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Response of strawberries exposed to Thymol and Carvacrol vapours

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ABSTRACT

Strawberry is a delicate fruit with short shelf life, primarily due to high nutrient and moisture content and decay caused by fungi. Traditionally, fungicides are used to control postharvest decay but natural phytochemicals (thymol and carvacrol volatiles) have also shown antimicrobial properties, which can reduce produce waste due to pathogens. The aim of the study was to test the efficacy of thymol and carvacrol volatiles to control fungal activity and maintain strawberry fruit quality, packed in two container types (airtight and clamshells). Fungal contamination of strawberries was reduced by thymol and carvacrol in both concentrations (30 & 60 ppm) and their combinations. However, weight loss was not affected by the volatile treatments. Titratable acidity (TA) and total soluble solids (TSS) was also affected by volatile treatments. Volatile-treated strawberries had higher TA than control fruits. Our results suggest that plant volatiles have the potential to extend the shelf life of strawberries after harvest.

Key words: *Fragaria* × *ananassa*, essential oils, volatiles, fruit quality, pathogens, package.

INTRODUCTION

Fresh fruits and vegetables are prone to fungal contamination due to their high water and nutrient content. The produce loss after harvest is very high ranging from 20-50% depending on the crop and the postharvest management of the produce (Agrios, 2). The world population and the demand for food is increasing (FAO, 6), therefore, it is crucial to reduce the produce waste, while increasing current agricultural production (USDA, 18). Traditionally, cooling facilities and fungicides are used to reduce produce waste and control fungal contaminations. However, cooling facilities are not adequate for prolonged storage during long distance shipments (Bhaskara Reddy *et al.,* 3; Perdones *et al.,* 11). Also, fungicide applications have raised a lot of concern over the chemical residue and safety of synthetic chemicals for human and the environment. Therefore, there is a lot of interest to develop eco-friendly alternatives to control fungal contaminations growing on fresh fruits and vegetables. Different physical and chemical approaches have been investigated such as UV-irradiation, heat treatment (Shafiee *et al.,* 15), modified atmosphere storage (Reyes and Smith, 12), and biological controls, where each of them has its own pros and cons.

Concern about the safety of fresh and processed strawberries has increased in recent years due to increased application of synthetic chemicals, placing strawberries on the "Dirty Dozen" fruit list (EWG, 5). Also, emergence of several outbreaks of foodborne pathogens linked to their consumption has increased the demand for strategies to control contaminants and development of novel alternatives to synthetic chemical disinfection. The technologies that do not affect the nutritional and organoleptic properties of fresh fruits and stimulate the production of valuable compounds in strawberries warrant further studies (Lafarga *et al.,* 8). Many plant phytochemicals such as volatiles have shown antibacterial and antifungal activities in *in vitro* conditions and there is a large number of research articles on this topic (Taghavi *et al.,* 16). However, the antimicrobial activity of plant volatiles has been tested in their natural liquid state and in physical contact with the microbial agents in *in vitro* conditions (petri dishes), which does not necessarily translate to a large fruit storage cabinet. Many of the active compounds present in essential oils are volatiles; therefore, their antimicrobial activity should also be evaluated in vapor phase.

There are very few publications on the efficacy of plant volatiles (in vapor phase), on fresh fruits and vegetables during storage (Perdones *et al.,* 11). Vapours of clove, mustard and lemon essential oils, had strong inhibitory effect against *B. cinerea* both in *in vitro* and *in vivo* conditions (Perdones *et al.,* 11). Essential oils of eucalyptus (*Eucalyptus globulus* L.), cinnamon (*Cinnamomum Zeylanicum*) and thyme also reduced strawberry fruit decay (including *Colletotrichum acutatum*, *Rhizopus stolonifer* and *Botrytis cinerea*) and improved fruit quality. (Tzortzakis, 17; Bhaskara Reddy *et al.,* 3; Wang *et al.,* 19).

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There are several mechanisms reported for the fruit resistance to fungal attack once essential oils are applied. One mechanism is the anti-proliferative activity and free radical scavenging capacity of essential oils. This has been reported in strawberries and kiwifruit (Wang et al., 19). The role of essential oils in plant self-defense and induced resistance as a second mechanism was also investigated. Essential oils also affect pathogens through secretion of cell protoplasts and can reduce spore production and germination (Santos *et al.,* 13). The ease of essential oil application in vapor phase and their effectiveness makes them attractive fumigants in small fruit that are not recommended for wet sanitation. However, their application may create off-flavor and phytotoxicity. Therefore, further research is needed to develop new techniques of their application. In this manuscript, the efficacy of two plant volatiles (thymol and carvacrol) were tested to control fruit decay and maintain strawberry fruit quality during storage in two container types (air-tight and clamshell).

MATERIALS AND METHODS

Strawberries were purchased from local supermarkets in Chester, Virginia, USA. They were used the same day of acquisition to avoid natural decomposition. Fruits were sorted and only firm fruits with uniform size and red color with no blemishes were selected for the experiment. Selected berries were randomized between treatments and 10-15 fruits were considered for each experimental unit and placed in each container. The experiment was a Completely Randomized Design (CRD) with two factors (container types and volatiles) and three replicates and was repeated two times as two separate experiments.

Two container types were selected, a 500 ml air-tight polypropylene container (Nalgen straight-side container cat no. 2118-0016) and the 1 lb clamshell containers commercially used for strawberries (Peninsula Packaging Company cat no. H7352-7). The clamshells have several holes on the sides and the top for cooling of strawberries.

Two volatiles (thymol, carvacrol) were purchased from Sigma Aldrich. Thymol was used at two concentrations of 30 and 60 (mgL^{-1} of container volume) and dissolved in 0.5 ml ethanol 70% (Serrano *et al.,* 14). Thymol was used as a control in the experiment because it has proven antifungal properties (Bhaskara Reddy *et al.,* 3). Carvacrol was used at two concentrations of 60 and 120 (mgL^{-1} of container volume) (Abd-Alla *et al.,* 1). Essential oils were not in physical contact with the fruits, but a cotton ball was placed in each container and essential oils were added to the cotton ball to be vaporized inside the container. For the mixture of Thymol and carvacrol, the lowest concentration (30 & 60 mgL^{-1} respectively) and the highest concentrations (60 & 120 mgL^{-1}, respectively) were added to the cotton balls. Ethanol was used in the first experiment to see if its vapor has any effect on strawberry fruit quality. However, it was deleted from the second experiment as there was no effect on fruit quality.

Fungicide Switch (positive control) with 40% active ingredients (Cyprodinil and Fludioxonil), was dissolved in distilled water and used at a rate of 30 mgL^{-1} of active ingredient (Mercier *et al.,* 10). Therefore, distilled water was used as a negative control. Both fungicide Switch and distilled water (negative control) were sprayed on strawberries and left on paper towels to dry at room temperature for 10-15 minutes.

Fruit containers were then kept at 4 ºC and 95% humidity. Fruit quality parameters (weight loss, total soluble solids, titratable acidity, and fruit decay) were assessed before storage and every week for 4 weeks using standard protocols as follow (Shafiee *et al.,* 15). Fruits were weighed after harvest and every 7 days for 28 days of storage at 4 °C. The results were expressed as percentage of weight loss relative to the initial weight. A subjective scale was used to assess the fruit decay on which 1 = no decay, 2 = less than 5% decay, 3 = 5–20% decay, 4 = 20–50% decay and 5 = >50% decay. Total soluble solids in the extracted juice of fruits was measured by a refractometer (ATAGO Brix = 0-32%) and the results were expressed as °Brix. Five ml of extracted fruit juice was diluted to 20 ml with distilled water and were titrated with 0.1N sodium hydroxide to a pH of 8.1. Titratable acidity was calculated as percentage of citric acid by this formula:

$$Z = \left(\frac{V\ N\ Meq}{Y}\right) 100$$

Where Z = percentage of acidity, V = volume of sodium hydroxide used (ml), N = sodium hydroxide normality (0.1), milliequivalent factor of citric acid=0.064 and Y = volume of sample (5ml). All the data were analyzed using analysis of variance ANOVA of SAS program (SAS Institute Inc. v 9.4, NC). Means were compared using Duncan multiple range test at $p \leq 0.05\%$.

RESULTS AND DISCUSSION

Very small amount of ethanol used to dissolve thymol did not have any effect on strawberry fruit quality, therefore, it was not considered nor reported in this study. Container type had an effect on fungal contamination. Fungal contamination was higher in clamshells compared to airtight containers in all treatments (Fig. 1A & B), except in fungicide treatment which was equal in both container types (score 2.5). The results suggest that, volatiles placed in airtight containers were more effective than in clamshells (Fig. 2 & 3) mainly because they had higher vapor pressure and could control fungal contamination more effectively than clamshells. However, the holes on the container did not affect the fungicide effectiveness sprayed on strawberries.

Although the vapor pressure of volatiles in the containers was not measured, the volatiles could easily escape through the holes on the clamshells as the volatiles could be smelled inside the storage cabinet. Therefore, the effectiveness of essential oils may have significantly reduced in clamshells, which was observed as higher fungal contamination in clamshells compared to airtight containers (Fig. 2 & 3).

Negative control treatment (water spray) had the highest fungal contamination among all treatments. Volatiles reduced fungal contamination significantly compared to negative control (water). In airtight containers, volatiles were as effective as fungicides in controlling the pathogens, while in clamshells, they reduced the fungal contamination compared to control, but were not as effective as fungicide. Also, the differences between volatiles were not significant in airtight containers, however, in clamshells, carvacrol-treated strawberries had lower fungal contamination than thymol, and at 120 ppm were similar to fungicide treatment. Combinations of thymol and carvacrol created lesions that resembled to decay, resulting to be scored with higher contamination compared to volatiles alone. However, the lesions need to be tested for their fungal presence (Fig. 2 & 3).

Titratable acidity (TA) decreased in control treatments, both in strawberries treated with water or fungicide regardless of the container type (Fig. 1C & D). This led to the higher TSS/TA ratio in these control treatments (data not presented). TA did not differ significantly among volatile treatments.

Strawberries treated with Thymol, carvacrol and their combination had higher TSS in airtight containers than those treated with water or fungicide Switch (Fig. 1E & F). However, this pattern was not observed in clamshells. In clamshells, water treated strawberries (negative control) had the highest and fungicide treated strawberries the lowest TSS among other treatments.

Weight loss did not change among treatments (Fig. 1G& H). Weight loss in clamshell containers was higher than airtight containers and was between 0.15-0.25%. The higher weight loss in clamshells is due to the fact that, there are several holes on the container that allows the water to evaporate. However, none of the volatile treatments had an effect on the weight loss of strawberries.

Our results have shown that fungal contamination of strawberries is affected by both volatile application and type of strawberry packaging. Antifungal properties of volatiles are affected by their active vapor pressure inside the packages (Gardini *et al.,* 7). Therefore, factors that affect vapor pressure such as packaging type or temperature can influence pathogen growth indirectly by changing the vapor pressure. More research is needed to identify the effective vapor pressure and the method of application of plant volatiles on biological activity of plant pathogens.

Ethanol vapor reduces the decrease of firmness and weight loss in cut pieces of strawberries; however, no report exists on its effect on fungal decay (Li *et al.,* 9). In our experiment, ethanol did not affect fungal decay and weight loss and will not be reported further.

Essential oil vapours have proven antimicrobial activity against a wide range of micro-organisms and rarely leave detectable residues (Tzortzakis, 17; Dorman and Deans, 4). Thymol has shown to be the best plant volatile in slowing fruit decay in strawberry (Wang *et al.,* 19), and against *Botrytis cinerea* and *Rizoctonia stolonifer* (Bhaskara Reddy *et al.,* 3). In our study, both thymol and carvacrol have shown antifungal properties on strawberries, however, depending on the container type, thymol was more effective in air-tight and carvacrol in clamshell containers.

Tzortzakis (17) reported antifungal activity of cinnamon and eucalyptus essential oils on strawberry and tomatoes. Wang et al. (19) also reported suppressed fungal growth of thymol, menthol and eugenol on strawberries, sweet cherries and table grapes. Two essential oils, carvacrol and cinnamic acid, delayed the spoilage of fresh-cut kiwifruit, honeydew melon and blueberry in cold storage. Mold retardation was also high by using p-cymen, linalool, carvacrol, anethole and perillaldehyde on blueberries (Wang *et al.,* 19).

Different mechanisms have been reported for the biological activity of plant volatiles. Plant volatiles may change the permeability of microbe membranes, disrupt cellular membrane and function and interfere with active sites of enzymes and cellular metabolism. The degeneration of the ion gradients adversely affects cell vital processes and results in cell death (Taghavi *et al*. 16).

Plant volatiles may act as a signaling compound that induce antioxidant capacity in fruit tissues. Antifungal properties of these plant volatiles (such as thymol, menthol and eugenol) may also be attributed to the improved fruit antioxidant capacity by increasing phenolic and flavonoid contents (Wang *et al.,* 19).

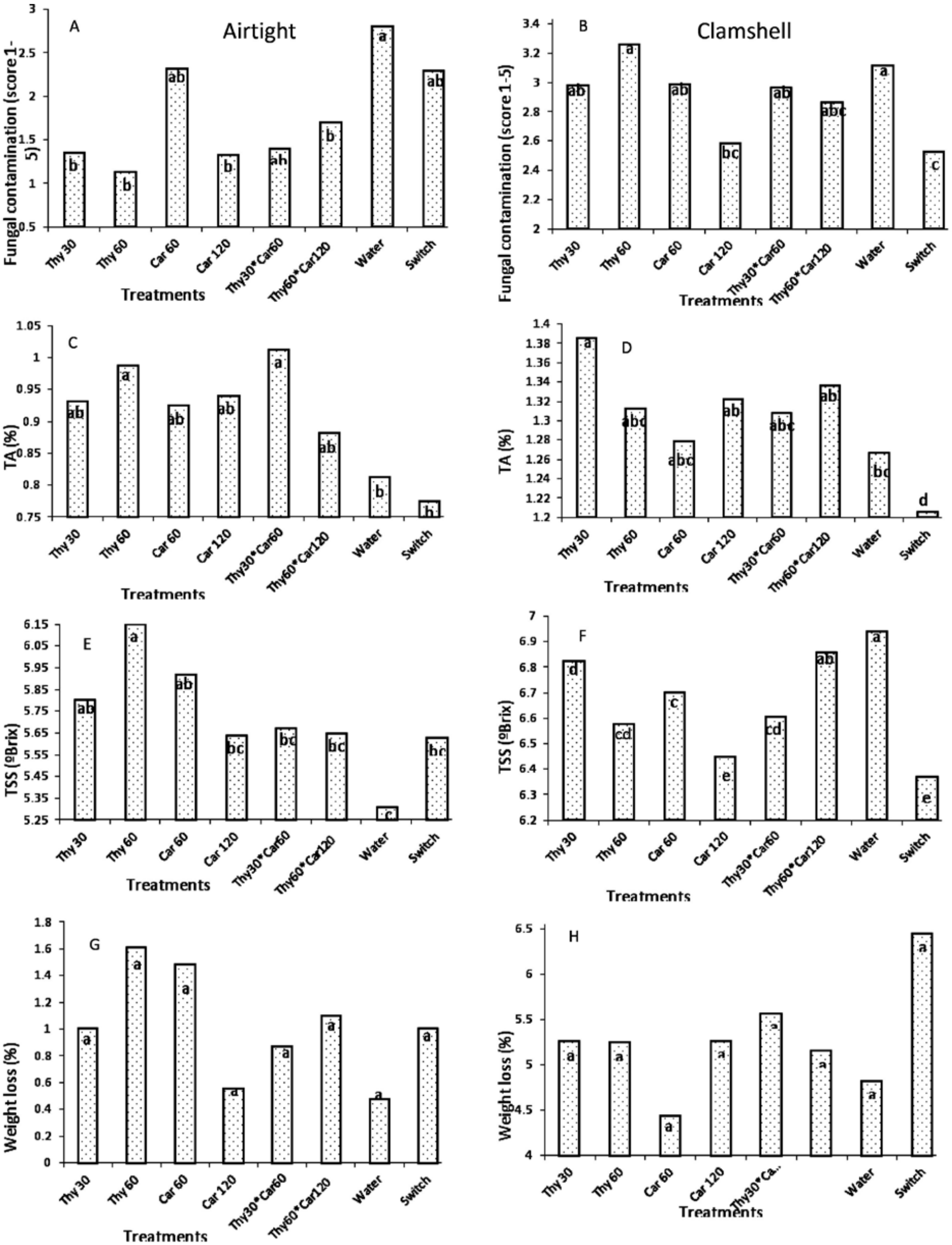
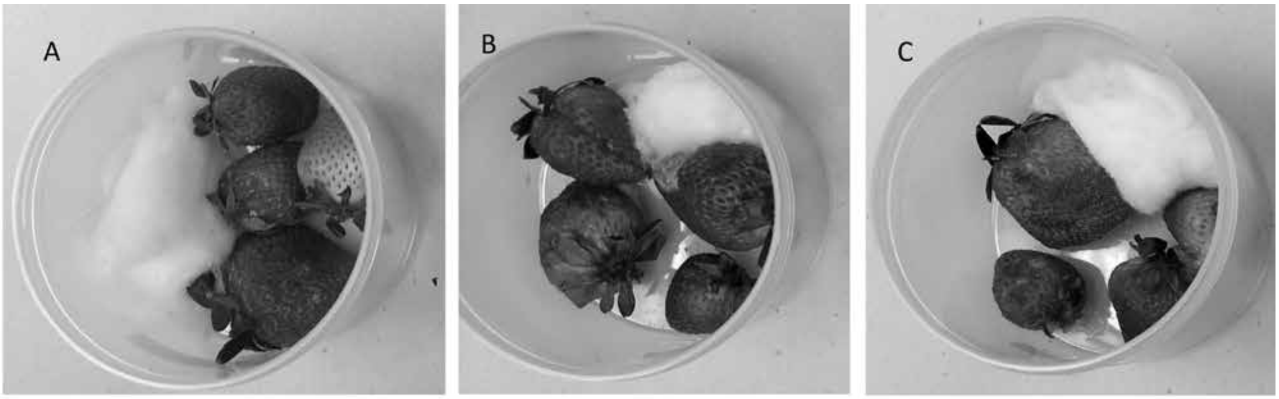
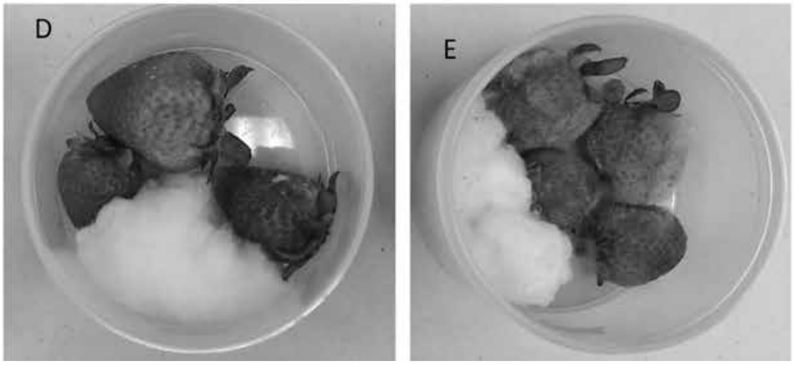


Fig. 1. Fruit quality parameters of strawberries treated with two essential oil volatiles Thymol (30 and 60 pmm), carvacrol (60 and 120 ppm) and their two combinations (Thymol 30 ppm + carcacrol 60 ppm and thymol 60 ppm+ carvacrol 120 ppm), distilled water (as negative control) and fungicide Switch (30 ppm active ingredient, as positive control). Figures A,C, E and G are airtight containers and B, D, F and H are clamshells.



Thymol (30 ppm) Carvacrol (60 ppm) Thymol & carvacrol (30*60 ppm)



Positive control fungicide Negative control water

Fig. 2. Strawberries stored in airtight containers (500m) and exposed to volatiles placed on cotton balls: A) Thymol 30 ppm, B) Carvacrol 60 ppm, C) mixture of Thymol and Carvacrol (30 & 60 ppm respectively), D) sprayed with fungicide Switch 30 ppm active ingredient and E) sprayed with distilled water (negative control). Lids were removed for photography.

Thymol (30 ppm) Carvacrol (60 ppm) Thymol & carvacrol (30*60 ppm)

Fig. 3. Strawberries stored in clamshells (722 ml, with holes on the sides and the top) and exposed to volatiles placed on cotton balls: A) Thymol 30 ppm, B) Carvacrol 60 ppm, and C) mixture of Thymol and Carvacrol (30 & 60 ppm respectively). Holes can be seen on the sides and bottom of the containers.

In this experiment, only strawberries in control treatments (sprayed with water and fungicide) had lower TA compared to thymol and carvacrol treated strawberries. Reports have shown that TA and organic acids either did not change or increase in essential oil treatments. Titratable acidity and pH of treated strawberries and tomatoes did not change during thymol vapor exposure, while cherry tomatoes increased, similar to grapes exposed to thymol and menthol vapours (Tzortzakis, 17). Pears treated with plant oils maintained TSS and TA in a concentration-dependent manner and papayas treated with methyl jasmonate retained higher organic acids than control. Also raspberries treated with methyl jasmonate and tea tree oil maintained higher sugars and organic acids compared to control. Strawberries treated with thymol, eugenol and menthol had higher sugars and organic acids (Wang *et al.,* 19).

Total soluble solids changed between 5.2 - 7 and is in the similar range reported by others for strawberries. Cinammon oil improved fruit quality attributes and TSS in strawberries and tomatoes, while basil oil spray did not change TSS in banana (Tzortzakis 17).

Researchers reported better strawberry, sweet cherry and grape quality with higher sugar and organic acids treated with thymol, menthol and eugenol (Santos *et al.,* 13; Serrano *et al.,* 14;). Thymol and eugenol also reduced grape quality loss in fruits when added to the packages. Blueberries treated with carvacrol, anethole and perillaldehyde had significantly higher levels of sugars and organic acids, which contributed to better quality fruits (Wang *et al.,* 19). Essential oils of *Origanum vulgare* inhibited fungal population of grapes during storage (Santos *et al.,* 13). While weight loss of strawberries treated with essential oils did not change in our experiment, there are three patterns reported by other researchers. Tzortzakis (17) reported higher weight loss on strawberry and tomato fruits pre-exposed to oil vapor. In contrast lower weight loss is reported in cherries and grapes and chitosan-bergamot oil coated grapes (Santos et al. 13; Serrano *et al.,* 14). Similar to our experiment, weight loss of thymol and eugenol treated grapes did not change as reported by Santos et al. (13). No further explanation has been given on the differences between essential oils and commodities. The differences also could be due to the experimental conditions, although container type (air tight or clamshells) did not affect water loss in our experiment.

The present research demonstrated that, fruit quality attributes have improved in exposure to thymol and carvacrol oils. This may have considerable commercial value to extend the shelf life and maintain fruit quality by oil vapours. Plant volatiles that have positive effects on TSS and acid content and negative effects on microbial growth deserve to be evaluated for their potential preservation activity to extend shelf life of fresh produce. However, efficacy of oil vapours and method of their application must be further determined in commercial settings, where fresh produce is exposed to oil vapours in commercial storage containers or modified atmosphere packaging.

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