

## Improvement of Table Grapes Quality and Safety by the Combination of Modified Atmosphere Packaging (MAP) and Eugenol, Menthol, or Thymol

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Table grape is a nonclimacteric fruit that shows a rapid loss of quality during storage and is very susceptible to colonization by fungi, especially *Botrytis cinerea*, which is considered the most important disease of this commodity. To solve this problem, synthetic fungicides have been used, although legal restrictions and consumer's concern demand the search of other safe means. In the present paper, and as an alternative of synthetic fungicides, an active packaging to improve MAP effectiveness on preserving table grape (cv. Crimson Seedless) quality and safety was developed by the addition of 0.5 mL of eugenol, thymol, or menthol inside the packages. Packages were stored at 1 °C for 35 days. The final gas composition inside the packages was 1.4–2.0 and 10.0–14.5 kPa of CO<sub>2</sub> and O<sub>2</sub>, respectively, with no significant slight differences among treatments. Results showed that the addition of eugenol, thymol, or menthol improved the beneficial effect of MAP in terms of delaying weight loss and color changes, retarding °Brix/acidity ratio evolution, and maintaining of firmness. Thereafter, these treatments showed additional benefit in terms of delayed rates of rachis deterioration and berry decay. Finally, the total viable counts for both mesophilic aerobics and especially yeast and molds were significantly reduced in the grapes packaged with the natural antimicrobial compounds. All of the above effects led to maintenance of table grape quality and safety for longer storage periods (3 additional weeks as compared to controls under MAP only).

**KEYWORDS:** Thymol; menthol; eugenol; active packaging; ethylene; mesophilic aerobics; yeast; molds

### INTRODUCTION

Table grape (*Vitis vinifera* L.) is a nonclimacteric fruit that shows severe problems during postharvest handling, storage, and marketing. As with other fruits, the losses of quality are based on weight loss, color changes, and accelerated softening. Additionally, the postharvest quality deterioration in table grapes is also attributed to rachis browning and high incidence of berry decay (1, 2). All of the above detrimental effects lead to quality losses and high occurrence of decay when storage is prolonged (3).

In table grape, gray mold caused by *Botrytis cinerea* is considered the most important disease, and the uncontrolled infections result in the development of aerial mycelium spreading rapidly to adjacent berries with severe economical repercussions. To solve this problem, several methods have been used. The most common synthetic fungicide is sulfur dioxide (SO<sub>2</sub>), which is highly effective in killing both spores and mycelia, but the necessary concentrations may induce injuries in both

rachis and berry, manifested as bleaching of color, accelerated water loss, and browning (4, 5). In addition, sulfite residue is another important problem associated with SO<sub>2</sub> fumigations. In fact, the Food and Drug Administration (FDA) has established the maximum tolerance to sulfite residues at 10 μL L<sup>-1</sup>, while the European Union has forbidden the use of SO<sub>2</sub> (EU Directive 95/2/CE). Other synthetic fungicides, anilopyridines, were found to be also effective against *B. cinerea*, although populations resistant to these chemicals have been reported recently (6). However, the associations of pesticide usage with the development of fungicide-resistant strains and the public's concern for the human health conditions and the environmental pollution have stimulated the search for new strategies as alternative means for controlling postharvest decay.

Thus, to avoid the use of chemicals, the storage with high CO<sub>2</sub> (15–25%) under controlled atmospheres was effective in reducing decay of "Thompson" and "Redglobe" grapes, although occurrence of injuries (rachis browning and off-flavors) existed, these symptoms being higher in early- than in late-cultivars (2, 7). Thereafter, hypobaric treatments, heated ethanol, and the use of natural occurring microorganisms (biocontrol) have been reported to reduce the incidence of gray mold in table grapes

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(8–10). Finally, the use of modified atmosphere packaging (MAP) has been shown to maintain berry quality (11) and to reduce decay alone or in combination with acetic acid (12), chlorine gas (13), or SO<sub>2</sub>-commercial generator (14).

Finally, among these tools, there is an increasing interest for the use of natural antimicrobial compounds in foods. The antimicrobial properties of essential oils derived from many plant organs have been empirically recognized for centuries, although scientific confirmation has been reviewed recently (15). Among these natural compounds, the antifungal activity of several essential oils belonging to genus *Thymus*, *Syzygium*, and *Mentha* is documented. The potential use of essential oils as natural preservatives has been reported in cheese (16), bakery products (17), and meat (18). In fruits, there is little evidence for the use of these compounds during postharvest storage to avoid fruit spoilage. Thus, several essential oils reduced the viable counts in fresh-cut kiwifruit and melon inside sealed jars (19), as well as in sweet cherry (20). The European Commission has registered a number of essential oils and their components to be applied in foodstuffs, in which eugenol, menthol, and thymol are included (CD, 23 January 2002, CE). These compounds appear also in EAFUS (Everything Added to Food in the US) and GRAS (Generally Recognized as Safe) lists.

The objective of this paper was to study the effects of the combination of MAP with eugenol, menthol, or thymol on maintenance of table grape quality parameters and the reduction of microbial spoilage during 35 days of cold storage. As far as we know from the literature, this is the first paper dealing on this issue in table grape, which would be interesting for commercial application.

## MATERIALS AND METHODS

**Plant Material and Experimental Design.** Table grapes (*Vitis vinifera* L. cv. "Crimson Seedless") were harvested from a commercial farm in Abarán (Murcia, Spain). In the laboratory, clusters were selected to obtain homogeneous batches based on color, size, absence of injuries, and healthy greenish rachises. Clusters were cut to obtain samples in the range of 150–170 g and packed in 20  $\mu$ m thickness nonperforated oriented polypropylene (N-OPP) bags (30  $\times$  20 cm), which had permeabilities at 1 °C of 1600 mL O<sub>2</sub> m<sup>-2</sup> d<sup>-1</sup> atm<sup>-1</sup> and 3600 mL CO<sub>2</sub> m<sup>-2</sup> d<sup>-1</sup> atm<sup>-1</sup>. This film was selected on the basis of previous experiments in table grapes (11). Treatments with eugenol, thymol, or menthol (99.5% purity and purchased from Sigma, Sigma-Aldrich, Madrid, Spain) were performed by placing 0.5 mL of these natural compounds on a sterile gauze inside the bag, avoiding contact with the berries, and the bags were immediately sealed to minimize vaporization. Clusters packed in the same conditions but without antimicrobial compounds served as control. All packages were stored at 1 °C and with RH of 90% in darkness, and weekly 5 bags for each treatment were sampled, in which the following analytical determinations were performed.

**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<sub>2</sub> and O<sub>2</sub> 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 Inc., 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 kPa O<sub>2</sub> and CO<sub>2</sub> inside the bags and were the mean  $\pm$  SE of two determinations for each of the five replicates ( $n = 10$ ). Another milliliter of the same atmosphere was used to quantify ethylene concentration using a Hewlett-Packard model 5890A gas chromatograph (Wilmington, DE) equipped with a flame ionization detector and a 3-m stainless steel column with an inner diameter of 3.5 mm containing activated alumina of 80/100 mesh. The

column temperature was 90 °C, and injector and detector temperatures were 150 °C. Results were the mean  $\pm$  SE of two determinations for each of the five replicates ( $n = 10$ ) and were expressed as  $\mu$ L L<sup>-1</sup> of ethylene inside the bags.

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

**Color.** Color was determined using the Hunter Lab System and a Minolta colorimeter CR200 model (Minolta Camera Co., Osaka, Japan). Following the recording of individual  $L^*$ ,  $a^*$ , and  $b^*$  parameters, color was expressed as  $L^*$  parameter and Hue angle, and results were the mean  $\pm$  SE of determinations made on 10 berries for each bag along the equatorial axis ( $n = 50$ ).

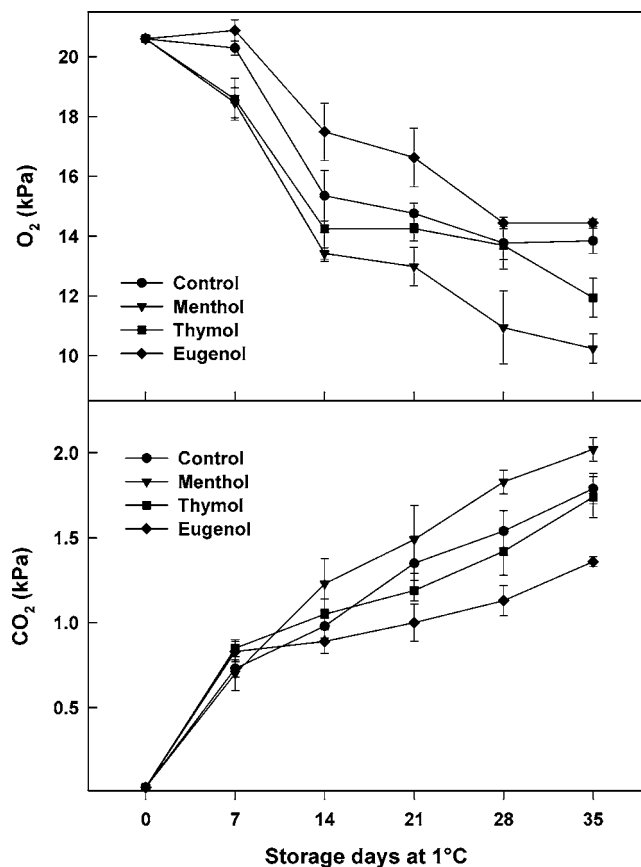
**Firmness Determination.** Texture, berry, and flesh firmness were determined using a TX-Xt2i Texture Analyzer (Stable Microsystems, Godalming, UK) interfaced to a personal computer, as previously reported (11). Berry firmness was measured using a flat steel plate mounted on the machine. For each berry, the diameter was measured, and then a force that achieved a 2% deformation of the berry diameter was applied. Results were expressed as the ratio between this force and the covered distance (N mm<sup>-1</sup>) and were the mean  $\pm$  SE of determinations made in 10 berries for each bag ( $n = 50$ ). For flesh firmness determination, 1 cm<sup>2</sup> of the skin was removed and penetration force measurement was individually recorded using a 2 mm diameter probe coupled on the same Texture Analyzer. The penetration rate was 20 mm min<sup>-1</sup> for 10 mm after contacting the flesh, and results were the mean  $\pm$  SE of determinations made on 10 berries for each bag ( $n = 50$ ) and expressed in N. In both cases, a beveled holder prevented bruising of the opposite side.

**Maturity Index Determination.** Maturity index was expressed as the ratio between total soluble solids concentration (TSS) and titratable acidity (TA), according to a previous report (11). TSS was determined in triplicate from the juice obtained from 10 berries for each bag with a digital refractometer Atago PR-101 (Atago Co. Ltd., Japan) at 20 °C, and results were expressed as the mean  $\pm$  SE of °Brix ( $n = 15$ ). The pH of the juice was recorded, and then titratable acidity (TA) was determined in triplicate from the above juice by potentiometric titration with 0.1 N NaOH up to pH 8.1, using 1 mL of diluted juice in 25 mL of distilled H<sub>2</sub>O, and results were the mean  $\pm$  SE expressed as g of tartaric acid equivalent per 100 g<sup>-1</sup> fresh weight ( $n = 15$ ).

**Rachis Quality Evaluation and Decay Analysis.** Rachis visual aspect of treated and control clusters was evaluated by 10 trained adults, aged 25–40 years (5 female and 5 male) in a laboratory of sensory analyses with an individual booth for each panelist. For rachis, symptoms of dehydration and browning for primary and secondary branches were evaluated (2) on a ranked scale of 1–4, where 4 = absence of these symptoms, 3 = slight occurrence, 2 = moderate browning and dehydration, and 1 = severe browning and dehydration. For each treatment and sampling date, the five branches were evaluated by each judge ( $n = 50$ ). For decay analysis, the number of decayed and faded grapes for each bunch was counted, and then the decay percentage from the total berries was calculated for each cluster and sampling date.

**Microbiological Analysis.** After 35 days at 1 °C, samples of 10 g from each bag were obtained under sterilized conditions (laminar fume cupboard, gloves, and scalpels), which were homogenized in 90 mL of sterile peptone water using a stomacher (Model Seward, Laboratory Blender Stomacher 400, London, UK). Serial dilutions were carried out, and 1 mL was added to plate count agar for mesophilic aerobic and for mold and yeast counts (PetriFilm Aerobic Count Plate, Laboratories 3M Santé, France). Samples were prepared in triplicate, and only counts of 30–300 colony forming units (CFU) were considered. The same procedure was carried out in recently harvested berries (day 0). All plates were incubated for 3 days at 30 °C.

**Statistical Analysis.** Data for the physical, chemical, microbiological, 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 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.



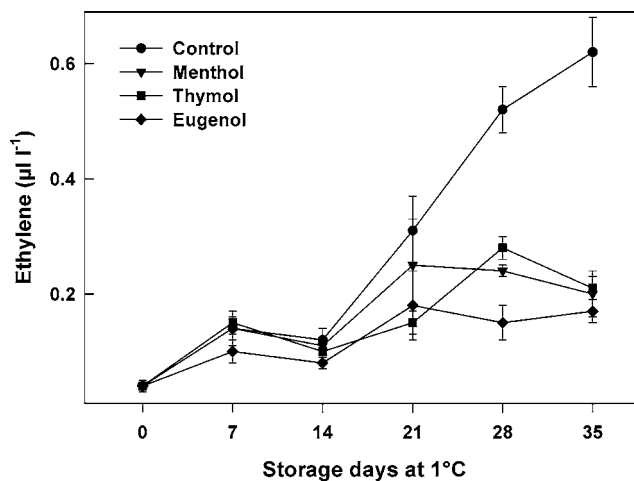
**Figure 1.** O<sub>2</sub> and CO<sub>2</sub> concentrations inside MAP-packages without (control) and with menthol, thymol, or eugenol during cold storage. Data are the mean  $\pm$  SE.

## RESULTS

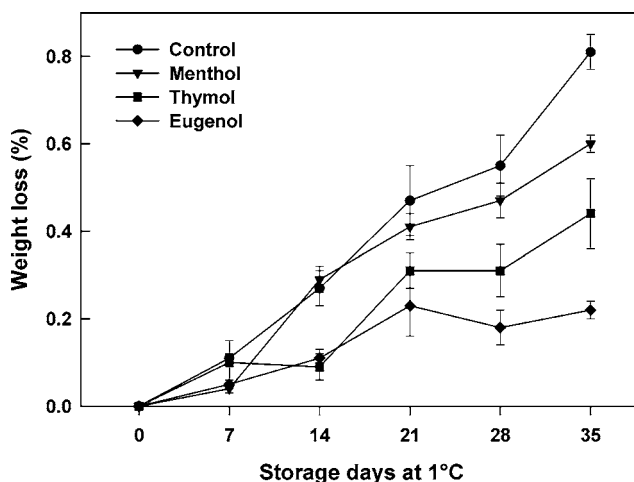
**Gas Composition Inside the Packages.** A significant accumulation of CO<sub>2</sub> and diminution of O<sub>2</sub> inside the packages was observed for all treatments over cold storage at 1 °C (Figure 1), these changes being higher during the first 2 weeks of storage. Slight differences on the final gas composition were observed between control and treated packages. Thus, clusters treated with menthol led to the highest CO<sub>2</sub> (~2 kPa) and the lowest O<sub>2</sub> (~10 kPa) concentrations inside the packages, while the contrary was detected for eugenol treatment (~1.4 and 14.5 kPa for CO<sub>2</sub> and O<sub>2</sub>, respectively). The atmosphere composition reached by control and thymol-treated clusters ranged between and was very similar to concentrations of ~1.8 kPa CO<sub>2</sub> and ~12 kPa O<sub>2</sub>. With respect to ethylene, a significant increase was obtained in control packages from day 14 of storage reaching final concentrations of  $0.62 \pm 0.06 \mu\text{L L}^{-1}$  (Figure 2). However, ethylene concentration was significantly lower than that of the controls in those bags treated with whatever antimicrobials, showing no significant differences among them over storage.

**Parameters Related to Berry Quality.** The percentage of weight loss was very low for all clusters under MAP conditions, with  $0.81 \pm 0.04\%$  for control clusters after 35 days of cold storage, these losses of weight being significantly reduced when antimicrobial compounds were added (Figure 3). Among treated clusters, significant differences were also obtained, for which eugenol treatment led to the lowest weight loss ( $0.22 \pm 0.02\%$ ).

With respect to firmness, two texture measurements were recorded: berry and flesh firmness. For both parameters, the same behavior was shown, that is, an accelerated softening



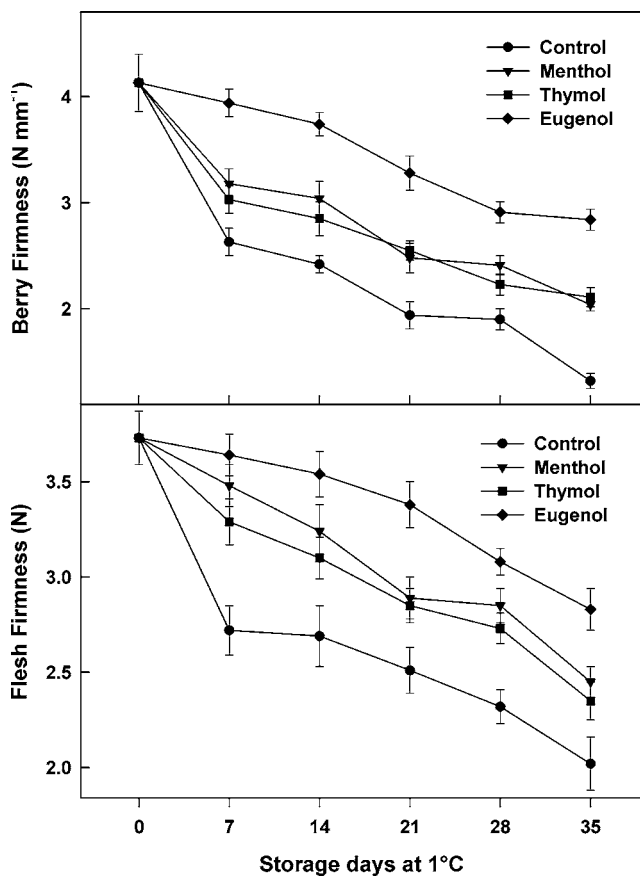
**Figure 2.** Ethylene concentration inside MAP-packages without (control) and with menthol, thymol, or eugenol during cold storage. Data are the mean  $\pm$  SE.



**Figure 3.** Weight loss of table grape clusters under MAP conditions without (control) and with menthol, thymol, or eugenol during cold storage. Data are the mean  $\pm$  SE.

process in control clusters as the storage increased, with final values of  $1.32 \pm 0.07 \text{ N mm}^{-1}$  and  $2.02 \pm 0.14 \text{ N}$  for berry and flesh firmness, respectively (Figure 4). Contrarily, the addition of the antimicrobials inside the packages significantly delayed the loss of firmness, but differentially depending on the compound incorporated. Thus, menthol and thymol behaved similarly, with the eugenol treatment being the most effective on maintaining both berry and flesh firmness, reaching final levels of  $2.84 \pm 0.10 \text{ N mm}^{-1}$  and  $2.83 \pm 0.11 \text{ N}$ , respectively.

Color attributes were drastically affected by treatments, because control clusters showed the greatest variations of color, in both Hue angle and lightness ( $L^*$ ). The level of Hue angle at harvest was  $\sim -5$ , which corresponded to a pink-light red color, and sharply increased in just 7 days of storage for all berries (Figure 5). However, Hue angle remained without significant changes throughout storage for treated berries, while in controls this color parameter showed further increases until day 14 ( $16.29 \pm 0.19$ ), which were unchanged afterward. With respect to parameter  $L^*$ , the addition of the antimicrobials inside the packages led to berries with significantly higher maintenance of lightness than controls, for which a continuous decrease in  $L^*$  values was observed as storage time advanced (Figure 5), reaching the lowest values at the end of the experiment (28.14



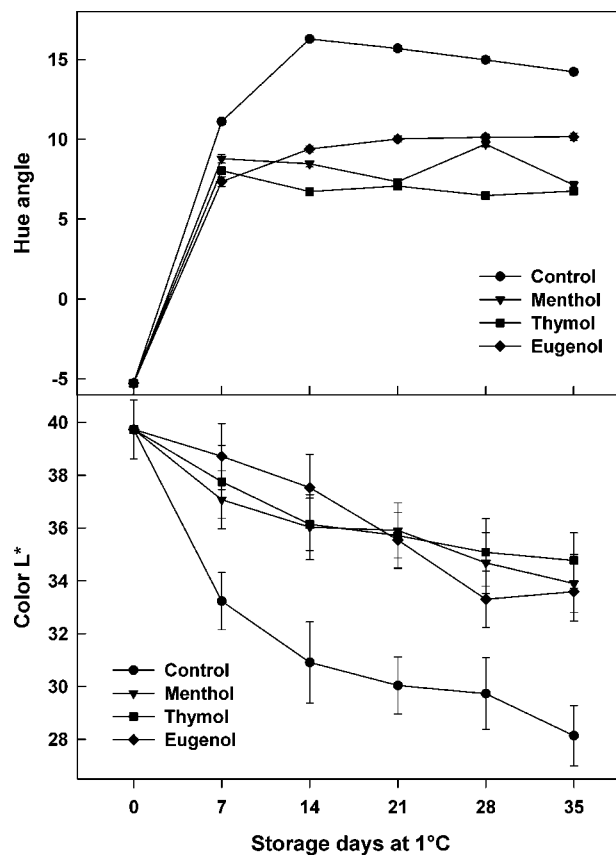
**Figure 4.** Berry and flesh firmness of table grape clusters under MAP conditions without (control) and with menthol, thymol, or eugenol during cold storage. Data are the mean  $\pm$  SE.

$\pm 1.14$ ). No significant differences were obtained among treatments with final  $L^*$  levels of  $\sim 34$ .

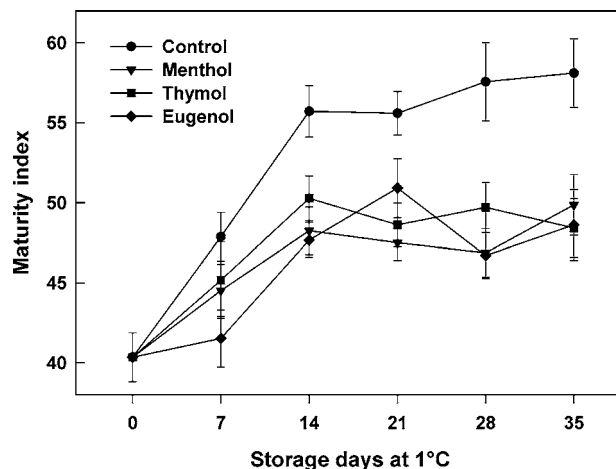
Levels of TSS and TA at harvest were  $20.59 \pm 0.10$  °Brix and  $0.52 \pm 0.02$  g  $100$  g<sup>-1</sup> tartaric acid equivalent, respectively, resulting in a maturity index (TSS/TA ratio) of  $40.35 \pm 1.53$ . TSS/TA experienced increases throughout storage, which were significantly higher in control than in treated berries (Figure 6). At the end of cold storage, control fruits showed levels of TSS/TA of  $58.10 \pm 2.15$ , while values of  $\sim 48$  were found in treated ones, showing no significant differences among treatments. The lower TSS/TA ratio in treated berries was due to both titratable acidity retention and lower accumulation of sugars (data not shown).

**Rachis Quality.** Panelists evaluated the visual aspect of the rachis and gave the lowest scores to those rachises of control clusters, which became significantly lower from day 21 of storage as compared to treated clusters (Figure 7). Among treatments, no significant differences were detected, although the addition of eugenol to packages led to rachises with slightly higher scores (scores = 3) after 35 days of storage (end of the experiment). These results indicated severe symptoms of dehydration and browning in control rachises (scores = 1) and slight-moderate in treated clusters.

**Berry Decay.** Incidence of decay was significantly affected by treatment. Thus, in control berries the fungal development appeared from the first week, with percentage levels increasing along storage. On the contrary, in treated clusters decay was delayed on time and significantly reduced. Thus, after 35 days of cold storage, occurrence of decay was 25.4% in control grapes, and significantly reduced by the addition of menthol



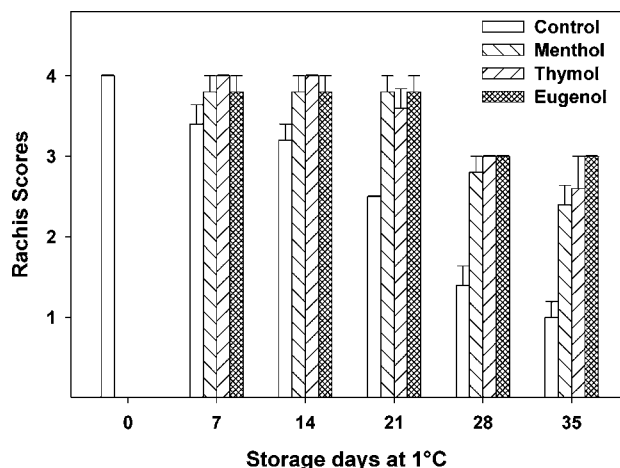
**Figure 5.** Color (Hue angle and  $L^*$ ) of table grape clusters under MAP conditions without (control) and with menthol, thymol, or eugenol during cold storage. Data are the mean  $\pm$  SE.



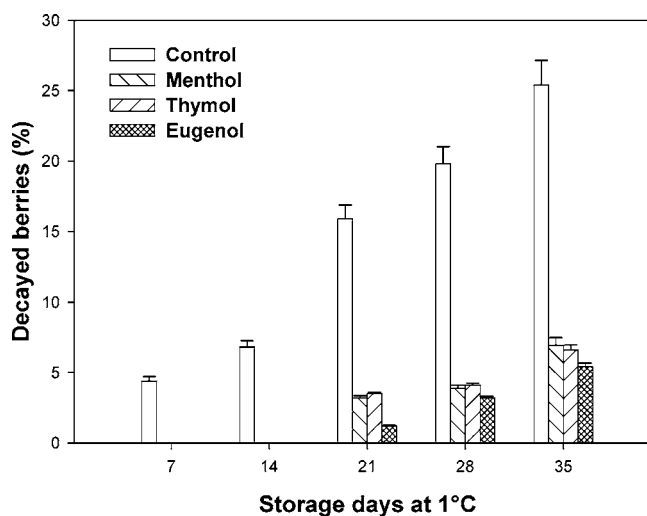
**Figure 6.** Maturity index (TSS/TA ratio) of table grape clusters under MAP conditions without (control) and with menthol, thymol, or eugenol during cold storage. Data are the mean  $\pm$  SE.

(6.93%), thymol (6.59%), and eugenol (5.41%), this treatment being the most effective in reducing decay (Figure 8).

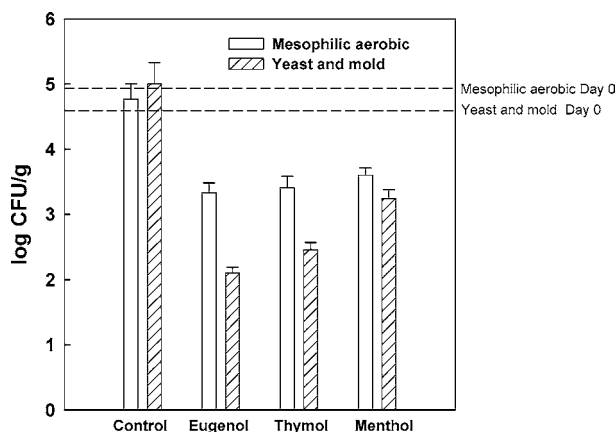
**Microbiological Population.** At harvest, table grape had  $4.9 \pm 0.3$  and  $4.7 \pm 0.2$  log CFU g<sup>-1</sup> for total mesophilic aerobic and mold and yeast counts, respectively. Following 35 days of cold storage, in all packages that contained eugenol, thymol, or menthol, the microbial populations were drastically reduced, the reduction being more effective for yeast and mold counts than for mesophilic aerobics. It has been noticed that the most effective compound in reducing the mold and yeast counts was eugenol ( $2.1 \pm 0.1$  log CFU g<sup>-1</sup>). On the contrary, increases ( $5.0 \pm 0.3$  log CFU g<sup>-1</sup>) in these microbial populations were



**Figure 7.** Scores of rachis quality evaluation of clusters under MAP conditions without (control) and with menthol, thymol, or eugenol during cold storage. Data are the mean  $\pm$  SE.



**Figure 8.** Percentage of decayed berries under MAP conditions without (control) and with menthol, thymol, or eugenol during cold storage. Data are the mean  $\pm$  SE.



**Figure 9.** Mesophilic aerobic and yeast and mold counts in berries at harvest and after 35 days of cold storage under MAP conditions without (control) and with the addition of menthol, thymol, or eugenol. Data are the mean  $\pm$  SE.

observed for control berries (**Figure 9**). For total mesophilic aerobic, a significant reduction was found in those packages treated with the antimicrobials, the eugenol also being the most effective ( $3.3 \pm 0.1$  log CFU  $g^{-1}$ ).

## DISCUSSION

Consumers demand safe products that avoid the use of chemicals as a mean of preservation, but unfortunately increasing incidence of foodborne illnesses from pathogenic microorganisms has occurred (21), resulting in a major public health impact around the world. On the other hand, considerable postharvest losses of fruit and vegetables are brought about by decay caused by fungal plant pathogens. In this sense, continuing efforts in processing, preservation, distribution, and marketing are being made worldwide to supply fresh fruits of high quality and safety to consumers.

In this work, an active packaging was developed to improve MAP effectiveness on preserving table grape quality and safety by the addition of menthol, thymol, or eugenol as an alternative of synthetic fungicides. The atmosphere composition inside the packages was not significantly affected by the addition of eugenol, thymol, or menthol. The final gas composition (10–14 O<sub>2</sub> and 1.3–2 kPa CO<sub>2</sub>) could be considered as suitable for “Crimson Seedless” table grape quality preservation, because a wide range of atmosphere compositions (5–15 O<sub>2</sub> and 3–15 kPa CO<sub>2</sub>) has been reported depending on cultivar and ripening stage at harvest for table grape storage under MAP conditions (11, 12, 14).

Weight loss of both berry and rachis is one of the most important physiological disorders affecting the shelf life of table grapes, which are associated with increasing fruit susceptibility to fungal decay. Values ranging from 8% to 20% were found during cold storage without MAP, depending on cultivar and storage conditions (11, 22). However, loss of weight in MAP packaged clusters was very low (<1%), mainly due to the effect of polypropylene film on increasing water vapor pressure. Moreover, weight losses were significantly reduced by the addition of eugenol, thymol, or menthol, as occurred in a similar experiment with sweet cherry (20), showing that these compounds lowered the dehydration process, although the intrinsic mechanism of this effect is still unclear.

Texture and color are also important attributes demanded by consumers and many times are the main parameters responsible for acceptance. Although table grape is a nonclimacteric fruit, significant reduction in firmness and color change acceleration occurred very rapidly during storage. Softening occurs normally during postharvest storage of fruits, contributing to quality loss and reducing the shelf life. MAP is an effective technique retarding the softening process in fruits such as nectarine, strawberry, and table grape, among others (11, 23, 24). In addition, results showed that the combination of antimicrobials with MAP led to an additional benefit by delaying both berry and flesh firmness losses, as previously reported for cherries (20). With the available literature, no relationship between the application of these compounds and retention of firmness has been found, although their synergistic effect together with MAP (25) in the reduction of  $\beta$ -galactosidase, polygalacturonase, and pectinmethylesterase activities, the main cell wall degrading-enzymes responsible for table grape softening (26), could not be discarded. Red table grapes, as “Crimson Seedless”, are rich in anthocyanin compounds, which have been associated with the contribution to consumer’s health and well-being (27, 28). The ripening process of berries has been correlated to the anthocyanin content (29), and changes in their profile, peonidin 3-glucoside being the major anthocyanin in “Crimson Seedless” (30). Evaluation of color on the basis of Hue angle and parameter  $L^*$  evolutions was a good indicator for berry skin color changes during postharvest storage, because a lower color increase during postharvest storage, because a skin color increase in Hue and decrease in  $L^*$  were obtained when eugenol,

thymol, or menthol was added to packages than in controls. Moreover, maintenance of color  $L^*$  could be also related to the lower weight losses above-described.

Other important parameters determining the quality of table grapes are the TSS, TA, and TSS/TA ratio, which affected the consumer acceptance (31, 32). The addition of the antimicrobials led to a lower increase in TSS/TA ratio with respect to controls, mainly due to maintenance of TSS and lower reduction in TA (data not shown) along storage, showing the effect of these treatments on maintaining TSS and TA contents close to those observed in berries at harvest. In "Flame Seedless" table grape under MAP conditions and with the same film, the same evolution in TSS and TA was reported, while storage on air led to an increase in TSS and maintenance of TA (11).

The beneficial effect of eugenol, menthol, or thymol was also clear in delaying the rachis browning, because these clusters received higher scores than controls from a trained panel. There is no evidence of the role of these natural compounds on this issue, but the well-known antioxidant activity reported for these compounds (33, 34) might probably reduce dehydration, chlorophyll degradation, and occurrence of browned polymers responsible for stem browning. These symptoms first appeared on pedicels following by lateral branches and finally on central axis, as has been reported for "Flame Seedless" table grapes and due to increased polyphenoloxidase activity (1). Thus, some effects of the added antimicrobial compounds on the activity of this enzyme could not be discarded.

The potential use of essential oils or their components in the preservation of food products has been recently reviewed (15). They were effective in reducing food spoiling microorganisms, food borne pathogens, spoilage, and mycotoxigenic fungi, pathogenic, and dimorphic yeasts (35). The addition of eugenol, menthol, or thymol inside the packages was effective in reducing the microorganism proliferation in table grape, the effect being higher for yeast and molds than for mesophilic aerobics. The total viable counts were  $<3 \log \text{CFU g}^{-1}$  for yeast and molds and  $<3.5 \log \text{CFU g}^{-1}$  for mesophilic aerobics, which meet the recommended microbiological criteria for nonheated fruit desserts (36), while counts  $\sim 5 \log \text{CFU g}^{-1}$  were observed for control berries. The lower counts for yeast and molds were related to the lower ethylene accumulation inside the packages, which could indicate that the accumulated ethylene in control packages proceeds from the fungal metabolism rather than from berry fruit. In fact, berries of control clusters showed the highest incidence of decay. It has been reported that *B. cinerea* produced greater amounts of ethylene as the concentration of conidia inoculated in vitro was increased (37). The mechanism of action of the essential oils is attributed to their hydrophobicity, which enables them to partition in the lipids of the cell membrane, thus disturbing its integrity and the inorganic ions equilibrium (38, 39). The presence of the phenolic ring may be necessary for their antimicrobial activity (40), which could explain the lower activity of menthol as compared to eugenol and thymol. Also, it has been reported that MAP could act synergistically with essential oils by inhibiting the microbial growth in beef (41), bakery products (42), and sweet cherry according to our previous work (43).

In conclusion, to our knowledge this is the first time that thymol, menthol, or especially eugenol improved the beneficial effect of MAP in reducing the microbial spoilage in table grape during storage. In addition, these compounds maintained berry quality properties as compared to MAP-control fruits, through a reduction of weight loss, softening, color changes, and TSS/TA ratio evolution, together with low rachis deterioration and

browning. A delay of 3 weeks on the evolution of these parameters was obtained between control and treated clusters. Thus, storability of 14 days could be considered for control clusters and extended up to 35 days for treated ones. From the three antimicrobials assayed, the best results were obtained with eugenol followed by thymol and finally menthol, which could be related to their different antioxidant activity, which followed the same sequence using in vitro systems (33). Although no sensory analyses were performed, slight odor of these compounds was detected immediately after opening the packages, which disappeared very rapidly on time due to evaporation at room temperature. However, after tasting the grapes, persistence of their characteristic aroma was detected, especially for menthol, while eugenol showed the lowest residual flavor. Thus, further studies are needed to gain a better understanding on the mechanism/s by which these essential oils affect the fruit physiology modulating the ripening process, as well as the establishment of appropriate doses to minimize their possible effects on sensorial fruit attributes but maintain both quality and antimicrobial functions.

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