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ORIGINAL PAPER

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Methyl jasmonate in conjunction with ethanol treatment increases antioxidant capacity, volatile compounds and postharvest life of strawberry fruit

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Abstract The antioxidant capacity, total anthocyanins, total phenolics, volatile compounds, and postharvest quality of strawberry fruit were evaluated after treatment with natural antimicrobial compounds and during storage at 7.5 °C. Strawberries treated with methyl jasmonate (MJ) in conjunction with ethanol (MJ-ETOH) showed higher antioxidant capacity, total phenolics, and anthocyanins than those treated with ethanol or control (non-treated). MJ-ETOH and ethanol treatments also increased volatile compounds during storage period. However, individual volatile compounds were affected differently. Methyl acetate, isoamyl acetate, ethyl hexanoate, butyl acetate, and hexyl acetate increased, while ethyl butanoate, 3-hexenyl acetate, and methyl hexanoate decreased during storage. The postharvest life was longer for those berries treated with MJ-ETOH and MJ than for those treated with ethanol or control fruit. In conclusion, strawberries treated with MJ-ETOH maintained an acceptable overall quality for the longest storage duration and retained higher levels of volatile compounds; also, berries treated with MJ showed the highest antioxidant capacity compared with other treatments during the postharvest period.

Keywords Antioxidant · Volatile compounds · Methyl jasmonate · Ethanol · Strawberry

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Abbreviations used AAPH 2′,2′-azobis (2-amidinopropane) dihydrocloride · ORAC oxygen radical absorbance capacity · R-PE (R)-phycoerithrin · Trolox 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxilic acid · TE Trolox equivalents

Introduction

Strawberries are good sources of natural antioxidants [1–3]. In addition to the usual nutrients, such as vitamins and minerals, strawberries are also rich in anthocyanins, flavonoids, and phenolic acids [2, 4]. Strawberries have shown a remarkably high scavenging activity toward chemically generated radicals, thus making them effective in inhibiting oxidation of human low-density lipoproteins [4]. Previous studies [3, 5] have shown that strawberries have high oxygen radical absorbance activity against peroxyl radicals (ROO), superoxide radicals (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radicals (OH), and singlet oxygen ($^1O_2$); and that antioxidant activities were different among varieties [5]. There is a positive correlation between antioxidant activity and total phenolic or anthocyanin content [1, 3].

The postharvest life of fruit and vegetables has been traditionally defined in terms of visual appearance (freshness, color, and absence of decay or physiological disorders) and texture (firmness, juiciness and crispness). Although this concept involves aesthetic appeal and mechanical properties associated with quality, it disregards flavor and nutritional quality. Flavor plays an important role in consumer satisfaction and influences further consumption of fruits and foods in general [6, 7]. In addition to their aesthetic qualities, fruits form an important part of our diet mainly as a source of energy, vitamins, minerals, and antioxidants.

Several natural volatile compounds have been reported to possess antimicrobial activity. Methyl jasmonate (MJ) either as a vapor or as an emulsion has been shown to reduce microbial contamination of fresh-cut celery and peppers [8], inhibit grey mould infection in strawberries [9] suppress green mould growth in grapefruit [10] and control *Botrytis rot* in cut rose flowers [11]. Methyl

jasmonate also decreased the severity of postharvest brown rot in sweet cherries when used as a co-fumigant with thymol or carvacrol [12]. Ethanol has also been found to have antimicrobial properties, and a postharvest ethanol dip eliminated most of the fungal and bacterial populations on surface of grapes without impairing bunch appearance or berry firmness [13]. Ethanol vapor also prevented scald development in apples and reduced leaf blackening in the stems of the cut flower Protea 'Pink Ice' [14]. Postharvest ethanol treatments can have beneficial effects on fruit physiology such as enhancing the sensory quality of apples [15], reducing astringency of persimmons and bananas [16, 17], delaying ripening of tomatoes [18], reducing postharvest decay of citrus and stonefruit [19, 20] and controlling scald in apples [21].

Interest in the role of antioxidants in human health has promoted research in the field of horticulture and food science to evaluate fruit and vegetable antioxidants and to determine how their content and activity can be maintained or even improved through crop breeding, cultural practices, and postharvest storage and processing. Preharvest factors, such as genetic background and cultural practices, have the potential to influence antioxidant capacity in crops. Strawberry fruit from a hill plasticulture system consistently had higher flavonoid content and antioxidant capacity than fruit from plants grown using the matted row system [22]. Postharvest storage can also affect anthocyanin, phenolic compound levels and antioxidant capacity in fruits and vegetables. Controlled atmosphere (CA) storage of strawberry fruit did not affect anthocyanin content in external tissues but decreased anthocyanin content in internal tissues [23]. Processing also has marked effects on phenolic content and antioxidant capacity in fruits. Strawberry processing to produce jams decreased the total ellagic acid content by 20% and the flavonoids by 15–20% [24]. It has also been reported that the freezing process decreased both the total phenolic content and free radical scavenging capacity by 4–20% in four cultivars of raspberries [25]. As antioxidant content is becoming an increasingly important parameter with respect to fruit and vegetable quality, it is of great interest to evaluate changes in antioxidant status during postharvest storage of horticultural crops. However, little information is available regarding the effects of storage conditions, such as exposure to natural antimicrobial compounds, on the changes of anthocyanins, phenolic compounds and antioxidant capacity in strawberry fruit. This study was undertaken to investigate the effects of natural antimicrobial compounds on total phenolics, total anthocyanins, and antioxidant capacity as well as the main volatile constituents and fruit quality in strawberry fruit during postharvest storage.

Materials and methods

Chemicals

R-Phycoerythrin (R-PE) from *Porphydium cruentum* was purchased from Sigma (St Louis, MO). 2′,2′-Azobis(2-amidinopropane) dihydrochloride (AAPH) was purchased from Wako Chemicals USA Inc. (Richmond, VA). 6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) was obtained from Aldrich (Milwaukee, WI). Methyl jasmonate from jasmine (*Officinale Linnaeus*) was purchased form Sigma-Aldrich (St Louis, MO).

Plant materials

Strawberry fruit (*Fragaria x ananassas* Duch. cv. Allstar) grown at Butler's Orchard in Germantown, MD, USA, were hand-harvested at a commercially mature stage, sorted to eliminate damaged, shriveled, and unripe fruit, and selected for uniform size and color. Seventy berries were placed in 3.8 l jars with lids, three jars per treatment. Volatile compounds used in this study include MJ (22.4 mg l^{-1}), absolute ethyl alcohol (ethanol, 400 μL l^{-1}), and its combination. The specified volume of each volatile compound was placed into individual small beakers, which were subsequently placed inside the jars just before the lids were covered. The volatile compounds were allowed to vaporize inside the containers spontaneously during 24 h at 20 °C. The containers were then transferred to 7.5 °C. Control samples were handled similarly with the exception of the volatile treatment. Aroma, antioxidant capacity, total anthocyanins, phenolic compounds, and quality were evaluated on days 0, 5, 7 and 11 after harvest.

Overall quality

Thirty fruits per treatment were used for each quality evaluation. Samples from each treatment were evaluated subjectively on the initial day and on day 5, 7, 11, and 13 during storage. Overall quality was evaluated on a 1–5 scale according to the overall condition of the fruit, where 1 = unacceptable, 2 = bad, 3 = acceptable, 4 = good, and 5 = excellent. Results were expressed as an overall quality index.

Fungal decay index

Fungal decay was visually inspected during the course of the experiment. Strawberry fruits showing surface mycelial development were considered decayed. Fungal decay was evaluated on a 1–5 scale, where 1 = normal, 2 = trace (up to 5% surface affected), 3 = slight (5–20% surface affected), 4 = moderate (20–50% surface affected), and 5 = severe (>50% surface affected). Results were expressed as overall decay index.

Total soluble solids and total titratable acidity

Twenty fruit from each replicate were wrapped in cheesecloth and squeezed with a hand press, and the juice was analyzed by triplicate for total soluble solids (TSS) and titratable acidity (TA). TSS was determined at 20 °C on an Atago DBX-55 refractometer (Atago Co. Ltd., Tokyo,

Japan). TA was determined by diluting each 5 ml aliquot of strawberry juice in 95 ml of distilled water and then titrated to pH 8.2 using 0.1 mol/l NaOH.

Surface color measurement

Fruit surface color was measured on 10 fruit from each replicate using a chromameter (CR 200, Minolta, Ramsey, NJ), which provided CIE $L*$, $a*$, and $b*$ values. Negative $a*$ values indicate green and higher positive $a*$ values red color. Higher positive $b*$ values indicate a more yellow skin color. These values were then used to calculate hue degree (h° = arctangent [$b*/a*$]), where 0 ° = red-purple; 90 ° = yellow; 180 ° = bluish green; and 270 ° = blue, and chroma ($C* = [a*^2 + b*^2]^{1/2}$), which indicates the intensity or color saturation.

Total phenolic compound analysis

Total soluble phenolics in the fruit juice extracts were determined with Folin–Ciocalteu reagent according to the method of Slinkard and Singleton [26] using gallic acid as a standard. Results were expressed as milligrams of gallic acid equivalent per 100 g of fresh weight.

Analysis of total anthocyanin content

Total anthocyanin contents in fruit juice were determined using the pH differential method [27]. Absorbance was measured in a Shimadzu spectrophotometer (Shimadzu UV-160) at 510 and 700 nm in buffers at pH 1.0 and 4.5, using $A = [(A_{510}-A_{700})_{\text{pH1.0}}-(A_{510}-A_{700})_{\text{pH4.5}}]$ with molar extinction coefficients of pelargonidin 3-glucoside (22,400) for strawberry fruit juice. Results were expressed as milligrams of pelargonidin 3-glucoside equivalents per 100 g of fresh weight.

Oxygen radical absorbance capacity ORAC assay

The procedures for the ORAC assay on strawberries were modified from a previously described method by Cao et al. [28]. This assay measures the effect of antioxidant components in fruit juices of strawberries on the decline in R-phycoerythrin (R-PE) fluorescence induced by a peroxyl radical generator, 2′,2′ azobis (2-amidinopropane) dihydrochloride (AAPH). The reaction mixture contained 1.7 ml of 75 mM phosphate buffer (pH 7.0), 100 µL of R-PE (3.4 mg/l), 100 µl of 320 mM AAPH, and 100 µl of sample. Phosphate buffer was used as a blank, and 1 µM of Trolox (a water-soluble α-tocopherol analogue) was used as a standard during each run. The final volume of 2 ml was used in a 10-mm-wide fluorometer cuvette. R-PE, phosphate buffer, and samples were preincubated at 37 °C for 15 min. The reaction was started by the addition of AAPH. Fluorescence was measured and recorded every 5 min at the emission of 570 nm and excitation of 540 nm using a Shimadzu RF-Mini 150 recording fluorometer (Columbia, MD) until the fluorescence of the last reading declined to less than 5% of the first reading (approximately 70 min). One blank, one standard, and a maximum of 10 samples were analyzed at the same time. Each sample was repeated three times. The ORAC value refers to the net protection area under the quenching curve of R-PE in the presence of an antioxidant. The final results (ORAC value) were calculated and expressed using Trolox equivalents (TE) per gram on a fresh weight basis [28]:

$$\text{ORACValue}(\mu\text{molTE/gF.W.}) = 20K(S_{\text{sample}}-S_{\text{blank}})/(S_{\text{trolox}}-S_{\text{blank}})$$

where K = sample dilution factor and S = the area under the fluorescence decay curve of the sample, Trolox, or blank. S is calculated as follows:

$$S = (0.5 + f_5/f_0 + f_{10}/f_0 + f_{15}/f_0 + f_{20}/f_0 + f_{25}/f_0 + f_{30}/f_0 + \cdots + f_{60}/f_0 + f_{65}/f_0 + f_{70}/f_0) \times 5$$

where f_0 = initial fluorescence at 0 min and f_i = fluorescence measurement at time i.

Analysis of volatile compounds

Strawberry fruit (100 g) were placed in a hermetically closed container (500 ml) housed within a thermostated water bath (25 °C). After 10 min equilibrium time period, volatile compounds were adsorbed on a SPME fiber (65 µm, poly(dimethylsiloxane)/DVB) (Supelco, Bellefonte, PA, USA). Sampling time was 20 min. Two replicates per day per treatment were obtained with this procedure. Desorption of volatile compounds trapped in the SPME fiber was carried out directly into the GC injector. Volatiles were analyzed using a GC HP-6890 (Hewlett-Packard, Rockville, MD, USA) equipped with a fused silica capillary column 5-HP (5 m × 0.25 mm). Oven temperature was initially held at 40 °C for 1.5 min and then a temperature ramp of 5 °C/min was programmed up to 250 °C. Authentic standards were used for identification of volatile compounds. Quantification was achieved by integrating the area under the curve of each identified compound [29].

Statistical analysis

Experiments were performed according to a completely randomized design. Analysis of variance (ANOVA) of data was performed for this experiment using NCSS Statistical Analysis System [30]. The effect of natural volatile compounds and time storage on fruit quality (decay, TSS, TA, fruit color, and aroma compounds) and the values of phenolics, anthocyanins, and their antioxidant capacity were evaluated by the Fischer test. Differences between means of data were compared by least significant

difference (LSD). Differences at $p \leq 0.05$ were considered to be significant.

Results and discussion

Overall quality and fungal decay indexes

Figure 1 shows the effect of antimicrobial compounds on overall quality and fungal decay of strawberries. Overall quality decreased continuously during storage at higher rate in untreated strawberries compared with those treated with ethanol, MJ-ETOH and MJ. Strawberry fruit has a very short shelf life, mostly due to their relatively high water content, high metabolic activity, and the susceptibility to microbial molds and rots. The control fruit in our experiment reached the limit of shelf life in 6 days. The combination of MJ-ETOH was the most effective treatment in maintaining the overall quality of strawberries during the storage period. Strawberries treated with this treatment maintained an acceptable quality for up to 11 days, which is an extension of 5 days over that of the control fruit.

Fungal decay index started to increase rapidly after 5 days in untreated strawberries (Fig. 1b). Berries treated with MJ or MJ+ETOH showed slight fungal decay during 7 days of storage. The combination MJ-ETOH was very effective in suppressing fungal decay of strawberries. Treatment with ethanol by itself was not as effective as treatment with MJ or its combination with MJ. Methyl jasmonate has been shown to induce the synthesis and expression of some stress proteins, such as heat-shock proteins and pathogenesis-related proteins, which lead to increased resistance to pathogens and decreased incidence of decay [31, 32].

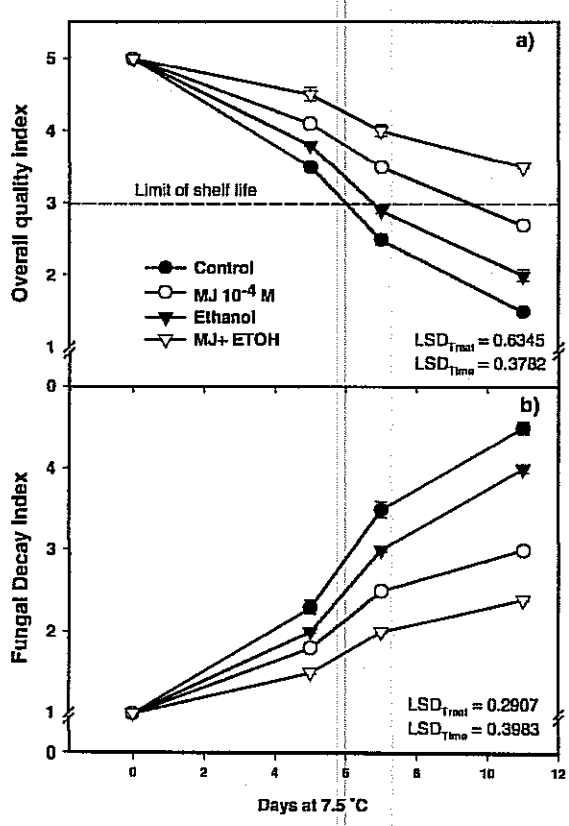

Fig. 1 Effect of ethanol, methyl jasmonate and their combination on (a) overall quality and (b) fungal decay index of strawberries (cv. Allstar) after 12 days of storage at 7.5 °C. Data points are means of three replicates and LSD at 0.05 levels for treatment and time are shown

Ethanol has been shown to effectively control quarantine pests such as *Tetranychus urticae* Koch [33], and *E. postvittana* [34]. These studies used ethanol vapor at ambient temperatures, ethanol dipping in conjunction with high temperatures to kill insects and mites. Buta and Moline [8] showed that MJ extended shelf life and reduced microbial contamination of fresh-cut celery and peppers. It has been reported that MJ inhibited bacterial growth only when relatively high concentrations such as 1×10^{-3} M were used [8]. Jasmonic acid, and derivatives such as MJ, have been described as signaling compounds that stimulated the expression of wound-inducible and defense related genes, as well as being involved in many developmental processes in plants [35]. The observed suppression of fungus decay of strawberry fruit stored at 7.5 °C suggests that the inherent chemical defense mechanisms of plant tissues were able to be activated sufficiently by low-concentration jasmonate and ethanol treatments to lengthen the postharvest life of the commodities. These jasmonates and ethanol treatments may be a practical means of increasing food safety of fresh fruits and vegetables by enhancing resistance to fungus and bacterial growth.

Total soluble solids and total titratable acidity

Ethanol vapors increased the total soluble solids content in strawberries stored at 7.5 °C (Fig. 2a). However, MJ-ETOH combination and MJ treatments maintained similar levels of total soluble solids as the initial samples at the beginning of the experiment. Total soluble solids decreased in the control samples during storage. Depletion of TSS in controls could be explained by a high metabolism of fruit and senescence processes. Comparatively, lower rates of respiration of strawberries treated with ethanol or the combination ethanol-MJ might have contributed to conserve higher levels of carbohydrates in tissue.

Figure 2b shows the effect of antimicrobial compounds on titratable acidity after 12 days of storage. Titratable acidity (TA) was higher in those berries treated with ethanol and MJ-ETOH, meanwhile, control berries showed the lowest TA values. However, at the end of storage period no significant differences were observed among the treatments evaluated.

High sugar and relatively high acid content are required for good strawberry flavor [36]. Although not all strawberries with high TSS are high quality, the absence of high TSS can not assure good quality. Galleta et al. [37] reported that TSS of strawberries generally ranged from 7 to 12% depending on genotype. It have been reported that fructose

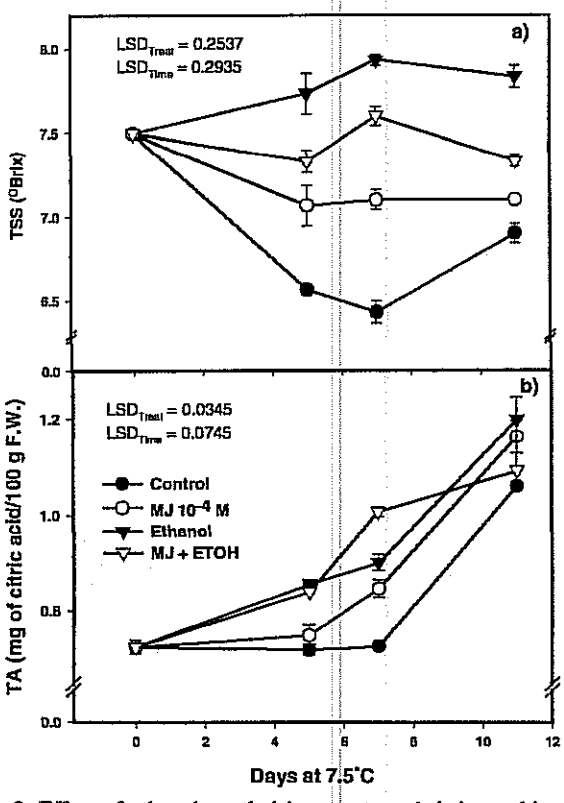

Fig. 2 Effect of ethanol, methyl jasmonate and their combination on (a) soluble solids content and (b) titratable acidity of strawberries (cv. Allstar) after 12 days of storage at 7.5 °C. Data points are means of three replicates and LSD at 0.05 levels for treatment and time are shown

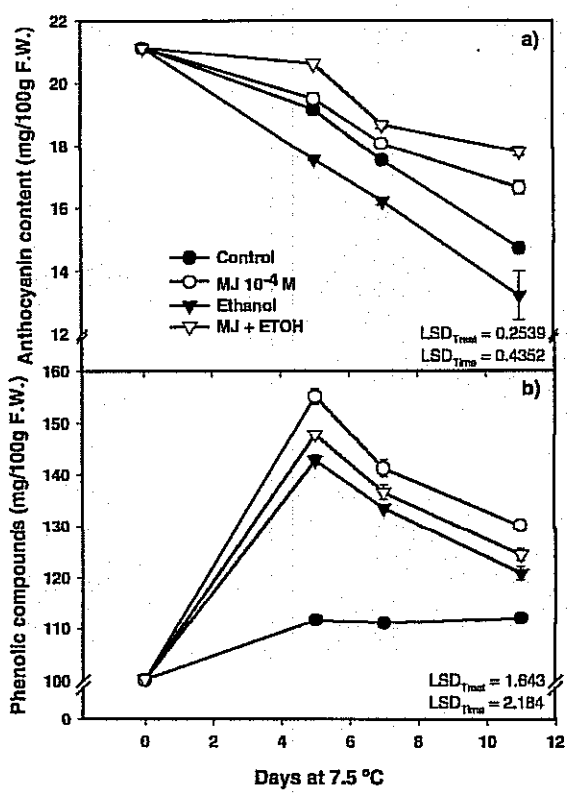

Fig. 3 Effect of ethanol, methyl jasmonate and their combination on total anthocyanin content (a) and total phenolic compounds (b) in strawberries (cv. Allstar) after 12 days of storage at 7.5 °C. Data points are means of three replicates and LSD at 0.05 levels for treatment and time are shown

and glucose are the two major sugars in strawberry fruit comprising more than 65% of the TSS [38].

Color

No differences were found in skin color L* parameter among different treatments (Table 1). However, there were differences in color values of C* and Hue angle. Fruit treated with MJ or MJ-ETOH were found to have the highest C* values as compared with controls and those treated with ethanol, indicating that berries from the treatment have more intense color than fruit from others treatments. The retention or promotion of fruit color by MJ treatment has also been reported in other fruits including apples [39] and mangoes [40].

Table 1 Effect of natural antimicrobial compounds on fruit color of strawberries after 11 days of storage at 7.5 °C

Treatment	Color measurement		
	*L**	*C**	*H°*
Control	38.92a[a]	38.68b	33.57a
MJ × 10^{-4} M	38.67a	40.07a	32.69ab
ETOH	38.54a	38.67b	33.56a
MJ-ETOH	38.02a	40.48a	32.08b

[a]Mean separation by LSD's test, $*p$=0.05

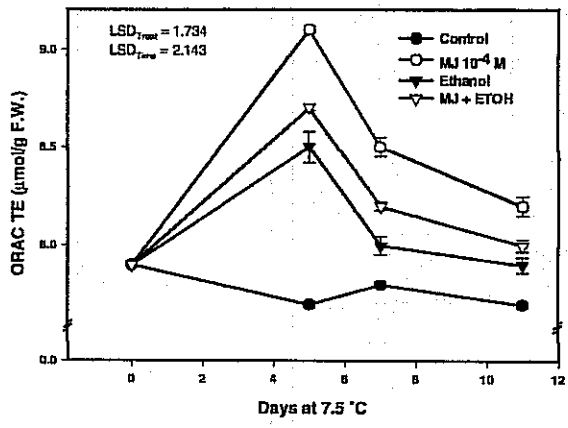

Fig. 4 Effect of ethanol, methyl jasmonate and their combination on antioxidant capacity in strawberries (cv. Allstar) after 12 days of storage at 7.5 °C. Data points are means of three replicates and LSD at 0.05 levels for treatment and time are shown

Anthocyanins are the major compounds present in strawberries. We observed differences in total anthocyanin content on treated fruit (Fig. 3a). This could be as a result of an increase in internal anthocyanin content in strawberry flesh. Changes in anthocyanins in external and internal tissues might not be necessary to be similar in response to different treatments. For example, high carbon dioxide

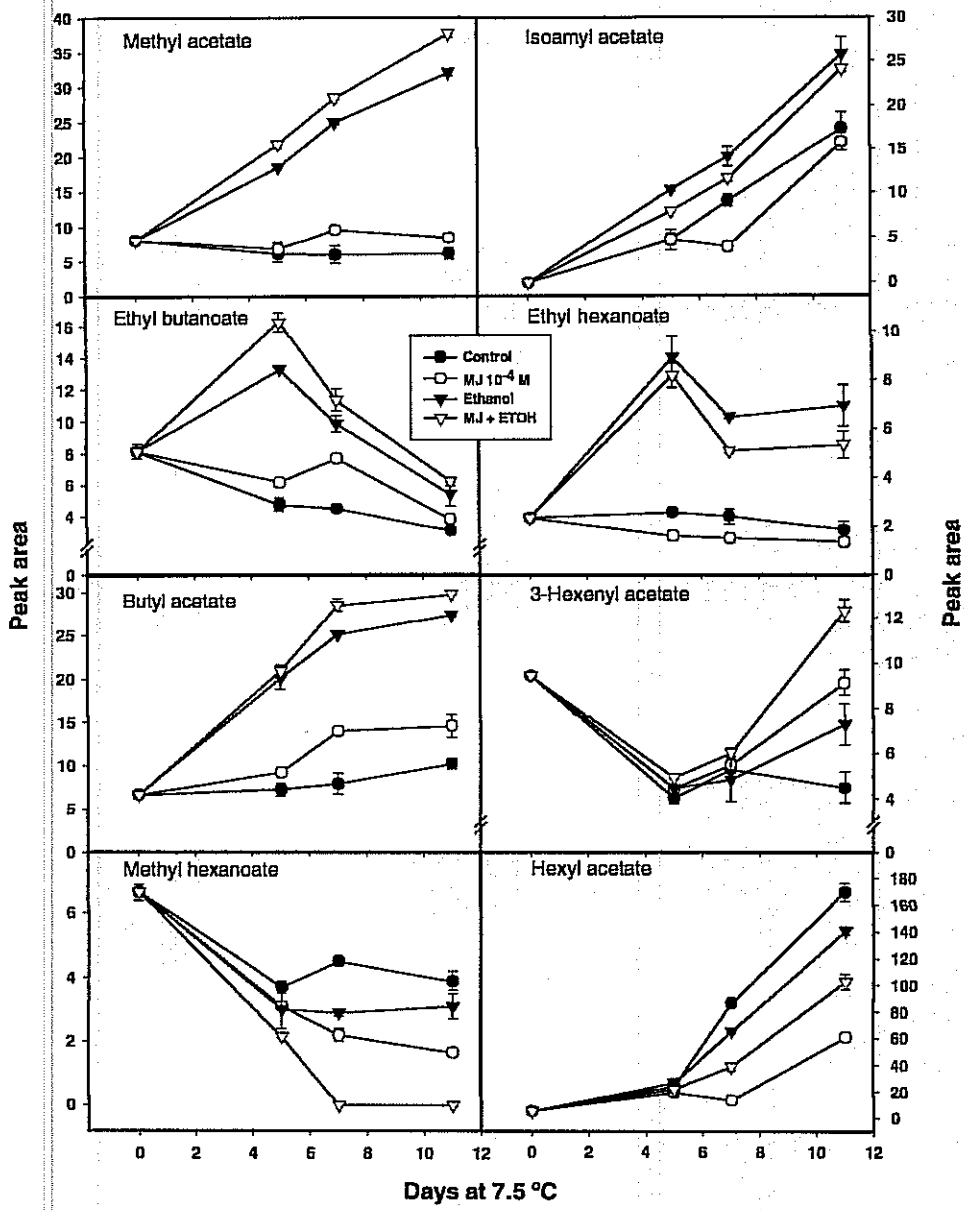

Fig. 5 Effect of ethanol, methyl jasmonate and their combination on main aroma constituents of 'Allstar' strawberry after 12 days of storage at 7.5 °C. Data points are means of two replicates and LSD at 0.05 levels for treatment and time are shown

decreased anthocyanins of internal tissues in strawberries while it did not affect the anthocyanins of external tissues [23]. Distribution of these pigments in the fruit tissues of different strawberry cultivars is not uniform; the internal color of 'Aromas' and 'Diamante' strawberries is mostly white, whereas it is light red in 'Selva' [6].

Total anthocyanin content and total phenolic compounds

Total anthocyanin content was significantly affected by treatment with these antimicrobials and the storage period. As observed in Fig. 3a, anthocyanin content decreased continuously in all treatments. However, anthocyanin content of fruits treated with ethanol vapors decreased at a higher rate. Strawberries treated with the combination MJ-ETOH showed the highest values at the end of storage period.

Figure 3b shows the effect of antimicrobial compounds on total phenolic compounds in strawberry fruit. A sharp increase on total phenolic compounds was observed during the first 5 days at 7.5 °C in berries treated with the volatiles used. Afterwards, a decrease was exhibited in these treatments. Untreated-berries showed the lowest values during the storage period. Both treatments and storage period showed a significant effect on the total phenolic compounds of strawberry fruit.

The anthocyanidin glycosides that contribute primarily to the red color of strawberries are: pelargonidin 3-glucoside and cyanidin 3-glucoside [41]. The antioxidant capacity of anthocyanidin may be one of their most significant biological properties [1]. Therefore, it is important to maintain

higher levels of these compounds during storage and shelf life period.

Oxygen radical absorbance capacity (ORAC) assay

In this study, we found that antimicrobials significantly affected the oxygen radical absorbance capacity of strawberry fruit (Fig. 4). ORAC values of control berries changed during storage at 7.5 °C. However, significant increases of ORAC values were observed in strawberries treated with MJ, MJ-ETOH and ethanol. One explanation for this difference could be associated with differences on total phenolic content.

Strawberry treated with MJ resulted in significant increase in total phenolic content (Fig. 3b). However, even though antioxidant activity was the highest in those berries treated with MJ, the combination MJ-ETOH was the most effective in extending the shelf life (Fig. 1). It appears that MJ and ETOH treatments had an additive effect in maintaining quality of strawberries but not in retaining high antioxidant activity. The possible mode of action of these compounds needs to be investigated when applied individually or in combination.

Volatile compounds

Strawberry volatile compounds were markedly affected by antimicrobial treatments (Fig. 5). Even though individual volatile compounds were affected to a different extent by the treatments, berries treated with ethanol and MJ-ETOH generally produced higher levels of these volatiles. Ethyl hexanoate, methyl acetate, and butyl acetate were the compounds most affected by these treatments. Methyl acetate, butyl acetate, and isoamyl acetate showed a steady increase in ethanol and ethanol-MJ treatments during storage period. Methyl hexanoate and hexyl acetate showed the highest values only in control fruit. A continuous decrease in methyl hexanoate was observed in all treatments. The 3-hexenyl acetate was increased by MJ-ETOH, MJ and ethanol treatments during the later part of storage.

The typical aroma of strawberries comes from not just one or a few impact volatile aroma compounds, but from numerous volatiles present at certain concentrations in a particular balance among them. Thus, strawberry aroma is the result of the combined perception of many aromatic constituents [42]. Although over 360 compounds have been identified in the aroma of strawberries [43, 44], only a few volatiles (primarily methyl and ethyl esters) appear to be the most important contributors to strawberry aroma [45, 44]. Generally, volatile esters that contribute to aroma, increased during storage [46]. Our study revealed that natural volatile compounds have a significant effect on the production of these volatile compounds. Strawberries treated with MJ-ETOH or ethanol produced higher levels of most of these methyl or ethyl esters during storage at 7.5 °C. Pelayo et al. [7] showed that storage temperatures affect aroma compounds production during storage period of strawberry fruit. In addition to storage temperature, other factors such as maturity, storage atmosphere, and light, have also been shown to affect the production of volatile aroma compounds in strawberries [47].

In conclusion, the data presented in this paper indicate that the treatment with MJ and ETOH significantly affected strawberry antioxidant capacity, anthocyanin, phenolic compounds, volatile compounds, and overall quality. Methyl jasmonate greatly increased the antioxidant capacity of strawberry and the combination of MJ-ETOH further extended shelf life of the fruit.

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