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Research Article

Preserving Quality and Nutrient Value of Blackberries by Essential Oil Wash and Vapor Treatments

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Abstract

Blackberries have a short life as they are highly susceptible to contamination of spoilage microorganisms. In this study, two essential oils were used to treat fresh blackberries for the extension of shelf-life. The application methods of essential oils on blackberries that were evaluated included: (1) Carvacrol (COL) used as a wash solution, (2) ally isothiocyanate (AIT) used as a vapor, and (3) COL wash followed by AIT vapor treatment (COL+AIT) was also investigated. The survival of total bacterial populations, yeast and mold populations, respiration rate, and quality and nutrient changes were determined during 12 d storage at 10° C. The washing treatment reduced microbial loads by 2 logs initially but could not inhibit their outgrowth during storage, while AIT vapor treatment and the combination of COL wash and AIT vapor treatment completely inhibited the growth of bacteria and mold and yeast. AIT vapor alone or in combination with COL wash significantly reduced the respiration rate of blackberries, hence, maintaining the quality and nutritional values through 12 d storage. The results suggest that the combination of COL washing and AIT vapor is not necessary and AIT vapor treatment alone appears to be very promising for extending the shelf-life of blackberries.

Keywords: Blackberries; Quality; Nutrition; Essential oils; Wash; Vapor

Introduction

Blackberries have a sweet, tart flavor, and are also rich in vitamins and antioxidants. They are consumed in foods such as salads, fruit smoothies, juices and yogurt. However, blackberries are highly perishable, and up to 40% of blackberries are lost from the post-harvest stage to the time they appear on the market due to improper handling [1]. Most blackberries destined for sale on the fresh market become unmarketable after two to three days due to decay [2]. In addition, possible pathogenic contamination is also a food safety concern since they are eaten fresh. Standard small fruit production practices discourage growers from washing these fruits during the post-harvest stage. The presence of water on the surface of small fruits may promote the growth of pathogenic bacteria, increasing the risk of foodborne disease [3]. It is necessary to develop an intervention method which inactivates populations of undesirable microorganisms and preserves the nutritional attributes of blackberries.

Essential oils (EO) are a complex of volatile, naturally occurring substances, such as terpenes and other aromatic compounds, which are produced in different parts of the plant and can be extracted from plant material [4]. Carvacrol (COL) is an example of an essential oil which is derived from the leaves of the *Origanum vulgare* plant [5]. It has been shown to be an effective antifungal agent against a wide range of fungi, including *Botrytis cinerea* [6], *Penicillium digitatum*, and *Penicillium italicum* [7]. Studies have shown that COL can reduce the rate of decay in peaches [8] and lemons [7], allowing for their shelf-life to be extended.

Allyl isothiocyanate (AIT) is another example of an essential oil derived from plants. It is a natural antimicrobial agent for food preservation and its volatility and strong antimicrobial activity are well known. Previous studies have shown that AIT can be incorporated into edible food coatings or antimicrobial packaging films in order to reduce populations of pathogenic bacteria and extend the shelf-life of various foods, such as cantaloupe [9], tomatoes [10], ready-to-eat meat [11-13], and shrimp. AIT and COL have been classified as generally-recognized-as-safe (GRAS) by the U.S. FDA (U.S. Food and Drug Administration) [14]. However, in order to achieve the desired antimicrobial effect, large amounts of AIT in the liquid phase must be directly applied on the surface of foods, which could negatively affect food sensory attributes due to the strong, pungent. The antimicrobial efficacy of essential oils in foods can be increased when the lowest possible concentrations are used. One way to do this is by application in the vapor phase. Several studies have demonstrated that application of essential oils in the vapor phase is highly effective against populations of spoilage microorganisms and foodborne pathogens, resulting in fewer detrimental effects on the sensory attributes of foods, since relatively lower concentrations are used as compared to application in the liquid phase [15-19]. Limited information is available on the use of AIT vapor treatment in combination with COL washing for small fruits such as blackberries. Therefore, in this study, we investigated the effects of various application methods (wash, gaseous application, or a combination of these methods) of essential oils on microbial populations in blackberries and on their shelf life.

Materials and Methods

Blackberries

Fresh blackberries were purchased from a local grocery store and stored in a refrigerator at 4°C for up to three days before experimental use. Blackberry fruits to be used in the study were selected based on uniform size, shape, color, and lack of physical injury or presence of disease.

Treatments, package and storage

Carvacrol washing treatment

Blackberries (100 g) were washed for 2 min in a solution consisting of 40 µl/100 ml (400 ppm) carvacrol (>98% purity, Sigma Aldrich, St. Louis, MO, USA)+2 mg/ml citric acid (Fisher Scientific, Fairlawn, NJ, USA), dried under a biological laminar flow hood for 30 min, placed in a 8 oz clam shell box (Genpak, Glens Falls, NY, USA) with a lid and stored in a refrigerator at 4°C. Blackberries washed with 2 mg/ml citric acid solution served as controls (CK).

Allyl isothiocyanate vapor treatment:

Blackberries (100 g) were placed in an 8 oz clam shell box with a lid. A 1.0×1.0 cm^2 piece of filter paper treated with 1.5 µl of liquid AIT (>95% purity, Sigma Aldrich, St. Louis, MO, USA) was inserted inside the plastic box, after which the lid of the box was immediately closed and blackberry samples were placed in a refrigerator at 4°C.

Combined treatment of carvacrol washing with allyl isothiocyanate vaporous release:

Blackberries (100 g) were washed for 2 min in a solution consisting of 40 µl/100 ml (400 ppm) carvacrol + 2 mg/ml citric acid, dried in the laminar flow hood for 30 min, and then placed in a 8 oz clam shell box with a lid. A 1.0×1.0 cm^2 piece of filter paper treated with 1.5 µl of liquid AIT (>95% purity) was also inserted inside the plastic box, after which the lid was immediately closed and blackberry samples were placed at 4°C.

The selected treatment conditions (concentrations of carvacrol, AIT, citric acid and treatment time) in this study were based on our previous trials in vitro.

Analyses of total phenolics, anthocyanins, antioxidant capacity

Ten grams of blackberries were first mixed with 30 ml of ethanol. After the mixture was centrifuged at 4000 x g for five minutes, the blackberry residue was extracted with 85% (v/v) ethanol twice, and the final volume of supernatant was 100 ml. The supernatant was used to determine total phenolic compounds and anthocyanins content.

Folin-Ciocalteu method was used for the determination of total phenolic compounds as described by Yu and others [20]. The contents of total phenolic compounds were calculated based on gallic acid standard solutions, and were expressed as gallic acid equivalent (GAE).

Anthocyanin content in the supernatant was determined using the pH differential method described by Lee and others [21]. The results were expressed as cyanidin-3-glucoside (C3G) mg/g dry matter. The antioxidant capacity of each fruit samples was determined using the DPPH method described by Jabbari and others [22] with some modifications. Twenty µl of each sample diluted in ethanol and 3 mL of DPPH (2, 2-diphenyl-2-picryl-hydrazyl) in ethanol (150 µM final concentration) were added to a test tube. The test tube for blank contained ethanol only. The mixture was vortexed for thirty seconds and incubated in the dark for one hour at room temperature. After one hour, a spectrophotometer (Genesys 10 UV, Thermo Electron Corporation, Pennsylvania, USA) at 515 nm was used to measure the absorbance of each sample. The antioxidant capacity of each sample was determined by calculating the decrease in absorbance. Gallic acid was used as an internal control, and the results were expressed as GAE.

Texture Analysis

Compression tests were performed to evaluate any changes in the hardness of blackberry samples. The hardness value (g) is the peak force that occurs during the first compression. A TA.XT Plus Texture Analyzer (Texture Technologies, Scarsdale, NY, USA) with a 3 mm diameter stainless cylinder probe TA-212 was used to evaluate the firmness of blackberries. Pretest speed was 2 mm/s, test speeds was 2 mm/s with an automatic trigger set to 5 grams of force, test distance into the samples was 5 mm. Each time a set of 10 fruits was measured, the results were the average of ten berries.

CO_2 and O_2 concentrations in headspace

The concentrations of CO_2 and O_2 in the plastic box was determined using a portable gas analyzer (Dansensor A/S, Ringsted, Denmark), following the manufacturer's operation manual.

Total soluble solid (TSS) and titratable acidity (TA)

TSS was determined using a digital refractometer at 25° C, and the results were reported as °Brix. TA was measured by titration using 0.1 M NaOH, and the results were expressed as g citric acid equivalents per liter.

Color measurement

Color measurements of blackberry were performed at 25° C using a General Colorimeter (JZ-300, Shengzhen Kingwell Instrument Col. Ltd., Guangdong and China). *L**, *a**, and *b** values were recorded. Three measurements for each sample were conducted and averaged.

Microbial analysis

Blackberry samples (10 g) were transferred into individual sterile stomacher bags containing 10 mL of sterile peptone water (0.1%) and stomached for 2 min. The resultant suspension was serially diluted in sterile peptone water and surface-plated (0.1 ml) in duplicate onto plate count agar (PCA, BBL/Difco ™) plates for aerobic mesophilic bacteria and dichloran rose Bengal chloramphenicol agar (DRBC, BBL/Difco ™) plates for yeasts and molds, respectively. PCA plates were incubated at 37° C for up to 48 hrs; DRBC plates were incubated at 25°C for 4 to 5 days. The colony forming units (CFU) were then counted.

Statistical analysis

Data were analyzed by ANOVA, and significant differences ($p<0.05$) between control and treated samples were determined by Fisher's Least Significant Difference

Test (LSD) via SAS Version 9.2 (SAS Institute, Inc., Cary, NC, USA).

Results and Discussion

Changes in natural microorganisms during storage

The results in Figure 1 show the changes in aerobic mesophilic bacterial populations and in yeast and mold populations throughout the twelve-day storage period at 10°C. Carvacrol washing (COL) and carvacrol washing combined with AIT vapor treatment (COL+AIT) immediately reduced aerobic mesophilic bacterial populations from 3.5 to 1.3 log CFU/g and maintained these populations at this level throughout storage. AIT vapor treatment gradually reduced aerobic bacterial populations to 1.3 log after 5 days and maintained them at this level throughout storage (Figure 1A). Similar changes in the populations of yeasts & molds were observed for those samples subject to AIT and the combination treatments, while the populations of yeasts & molds after COL washing increased from 1.5 to 4.5 log CFU/g (Figure 1B) during 12 days of storage at 10°C. It suggests that COL washing is not enough to inhibit the growth of yeasts & molds in blackberries.

Similar results were observed in several studies using vapor phase of essential oils. Aguilar-González and others [34] found that the minimum inhibitory concentration of AIT in the vapor phase needed to prevent the growth of gray mold (*Botrytis cinerea*) in strawberries was 15.42 µL/L_{air}. Chang and others [23] investigated the antifungal activity of sachets containing oregano microcapsules against the growth of molds and yeasts in iceberg lettuce. They observed that the populations of molds and yeasts in iceberg lettuce subject to treatment with a sachet containing 2 g of oregano microcapsule decreased by 1.4 log after storage for 5 days at 20°C. Gao and others [24] developed PLA films which incorporated AIT vapor and found that these films significantly reduced *E. coli* and *G. candidum* populations in tomatoes stored at 4 and 10°C for 21 days. Gamage and others [25] developed soy protein isolate films which incorporated AIT vapor and found that these films significantly reduced populations of *E. coli*, *L. monocytogenes*, and *S. typhimurium* in radish, broccoli, and alfalfa sprouts stored at 10°C for five days. Lin and others [26] studied the bactericidal activity of AIT vapor against various pathogens artificially inoculated on fresh produce. They observed that treatment with vapor generated from 400 µl AIT caused an 8-log reduction in populations of *E. coli* O157:H7 artificially inoculated on lettuce

Changes in O_2 and CO_2 concentrations during storage

Figure 2 shows the O_2 and CO_2 concentrations in the box headspace during storage. O_2 and CO_2 concentrations in AIT treated samples (AIT and COL+AIT) were maintained at relatively stable levels throughout the 12 d storage period.

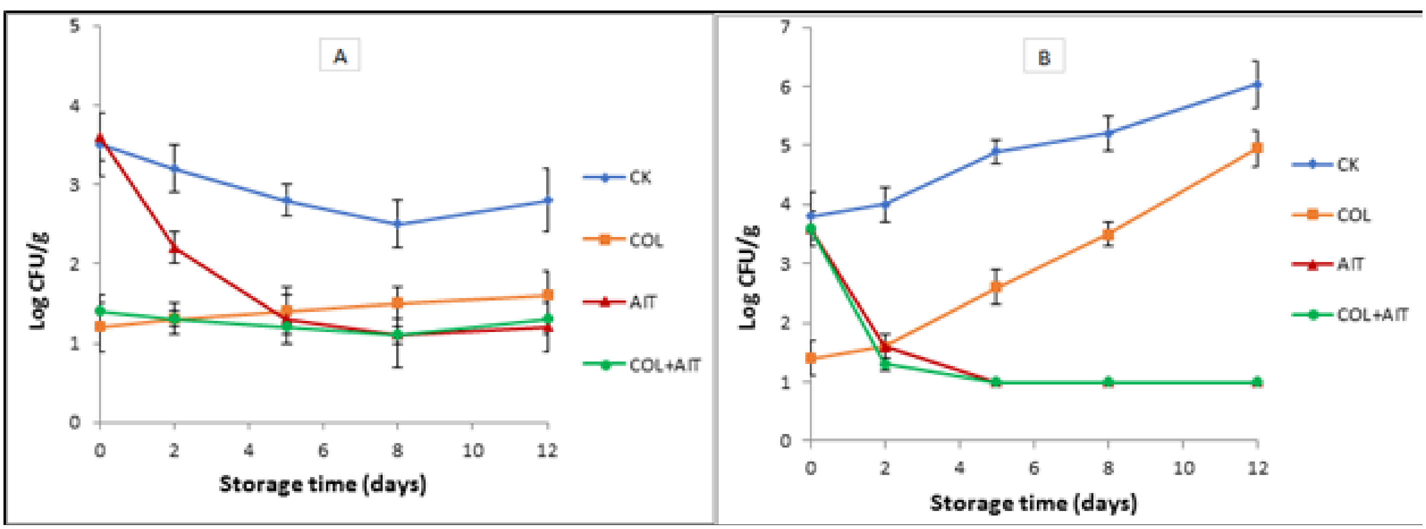

Figure 1: Changes in natural microorganisms during storage at 10°C. A: Total aerobic bacteria; B: Yeasts and molds. CK: control, COL: COL washing; AIT: AIT vapor; COL+AIT: COL washing followed by AIT vapor. Error bars represent for the standard deviation of the mean. The minimum detection limit was log 1.0 CFU/g.

The control samples had the lowest O_2 concentration at day 5 and then increased thereafter, corresponding to the highest CO_2 concentration at day 5 and then decreased thereafter. Similarly, samples after the washing treatment (COL) gradually decreased in O_2 concentrations and increased in CO_2 concentrations throughout the storage period.

Fresh or minimally processed vegetables are alive even after treatments, thus continue their respiration, causing the consumption of atmospheric oxygen (O_2) and production of carbon dioxide (CO_2) in a package. AIT treated samples (AIT and COL+AIT) had fewer changes in O_2 and CO_2 concentrations throughout storage than the control samples, which indicates that the presence of AIT vapor in the headspace of the package reduced the physiological activity of blackberry fruits during storage. Therefore, longer shelf life for treated fruits could be expected.

Sánchez-González and others [27] studied the effects of the direct application of chitosan coatings alone or in combination with bergamot essential oil on the quality and safety of table grapes during postharvest cold storage and found that chitosan coatings containing bergamot essential oil were the most effective in controlling the respiration rate and water loss in table grapes throughout postharvest cold storage. These coatings also maintained populations of aerobic mesophilic bacteria and yeasts and molds at low levels throughout storage. Santos and others [28] also found that the direct application of chitosan coatings infused with *O. vulgare* essential oil were also highly effective in inhibiting the growth of fungi in grapes, allowing for their quality to be maintained throughout postharvest storage, as shown by measurement of their physical, physicochemical, and sensory attributes.

Changes in contents of total phenolics, anthocyanins and antioxidant capacity

AIT vapor alone and in combination with COL wash caused fewer changes in the concentrations of total phenolics (Figure 3A), anthocyanins (Figure 3B), and in the antioxidant capacity (Figure 3C) of blackberries during 12 days of storage at 10°C, suggesting that AIT vapor treatments were able to maintain the nutritional value of blackberries.

Wang and others [29] investigated the effects of several essential oils on the shelf life of strawberries stored at 10°C and found that treatment with thymol and eugenol allowed for these strawberries to have increased free radical scavenging properties and oxygen radical absorbance properties. Strawberries subject to treatment with thymol and eugenol also had higher levels of sugars, flavonoids, anthocyanins, organic acids, and phenolic compounds, enhancing their resistance to spoilage and deterioration and allowing for their shelf life to be extended.

Previous studies have evaluated the effects of essential oil treatment on the antioxidant capacities and shelf life of small fruits. Jin and others [10] evaluated the effects of several essential oils on the antioxidant capacities of raspberries during the postharvest stage and found that perillaldehyde was the most effective in enhancing the activities of antioxidant enzymes and antioxidant ca-

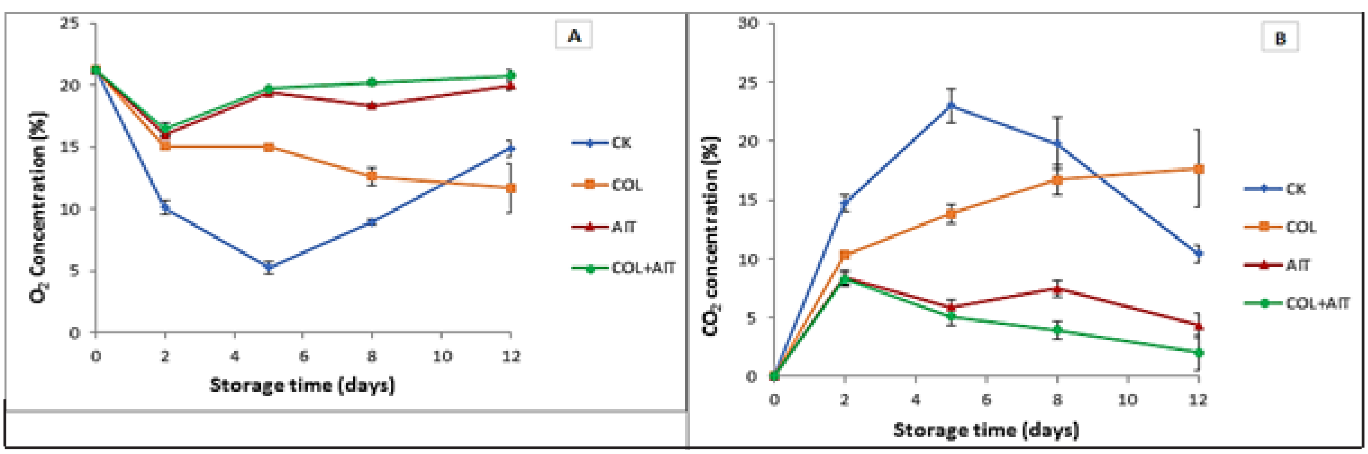

Figure 2: Changes in CO_2 and O_2 concentrations in headspace during storage at 10°C. A: O2; B: CO2; CK: control, COL: COL washing; AIT: AIT vapor; COL+AIT: COL washing followed by AIT vapor. Error bars represent for the standard deviation of the mean.

pacities of raspberries. Wang and others [30] evaluated the effectiveness of several essential oils on the antioxidant levels, antioxidant activities, and shelf life of blueberries and found that blueberries treated with carvacrol, anethole, and perillaldehyde had higher levels of total anthocyanins and phenolic compounds, enhanced antioxidant activity, and increased oxygen radical absorbance properties, allowing for their shelf life to be extended.

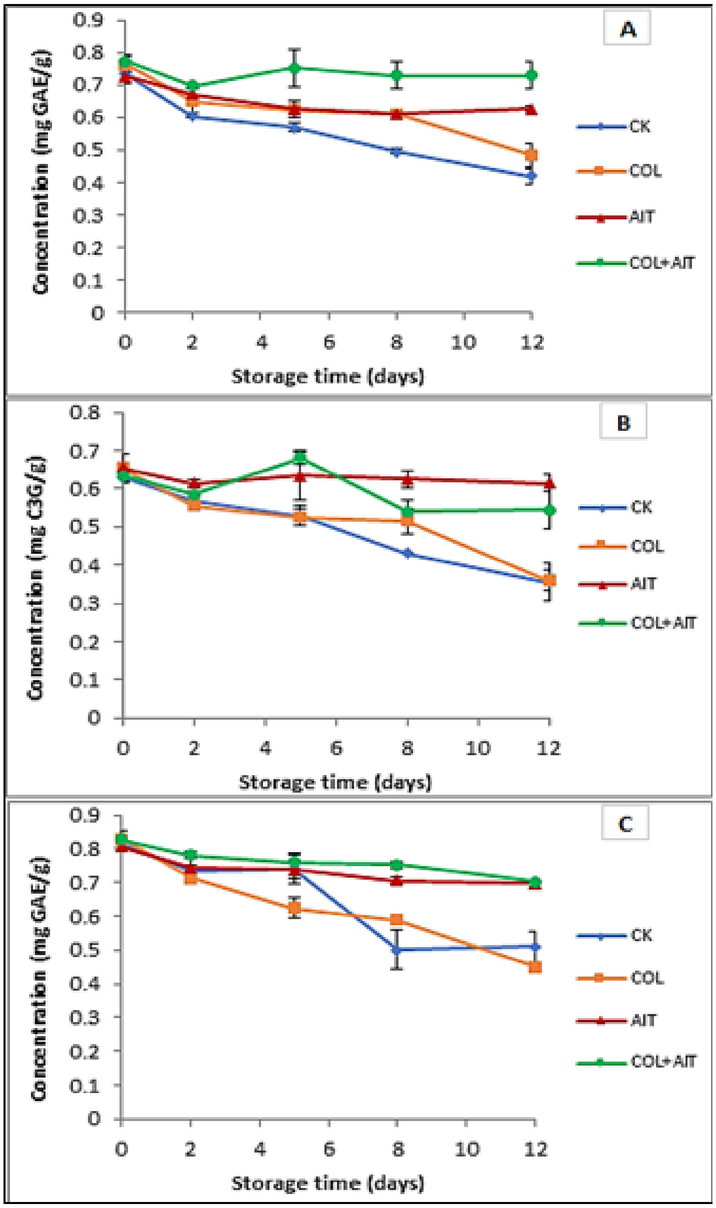

Figure 3: Changes in total phenolics (A), anthocyanins (B), and antioxidant capacity (C) during storage at 10°C. CK: control, COL: COL washing; AIT: AIT vapor; COL+AIT: COL washing followed by AIT vapor. Error bars represent for the standard deviation of the mean.

Changes in titratable acidity (TA), and total soluble solid (TSS)

Changes in TA and TSS contents in blackberries throughout storage are shown in Figure 4. The TA contents of all blackberry samples stored at 4°C were maintained at ca. 0.8% throughout the 12 d storage period, and no significant ($P<0.05$) differences were observed among the samples (Figure 4A). Although the TSS contents of all blackberry samples decreased slightly throughout storage, the TSS contents of AIT and AIT+COL treated samples were higher than those of the samples subject to COL treatment and the controls by the end of storage (Figure 4B). Chen and others [35] also observed that mulberry fruit subject to treatment with AIT vapor had higher levels of TA and TSS throughout the 15d storage period at 5°C. The TSS/TA ratio has been used to evaluate the quality of small fruits. A high TSS/TA ratio indicates that the fruit is of high quality and fit for human consumption [31]. As shown in Figure 4C, the AIT and COL+AIT treated blackberries maintained higher TSS/TA ratio than COL treated and control samples after 12-days of storage, suggesting that these treatments had the least effect on the eating quality of blackberries.

Changes in color and texture

Table 1 shows the changes in the color of blackberries throughout storage. Throughout storage, AIT and COL+AIT samples-maintained L* values (whiteness) while COL and control samples decreased the L* values. There were no significant differences in a* value (redness) and b* values (yellowness) among the three treatments and control after 5 days storage. During further storage, AIT vapor-treated samples (AIT and COL+AIT) had higher a* value and b* values than COL and control samples. Although there were no color differences among all samples in fruit appearance by visual observation, the hunter color parameters indicate that AIT vapor treatment could slightly increase the redness of fruits.

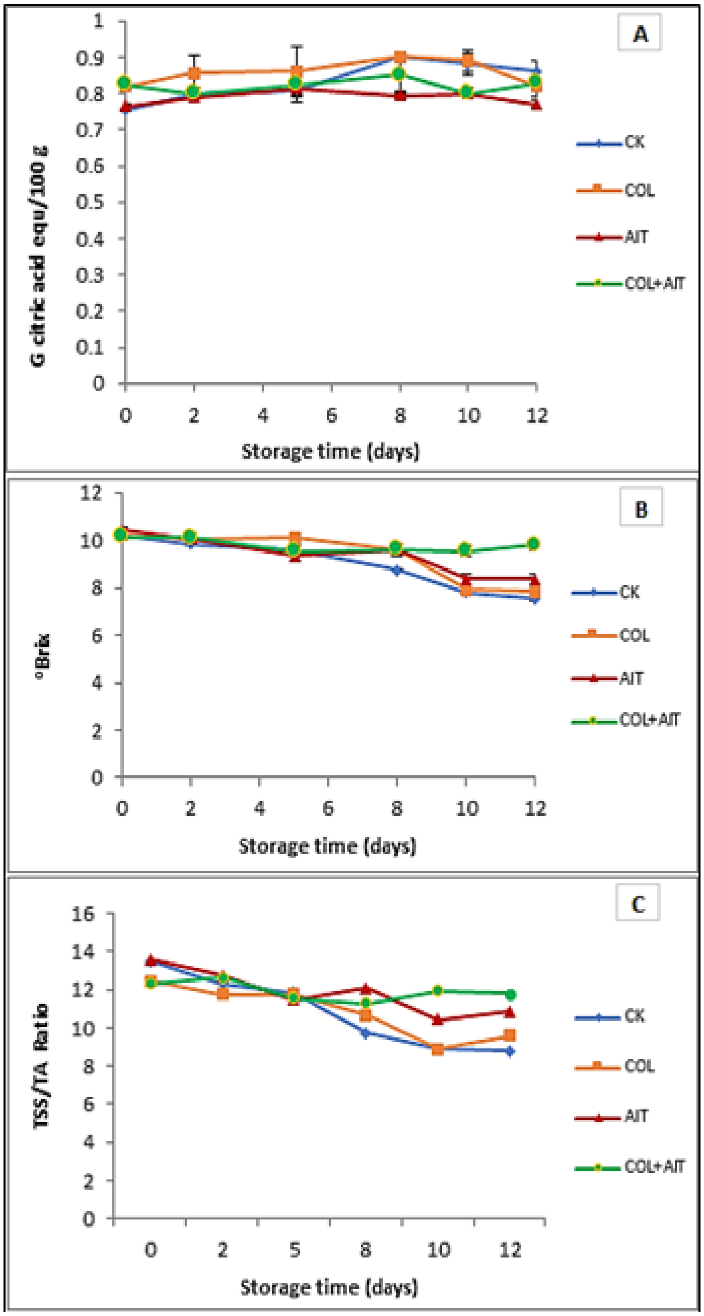

Figure 4: Changes in titrable acidity (A), total soluble solid (B), and TSS/TA ratio (C) during storage at 10˚C. CK: control, COL: COL washing; AIT: AIT vapor; COL+AIT: COL washing followed by AIT vapor. Error bars represent for the standard deviation of the mean.

The firmness of blackberry texture was shown in Figure 5. Three treated samples had similar changes in firmness throughout storage and no significant differences (P>0.05) among them were observed. However, the control samples had significantly (P<0.05) higher firmness than the other treated samples. Previous studies have evaluated the effects of essential oil vapor treatment on the texture and color characteristics of fresh produce. Chang and others [23] evaluated the effect of oregano essential oil vapor on the quality and shelf life of iceberg lettuce throughout storage at room temperature for five days and found that essential oil treatment did not affect the texture and color characteristics of iceberg lettuce throughout storage. Yun and others [32] investigated the effectiveness of zein based coatings containing up to 20% cinnamon essential oil on the quality of cherry tomatoes stored at 10˚C for up to three weeks and found that the coating treatment did not affect the texture and color of cherry tomatoes throughout storage.

For successful commercialization of new blackberry cultivars, it is critical to maintain postharvest quality and shelf life of blackberries after long-distance shipping. Disinfection is crucial in the processing of fresh fruits and directly affects the product quality, safety, and shelf-life. The purpose of washing fresh produce is to remove dirt and pesticide residues and to reduce populations of spoilage microorganisms [33-35]. It is important to wash fresh produce with sanitizers so that populations of possible foodborne pathogens can be reduced. In this study, washes with the EO COL initially reduced total bacterial and yeast & mold populations in blackberries, however, the wash treatment could not inhibit the outgrowth of these microorganisms throughout storage. In contrast, AIT vapor or its combination with wash reduced the initial microbial loads as well completely inhibited their growth during the storage.

Standard small fruit production practices discourage growers from washing fruits after harvest. The results from this study show that the use of essential oils in the vapor phase have high antimicrobial activity and are better suited for the decontamination of small fruits during the postharvest stage. Except the initial microbial reduction after the treatments (Figure 1), AIT vapor treatment are not significantly different from COL+AIT treatment in microbial reduction and nutrition and quality maintenance, suggesting that AIT vapor alone could be good enough for blackberries without an additional washing procedure.

This study demonstrated a prototype for AIT vapor

Table 1: Changes in color of blackberries after treatments and during storage at 10° C.

		Storage days				
		0	**2**	**5**	**8**	**12**
L*	**CK**	18.77±1.20a	18.63±0.40a	14.80±0.70b	15.46±1.47b	16.27±0.62b
	COL	19.67±0.88a	16.83±0.65b	17.83±1.96a	16.10±1.38b	16.12±0.68b
	AIT	18.77±1.20a	18.20±0.80ab	18.56±0.92a	19.63±1.07a	18.57±0.54a
	COL+AIT	19.67±0.88a	17.75±1.38ab	17.33±1.15a	17.03±1.32b	18.30±0.86a
a*	**CK**	1.60±1.73b	1.63±0.66a	2.83±0.63a	2.90±0.34b	2.37±0.61c
	COL	3.82±1.00a	3.40±1.51a	3.23±1.15a	3.63±0.21b	4.95±0.96bc
	AIT	1.60±1.73b	1.97±1.82a	1.96±1.04a	2.70±0.52b	6.83±2.74ab
	COL+AIT	3.82±1.00a	3.47±1.82a	4.80±2.61a	6.50±2.06a	8.07±2.15a
b*	**CK**	1.50±0.84a	1.40±0.98a	1.20±0.20a	1.30±0.26b	1.22±0.54b
	COL	1.67±0.45a	1.40±0.85a	1.30±0.98a	1.46±0.61b	1.40±0.92b
	AIT	1.50±0.84a	1.41±0.29a	0.90±0.52a	1.30±0.17b	2.57±1.18ab
	COL+AIT	1.67±0.45a	1.77±0.68a	1.50±0.95a	2.46±0.64a	3.60±1.31a

Data having a common letter in each column are not significantly different ($p > 0.05$).

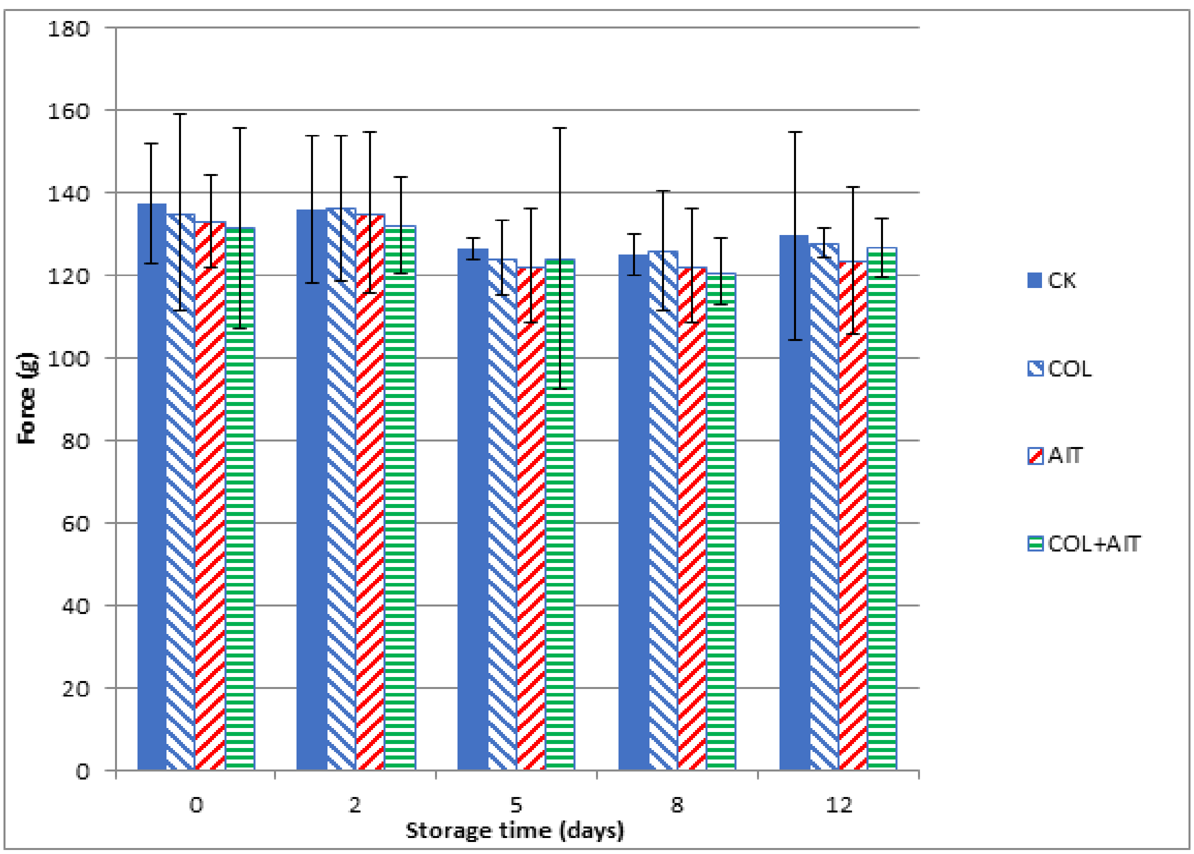

Figure 5: Changes in Texture firmness during storage at 10° C. CK: control, COL: COL washing; AIT: AIT vapor; COL+AIT: COL washing followed by AIT vapor. Error bars represent for the standard deviation of the mean.

treatment, as only filter paper was used as the AIT carrier. Future studies will focus on the development of sachets/pads or the use of a transparent packaging plastic material as carriers for essential oil vapor. The packaging plastic material can be used as a plastic container or applied as a "patch" placed on the inside of the package or container.

Conclusion

Carvacrol EO solution washing could reduce the initial microbial load but did not inhibit microbial growth during storage. In contrast, gaseous AIT in the package headspace shows a strong antimicrobial effect against bacteria as well as yeasts & molds. In addition, the AIT vapor in the headspace of the package reduces the fruit respiration rate and resulted in less quality changes during storage. Therefore, the use of AIT essential oil vapor is one approach to extend the shelf-life of blackberries and maintain their nutritional quality throughout postharvest storage.

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Authors' Contributions

T. Jin designed the study, interpreted the results, and prepared the manuscript. Y. Yu conducted experiments, collected data, and drafted the manuscript. X. Fan and J. Gurtler assisted quality and microbial analyses.

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