



## ARCHIVIO ISTITUZIONALE DELLA RICERCA

### Alma Mater Studiorum Università di Bologna Archivio istituzionale della ricerca

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

This is the final peer-reviewed author's accepted manuscript (postprint) of the following publication:

*Published Version:*

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 / Patrignani, Francesca; Siroli, Lorenzo; Serrazanetti, Diana I.; Gardini, Fausto; Lanciotti, Rosalba. - In: TRENDS IN FOOD SCIENCE & TECHNOLOGY. - ISSN 0924-2244. - STAMPA. - 46:2, Part B(2015), pp. 311-319. [10.1016/j.tifs.2015.03.009]

This version is available at: <https://hdl.handle.net/11585/552031> since: 2016-07-14

*Published:*

DOI: <http://doi.org/10.1016/j.tifs.2015.03.009>

*Terms of use:*

Some rights reserved. The terms and conditions for the reuse of this version of the manuscript are specified in the publishing policy. For all terms of use and more information see the publisher's website.

(Article begins on next page)

This item was downloaded from IRIS Università di Bologna (<https://cris.unibo.it/>).  
When citing, please refer to the published version.

# Accepted Manuscript

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

Francesca Patrignani, Lorenzo Siroli, Diana I. Serrazanetti, Fausto Gardini, Rosalba Lanciotti

PII: S0924-2244(15)00076-X

DOI: [10.1016/j.tifs.2015.03.009](https://doi.org/10.1016/j.tifs.2015.03.009)

Reference: TIFS 1644

To appear in: *Trends in Food Science & Technology*

Received Date: 31 December 2014

Revised Date: 17 March 2015

Accepted Date: 18 March 2015

Please cite this article as: Patrignani, F., Siroli, L., Serrazanetti, D.I., Gardini, F., Lanciotti, R., 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, *Trends in Food Science & Technology* (2015), doi: <https://doi.org/10.1016/j.tifs.2015.03.009>

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

© 2015 Elsevier. This manuscript version is made available under the Creative Commons Attribution-NonCommercial-NoDerivs (CC BY-NC-ND) 4.0 International License (<http://creativecommons.org/licenses/by-nc-nd/4.0/>)

**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**

Francesca Patrignani<sup>1</sup>, Lorenzo Siroli<sup>1</sup>, Diana I. Serrazanetti<sup>2</sup>, Fausto Gardini<sup>1,2</sup>, Rosalba Lanciotti<sup>1</sup>

<sup>2\*</sup>

<sup>(1)</sup> Department of Agricultural and Food Sciences, Alma Mater Studiorum, University of Bologna, Campus of Food Science, Piazza Goidanich 60, 47521 Cesena, Italy

<sup>(2)</sup> Interdepartmental Center for Industrial Agri-Food Research, University of Bologna, Piazza Goidanich 60, 47521 Cesena (FC), Italy

\*Corresponding Author:

Rosalba Lanciotti, p.zza Goidanich 60, 47521, Cesena (FC), Italy

rosalba.lanciotti@unibo.it

Phone +39 0547 338132

Fax +39 0547 382348

**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

## 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 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

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 *Escherichia 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 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.*,

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 been investigating 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 Martín-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 Martín-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-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<sub>2</sub> and 0% CO<sub>2</sub>) increased the shelf-life of apples treated with the mixture hexanal/2-(E)-hexenal up to 35 days of storage (Figure 2).

Valero, Valverde, Martinez-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, N-acetyl-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,

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, & Karapinar, 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<sup>-1</sup>) 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

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  $\mu$ l/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  $\mu\text{L}$  / mL of EO of *Origanum compactum*, 1.6  $\mu\text{L}$  / mL of EO of *Coriandrum sativum*, > 8  $\mu\text{L}$  / 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



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 <sup>1</sup>H-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 studies 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 citrus 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.

## Acknowledgment

Several data described in this review were obtained in the framework of the "AGER - Agroalimentare e Ricerca Project 2010 2370"

## 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 hexenal/2-(E)-hexenal (c) (125mg/L each one).

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

## References

- Abadias, M., Alegre, I., Usall J., Torres, R., & Vinas, I. (2011). Evaluation of alternative sanitizers to chlorine disinfection for reducing foodborne pathogens in fresh-cut apple. *Postharvest Biology and Technology*, 59, 289-297.
- Alegre, I., Abadias, M., Anguera, M., Oliveira, M., & Viñas, I. (2010). Factors affecting growth of foodborne pathogens on minimally processed apples. *Food Microbiology*, 27, 70-76.

- 457 Allende, A., Martinez, B., Selma, V., Gil, M. I., Suarez, J. E., & Rodriguez, A. (2007). Growth and  
458 bacteriocin production by lactic acid bacteria in vegetable broth and their effectiveness at  
459 reducing *Listeria monocytogenes* in vitro and in fresh-cut lettuce. *Food Microbiology*, 24,  
460 759–766.
- 461 Bhalla, Y., Gupta, V.K., & Jaitak, V. (2013). Anticancer activity of essential oils: a review. *Journal*  
462 *of the Science of Food and Agriculture*, 93, 3643–3653.
- 463 Bakkali, F., Averbeck, S., Averbeck, D., & Idaomar, M. (2008). Biological effects of essential oils.  
464 *Reviews in Food Chemistry & Toxicology*, 46, 446–475.
- 465 Becerril, R., Gomes-Lus, R., Goni, P., Lopez, P., & Nerin, C. (2007). Combination of analytical  
466 and microbiological techniques to study the antimicrobial activity of a new active food  
467 packaging containing cinnamon or oregano against *E. coli* and *S. aureus*. *Analytical and*  
468 *Bioanalytical Chemistry*, 388, 1003-1011.
- 469 Belletti, N., Lanciotti, R., Patrignani, F., & Gardini, F. (2008). Antimicrobial efficacy of citron  
470 essential oil on spoilage and pathogenic microorganisms in fruit-based salads. *Journal of*  
471 *Food Science*, 73, 331-338.
- 472 Belletti, N., Sado Kamdem, S., Patrignani, F., Lanciotti, R., Covelli, A., & Gardini, F. (2007).  
473 Antimicrobial activity of aroma compounds against *Saccharomyces cerevisiae* and  
474 improvement of microbiological stability of soft drinks as assessed by logistic regression.  
475 *Applied and Environmental Microbiology*, 73, 5580-5586.
- 476 Brewer, M.S. (2011). Natural antioxidants: sources, compounds, mechanisms of action, and  
477 potential applications. *Comprehensive Reviews in Food Science & Food Safety*, 10, 221–  
478 247.
- 479 Burt, S. (2004) Essential oils: their antibacterial properties and potential applications in foods.  
480 *Review in Journal of Food Microbiology*, 94, 223-253.

- 481 Carocho, M., Barreiro, M.F., Morales, P., & Ferreira, I.C.F.R. (2008). Adding Molecules to Food,  
 482 Pros and Cons: A Review on Synthetic and Natural Food Additives. *Comprehensive*  
 483 *Reviews in Food Science and Food Safety*, 13, 377-399.
- 484 Centers for Disease Control and Prevention, CDC. (2006). E. coli outbreak from fresh spinach  
 485 <http://www.cdc.gov/ecoli/2006/september/>
- 486 Cook, R. (2008). The dynamic U.S. fresh produce industry: an industry in transition. Fresh Fruit  
 487 and Vegetable Marketing and Trade Information.  
 488 <http://are.ucdavis.edu/en/people/faculty/roberta-cook/articles-and-presentations/>
- 489 Corbo, M. R., Lanciotti, R., Gardini, F., Sinigaglia, M., & Guerzoni, M. E. (2000). Effects of  
 490 hexanal, (E)-2-hexenal, and storage temperature on shelf life of fresh sliced apples. *Journal*  
 491 *of Agriculture and Food Chemistry*, 48, 2401-2408.
- 492 Cristiani, M., D'Arrigo, M., Mandalari, G., Castelli, F., Sarpietro, M.G., Micieli, D. et al., (2007).  
 493 Interaction of four monoterpenes contained in essential oils with model membranes:  
 494 Implication for their antibacterial activity. *Journal of Agriculture & Food Chemistry*, 55,  
 495 6300-6308.
- 496 de Azeredo, G. A., Stamford, T. L. M., Campos Nunes, P., Gomez Neto, N. J., de Oliveira, M. E.  
 497 G., de Souza, E. L. (2011). Combined application of essential oils from *Origanum vulgare*  
 498 L. and *Rosmarinus officinalis* L. to inhibit bacteria and autochthonous microflora associated  
 499 with minimally processed vegetables. *Food Research International*, 44, 1541-1548.
- 500 EAFUS, a food additive database. (1998). U.S. Food and Drug Administration.  
 501 [www.usc.es/caa/EdulcWeb/EAFUS.pdf](http://www.usc.es/caa/EdulcWeb/EAFUS.pdf)
- 502 Espina, L., Somolinos, M., Loran, S., Conchello, P., Garcia, D., & Pagan, R. (2011). Chemical  
 503 composition of commercial citrus fruit essential oils and evaluation of their antimicrobial  
 504 activity acting alone or in combined processes. *Food Control*, 22, 896-902.

- European Food Safety Authority. (EFSA). (2011). Shiga toxin-producing *E. coli* (STEC) o104:H4 2011 outbreaks in Europe: taking stock. *EFSA Journal*, 9 (10), 2390. <http://dx.doi.org/10.2903/j.efsa2011.2390>, [22pp.].
- Fisher, K., & Phillips, C. (2008). Potential antimicrobial uses of essential oils in food: is citrus the answer? *Trends in Food Science & Technology*, 19, 156-164.
- Gardini, F., Lanciotti, R., & Guerzoni, M. E. (2001). Effect of (E)-2-hexenal on the growth of *Aspergillus flavus* in relation to its concentration, temperature and water activity. *Letters in Applied Microbiology*, 33, 50-55.
- Gil, M. I., Selma, M. V., López-Gálvez, F., & Allende, A. (2009). Fresh-cut product sanitation and wash water disinfection: Problems and solutions. *International Journal of Food Microbiology*, 134, 37-45.
- Gill, A. O., & Holley, R. A. (2006). Inhibition of membrane bound ATPases of *Escherichia coli* and *Listeria monocytogenes* by plant oil aromatics. *International Journal of Food Microbiology*, 111, 170–174.
- Gunduz, G.T., Gonul, S.A., & Karapinar, W. (2010). Efficacy of oregano oil in the inactivation of *Salmonella typhimurium* on lettuce. *Food Control*, 21, 513–517.
- Gunes, G. G., & Hotchkiss, J. H. (2002). Growth and survival of *Escherichia coli* O157: H7 on fresh-cut apples in modified atmospheres at abusive temperatures. *Journal of Food Protection*, 65, 1641-1645.
- Gutierrez, J., Barry-Ryan, C., & Bourke, P. (2008). Antimicrobial activity of plant essential oils using food model media: Efficacy, synergistic potential and interactions with food components. *Food Microbiology*, 26, 142-150.

- 527 Gutierrez, J., Bourke, P. , Lonchamp, J., & Barry-Ryan, C. (2009). Impact of plant essential oils on  
 528 microbiological, organoleptic and quality markers of minimally processed vegetables.  
 529 *Innovative Food Science and Emerging Technologies* , 10, 195–202.
- 530 Gyawali, R., & Ibrahim, S.A. (2014). Natural products as antimicrobial agents. *Food Control*, 46,  
 531 412-429.
- 532 Harris, L. J., Farber, J. N., Beuchat, L. R., Parish, M. E., Suslow, T. V., Garrett, E. H., *et al.* (2003).  
 533 Outbreaks associated with fresh produce: incidence, growth, and survival of pathogens in  
 534 fresh and fresh-cut produce. *Comprehensive Reviews in Food Science and Food Safety*, 2,  
 535 78-141.
- 536 Holley, R. A, & Patel, D. (2005). Improvement in shelf-life and safety of perishable foods by plant  
 537 essential oils and smoke antimicrobials. *Food Microbiology*, 22, 273–292.
- 538 Khan, A., Huq, T., Khan, R.A., Riedl, B., & Lacroix, M. (2014). Nanocellulose-Based  
 539 Composites and Bioactive Agents for Food Packaging. *Critical Reviews in Food Science*  
 540 *and Nutrition*, 54, 163-174.
- 541 Kubo, I., & Fujita, K. (2001). Naturally occurring anti-*Salmonella* agents. *Journal of Agriculture*  
 542 *and Food Chemistry*, 49, 5750-5754.
- 543 Kykkidou, S., Giatrakou, V., Papavergou, A., Kontominas, M.G., & Savvaidis, I.N. (2008). Effect  
 544 of thyme essential oil and packaging treatments on fresh Mediterranean swordfish fillets  
 545 during storage at 4 °C. *Food Chemistry*, 115, 169-175.
- 546 Lanciotti, R., Belletti, N., Patrignani, F., Gianotti, A., Gardini, F., & Guerzoni, M. E. (2003).  
 547 Application of hexanal, (E)-2-hexenal, and hexyl acetate to improve the safety of fresh-  
 548 sliced apples. *Journal of Agriculture & Food Chemistry*, 51,2958-2963.



- 549 Lanciotti, R., Gianotti, A., Patrignani, F., Belletti, N., Guerzoni, M. E., & Gardini, F., (2004). Use  
 550 of natural aroma compounds to improve shelf-life and safety of minimally processed fruits.  
 551 *Trends in Food Science & Technology*, 15, 201–208.
- 552 Liolios C.C., Gortzi O., Lalas S., Tsaknis J., & Chinou, I.( 2009). Liposomal incorporation of  
 553 carvacrol and thymol isolated from the essential oil of *Origanum dictamnus* L. and in vitro  
 554 antimicrobial activity. *Food Chemistry*, 112, 77–83.
- 555 López-Gálvez, F., Allende, A., Selma, M.V., & Gil, M.I. (2009). Prevention of *Escherichia coli*  
 556 cross-contamination by different commercial sanitizers during washing of fresh-cut lettuce.  
 557 *International Journal of Food Microbiology*, 133, 167-171.
- 558 Lynch, M.F., Tauxe, & R.V., Hedberg, C.W. (2009). The growing burden of foodborne outbreaks  
 559 due to contaminated fresh produce: risks and opportunities. *Epidemiological Infection*, 137,  
 560 307-315.
- 561 Manzocco, L., Da Pieve, S., Bertolini, A., Bartolomeoli, I., Maifreni, M., Vianello, A., *et al.* (2011).  
 562 Surface decontamination of fresh-cut apple by UV-C light exposure: Effects on structure,  
 563 colour and sensory properties. *Postharvest Biology and Technology*, 61, 165-171.
- 564 Muriel-Galet, V., Cerisuelo, J.P., López-Carballo, G-, Aucejo, S., Gavara, R., & Hernández-Muñoz,  
 565 P. (2013). Evaluation of EVOH-coated PP films with oregano essential oil and citral to  
 566 improve the shelf-life of packaged salad. *Food Control*, 30,137-143.
- 567 Muriel-Galet, V., Cerisuelo, J.P., López-Carballo, G., Aucejo, S., Gavara, R., & Hernández-  
 568 Muñoz, P. (2013). Evaluation of EVOH-coated PP films with oregano essential oil and citral  
 569 to improve the shelf-life of packaged salad. *Food Control*, 30, 137-143.
- 570 Nazzaro F., Fratianni, F., De Martino, L., Coppola, R., & De Feo, V. (2013). Effect of Essential  
 571 Oils on Pathogenic Bacteria. *Pharmaceuticals*, 6, 1451-1474.

- 572 Nielsen, P. V., & Rios, R.. (2000). Inhibition of fungal growth on bread by volatile components  
573 from species and herbs, and the possible application in active packaging, with special  
574 emphasis on mustard essential oil. *International Journal of Food Microbiology*, 60, 219–  
575 229.
- 576 Olaimat, A. N., & Holley, R. A. (2012). Factors influencing the microbial safety of fresh produce: a  
577 review. *Food Microbiology*, 32, 1-19.
- 578 Oliveira, M. Viñas, I., Anguera, M., & Abadias, M. (2012). Fate of *Listeria monocytogenes* and  
579 *Escherichia coli* O157:H7 in the presence of natural background microbiota on conventional  
580 and organic lettuce. *Food Control*, 25, 678-683.
- 581 Oussalah M. , Caillet, S., Saucier, L., Lacroix, M. (2007). Inhibitory effects of selected plant  
582 essential oils on the growth of four pathogenic bacteria: *E. coli* O157:H7, *Salmonella*  
583 Typhimurium, *Staphylococcus aureus* and *Listeria monocytogenes*. *Food Control*, 18, 414-  
584 420.
- 585 Picone, G., Laghi, L., Gardini, F., Lanciotti, R., Siroli, L., & Capozzi, F. (2013). Evaluation of the  
586 effect of carvacrol on the *Escherichia coli* 555 metabolome by using 1H-NMR  
587 spectroscopy. *Food Chemistry*, 141, 4367–4374 .
- 588 Pillai, P., & Ramaswamy, K. (2012). Effect of naturally occurring antimicrobials and chemical  
589 preservatives on the growth of *Aspergillus parasiticus*. *Journal of Food Science &*  
590 *Technology*, 49, 228–33.
- 591 Powell, D., & Luedtke, A. (2000). Fact sheet: A timeline of fresh juice outbreaks.  
592 <http://www.foodsafety.ksu.edu/en/article-details.php?a%42&c%46&sc%437&id%4427>.
- 593 Quintavalla, S., & Vicini, L. (2002). Antimicrobial food packaging in meat industry. *Meat Science*,  
594 62, 373-380.

- 595 Ragaert, P., Verbeke, W., Devlieghere, F., & Debevere, J. (2004). Consumer perception and choice  
596 of minimally processed vegetables and packaged fruits. *Food Quality and Preference*, 15,  
597 259-270.
- 598 Rasooli, I. (2007). Food Preservation – A Biopreservative Approach. *Food I*, 111-136.
- 599 Rico, D., Martín-Diana, A.B., Barat, J.M., & Barry-Ryan, C. (2007). Extending and measuring the  
600 quality of fresh-cut fruit and vegetables: a review. *Trends in Food Science & Technology*,  
601 18, 373-386.
- 602 Ríos, J.L., & Recio, M.C. (2005). Medicinal plants and antimicrobial activity *Journal of*  
603 *Ethnopharmacology*, 100, 80–84.
- 604 Rojas-Grau M.A., Raybaudi-Massilia, R. M., Soliva-Fortuny, R. C., Avena-Bustillos, R. J.,  
605 McHugh T. H., & Martín-Belloso, O. (2007). Apple puree-alginate edible coating as carrier  
606 of antimicrobial agents to prolong shelf-life of fresh-cut apples. *Postharvest Biology and*  
607 *Technology*, 45, 254–264.
- 608 Runyoro, D., Ngassapa, O, Vagionas, K., Aligiannis, N., Graikou, K., & Chinou, I.(2010).  
609 Chemical composition and antimicrobial activity of the essential oils of four *Ocimum*  
610 species growing in Tanzania. *Food Chemistry*, 119, 311–316.
- 611 São José J. F. B. & Vanetti M.C.D. (2012). Effect of ultrasound and commercial sanitizers in  
612 removing natural contaminants and *Salmonella enterica* Typhimurium on cherry tomatoes.  
613 *Food Control*, 24, 95-99.
- 614 Sellamuthu, P.S., Mafune, M., Sivakumar, D., & Soundy, P. (2013). Thyme oil vapour and  
615 modified atmosphere packaging reduce anthracnose incidence and maintain fruit quality in  
616 avocado. *Journal of Science & Food Agriculture* 93, 3024-3031

- 617 Serrano, M., Martinez-Romero D., Castillo, S., Guillen, F., & Valero, D. (2005). The use of natural  
 618 antifungal compounds improves the beneficial effect of MAP in sweet cherry storage.  
 619 *Innovative Food Science and Emerging Technologies*, 6, 115– 123.
- 620 Serrano, M., Martinez-Romero, D., Guillen, F., Valverde, J.M., Zapata, P.J., Castillo, S., & Valero  
 621 D. (2008). The addition of essential oils to MAP as a tool to maintain the overall quality of  
 622 fruits. *Trends in Food Science & Technology*, 19, 464-471.
- 623 Settanni, L., Palazzolo, E., Guarrasi, V., Aleo, A., Mammina, C., Moschetti, G., et al. (2012).  
 624 inhibition of foodborne pathogen bacteria by essential oils extracted from citrus fruits  
 625 cultivated in Sicily. *Food Control*, 26, 326-330.
- 626 Siroli, L., Patrignani, F., Serrazanetti, D.I., Tabanelli, G., Montanari, C., Tappi, S., et al. (2014).  
 627 Efficacy of natural antimicrobials to prolong the shelf-life of minimally processed apples  
 628 packaged in modify atmosphere. *Food Control*, 46, 1-9.
- 629 Siroli, L., Patrignani, F., Serrazanetti, D.I., Tabanelli, G., Montanari, C., Tappi, S., et al. (2015a).  
 630 Potential of natural antimicrobials for the production of minimally processed fresh-cut  
 631 apples. *Journal of Food Processing & Technology*, 6, 1-9.
- 632
- 633 Siroli, L., Patrignani, F., Serrazanetti, D.I., Tappi, S., Rocculi, P., Gardini, F., Lanciotti, R.  
 634 (2015b). Natural antimicrobials to prolong the shelf-life of minimally processed lamb's  
 635 lettuce. *Postharvest Biology & Technology*, 103, 35-44.
- 636 Siroli, L., Patrignani, F., Serrazanetti, D.I., Tabanelli, G., Montanari, C., Gardini, F., Lanciotti, R.  
 637 (2015c). Lactic acid bacteria and natural antimicrobials to improve the safety and shelf-life  
 638 of minimally processed sliced apples and lamb's lettuce. *Food Microbiology*, 47, 74-84
- 639 Skandamis, P.N., and Nychas, G.J.E. (2000). Development and evaluation of a model predicting the  
 640 survival of *Escherichia coli* O157:H7 NCTC 12900 in homemade eggplant salad at various

temperatures, pHs, and oregano essential oil concentrations. *Applied and Environmental Microbiology*, 66, 1646-1653.

Soliva-Fortuny, R.C, & Martín-Belloso, O.(2003). New advances in extending the shelflife of fresh-cut fruits: a review. *Trends in Food Science & Technology*, 14, 341-353.

Somolinos, M., García, D., Pagan, R., & Mackey, B. (2008). Relationship between Sublethal Injury and Microbial Inactivation by the Combination of High Hydrostatic pressure and Citral or *tert*-Butyl Hydroquinone. *Applied and Environmental Microbiology*, 74, 7570–7577.

Tassou, C. C., Koutsoumanis, K., & Nychas, G.J. E. (2000). Inhibition of *Salmonella enteritidis* and *Staphylococcus aureus* in nutrient broth by mint essential oil. *Food Research International*, 33, 273–280

Tiwari, B.K., Valdramidis, V.P., O'Donnell, C.P., Muthukumarappan, K., Bourke, P., & Cullen, P.J. (2009). Application of natural antimicrobials to food preservation. *Journal of Agriculture & Food Chemistry*, 57, 5987–6000.

Valero, D., Valverde, J.M., Martínez-Romero, D., Guillen, F., Castillo, S., & Serrano, M. (2006). The combination of modified atmosphere packaging with eugenol or thymol to maintain quality, safety and functional properties of table grapes. *Postharvest Biology and Technology*, 41, 317–327.

Valero, M., & Giner, M. (2006). Effects of antimicrobial components of essential oils on growth of *Bacillus cereus* INRA L2104 in and the sensory qualities of carrot broth. *International Journal of Food Microbiology*, 106, 90 – 94.

Van Boxtael, S., Habib, I., Jacxsens, L., De Vocht, M., Baert, L., Van De Perre, E., et al. (2013). Food safety issues in fresh produce: bacterial pathogens, viruses and pesticide residues indicated as major concerns by stakeholders in the fresh produce chain. *Food Control*, 32, 190-197.

- 665 Vandekinderen, I., Devlieghere, F., De Meulenaer, B., Ragaert, P., & Van Camp, J. (2009).  
666 Decontamination strategies for fresh-cut produce. *Stewart Postharvest Review*, 5, 1-8.
- 667 Vazquez, B. I., Fente, C., Franco, C. M., Vazquez, M. J., & Cepeda, A. (2001). Inhibitory effects of  
668 eugenol and thymol on *Penicillium citrinum* strains in culture media and cheese.  
669 *International Journal of Food Microbiology*, 67, 157–163.
- 670 Viuda-Martos, M., Ruiz-Navajas, Y., Fernandez-Lopez, J., & Perez-Alvarez, J. (2008). Antifungal  
671 activity of lemon (*Citrus lemon* L.), mandarin (*Citrus reticulata* L.), grapefruit (*Citrus*  
672 *paradisi* L.) and orange (*Citrus sinensis* L.) essential oils. *Food Control*, 19, 1130–1138.
- 673 Zheng, L., Bae, Y.M., Jung, K.S., Heu, S., & Lee, S.Y. (2013). Antimicrobial activity of natural  
674 antimicrobial substances against spoilage bacteria isolated from fresh produce. *Food Control*  
675 32, 665-672.

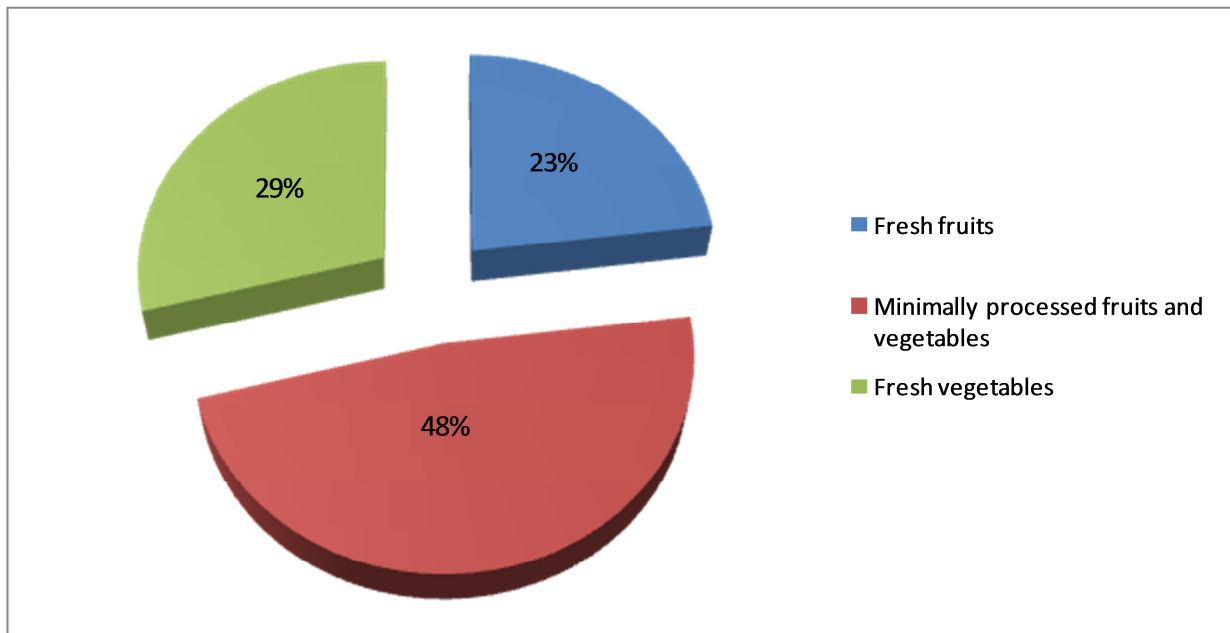
**Table 1.** Overview of cases testing the antimicrobial activity of essential oils (EO) or their components in minimally processed fruits and vegetables.

	Food	EO or component	Concentration applied	Microbial targets	References
<b>Fruits</b>	<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	1000µL in gas used for MAP	Natural microflora	Serrano et al., 2005
	<i>Table Grape</i>	eugenol thymol	75-150µL 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 µL 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
<b>Vegetables</b>	<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.

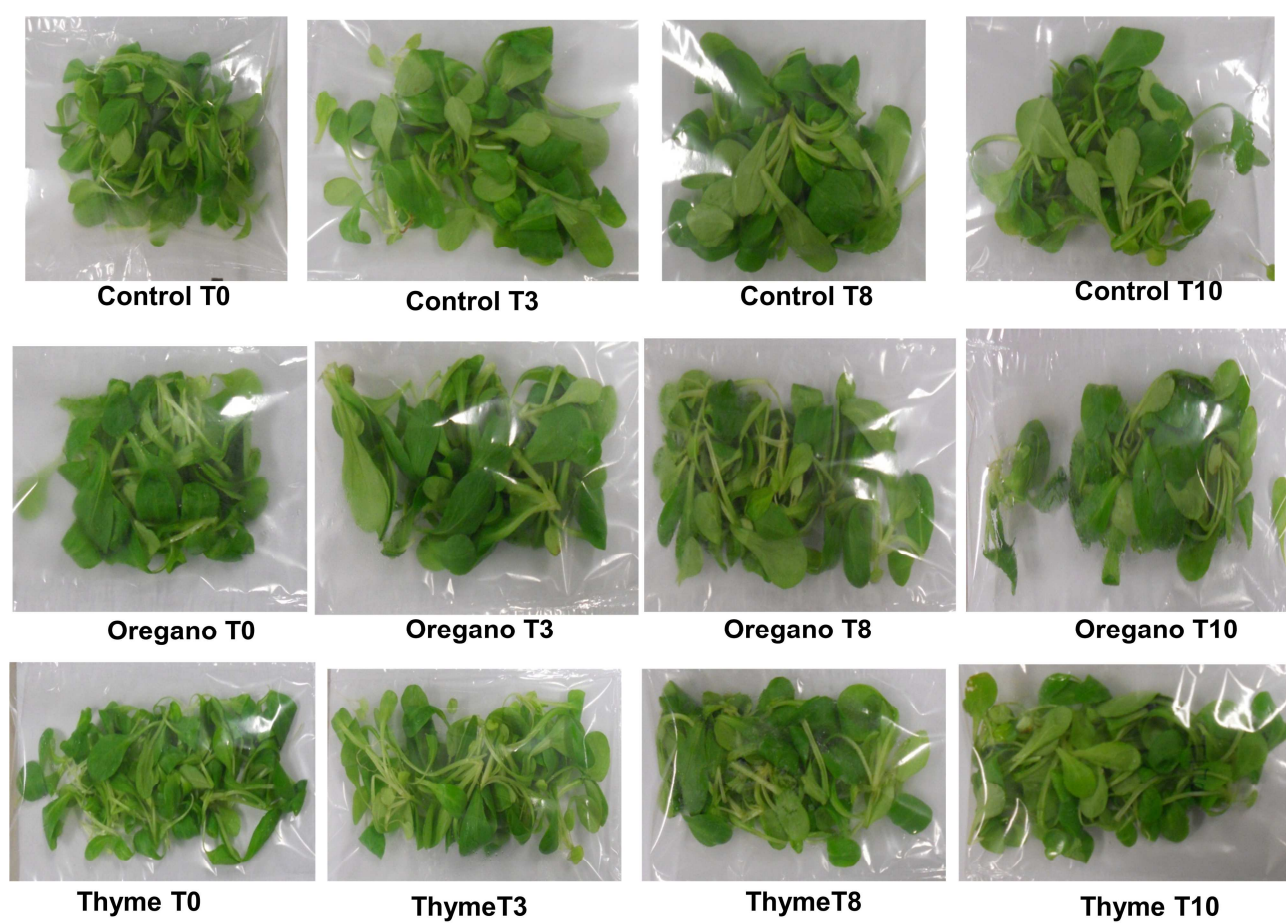


Figure 3.

- 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