Natural volatile treatments increase free-radical scavenging capacity of strawberries and blackberries

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Abstract: Free-radical scavenging capacities of strawberries and blackberries treated with methyl jasmonate (MJ), allyl isothiocyanate (AITC), essential oil of *Melaleuca alternifolia* (tea-tree oil or TTO), and ethanol (EtOH) were investigated. All of these natural volatiles tested reduced the severity of decay in both strawberries and blackberries during storage at 10 °C as compared to the control. Most of these compounds enhanced antioxidant capacity and free-radical scavenging capacity, except the AITC treatment. The MJ treatment for strawberries and blackberries had the highest antioxidant capacity, expressed as oxygen radical absorbance capacity (ORAC) values, after 7 days of storage. Moreover, the MJ treatment promoted the antioxidant capacity in strawberries and blackberries as measured by the radical 2,2-di (4-tert-octylphenyl) -1-picrylhydrazyl (DPPH) and the radical cation 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt ($ABTS^{\bullet+}$) scavenging activity in both 7 and 14 days after storage. The MJ treatment also increased scavenging capacities on the superoxide radical ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), hydroxyl radical ($^{\bullet}OH$), and singlet oxygen ($^1O_2$) in strawberries and blackberries. Treatment with TTO or EtOH enhanced most of these free-radical scavenging capacities, except for H_2O_2 in strawberries, and for $O_2^{\bullet-}$ and $^1O_2$ in blackberries. These results indicated that all of the natural volatile compounds tested in this study, except AITC, promoted the antioxidant capacity and scavenging capacity of most major free radicals and, thus, helped to improve the physiology of berry fruits and enhanced their resistance to decay. While AITC was also very effective in reducing decay, its effect on free-radical scavenging capacity was inconsistent, suggesting that additional mechanisms may be involved in its inhibition of fungal growth.

Keywords: blackberries; strawberries; antioxidants; free-radical scavenging; natural volatile

INTRODUCTION

One important source of healthful antioxidants comes from fruits. Increasing evidence has shown that a high consumption of fruits and vegetables can reduce the risk of cancer and other degenerative diseases caused by oxidative stress.[1] It has been reported that berry fruits, which include strawberries and blackberries, contain high antioxidant capacities.[2] The antioxidants in fruit, such as anthocyanin and phenolic acid may help protect cells against the oxidative damage caused by free radicals.[3]

Reactive oxygen species (ROS), such as superoxide radicals ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), hydroxyl radicals ($^{\bullet}OH$) and singlet oxygen ($^1O_2$) are by-products of normal metabolism and generated when metabolism interacts with oxygen. Under stress conditions, those free radicals can be produced in large quantities. Excessive ROS can damage proteins, lipids, enzymes, DNA and RNA. This can lead to cell or tissue injury associated with degenerative diseases, and potentially disrupt functions and cause mutations.[4–6] In plants, a high ROS concentration leads to cellular damage and ultimately cell death, primarily through damage to the photosystem reaction center and to the lipid membrane. Therefore, plant cells need to be able to control the extra accumulation of ROS, which can be cytotoxic. To protect themselves against the harmful action of ROS, plants have developed detoxifying mechanisms or free-radical scavenging systems to keep low concentrations of ROS.[7,8]

Natural volatile compounds, such as methyl jasmonate (MJ), allyl isothiocyanate (AITC), ethanol (EtOH) and tea tree oil (TTO), have been reported to be effective in reducing decay and maintaining the quality of some fruits and vegetables. For example, when MJ was applied to fruits, such as grapefruit, papaya and guava, it prevented microbial invasion and chilling injury development.[9–11] MJ has been found to occur naturally in a wide range of higher plants.[12,13] Jasmonates were first detected as fragrant constituents of essential oils in *Jasminum*[14] and *Rosmarinum*.[15] MJ is a product of the enzymatic oxidation of polyunsaturated fatty acids with lipoxygenase as a pivotal enzyme in this biosynthetic pathway.[16] MJ has been shown to increase chilling tolerance of several plant species.[17,18] In addition, post-harvest decay of

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(*Received 15 March 2006; revised version received 21 August 2006; accepted 30 August 2006*)
Published online 19 April 2007; DOI: 10.1002/jsfa.2841

strawberries caused by *Botrytis cinerea* was reduced by exposure of the fruit to MJ vapor.[19] The storage life of fresh-cut celery sticks and bell pepper slices was extended because of the reduction of microbial growth and less physiological deterioration resulted from MJ treatment.[20] The effects of AITC, EtOH and TTO on fruit decay and antifungal activity have also been reported.[21,22] AITC is a constituent of cruciferous vegetables and is produced during the enzymatic degradation of glucosinolate.[23] It has been shown to have strong antimicrobial activity in both liquid and vapor forms.[24,25] The bactericidal activity of AITC against pathogens on iceberg lettuce and tomatoes has also been reported.[21] TTO is the essential oil of *Melaleuca alternifolia*. It is a naturally occurring antimicrobial agent and is effective in inhibiting the storage pathogen *Botrytis cinerea* on Dutch white cabbage (*Brassica oleracea*).[26] Its vapor phase has shown a high level of antifungal activity in 15 common post-harvest pathogens on a variety of crops.[22] However, little information is available on the relationship between these natural volatile compounds and their antioxidant activity and free-radical scavenging capacity in strawberries and blackberries.

The aim of this study was to evaluate the effect of various natural volatiles on decay and the antioxidant system such as oxygen radical absorbance capacity (ORAC), radical 2,2-di-(4-tert-octylphenyl)-1-picrylhydrazyl (DPPH), radical cation 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt ($ABTS^{\bullet+}$) in strawberries and blackberries. The changes of free-radical scavenging capacity in strawberries and blackberries treated with natural volatile compounds were also determined.

MATERIALS AND METHODS

Chemicals

Ascorbate, chlorogenic acid, β-carotene, ferrous sulfate (heptahydrate), histidine, hydrogen peroxide (30% w/w), hydroxylamine hydrochloride, *N*,*N*-dimethyl-*p*-nitrosoaniline, β-naphthyamine, sodium nitrite, sodium tungstate dihydrate, sulfanilic acid, xanthine, and xanthine oxide were purchased from Sigma (St Louis, MO, USA). Ether, sodium hypochlorite, α-tocopherol, and titanium (IV) chloride were purchased from Aldrich (Milwaukee, WI, USA). Salicylic acid was purchased from Fisher (Pittsburgh, PA, USA).

Plant materials and treatments with natural volatile compounds

Strawberries (*Fragaria x ananassas* cv. Earliglow) and blackberries (*Rubus* sp. cv. Triple Crown) 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. The selected fruit were randomized before being used for treatments. Thirty fruit of blackberries and 15 fruit of strawberries were placed into 1 L polystyrene containers with snap-on lids. The volatile compounds used in this study include methyl jasmonate ($22.4\,\mu L\,L^{-1}$), essential oil of the tea tree (*Melaleuca alternifoli*, $100\,\mu L\,L^{-1}$), absolute ethyl alcohol ($200\,\mu L\,L^{-1}$), and allyl isothiocyanate (AITC, $5\,\mu L\,L^{-1}$). Concentrations of these volatiles employed in this experiment were previously demonstrated as the optimum strength for reducing decay in various commodities.[17,19,20,27] The specified volume of each volatile compound was spotted onto a piece of filter paper which was subsequently hung inside the plastic containers just before the lids were closed. The containers were then stored at 10 °C. There were three containers for each treatment. Control samples were handled similarly but without the volatile treatment. The antioxidant capacity was determined after 7 and 14 days of storage at 10 °C and the severity of decay was visually evaluated after 14 days of storage.

Berry fruit sample preparation for assay

For the assays of ORAC, DPPH and $ABTS^{\bullet+}$ scavenging activity, blackberries and strawberries were extracted with 80% acetone (containing 0.2% formic acid) using a Polytron (Brinkmann Instruments, Inc., Westbury, NY, USA). The homogenized samples from the acetone extraction were then centrifuged at $14\,000 \times g$ for 20 min at 4 °C. The supernatants were transferred to vials, stored at −80 °C, and later used for ORAC, DPPH and $ABTS^{\bullet+}$ analysis.

To prepare the juice samples for superoxide radicals ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), hydroxyl radicals ($^{\bullet}OH$), and singlet oxygen ($^1O_2$), blackberries and strawberries from each treatment were pulverized and then centrifuged at $14\,000 \times g$ for 20 min at 4 °C. The supernatants were transferred to vials stored at −80 °C until used for analysis.

Free-radical measurements

The ORAC assay was carried out using a high-throughput instrument platform consisting of a robotic eight-channel liquid handling system and a microplate fluorescence reader.[28] The automated sample preparation was performed using a Precision 2000 instrument (Bio-Tek Instruments, Inc., Winooski, VT, USA). The sample series dilution sequence was programmed and controlled by the Precision Power software. The free-radical DPPH scavenging capacity of berry juice was evaluated as previously described.[29] Using a calibration curve with different amounts of DPPH, the ED_{50} was calculated. The ED_{50} is the concentration of an antioxidant that is required to quench 50% of the initial DPPH radicals under the experimental conditions given. Radical cation scavenging capacity of berry extract was examined against $ABTS^{\bullet+}$ generated by chemical methods.[30]

The assay for superoxide radicals ($O_2^{\bullet-}$) was performed using the methods of Gutteridge.[31] The assay

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DOI: 10.1002/jsfa

for hydrogen peroxide was carried out following procedures previously described by Patterson *et al*.[32] The assay for hydroxyl radicals ($^{\bullet}OH$) was performed using the methods of Richmond *et al*.[33] The production of singlet oxygen ($^1O_2$) by sodium hypochloride and H_2O_2 was determined by using a spectrophotometric method according to Chakraborty and Tripathy.[34]

Statistical analysis

Data were subjected to analysis of variance using NCSS (NCSS 97, Kaysville, UT, USA). The values of oxygen radical absorbance capacity ORAC, DPPH, $ABTS^{\bullet+}$, $O_2^{\bullet-}$, H_2O_2, $^{\bullet}OH$, and $^1O_2$ radical absorbance capacity and their relative scavenging efficiency were evaluated by the Tukey–Kramer multiple-comparison test used in NCSS. Differences at $P \leq 0.05$ were considered significant.

RESULTS AND DISCUSSION

Decay evaluation

The severity of decay in both strawberries and blackberries after 14 days of storage at 10 °C was reduced by all the natural volatiles tested in this study (Table 1). AITC was the most effective compound among all the treatments used in retarding decay. AITC is a natural component in plant tissue and has been used as a food additive responsible for antimicrobial activity.[14] MJ also significantly inhibited the development of fungal decay as compared to the control. Although not as effective, EtOH and TTO also had a lower percentage of decay than the control in both strawberries and blackberries. Concentrations of O_2 and CO_2 in the containers were measured and found that O_2 generally stayed above 19% and CO_2 stayed below 1% during storage (data not shown), therefore they were not a factor in affecting the decay.

Antioxidant capacity

Common oxidants produced in organisms include reactive oxygen species (ROS), such as superoxide radicals ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), hydroxyl radicals ($^{\bullet}OH$) and singlet oxygen ($^1O_2$). A variety of stress conditions, both biotic and abiotic, trigger the increase of ROS. The imbalance in production and metabolism of ROS can cause oxidative stress and lead to cell death.[35] For this reason, recent studies are interested in antioxidants because they may help protect the body against ROS damage.[36] There are many techniques used to evaluate antioxidant capacity in plants. In our study, we used ORAC, $ABTS^{\bullet+}$, DPPH and also evaluated the free-radical scavenging capacity of $O_2^{\bullet-}$, H_2O_2, $^{\bullet}OH$ and $^1O_2$ in strawberries and blackberries treated with different natural volatile compounds. Strawberries treated with MJ showed the highest ORAC values among all the treatments both after 7 and 14 days of storage at 10 °C (Fig. 1). In contrast, AITC treatment had the lowest ORAC values when strawberries were stored for 7 and 14 days. These natural volatile compounds had similar effects on blackberries. However, the ORAC values of blackberries were higher overall when compared to strawberries. The results are consistent with a previous study, in that blackberries at ripe stage had higher ORAC values than strawberries of the same maturity stage.[37]

The other assay used for determining antioxidant activities of strawberries and blackberries was the DPPH scavenging capacity assay. In our study, the scavenging capacity for the DPPH radical was expressed as the ED_{50}, which is the concentration of an antioxidant that is required to quench 50% of the initial DPPH radicals under the experimental conditions given and also expressed as percent inhibition as shown in Figs 2 and 3, respectively. In both strawberries and blackberries, the MJ treatment had the best result for DPPH scavenging capacity. The result showed that strawberries need higher amounts of antioxidant to quench 50% of the initial DPPH radicals than in blackberries. Additionally, strawberries treated with MJ needed the lowest amount of antioxidant to quench 50% of initial DPPH. In percent inhibition of DPPH, both strawberries and blackberries showed the same pattern and the results were similar for both 7 and 14 days of storage.

The $ABTS^{\bullet+}$ scavenging activity was another method used in our study to measure the antioxidant activity in strawberries and blackberries. In our study, we used the decolorization assay based on the inhibition by antioxidants of absorbance of radical cation ($ABTS^{\bullet+}$), which was generated through chemical reduction by manganese dioxide.[38] Strawberries and blackberries treated with MJ stored for 7 days had the highest radical cation $ABTS^{\bullet+}$ scavenging capacity, followed by EtOH, AITC and TTO (Fig. 4). The results indicate that these natural volatile compounds significantly changed the $ABTS^{\bullet+}$ scavenging capacity in blackberries and strawberries. In comparison, the $ABTS^{\bullet+}$ scavenging activities of blackberries was higher than those of strawberries after both 7 and 14 days of storage. After 14 days of storage, $ABTS^{\bullet+}$ scavenging activity decreased in every treatment in both strawberries and blackberries.

Table 1. Effect of various volatile compounds on the severity of decay in strawberries and blackberries after 14 days of storage at 10 °C

Volatile compound	Concentration (μL L^{-1})	% Decay[a,b] Strawberries	% Decay[a,b] Blackberries
Control	–	37.4[a]	58.6[a]
Methyl jasmonate	22.4	19.8[c]	36.3[c]
Ethanol	200	22.9[bc]	47.2[b]
Tea tree oil	100	24.7[b]	45.8b
Allyl isothiocyanate	5	15.2[d]	29.4[d]

[a] Severity of decay is expressed as percent of fruit showing fungal symptoms.
[b] Means in a column followed by the same letter are not significantly different at $P \leq 0.05$.

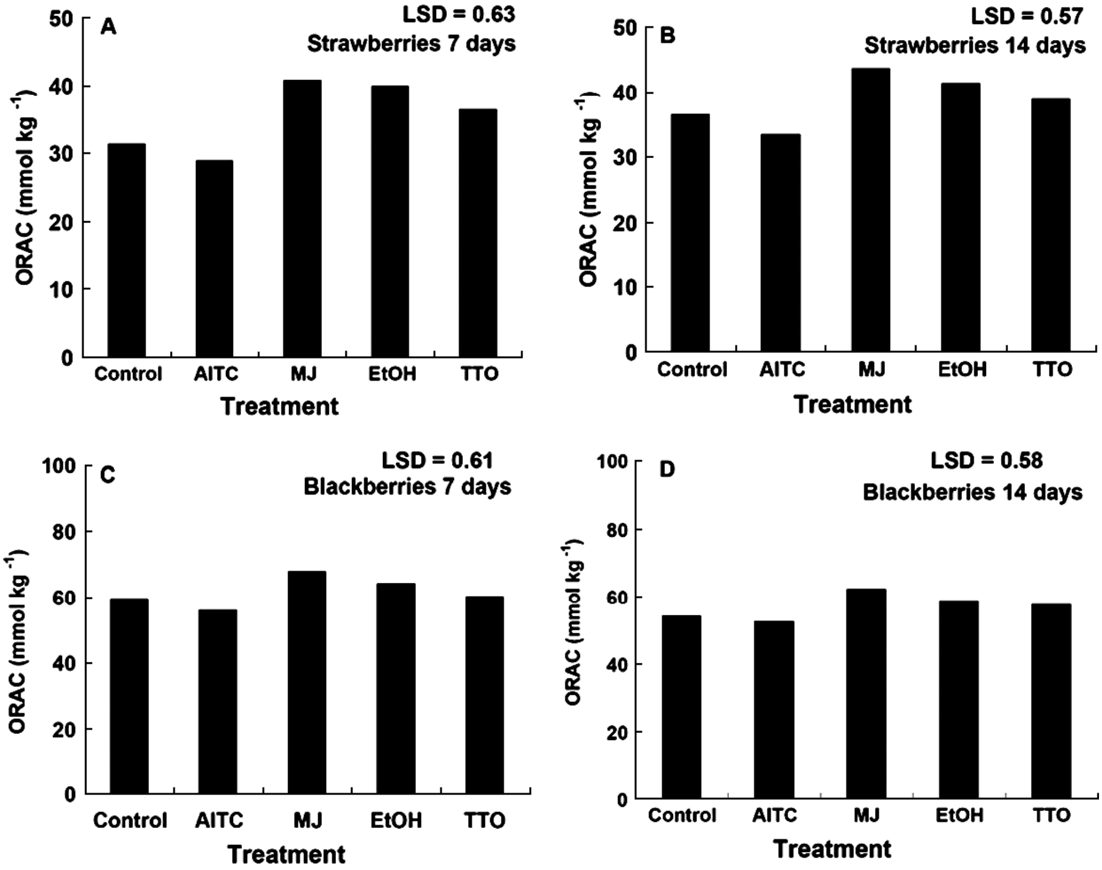

Figure 1. Oxygen radical absorbance capacity (ORAC) values in strawberries and blackberries treated with different natural volatile compounds and stored at 10 °C for 7 and 14 days.

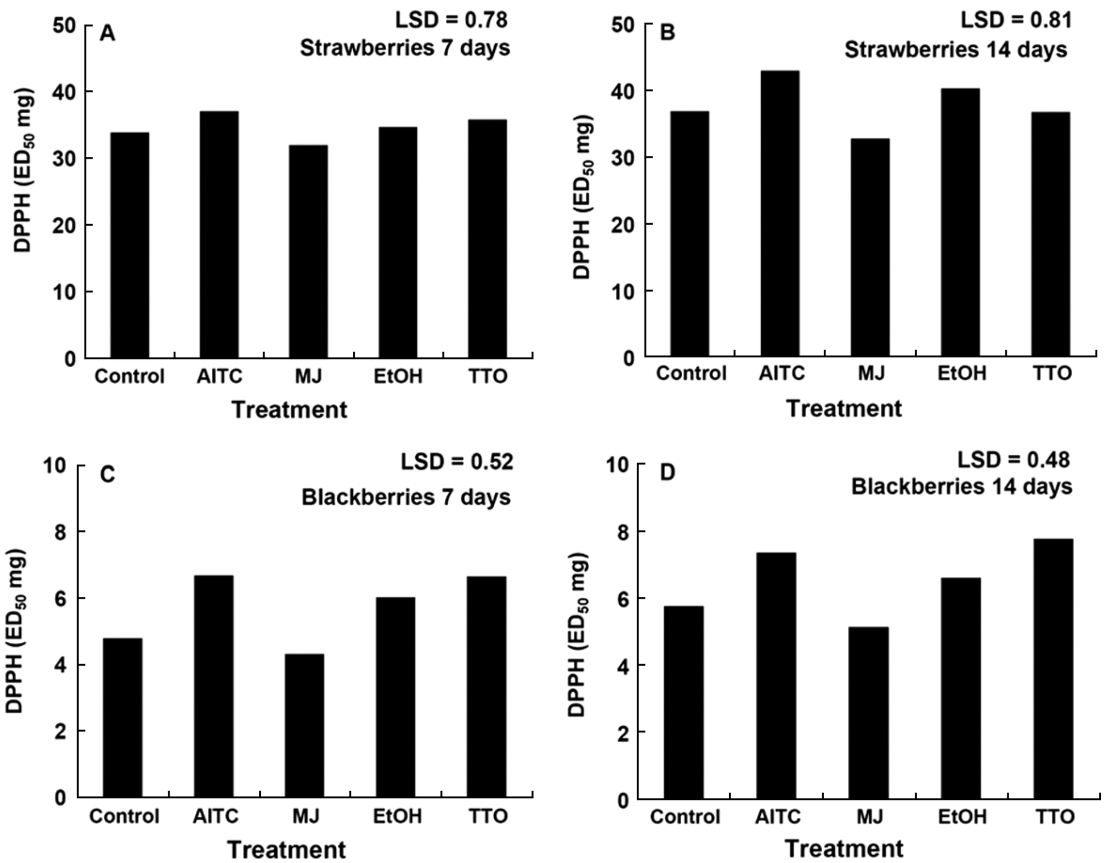

Figure 2. The radical 2,2-di-(4-tert-octylphenyl)-1-picrylhydrazyl (DPPH) scavenging capacity expressed as ED_{50} in strawberries and blackberries treated with various natural volatile compounds and stored at 10 °C for 7 and 14 days.

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DOI: 10.1002/jsfa

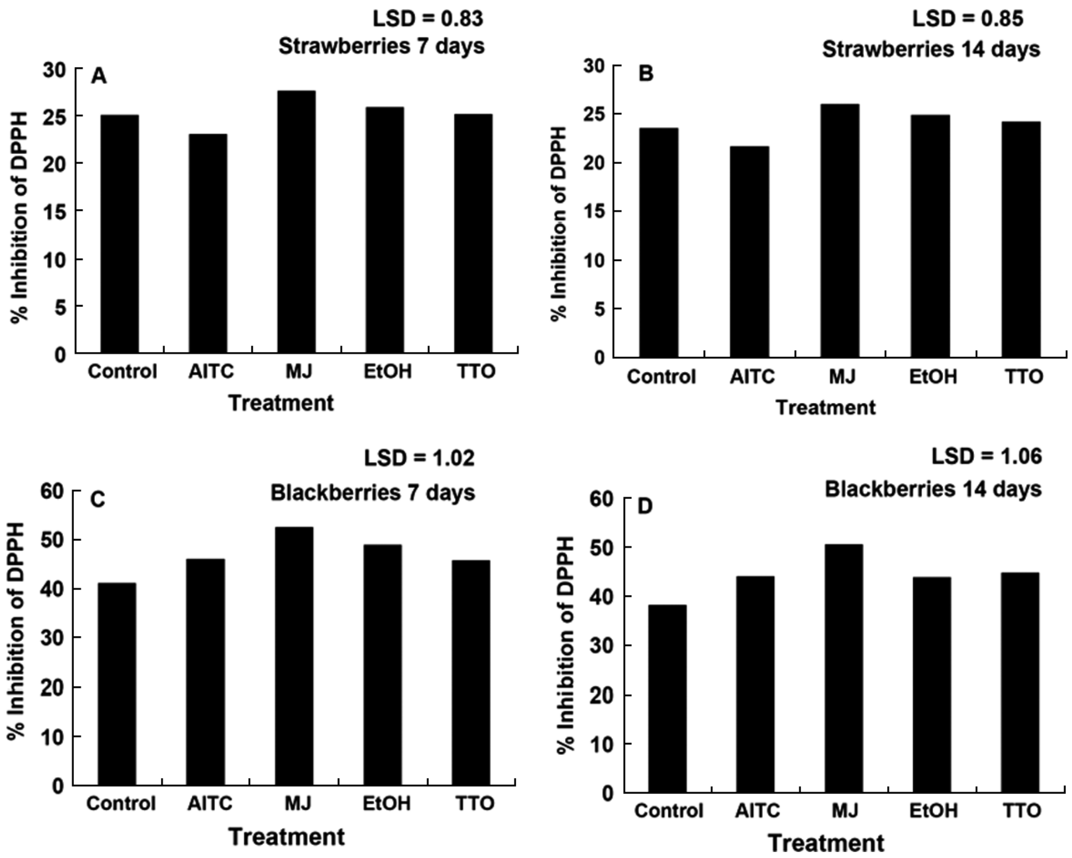

Figure 3. The radical 2, 2-di-(4-tert-octylphenyl)-1-picrylhydrazyl (DPPH) scavenging capacity expressed as the percent inhibition in strawberries and blackberries treated with various natural volatile compounds and stored at 10 °C for 7 and 14 days.

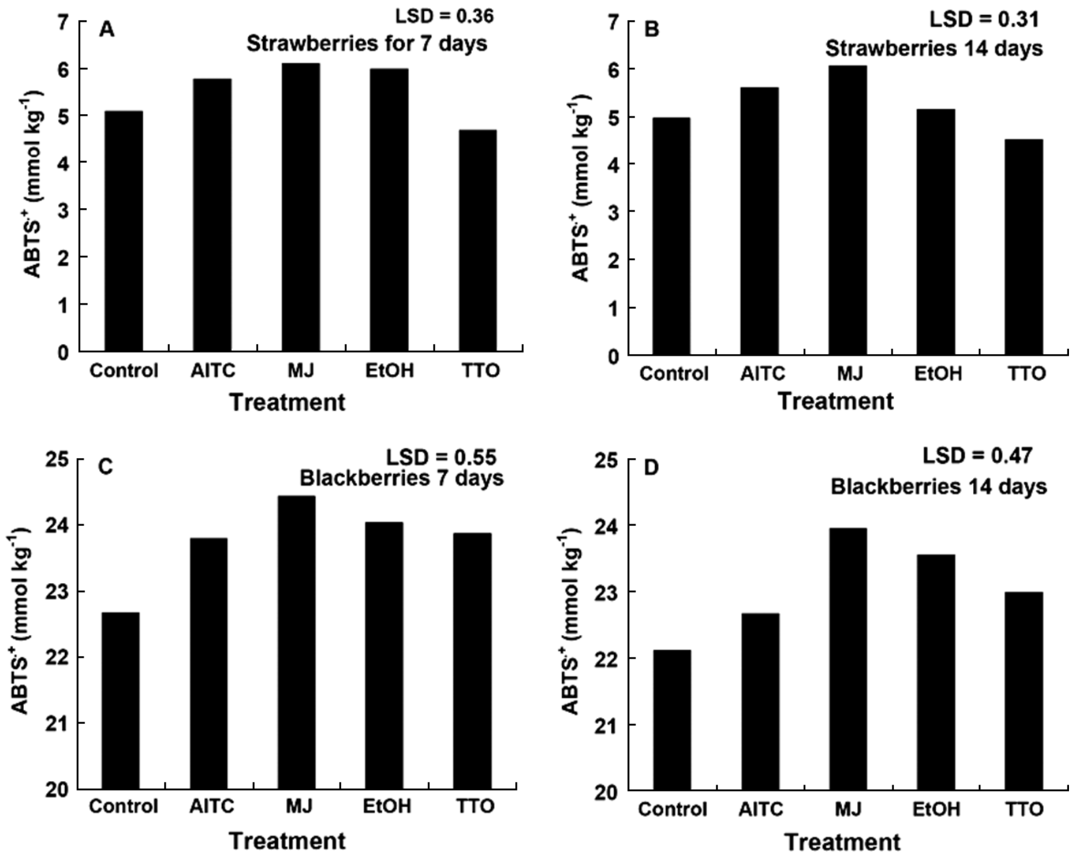

Figure 4. The radical cation 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid diammonium salt ($ABTS^{\bullet+}$) scavenging capacity in strawberries and blackberries treated with different natural volatile compounds and stored at 10 °C for 7 and 14 days.

DOI: 10.1002/jsfa

The $O_2^{\bullet-}$ is one of the important radicals which has been studied in plants. $O_2^{\bullet-}$ is protonated from hydroperoxyl ($HO_2^{\bullet}$) and hydroxyl radicals ($^{\bullet}OH$) which are relatively short-lived.[39] Our study showed that different natural volatile compounds significantly affected the $O_2^{\bullet-}$ scavenging capacity in strawberries and blackberries as shown in Fig. 5 and Table 2.

After both 7 and 14 days of storage, strawberries treated with MJ, EtOH and TTO had a higher scavenging capacity (Fig. 5) and percent inhibition (Table 2) for superoxide radical ($O_2^{\bullet-}$) than for AITC and control. Blackberries showed much higher levels of superoxide radical ($O_2^{\bullet-}$) scavenging capacity than strawberries in every treatment. Blackberries treated with AITC and MJ had higher superoxide radical ($O_2^{\bullet-}$) scavenging capacity than other treatments.

Table 2. Percent inhibition for superoxide radical ($O_2^{\bullet-}$) of strawberries and blackberries treated with allyl isothiocyanate (AITC), methyl jasmonate (MJ), ethanol (EtOH) and tea-tree oil (TTO) stored at 10 °C for 7 and 14 days

	% Inhibition			
	Strawberries		Blackberries	
Treatment	7 days	14 days	7 days	14 days
Control	15.93	12.85	45.79	47.54
AITC	17.25	13.07	46.23	47.98
MJ	23.2	18.57	48.64	51.93
EtOH	19.01	17.25	46.23	46.45
TTO	19.45	15.05	47.32	46.67
LSD	3.74	4.64	4.27	4.01

The H_2O_2 scavenging capacity in this study was measured by the direct reaction of H_2O_2 and titanium(IV) and the precipitation of the Ti–H_2O_2 complex dissolved in sulfuric acid and measured at 410 nm as in the method used by Wang and Jiao.[2] Strawberries and blackberries treated with different natural volatile treatment showed varying results in hydrogen peroxide scavenging capacity. Strawberries treated with MJ and stored for 7 days had the highest capacity for scavenging hydrogen peroxide as compared with other treatments (Fig. 6). The generation of H_2O_2 is known to play an important part in the killing of several bacterial and fungal strains as mention by Sánchez-Moreno.[38] The results show that the compounds tested could change the H_2O_2 scavenging capacity for strawberries and blackberries. The TTO and EtOH treatments had similar capacities for scavenging hydrogen peroxide. The hydrogen peroxide scavenging capacity for most of the natural volatile treatments decreased when strawberries were kept for a longer period of time, except the MJ treatment (Fig. 6). The percent inhibition for hydrogen peroxide in both strawberries and blackberries treated with various natural volatile compounds also decreased with storage duration as shown in Table 3. Blackberries had a higher hydrogen peroxide scavenging capacity as compared to strawberries after both 7 and 14 days of storage (Fig. 6).

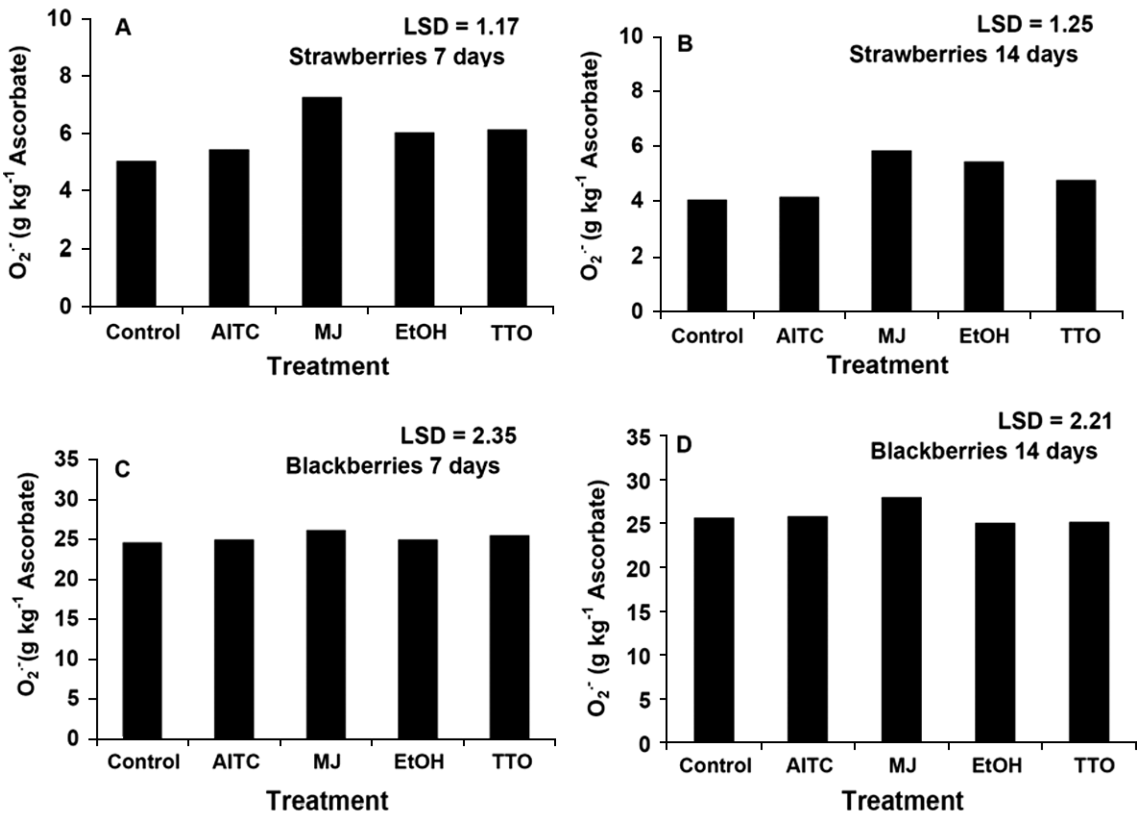

Figure 5. Superoxide radicals ($O_2^{\bullet-}$) scavenging capacity in strawberries and blackberries treated with different natural volatile compounds and stored at 10 °C for 7 and 14 days.

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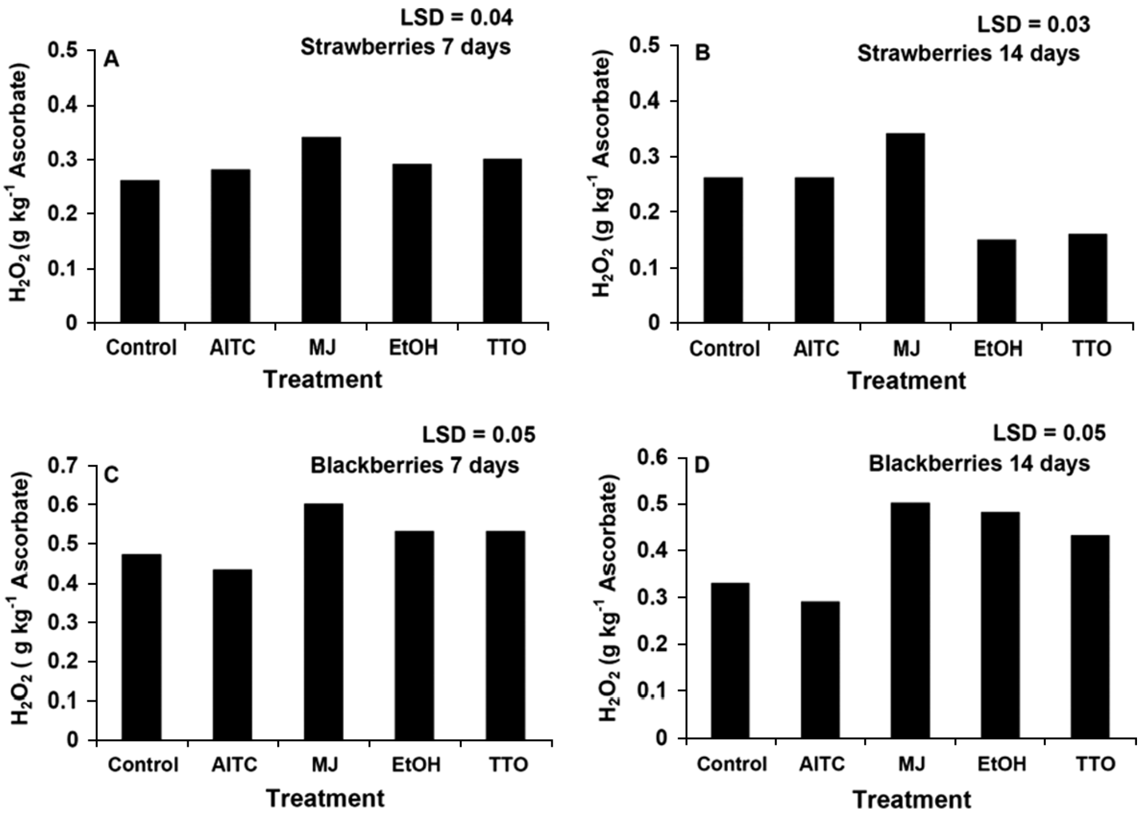

Figure 6. Hydrogen peroxide (H_2O_2) scavenging capacity in strawberries and blackberries treated with various natural volatile compounds and stored at 10 °C for 7 and 14 days.

Table 3. Percent inhibition for hydrogen peroxide (H_2O_2) of strawberries and blackberries treated with allyl isothiocyanate (AITC), methyl jasmonate (MJ), ethanol (EtOH) and tea-tree oil (TTO) after 7 and 14 days of storage at 10 °C

	% Inhibition			
	Strawberries		Blackberries	
Treatment	7 days	14 days	7 days	14 days
Control	12.57	12.38	12.55	8.97
AITC	13.42	12.29	11.51	7.83
MJ	16.23	16.32	16.04	13.40
EtOH	14.07	7.03	14.34	12.83
TTO	14.35	7.87	14.15	11.42
LSD	2.10	1.56	2.32	1.42

Among ROS, the •OH is toxic due to its ability to react spontaneously with organic molecules such as phenols, fatty acids, proteins and nucleic acids.[39–41] In our study, strawberries treated with MJ had the highest scavenging capacity for hydroxyl radical (Fig. 7). The scavenging capacities for the hydroxyl radical in all other treatments were comparable to that of the control after 7 days of storage. The •OH scavenging capacity decreased with time in storage in every treatment as shown in Fig. 7. Similarly, treated blackberries showed a similar scavenging capacity as compared to control with the exception of the MJ treatment. The •OH scavenging capacity of blackberries also showed a significant decrease in every treatment after a period of storage. The MJ treatment had the highest •OH scavenging capacity in blackberries after both 7 days and 14 days in storage. The percent inhibition for hydroxyl radical in strawberries and blackberries also showed significant decreases in every treatment during storage (Table 4).

Different from the other free-radical scavenging capacities measured, the singlet oxygen ($^1O_2$) scavenging capacity was higher in strawberries than in blackberries. Strawberries treated with MJ had the highest $^1O_2$ scavenging capacity, followed by TTO and EtOH treatments (Fig. 8). AITC treatment had the lowest $^1O_2$ scavenging capacity which was comparable to that of the control. The $^1O_2$ scavenging

Table 4. Percent inhibition for hydroxyl radical (•OH) of strawberries and blackberries treated with allyl isothiocyanate (AITC), methyl jasmonate (MJ), ethanol (EtOH) and tea-tree oil (TTO) stored at 10 °C for 7 and 14 days

	% Inhibition			
	Strawberries		Blackberries	
Treatment	7 days	14 days	7 days	14 days
Control	44.11	24.10	64.78	18.84
AITC	37.69	22.59	57.89	24.20
MJ	52.04	42.98	67.08	52.15
EtOH	42.22	35.05	54.83	33.39
TTO	41.84	32.02	55.59	36.83
LSD	8.52	9.34	7.27	7.89

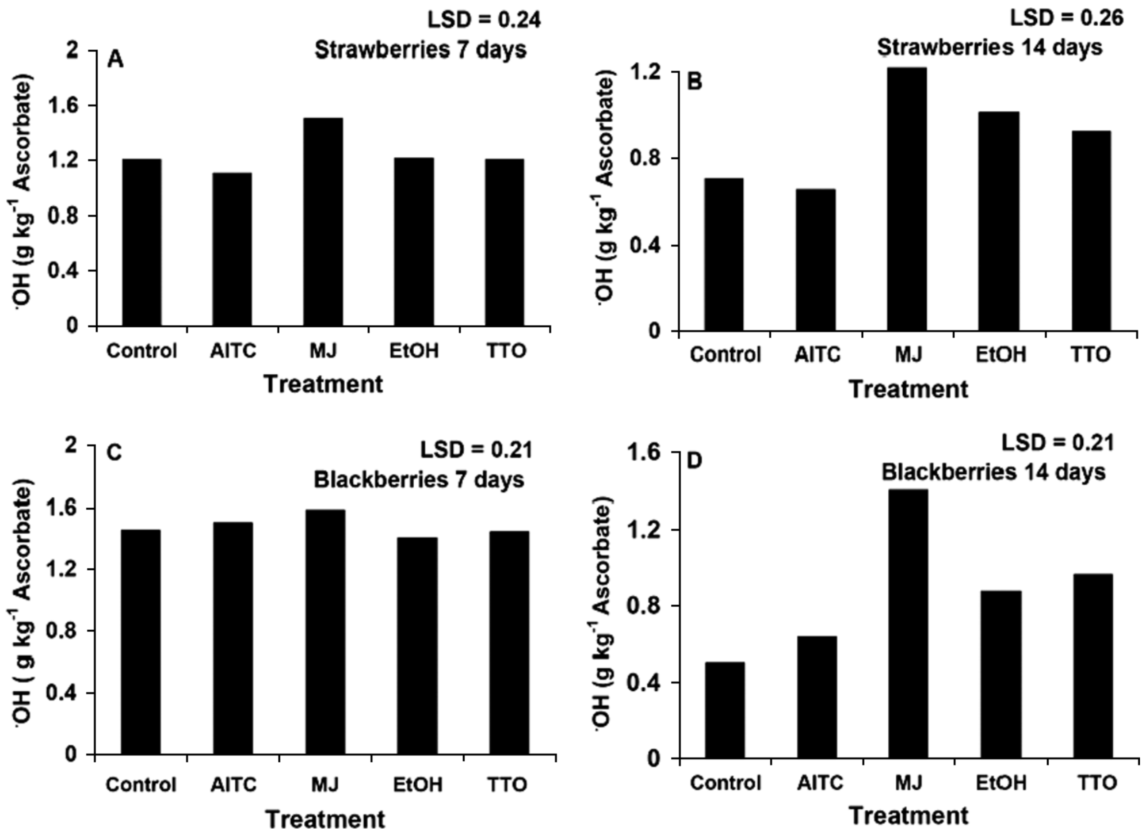

Figure 7. Hydroxyl radicals ($\bullet OH^-$) scavenging capacity in strawberries and blackberries treated with different natural volatile compounds and stored at 10 °C for 7 and 14 days.

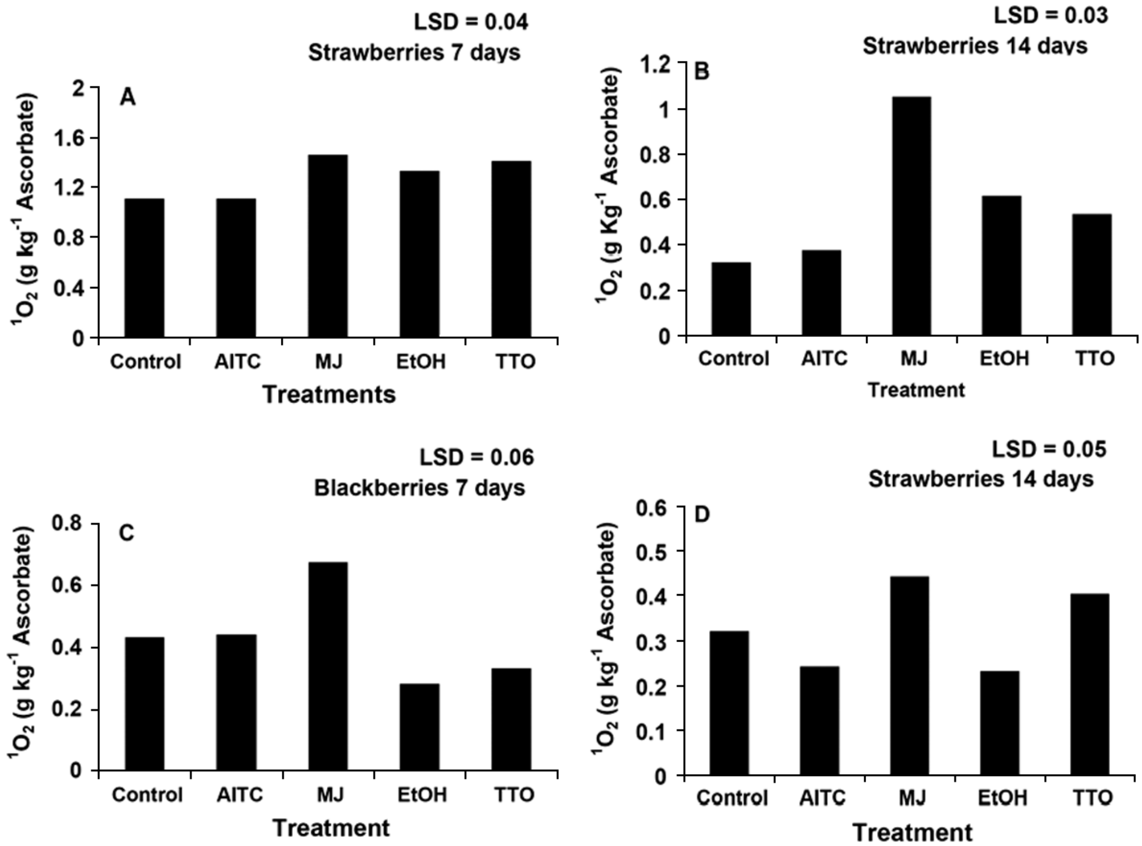

Figure 8. Singlet oxygen ($^1O_2$) scavenging capacity in strawberries and blackberries treated with various natural volatile compounds and stored at 10 °C for 7 and 14 days.

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Table 5. Percent inhibition for singlet oxygen ($^1O_2$) of strawberries and blackberries treated with allyl isothiocyanate (AITC), methyl jasmonate (MJ), ethanol (EtOH) and tea-tree oil (TTO) after 7 and 14 days of storage at 10 °C

	% Inhibition			
	Strawberries		Blackberries	
Treatment	7 days	14 days	7 days	14 days
Control	17.47	5.45	12.88	8.98
AITC	18.27	6.48	13.34	7.26
MJ	25.25	18.16	20.46	10.93
EtOH	22.85	10.6	8.52	7.03
TTO	24.22	9.11	10.01	10.13
LSD	4.78	4.81	2.26	3.56

capacity of all treated strawberries decreased noticeably from 7 days of storage to 14 days of storage. The $^1O_2$ scavenging capacity in treated blackberries showed the same trend as those in strawberries. The MJ treatment in blackberries stored for 7 days had the highest $^1O_2$ scavenging capacity, followed by AITC, TTO, and EtOH treatments (Fig. 8). The scavenging capacity and the percent of inhibition (Table 5) for $^1O_2$ decreased in every treatment in blackberries from 7 days of storage to 14 days of storage.

CONCLUSIONS

All of the natural volatiles tested reduced the severity of decay in both strawberries and blackberries during storage at 10 °C. The MJ treatment was the most effective in promoting the antioxidant capacity and free-radical scavenging capacity among all the treatments in both strawberries and blackberries. Treatments with TTO or EtOH also enhance the antioxidant system. It is possible that the elevated capacity in scavenging various free radicals by these natural volatile compounds may increase the resistance of tissues to decay. Although AITC treatment promoted the $O_2^{\bullet -}$ scavenging capacity in blackberries after 14 days of storage, it had little effect on the scavenging capacities of other radicals. It is most likely that AITC may retard fungal growth through additional mechanisms. Overall, our study showed that it is possible to reduce decay and extend the storage life of strawberries and blackberries by treatment with these natural volatile compounds.

ACKNOWLEDGEMENT

The authors would like to thank Sue Kim for her technical assistance.

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