, 8).

1.1 New alternatives for food preservation

One of the alternatives more studied is "natural conservation", which is the use of natural antimicrobial preservatives present in plants, animals or microorganisms, and especially those derived from extracts of various types of plants and parts of plants that are used as flavouring agents in some foods (6-9).

Antimicrobials are used in food to control natural spoilage processes (food preservation) and to control microbial growth (food safety) (10). The difficulty is to extract, purify, stabilise and incorporate these antimicrobial into food products without affecting its sensorial quality and safety (1).

The FDA (Food and Drug Administration) treats antimicrobial agents of natural origin as GRAS (Generally Recognized As Safe) type products, including plant products from which essential oils (EOs), oleoresins and natural extracts, as well as their distillates, are obtained (1, 11).

Recently, the food industry has shown a considerable interest in the extracts and EOs of aromatic plants due to their ability to control the growth of pathogenic microorganisms (1-14).

1.2 Medicinal and aromatic plants and EOs

Medicinal and aromatic plants (MAPs) are very important in various fields, such as the pharmaceutical, perfumery and cosmetic industries (15). MAPs constitute a high percentage of the natural flora, 80% of the world's population uses traditional MAPs-based medicines to treat various human health problems. More than 9000 native plants have been identified and recorded for their curative properties, and about 1500 species are known for their aroma and flavour (15, 16).

EOs are volatile substances, although is presented as a natural liquid that is extracted from different parts of medicinal and aromatic plant (flowers, buds, seeds, leaves, twigs, bark, herbs, wood, fruits and roots) (17). EOs are important for plant protection, because they have antimicrobial, antiparasitic, insecticidal, antiviral, antifungal and antioxidant properties (18). There has been an increasing demand in medicine, perfumery and cosmetic industries.

Furthermore, the use of EOs in food industry, as antioxidants, flavourings or colorants, has also increased and they can also be used for food preservation (17, 19).

Driven by the growing interest of consumers for natural ingredients and their concern about potentially harmful synthetic additives, the global demand for EOs is increasing nowadays, more than 250 types of EOs are marketed annually on the international market (15, 20, 21).

Before these products can be used, they have to be extracted from the plant matrix. Different methods can be used for this purpose; expression, distillation, solvent extraction and emergent methods (21).

Expression or cold pressing is mainly used for the isolation of citrus peel oils because of the thermal instability of the aldehydes contained therein. This method has the advantage of low heat generation during the process, but the disadvantages are that it gives low yields and low purity (21).

Distillation process consists basically in placing the plant material in boiling water or heating by steam, the heat applied causing break down of cell structure of plant material and releasing the EOs. The advantage of this method is that the volatile components can be distilled at temperatures lower than the boiling points of their individual constituents and are easily separated from the condensed water. Considering the manner in which the contact between water and the original matrix is promoted, distinguishes three types of distillation has been proposed: hydrodistillation, steam distillation and water/steam distillation (21, 22).

Solvent extraction allows to extract EOs with organic solvents (acetone, hexane, petroleum ether, methanol or ethanol), because of their hydrophobic and nonpolar character. In this method, solvent is mixed with the plant material and heated to extract the essential oil. Then, it is filtrated and concentrated by solvent evaporation, later it is mixed with pure alcohol to extract the oil at low temperatures and finally the alcohol is evaporated and the EO stays pure. This method has some disadvantages, such as the presence of traces of the solvents in the essential oil (dangerous for health) and the production of effluents (dangerous for the environment) (21).

Due to concerns about the environment, new separation techniques are emerging that reduce energy consumption and CO_2 emissions. Emergent methods for extracting OE are microwave assisted extraction, ultrasonic assisted extraction and supercritical fluid extraction.

Once the EOs are extracted, it is necessary to know their chemical composition, because their antimicrobial activity depends on their chemical composition.

There are different methods to analyse EOs, but gas chromatography (GC) has been described as the most suitable method to the analysis of EOs (21, 23). Nowadays, GC coupled with mass spectrometry is used to improve the separation of the different EOs compounds.

2. Composition of EOs

The chemical composition of EOS is complex; there may be around 20 to 60 different bioactive components in each EO. However, generally only 2-3 major components are present at a fairly high concentration (20-70%) compared to other components present in traces (16). Some factors that may affect these constituents include the geographic location; the environment, the stage of maturity harvest season or extraction method (15, 16).

EOs are secondary metabolites formed by plants, these metabolites protect to the plant against conditions of biotic and abiotic stress. Most EOs are composed of terpenes, and other aromatic and aliphatic constituents with low molecular weights (11, 15).

Terpenes are represented by the chemical formula $(C_5H_8)_n$ and are composed of isoprene units. These compounds are classified into several groups, such as monoterpenes ($C_{10}H_{16}$), sesquiterpenes ($C_{15}H_{24}$), diterpenes ($C_{20}H_{32}$), and triterpenes ($C_{30}H_{40}$).

The major bioactive components (~90%) of EOs oils are monoterpenes, that are synthesized within the cytoplasm of the cell through the mevalonic acid pathway (15). Some compounds include monoterpene hydrocarbons (p-cymene, α-pinene and α-terpinene), oxygenated monoterpenes (camphor, carvacrol, eugenol and thymol), diterpenes (kaurene and camphorene), oxygenated sesquiterpenes (spathulenol and caryophyllene oxide), monoterpene alcohols (geraniol, linalool and nerol), sesquiterpene alcohol (patchoulol), aldehydes (citral and cuminal), phenols (eugenol, thymol, carvacrol, and catechol) and coumarins (fumarin and benzofuran) (15, 16).

Generally, the EOs possessing the strongest antibacterial properties against foodborne pathogens contain a high percentage of phenolic compounds, such as carvacrol, eugenol and thymol. Phenolic compounds possess great structural variations and are one of the most diverse groups of secondary metabolites. The hydroxyl (-OH) groups in phenolic compounds are thought to cause inhibitory action as these groups can interact with the cell membrane of bacteria to disrupt membrane structures and cause the leakage of cellular components (8).

The major components of a number of EOs with antibacterial properties are presented in Table 1.

Table 1 Major components of selected[a] EOs that exhibit antibacterial properties.

Common name of EO	Latin name of plant source	Major components	References
Cinnamon	*Cinnamomum zeylandicum*	Trans-cinnamaldehyde	(24)
Cinnamon	*Cinnamon casia*	Trans-cinnamaldehyde Eugenol	(25) (26)
Clove	*Syzygium aromaticum*	Eugenol Eugenyl acetate	(27)
Oregano	*Origanum vulgare*	Carvacrol Thymol γ-terpinene p-cymene	(25, 28-30) (31-33) (34-36) (37-39)
Rosemary	*Rosmarinus officinalis*	α-pinene Bornyl acetate Camphor 1,8-cineole	(37, 40-42)
Sage	*Salvia officinalis L.*	Camphor α-pinene β-pinene 1,8-cineole α-tujone	(39)
Sage	*Salvia triloba*	Eucalyptol	(42)
Thyme	*Thymus vulgaris*	Thymol Carvacrol γ-terpinene p-cymene Linalool	(2, 24) (41, 43) (44, 45) (37, 46, 47) (25)

Adapted from Burt *et al.* (11)

[a] EOs which have been shown to exert antibacterial properties *in vitro* or in food models.

According to the study carried out by different authors, the percentages of main bioactive compounds of EOs named above are: cinnamon ~65 % Trans-cinnamaldehyde (24), clove ~75-85 % eugenol (8), oregano ~64 % thymol (31), oregano ~15 % p- Cymene and ~30 % carvacrol (30%) (48), rosemary ~14 % 1,8-cineole (37), sage ~42 % α-tujone and ~15 % camphor (39), thyme ~64 % thymol (2), and thyme ~44 % linalool (49).

EOs present different bioactive compounds, as well as different percentage of them, this explain the differences that exist between them in their behaviour against microorganisms, that is to say, their antimicrobial activity (8, 50).

3. EOs antimicrobial activity

The antimicrobial activity is mediated by a series of biochemical reactions, which are dependent on the type of chemical constituents present in the EOs (15, 50).

3.1 Mechanism of antimicrobial action of EOs

The mechanisms of antimicrobial action of EOs are not only different depending on the main chemical of EOs, also if the action is on Gram-positive or Gram-negative bacteria, or on fungi, that are found in food (11).

3.1.1 Mechanism of antibacterial action

Firstly, EOs destabilize the cellular structure, destroying membrane integrity and increasing permeability, and disrupting cellular activities, as for example energy production and membrane transport (15).

The disruption of the cell membrane causes the alteration of various vital processes, such as nutrient processing, the synthesis of structural macromolecules, and the growth regulators (15).

Owing to their lipophilic nature, EOs are easily penetrable through the bacterial cell membranes, causing to the leakage of cellular components and loss of ions (15, 51). Also antibacterial effects of EOs produce the alteration of proton pumps, and the depletion of the ATP (50), this alteration may cause a cascade effect, resulting in other cell organelles being affected.

The antibacterial effect of EOs constituents, such as thymol, menthol and linalyl acetate, is due to a perturbation of the lipid fraction of bacterial plasma membranes (52), while carvacrol changes the composition of fatty acids, which affects the membrane fluidity and permeability, and trans-cinnamaldehyde enters the periplasm of the cell and disrupts cellular functions (53).

3.1.2 Mechanism of antifungal action

The antifungal actions of EOs are similar to that of antibacterial mechanisms by direct contact with the fungus, but also EOs have antifungal activity in vapour phase, this effect is mainly for moulds (21, 54).

By contact, EOs penetrate and altered the fungal cell wall and cytoplasmic membranes by a permeabilisation process, which disintegrate the mitochondrial membranes (15).

The vapour phase generated by EOs attack the life cycle of some moulds in the stage of germination, affecting the growth of hypha and sporulation. The inactivation of conidia by EOs is the key process of inhibition, because conidia are stable to heat, light and chemical compounds, being difficult to eliminate (21).

3.2 Antimicrobial effects of EOs

EOs may inhibit the growth of bacteria (bacteriostatic) and of fungus (fungistatic) or destroy bacterial cells (bactericidal) and fungus (fungicide), but it is difficult to distinguish it. Thus, antimicrobial activity can be measured as the minimum inhibitory concentration (MIC), which is the lowest concentration of an antimicrobial that inhibits the growth of a microorganism after incubation (11).

3.2.1 Antibacterial effects

Some of the most severe foodborne bacteria are *Bacillus cereus*, *Escherichia coli*, *Listeria monocytogenes*, *Salmonella spp*. and *Staphylococcus aureus* (25).

Bacillus cereus is a Gram-positive bacterium producing spores and forming both thermostable and thermolabile toxins widely distributed in the environment, which can be transmitted to humans through contaminated foods. Studies have shown that EOs of rosemary inhibited the growth of this bacterium with a MIC of 0.2 µL mL^{-1} (11, 55).

According to the study of different authors on antimicrobial activity, EOs of clove, oregano, rosemary, sage and thyme were good inhibitors of the growth of *E. coli*, with a MIC range of 0.4-10 µL mL^{-1} (11, 56-59). Although *E. coli* (Gram-negative bacilli) lives in the intestine, it can reach food by poor manipulation or hygiene.

EOs of clove, oregano, rosemary, sage and thyme also presented antimicrobial action in *L. monocytogenes* (Gram-positive) with a MIC value of 0.15-0.45 µL mL^{-1} (11). *L. monocytogenes* causes listeriosis, which is a foodborne disease, but that occurs in sporadic cases or in outbreaks.

Salmonella spp. (Gram-negative bacilli) leads to salmonellosis, which is the most common cause of foodborne illness and it is transmitted by direct contact or cross-contamination during handling. EOs of clove, oregano, rosemary, sage and thyme produced great inhibition in the growth of this bacterium, with MIC value of 1.2- >20 µL mL^{-1} (11, 56), being oregano the one that presented greater inhibitory power.

Shan *et al.* (58), the EOs of cinnamon, oregano and clove were found to be effective against *Salmonella enterica*, but the clove extracts possessed the highest antibacterial activity (15).

S. aureus (Gram-positive bacilli) can produce a wide range of diseases, ranging from skin and mucosal infections, but it may also affect the gastrointestinal tract, through the intake of food products contaminated with staphylococcal enterotoxins.

Different studies confirmed that EOs clove, oregano, rosemary, sage and thyme were effective in inhibiting *S. aureus* growth, on MIC value of 0.2-10 µL mL^{-1} (11, 44, 56, 59).

In a study by Radaelli *et al.* (60), a major foodborne disease-causing agent, *Clostridium perfringens* (Gram-positive), was inhibited by EOs of rosemary with a MIC value of 10 mg mL^{-1}. *C. perfringens* is one of the most common causes of food poisoning. It often happen when food is prepared in large quantities and kept warm for a long time before serving them, which is why outbreaks of these infections are usually related to catering events.

EOs of MAPs as is showed in the Table 2, have antimicrobial activity on other pathogenic bacteria.

Table 2 Antibacterial activity against human pathogens.

Common name of EO	Inhibited microorganisms	References
Cinnamon	Enterobacteriaceae *Streptococcus pyogenes* *Streptococcus pneumoniae* *Enterococcus faecium* *Acinetobacter lwoffii* *Enterobacter aerogenes* *Klebsiella pneumoniae* *Proteus mirabilis* *Pseudomonas aeruginosa*	(15, 61)

	Mycobacterium smegmatis	
Oregano	*Clostridium perfringens* *Salmonella choleraesuis* *Bacillus subtilis* *Pseudomonas aeruginosa* *Shigella sonnei* *Sarcina lutea*	(15, 21)
Rosemary	*Bacillus subtilis* *Streptococcus agalactiae* *Streptococcus epidermidis* *Proteus vulgaris* *Proteus aeruginosa* *Klebsiella pneumoniae* *Enterococcus faecalis*	(41, 62)
Sage	*Providencia stuartii* *Shigella sonnei* *Sarcina lutea* *Brochothrix thermosphacta*	(15, 62)
Thyme	*Clostridium perfringens* *Shigella sonnei* *Sarcina lutea* *Brochothrix thermosphacta*	(21, 41)

3.2.2 Antifungal effects

In the food industry there is great interest in EOs derived from MAPs due to their property of controlling the growth of pathogenic microorganisms, such as *Fusarium spp*., *Aspergillus spp*., among others, which have been reported as causative agents of diseases caused by food and/or decomposition of the same (1).

The EOs have been used against a broad range of fungal pathogens, as is showed Table 3.

Clove essential oil showed a MIC value of 0.062 and 0.125 % (v/v) against *Candida albicans* and *Aspergillus niger*, respectively. Rosemary EO is also effective against *C. albicans* and *A. niger*, but with MIC values of 0.25 and 1.0 % (v/v), respectively (15).

The EOs of thyme and clove completely inhibited the mycelium growth of *Aspergillus flavus* (14).

The major chemical compound of clove, eugenol, was proved to produce permanent damage to the cells of *C. albicans* and was considered an efficient antifungal agent, with a MIC value of 1.0% v/v (63).

EOs of clove, cinnamon and oregano were effective against *Aspergillus parasiticus* and *Fusarium moniliforme*, because they caused damage in mycelium growth and mycotoxin-producing ability (64).

Table 3 Antifungal activity against human pathogens.

Common name of EO	Inhibited microorganisms
Cinnamon	*Candida albicans* *C. parapsilosis* *C. krusei* *Aspergillus flavus*
Clove	*Fusarium spp*
Oregano	*Phytophthora infestans* *Botrytis cinerea*
Rosemary	*Phytophthora infestans* *B. cinerea*
Thyme	*Fusarium oxysporum* *F. verticillioides* *Penicillium expansum* *P. brevicompactum* *Aspergillus flavus* *A. fumigatus* *Alternaria alternata*

Adapted from da Cruz-Cabral *et al.* (65)

4. Use of EOs in food industry

4.1 Meat and meat products

Usually, the antimicrobial effect of EOs is higher *in vitro* than in food products. This might be explained by the availability of more nutrients in food products than in laboratory media. Nutrient-rich matrices, such as meat and meat products, may enhance bacterial repair and turnover of cellular components, which may increase the resistance of bacterial populations to many different stresses (66). Still, there are a number of other reasons that may influence the antimicrobial effect of EOs in foods (67). The fat contents of traditional meat products are considerably high and EOs are soluble in lipids, which taken together with the usual low pH that increases the solubility of the EOs and the typical low a_W that reduces the aqueous phase of food products increasing the contact between EOs and spoilage or foodborne microbiota, enhances their antimicrobial effect.

The use of EOs to improve both food safety and shelf-life of meat products has been reported mainly in beef, chicken, lamb or rabbit fresh meat (2, 47, 68, 69).

However, only a few studies have described the use of EOs as food ingredients (70-72). Application of EOs to food products is uncommon and mainly limited to cooked meat products. The application of thyme and cinnamon EOs in ham has been shown to significantly decrease the *L. monocytogenes* population (70). Furthermore the shelf-life of mortadella has been extended with the use of rosemary/thyme EOs (72), while the shelf-life of bologna sausages was extended using oregano EO (71). García-Díez and colleagues (73) reported the improved safety of dry-cured sausages through the antibacterial effect of garlic and oregano EOs against *Salmonella* spp., *L. monocytogenes* and *S. aureus*. At concentrations of 0.5% and 1% clove EO restricted the growth of *L. monocytogenes* in meat at both 30 °C and 7 °C (74).

The antimicrobial effect of EOs is associated with their composition, the characteristics of each food product and the specific microorganisms to be eliminated. Additionally, several other factors may affect the antimicrobial effect of EOs in food products, namely heat treatments, smoking, chemical preservatives and packaging (11).

The use of EOs in combination with other technologies, such as thermal treatments, high isostatic pressures (HIP), pulsed electric field and active packaging against foodborne pathogens have also been reported (75, 76). For example, low concentrations of orange, lemon and mandarin EOs (0.2 mL/mL) combined with a mild heat treatment (54 °C/10') showed synergistic lethal effects, inactivating more than 5 log units of bacterial cells, thus demonstrating the potential of successful combined treatments for food preservation (77).

The use of EOs as natural preservatives in food industries meets the current consumer trends of "green", "biological", "natural" and "no chemicals added" labels. However, their organoleptic impact must be assessed in each product, since the concentrations needed to obtain the desired antimicrobial effect might be negatively perceived by consumers. For example, Viuda-Martos *et al.* (71) reported a marked aroma of oregano in bologna sausages, which however was not considered unpleasant by the panellists. Likewise, other authors described the use of oregano EO in a Spanish fermented dry-cured *salchichón*, which did not significantly affect sensory properties, but improved texture, with the consequent possible reduction in the ripening time (78). Furthermore, the addition of rosemary or marjoram EOs at a concentration of 200 mg/kg in beef patties has been shown to reduce lipid oxidation, due to the EOs' antioxidant properties, thus improving the flavour of the patties (79). Similarly good acceptance has been reported for foal meat after 10 days active packaging with 2% oregano EO, due to reduced lipid oxidation shown by lower TBARS values (80).

The references to recommended concentrations in food products are scarce in the literature, but Dussault and colleagues (70) have proposed a 5000 ppm limit.

Furthermore, the legal regulations for food additives consider EOs as flavouring agents (81). Rosemary extract is the only one included in EC Regulation No. 1333/2008 with a legal limit of 100 mg/kg for dry-cured meat products (81).

The combined use of different EOs, in lower concentrations, may satisfy the safety of food products not depreciating their sensory characteristics (10).

A mixture of cinnamon and clove EOs was found to have a good potential to inhibit growth of spoilage fungi, yeasts and bacteria usually found on intermediate moisture foods (a_W between 0.65 and 0.90), and thus seems to be an interesting alternative to chemical preservatives well suited to be used in active packaging systems (82).

An active packaging system for chicken meat with 4% rosemary EO has been shown to inhibit the increase of biogenic amines, namely putrescine, cadaverine and histamine, as well as enterobacteria, *Pseudomonas* spp. and *Brochothrix thermosphacta* involved in their production (83).

Combined use of EOs and starter cultures in the manufacture of traditional Chinese smoked horsemeat sausages showed that both EOs (cinnamon, cloves, ginger and anise) and starter cultures (*L. sakei* and *S. xylosus*) inhibited the accumulation of biogenic amines, namely tryptamine, putrescine, cadaverine, histamine and tyramine, and the growth of enterobacteria. It is noteworthy that the inhibitory effects of EOs were stronger than those of starter cultures.

Additionally, the synergistic effects between EOs and starter cultures against the accumulation of the abovementioned biogenic amines and the growth of enterobacteria were observed (84).

4.2 Cheeses

The antimicrobial efficacy of the different EOs in dairy products can be influenced by several factors, such as the chemical composition of these products, the concentration in which the oils are used and the microorganisms that are intended to be reduced or eliminated.

Comparing the anti-microbial effect of clove, cinnamon, bay and thyme EOs in different concentrations (0.1%, 0.5% and 1%) in low-fat and full-fat soft cheese, Smith-Palmer *et al.* (85) found that 1% was the most effective concentration for all EOs. The anti-*Listeria monocytogenes* effect was more pronounced in the low-fat cheese, but clove EO at 1% was more effective in the two types of cheese. This essential oil, at the same concentration, was more active against *Salmonella* Enteritidis in full-fat cheese than in low-fat cheese. When used at a concentration of 0.5%, the population of *Salmonella* Enteritidis recovered in the low-fat cheese, but not in full-fat cheese.

In fact, fat plays a protective role of the bacterial cells over antimicrobial agents. However, the protein content is higher in low-fat cheeses and can contribute to the reduction of the activity of the EOs due to the formation of complexes between the phenolic compounds of the EOs and the proteins of these products (67).

Govaris *et al.* (86) noted that the antimicrobial activity of oregano and thyme EOs was equivalent for *L. monocytogenes* and *Escherichia coli* O157: H7 in Feta cheese. However, they observed that *L. monocytogenes* reduced faster than *E. coli* O157: H7, most likely because the EOs have a more pronounced effect on Gram-positive than on Gram-negative bacteria (11).

This may have other consequences, for example in product safety, in processing and conservation phenomena, since the predominant microbiota, as well as starter cultures, are mostly made up of Gram-positive bacteria.

EOs can increase the shelf-life of dairy products, not only eliminating unwanted microorganisms, but also decreasing the degree of chemical deterioration during storage and marketing periods.

The addition of oregano and rosemary EOs has affected the number of mesophilic microorganisms in cream cheese.

This occurrence caused a lower pH reduction and a less pronounced acidity. In addition, the rancid and fermented flavours which determined a shorter shelf-life of the product, were less pronounced in products with added oregano and rosemary EOs, since the oxidative and fermentative processes were inhibited (36, 87).

The addition of marjoram and rosemary EOs has affected the population of mesophilic bacteria in full cream cheese, which resulted in a lower pH reduction and a less pronounced acidity.

Clove EO (0.5% and 1%) restricted the growth of *L. monocytogenes* in cheese both at 30 °C and 7 °C (74).

The reduction of the microbial population is also associated with reduced production of biogenic amines in fermented products. In Gouda cheese added with *Zataria multiflora* (thyme-like plant) EO, there was a significant reduction in the production of histamine and tyramine in cheeses with different EO concentrations. These reductions amounted to 44% and 46% for tyramine and histamine, respectively, with an EO concentration of 0.4%, although the preferred concentration for the sensory panel was 0.2%. In this case, the reduction of tyramine and histamine was 22% and 29%, respectively (88).

4.3 Fruits

Fruits and vegetables are perishable products, characterised by a short shelf-life due to weight loss and decay, this one caused mainly by fungal activity. This is a huge problem for producers, stakeholders and consumers. Part of the problem has been solved, or at least minimised, using low temperatures throughout the postharvest period, storage and transportation over long distances. However, the use of low temperatures is insufficient for consumers and commercial requirements. So a range of complementary techniques are therefore used to extend fruits' shelf-life, such as Modified Atmosphere (MAP) and Controlled Atmosphere (CA). Chemical agents have helped controlling pathogens during the postharvest period, however there are restrictions on their use related to environmental impact and food safety that should be considered. Thereby, in recent years, there has been a significant increase in research works about postharvest control of phytopathogens through alternative natural processes, such as the use of EOs from aromatic plants. EOs of various plant species have been studied, because of their antimicrobial properties, being effective in the control of fungi first of all *in vitro* and later on *in vivo* in vegetables and fruits.

Many research works demonstrated the real effectiveness of EOs action in some diseases' control. The role of EOs as well as their use in the active packaging of fruits and other foodstuffs, was considered in several studies (4, 8, 11, 17, 89-95).

An important systematic work was done by Wilson *et al.* (96) testing numerous extracts from plants and 49 EOs, that were evaluated for their antifungal activity against *Botrytis cinerea*, and among all the EO tested, palmarosa (*Cymbopogon martini*), red thyme (*Thymus zygis*), cinnamon leaf (*Cinnamomum zeylanicum*) and clove buds (*Eugenia caryophyllata*) demonstrated the best results controlling the fungus. The same authors stated that the most frequently occurring constituents in EOs showing high antifungal activity were *D*-limonene, cineole, β-myrcene, α-pinene, β-pinene and camphor.

Recently, Campos *et al.* (12) using thyme and sage EOs, in strawberries, in the head space of the package, observed the decreasing in the amount of fungi existing with these treatments, when compared with the control samples. Braga (97) also tested different EO in strawberry with promising results. Moghaddam *et al.* (98) presented results that proved the effect of the EO of *Echinophora platyloba* in relation to different fungi, highlighting its great antifungal power. Feliziani & Romanazzi (99) verified that the presence of some EOs has an effect on some fungi, especially on *Botrytis cinerea*. Reminding that this fungus appears very frequently in fruits in the postharvest period. Vitoratos *et al.* (100) confirmed the effect of EO of thyme, oregano and lemon with different concentrations, in strawberry, tomato and cucumber, to evaluate the reduction of propagation of different fungi. Scariot (101) obtained EOs from *Mentha arvensis, Citrus limon, Zingiber officinalis, Thymus vulgaris* and tested *in vitro* and *in vivo* in strawberries and verified that all of them would have some effect on the growth of the fungus *Botrytis cinerea*. Mohammadi *et al.* (92) tested *in vitro* the fungicide effect of four EOs (fennel, black caraway, peppermint and thyme) at five different concentrations and found that they presented effect on the growth of the fungus *Botrytis cinerea* and *Rhizopus stolonifer*.

EOs have been recently used in table grapes, in order to improve shelf-life without changing their organoleptic characteristics. Several authors tested a new package, with grapes wrapped with two distinct films, with the addition of a mixture of eugenol, thymol and carvacrol, and observed that microbial counts were drastically decreased as well as lower occurrence of berry decay (102, 103). Several works on 'Crimson Seedless' table grapes, using MAP and eugenol, thymol or menthol inside the packages, showed that counts for yeasts and moulds were significantly reduced in the grapes' packages with natural antimicrobial compounds. Valero *et al.* (102) tested table grapes on MAP conditions and active packaging by adding eugenol or thymol and obtained reduced losses of quality, considering sensory, nutritional and functional properties, and also lower spoilage counts, in active packaging with added EO. According to Ricardo-Rodrigues *et al.* (94), 'Crimson seedless' table grapes treated with eugenol and menthol EOs had better results during the storage time compared to grapes without such treatments.

Waithaka *et al.* (95) tested the controlling effect of EOs against other fungi, like *Alternaria* spp., *Fusarium* spp., *Colletotrichum* spp. and *Penicillium* spp., present in species like *Passiflora edulis* Sims (Passion fruit). They extracted EOs from rosemary and eucalyptus (*Eucalyptus agglomerata*). The main conclusion was that those EOs are capable of controlling the above referred passion fruits' fungal pathogens.

The use of edible coatings, like chitosan, combined with EOs, allows the synergetic effect of reducing weight loss and maintaining overall quality of fruits. Munhuweyi *et al.* (93) investigated the *in vitro* and *in vivo* inhibitory effects of chitosan EOs, with different concentrations of lemongrass, cinnamon, and oregano oils, using vapour emission and direct coating against *Botrytis s*p., *Penicillium* sp. and *Pilidiella granati* pathogens of pomegranate fruit. Chitosan film incorporated with oregano EO had the highest antifungal activity, followed by cinnamon and lemongrass EOs. The inhibitory effect was higher for fruit directly dipped into the chitosan-EO emulsions than those exposed to vapour.

Recently, the idea of using the potential antimicrobial effect of EOs has served as a basis for the development of innovative approaches to increase shelf-life of fresh-cut fruits and vegetables, referred to as minimally processed (MP) products (104), such as the use of EOs in addition to edible films (105). The more enlightened consumers show great interest in acquiring MP products, "easy to eat", and at the same time seeking to ensure that these products correspond to the concept of clean label, natural and environmentally "friendly".

5. Conclusions

EOs are natural substances extracted from medicinal and aromatic plants, commonly by distillation processes. These compounds have an important role in food preservation contributing to safety and shelf-life extension of food products.

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.

The EOs 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 EOs.

Driven by the growing interest of consumers for natural ingredients and their concern about potentially harmful synthetic additives, the global demand for EOs is increasing nowadays, and more than 250 types of EOs are marketed annually worldwide.

Most EOs are composed of terpenes, and other aromatic and aliphatic constituents with low molecular weights. The major bioactive fraction (~90%) of EOs is composed by monoterpenes. Generally, the EOs with the strongest antibacterial properties against foodborne pathogens contain a high percentage of phenolic compounds, such as carvacrol, eugenol and thymol.

The antagonistic effect of the major compounds of EOs are studied against bacteria (*B. cereus*, *E. coli*, *L. monocytogenes*, *Salmonella spp*., *S. aureus, C. perfringens,* among others*),* moulds (*Fusarium spp*. and *Aspergillus spp*., among others) and yeasts (for example *C. albicans*).

Besides the richness in nutrients, which increases microbiota resistance to EOs, factors as lipid contents, once EOs are soluble in lipids, and low a_W, that reduces the aqueous phase of food products increasing the contact between EOs and spoilage or foodborne microbiota, enhances their antimicrobial effect.

However, it must be taken into account that EOs have an intense taste and smell, which can modify the taste and aroma of food products. Therefore, studies should focus on the minimum necessary EO amount, which still maintains antimicrobial activity without changing the organoleptic characteristics of food products. Besides, the combined use of different EOs, in lower concentrations, may satisfy the safety of food products not depreciating their sensory characteristics.

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