Trends in Food Science & Technology 19 (2008) 464–471

Review

The addition of essential oils to MAP as a tool to maintain the overall quality of fruits

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This paper covers the recent literature on fruit ripening and problems related to quality loss during postharvest storage, as well as the use of essential oils as antioxidants and antimicrobials. This review sets the principles for the creation of innovative technological developments by using an active packaging based on the combination of modified atmosphere packaging (MAP) with natural antimicrobial compounds. The use of this active packaging on the delay of fruit ripening and the extension of shelf-life based on safety and the preservation of sensory attributes and bioactive compounds with functional properties will be provided.

Introduction

For decades the postharvest management of fruit related to the overall quality maintenance has been a challenge. It is believed that quality can only be maximised when the commodity is harvested mature or ripe, and on the contrary shelf-life is usually extended for less mature or unripe products (Toivonen, 2007). However, produce is harvested at commercially mature stage (not over-ripe or senescent) but shows a large number of problems that lead to a net reduction of shelf-life or so-called "postharvest losses" due to fungal attack and quality deterioration.

There is a wide range of research on the possibilities to reduce the acceleration of the ripening process and microbial spoilage, and in turn to increase fruit shelf-life. Some of the postharvest technologies include the application of low doses of UV radiation for protecting fruits against attack by pathogenic fungi (Shama & Alderson, 2005), the use of exogenous polyamines which compete with ethylene and extend the fruit shelf-life (Valero, Martínez-Romero, & Serrano, 2002) or the application of calcium to increase fruit texture (Martín-Diana *et al.*, 2007). Modified atmosphere packaging (MAP) has been used since the late 1990s to preserve the quality of vegetable products (Kader, Zagory, & Kerbel, 1989). However, traditional MAP is not enough to ensure quality and safety preservation to fulfil consumer demand. In this sense, active packaging is an innovative concept for food packaging as a response to the continuous changes in current consumer demands and market trends (Vermeiren, Devlieghere, van Beest, de Kruijf, & Debevere, 1999).

Literature data indicate that volatile compounds can represent a useful tool to increase the shelf-life of plant products. In fruits, the use of natural compounds such as hexanal, 2-(E)-hexenal, and hexyl acetate improved shelf-life and safety of minimally processed fruits (Lanciotti *et al.*, 2004). In this review, authors postulated that future trends in the use of natural compounds would be focused on the use of specific active packaging able to release the active molecules in the head space slowly over time.

Thus, the aim of this review is to give an overview on the use of natural antimicrobial compounds combined with MAP to obtain active packaging. The effect of this technology, which is considered safe and environmentally friendly, on the delay of the ripening process and improvement of fruit quality and safety will be evaluated.

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0924-2244/$ - see front matter
doi:10.1016/j.tifs.2008.01.013

Fruit ripening and quality

According to Brady (1987), fruit ripening is a highly coordinated, genetically programmed process occurring at the later stages of maturation and involving a series of physiological, biochemical and sensory changes leading to the development of an edible ripe fruit with desirable quality parameters (Giovannoni, 2001). The spectrum of biochemical changes is wide including chlorophyll degradation, increased activity of cell wall-degrading enzymes, biosynthesis of carotenoids, anthocyanins, flavour and aroma components, accumulation of sugars and diminution of acidity. Two major classifications of ripening fruit (climacteric and non-climacteric) based on the respiration and ethylene production rates have been distinguished.

Climacteric fruit, such as apple, apricot, avocado, banana, peach, plum, and tomato are characterised by their increased respiration and ethylene biosynthesis rates during ripening. In this sense, ethylene is considered the plant hormone responsible for the ripening process in climacteric fruit. Ethylene biosynthesis, perception, signal transduction and its regulation are well documented (Adams-Phillips, Barry, & Giovannoni, 2004; Giovannoni, 2001; Martínez-Romero, Bailén, *et al.*, 2007). Contrarily, in non-climacteric fruit, such as citrus, eggplant, sweet cherry, grape, pepper, and strawberry, ethylene is not required for the coordination and completion of ripening (Lelièvre, Latché, Jones, Bouzayen, & Pech, 1997; Prassana, Prabha, & Tharanathan, 2007).

Fruit quality refers to a range of attributes that in the case of fruits are related to appearance, colour, texture, flavour and aroma (Shewfelt, 1999). These attributes are considered at maximum level with the optimum ripening stage and immediately after harvest. However, during postharvest storage these parameters change, the diminution or increase being related to quality loss.

Colour is one of the main fruit attributes for consumer acceptance and during ripening there is a degradation of chlorophyll and accumulation of either anthocyanins or carotenoids. Anthocyanins are polyphenolic pigments responsible for the red to blue colours of a wide range of fruits including red apples, plums, sweet cherries and grapes (Wrolstad, Durst, & Lee, 2005). Carotenoids are a group of *ca.* 600 natural lipid-soluble pigments responsible for a broad variety of colours (Sagilata & Singhal, 2006), such as yellow (plums and lemons), orange (apricots, oranges and mandarins), and red (tomatoes). Apart from their role in colouring fruits, both pigment groups have been claimed to show health benefits as biomolecules with functional properties (Stintzing & Carle, 2004; Temple & Gladwin, 2003).

Fruit softening occurs during ripening due to the activity of the enzymes polygalacturonase (PG), pectin-methyl-esterase (PME), endo-β-mannase, α- and β-galactosidases and ß-glucanases, which alter the structural components of the cell wall and diminish the cell adhesion (Brummel & Harpster, 2001; Rose & Bennet, 1999; Seymour, Manning, Eriksson, Popovich, & King, 2002). Cell wall modifications determine practical shelf-life by altering fruit quality, which influences consumer palatability and facilitates the fungal infections (Lashbrook, 2005).

During ripening, accumulation of sugars and diminution of organic acids occurs, conferring to the fruit a good balance of sweetness/sourness in terms of taste acceptability together with volatile compounds. For commercial purposes the ratio between soluble solids concentration (°Brix) and titratable acidity is often used as a quality criterion for fruits before marketing and commonly named as ripening index (Crisosto, 1994).

Problems during postharvest storage of fruits

Fruits deteriorate rapidly after harvest and in some cases do not reach consumers at optimal quality after transport and marketing. The main causes of fruit deterioration are dehydration, with the subsequent weight loss, colour changes, softening, surface pitting, browning, loss of acidity and microbial spoilage, among others. However, deterioration rate is affected by different factors, such as intrinsic characteristics of the product and storage conditions in terms of temperature, relative humidity, storage atmosphere composition, etc. The main objective of postharvest technology is the quality optimisation and reduction of losses along the postharvest chain.

Since fruit ripening and deterioration are accelerated as temperature increases, cold storage is therefore necessary to slow down the above processes. However, chilling injury (CI) limits the storage life of sensitive fruits under low temperature with occurrence of browning (both flesh and internal) and decay especially when produce is transferred at ambient temperature (Lurie & Crisosto, 2005).

Decay occurrence is mainly due to species of genera *Penicillium*, *Botrytis* and *Monilia* causing great economic losses, although its severity has been reported to be dependent on cultivar and ripening stage at harvest. Fruit quality losses have been reduced mainly by the usage of postharvest fungicides although their application has becoming very restrictive, mainly due to development of pathogen resistance, lack of new fungicides, and public perception that pesticides are harmful to human health and environment. This negative perception has promoted governmental policies restricting use of fungicides (Tripathi & Dubey, 2004). One clear example is the sulphur dioxide (SO_2) usage in table grapes, which is highly effective in killing both spores and mycelia of *Botrytis cinerea*. In the last years, SO_2 has been removed from the list of general recognise as safe (GRAS) by the United States Food and Drug Administration (FDA) and has been classified as a pesticide by the United States Environmental Protection Agency (EPA) with a 10 ppm residue tolerance (*Pesticide tolerance for sulphur dioxide*, 1989). This change was due to excessive residues of SO_2 (sulphites) that can occur along food chain causing hypersensitive reactions in certain consumers. Finally, the use of SO_2 is banned for organic produce, and thus new alternatives are therefore necessary. In this sense, fruit exposure to low doses of UV-C light has been effective on reducing postharvest decay in a wide range of fruits, such as table grapes, apple, peaches, tangerines, mango, pepper, and tomato, among others, with additional effects on delaying the ripening process and maintaining fruit quality (González-Aguilar, Zavaleta-Gatica, & Tiznado-Hernández, 2007; Lichter, Gabler, & Smilanick, 2006; Shama & Alderson, 2005; Stevens *et al.*, 2005; Vicente *et al.*, 2005). However, it seems that UV-C alone is not sufficiently effective in prevention of fruit decay, but rather it might enhance the effectiveness of other postharvest treatments. Another alternative to solve decay and fruit quality losses during postharvest storage could be the use of essential oils, since they have antimicrobial and antioxidant properties.

Essential oils as natural antioxidant and antimicrobial agents

Essential oils or the so-called volatile or ethereal oils (Guenther, 1948) are aromatic oily liquids obtained from plant organs: flower, bud, seed, leaf, twig, bark, herb, wood, fruit and root. The term of ‘‘essential oil’’ is thought to derive from the word *Quinta essentia* with medical use attributed to Paracelsus. Until recently, essential oils have been used as food flavourings due to their flavour and fragrance, but nowadays essential oils and their pure components are gaining increasing interest from the point of view of their safe status, wide acceptance by consumers and their exploitation for multi-purpose uses (Cowan, 1999).

The utilisation of natural antioxidants as substitutes for those from chemical synthesis has encouraged the search of new sources including essential oils (Capecka, Mareczek, & Leja, 2005; Ruberto & Baratta, 2000). In addition, the antimicrobial properties of essential oils derived from many plant organs have been empirically recognised for centuries, but only came to scientific attention recently (Appendini & Hotchkiss, 2002; Burt, 2004). Table 1 shows some of the most common essential oils as well as the major component used in the food industry with description of antioxidant or antimicrobial properties *in vitro*. These natural compounds belong to genus *Thymus*, *Origanum*, *Syzygium*, *Mentha* and *Eucalyptus*. The whole essential oils show antioxidant activity, but their fractionation has indicated that the main component responsible for the antioxidant effect is carvacrol for oregano (Milos, Mastelic, & Jerkovic, 2000), thymol for thyme (Lee, Umano, Shibamoto, & Lee, 2005), eugenol for clove (Lee & Shibamoto, 2001), menthol for mint (Shan, Cai, Sun, & Corke, 2005), and eucalyptol for eucalyptus (Amakura *et al.*, 2002). The chemical structures of these natural essential oils are shown in Fig. 1, in which eugenol, thymol and carvacrol are phenols, while eucalyptol and menthol are terpenoids. For these compounds, the activity as antioxidants has been reported to be close to that of α-tocopherol (Ruberto & Baratta, 2000; Sacchetti *et al.*, 2005) or vitamin C (Kim & Lee, 2004), and mainly due to the presence of hydroxyl groups in the benzene ring (Shahidi, Janitha, & Wanasundara, 1992).

However, the main biological activity and the possible use of the essential oils in the food industry are derived from their capacity to kill microorganisms. In this sense,

Table 1. Some common essential oils and their components used as flavouring in the food industry that exhibit antioxidant, antifungal and antibacterial activity *in vitro* systems

Common name	Latin name	Source	Major component	Antioxidant	Antifungal	Antibacterial	Reference
Clove	*Syzygium aromaticum*	Bud Leaf	Eugenol	Lipid	*Penicillium* *Aspergillus*	*Listeria monocytogenes* *Lactobacillus sakei*	Gill and Holley (2004), Lee and Shibamoto (2001) and Suhr and Nielsen (2003)
Eucalyptus	*Eucalyptus globulus*	Leaf Wood	Eucalyptol Eucalyptone	Thiobarbituric acid DPPH	Moulds and yeasts *in vivo*	Pathogenic bacteria	Amakura *et al.* (2002), González, Cruz, Domínguez, and Parajó (2004) and Ponce, Del Valle, and Roura (2004)
Mint	*Mentha canadensis*	Leaf	Menthol	$ABTS^{++}$	*Botrytis*	Pathogenic bacteria	Bouchra, Achouri, Hassani, and Hmamouchi (2003), Īşcan, Kirimer, Kürkcüoğlu, Başer, and Demirci (2002), Özkan, Sağdiç, and Özcan (2002) and Shan *et al.* (2005)
Oregano	*Origanum vulgare*	Leaf Flower	Eugenol Carvacrol Thymol	Peroxidase	*Botrytis* *Fusarium* *Clavibacter*	*Shigella* sp.	Bagamboula *et al.* (2004), Daferera, Ziogas, and Polissiou (2003) and Milos *et al.* (2000)
Thyme	*Thymus vulgaris*	Leaf	Carvacrol *p*-Cymene Thymol	Aldehyde/ Carboxylic acid	*Aspergillus*	Pathogenic bacteria	Lee *et al.* (2005), Özkan *et al.* (2002) and Rasooli and Abyaneh (2004)

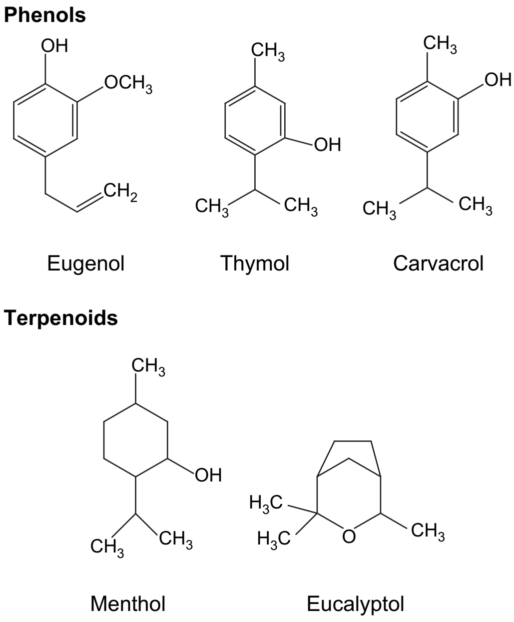

Fig. 1. Chemical structures of pure essential oils.

the *in vitro* antimicrobial activity of eugenol, thymol and carvacrol has been reported against bacteria (Periago, Delgado, Fernández, & Palop, 2004), yeasts (Arora & Kaur, 1999) and fungi (Vázquez, Gente, Franco, Vázquez, & Cepeda, 2001). Accordingly, menthol, eugenol, carvacrol and especially thymol were also effective in reducing the percentage of damaged berries caused by the development of *B. cinerea* artificially inoculated, while eucalyptol failed. Moreover, in those berries that showed fungal infection the colony diameter of *Botrytis* was significantly lower in treated than in control berries, even when eucalyptol was used. Additionally, some physiological parameters of grapes such as ethylene production and respiration showed rates at much lower levels in treated berries than in control ones (Fig. 2).

Since grape has been reported as a non-climacteric fruit, and its ripening process is not associated with enhancements in ethylene production and respiration rate (Martínez-Romero, Guillén, Castillo, Valero, & Serrano, 2003), the higher ethylene production observed in control fruits might be a consequence of the fungal growth. In fact, in the experiment about berries artificially inoculated with *B. cinerea* and treated with essential oils (Fig. 2), high correlations ($r^2 = 0.83–0.95$) were found between percentage of damaged berries or colony diameter and ethylene production or respiration rate. Accordingly, it has been reported that *B. cinerea* produced higher amounts of ethylene as concentration of conidia increased (Cristescu, De Martinis, Hekkert, Parker, & Harren, 2002).

Although the exact mechanism of action of the essential oils was not completely clarified, some authors have attributed it to their hydrophobicity, which enables them to partition in the lipids of the cell membrane disturbing its integrity and the inorganic ions equilibrium (Bagamboula, Uyttendaele, & Debevere, 2004; Lambert, Skandamis, Coote, & Nychas, 2001). Additionally, it has been postulated that the presence of the phenolic ring may be necessary for the antimicrobial activity of eugenol and thymol (Ultee, Bennik, & Moezelaar, 2002). Moreover, the site(s) and number of hydroxyl groups on the phenol ring are thought to be related to their relative toxicity to microorganisms, with evidence that increased hydroxylation results in increased toxicity (Cowan, 1999). For those essential oils with absence of phenolic groups like menthol, it has been speculated that the mechanism of action involves membrane disruption by the lipophilic compounds (Mendoza, Wilkens, & Urzua, 1997).

In foods, the potential use of essential oils as natural preservatives has been reported in cheese (Smith-Palmer,

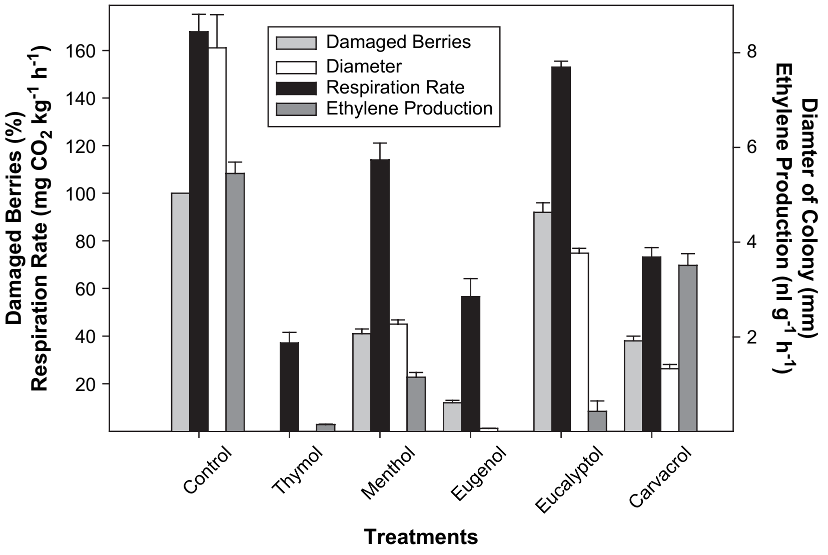

Fig. 2. Effect of several essential oils at 0.5 ml l^{-1} dose inside hermetically-sealed packages in the percentage of damaged berries, dimension of infection (diameter), respiration rate (expressed as CO_2 production) and ethylene production after 4 days at 25 °C. Berries were injured (2 × 2 mm) and further artificially-inoculated with 75 spores of *Botrytis cinerea*. Data adapted from Martínez-Romero, Guillén, *et al.* (2007) and Valverde (2005).

Stewart, & Fyfe, 2001), bakery products (Guynot *et al.*, 2003) and meat (Quintavalla & Vicini, 2002), among others. The main disadvantages for the use of these natural compounds are related to persistence of strong aromas which sometimes would affect the organoleptic properties of food adversely.

Particularly in fruits, the use of essential oils as preservatives is limited although there is little evidence. Thus, carvacrol was very effective in reducing the viable count of the natural flora on fresh-cut kiwifruit when used at 0.15 μl $\mathrm{ml^{-1}}$ in dipping solution but failed in honeydew melon (Roller & Seedhar, 2002). Eugenol and carvacrol were found to be the most active compounds from 17 evaluated essential oils against *Escherichia coli* O157:H7 and *Salmonella enterica* in apple juices, the activity being greater for *S. enterica* (Friedman, Henika, Levin, & Mandrell, 2004). Thyme or oregano and their respective major components (thymol and carvacrol) as dip treatment reduced disease development in tomatoes inoculated with *Botrytis* and *Alternaria* fungi (Plotto, Roberts, & Roberts, 2003). In addition, carvacrol was found also effective in reducing the fungal growth of *B. cinerea* in grapes and alleviated the injuries showing better tissue integrity (Martínez-Romero, Guillén, *et al.*, 2007). Similarly, strawberry exposed to eucalyptol essential oil vapour at 50 or 500 ppm decreased fruit decay during storage (Tzortzakis, 2007).

Active packaging using essential oils combined with MAP to preserve fruit quality and safety

With MAP technology, an increase in CO_2 and decrease in O_2 occur inside the packages taking into account the film permeability and product respiration (Fonseca, Oliveira, & Brecht, 2002). The choice of packaging system/method is a key factor in order to obtain optimum modifications of the atmosphere and to avoid extremely low levels of O_2 and/or high levels of CO_2, which could induce anaerobic metabolism with possibility of off-flavour generation, and/or the risk of anaerobic microorganism proliferation. The use of MAP in fruits has been found to be effective on quality maintenance, but the CO_2 concentration inside packages could not be high enough to act as fungicide or bactericide. In this sense, and taking into account the pressure by consumers about the use of synthetic chemicals, there has been an increasing interest about the use of natural compounds. However, there is almost no evidence on the use of essential oils or their components in combination with MAP in fruits, apart from our previous work on sweet cherry and several cultivars of table grapes such as 'Crimson', 'Autumn Royal' and 'Aledo' (Guillén *et al.*, 2007; Serrano, Martínez-Romero, Castillo, Guillén, & Valero, 2005; Valero *et al.*, 2006; Valverde *et al.*, 2005). With this active packaging, the overall quality of products can be improved in terms of maintenance of organoleptic and functional properties together with safety. This active packaging was performed using bags (30 × 20 cm) of non-perforated oriented polypropylene (N-OPP) film (20 μm thickness), which had permeabilities at 1 °C of 1600 ml $O_2\ \mathrm{m^{-2}\,d^{-1}\,atm^{-1}}$ and 3600 ml $CO_2\ \mathrm{m^{-2}\,d^{-1}\,atm^{-1}}$. The essential oils (99.5% purity and purchased from Sigma, Sigma–Aldrich, Madrid, Spain), were placed on a sterile gauze inside the bag avoiding the contact with fruits, and the bags were immediately sealed to minimize vaporization. This active packaging led to reduced softening and colour evolution compared to those control fruits under MAP conditions. Thus, the combination of MAP and essential oils delays the evolution of these parameters related to postharvest ripening more than MAP alone. In fact, these effects could be attributable to the essential oils added, since gas composition were similar (11–12 O_2 and 2–3 kPa CO_2 for sweet cherry, and 10–14 O_2 and 1.3–2.0 kPa CO_2 for table grapes) in both control bags and active packages.

The compounds responsible for the human health beneficial properties associated with fruit and vegetable intake are those with antioxidant activity, including carotenoids, ascorbic acid, flavonoids, anthocyanins and other phenolic compounds (Kalt, 2005; Tomás-Barberán & Espín, 2001). Little is known about the behaviour of these bioactive compounds during fruit storage, although some evidence shows different evolution depending on fruit specie. Thus, in sweet

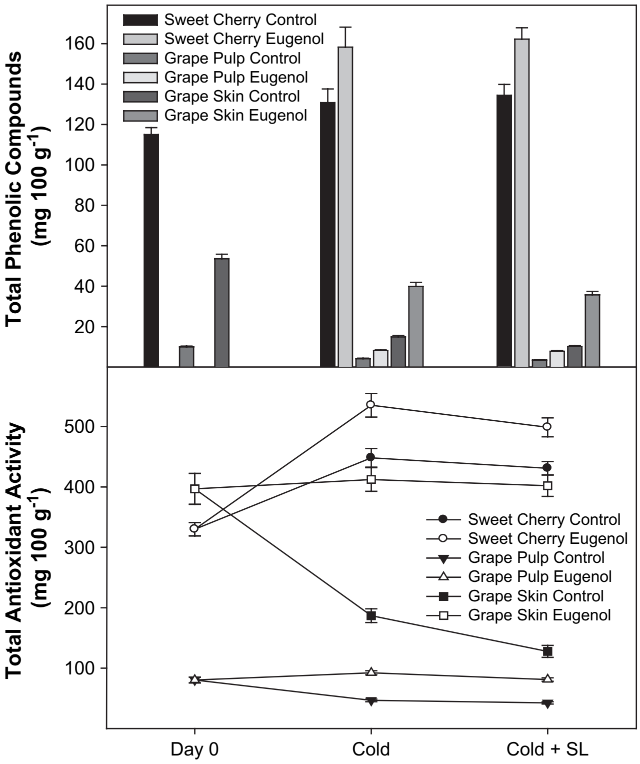

Fig. 3. Results showing concentration of total phenolic compounds and total antioxidant activity (TAA) at harvest (day 0) and after 16 or 35 days of cold storage for sweet cherry and table grapes, respectively, or a subsequent period of 2 days at 20 °C, shelf-life (SL), under MAP without (control) or with eugenol as essential oil (0.5 ml $\mathrm{l^{-1}}$ inside bags). Determinations were made according to previous work (Serrano, Guillé, *et al.*, 2005). Data adapted from Valero *et al.* (2006) and Valverde (2005).

cherry total polyphenols showed slight increases during storage while significant decreases were found in both skin and pulp of table grapes under MAP conditions (Fig. 3). Total antioxidant activity (TAA) evolved in a similar way to phenolic compounds, that is enhancements in sweet cherry and diminution in table grapes, especially in the skin. However, the addition of eugenol to MAP led to higher increases in both total phenolics and TAA than those found in control sweet cherry, and remained unchanged during storage of table grapes. The effect of essential oils on increasing or maintaining total phenolics and TAA has been also found using thymol in table grapes (Valero *et al.*, 2006) or thymol, eugenol and menthol in strawberry (Wang, Wang, Yin, Parry, & Yu, 2007). In addition, the content of total phenolics was correlated to TAA ($r^2 > 0.82$) in sweet cherry and skin or pulp of table grapes, according to previous reports (Dávalos, Bartolomé, & Gómez-Cordovés, 2005; Kallithraka, Mohdaly, Makris, & Kefalas, 2005; Serrano, Guillén, Martínez-Romero, Castillo, & Valero, 2005).

The essential oils also show antimicrobial effects, as can be seen in Fig. 4, since microbial spoilage increased during fruit storage under MAP conditions only, especially moulds and yeasts in sweet cherry. However, the addition of thymol, eugenol or menthol led to a significant reduction of mesophillic aerobics and moulds and yeasts after cold or cold + SL storage with respect to their levels at harvest. This reduction in microbial population obtained confirms the antimicrobial effects described for these compounds (Appendini & Hotchkiss, 2002; Burt, 2004), since the concentration of CO_2 achieved inside the bags (below 3 kPa) was not high enough to act as antimicrobial.

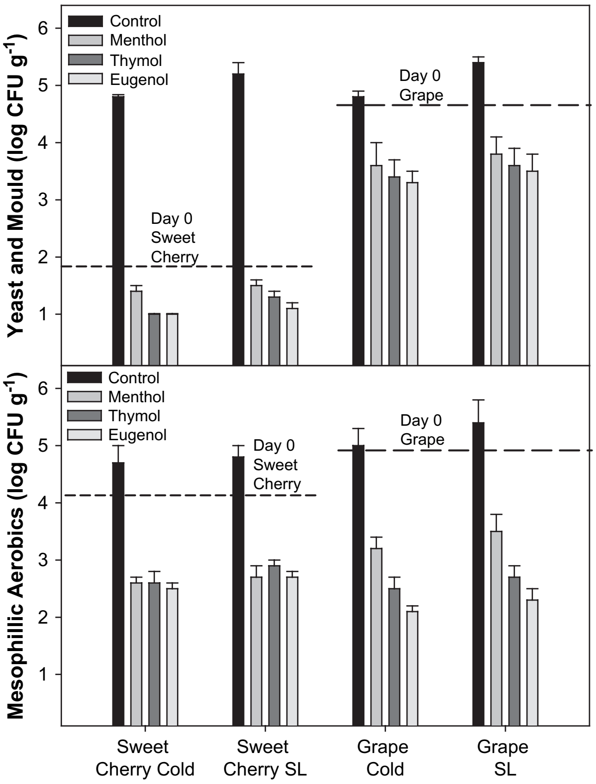

Fig. 4. Total viable counts for yeast and mould and mesophillic aerobic in sweet cherry or table grapes under MAP packages without (control) or with essential oils (0.5 ml l^{-1} inside bags) after 16 or 35 days of cold storage for sweet cherry and table grapes, respectively, or a subsequent period of 2 days at 20 °C, shelf-life (SL). Dotted lines represent values at harvest (day 0). Determinations were made according to previous work (Valverde, 2005). Data adapted from Guillén *et al.* (2007), Serrano, Martínez-Romero, *et al.* (2005), Valero *et al.* (2006), and Valverde *et al.* (2005).

Conclusions and future trends

The development of the active packaging based on essential oils added to MAP was designed to respond to a number of issues related to fruit quality deterioration during postharvest storage. The use of this active packaging improves the benefits of MAP only, based on maintenance of organoleptic parameters and controlling the microbial spoilage. From the results reported herein and the reviewed literature, it can be concluded that the use of pure essential oils (eugenol, thymol or menthol) in combination with MAP is an innovative and useful tool as alternative to the use of synthetic fungicides in fruits and vegetables, especially for those which are highly perishable and have a reduced shelf-life. Given the phenolic nature of some of the essential oils, such as eugenol, the bioactive compounds with antioxidant activity were also enhanced during prolonged storage.

In future, a strong impulse in the development of this technology is required for commercial application, since active packaging is an emerging and exciting concept in food technology conferring many benefits which fulfils consumer demand for safe products avoiding the use of chemicals as a means of preservation. Further studies are needed to better understand the mechanism(s) by which these essential oils affect the fruit physiology modulating the ripening process as well as their ability to kill microorganisms. Finally, other possible use of essential oils could be their combination with other tools that modified the atmosphere surrounding the fruit such as edible coatings.

Acknowledgements

This work has been co-funded by Spanish Ministry of Science and Technology through projects AGL2003-03257/ALI and AGL2006-04359/ALI and European Commission with FEDER funds.

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