Available online at www.sciencedirect.com

SCIENCE DIRECT®

Innovative Food Science and Emerging Technologies xx (2005) xxx–xxx

Innovative Food Science & Emerging Technologies

www.elsevier.com/locate/ifset

The use of natural antifungal compounds improves the beneficial effect of MAP in sweet cherry storage

M. Serrano[a,*], D. Martínez-Romero[b], S. Castillo[b], F. Guillén[b], D. Valero[b]

[a]*Department of Applied Biology, EPSO, University Miguel Hernández. Ctra. Beniel km 3,2, 03312 Orihuela, Alicante, Spain*
[b]*Department of Food Technology, EPSO, University Miguel Hernández, Ctra. Beniel km 3,2, 03312 Orihuela, Alicante, Spain*

Received 14 July 2004; accepted 21 September 2004

Abstract

Sweet cherry shows severe problems for commercialisation mainly due to incidence of decay and a fast loss of sensory quality, both for fruit and stem. A package has been developed based on the addition of eugenol, thymol, menthol or eucalyptol (pure essential oils) separately to trays sealed with polypropylene bags to generate a modified atmosphere (MAP). In addition, cherries in MAP (without essential oils) were selected and served as controls. All cherries were stored during 16 days at 1 °C and 90% RH. Steady-state atmosphere was reached after 9 days of cold storage with 2–3% of CO_2 and 11–12% of O_2 with no significant differences between treated and control, with the exception of eucalyptol, in which significant increases in CO_2 and decreases of O_2 were obtained. When fruit quality parameters were determined, those treated with eugenol, thymol or menthol showed benefits in terms of reduced weight loss, delayed colour changes and maintenance of fruit firmness compared with control. Stem remained green in treated cherries while they became brown in control. However, cherries packaged with eucalyptol behaved even worst than control cherries, with generation of off-flavours, loss of quality and stem browning. Finally, the microbial analysis showed that all essential oils reduced moulds and yeasts and total aerobic mesophilic colonies by 4- and 2-log CFU compared with control, respectively. In conclusion, the use of MAP in combination with eugenol, thymol or menthol is an effective tool on maintaining cherry fruit quality and reducing the occurrence of decay.

Keywords: *Prunus avium* L.; Essential oils; Fruit quality; Decay; Ripening; MAP

Industrial relevance: The data presented in this work suggest that the use of pure essential oils (eugenol, thymol or menthol) in combination with modified atmosphere packaging (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 short shelf-life, as cherries. These compounds have been included in the list of generally recognized as safe (GRAS) compounds by FDA. As far as we know, this is the first paper dealing on the use of natural antifungal compounds and MAP and that these combined technologies confer benefits in fruit storage and retailing, with reduction in spoilage microorganisms, maintenance of cherry quality attributes and extension of shelf-life. The effects of these natural compounds on individual microorganisms, both responsible for spoilage and food-borne pathogens, as well as the minimum concentration to gain effectiveness deserve further research.

1. Introduction

Spain is one of the main cherry producers in Europe, with production of 115 000 metric tonnes in 2003, which represents a 20% of the total in the European Union (MAPYA, 2003). Sweet cherry is considered one of the most appreciated fruit by consumers since it is an early season fruit and has an excellent quality. The main sensory attributes are: colour, sweetness, sourness and firmness. Skin colour is considered one of the main quality indexes and is related to fruit ripening and affected by anthocyanin concentration (Gao & Mazza, 1995). Sweetness is mainly due to glucose and fructose

* Corresponding author. Tel.: +34 96 6749616; fax: +34 96 6749676.
E-mail address: m.serrano@umh.es (M. Serrano).

1466-8564/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.ifset.2004.09.001

and lower presence of sucrose and sorbitol, with a range of total soluble solids (TSS) of 11–20 °Brix, depending on cultivar. Acidity (TA) depends also on cultivar, with levels of 0.4–1.5%, the main organic acid being malic acid (Bernalte, Hernández, Vidal-Aragón, & Sabio, 1999; Bernalte, Sabio, Hernández, & Gervasini, 2003; Esti, Cinquanta, Sinesio, Moneta, & Di Matteo, 2002). Fruit firmness is also an important quality attribute and is directly related to enhance the storability potential and to induce greater resistance to decay and mechanical damage (Barret & González, 1994). In addition, the TSS/TA ratio at harvest has been shown to be a predominant parameter for consumer acceptance together with the absence of stem browning (Crisosto, Crisosto, & Metheny, 2003).

Sweet cherry fruits deteriorate rapidly after harvest with a reduced shelf life and in some cases do not reach the consumer at optimal quality after transport and marketing. The main causes of cherry deterioration are weight loss, colour changes, softening, surface pitting, stem browning and loss of acidity, while low variations occur in TSS (Barret & González, 1994; Batisse, Buret, & Coulomb, 1996; Bernalte et al., 2003). Finally, special care is needed with the occurrence of decay, which is responsible for the high percentage of losses during postharvest storage. In cherry, the fungal spoilage is mainly due to species of genera *Penicillium*, *Botrytis* and *Monilia*, which are responsible for blue rot, gray mold and brown rot, respectively (Venturini, Oria, & Blanco, 2002). The development of these fungi during postharvest storage can cause great economic losses, and thereafter a fermentative metabolism with generation of "off-flavours" due to ethanol and acetaldehyde (Esti et al., 2002). The occurrence of rots and their influence on cherry quality have been reported to be dependent on cultivar (Kappel, Toivonen, McKenzie, & Stam, 2002) and ripening stage at harvest (Drake & Elfving, 2002). Several pre- and postharvest technologies have been used to control decay, but the postharvest use of chemicals as fungicides is restricted in most countries. Besides, consumers demand agricultural commodities without pesticide residues. Thus, new preservation technologies are needed, which have to be considered as human-safe and environmentally friendly.

Among these technologies, the use of modified atmosphere packaging (MAP) has been reported to be effective in cherry storage. MAP induces a delay in the physicochemical changes related to fruit quality loss by increasing the level of CO_2 and decreasing the O_2 content. However, different O_2 (2–10%) and CO_2 (5–20%) concentrations have been reported to be optimal for different cherry cultivars (Kupferman & Sanderson, 2001; Meheriuk et al., 1997; Remón, Ferrer, Maquina, Burgos, & Oria, 2000; Remón, Venturini, López-Buesa, & Oria, 2003; Spotts, Cervantes, & Facteau, 2002; Tian, Fan, Xu, Wang, & Jiang, 2001). These discrepancies could be related to cultivar itself or ripening stage at harvest. Thus, for each cultivar the optimum atmospheric composition should be carefully evaluated.

In recent years, there is an increasing interest in the possible use of natural compounds to prevent microbial growth in the food items, thus answering to consumer's pressure to reduce chemical additives in foods. Plants have an almost limitless ability to synthesise aromatic substances, most of which are phenols or derivatives. Many compounds are responsible for plant flavour, and some of the herbs and spices used by human to season food yield useful medicinal compounds (Cowan, 1999). Among these natural compounds, the antifungal activity of several essential oils belonging to genus *Thymus*, *Syzygium*, *Mentha* and *Eucalyptus*, is well documented (see review of Appendini & Hotchkiss, 2002). In food products, these essential oils have been used in bakery (Nielsen & Ríos, 2000), cheese (Vázquez, Fente, Franco, Vázquez, & Cepeda, 2001), meat (Quintavalla & Vicini, 2002) and fruit (Lanciotti et al., 2004), among others. The advantage of essential oils is their bioactivity in the vapour phase, a characteristic that makes them useful as possible fumigants for stored commodity protection. In this sense, fumigation of sweet cherry with thymol (the main active ingredient of thyme) was effective controlling gray mold and brown rot caused by previous inoculation with spores of *B. cinerea* (Chu, Liu, Zhou, & Tsao, 1999).

There are evidences that some of these compounds have been added to polymeric films in their structure (Appendini & Hotchkiss, 2002). However, no data are available of these essential oils inside the packages and their role on controlling cherry quality and spoilage. Thus, the aim of this paper was to develop a package using some natural antifungal compounds to improve the beneficial effect of MAP on maintaining cherry fruit quality during cold storage and extending its shelf life. The natural antifungal compounds used were eugenol, thymol, menthol and eucalyptol.

2. Material and methods

2.1. Plant material and experimental design

Sweet cherries (*Prunus avium* L. cv. 'StarKing') were harvested from a commercial farm belonging to Denominación Específica "Cerezas de la Montaña de Alicante" (Alicante, Spain). At laboratory, fruit were selected to obtain homogeneous batches based on colour, size, absence of injuries and healthy greenish stems. Twenty cherries (average mass of 135.91±0.23 g) were packed in polypropylene trays. Five sets of 30 trays were introduced in non-perforated oriented polypropylene (N-OPP) bags (20×15 cm) for the natural antifungal treatments: eugenol, thymol, menthol or eucalyptol (99.5% purity and purchased from Sigma, Sigma-Aldrich, Madrid, Spain). Sweet cherries packed in the same conditions but without essential oils

served as control. Plastic films had 20 μm thickness and permeabilities at 1 °C of 1600 ml O_2 m^{-2} day^{-1} atm^{-1} and 3600 ml CO_2 m^{-2} day^{-1} atm^{-1}. Treatments were performed by placing 1 ml of the above compounds on sterile gauze, and then immediately sealed to avoid vaporisation. Gauzes were deposited outside the trays to avoid the contact with cherries.

All packages were stored at 1 °C and with RH of 90% in darkness. Fruits were analysed at three time points: at harvest (day 0), immediately after refrigeration (2-, 6-, 9-, 13- and 16-day intervals) and with one additional day of storage at 20 °C (shelf life, SL). For each sampling date, five trays were selected in which the following analytical determinations were performed.

2.2. Gas composition

A silicone septum was provided on the bag surface for sampling gas inside the package. One milliliter of the headspace atmosphere was withdrawn using a gas syringe and injected into the GC, 14B (Shimadzu, Tokyo, Japan) to quantify CO_2 and O_2 concentrations inside the packages. GC was equipped with a thermal conductivity detector (TCD) and a molecular sieve 5A column, 80–100 mesh (Carbosieve SII, Supelco, Bellefonte, USA), of 2 m length and 3 mm i.d. Oven and injector temperatures were 50 and 110 °C, respectively. Helium was used as carrier gas at a flow rate of 50 mL/min. Results were expressed as % O_2 and % CO_2 inside the bags.

2.3. Weight loss

Weight of individual trays was recorded on the day of harvesting and after the different sampling dates. Cumulative weight losses were expressed as percentage loss of original weight.

2.4. Colour

Colour was determined using the Hunter Lab System and a Minolta colorimeter CR200™ model (Minolta Camera, Osaka, Japan). Following the record of individual L^*, a^* and b^* parameters, colour was expressed as chroma $[(a^2+b^2)^{1/2}$ index and results are the means of two determinations for each cherry along the equatorial axis.

2.5. Firmness determination

Texture was determined using a TX-XT2i Texture Analyzer (Stable Microsystems, Godalming, UK) interfaced to a personal computer. Cherry firmness was measured using a flat steel plate mounted on the machine. For each fruit, the diameter was measured and then a force that achieved a 2% deformation of the fruit diameter was applied. Results were expressed as the ratio between the force that achieved the 2% deformation of the fruit and the fruit diameter (N mm^{-1}) multiplied by 100. A bevelled holder prevented bruising of the opposite side.

2.6. Total soluble solids content and acidity determination

Total soluble solids concentration (TSS) was determined in the juice from five fruits from each tray with a digital refractometer Atago PR-101 (Atago, Japan) at 20 °C and results expressed as the mean±S.E. of °Brix. The pH of the juice was recorded and then titratable acidity (TA) was determined by potentiometric titration with 0.1 N NaOH up to pH 8.1, using 1 ml of diluted juice in 25 ml distilled H_2O and results were the mean±S.E. expressed as g of malic acid equivalent per 100 g^{-1} fresh weight.

2.7. Stem quality evaluation

The stems of the cherry were rated for colour using a 5-point index based on percentage of the surface being brown: 5=0%, 4=1–25%, 3=25–50%, 2=50–75% and 1=75–100%.

2.8. Microbiological analysis

Five packages from each treatment were sampled at the end of the experiment to obtain under sterilised conditions (laminar fume cupboard, gloves and scalpels) samples (free of stones) of 10 g, which were homogenized in 90 ml of sterile peptone water using a stomacher (Model Seward, Laboratory Blender Stomacher 400, London, UK). Serial dilutions with the same diluent were carried out and the media used were plate count agar for mesophilic aerobic and for mould and yeast counts (Petrifilm™ Aerobic Count Plate, Laboratoires 3M™ Santé, France). Samples were prepared in triplicate and only counts of 30 to 300 colony forming units (CFU) were considered. The same procedure was carried out in recently harvested cherries (day 0). All plates were incubated for 3 days at 30 °C.

2.9. Statistical analysis

Data for the physical, chemical and sensory parameters were subjected to analysis of variance (ANOVA). Sources of variation were time of storage and treatments. Mean comparisons were performed using HSD the Tukey's test to examine if differences between treatments and storage time were significant at $P<0.05$. All analyses were performed with SPSS software package v. 11.0 for windows.

3. Results

3.1. Gas composition inside packages

Cherries packed with N-OPP film modified passively the internal atmosphere with reductions in O_2 and increases in

CO_2. The steady-state atmosphere was reached after 9 days of cold storage with levels of 2–3% for CO_2 and 11–12% for O_2, without significant differences among treatments, with the exception of eucalyptol, for which the atmosphere modification was significantly higher, reaching levels of ca. 7% and 3.5%, for O_2 and CO_2, respectively (Fig. 1A and C). When cherries were transferred to 20 °C, a similar behaviour was found. For all treatments, the CO_2 concentration inside the packages increased and the O_2 decreased, this modification being significantly higher when eucalyptol was added (Fig. 1B and D).

3.2. Weight loss

Weight loss was very low under MAP conditions (below 3% and 3.5% for cold storage and further SL, respectively). However, these weight losses were affected by the addition of natural antifungals (Fig. 2). Thus, the highest weight losses in cherry after 16 days of cold storage plus SL were observed when eucalyptol was added (3.47±0.14%), whereas significant reductions were obtained after the addition of eugenol (0.68±0.10%), menthol (1.28±0.06%) or thymol (1.29±0.17%) compared to controls (1.76±0.05%).

3.3. Colour changes

Chroma index value at harvest was 39.67±1.23 and did not significantly change during the first 13 days of cold storage for eugenol treatment, and then slightly decreased until the end of the experiment. For those cherries treated with thymol or menthol, the decrease in chroma index started after 9 days of cold storage, and after 6 days for control cherry and those treated with eucalyptol, in which the highest reduction was found (Fig. 3A). The same behaviour was observed when packages were transferred at 20 °C, although these changes were accelerated, especially when eucalyptol was added. However, the addition of eugenol, thymol or menthol led to less reduction in chroma index, as compared to control cherries (Fig. 3B).

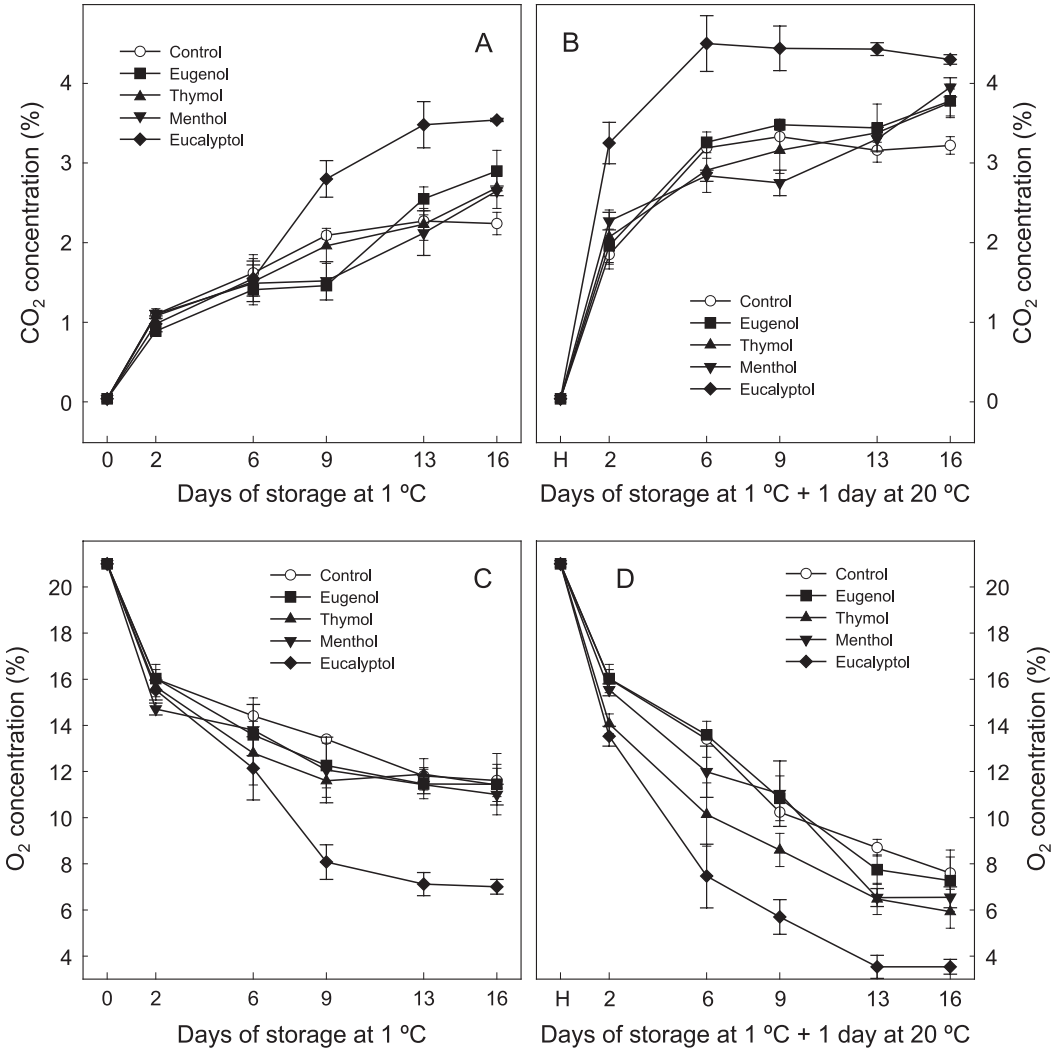

Fig. 1. Gas evolution inside the packages, CO_2 and O_2 during cold storage (A and C, respectively) and subsequent SL at 20 °C (B and D, respectively). Data are the mean±S.E. of determinations made in five bags. H corresponds to value at harvest.

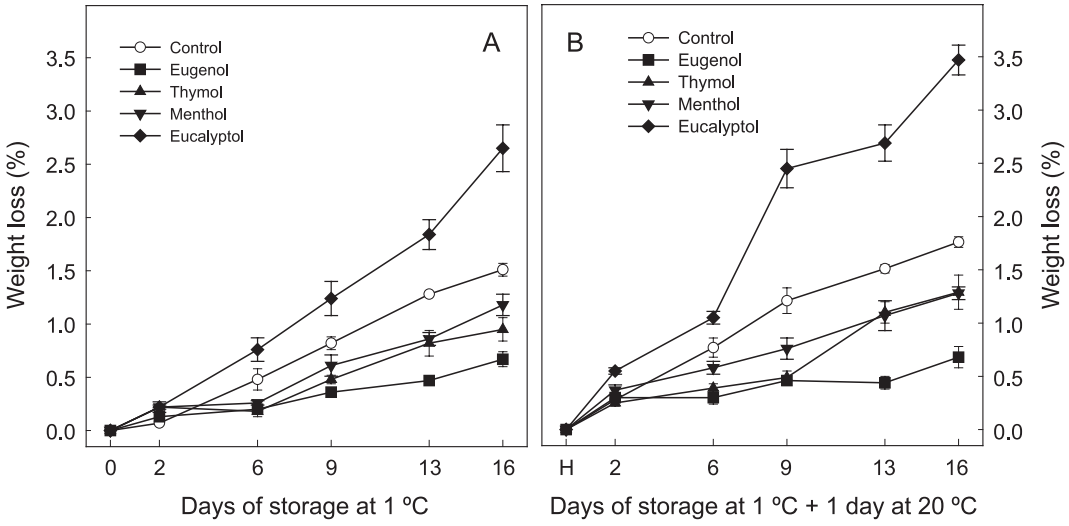

Fig. 2. Weight loss evolution during cold storage (A) and subsequent SL (B). Data are the mean±S.E. of determinations made in five bags. H corresponds to value at harvest.

3.4. Fruit firmness

Firmness at harvest was 1.48±0.05 N mm^{-1} and during cold storage, a reduction of this parameter was observed, with the exception of those treated with eugenol, in which cherry firmness remained unchanged. Moreover, the firmness diminution was significantly lower in those treated with thymol or menthol than in controls. The greatest reduction of cherry firmness was obtained for those cherries treated with eucalyptol (Fig. 4A). Following the transfer of packages at 20 °C, a higher decrease of firmness for all fruit was obtained, although significant differences depending on treatment could be observed (Fig. 4B). Thus, final values of cherry firmness from high to low were: eugenol (1.40±0.05 N mm^{-1}), thymol (1.24±0.03 N mm^{-1}), menthol (1.12±0.04 N mm^{-1}), control (0.93±0.05 N mm^{-1}) and eucalyptol (0.71±0.03 N mm^{-1}).

3.5. TSS, TA and pH

At harvest, the levels of TSS were 16.57±0.57 °Brix, and no significant modifications were observed during cold storage or further SL at 20 °C irrespective of treatments. TA (0.91±0.01 g malic acid equivalent 100 g^{-1}) and pH (3.70±0.04) at harvest evolved differentially depending on treatments. Thus, control cherries showed significant decreases and increases along the storage period and subsequent SL, reaching levels of 0.44±0.01 g malic acid equivalent 100 g^{-1} and 4.50±0.05, for TA and pH, respectively, at the end of the experiment. In cherries treated

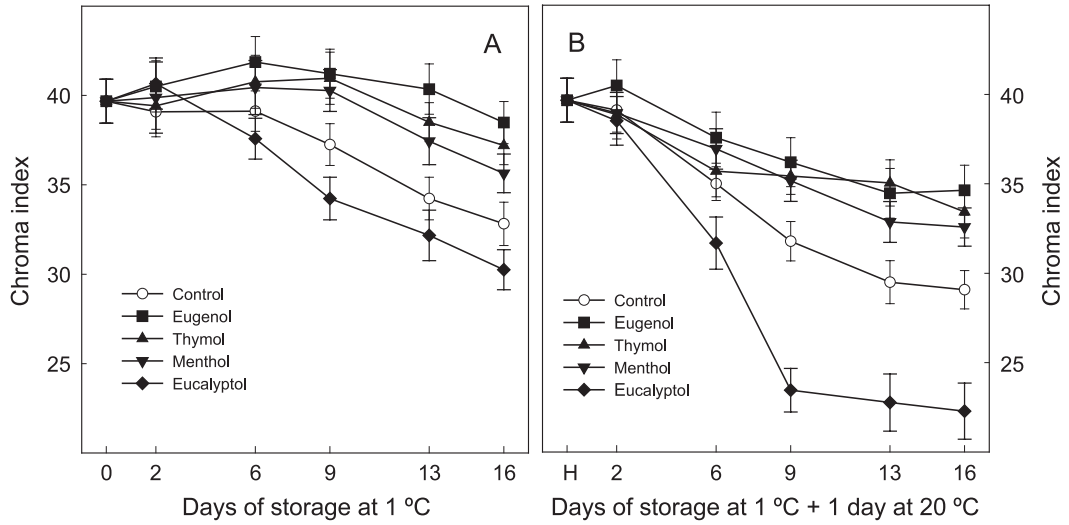

Fig. 3. Chroma index evolution during cold storage (A) and subsequent SL (B). Data are the mean±S.E. of determinations made in five samples of 20 cherries. H corresponds to value at harvest.

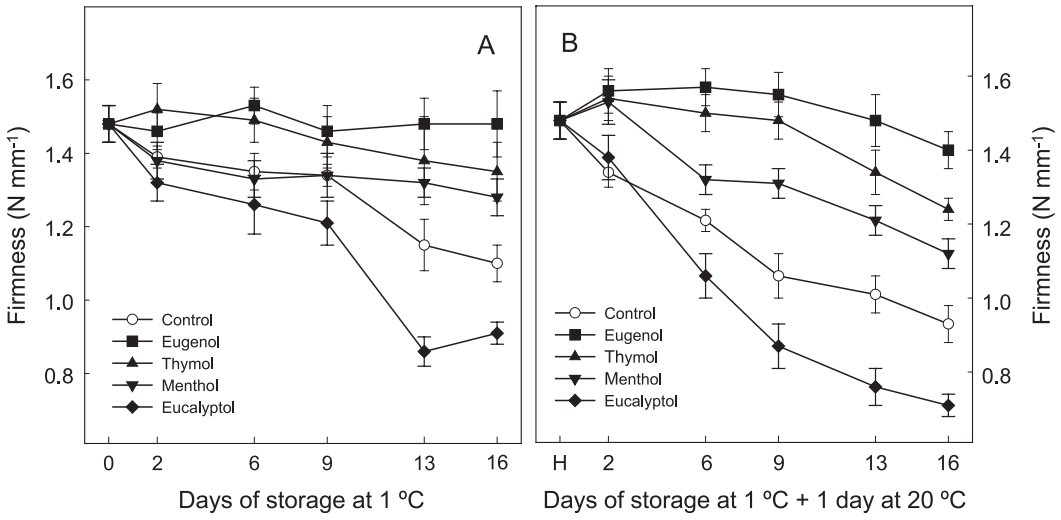

Fig. 4. Fruit firmness evolution during cold storage (A) and subsequent SL (B). Data are the mean±S.E. of determinations made in five samples of 20 cherries. H corresponds to value at harvest.

with eugenol, menthol or thymol, lower variations than in control were obtained (ranging from 0.57–0.67 and 4.10–4.27 for TA and pH, respectively) and with no significant differences among them. Conversely, in cherries treated with eucalyptol the highest modifications of both parameters were detected, with final values of 0.36±0.09 g malic acid equivalent 100 g^{-1} and 5.85±0.12, for TA and pH, respectively.

3.6. Stem quality

Cherries were evaluated after cold storage and subsequent SL to check stem browning and dehydration. Stem browning typically developed during cherry storage, but was differentially affected by treatments (Fig. 5). Thus, the incidence of brown stem was negligible when eugenol was added to packages with final scores of 4.07±0.12 at the end of the experiment. Packages with thymol or menthol received also significantly higher scores (3.67±0.14 and 3.37±0.14, respectively) than control cherries (2.32±0.12). On the contrary, the occurrence of stem browning was extremely high when eucalyptol was added to packages, and received the lower scores (1.00±0.10).

3.7. Microbial analyses

At harvest, cherry fruit had 4.2 and 2.1 log CFU g^{-1} for total mesophilic aerobic and mould and yeast counts, respectively. Following 16 days of cold storage plus SL,

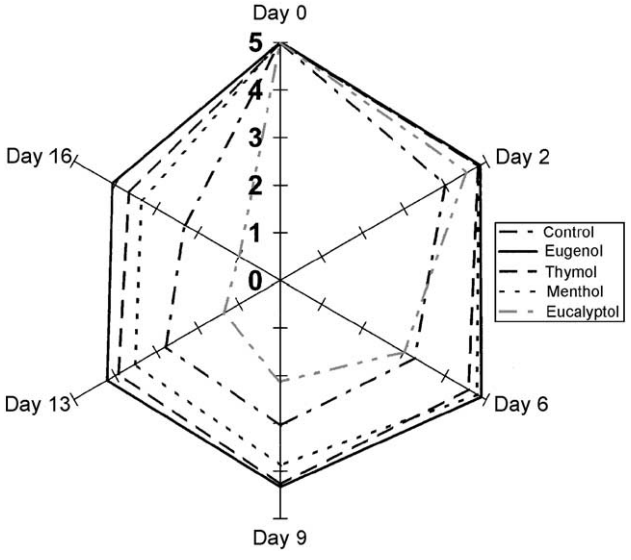

Fig. 5. Stem quality evolution after cold storage and subsequent SL. Data are the mean±S.E. of determinations made in five samples of 20 cherries.

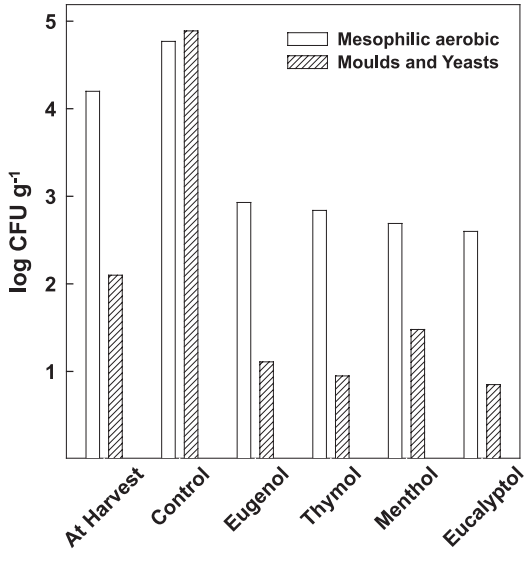

Fig. 6. Mesophilic aerobic and mould and yeast counts, at harvest and after 16 days of cold storage +1 day at 20 °C, for control and treated cherries. Data are the mean±S.E. of determinations made in five samples.

in all packages that contained eugenol, thymol, menthol or eucalyptol the microbial populations were drastically reduced, the reduction being more effective for mould and yeast counts (below 1.5 log CFU g^{-1}). On the contrary, increases in microbial populations were observed for control cherries (Fig. 6), especially for mould and yeast counts, which were 4.9 log CFU g^{-1}.

4. Discussion

Continuing efforts in processing, preservation, distribution and marketing are being made worldwide to supply fresh fruits of high quality to consumers. Thereafter, consumers demand safe products avoiding the use of chemicals as a mean of preservation, but unfortunately increasing incidence of human illnesses from pathogenic microorganisms is being occurred as availability of fresh foods is increasing (Beuchat, 1998). In this work, and as alternative of synthetic fungicides, an active packaging to improve MAP effectiveness on preserving cherry fruit quality and safety was developed by the addition of pure essential oils (eugenol, thymol, menthol or eucalyptol) inside the packages. Results revealed that the addition of eugenol, thymol or menthol improved the beneficial effect of MAP in terms of delaying weight loss, softening, colour changes and stem deterioration, and in turn maintenance of cherry quality for longer storage periods was achieved. On the contrary, packages with eucalyptol gave worse results compared to MAP control.

The steady-state atmosphere inside the packages did not change by the addition of the essential oils eugenol, thymol or menthol. No references are available for comparative purposes, but from the results it could be inferred that maintenance of cherry quality was not due to modification of the internal atmosphere but rather by the beneficial effects of these natural compounds themselves. The final atmospheric composition could be considered as optimum for 'StarKing' cherry quality preservation, as has been reported for other cherry cultivars (Meheriuk et al., 1997; Spotts et al., 2002), although a wide range of atmosphere compositions have been recommended depending on cultivar and ripening stage at harvest.

Loss of weight is one of the most important causes responsible for cherry quality deterioration, which increases the fruit susceptibility to fungal decay. In fact, weight loss in cherries is higher than in other commodities due to their low skin diffusion resistance (Crisosto, 1992). Weight loss in MAP packages was very low (<2%), mainly due to the effect of polypropylene film on increasing water vapour pressure. Weight losses were significantly reduced with the addition of eugenol, thymol or menthol. In addition, the high relative humidity generated inside the MAP packages might be responsible for the delay in fruit softening as compared to cherries stored in air (Kappel et al., 2002; Meheriuk et al., 1997). Softening process in cherries has been reported to be dependent on the increase in polygalacturonase, β-galactoxidase and pectinmethylesterase activities (Barret & González, 1994; Batisse et al., 1996; Remón et al., 2003). An effect of high CO_2 and low O_2 concentrations on reducing the activities of cell-wall degrading enzymes has been also proposed in other fruits (Femenia, Sánchez, Simal, & Roselló, 1998; Salunkhe, Boun, & Reddy, 1991). Moreover, when eugenol, thymol or menthol were added to packages, fruit firmness remained significantly higher than in control MAP, especially when cherries were transferred at 20 °C, showing that these natural compounds somehow could reduce the action of cell-wall degrading enzymes. These results show beneficial effects of these essential oils on increasing the cherry SL, since it has been postulated that fruit softening and texture changes during cherry storage determine fruit storability and shelf life, as well as reduced incidence of decay and less susceptibility to mechanical damage (Batisse et al., 1996; Vidrih, Zavrtanik, & Hribar, 1998). However, the mechanism by which these essential oils led to a reduction in weight losses and delay of the softening process is still unknown.

It is widely accepted that the most important parameters determining cherry acceptability by consumers are colour, for both fruit and stem, and ratio between TSS and TA (Crisosto et al., 2003). Consumers demand cherry fruit with a bright red colour and green stems. In this work, evaluation of colour based on chroma index evolution was a good indicator for cherry skin darkening during postharvest storage, since a reduction of chroma index was obtained. However, when eugenol, thymol or menthol were added to packages, a net delay in skin colour changes was observed compared to control cherries. It has been reported that chroma is an optimum indicator for the colour changes during cherry ripening and hence is correlated to the anthocyanin content (Mozetiĉ, Trebše, Simĉiĉ, & Hribar, 2004). Nevertheless, discrepancies exist on the evolution of anthocyanins during postharvest cherry storage, since either increases or decreases have been reported in different cultivars (Bernalte et al., 2003; Esti et al., 2002; Mozetiĉ et al., 2004). The beneficial effects of eugenol, menthol or thymol was also evident in delaying the stem browning, since these cherries received higher scores than controls from a semi-trained panel. There are no evidences of the role of these natural compounds on this issue, but the well-known antioxidant activity reported for these essential oils might probably reduce dehydration, chlorophyll degradation and occurrence of browned polymers responsible for stem browning and shrivel (Drake, Kupferman, & Fellman, 1988). Other quality attributes, such as TSS did not change along storage irrespective of treatments. Accordingly, previous reports have shown that in cherry, the use of MAP has a slight or no effect on the evolution of these chemical parameters (Neven & Drake, 2000; Remón et al., 2000, 2003; Tian, Jiang, Xu and Wang, 2004; Tian, Xu, Jiang, & Gong, 2002). However, treatments were effective

on retarding the TA loss or the increasing in pH observed for control cherries.

All the beneficial effects above mentioned for eugenol, thymol or menthol were not observed when eucalyptol was added inside the packages. Quality parameters were even worst when compared to control cherries. Eucalyptol led to the highest CO_2 and lowest O_2 concentrations, probably due to an increase of the oxidative metabolism. This was confirmed by the greater weight losses (Fig. 2), the accelerated colour changes detected by the sharp decrease in chroma index (Fig. 3), and the extremely high occurrence of stem browning detected just after 6 days of storage (Fig. 5). Additionally, packages treated with eucalyptol experienced a great reduction in TA and the highest increase in the final pH of the juices. Since all the essential oils were applied at same dose, that is 1 ml inside the packages, it could be inferred that eucalyptol induced a certain oxidation process, with occurrence of phytotoxicity, and acceleration of the physico-chemical and physiological changes related to cherry ripening and senescence.

Finally, all the essential oils were effective in reducing the microorganism proliferation, the effect being higher for moulds and yeasts than for mesophillic aerobics. The antimicrobial effects of these natural compounds are well established and their mechanism of action has been related to damages in membrane integrity (Bagamboula, Uyttendaele, & Debevere, 2004; Lambert, Skandamis, Coote, & Nychas, 2001). Indeed, their potential use in food products has been recently reviewed (Burt, 2004), since essential oils were effective in reducing food-spoiling microorganisms, food-borne pathogens, spoilage and mycotoxigenic fungi, pathogenic and dimorphic yeasts (Dorman & Deans, 2000). The essential oils reported in this work led to total viable counts to <2 log CFU g^{-1} for moulds and yeasts and to <3 log CFU g^{-1} for mesophillic aerobics, which meet the recommended microbiological criteria for non-heated fruit desserts (IFST, 1999), while counts near 5 log CFU g^{-1} were observed for control cherries. In addition, it has been reported that MAP could act synergistically with essential oils by inhibiting the microbial growth in fish and beef (Skandamis & Nychas, 2001). However, to our knowledge this is the first time that thymol, menthol or especially eugenol improved the beneficial effect of MAP in preserving cherry quality during storage.

References

Appendini, P. & Hotchkiss, J. H. (2002). Review of antimicrobial food packaging. *Innovative Food Science & Emerging Technologies*, *3*, 113–126.

Bagamboula, C. F., Uyttendaele, M., & Debevere, J. (2004). Inhibitory effect of thyme and basil essential oils, carvacrol, thymol, estragol, linalool and *p*-cymene towards *Shigella sonnei* and *S. flexneri*. *Food Microbiology*, *21*, 33–42.

Barret, D. M. & González, C. (1994). Activity of softening enzymes during cherry maturation. *Journal of Food Science*, *59*, 574–577.

Batisse, C., Buret, M., & Coulomb, P. J. (1996). Biochemical differences in cell wall of cherry fruit between soft and crisp fruit. *Journal of Agricultural and Food Chemistry*, *44*, 453–457.

Bernalte, M. J., Hernández, M. T., Vidal-Aragón, M. C., & Sabio, E. (1999). Physical, chemical, flavor and sensory characteristics of two sweet cherry varieties grown in "Valle del Jerte" (Spain). *Journal of Food Quality*, *22*, 403–416.

Bernalte, M. J., Sabio, E., Hernández, M. T., & Gervasini, C. (2003). Influence of storage delay on quality of "Van" sweet cherry. *Postharvest Biology and Technology*, *28*, 303–312.

Beuchat, L. R. (1998). Surface decontamination of fruits and vegetables eaten raw: A review. *WHO/FSF/FOS/98.2*. Geneva: World Health Organization.

Burt, S. (2004). Essential oils: their antibacterial properties and potential applications in foods—a review. *International Journal of Food Microbiology*, *94*, 223–253.

Chu, C. L., Liu, W. T., Zhou, T., & Tsao, R. (1999). Control of postharvest grey mold rot of modified atmosphere packaged sweet cherries by fumigation with thymol and acetic acid. *Canadian Journal of Plant Science*, *79*, 685–689.

Cowan, M. M. (1999). Plant products as antimicrobial agents. *Clinical Microbiology Reviews*, *12*, 564–582.

Crisosto, C. H. (1992). Sweet cherry harvest, post-harvest handling and storage. *WSU Tree Fruit Postharvest Journal*, *3*, 3–6.

Crisosto, C. H., Crisosto, G. M., & Metheney, P. (2003). Consumer acceptance of "Brooks" and "Bing" cherries is mainly dependent on fruit SSC and visual skin color. *Postharvest Biology and Technology*, *28*, 159–167.

Dorman, H. J. D. & Deans, S. G. (2000). Antimicrobial agents from plants: antibacterial activity of plant volatile oils. *Journal of Applied Biology*, *88*, 308–316.

Drake, S. R. & Elfving, D. C. (2002). Indicators of maturity and storage quality of "Lapins" sweet cherry. *HortTechnology*, *12*, 687–690.

Drake, S. R., Kupferman, E. M., & Fellman, J. K. (1988). "Bing" sweet cherry (*Prunus avium* L.) quality as influenced by wax coatings and storage temperatures. *Journal of Food Science*, *53*, 124–126.

Esti, M., Cinquante, L., Sinesio, F., Moneta, E., & Di Matteo, M. (2002). Physicochemical and sensory fruit characteristic of two sweet cherry cultivars after cool storage. *Food Chemistry*, *76*, 399–405.

Femenia, A., Sánchez, E. S., Simal, S., & Roselló, C. (1998). Modification of cell wall composition of apricot (*Prunus armeniaca*) during drying and storage under modified atmospheres. *Journal of Agricultural and Food Chemistry*, *46*, 5253–5428.

Gao, L. & Mazza, G. (1995). Characterization, quantification, and distribution of anthocyanins and colorless phenolic in sweet cherries. *Journal of Agricultural and Food Chemistry*, *43*, 343–346.

IFST (1999). Microbiological criteria for food. London: Institute of Food Science and Technology.

Kappel, F., Toivonen, P., McKenzie, D. L., & Stan, S. (2002). Storage characteristics of new sweet cherry cultivars. *HortScience*, *37*, 139–143.

Kupferman, E. & Sanderson, P. (2001, July). Temperature management and modified atmosphere packing to preserve sweet cherry quality. *Postharvest Information Network*, , 1–9.

Lambert, R. J. W., Skandamis, P. N., Coote, P. J., & Nychas, G. J. E. (2001). A study of the minimum inhibitory concentration and mode of action of oregano essential oil, thymol and carvacrol. *Journal of Applied Microbiology*, *91*, 453–462.

Lanciotti, R., Gianotti, A., Patrignani, F., Belleti, N., Guerzoni, M. E., & Gardini, F. (2004). Use of natural aroma compounds to improve shelf life and safety of minimally processed fruits. *Trends in Food Science and Technology*, *15*, 201–208.

MAPYA. (2003). Statistics from the Spanish Ministry of Food, Agriculture and Fisheries (www.mapya.es).

Meheriuk, M., McKenzie, B., Girard, B., Molys, A. L., Weintraub, S., Hocking, R., et al. (1997). Storage of "Sweetheart" cherries in sealed plastic film. *Journal of Food Quality*, *20*, 189–198.

Mozetič, B., Trebše, P., Simčič, M., & Hribar, J. (2004). Changes of anthocyanins and hydroxycinnamic acids affecting the skin colour during maturation of sweet cherries (*Prunus avium* L.). *Lebensmittel-Untersuchung und-Technology*, *37*, 123–128.

Neven, L. G. & Drake, S. R. (2000). Comparison of alternative postharvest quarantine treatments for sweet cherries. *Postharvest Biology and Technology*, *20*, 107–114.

Nielsen, P. V. & Ríos, R. (2000). Inhibition of fungal growth on bread by volatile components from spices and herbs, and the possible application in active packaging, with special emphasis on mustard essential oil. *International Journal of Food Microbiology*, *60*, 219–229.

Quintavalla, S. & Vicini, L. (2002). Antimicrobial food packaging in meat industry. *Meat Science*, *62*, 373–380.

Remón, S., Ferrer, A., Maquina, P., Burgos, J., & Oria, R. (2000). Use of modified atmospheres to prolong the postharvest life of Burlat cherries at two different degrees of ripeness. *Journal Asian of Food and Agriculture*, *80*, 1545–1552.

Remón, S., Venturini, M. E., López-Buesa, P., & Oria, R. (2003). Burlat cherry quality after long range transport: optimisation of packaging conditions. *Innovative Food Science & Emerging Technologies*, *4*, 425–434.

Salunkhe, D. K., Boun, H. R., & Reddy, N. R. (1991). *Storage Processing and Nutritional Quality of Fruits and Vegetables*. *Fresh Fruits and Vegetables*, *vol. 1*. Boston: CRC Press.

Skandamis, P. N. & Nychas, G. J. E. (2001). Effect of oregano essential oil on microbiological and physico-chemical attributes of minced meat stored in air and modified atmospheres. *Journal of Applied Microbiology*, *91*, 1011–1022.

Spotts, R. A., Cervantes, L. A., & Facteau, T. J. (2002). Integrated control of brown rot of sweet cherry fruit with a preharvest fungicide, a postharvest yeast, modified atmosphere packaging, and cold storage temperature. *Postharvest Biology and Technology*, *24*, 251–257.

Tian, S. P., Fan, Q., Xu, Y., Wang, Y. S., & Jiang, A. L. (2001). Evaluation of the use of high CO_2 concentrations and cold storage to control *Monilia fruticola* on sweet cherries. *Postharvest Biology and Technology*, *21*, 53–60.

Tian, S. P., Jiang, A. L., Xu, Y., & Wang, Y. S. (2004). Responses of physiology and quality of sweet cherry fruit to different atmospheres in storage. *Food Chemistry*, *87*, 43–49.

Tian, S. P., Xu, Y., Jiang, A. L., & Gong, Q. Q. (2002). Physiological and quality responses of longan fruits to high-O_2 or high-CO_2 atmospheres in storage. *Postharvest Biology and Technology*, *24*, 335–340.

Vázquez, B. I., Fente, C., Franco, C. M., Vázquez, M. J., & Cepeda, A. (2001). Inhibitory effect of eugenol and thymol on *Penicillium citrinum* strains in culture media and cheese. *International Journal of Food Microbiology*, *67*, 157–163.

Venturini, M. E., Oria, R., & Blanco, D. (2002). Microflora of two varieties of sweet cherries: Burlat and Sweetheart. *Food Microbiology*, *19*, 15–21.

Vidrih, R., Zavrtanik, M., & Hribar, J. (1998). Effect of low O_2, high CO_2 or added acetaldehyde and ethanol on postharvest physiology of cherries. *Acta Horticulturae*, *2*, 693–695.