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

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Abstract

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

34
35 **Keywords:** minimally processed fruits and vegetables, natural antimicrobials, essential oils, shelf-
36 life

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47 Introduction

48 The market for minimally processed fruits and vegetables has significantly increased in recent years
49 and their appeal derives from their convenience and the decrease of generated waste (Rico, Martín-
50 Diana, Barat, & Barry-Ryan, 2007). In particular, as reported by Figure 1, in USA, their
51 consumption covers about 48% of the market. Moreover, because characterized by high levels of
52 vitamins, fibres, minerals and antioxidants (Siroli *et al.*, 2014, 2015a,b,c), they can represent a
53 convenient way of preventing cancers and chronic illnesses such as coronary heart disease (Bhalla,
54 Gupta, & Jaitak, 2013). In fact, the recommended fruit and vegetable consumption for this purpose
55 should be higher than 400 g/day and consumers should be encouraged to eat at least five servings of
56 fruit and vegetables each day (Ragaert, Verbeke, Devlieghere, & Debevere, 2004). However,
57 minimally processed fruits and vegetables are susceptible to microbial proliferation due to the loss
58 of their natural resistance and their high water and nutrient content (Rico *et al.*, 2007; Serrano *et al.*,
59 2008). In addition, the fresh produce during processing are subjected to several processing steps
60 such as peeling, cutting or slicing favouring the microbial growth due to the release of nutritive
61 substances and the transport of the microbiota located on fruit and vegetable surfaces to the cut
62 surfaces (Lanciotti, Belletti, Patrignani, Gianotti, Gardini, & Guerzoni, 2003; Rojas-Grau,
63 Raybaudi-Massilia, Soliva-Fortuny, Avena-Bustillos, McHugh, & Martín-Belloso, 2007; Siroli *et al.*,
64 *et al.*, 2014; Siroli *et al.*, 2015a,b). Also the active metabolism of fruit tissue, and the confinement of
65 final product inside the packaging favour the growth of the naturally occurring microorganisms
66 (Lanciotti, Gianotti, Patrignani, Belletti, Guerzoni, & Gardini, 2004). Between 1996 and 2004, the
67 Food Drug and Administration (FDA) responded to 14 outbreaks of foodborne illness for which
68 lettuce or tomatoes were confirmed to be the source, where there were 859 cases of reported illness.
69 In 2006 in the United States, there was a multi-state outbreak of *E. coli* 0157:H7 implicating
70 spinach, 276 cases of foodborne illness and three deaths were reported (Centers for Disease Control
71 and Prevention (CDC, 2006). In May 2011, Germany reported an ongoing outbreak of Shiga-toxin

72 producing *E. coli* (STEC), serotype O104:H4, reporting 3785 cases of illness and 45 deaths. Other
73 illness and deaths attributed to this outbreak were reported outside of Germany and sprouted seeds
74 were later identified as the outbreak vehicle (EFSA, 2011). The lack of processing steps or factors
75 able to eradicate microbial contaminants make necessary an efficient temperature control during
76 manufacture, distribution and retailing to ensure the maintaining of the microbiological quality and
77 the safety of minimally processed fruits and vegetables (Siroli *et al.*, 2014 2015a,b). Currently, for
78 minimally processed vegetables, the washing step, performed with sanitizing solution, is the only
79 phase able to reduce the number of pathogenic and spoilage microorganisms (Sao José & Vanetti,
80 2012) and nowadays, chlorine is the most common decontaminant used at industrial level (Rico *et*
81 *al.*, 2007), although its use is prohibited in some European countries such as the Netherlands,
82 Sweden, Germany and Belgium (Gil, Selma, López-Gálvez, & Allende, 2009). In addition,
83 chlorine-based compounds are corrosive, cause skin and respiratory tract irritation and react with
84 the organic matter present in the water leading to the formation of potentially harmful
85 trihalomethanes (López-Gálvez, Allende, Selma, & Gil, 2009). However, at the concentration
86 normally employed (50-200 mg/L) it does not achieve more than a 1-2 log reduction in bacterial
87 populations and it is ineffective in reducing pathogens on vegetables (Oliveira, Viñas, Anguera, &
88 Abadias, 2012). Also the control and maintenance of the cold chain of raw materials are not
89 sufficient to eliminate and significantly delay the microbial spoilage and to guarantee the safety of
90 these products (Soliva-Fortuny & Martín-Belloso, 2003). In fact, foodborne illness relating to the
91 consumption of minimally processed fruit and vegetables is widely reported (Lynch, Tauxe, &
92 Hedberg, 2009) and a wide literature shows the presence on fresh fruit and related minimally
93 processed products of pathogenic species such as *Listeria monocytogenes*, *Salmonella* spp.,
94 *Yersinia enterocolitica*, *Aeromonas hydrophila*, *Staphylococcus aureus* and *Escherichia coli*
95 O157:H7 (Abadias, Alegre, Usall, Torres, & Vinas, 2011; Alegre, Abadias, Anguera, Oliveira, &
96 Vinas, 2010; Gunes & Hotchkiss, 2002; Harris *et al.*, 2003; Olaimat & Holley, 2012; Powell &
97 Luedtke, 2000; Van Boxtael *et al.*, 2013). In addition, some literature reports show that emerging

98 pathogens are more resistant to chlorinated compounds raising further concerns about the
99 effectiveness and the use of chlorine in the minimally processed food industry (Abadias *et al.*, 2011;
100 Allende, Martinez, Selma, Gil, Suarez, & Rodriguez, 2007). These drawbacks have stimulated the
101 research towards non-traditional sanitizers (hydrogen peroxide, peroxyacetic acid and ozone) and
102 other alternative technologies such as physical treatments (UV-C light, ultrasound and gamma rays)
103 (Gil *et al.*, 2009; Manzocco *et al.*, 2011). At the same time, the era of natural food additives has
104 encouraged by the consumers oriented to low and/or natural additives (Carocho, Barreiro, Morales,
105 & Ferreira, 2014; Gyawali, & Ibrahim, 2014; Zheng, Bae, Jung, Heu, & Lee, 2013). Although the
106 latter does not always represent a benefit compared to chemical ones, in most cases they are
107 believed to be healthier and able to confer functionality. Natural additives are compounds or groups
108 of compounds that are already used empirically by the population for taste purposes. Fungi,
109 seaweeds, and algae are also interesting sources of natural additives. These natural compounds have
110 been around for some time, but in recent years they have gained more interest from the food
111 industry for direct application or in synergy with other natural or chemical additives. Among natural
112 additives, essential oils (EOs), complex mixture of volatile compounds characterized by a strong
113 sensorial impact, are produced by many plants as secondary metabolites. Also called volatile oils,
114 they may be obtained from all the organs of the plant, i.e. flowers, buds, seeds, leaves, roots, wood,
115 stems, twigs, fruits or bark, and they are stored in secretory cells, cavities, canals, epidermis cells or
116 glandular trichomes (Bakkali, Averbeck, Averbeck, & Idaomar, 2008). EOs are extracted from
117 various aromatic plants generally located in warm temperate countries such as the Mediterranean
118 and tropical countries where they represent an important part of the traditional medicine and their
119 function in nature may be different. In fact, they can act as internal messengers, as defensive
120 substances against herbivores or as volatiles directing not only natural enemies to these herbivores
121 but also attracting pollinating insects to their host (Bakkali *et al.*, 2008). Among the many effects,
122 they are important for their antimicrobial and antioxidant properties (Brewer, 2011; Carocho *et al.*,

123 2014; Pillai & Ramaswamy, 2012; Tiwari, Valdramidis, O'Donnell, Muthukumarappan, Bourke, &
124 Cullen, 2009; Rasooli, 2007; Rios & Recio, 2005).

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

132

133 **Applicative potential**

134 In these last two decades, essential oils (EOs) and their components have gained much attention
135 from researcher because gifted of several properties such antimicrobial, antioxidant and anticancer
136 activities (Khan, Huq, Khan, Riedl, & Lacroix, 2014; Runyoro, Ngassapa, Vagionas, Aligiannis,
137 Graikou, & Chinou, 2010). Moreover, recent development in natural food preservatives has led to
138 increase interesting application of EOs or their components into food packaging. Active packaging
139 involves the support, coat, or absorption of active components on a solid matrix from which they
140 can be released to the atmosphere and act as food preservation agents. Antimicrobial active
141 packaging is aimed both to extend the fruit and vegetable shelf-life and to improve consumer safety
142 by reducing, inhibiting, and/or retarding the growth of spoilage and pathogenic bacteria in packed
143 foods and packaging materials (Khan *et al.*, 2014). The highly volatile and antimicrobial nature of
144 natural plant EOs or their components make them attractive candidates for this purpose, and provide
145 an alternative to less desirable synthetic additives (Becerril, Gomes-Lus, Goni, Lopez, & Nerin,
146 2007). Also antioxidant activity is one of the most intensively studied property in EO research,
147 because oxidation damages various biological substances and subsequently causes many diseases,

148 including cancer, liver, Parkinson's and Alzheimer's disease, aging, arthritis, inflammation,
149 diabetes, atherosclerosis and AIDS. As a result, many illnesses have been treated with antioxidants
150 to prevent oxidative damage and many researchers have being investigated the antioxidant activity
151 of different EOs and their components in order to search for safe natural antioxidants. However, the
152 largest amount of papers are referred to the study of the antimicrobial activity of EOs and their
153 constituents for a potential application in food sector. Although, most of the papers regard the EO
154 antimicrobial activity in vitro systems, and the application of EOs for antimicrobial purpose in real
155 system is still limited, EOs have been used at lab-scale in bakery (Nielsen & Rios, 2000), cheese
156 (Vazquez, Fente, Franco, Vazquez, & Cepeda, 2001), meat production (Quintavalla & Vicini,
157 2002), seafoods (Kykkidou *et al.*, 2009) and minimally processed fruits and vegetables (Lanciotti *et*
158 *al.*, 2004; Serrano *et al.*, 2008; Siroli *et al.*, 2014, 2015a,b,c; Soliva-Fortuny & Olga Martin-
159 Beloso, 2003).

160 Despite the strong antimicrobial activity against foodborne pathogens and spoilage microorganisms
161 shown by EOs (Oussalah *et al.*, 2007; Rhayour *et al.*, 2003; Tassou, Koutsoumanis, & Nychas,
162 2000), their practical application is currently limited due to their strong impact and changes they
163 cause in food products (Gutierrez, Barry-Ryan, & Bourke, 2008).

164 Moreover, the limited use is due to i) the variability of the composition of EOs (due to the
165 geographic origin, agricultural techniques, season, methods of extraction, etc..) able to influence
166 their effective overall antimicrobial activity (Burt, 2004); ii) the interaction of bioactive molecules
167 with the food matrix (in particular with proteins, lipids, starch, etc..) limiting the contact of these
168 molecules with the microbial cells, thereby reducing the effects on cell viability (Gutierrez *et al.*,
169 2008); iii) the lack of knowledge of the mechanisms by which these molecules exert their
170 antimicrobial activity; iv) the lack of knowledge of the interaction between technological and
171 composition parameters and their activity.

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

174

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

177 Several investigations have focused on the search for natural antimicrobials able to increase the
178 quality and safety of the minimally processed fruits (Allende *et al.*, 2007; De Azeredo *et al.*, 2011;
179 López-Gálvez *et al.*, 2009; Siroli *et al.*, 2014, 2015a,b; Soliva-Fortuny & Olga Martín-Belloso,
180 2003; Vandekinderen, Devlieghere, De Meulenaer, Ragaert, & Van Camp, 2009) (Table 1). A wide
181 literature shows the great potential as antimicrobials in model and food systems of EOs deriving
182 from citrus fruit peels (Espina, Somolinos, Loran, Conchello, Garcia, & Pagan, 2011; Fisher, &
183 Phillips, 2008; Settanni *et al.*, 2012). In particular, citral (3,7-dimethyl-2-octadienal), is an acyclic
184 unsaturated monoterpene aldehyde found naturally in the volatile oils of citrus fruits, lemongrass,
185 and other herbs and spices. It consists of a mixture of two isomers, geranial and neral, and is used
186 for flavoring citrus-based beverages. Its antimicrobial properties and pleasant fruity scent could
187 make citral a suitable antimicrobial ingredient for wider use in the food industry (Somolinos,
188 García, Pagan, & Mackey, 2008). Citral and citron EO, at concentrations compatible with sensorial
189 features of fruits, were able to significantly prolong the shelf-life of the fruit-based salads in syrup
190 (Belletti, Lanciotti, Patrignani, & Gardini, 2008), and the stability of fruit based soft drink (Belletti,
191 Sado Kamdem, Patrignani, Lanciotti, Covelli, & Gardini, 2007). Also, the antimicrobial activity of
192 hexanal and 2-(*E*)-hexenal, which are components of the aroma of many fruits and vegetables, has
193 been tested in model (Gardini, Lanciotti, & Guerzoni, 2001; Kubo & Fujita, 2001) and real systems
194 (Corbo, Lanciotti, Gardini, Sinigaglia, & Guerzoni, 2000; Lanciotti *et al.*, 2003; Lanciotti *et al.*,
195 2004). Hexanal, 2-(*E*)-hexenal, and hexyl acetate improved shelf-life and safety of minimally
196 processed fruits (Lanciotti *et al.*, 2004; Serrano *et al.*, 2008). In particular, the addition of hexanal
197 and 2-(*E*)-hexenal in storage atmosphere of fresh-cut apples resulted in a positive effect on shelf-
198 life, due to their antimicrobial activity against naturally occurring spoilage yeasts, and also when
199 deliberately inoculated at levels of 10^3 cfu/g. Moreover, these molecules determined the

200 enhancement of the sensorial properties, as well as the retention of the original colour of the
201 packaged products (Corbo *et al.*, 2000). These aldehydes showed a great potential as antimicrobials
202 also against pathogens such as *Salmonella* spp., *E. coli* and *Pseudeomonas aeruginosa* (Kubo *et al.*,
203 2001). Little information is available on the relationship between the outgrowth of spoilage
204 microorganisms, their volatilome, and the perception of the decay of minimally processed
205 vegetables by consumers. Also Siroli *et al.* (2015a), to increase the shelf-life and quality parameters
206 of sliced apples, proposed the use of these antimicrobials and citron EO in apple dipping solution,
207 alone or in combination, as alternative to the traditional sanitization methods. The use of these
208 antimicrobials changed the naturally occurring yeast growth parameters with respect to the control.
209 Samples treated with hexanal/2-(E)-hexenal and citral showed better colour and texture attributes
210 compared to the controls. Siroli *et al.* (2014) demonstrated also that the optimization of the process
211 and the package in active modified atmosphere (7% O₂ and 0% CO₂) increased the shelf-life of
212 apples treated with the mixture hexanal/2-(E)-hexenal up to 35 days of storage (Figure 2).

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

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

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

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

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

240 *Potential use of natural antimicrobials to prolong shelf-life and safety of minimally processed* 241 *vegetables*

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

246 As previously reported, the safety and shelf-life of minimally processed vegetables are based on
247 few tools such as modified atmosphere packaging and maintaining of refrigeration chain. Although
248 chlorine is the most common decontaminant used in these products, the concentrations used are
249 quite ineffective in reducing pathogens on vegetables. In addition, chlorine-based compounds bring
250 to the formation of potentially harmful chlorinated by-products such as trihalomethanes. For this,

251 plant EOs and their components have been investigated as natural sanitizer alternative to chlorine to
252 control of foodborne pathogens and spoilage bacteria associated with minimally processed
253 vegetables (2011Gunduz, Gonul, & Karapınar, 2010; Gutierrez, *et al.*, 2008; 2009). The *in vitro*
254 antimicrobial activity of oregano (*Origanum vulgare*), thyme (*Thymus vulgaris*) EOs and their main
255 components (carvacrol and thymol) against a huge variety of Gram-positive, Gram-negative
256 bacteria, yeasts and moulds is well documented (Burt, 2004; Lanciotti *et al.*, 2004; Viuda-Martos,
257 Ruiz-Navajas, Fernandez-Lopez, & Perez-Alvarez, 2007). However, there are very limited studies
258 that investigate the antimicrobial efficacy of these natural antimicrobials alone or in combinations
259 with other hurdles on vegetable produce. (De Azeredo *et al.*, 2011; Gutierrez *et al.*, 2008; Gutierrez
260 *et al.*, 2009; Muriel-Galet *et al.*, 2013; Siroli *et al.*, 2015 b,c). In particular, as reported by Table 1,
261 oregano and thyme were the most studied for this application. Siroli *et al.* (2015b,c) evaluated the
262 efficacy of oregano and thyme EOs in comparison with chlorine for lamb's lettuce decontamination
263 using them in the product washing solution. The data obtained showed that these EOs were able to
264 assure a product shelf-life similar to that obtained with chlorine. Moreover, Siroli *et al.* (2015b)
265 demonstrated that increasing the temperature of the washing solution up to 13 °C, the EOs could be
266 better exploited. In fact, it is well known that the temperature increase results in the vapour pressure
267 increase of volatile molecules enhancing, consequently, their affinity for the cell membranes, main
268 and primary target of antimicrobials (Gardini *et al.*, 1997). In fact, while in the first experimental
269 phase chlorine (120 mg L⁻¹) and the natural antimicrobial showed the same reduction of the
270 naturally occurring microbial population, in the second trial, thyme and oregano reduced the cell
271 loads of mesophilic aerobic bacteria of about 1 log cfu/g more than the chlorine solution. In the
272 experimental conditions of Siroli *et al.* (2015b,c), the initial reduction of the naturally occurring
273 microbiota due to the use of EOs did not affect negatively the safety of the products. In fact, the
274 pathogenic species, most frequently associated to minimally processed vegetables, such as *L.*
275 *monocytogenes*, *E. coli*, *S. enteritidis* and *S. aureus*, were not detected after 14 d of storage at 6°C,
276 also when inoculated. Also the colour and the withering data showed that the treatments applied can

277 guarantee the maintenance of the main quality parameters affecting the consumer choice. In fact, by
278 improving the condition of the washing process, the products treated with thyme and oregano,
279 similarly to chlorine, were able to maintain good colour and turgidity attributes over 12 days of
280 storage at 6°C (Figure 3). Also the sensorial analysis confirmed that the organoleptic features of the
281 Lamb's lettuce treated with oregano and thyme instead of chlorine was not significantly affected.
282 Gunduz *et al.* (2010) conducted a study aimed to determine the efficacy of oregano oil in the
283 inactivation of *Salmonella typhimurium* inoculated onto iceberg lettuce. The effects of washing with
284 oregano oil (*Oreganum onites*), typical of Turkey, at three different concentrations and four
285 different treatment times on survival of *S. typhimurium* inoculated to fresh cut iceberg lettuce were
286 determined at 20 °C storage temperature and compared with chlorine. Reductions of *S. typhimurium*
287 by washing with oregano did not exceed 1.92 logarithmic units regardless of the washing times and
288 concentrations. The effectiveness of washing lettuce with 75 ppm oregano oil on inactivation of *S.*
289 *typhimurium* was comparable with that affected by 50 ppm chlorine.

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

301 Gutierrez *et al.* (2009) studied the efficacy of plant EOs for control of the natural spoilage
302 microflora on ready-to-eat lettuce and carrots whilst also considering their impact on sensory

303 properties. Initial decontamination effects, achieved using EOs, were comparable to that observed
304 with chlorine and solution containing oregano recorded a significantly lower initial total count level
305 than the water treatment on carrots. No significant difference was found between the EO treatments
306 and chlorine considering gas composition, colour, texture and water activity of samples. The
307 sensory panel found EO treatments acceptable for carrots throughout storage, while lettuce washed
308 with the EO solutions were rejected for overall appreciation by day 7.

309 Valero & Giner (2006) studied the possible use of antimicrobials from seven plant EOs as food
310 preservatives by examining their effects on the growth kinetics of activated *Bacillus cereus* INRA
311 L2104 spores inoculated into tyndallized carrot broth. The effects of various concentrations of
312 borneol, carvacrol, cinnamaldehyde, eugenol, menthol, thymol, and vanillin were determined.
313 Lower concentrations of the three antimicrobials prolonged the lag phase and reduced both the
314 exponential growth rate and the final population densities of cultures. The study of the sensory
315 characteristics of the supplemented broths suggested that low concentration of cinnamaldehyde
316 enhanced the taste of carrot broth, and that it did not have any adverse effect on the taste and smell
317 of carrot broth at concentrations less than 6 $\mu\text{l}/100\text{ mL}$.

318

319 **Mechanisms of action**

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

327 The antimicrobial activity of the EOs seems to be related to their composition, to the structural
328 configuration of the constituents and to their functional groups, as well as to the possible synergistic

329 interactions among the components. Consequently, the chemical structure of the individual
330 compounds present in the EOs affects their precise mode of action and their antibacterial activity
331 (Picone *et al.*, 2013; Viuda-Martos *et al.*, 2008). Some works on *Saccharomyces cerevisiae* have
332 shown that the cytotoxicity of some EOs, on the basis of the ability to form colonies, was
333 considerably different depending on their chemical composition. Treatments with EOs on cells in
334 stationary growth phase showed 50% mortality with 0.45 $\mu\text{L} / \text{mL}$ of EO of *Origanum compactum*,
335 1.6 $\mu\text{L} / \text{mL}$ of EO of *Coriandrum sativum*, $> 8 \mu\text{L} / \text{mL}$ of EO of *Cinnamomum camphora*,
336 *Artemisia herba-alba* and *Helichrysum italicum* (Bakkali *et al.*, 2008).

337 As previously mentioned, the aromatic molecules among the various physical properties, are
338 characterized by a poor solubility in water and a high hydrophobicity. For this reason, many studies
339 indicate their antimicrobial effects as dependent on these characteristics and on their ability to act
340 on the cell membrane. In addition, the bioactivity of many aromatic compounds may depend on the
341 vapour pressure, which can be considered an indirect measure of hydrophobicity (Picone *et al.*,
342 2013). The factors responsible for the increase of the vapour pressure of the aromatic molecules
343 lead to a rise of the antimicrobial activity, since it increases their solubility in cell membranes
344 (Picone *et al.*, 2013). Precisely, their hydrophobicity permits them to have a good partition in the
345 lipids of cell membranes and mitochondria, altering the structures and making them more
346 permeable and leading to the loss of ions and other cell contents (Nazzarro *et al.*, 2013). The
347 bacterial cell can tolerate, up to a certain limit, the loss of some cell contents, but their excessive
348 leakage or the loss of critical molecules and ions lead to the cell death.

349 Many studies indicate the cell membrane as the primary target of bioactive aromatic compounds.
350 Membranes disrupted by the action of terpenes can be observed both on bacteria and fungi (Holey
351 & Patel, 2005; Lanciotti *et al.*, 2004, Liolios *et al.*, 2009; Nazzarro *et al.*, 2013; Viuda-Martos *et al.*,
352 2008). The antimicrobial action of many EOs appears to be connected with the presence of phenolic
353 compounds. Various studies, concerning oregano species have shown that their oils possess strong
354 antimicrobial activity; this activity could be attributed to their high percentage of phenolic

355 compounds and, specifically, carvacrol, thymol, p-cymene and their precursor c-terpinene (Liolios
356 *et al.*, 2009). It was hypothesized that the inhibition against *S. typhimurium* and *S. aureus* by the
357 thyme EO, was dependent on the hydrophobicity and the nature of the present phenolic constituents,
358 which determined alteration of the functionality of membrane proteins after partitioning in the
359 phospholipid bilayer. The inhibitory effect of phenols can be explained with the interaction with the
360 cell membrane of microorganisms, and it is often correlated with the hydrophobicity of the
361 compounds (Liolios *et al.*, 2009). The lipophilic structure of cyclic monoterpenes promotes their
362 partition from the aqueous phase to cell membranes resulting in expansion and increase in fluidity
363 and permeability of the membrane, which leads ultimately to an inhibition of membrane enzymes
364 (Nazzarro *et al.*, 2013). In some microorganisms, mild heat treatments increase the inhibitory effect
365 of carvone, altering the membrane composition, the fluidity and favouring the partition of these
366 molecules in the membrane phospholipids. In bacteria, the permeabilization of membranes is
367 associated with the loss of ions and the reduction of the membrane potential, the collapse of the
368 proton pump and the depletion of the ATP pool (Nazzarro *et al.*, 2013). EOs can coagulate the
369 cytoplasm (Picone *et al.*, 2013) and cause damage to lipids and proteins (Burt, 2004). The damages
370 to the cell wall and in the membranes, may lead to loss of macromolecules up to cell lysis. In
371 particular, the loss of specific ions, due to the action of the aromatic molecules on the cell
372 membrane, has dramatic effects on the proton motive force, by decreasing the content of
373 intracellular ATP. In this manner, the total activity of the cells is greatly compromised, as well as
374 the cellular turgor (osmotic pressure), the transport of solutes and the regulation of metabolism
375 (Lanciotti *et al.*, 2004). The oregano EO, for example, creates an alteration of membrane
376 permeability with a consequent loss of protons, phosphorus and potassium. Carvacrol leads to a
377 dissipation of intracellular ATP in *B. cereus* due to the reduction of the synthesis or hydrolysis,
378 accompanied by the increase of permeability of the membrane to ATP. Also Picone *et al.* (2013),
379 who evaluated the effect of four levels of carvacrol (0–2 mM) on *Escherichia coli* 555 metabolome
380 by using ¹H-NMR spectroscopy, showed clearly that the cells exposed to the highest carvacrol

381 concentrations were unable to recover the damages caused by the terpenic molecule. In fact, the
382 interaction of hydrophobic molecules with cell membranes is known to affect the activity of
383 membrane bound or embedded enzymes. The inhibition of membrane bound ATPases in *Listeria*
384 *monocytogenes* and *E. coli* was also demonstrated (Gill & Holley, 2006). Moreover, dissipation of
385 pH gradients, K and P leakage from *Pseudomonas aeruginosa* and *Staphylococcus aureus* were
386 reported following treatment with carvacrol-containing oregano EO. The result of the NMR
387 experiments showed a chemical shift of metabolites as a consequence of the perturbation induced
388 by increasing amount of carvacrol. The first striking evidence involved glucose, which tends to be
389 accumulated in the cells treated with carvacrol.

390 Some studies have examined the antifungal activity of citrus EOs (Viuda-Martos *et al.*, 2008).
391 Citrus EOs are a complex mixture of volatile compounds that show, among other properties,
392 antifungal activity by reducing or totally inhibiting fungal growth in a dose-response manner. Some
393 authors have attributed the antifungal capacity of citrus EOs to the presence of components such as
394 D-limonene, linalool or citral (Siroli *et al.*, a,b) which are present in differing concentrations in
395 citrus EOs, although this function can be attributed to the phenolic compounds. The hydrophilic part
396 of the molecule interacts with the polar part of the membrane, while the hydrophobic benzene ring
397 and the aliphatic side chains are buried in the hydrophobic inner part of the bacterial membrane.
398 Furthermore, the involvement of the hydroxyl group in the formation of hydrogen bonds and the
399 acidity of these phenolic compounds may have other possible explanations (Nazzarro *et al.*, 2013).
400 Possible action mechanisms by which mycelial growth may be reduced or totally inhibited have
401 been proposed. It is commonly accepted that it is the toxic effects of the EO components on the
402 functionality and structure of the cell membrane that is responsible for the aforesaid activity.
403 Serrano *et al.*, (2013) related low EO concentrations with changes in the cell structure, which would
404 inhibit respiration and alter the permeability of the microbe cell membrane, while high
405 concentrations would provoke severe damage to the membrane and the loss of homeostasis, leading
406 to cell death. Viuda-Martos *et al.* (2007) suggested that components of the EOs cross the cell

407 membrane, interacting with the enzymes and proteins of the membrane, so producing a flux of
408 protons towards the cell exterior which induces changes in the cells and, ultimately, their death.
409 Cristani et al. (2007) reported that the antimicrobial activity is related to ability of terpenes to affect
410 not only permeability but also other functions of cell membranes, these compounds might cross the
411 cell membranes, thus penetrating into the interior of the cell and interacting with critical
412 intracellular sites.

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

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

429 **Future perspective**

430 The results reported in this review provided encouraging information concerning the use of EO in
431 food sector. However, their effect on food constituents and microorganisms remains a focal area for
432 future research. Moreover, a deeper knowledge of the effects of their physicochemical properties

433 on their bio-activities needs to be better investigated. The study of the synergistic effects among
434 EOs and/or their components could be utilized both to make best use of their antibacterial activity
435 and to reduce their concentrations required to achieve a particular antibacterial effect for food safety
436 and for health purposes.

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

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442 **Figure Caption**

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

445 **Figure 2.** Evolution of colour in minimally processed apples treated with traditional dipping in
446 0.5% ascorbic acid and 1% citric acid (a), citral/2-(E)-hexenal (b) (125mg/L each one) and
447 hexanal/2-(E)-hexenal (c) (125mg/L each one).

448 **Figure 3.** Evolution of colour in minimally processed lamb's lettuce treated with chlorine 120 mg/L
449 (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	<i>Kiwifruits</i>	Carvacrol	1mM in dipping solution	Natural microflora	Roller and Seedhar, 2002
	<i>Honeydew melon</i>	Carvacrol and cinnamic acid	1mM in dipping solution	Natural microflora	Roller and Seedhar, 2002
	<i>Fresh sliced apples</i>	Hexanal, hexyl acetate E(2)hexenal,	50-250 ppm 20-200 ppm	<i>Salmonella enteritidis</i> , <i>Escherichia coli</i> , <i>Listeria monocytogenes</i>	Lanciotti et al., 2003
	<i>Sweet cherries</i>	Eugenol, thymol, menthol, eucalyptol	1000 μ L in gas used for MAP	Natural microflora	Serrano et al., 2005
	<i>Table Grape</i>	eugenol thymol	75-150 μ L in gas used for MAP	Natural microflora	Valero et al., 2006
	<i>Fresh sliced apples</i>	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 <i>L. innocua</i>	Rojas-Grau et al., 2007
	<i>Fruit salads</i>	Citral	25-125 ppm	<i>Salmonella enteritidis</i> ,	Belletti et al., 2008

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

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

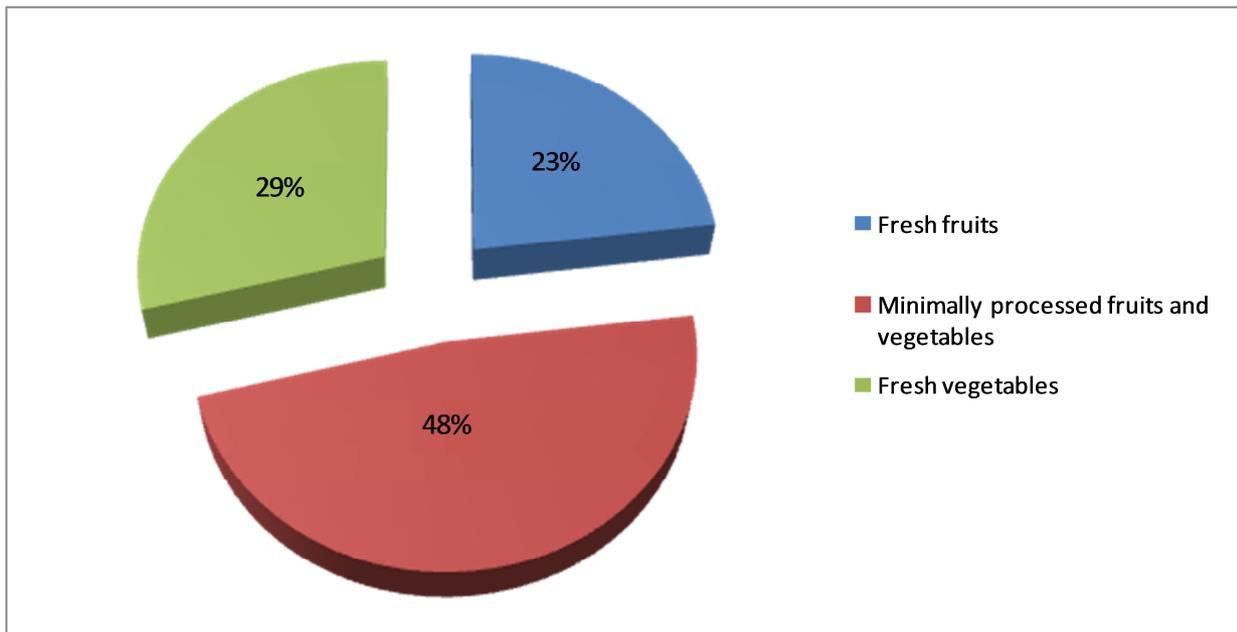


Figure 1.



Figure 2.

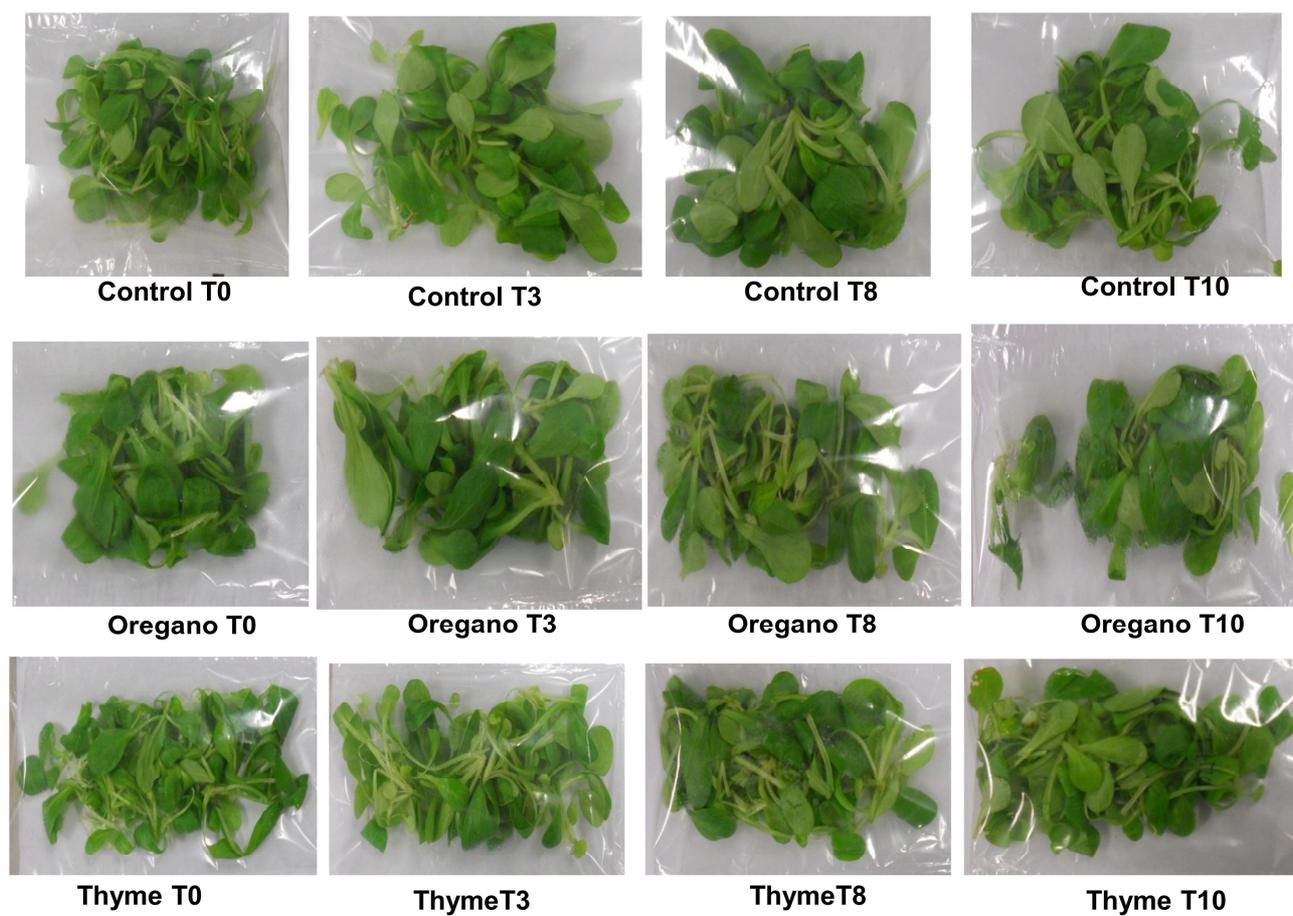


Figure 3.

- 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.

ACCEPTED MANUSCRIPT