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Innovative strategies based on the use of essential oils and their components to improve safety, shelf-life and quality of minimally processed fruits and vegetables

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Abstract

Minimally processed fruits and vegetables are one of the major growing sector in food industry. Although important for their nutritional values and convenience, their composition and physicochemical properties affect their microbiological shelf life and overall quality. On the other hand, processing steps as washing, if well performed, can partially reduce the occurring microflora and the use of sanitizers are perceived negatively by the consumers. For this reasons, researchers have proposed some alternatives to the use of traditional sanitizers, such as essential oils which are complex mixtures of volatile compounds, characterized by a strong sensorial impact and produced by many plants as secondary metabolites. In this perspective, this review discusses the growing importance of minimally processed fruits and vegetables and the potential application of essential oils and their components as natural antimicrobial. Finally, the mechanisms of action of these molecules have being reviewed taking into account their use in food systems.

Keywords: minimally processed fruits and vegetables, natural antimicrobials, essential oils, shelf-life

Introduction

The market for minimally processed fruits and vegetables has significantly increased in recent years and their appeal derives from their convenience and the decrease of generated waste (Rico, Martín-Diana, Barat, & Barry-Ryan, 2007). In particular, as reported by Figure 1, in USA, their consumption covers about 48% of the market. Moreover, because characterized by high levels of vitamins, fibres, minerals and antioxidants (Siroli *et al.*, 2014, 2015a,b,c), they can represent a convenient way of preventing cancers and chronic illnesses such as coronary heart disease (Bhalla, Gupta, & Jaitak, 2013). In fact, the recommended fruit and vegetable consumption for this purpose should be higher than 400 g/day and consumers should be encouraged to eat at least five servings of fruit and vegetables each day (Ragaert, Verbeke, Devlieghere, & Debevere, 2004). However, minimally processed fruits and vegetables are susceptible to microbial proliferation due to the loss of their natural resistance and their high water and nutrient content (Rico *et al.*, 2007; Serrano *et al.*, 2008). In addition, the fresh produce during processing are subjected to several processing steps such as peeling, cutting or slicing favouring the microbial growth due to the release of nutritive substances and the transport of the microbiota located on fruit and vegetable surfaces to the cut surfaces (Lanciotti, Belletti, Patrignani, Gianotti, Gardini, & Guerzoni, 2003; Rojas-Grau, Raybaudi-Massilia, Soliva-Fortuny, Avena-Bustillos, McHugh, & Martın-Belloso, 2007; Siroli *et al.*, 2014; Siroli *et al*., 2015a,b). Also the active metabolism of fruit tissue, and the confinement of final product inside the packaging favour the growth of the naturally occurring microorganisms (Lanciotti, Gianotti, Patrignani, Belletti, Guerzoni, & Gardini, 2004). Between 1996 and 2004, the Food Drug and Administration (FDA) responded to 14 outbreaks of foodborne illness for which lettuce or tomatoes were confirmed to be the source, where there were 859 cases of reported illness. In 2006 in the United States, there was a multi-state outbreak of *E. coli* 0157:H7 implicating spinach, 276 cases of foodborne illness and three deaths were reported (Centers for Disease Control and Prevention (CDC, 2006). In May 2011, Germany reported an ongoing outbreak of Shiga-toxin producing *E. coli* (STEC), serotype O104:H4, reporting 3785 cases of illness and 45 deaths. Other illness and deaths attributed to this outbreak were reported outside of Germany and sprouted seeds were later identified as the outbreak vehicle (EFSA, 2011). The lack of processing steps or factors able to eradicate microbial contaminants make necessary an efficient temperature control during manufacture, distribution and retailing to ensure the maintaining of the microbiological quality and the safety of minimally processed fruits and vegetables (Siroli *et al.*, 2014 2015a,b). Currently, for minimally processed vegetables, the washing step, performed with sanitizing solution, is the only phase able to reduce the number of pathogenic and spoilage microorganisms (Sao José & Vanetti, 2012) and nowadays, chlorine is the most common decontaminant used at industrial level (Rico *et al.*, 2007), although its use is prohibited in some European countries such as the Netherlands, Sweden, Germany and Belgium (Gil, Selma, López-Gálvez, & Allende, 2009). In addition, chlorine-based compounds are corrosive, cause skin and respiratory tract irritation and react with the organic matter present in the water leading to the formation of potentially harmful trihalomethanes (López-Gálvez, Allende, Selma, & Gil, 2009). However, at the concentration normally employed (50-200 mg/L) it does not achieve more than a 1-2 log reduction in bacterial populations and it is ineffective in reducing pathogens on vegetables (Oliveira, Viñas, Anguera, & Abadias, 2012). Also the control and maintenance of the cold chain of raw materials are not sufficient to eliminate and significantly delay the microbial spoilage and to guarantee the safety of these products (Soliva-Fortuny & Martín-Belloso, 2003). In fact, foodborne illness relating to the consumption of minimally processed fruit and vegetables is widely reported (Lynch, Tauxe, & Hedberg, 2009) and a wide literature shows the presence on fresh fruit and related minimally processed products of pathogenic species such as *Listeria monocytogenes*, *Salmonella* spp., *Yersinia enterocolitica*, *Aeromonas hydrophila*, *Staphylococcus aureus* and E*scherichia coli* O157:H7 (Abadias, Alegre, Usall, Torres, & Vinas, 2011; Alegre, Abadias, Anguera, Oliveira, & Vinas, 2010; Gunes & Hotchkiss, 2002; Harris *et al.*, 2003; Olaimat & Holley, 2012; Powell & Luedtke, 2000; Van Boxstael *et al.*, 2013). In addition, some literature reports show that emerging pathogens are more resistant to chlorinated compounds raising further concerns about the effectiveness and the use of chlorine in the minimally processed food industry (Abadias *et al.,* 2011; Allende, Martınez, Selma, Gil, Suarez, & Rodrıguez, 2007). These drawbacks have stimulated the research towards non-traditional sanitizers (hydrogen peroxide, peroxyacetic acid and ozone) and other alternative technologies such as physical treatments (UV-C light, ultrasound and gamma rays) (Gil *et al.*, 2009; Manzocco *et al.*, 2011). At the same time, the era of natural food additives has encouraged by the consumers oriented to low and/or natural additives (Carocho, Barreiro, Morales, & Ferreira, 2014; Gyawali, & Ibrahim, 2014; Zheng, Bae, Jung, Heu, & Lee, 2013). Although the latter does not always represent a benefit compared to chemical ones, in most cases they are believed to be healthier and able to confer functionality. Natural additives are compounds or groups of compounds that are already used empirically by the population for taste purposes. Fungi, seaweeds, and algae are also interesting sources of natural additives. These natural compounds have been around for some time, but in recent years they have gained more interest from the food industry for direct application or in synergy with other natural or chemical additives. Among natural additives, essential oils (EOs), complex mixture of volatile compounds characterized by a strong sensorial impact, are produced by many plants as secondary metabolites. Also called volatile oils, they may be obtained from all the organs of the plant, i.e. flowers, buds, seeds, leaves, roots, wood, stems, twigs, fruits or bark, and they are stored in secretory cells, cavities, canals, epidermis cells or glandular trichomes (Bakkali, Averbeck, Averbeck, & Idaomar, 2008). EOs are extracted from various aromatic plants generally located in warm temperate countries such as the Mediterranean and tropical countries where they represent an important part of the traditional medicine and their function in nature may be different. In fact, they can act as internal messengers, as defensive substances against herbivores or as volatiles directing not only natural enemies to these herbivores but also attracting pollinating insects to their host (Bakkali *et al.*, 2008). Among the many effects, they are important for their antimicrobial and antioxidant properties (Brewer, 2011; Carocho *et al.*, 2014; Pillai & Ramaswamy, 2012; Tiwari, Valdramidis, O'Donnell, Muthukumarappan, Bourke, & Cullen, 2009; Rasooli, 2007; Rios & Recio, 2005).

EOs are usually extracted from plants through several different methods, including steam, hydro-distillation or supercritical carbon dioxide. Most of these substances have been recognized as safe (GRAS) (EAFUS, 1998). Initially, EOs have been used to enhance the aroma of foods, but several researches have proved they can be useful for the prolongation of the shelf-life of different food systems, although, for this purpose, it is necessary understanding their mechanisms of action (Belletti, Lanciotti, Patrignani, & Gardini, 2008; Serrano, Martinez-Romero, Castillo, Guillen, & Valero, 2005)

Applicative potential

In these last two decades, essential oils (EOs) and their components have gained much attention from researcher because gifted of several properties such antimicrobial, antioxidant and anticancer activities (Khan, Huq, Khan, Riedl, & Lacroix, 2014; Runyoro, Ngassapa, Vagionas, Aligiannis, Graikou, & Chinou, 2010). Moreover, recent development in natural food preservatives has led to increase interesting application of EOs or their components into food packaging. Active packaging involves the support, coat, or absorption of active components on a solid matrix from which they can be released to the atmosphere and act as food preservation agents. Antimicrobial active packaging is aimed both to extend the fruit and vegetable shelf-life and to improve consumer safety by reducing, inhibiting, and/or retarding the growth of spoilage and pathogenic bacteria in packed foods and packaging materials (Khan *et al.*, 2014). The highly volatile and antimicrobial nature of natural plant EOs or their components make them attractive candidates for this purpose, and provide an alternative to less desirable synthetic additives (Becerril, Gomes-Lus, Goni, Lopez, & Nerin, 2007). Also antioxidant activity is one of the most intensively studied property in EO research, because oxidation damages various biological substances and subsequently causes many diseases, including cancer, liver, Parkinson's and Alzheimer's disease, aging, arthritis, inflammation, diabetes, atherosclerosis and AIDS. As a result, many illnesses have been treated with antioxidants to prevent oxidative damage and many researchers have being investigated the antioxidant activity of different EOs and their components in order to search for safe natural antioxidants. However, the largest amount of papers are referred to the study of the antimicrobial activity of EOs and their constituents for a potential application in food sector. Although, most of the papers regard the EO antimicrobial activity in vitro systems, and the application of EOs for antimicrobial purpose in real system is still limited, EOs have been used at lab-scale in bakery (Nielsen & Rios, 2000), cheese (Vazquez, Fente, Franco, Vazquez, & Cepeda, 2001), meat production (Quintavalla & Vicini, 2002), seafoods (Kykkidou *et al.*, 2009) and minimally processed fruits and vegetables (Lanciotti *et al.,* 2004; Serrano *et al.,* 2008; Siroli *et al.*, 2014, 2015a,b,c; Soliva-Fortuny & Olga Martın-Belloso, 2003).

Despite the strong antimicrobial activity against foodborne pathogens and spoilage microorganisms shown by EOs (Oussalah *et al.*, 2007; Rhayour *et al.*, 2003; Tassou, Koutsoumanis, & Nychas, 2000), their practical application is currently limited due to their strong impact and changes they cause in food products (Gutierrez, Barry-Ryan, & Bourke, 2008).

Moreover, the limited use is due to i) the variability of the composition of EOs (due to the geographic origin, agricultural techniques, season, methods of extraction, etc..) able to influence their effective overall antimicrobial activity (Burt, 2004); ii) the interaction of bioactive molecules with the food matrix (in particular with proteins, lipids, starch, etc..) limiting the contact of these molecules with the microbial cells, thereby reducing the effects on cell viability (Gutierrez *et al.*, 2008); iii) the lack of knowledge of the mechanisms by which these molecules exert their antimicrobial activity; iv) the lack of knowledge of the interaction between technological and composition parameters and their activity.

In this Review, the use of EOs and their components in minimally processed fruits and vegetables will be discussed.

Potential use of natural antimicrobials to prolong shelf-life and safety of minimally processed fruits.

Several investigations have focused on the search for natural antimicrobials able to increase the quality and safety of the minimally processed fruits (Allende *et al.*, 2007; De Azeredo *et al.*, 2011; López-Gálvez *et al.*, 2009; Siroli *et al.*, 2014, 2015a,b; Soliva-Fortuny & Olga Martın-Belloso, 2003; Vandekinderen, Devlieghere, De Meulenaer, Ragaert, & Van Camp, 2009) (Table 1). A wide literature shows the great potential as antimicrobials in model and food systems of EOs deriving from citrus fruit peels (Espina, Somolinos, Loran, Conchello, Garcia, & Pagan, 2011; Fisher, & Phillips, 2008; Settanni *et al.*, 2012). In particular, citral (3,7-dimethyl-2-7-octadienal), is an acyclic unsaturated monoterpene aldehyde found naturally in the volatile oils of citrus fruits, lemongrass, and other herbs and spices. It consists of a mixture of two isomers, geranial and neral, and is used for flavoring citrus-based beverages. Its antimicrobial properties and pleasant fruity scent could make citral a suitable antimicrobial ingredient for wider use in the food industry (Somolinos, García, Pagan, & Mackey, 2008). Citral and citron EO, at concentration compatibles with sensorial features of fruits, were able to significantly prolong the shelf-life of the fruit-based salads in syrup (Belletti, Lanciotti, Patrignani, & Gardini, 2008), and the stability of fruit based soft drink (Belletti, Sado Kamdem, Patrignani, Lanciotti, Covelli, & Gardini, 2007). Also, the antimicrobial activity of hexanal and 2-(*E*)-hexenal, which are components of the aroma of many fruits and vegetables, has been tested in model (Gardini, Lanciotti, & Guerzoni, 2001; Kubo & Fujita, 2001) and real systems (Corbo, Lanciotti, Gardini, Sinigaglia, & Guerzoni, 2000; Lanciotti *et al.*, 2003; Lanciotti *et al.*, 2004). Hexanal, 2-(*E*)-hexenal, and hexyl acetate improved shelf-life and safety of minimally processed fruits (Lanciotti *et al.*, 2004; Serrano *et al.*, 2008). In particular, the addition of hexanal and 2-(*E*)-hexenal in storage atmosphere of fresh-cut apples resulted in a positive effect on shelf-life, due to their antimicrobial activity against naturally occurring spoilage yeasts, and also when deliberately inoculated at levels of 10^3 cfu/g. Moreover, these molecules determined the enhancement of the sensorial properties, as well as the retention of the original colour of the packaged products (Corbo *et al.,* 2000). These aldehydes showed a great potential as antimicrobials also against pathogens such as *Salmonella* spp., *E. coli* and *Pseudeomonas aeruginosa* (Kubo *et al.,* 2001). Little information is available on the relationship between the outgrowth of spoilage microorganisms, their volatilome, and the perception of the decay of minimally processed vegetables by consumers. Also Siroli et al. (2015a), to increase the shelf-life and quality parameters of sliced apples, proposed the use of these antimicrobials and citron EO in apple dipping solution, alone or in combination, as alternative to the traditional sanitization methods. The use of these antimicrobials changed the naturally occurring yeast growth parameters with respect to the control. Samples treated with hexanal/2-(E)-hexenal and citral showed better colour and texture attributes compared to the controls. Siroli et al. (2014) demonstrated also that the optimization of the process and the package in active modified atmosphere (7% O_2 and 0% CO_2) increased the shelf-life of apples treated with the mixture hexanal/2-*(E)*-hexenal up to 35 days of storage (Figure 2).

Valero, Valverde, Martınez-Romero, Guillen, Castillo, & Serrano (2006) developed an active packaging by adding eugenol or thymol to table grapes stored 56 days under modified atmosphere (MAP) showing lower microbial spoilage counts in for samples stored in active packaging.

Also Serrano et al. (2005) developed a package based on the addition of eugenol, thymol, menthol or eucalyptol in MAP. The results showed that all EOs reduced moulds and yeasts and total aerobic mesophilic colonies by 4 and 2 log cfu/g, respectively, compared with control.

Rojas-Grau et al. (2007) investigated the effect of lemongrass, oregano oil and vanillin incorporated in apple puree-alginate edible coatings, on the shelf-life of fresh-cut ‘Fuji’ apples. All antimicrobial coatings significantly inhibited the growth of psychrophilic aerobes, yeasts and moulds. The antimicrobial effect of EOs against *L. innocua* inoculated into apple pieces before coating was also examined. Lemongrass (1.0 and 1.5% w/w) and oregano oil containing coatings (0.5% w/w) exhibited the strongest antimicrobial activity against *L. innocua* (4 log reduction).

Abadias *et al.* (2011) studied alternative agents in order to prevent the risk of undesirable by-products from chlorine disinfection in fresh-cut industries. Carvacrol, vanillin, peroxyacetic acid, hydrogen peroxide, Nacetyl-l-cysteine and Citrox were selected for their results *in vitro* assays against *E. coli* O157:H7 and *Listeria* spp., to be tested on fresh-cut apple plugs. Apple flesh was inoculated by dipping in a suspension of a mix of the studied pathogens at 10^6 cfu/mL, and then treated with the antimicrobial substances. All treatments were compared to deionized water and a standard sodium hypochlorite treatment (SH, 100mg/L, pH6.5). Pathogen population on apple plugs was monitored for up to 6 days at 10 °C. Bacterial reductions obtained by peroxyacetic acid, vanillin, hydrogen peroxide and N-acetyl-l-cysteine were similar or higher than reduction obtained by SH.

Carvacrol and cinnamaldehyde were very effective at reducing the viable count of the natural flora on kiwifruit when used at 0.15 μl/mL in dipping solution, but less effective on honeydew melon. It is possible that this difference has to do with the difference in pH between the fruits; the pH of kiwifruit was 3.2–3.6 and of the melon 5.4–5.5 (Rasooli, 2007).

Potential use of natural antimicrobials to prolong shelf-life and safety of minimally processed vegetables

The antimicrobial activity of EOs in vegetable dishes is promoted by the decrease of temperature storage and/or the decrease in pH (Skandamis & Nychas, 2000). Vegetables generally have a low fat content, which may contribute to the successful results obtained with EOs. In fact, due to their lipophilic nature, they could share in fat missing the microbial targets.

As previously reported, the safety and shelf-life of minimally processed vegetables are based on few tools such as modified atmosphere packaging and maintaining of refrigeration chain. Although chlorine is the most common decontaminant used in these products, the concentrations used are quite ineffective in reducing pathogens on vegetables. In addition, chlorine-based compounds bring to the formation of potentially harmful chlorinated by-products such as trihalomethanes. For this, plant EOs and their components have been investigated as natural sanitizer alternative to chlorine to control of foodborne pathogens and spoilage bacteria associated with minimally processed vegetables (2011Gunduz, Gonul, & Karapınar, 2010; Gutierrez, *et al.*, 2008; 2009). The *in vitro* antimicrobial activity of oregano (*Origanum vulgare*), thyme (*Thymus vulgaris*) EOs and their main components (carvacrol and thymol) against a huge variety of Gram-positive, Gram-negative bacteria, yeasts and moulds is well documented (Burt, 2004; Lanciotti *et al.*, 2004; Viuda-Martos, Ruiz-Navajas, Fernandez-Lopez, & Perez-Alvarez, 2007). However, there are very limited studies that investigate the antimicrobial efficacy of these natural antimicrobials alone or in combinations with other hurdles on vegetable produce. (De Azeredo *et al*., 2011; Gutierrez *et al.*, 2008; Gutierrez *et al.*, 2009; Muriel-Galet *et al*., 2013; Siroli *et al.*, 2015 b,c). In particular, as reported by Table 1, oregano and thyme were the most studied for this application. Siroli et al. (2015b,c) evaluated the efficacy of oregano and thyme EOs in comparison with chlorine for lamb's lettuce decontamination using them in the product washing solution. The data obtained showed that these EOs were able to assure a product shelf-life similar to that obtained with chlorine. Moreover, Siroli et al (2015b) demonstrated that increasing the temperature of the washing solution up to 13 °C, the EOs could be better exploited. In fact, it is well known that the temperature increase results in the vapour pressure increase of volatile molecules enhancing, consequently, their affinity for the cell membranes, main and primary target of antimicrobials (Gardini *et al.*, 1997). In fact, while in the first experimental phase chlorine (120 mg L^{-1}) and the natural antimicrobial showed the same reduction of the naturally occurring microbial population, in the second trial, thyme and oregano reduced the cell loads of mesophilic aerobic bacteria of about 1 log cfu/g more than the chlorine solution. In the experimental conditions of Siroli et al. (2015b,c), the initial reduction of the naturally occurring microbiota due to the use of EOs did not affect negatively the safety of the products. In fact, the pathogenic species, most frequently associated to minimally processed vegetables, such as *L. monocytogenes*, *E. coli, S. enteritidis* and *S. aureus*, were not detected after 14 d of storage at 6°C, also when inoculated. Also the colour and the withering data showed that the treatments applied can guarantee the maintenance of the main quality parameters affecting the consumer choice. In fact, by improving the condition of the washing process, the products treated with thyme and oregano, similarly to chlorine, were able to maintain good colour and turgidity attributes over 12 days of storage at 6°C (Figure 3). Also the sensorial analysis confirmed that the organoleptic features of the Lamb's lettuce treated with oregano and thyme instead of chlorine was not significantly affected.

Gunduz *et al.* (2010) conducted a study aimed to determine the efficacy of oregano oil in the inactivation of *Salmonella typhimurium* inoculated onto iceberg lettuce. The effects of washing with oregano oil (*Oreganum onites*), typical of Turkey, at three different concentrations and four different treatment times on survival of *S. typhimurium* inoculated to fresh cut iceberg lettuce were determined at 20 °C storage temperature and compared with chlorine. Reductions of *S. typhimurium* by washing with oregano did not exceed 1.92 logarithmic units regardless of the washing times and concentrations. The effectiveness of washing lettuce with 75 ppm oregano oil on inactivation of *S. typhimurium* was comparable with that affected by 50 ppm chlorine.

Muriel-Galet, Cerisuelo, López-Carballo Aucejo, Gavara, & Hernández-Muñoz (2013) tried to improve the packaging of salad by combining modified atmosphere packaging with a new antimicrobial active bag consisting of PP/EVOH film with oregano EO or citral, with the purpose of extending shelf-life and reducing possible microbiological risks. The results showed that microorganism counts decreased especially at the beginning of the storage period. Oregano and citral samples had reductions of 1.38 log and 2.13 log respectively against enterobacterias, about 2 log against yeasts and moulds. The total aerobic counts reduced 1.08 log with oregano EO and 1.23 log with citral and the reduction of lactic acid bacteria and psychrotrophic was about 2 log. Citral-based films appeared to be more effective than materials containing oregano EO in reducing spoilage flora during storage time. Sensory studies also showed that the package with citral was the most accepted by customers at the end of the shelf-life.

Gutierrez *et al.* (2009) studied the efficacy of plant EOs for control of the natural spoilage microflora on ready-to-eat lettuce and carrots whilst also considering their impact on sensory properties. Initial decontamination effects, achieved using EOs, were comparable to that observed with chlorine and solution containing oregano recorded a significantly lower initial total count level than the water treatment on carrots. No significant difference was found between the EO treatments and chlorine considering gas composition, colour, texture and water activity of samples. The sensory panel found EO treatments acceptable for carrots throughout storage, while lettuce washed with the EO solutions were rejected for overall appreciation by day 7.

Valero & Giner (2006) studied the possible use of antimicrobials from seven plant EOs as food preservatives by examining their effects on the growth kinetics of activated *Bacillus cereus* INRA L2104 spores inoculated into tyndallized carrot broth. The effects of various concentrations of borneol, carvacrol, cinnamaldehyde, eugenol, menthol, thymol, and vanillin were determined. Lower concentrations of the three antimicrobials prolonged the lag phase and reduced both the exponential growth rate and the final population densities of cultures. 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.

Mechanisms of action

Although the antimicrobial properties of EOs and their components have been tested in the past (Holley & Patel, 2005), their mechanisms of action has not being studied in detail (Nazzaro, Fratianni, De Martino, Coppola, & De Feo, 2013). Considering the large number of different groups of chemical compounds present in the EOs, it is likely that their antibacterial activity is not attributable to a specific mechanism but there are more ways and targets in the microbial cell. The locations or mechanisms inside the bacterial cells seem to be the major sites of action of the components of the EOs (Picone, Laghi, Gardini, Lanciotti, Siroli, & Capozzi, 2013).

The antimicrobial activity of the EOs seems to be related to their composition, to the structural configuration of the constituents and to their functional groups, as well as to the possible synergistic interactions among the components. Consequently, the chemical structure of the individual compounds present in the EOs affects their precise mode of action and their antibacterial activity (Picone *et al.*, 2013; Viuda-Martos *et al.*, 2008). Some works on *Saccharomyces cerevisiae* have shown that the cytotoxicity of some EOs, on the basis of the ability to form colonies, was considerably different depending on their chemical composition. Treatments with EOs on cells in stationary growth phase showed 50% mortality with 0.45 µL / mL of EO of *Origanum compactum*, 1.6 µL / mL of EO of *Coriandrum sativum*, > 8 µL / mL of EO of *Cinnamomum camphora*, *Artemisia herba-alba* and *Helichrysum italicum* (Bakkali *et al.*, 2008).

As previously mentioned, the aromatic molecules among the various physical properties, are characterized by a poor solubility in water and a high hydrophobicity. For this reason, many studies indicate their antimicrobial effects as dependent on these characteristics and on their ability to act on the cell membrane. In addition, the bioactivity of many aromatic compounds may depend on the vapour pressure, which can be considered an indirect measure of hydrophobicity (Picone *et al.*, 2013). The factors responsible for the increase of the vapour pressure of the aromatic molecules lead to a rise of the antimicrobial activity, since it increases their solubility in cell membranes (Picone *et al.*, 2013). Precisely, their hydrophobicity permits them to have a good partition in the lipids of cell membranes and mitochondria, altering the structures and making them more permeable and leading to the loss of ions and other cell contents (Nazzarro *et al.*, 2013). The bacterial cell can tolerate, up to a certain limit, the loss of some cell contents, but their excessive leakage or the loss of critical molecules and ions lead to the cell death.

Many studies indicate the cell membrane as the primary target of bioactive aromatic compounds. Membranes disrupted by the action of terpenes can be observed both on bacteria and fungi (Holey & Patel, 2005; Lanciotti *et al.*, 2004, Liolios *et al.*, 2009; Nazzarro *et al.*, 2013; Viuda-Martos *et al.*, 2008). The antimicrobial action of many EOs appears to be connected with the presence of phenolic compounds. Various studies, concerning oregano species have shown that their oils possess strong antimicrobial activity; this activity could be attributed to their high percentage of phenolic compounds and, specifically, carvacrol, thymol, p-cymene and their precursor c-terpinene (Liolios *et al.*, 2009). It was hypothesized that the inhibition against *S. typhimurium* and *S. aureus* by the thyme EO, was dependent on the hydrophobicity and the nature of the present phenolic constituents, which determined alteration of the functionality of membrane proteins after partitioning in the phospholipid bilayer. The inhibitory effect of phenols can be explained with the interaction with the cell membrane of microorganisms, and it is often correlated with the hydrophobicity of the compounds (Liolios *et al.*, 2009). The lipophilic structure of cyclic monoterpenes promotes their partition from the aqueous phase to cell membranes resulting in expansion and increase in fluidity and permeability of the membrane, which leads ultimately to an inhibition of membrane enzymes (Nazzarro *et al.*, 2013). In some microorganisms, mild heat treatments increase the inhibitory effect of carvone, altering the membrane composition, the fluidity and favouring the partition of these molecules in the membrane phospholipids. In bacteria, the permeabilization of membranes is associated with the loss of ions and the reduction of the membrane potential, the collapse of the proton pump and the depletion of the ATP pool (Nazzarro *et al.*, 2013). EOs can coagulate the cytoplasm (Picone *et al.*, 2013) and cause damage to lipids and proteins (Burt, 2004). The damages to the cell wall and in the membranes, may lead to loss of macromolecules up to cell lysis. In particular, the loss of specific ions, due to the action of the aromatic molecules on the cell membrane, has dramatic effects on the proton motive force, by decreasing the content of intracellular ATP. In this manner, the total activity of the cells is greatly compromised, as well as the cellular turgor (osmotic pressure), the transport of solutes and the regulation of metabolism (Lanciotti *et al.*, 2004). The oregano EO, for example, creates an alteration of membrane permeability with a consequent loss of protons, phosphorus and potassium. Carvacrol leads to a dissipation of intracellular ATP in *B. cereus* due to the reduction of the synthesis or hydrolysis, accompanied by the increase of permeability of the membrane to ATP. Also Picone et al. (2013), who evaluated the effect of four levels of carvacrol (0–2 mM) on *Escherichia coli* 555 metabolome by using 1H-NMR spectroscopy, showed clearly that the cells exposed to the highest carvacrol concentrations were unable to recover the damages caused by the terpenic molecule. In fact, the interaction of hydrophobic molecules with cell membranes is known to affect the activity of membrane bound or embedded enzymes. The inhibition of membrane bound ATPases in *Listeria monocytogenes* and *E. coli* was also demonstrated (Gill & Holley, 2006). Moreover, dissipation of pH gradients, K and P leakage from *Pseudomonas aeruginosa* and *Staphylococcus aureus* were reported following treatment with carvacrol-containing oregano EO. The result of the NMR experiments showed a chemical shift of metabolites as a consequence of the perturbation induced by increasing amount of carvacrol. The first striking evidence involved glucose, which tends to be accumulated in the cells treated with carvacrol.

Some studied have examined the antifungal activity of citrus EOs (Viuda-Martos *et al.*, 2008). Citrus EOs are a complex mixture of volatile compounds that show, among other properties, antifungal activity by reducing or totally inhibiting fungal growth in a dose-response manner. Some authors have attributed the antifungal capacity of citrus EOs to the presence of components such as D-limonene, linalool or citral (Siroli *et al*., a,b) which are present in differing concentrations in citric Eos, although this function can be attributed to the phenolic compounds. The hydrophilic part of the molecule interacts with the polar part of the membrane, while the hydrophobic benzene ring and the aliphatic side chains are buried in the hydrophobic inner part of the bacterial membrane. Furthermore, the involvement of the hydroxyl group in the formation of hydrogen bonds and the acidity of these phenolic compounds may have other possible explanations (Nazzarro *et al*., 2013). Possible action mechanisms by which mycelial growth may be reduced or totally inhibited have been proposed. It is commonly accepted that it is the toxic effects of the EO components on the functionality and structure of the cell membrane that is responsible for the aforesaid activity. Serrano et al., (2013) related low EO concentrations with changes in the cell structure, which would inhibit respiration and alter the permeability of the microbe cell membrane, while high concentrations would provoke severe damage to the membrane and the loss of homeostasis, leading to cell death. Viuda-Martos *et al.* (2007) suggested that components of the EOs cross the cell membrane, interacting with the enzymes and proteins of the membrane, so producing a flux of protons towards the cell exterior which induces changes in the cells and, ultimately, their death. Cristani et al. (2007) reported that the antimicrobial activity is related to ability of terpenes to affect not only permeability but also other functions of cell membranes, these compounds might cross the cell membranes, thus penetrating into the interior of the cell and interacting with critical intracellular sites.

A number of characteristics of Gram-negative bacteria including the virulence and pathogenicity are regulated through the quorum sensing, a mechanism by which the bacterial population measure its cell density. It is a communication from cell to cell, based on the synthesis, the exchange and the perception of small signal molecules between bacteria, and that regulate the expression of certain sets of genes. Its interruption is an example of anti-pathogenic effect. Several EOs including cinnamon (*Cinnamomum zeylanicum*), mint (*Mentha piperita*) and lavender (*Lavandula officinalis*) have shown a potential anti-quorum sensing activity on pigment production by *C. violaceum* (Khan *et al.*, 2009). It is not clear if acting on the quorum sensing system are the larger or the smaller constituent of the EOs. The common mechanism of interference with quorum sensing includes:

i) the inhibition of the biosynthesis of signals or the inhibition of the activity of enzymes that produce N-acyl-homoserine lactones (AHLs) (small molecules that act as signals for the quorum sensing).
ii) the degradation of enzymatic signals;
iii) the inhibition of molecules of signal reception. It is also possible that the final effect on the inhibition of particular traits related to quorum sensing may be the result of an action of the various multi-target components of EOs on bacterial quorum sensing system.

Future perspective

The results reported in this review provided encouraging information concerning the use of EO in food sector. However, their effect on food constituents and microorganisms remains a focal area for future research. Moreover, a deeper knowledge of the effects of their physicochemical properties on their bio-activities needs to be better investigated. The study of the synergistic effects among EOs and/or their components could be utilized both to make best use of their antibacterial activity and to reduce their concentrations required to achieve a particular antibacterial effect for food safety and for health purposes.

In addition, also the role of EOs on the human gut needs to be investigated since the publications on this topic are still few.

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Figure Caption

Figure 1. Market of fresh and minimally processed fruits and vegetables in USA. Adapted from (Cook, 2008).

Figure 2. Evolution of colour in minimally processed apples treated with traditional dipping in 0.5% ascorbic acid and 1% citric acid (a), citral/2-(E)-hexenal (b) (125mg/L each one) and hexanal/2-(E)-hexenal (c) (125mg/L each one).

Figure 3. Evolution of colour in minimally processed lamb's lettuce treated with chorine 120 mg/L (control), thyme 250 mg/L and oregano 250 mg/L.

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Table 1. Overview of cases testing the antimicrobial activity of essential oils (EO) or their components in minimally processed fruits and vegetables.

	Food	EO or component	Concentration applied	Microbial targets	References
Fruits	*Kiwifruits*	Carvacrol	1mM in dipping solution	Natural microflora	Roller and Seedhar, 2002
	Honeydew melon	Carvacrol and cinnamic acid	1mM in dipping solution	Natural microflora	Roller and Seedhar, 2002
	Fresh sliced apples	Hexanal, hexyl acetate E(2)hexenal,	50-250 ppm 20-200 ppm	*Salmonella enteritidis, Escherichia coli, Listeria monocytogenes*	Lanciotti et al., 2003
	Sweet cherries	Eugenol, thymol, menthol, eucalyptol	1000µL in gas used for MAP	Natural microflora	Serrano et al., 2005
	Table Grape	eugenol thymol	75-150µL in gas used for MAP	Natural microflora	Valero et al., 2006
	Fresh sliced apples	Oregano lemongrass vanillin used encapsulated	0.1 and 0.5% (w/w) 1 and 1.5% (w/w) 0.2 and 0.6% (w/w)	Natural microflora and inoculated *L. innocua*	Rojas-Grau et al., 2007
	Fruit salads	Citral	25-125 ppm	*Salmonella enteritidis,*	Belletti et al., 2008

		Citron EO	300-600 ppm	*Escherichia coli, Listeria monocytogenes*	
	Fresh cut apples	Vanillin	12 g/L	*E. coli* O157:H7 *Listeria* spp	Abadias et al. 2011
	Avocado	Thyme EO in MAP	75 µL in filter	Natural microflora	Sellamuthu et al., 2013
	Fresh cut apples in MAP	Citron EO, Hexanal E(2)hexenal, Citral carvacrol	Alone 250 mg/L Combination 125 mg/L	Natural microflora *Listeria monocytogenes, Escherichia coli, Salmonella enteritidis*	Siroli et al., 2014 Siroli et al., 2015 Siroli et al., 2015
Vegetables	*Lettuce*	Thyme EO	0.1-10 mL/L	*E. coli* O157:H7	Singh et al., 2002
	Carrots	Thyme EO	0.1-10 mL/L	*E. coli* O157:H7	Singh et al., 2002
	Eggplant salad	Oregano EO	0.7-2.1% v/w	*E. coli* O157:H7	Skandamis and Nychas, 2000
	Lettuce and carrots	Oregano and thyme EOs	Alone 250 mg/L Combination 125 mg/L	Natural microflora	Gutierrez et al., 2009
	Lettuce	Oregano EO	25-75 mg/L	*Salmonella tiphymurium*	Gunduz et al., 2010

	Iceberg lettuce	Oregano and rosemary	0.003 to 80 µL/m	*Listeria monocytogenes, Yersinia enterocolitica* and *Aeromonas hydrophilla*	De Azeredo et al., 2011
	Four season salad	Oregano EO and citral in packaging	7.5% w/w	Natural microflora	Muriel-Galet et al., 2013
	Lamb's lettuce	Oregano and Thyme EOs	250 mg/L	Natural microflora *Listeria monocytogenes, Escherichia coli*	Siroli et al., 2015a, b

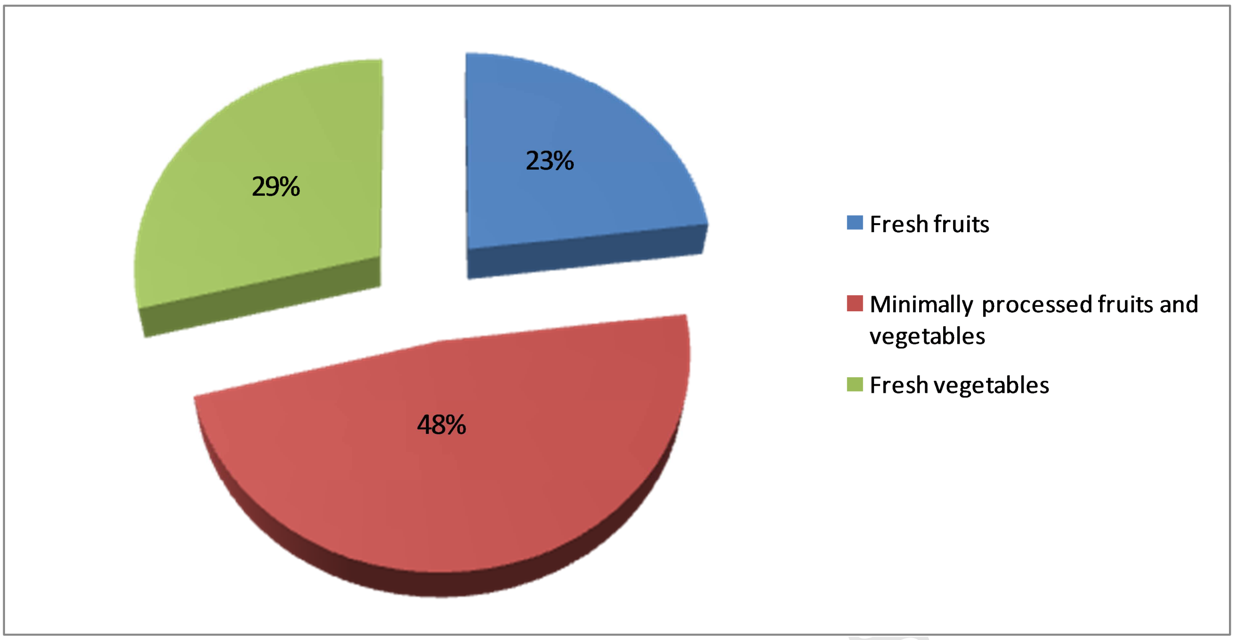
23%
29%
48%
Fresh fruits
Minimally processed fruits and vegetables
Fresh vegetables

Figure 1.


a
T=0
a
T=7
a
T=14
a
T=21
b
T=0
b
T=7
b
T=14
b
T=21
b
T=28
c
T=0
c
T=7
c
T=14
c
T=21
c
T=35

Figure 2.

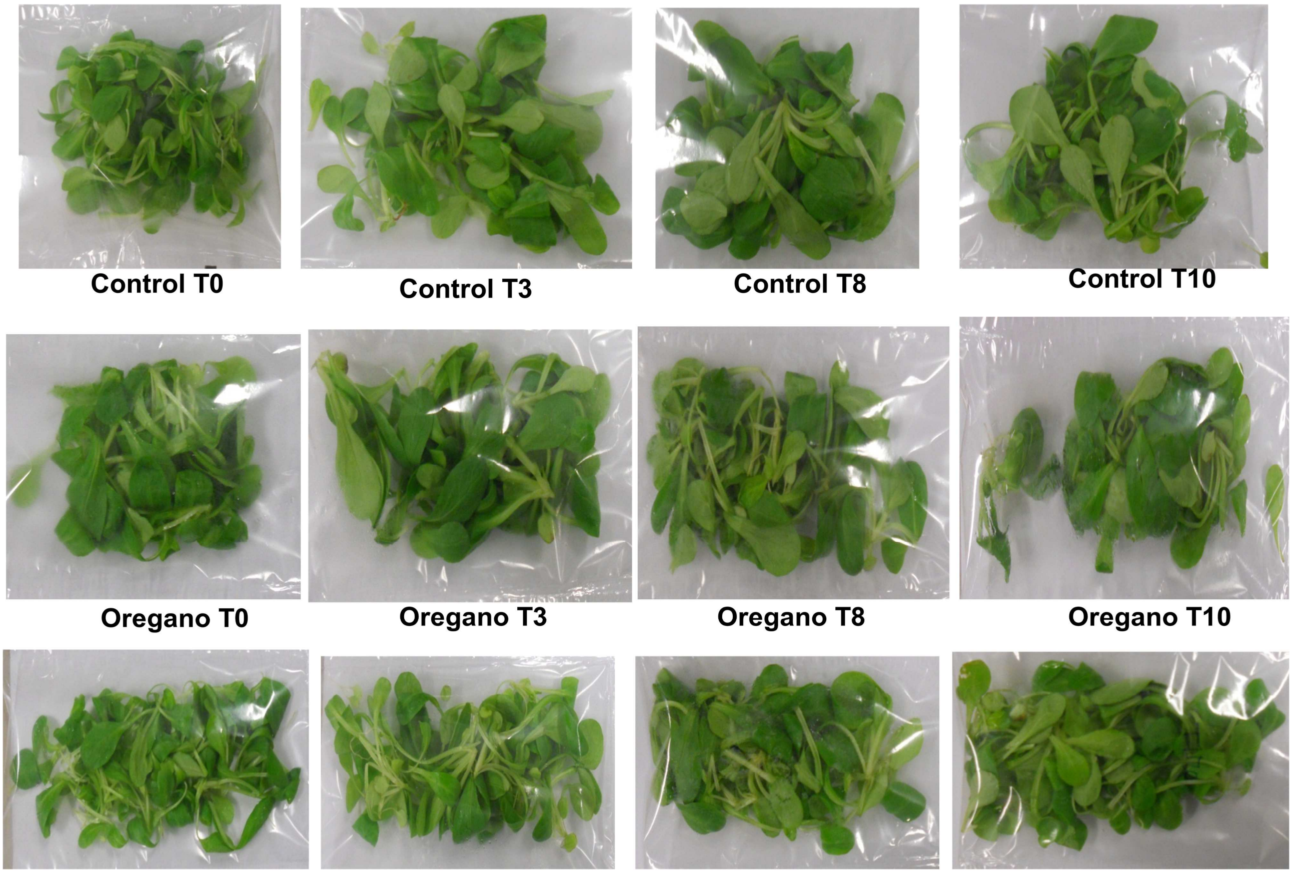
Control T0
Control T3
Control T8
Control T10
Oregano T0
Oregano T3
Oregano T8
Oregano T10
Thyme T0
ThymeT3
ThymeT8
Thyme T10

Figure 3.

- Natural antimicrobials represents a useful tool for decontamination of minimally processed fruits
- Essential oils represent a useful tool for decontamination of minimally processed vegetables
- Mechanisms of action of Essential oils need to be more investigated.