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The Contribution of Basil Essential Oil and Ascorbic Acid Application to the Preservation of Fresh Basil During Shelf Life

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ABSTRACT

Fresh basil is a widely used herb with distinct attributes (aroma and taste); however, it has a quite limited shelf life. The investigation and use of natural products to preserve fresh commodities is gradually increasing over the years. The current work aimed to investigate the impacts of basil essential oils (EOs) and ascorbic acid (AA) (at varying concentrations and times of submersion) on the quality characteristics of fresh basil stored for 6 days at 4°C. Basil respiration rates were increased with AA application (0.5%-5 min). Basil's EO profile was changed during the storage period, with the three main components to be linalool, eucalyptol and eugenol. Among the applied treatments, basil EO 1 min (0.001% and 0.01%), AA 1%-1 min, and AA 0.5%-5 min resulted in increased antioxidant capacity and total flavonoids content of fresh basil. This increase suggests a product with increased nutritional value. In addition, both AA and basil EO applications were able to lower the microbial load (aerobic plate count, yeast and filamentous fungi) of fresh basil, contributing to the product's storability. Overall, the examined natural products (AA and basil EO) could be considered as putative postharvest preservative means for fresh basil. However, such applications should be further investigated for commercialization and upscaling as well as for other types of commodities and herbs.

1 | Introduction

Culinary herbs are utilized for their organoleptic attributes as they give a unique flavor (taste and aroma) to food and beverages, rendering them more alluring and tastier. The food industry, spirits production, and confectionary utilize herbs as flavoring agents as well as preservation agents (Curutchet et al. 2014). *Ocimum basilicum* L. also called basil or sweet basil, is an important culinary herb of the Lamiaceae family (Aminian et al. 2022). Due to its distinct aroma and the plethora of health benefits that it presents, basil is utilized as fresh, dried, and/or frozen by the food, pharmaceutics, perfumes, and cosmetics industrial sectors (Bernhardt et al. 2015; Brindisi and Simon 2023). In addition, its essential oil (EO) is also used likewise. Basil is known to possess many benefits, like antioxidant (Noor et al. 2019), antimicrobial (antibacterial, antifungal) (Verrillo et al. 2023; Zhakipbekov et al. 2024), anti-inflammatory (Prasongdee et al. 2024), insecticidal (Kačániová et al. 2022), and hepatoprotective activity (Pandey et al. 2021), while it has also been used as folklore remedy for respiratory and gastrointestinal disorders (Bernhardt et al. 2015; Aminian et al. 2022). These activities are mainly attributed to the rich phytochemical content of this herb (especially

polyphenols and flavonoids) (Carro et al. 2013; Bernhardt et al. 2015).

The global vegetable production rose up by 21.50% within 10 years (2008-2018) (FAOSTAT and Food 2021). Furthermore, it is estimated that the worldwide fresh produce production will increase to 60% by the year 2025 compared to 2012 due to increased health awareness and healthy living approaches (Maringgal et al. 2020). Consumers' demands for healthier, full-flavor food with lower sodium content lead to the substitution of salt with various herbs and spices (Cozzolino et al. 2016). The majority of herbs are retailed as fresh and dried products, with basil being the most commonly marketed and consumed fresh herb. However, fresh basil is a very delicate leafy herb with a relatively short shelf life (Kusuma et al. 2024).

Poor harvesting practices, as well as improper handling, storage, and transportation, contribute to the fresh produce losses (Li and Thomas 2014; Al-Dairi et al. 2022). Fresh culinary herbs, such as basil, present prominent signs of senescence like the degradation of proteins and chlorophylls (i.e. yellowing). With limited success, several studies have been conducted trying to regulate the postharvest quality and prolong the storage of fresh basil; especially via controlling the storage temperature (Kusuma et al. 2024). It is important to investigate alternate techniques to extend the shelf life and preserve the quality of fresh basil.

The current practices followed in the food industry include the use of synthetic chemicals, which could have adverse effects on both human health as well as the environment according to previous mentions (Poimenidou et al. 2016; Anand and Sati 2013). In addition, consumers tend to show high preference for food prepared and/or treated with natural (less synthetic) and environmentally friendly products such as organic acids (acetic, ascorbic, oxalic acid), plant extracts, EOs, as well as pure natural compounds such as eugenol, eucalyptol, and limonene (Jackson-Davis et al. 2023). Ascorbic acid (AA) also known as vitamin C, and E300; is a water-soluble vitamin known for its high antioxidant activity. This organic acid is a key player in the suppression of reactive oxygen species (ROS) accumulated in plants when they are subjected to any stress (biotic and abiotic). According to earlier research, the pre- and postharvest application of exogenous AA on various fresh products resulted in their preservation and high quality (Alaey et al. 2011; Bilgin 2021). For instance, dipping fresh rosemary in AA solutions (1 and 2%) resulted in increased respiration rates of the product while reduced its microbial load (Xylia et al. 2022).

Great interest has been given to the EOs as natural agents to preserve fresh commodities. The EOs are complex mixtures of oxygenated monoterpenes, oxygenated sesquiterpenes, and hydrocarbons, among others (Pizzo et al. 2023). Their distinct aroma as well as the plethora of beneficial properties (i.e. antioxidant, antimicrobial, anti-inflammatory, insecticidal, among others) contributed to their numerous applications in the food and beverage, medicine, cosmetics, and aromatherapy sectors (Bhavaniramya et al. 2019; Jackson-Davis et al. 2023; Xylia et al. 2022). The Food and Drug Administration (FDA) designated the majority of EOs, including cinnamon, basil, clove, oregano, thyme, and nutmeg; under the generally recognized as safe (GRAS) category. The EOs are secondary metabolites of herbs (also medicinal and aromatic plants) stored in various plant tissues, including leaves, flowers, stems, roots etc., consisting of a mixture of lipophilic compounds that exhibit both antioxidant and antimicrobial (antibacterial and antifungal) activities (Pizzo et al. 2023; Jackson-Davis et al. 2023). In the food industry, the EOs are applied and/or delivered in various ways, such as active packaging, edible films, encapsulation, vapors and antimicrobial gaseous atmosphere, among others (Jackson-Davis et al. 2023). While many previous studies highlighted the EOs efficacy to preserve fresh produce, there are some limitations that need to be considered when using such products. These include the organoleptic properties and the most suitable combination of EO and produce like apple with cinnamon/clove EO. In addition, lower EO concentrations should be considered in order for the EO to complement the product's attributes without negatively influencing the consumer's sensory perception whilst preserving the EOs' antioxidant/antimicrobial capacity (Jackson-Davis et al. 2023).

This work aimed to examine the impact of basil EO and AA (at varying concentrations and times of submersion) on the qualitative attributes of fresh basil while it was stored at chilled conditions (4°C as the typical set temperature in a retail refrigerator). For this, a number of measurements was carried out, including respiration rate, total phenols and flavonoids, antioxidant capacity, color and chlorophylls content. This study's innovation was the use of the plant's EO for the preservation of the same plant in an effort to maintain and/or improve its quality attributes and aromatic profile.

2 | Materials and Methods

2.1 | Plants, Distillation of Essential Oils and Experimental Set Up

For the EO production to be used in the present study, basil plants that organically grown in soil at Cyprus University of Technology (Limassol, Cyprus) farm, were collected. The plants were air-dried at 42°C in an air-ventilated oven and were hydrodistilled for 3 h by using a Clevenger apparatus. After extraction, the EO was kept at −20°C until use. The basil's EO chemical profile was conducted as outlined by Chrysargyris, Panayiotou et al. (2016), where the collected mass spectra were referenced with mass spectra from the literature (Adams 2012) and the NIST08 mass spectral library of the GC–MS data system (ShimadzuGC2010 gas chromatograph interfaced Shimadzu GC/MS QP2010plus mass spectrometer). The main constituents of basil EO utilized in the postharvest treatments as identified were linalool (33.00%), eucalyptol (15.70%), and eugenol (11.66%).

For the postharvest study, fresh basil (*Ocimum basilicum*) was provided by the Cyprus University of Technology greenhouse. Plants were hydroponically cultivated in a nutrient film technique (NFT) infrastructures as previously mentioned (de Oliveira et al. 2024). Following harvest, plants were brought into the laboratory and arranged into small bundles (approximately 70–90 g). Considering uniformity, in terms of size and appearance,

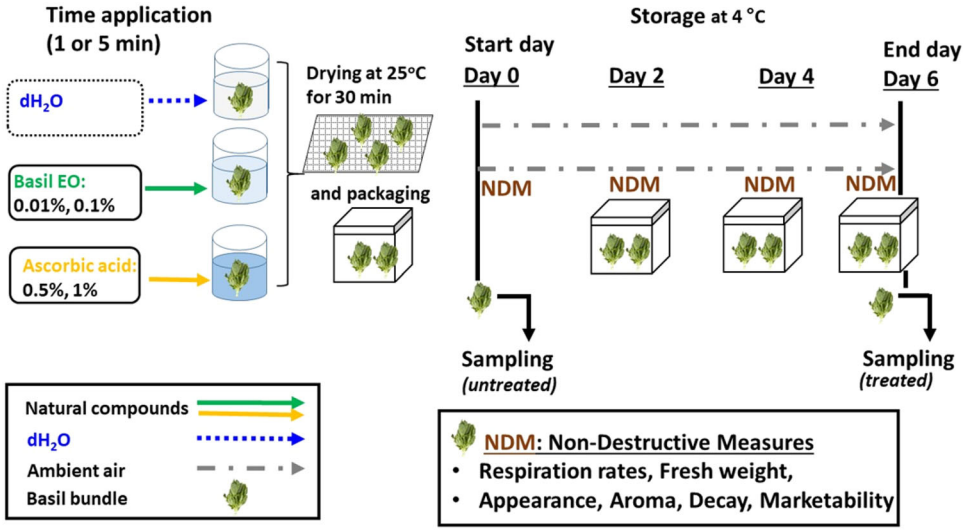

FIGURE 1 | Experimental illustration. Fresh basil bundles were submerged in treatment solutions of different concentrations (basil EO: 0.001% and 0.01%; ascorbic acid: 0.5% and 1%) and different time (1 min and 5 min). Bundles were kept at 4°C for 6 days.

and the lack of physical flaws such as wilting and wounds; a total number of 80 bundles were prepared and used promptly.

2.2 | Immersion in Treatment Solutions

Each bundle of basil was submerged in each of the following 1.5 L treatment solutions (total of eight bundles per treatment) for the appropriate time (as indicated): 1) distilled H_2O (control), 2) basil EO 0.001% for 1 min, 3) basil EO 0.01% for 1 min, 4) basil EO 0.001% for 5 min 5) basil EO 0.01% for 5 min. 6) AA 0.5% for 1 min, 7) AA 1% for 1 min, 8) AA 0.5% for 5 min, and 9) AA 1% for 5 min (Figure 1). Following a 30 min drying period at ambient temperature (25°C), the bundles were put in a 5 L polypropylene (PP) plastic container (two bundles per container; four replicated containers per treatment) and kept for 6 days at 4°C and 85–90% relative humidity (RH). To maintain the RH at high levels, a wet filter paper was added in each container, and was kept moist every 48 h (Chrysargyris, Nikou et al. 2016). To eliminate irregularities in air's composition (i.e. low O_2 and high CO_2 levels), the container lids were opened every second day and aerated. Four biological replicates with each replicate to be a pool of two bundles, were collected for each treatment and kept at −20°C until use.

2.3 | Measurements

2.3.1 | Weight Loss and Respiration Rate

The percentage (%) of overall weight decrease (i.e. weight loss) was computed for each respective day after the weight of each bundle was recorded (day 0, 2, 4, and 6). The impact on basil's respiration rate was investigated every second day with a dual gas analyzer (GCS 250 Analyzer, International Control Analyser Ltd., Kent, UK) and results were expressed results as mL of the emitted CO_2 per kg of plant tissue per hour (Xylia et al. 2024).

2.3.2 | Essential Oil Yield and Composition From Treated Basil Bundles

The EOs were extracted from treated basil plants on each sampling day (day 0 and 6), analyzed and their individual components were identified as described in Section 2.1.

2.3.3 | Basil Color and Leaf Pigments

The basil leaf's color (chromatic coordinates L*, a* and b*) was determined with a colorimeter (Chroma meter CR400 Konica Minolta, Tokyo, Japan) and hue (h) (Bolin and Huxsoll 1991; Goyeneche et al. 2014), chroma value (*C*), and color index (CI) were computed (Goyeneche et al. 2014).

Chlorophylls (Chl a, Chl b, total Chls) and carotenoids (total Car) were determined as reported before (Wellburn 1994) by measuring the absorbance at 470, 653, and 666 nm. The results were calculated as mg per g of fresh weight (mg/g).

2.3.4 | Total Polyphenols, Antioxidant Activity, Ascorbic Acid, and Total Flavonoids

The extraction procedure for total polyphenols and antioxidants was followed according to Chrysargyris, Panayiotou et al. (2016). Total polyphenols were determined by the Folin–Ciocalteu method. The antioxidant activity of basil extracts was determined with three different methods: i) the 2,2-diphenyl-1-picrylhydrazyl (DPPH), ii) the ferric reducing antioxidant power (FRAP) assay, and iii) the 2,2'-azinobis-(ethylbenzothiazoline-6-sulfonic acid) (ABTS). The DPPH and FRAP assays were conducted as indicated by the protocols mentioned by Chrysargyris, Panayiotou et al. (2016). The ABTS assay was determined based on Wojdyło et al. (2007). Ascorbic acid (AA) was determined according to Dinesh et al. (2015) and total flavonoids according to a modified method of Meyers et al. (2003) (adapted modifications by Chrysargyris, Panayiotou et al. 2016).

2.3.5 | Total Soluble Solids, Titratable Acidity, and Damage Indexes

Total soluble solids content (TSS) was estimated with a digital pocket refractometer (Sper Scientific 300017, Scottsdale, Arizona, USA) and results were presented as °Brix. Titratable acidity (TA) was determined titrimetrically (AOAC International 2007), and results were presented as g of citric acid per g of fresh weight.

The determination of damages indexes, such as hydrogen peroxide-H_2O_2 and lipid peroxidation (as malondialdehyde-MDA) was performed according to Loreto and Velikova (2001) and De Azevedo et al. 2006), respectively. For the determination of H_2O_2 production, the iodometric method was followed measuring the reaction's absorbance at 290 nm and expressing the results as μmol H_2O_2 per g of fresh weight. For the estimation of the produced MDA, the 2-thiobarbituric acid reactive substances (TBARS) method was employed, where the absorbance of the reaction was read at 532 and 600 nm and the results were calculated as nmol MDA per g of fresh weight.

2.3.6 | Microbial Load

The microbial load (i.e. aerobic plate count-APC, yeast and filamentous fungi) of basil was recorded after use of Plate count agar (PCA, Merck, Darmstadt, Germany) and Dichloran-rose Bengal chloramphenicol Agar (DRBC agar, Merck, Darmstadt, Germany), respectively (Xylia et al. 2022). Briefly, after homogenization of the plant tissue, serial decimal dilutions were prepared and plated on the aforementioned culturing media and incubated under proper conditions (48 h at 30°C for APC; 5 days at 25°C for yeast and filamentous fungi). After analysis, results were expressed as log of colony forming units (CFU) per g of fresh weight (log CFU/g).

2.4 | Data Analysis

Four biological replications per treatment were used in this study (except for EOs analysis where three biological replications per treatment were used). A completely randomized design (CRD) was used for this work. IBM SPSS version 29.0.2.0 was employed to statistically analyze the data by performing one-way analysis of variance (ANOVA). Means were compared using the Duncan's multiple range test (significance level of $p = 0.05$). Additionally, the non-treated samples (i.e. control) from day 0 (initial) and 6 (last) were compared with an independent samples *t*-test.

3 | Results

3.1 | Weight Loss and Respiration Rate

Figure 2 shows the impacts of basil EO and AA applications on basil's weight loss and respiration rate. The greatest weight loss throughout storage was observed with AA 0.5% (1 and 5 min) (up to 6.70 %) at the end of storage ($P = 0.002$). All basil EO treatments caused decreased weight loss (especially 0.01%-1 min, 0.001%-5 min, and 0.01%-5 min) compared to AA 0.5%. The application of AA 0.5%-5 min showed the highest respiration rate on the second and fourth day (102.61 and 90.96 mL CO_2/kg/h) ($P = 0.004$ and 0.010, respectively); whilst at the end of storage (day 6) AA 0.5%-1 min caused a decrease in basil's respiration rate as to basil EO 5 min applications (both concentrations) and the rest of AA concentrations ($P = 0.051$).

3.2 | Essential Oil Yield and Profile From Treated Basil Bundles

Table 1 displays the impact of applying different concentrations and application time of basil EO and AA on the yield and quality of EOs of fresh basil, after 6 days of storage. In general, thirty two components have been identified, representing the 98.58–99.45% of the total chromatograph. The oxygenated monoterpenes were the dominant group of compounds, followed by sesquiterpenes and monoterpenes. Comparing the basil EO obtained from control plants on day 0 and day 6, there was a significant decline in the percentage of the three major components of the basil EO: linalool, eucalyptol and eugenol. The only major compound that was significantly increased after 6 days of storage was *trans*-α-bergamotene (from 6.58% to 10.80%). The application of basil EO, significantly increased the percentage of the EO's major compound (linalool), in all applications, apart from the application of basil EO 0.001%-5 min, compared to the untreated (control) samples. Eucalyptol has also been measured increased in all cases of basil EO application (especially at 5 min), compared to the control after 6 days (Table 1). On the other hand, eugenol remained unaffected after the treatment with basil EO 0.001%-1 min, while all the other basil EO applications significantly decreased the eugenol from 14.678% to 10.35–11.65%. The fourth most abundant compound (*trans*-α-bergamotene) remained stable only at the application of basil EO 0.001% for 5 min, while the rest of EO treatments reduced its participation to the EO profile of fresh basil (especially 0.001%-1 min).

As for the different applications of ascorbic acid, they all increased the levels of linalool, compared to the control, reaching as high as 43.31% with AA 1%-5 min (Table 1). The same effect was revealed for eucalyptol, which was found increased after all treatments, while only the application of AA 0.5%-5 min, kept the eugenol's content stable at 14.34%, compared to the rest AA application that reduced the corresponding value. Finally, *trans*-α-bergamotene was reduced by all the tested AA applications. The rest of the identified compounds, with low participation in the total EO profile have been diversely affected, in cases. As for the EO yield, the 6 days of storage of basil plants without any application had no significant effect (EO yield at 1.05%) compared to the EO yield of plants at Day 0 (1.13%). This value was kept constant only after the application of basil EO 0.001%-1 min and AA 0.5%-5 min, while the rest of the treatments tested reduced the EO yield to an average of 0.83% (Table 1).

3.3 | Color and Leaf Pigments

The impacts of basil EO and AA treatments on basil's leaf color and pigment content (i.e. chlorophylls and carotenoids) are presented in Table 2. Increased hue angle (h) value was observed on basil treated with basil EO 0.001%-5 min as to the

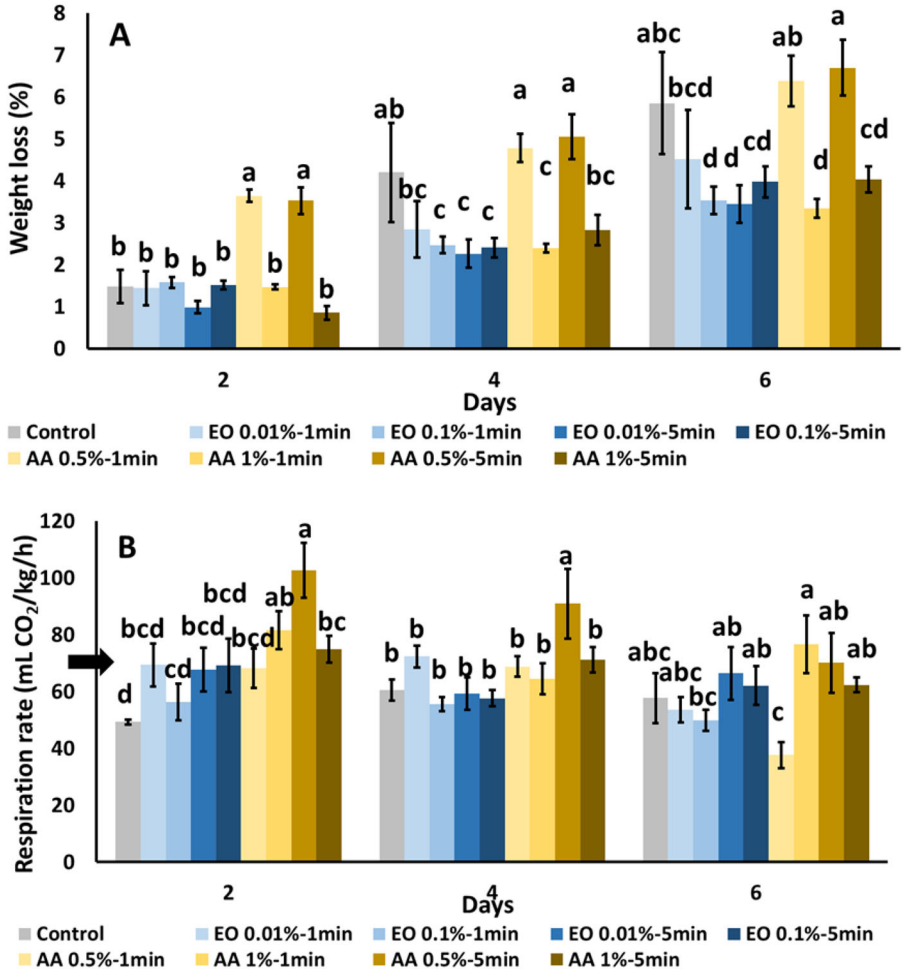

FIGURE 2 | Effects of basil EO and ascorbic acid (AA) application on basil's (A) weight loss percentage (%) and (B) respiration rate kept at 4°C for 6 days. Shown values are the mean ± standard error ($n = 4$). Control (non-treated, 0.00%) values for the initial day (day 0) are illustrated with an arrow (→). Significant differences ($p < 0.05$) are highlighted with different Latin letters on individual days.

ones treated with basil EO 0.001%-1 min and AA 0.5%-5 min ($P = 0.078$), while AA 0.5%-5 min resulted in higher color index (CI) compared to basil EO 0.001%-5 min ($P = 0.092$). Chroma value (C) was not changed by the tested applications ($P = 0.470$). No significant differences for the Chl a, Chl b, Total Chl, and Tot Car were found among treatments ($P = 0.953$, 0.794, 0.929, and 0.467, respectively), where they averaged as 0.62 mg Chl a/g, 0.28 mg Chl b/g, 0.90 mg total Chl/g, and 0.09 mg Tot Car/g, respectively. A representative image of the applied treatment in basil is illustrated in Figure 3.

3.4 | Total Polyphenols, Antioxidant Activity, Ascorbic Acid and Total Flavonoids

As shown on Table 3, treatment with AA 1%-1 min and 5 min (both AA concentrations) resulted in higher total phenols of fresh basil compared to other treatments after 6 days of storage at 4°C ($P = 0.000$). However, one min application of basil EO (0.001% and 0.01%) as well as AA 1%-1 min, and AA 0.5%-5 min were found to increase basil's antioxidant activity after 6 days of storage as opposed to the control, AA 0.5%-1 min and AA 1%-5 min (DPPH, FRAP, ABTS) ($P = 0.000$). Similarly, higher total flavonoids were evidenced with basil EO one min application (both concentrations), AA 1%-1 min, and AA 0.5%-5 min compared to AA 0.5%-1 min and AA 1%-5 min and the control ($P = 0.000$) (Table 3). The use of AA 1%-1 min (followed by AA 0.5%-5 min) resulted in increased AA content ($P = 0.000$).

3.5 | Total Soluble Solids, Titratable Acidity, and Damage Indexes

The effects of basil EO and AA application on basil's TSS and TA are shown in Table 4. Increased TSS were reported with AA 1%-1 min and 5 min (0.5 and 1%) compared to the control and basil EO 0.001%-5 min ($P = 0.010$). Higher TA was observed in basil treated with AA 0.5%-1 min and basil EO 0.001%-5 min as opposed to control, basil EO 1 min (both concentrations) and basil EO 0.01%-5 min ($P = 0.014$). A decline in H_2O_2 levels was marked with basil EO 0.001%-5 min and AA 0.5%-1 min as to basil EO 0.01%-5 min, AA 1%-1 min and 5 min (both concentrations) ($P = 0.026$) (Table 4). Moreover, treatment with AA increased the MDA levels as to basil EO and control, with the highest values recorded at 1 min applications (up to 6.37 and 7.16 nmol/g for AA 0.5 and AA 1%, respectively) ($P = 0.000$).

TABLE 1 | Effects of basil EO and ascorbic acid (AA) application on fresh basil's essential oil yield and composition, after storage at 4°C for 6 days. RI[a]: Calculated retention index on the ZB5 column; RI[b]: Relative retention index taken from Adams (Adams 2012).

			Day 0	Day 6								
					EO				AA			
Time (min)			**0**	**0**	**1**		**5**		**1**		**5**	
Concentration			**0.00%**	**0.00%**	**0.001%**	**0.01%**	**0.001%**	**0.01%**	**0.50%**	**1.00%**	**0.50%**	**1.00%**
Compound	**RI[a]**	**RI[b]**										
α pinene	933	932	0.87 ± 0.02A	0.92 ± 0.04bA	0.94 ± 0.06b	0.92 ± 0.04b	1.28 ± 0.10a	1.01 ± 0.09b	1.05 ± 0.08b	1.08 ± 0.10ab	0.94 ± 0.00b	0.89 ± 0.00b
Sabinene	973	969	0.86 ± 0.00A	0.95 ± 0.04bA	0.96 ± 0.08b	0.93 ± 0.02b	1.34 ± 0.13a	1.03 ± 0.08b	1.10 ± 0.04b	1.05 ± 0.08b	0.95 ± 0.03b	0.89 ± 0.03b
β pinene	977	974	2.00 ± 0.01A	2.22 ± 0.10bA	2.23 ± 0.14b	2.16 ± 0.03b	3.03 ± 0.25a	2.38 ± 0.15b	2.52 ± 0.11b	2.51 ± 0.19b	2.26 ± 0.02b	2.18 ± 0.01b
β myrcene	989	988	1.34 ± 0.02B	1.71 ± 0.06bA	1.49 ± 0.07bc	1.62 ± 0.02bc	2.14 ± 0.23a	1.46 ± 0.06bc	1.60 ± 0.03bc	1.70 ± 0.08b	1.33 ± 0.03c	1.46 ± 0.07bc
α terpinene	1017	1014	0.05 ± 0.00A	0.05 ± 0.00abA	0.05 ± 0.05ab	0.05 ± 0.05ab	0.06 ± 0.06a	0.05 ± 0.05ab	0.04 ± 0.04b	0.05 ± 0.05ab	0.05 ± 0.05ab	0.04 ± 0.04b
D limonene	1028	1024	0.59 ± 0.02A	0.65 ± 0.01bcdA	0.59 ± 0.01cd	0.65 ± 0.00bcd	0.77 ± 0.05a	0.72 ± 0.03ab	0.73 ± 0.03ab	0.69 ± 0.07abc	0.56 ± 0.01d	0.63 ± 0.01bcd
Eucalyptol	**1031**	**1026**	**20.86 ± 0.24A**	**18.61 ± 0.09cB**	**20.52 ± 0.05b**	**19.49 ± 0.15b**	**21.86 ± 0.33a**	**21.39 ± 0.01a**	**20.87 ± 0.24b**	**20.61 ± 0.20b**	**20.70 ± 0.26b**	**19.62 ± 0.15b**
Trans-ocimene	1046	1044	0.67 ± 0.04A	0.49 ± 0.04abB	0.68 ± 0.17a	0.36 ± 0.02b	0.62 ± 0.06a	0.26 ± 0.03c	0.29 ± 0.02c	0.40 ± 0.04b	0.60 ± 0.09a	0.31 ± 0.04c
γ terpinene	1058	1054	0.10 ± 0.00A	0.11 ± 0.00A	0.11 ± 0.01a	0.10 ± 0.01a	0.13 ± 0.01a	0.11 ± 0.01a	0.10 ± 0.00a	0.12 ± 0.01a	0.11 ± 0.00a	0.11 ± 0.01a
Cis-sabinene hydrate	1066	1065	0.05 ± 0.00A	0.07 ± 0.00A	0.05 ± 0.00a	0.07 ± 0.01a	0.10 ± 0.01a	0.08 ± 0.02a	0.11 ± 0.00a	0.04 ± 0.00a	0.05 ± 0.02a	0.06 ± 0.01a
Terpinolene	1089	1086	0.04 ± 0.00A	0.04 ± 0.00A	0.04 ± 0.01a	0.04 ± 0.00a	0.03 ± 0.00a	0.04 ± 0.00a	0.04 ± 0.00a	0.02 ± 0.02a	0.04 ± 0.01a	0.04 ± 0.01a
Linalool	**1100**	**1095**	**34.95 ± 0.75A**	**32.97 ± 0.71cA**	**41.30 ± 0.05a**	**40.63 ± 0.34b**	**35.17 ± 0.61c**	**39.33 ± 0.36b**	**39.70 ± 1.02b**	**39.65 ± 0.45b**	**40.75 ± 0.30b**	**43.31 ± 0.03a**
Camphor	1145	1141	0.04 ± 0.02B	0.17 ± 0.01aA	0.09 ± 0.03b	0.12 ± 0.01b	0.00 ± 0.00d	0.05 ± 0.05c	0.15 ± 0.02b	0.13 ± 0.03b	0.00 ± 0.00d	0.18 ± 0.03a

(Continues)

TABLE 1 | (Continued)

			Day 0	Day 6							
					EO				AA			
Time (min)			**0**	**0**	**1**		**5**		**1**		**5**	
Concentration			**0.00%**	**0.00%**	**0.001%**	**0.01%**	**0.001%**	**0.01%**	**0.50%**	**1.00%**	**0.50%**	**1.00%**
δ terpineol	1162	1162	0.30 ± 0.00A	0.28 ± 0.01A	0.28 ± 0.01a	0.27 ± 0.03a	0.31 ± 0.02a	0.34 ± 0.04a	0.32 ± 0.01a	0.30 ± 0.00a	0.29 ± 0.01a	0.31 ± 0.03a
Terpinen-4-ol	1178	1174	0.21 ± 0.01A	0.21 ± 0.01A	0.22 ± 0.01a	0.21 ± 0.00a	0.23 ± 0.01a	0.22 ± 0.00a	0.21 ± 0.00a	0.25 ± 0.01a	0.24 ± 0.01a	0.25 ± 0.02a
α terpineol	1191	1186	1.78 ± 0.04A	1.79 ± 0.04A	1.79 ± 0.02a	1.74 ± 0.01a	1.84 ± 0.00a	1.86 ± 0.06a	1.93 ± 0.04a	1.87 ± 0.01a	1.80 ± 0.09a	1.88 ± 0.08a
Octanol acetate	1210	1211	0.25 ± 0.02A	0.25 ± 0.03abA	0.19 ± 0.03ab	0.26 ± 0.00a	0.25 ± 0.02ab	0.20 ± 0.04ab	0.16 ± 0.01b	0.21 ± 0.03ab	0.18 ± 0.00ab	0.23 ± 0.01ab
Isobornyl acetate	1285	1283	0.59 ± 0.15A	0.42 ± 0.03bcA	0.16 ± 0.04c	0.78 ± 0.20a	0.20 ± 0.05c	0.50 ± 0.00b	0.37 ± 0.07bc	0.36 ± 0.06bc	0.16 ± 0.00c	0.38 ± 0.02bc
Eugenol	**1356**	**1356**	**19.65 ± 0.58A**	**14.78 ± 0.27aB**	**13.08 ± 0.55ab**	**11.65 ± 1.01b**	**10.44 ± 0.44c**	**10.35 ± 0.07c**	**9.14 ± 0.22c**	**11.92 ± 0.28b**	**14.34 ± 0.37a**	**10.57 ± 0.34c**
β elemene	1393	1389	0.27 ± 0.02B	0.37 ± 0.01aA	0.29 ± 0.00b	0.28 ± 0.03b	0.24 ± 0.04b	0.26 ± 0.00b	0.28 ± 0.02b	0.23 ± 0.02b	0.22 ± 0.03b	0.25 ± 0.02b
***Trans*-α-bergamotene**	**1444**	**1432**	**6.58 ± 0.49B**	**10.80 ± 0.26aA**	**7.02 ± 0.28c**	**8.27 ± 0.41b**	**10.13 ± 0.73a**	**8.81 ± 0.02b**	**9.17 ± 0.34b**	**8.10 ± 0.62b**	**6.89 ± 0.49c**	**7.40 ± 0.32c**
α guaiene	1445	1437	0.16 ± 0.02B	0.27 ± 0.01aA	0.20 ± 0.01ab	0.20 ± 0.03ab	0.17 ± 0.01b	0.17 ± 0.02b	0.19 ± 0.03b	0.18 ± 0.03b	0.18 ± 0.03b	0.19 ± 0.01b
α humulene	1461	1452	0.21 ± 0.03B	0.36 ± 0.02aA	0.20 ± 0.01b	0.23 ± 0.04b	0.29 ± 0.04ab	0.23 ± 0.02b	0.27 ± 0.04b	0.20 ± 0.00b	0.16 ± 0.02c	0.21 ± 0.01b
Trans-β-farnesene	1464	1454	0.08 ± 0.01 A	0.14 ± 0.01aA	0.07 ± 0.00b	0.10 ± 0.02ab	0.13 ± 0.03a	0.11 ± 0.01ab	0.10 ± 0.02ab	0.08 ± 0.00b	0.08 ± 0.01b	0.08 ± 0.01b
Cis-cadina-1 (6), 4-diene	1476	1461	0.14 ± 0.02A	0.18 ± 0.01A	0.12 ± 0.01a	0.15 ± 0.02a	0.11 ± 0.01a	0.16 ± 0.01a	0.14 ± 0.02a	0.12 ± 0.01a	0.11 ± 0.02a	0.13 ± 0.01a
Germacrene D	1497	1484	0.63 ± 0.08A	0.70 ± 0.04aA	0.47 ± 0.01b	0.63 ± 0.04a	0.48 ± 0.09b	0.63 ± 0.05a	0.61 ± 0.07a	0.48 ± 0.03b	0.31 ± 0.01c	0.44 ± 0.02b
Bicyclogermacrene	1512	1500	0.51 ± 0.06A	0.74 ± 0.03aA	0.56 ± 0.03b	0.57 ± 0.06b	0.50 ± 0.01b	0.52 ± 0.02b	0.55 ± 0.08b	0.52 ± 0.05b	0.48 ± 0.05b	0.28 ± 0.26bc

(Continues)

TABLE 1 | (Continued)

			Day 0	Day 6							
					EO				AA			
Time (min)			**0**	**0**	**1**		**5**		**1**		**5**	
Concentration			**0.00%**	**0.00%**	**0.001%**	**0.01%**	**0.001%**	**0.01%**	**0.50%**	**1.00%**	**0.50%**	**1.00%**
γ cadinene	1525	1513	1.46 ± 0.15B	2.18 ± 0.02aA	1.46 ± 0.00b	1.63 ± 0.15b	1.55 ± 0.11b	1.74 ± 0.02b	1.79 ± 0.21b	1.51 ± 0.09b	1.42 ± 0.16b	1.59 ± 0.03b
β sesquiphellandrene	1532	1521	0.29 ± 0.04B	0.49 ± 0.02aA	0.29 ± 0.00b	0.37 ± 0.03ab	0.37 ± 0.05ab	0.39 ± 0.01ab	0.39 ± 0.06ab	0.32 ± 0.04ab	0.25 ± 0.04b	0.30 ± 0.01ab
Spathulenol	1581	1577	0.13 ± 0.02B	0.42 ± 0.03aA	0.25 ± 0.06b	0.24 ± 0.03b	0.53 ± 0.04a	0.20 ± 0.03b	0.35 ± 0.04ab	0.25 ± 0.05b	0.24 ± 0.01b	0.29 ± 0.03b
1,10-di-epi-cubenol	1625	1618	0.27 ± 0.04A	0.41 ± 0.01A	0.27 ± 0.02a	0.34 ± 0.02a	0.36 ± 0.02a	0.35 ± 0.01a	0.38 ± 0.05a	0.33 ± 0.02a	0.29 ± 0.02a	0.36 ± 0.02a
Epi-α-cadinol	1641	1638	3.50 ± 0.39A	4.81 ± 0.21aA	3.48 ± 0.08b	4.06 ± 0.01a	4.13 ± 0.08a	4.25 ± 0.06a	4.45 ± 0.17a	4.06 ± 0.09ab	3.57 ± 0.14b	4.22 ± 0.05a
Total >0.05%			99.39 ± 0.10A	98.58 ± 0.08A	99.39 ± 0.02a	99.01 ± 0.10a	98.71 ± 0.19a	99.13 ± 0.01a	99.04 ± 0.17a	99.26 ± 0.03a	99.45 ± 0.02a	99.01 ± 0.28a
Monoterpenes			6.51 ± 0.04A	7.14 ± 0.29bA	7.08 ± 0.53b	6.81 ± 0.12b	9.37 ± 0.81a	7.04 ± 0.45b	7.46 ± 0.28b	7.61 ± 0.55b	6.80 ± 0.00b	6.54 ± 0.08b
Sesquiterpenes			10.30 ± 0.93B	16.23 ± 0.42aA	10.67 ± 0.22c	12.39 ± 0.80b	13.95 ± 1.09b	13.00 ± 0.08b	13.47 ± 0.87b	11.72 ± 0.07c	10.08 ± 0.84c	10.85 ± 0.67c
	Oxygenated monoterpenes		78.09 ± 1.66A	69.14 ± 0.01bB	77.50 ± 0.44a	74.42 ± 0.73ab	70.18 ± 0.55b	73.80 ± 0.45ab	72.57 ± 1.84ab	74.95 ± 1.54ab	78.32 ± 1.00a	76.40 ± 0.38ab
	Oxygenated sesquiterpenes		3.90 ± 0.44B	5.65 ± 0.25aA	3.99 ± 0.15b	4.63 ± 0.15ab	5.01 ± 0.03ab	4.80 ± 0.09ab	5.19 ± 0.46ab	4.63 ± 0.25	4.10 ± 0.15b	4.86 ± 0.09ab
Others			0.59 ± 0.15A	0.42 ± 0.03bA	0.16 ± 0.04c	0.78 ± 0.20a	0.20 ± 0.05c	0.50 ± 0.00b	0.37 ± 0.07b	0.36 ± 0.06b	0.16 ± 0.00c	0.38 ± 0.02b
EO yield %			**1.12 ± 0.13A**	**1.05 ± 0.02aA**	**1.14 ± 0.17a**	**0.86 ± 0.02b**	**0.78 ± 0.09b**	**0.86 ± 0.04b**	**0.81 ± 0.09b**	**0.87 ± 0.06b**	**0.97 ± 0.06ab**	**0.81 ± 0.04b**

Values are presented as the mean ± standard error ($n = 3$). The values for day 0 allude to the control (non-treated, 0.00%). Significant differences ($p < 0.05$) are highlighted with different lowercase Latin letters on each row. Different capital Latin letters highlight significant differences between the initial (day 0) and last day of storage of control (non-treated, 0.00%).

TABLE 2 | Effects of basil EO and ascorbic acid (AA) application on fresh basil's color parameters (hue angle-h (°), chroma value-C, and color index-CI) pigments (chlorophylls and total carotenoids), after storage at 4°C for 6 days.

	Time (min)	Concentration	h (°)	C	CI	Chl a (mg/g)	Chl b (mg/g)	Tot Chl (mg/g)	Tot Car (mg/g)
Day 0	0	0.00%	123.54 ± 0.97A	35.62 ± 0.88A	−13.36 ± 0.41A	0.64 ± 0.04A	0.30 ± 0.02A	0.95 ± 0.05A	0.07 ± 0.01A
Day 6	0	0.00%	125.22 ± 0.13abA	34.81 ± 0.51aA	−15.39 ± 0.20abB	0.63 ± 0.05aA	0.28 ± 0.03aA	0.91 ± 0.08aA	0.09 ± 0.003aA
EO	1	0.001%	124.07 ± 0.62b	33.28 ± 1.00a	−14.77 ± 0.59ab	0.65 ± 0.02 a	0.29 ± 0.01a	0.94 ± 0.03a	0.11 ± 0.006a
		0.01%	125.02 ± 0.53ab	34.32 ± 0.88a	−15.02 ± 0.73ab	0.57 ± 0.03 a	0.24 ± 0.01a	0.82 ± 0.03a	0.08 ± 0.006a
	5	0.001%	126.00 ± 0.37a	33.48 ± 0.64a	−16.34 ± 0.42b	0.62 ± 0.14 a	0.27 ± 0.06a	0.89 ± 0.20a	0.08 ± 0.018a
		0.01%	125.18 ± 0.59ab	32.66 ± 0.74a	−15.12 ± 0.44ab	0.65 ± 0.03 a	0.30 ± 0.02a	0.95 ± 0.05a	0.10 ± 0.013a
AA	1	0.50%	125.06 ± 0.37ab	34.89 ± 1.24a	−15.05 ± 0.32ab	0.68 ± 0.06 a	0.31 ± 0.03a	0.98 ± 0.10a	0.10 ± 0.011a
		1.00%	124.61 ± 0.38ab	34.83 ± 0.65a	−14.92 ± 0.42ab	0.62 ± 0.06 a	0.26 ± 0.03a	0.88 ± 0.09a	0.10 ± 0.009a
	5	0.50%	123.85 ± 0.81b	35.18 ± 1.56a	−14.38 ± 0.74a	0.62 ± 0.07 a	0.27 ± 0.03a	0.89 ± 0.10a	0.10 ± 0.009a
		1.00%	124.21 ± 0.78b	35.69 ± 1.36a	−14.85 ± 0.80ab	0.56 ± 0.06 a	0.24 ± 0.03a	0.80 ± 0.09a	0.09 ± 0.010a

Values are the mean ± standard error ($n = 4$). The values for day 0 allude to the control (non-treated, 0.00%). Significant differences ($p < 0.05$) are highlighted with different lowercase Latin letters on each column. Different capital Latin letters highlight significant differences between the control on day 0 and 6.

FIGURE 3 | Effects of basil EO and ascorbic acid (AA) application on basil kept at 4°C for 6 days. Selected cases of basil deterioration are presented in the right column.

3.6 | Microbial Load

As shown on Table 5, all applied basil EO and AA treatments were found to decrease the APC numbers (up to 0.59 log reduction with AA 1%-1 min) ($P = 0.000$). Yeasts and filamentous fungi were reported to be more susceptible to the basil EO treatments (both concentrations and both times of application), with basil EO 0.001%-1 min showing the greatest reduction (up to 1.6 log reduction) ($P = 0.040$).

4 | Discussion

Fresh sweet basil is marketed in small bundles of freshly cut shoots and is preferred by the consumers for its distinct aroma and taste as well as its high nutritional value (rich in retinol-vitamin A, calcium, and potassium) (Changsawake et al. 2017). However, basil is considered a very delicate leafy herb with a limited shelf life (approx. 6–7 days), while deterioration symptoms appear at very early postharvest stages, especially due to improper handling and storage (Iakimova et al. 2024; Brindisi and Simon 2023). Fresh leafy vegetables/herbs after harvest are typically stored at chilled temperatures to minimize the metabolic changes and ensure high-quality goods (Wang et al. 2015; Cozzolino et al. 2016). Perishable products can be subjected to a numerous physical and chemical treatments for the preservation of their quality.

TABLE 3 | Effects of spearmint EO and ascorbic acid (AA) application on fresh basil's total phenols, antioxidants (DPPH, FRAP and ABTS), total flavonoids, and ascorbic acid (AA) content after storage at 4°C for 6 days.

	Time (min)	Concentration	Total phenols (mg GEA/g)	DPPH (mg trolox/g)	FRAP (mg trolox/g)	ABTS (mg trolox/g)	Total flavonoids (mg rutin/g)	AA (mg AA/100 g)
Day 0	0	0.00%	1.86 ± 0.13A	2.95 ± 0.15A	4.25 ± 0.40A	4.99 ± 0.59A	1.44 ± 0.11A	17.30 ± 0.31A
Day 6	0	0.00%	0.96 ± 0.10dB	1.61 ± 0.10cdB	2.69 ± 0.03dbA	2.28 ± 0.39dB	0.60 ± 0.04dB	16.51 ± 1.22bcA
EO	1	0.001%	1.76 ± 0.14c	3.24 ± 0.32a	4.39 ± 0.31ab	6.04 ± 0.97abc	1.18 ± 0.14bc	11.24 ± 2.45c
		0.01%	1.74 ± 0.13c	2.96 ± 0.29ab	4.53 ± 0.38ab	6.58 ± 0.87ab	1.25 ± 0.15bc	13.67 ± 2.14c
	5	0.001%	1.30 ± 0.06cd	2.04 ± 0.15bc	3.47 ± 0.21bcd	3.51 ± 0.33cd	0.71 ± 0.03d	14.77 ± 1.43c
		0.01%	1.65 ± 0.21c	2.54 ± 0.41abc	3.82 ± 0.53bc	4.34 ± 1.48bcd	1.18 ± 0.21bc	15.70 ± 0.23bc
AA	1	0.50%	1.56 ± 0.06c	1.75 ± 0.22cd	3.10 ± 0.22cd	2.81 ± 0.73d	0.76 ± 0.09d	14.66 ± 1.06c
		1.00%	2.86 ± 0.16a	3.22 ± 0.20a	5.43 ± 0.38a	8.08 ± 0.76a	1.54 ± 0.10ab	30.00 ± 3.20a
	5	0.50%	2.64 ± 0.24ab	2.86 ± 0.40ab	5.22 ± 0.47a	7.44 ± 0.80a	1.70 ± 0.19a	20.84 ± 0.30b
		1.00%	2.26 ± 0.10b	1.32 ± 0.52d	3.95 ± 0.27bc	3.45 ± 0.64cd	0.93 ± 0.10cd	16.60 ± 0.83bc

Values are the mean ± standard error ($n = 4$). The values for day 0 allude to the control (non-treated, 0.00%). Significant differences ($p < 0.05$) are highlighted with different lowercase Latin letters on each column. Different capital Latin letters highlight significant differences between the control on day 0 and 6.

TABLE 4 | Effects of basil EO and ascorbic acid (AA) application on fresh basil's total soluble solids (TSS) and titratable acidity (TA), hydrogen peroxide (H_2O_2) production, and lipid peroxidation (MDA), stored at 4°C for 6 days.

	Time (min)	Concentration	TSS (°Brix)	TA (g citric acid/g)	H_2O_2 (μmol/g)	MDA (nmol/g)
Day 0	0	0.00%	0.18 ± 0.01A	0.18 ± 0.01A	0.38 ± 0.04A	6.86 ± 0.48A
Day 6	0	0.00%	0.27 ± 0.03dA	0.17 ± 0.003bcA	0.16 ± 0.05abB	4.55 ± 0.28dB
EO	1	0.001%	0.33 ± 0.03bcd	0.17 ± 0.015bc	0.17 ± 0.02ab	4.93 ± 0.35cd
		0.01%	0.33 ± 0.03bcd	0.18 ± 0.003bc	0.16 ± 0.02ab	3.26 ± 0.03e
	5	0.001%	0.30 ± 0.00cd	0.23 ± 0.020a	0.06 ± 0.01bc	4.97 ± 0.26cd
		0.01%	0.40 ± 0.00abc	0.16 ± 0.009c	0.17 ± 0.04a	5.16 ± 0.34cd
AA	1	0.50%	0.37 ± 0.03abcd	0.24 ± 0.017a	0.05 ± 0.02c	6.37 ± 0.20ab
		1.00%	0.43 ± 0.03ab	0.21 ± 0.009abc	0.21 ± 0.03a	7.16 ± 0.41a
	5	0.50%	0.47 ± 0.03a	0.19 ± 0.026abc	0.21 ± 0.05a	5.72 ± 0.11bc
		1.00%	0.43 ± 0.07ab	0.21 ± 0.022ab	0.19 ± 0.05a	5.93 ± 0.59bc

Values are the mean ± standard error ($n = 4$). The values for day 0 allude to the control (non-treated, 0.00%). Significant differences ($p < 0.05$) are highlighted with different lowercase Latin letters on each column. Different capital Latin letters highlight significant differences between the control on day 0 and 6.

Sweet basil has a comparatively shorter shelf life than other varieties of basil and other leafy vegetables (Ciriello et al. 2023; Brindisi and Simon 2023). One frequent aspect that negatively influences the shelf life and quality of the majority of fresh produce is water loss. Water loss in fresh commodities is linked with higher metabolic processes including respiration and transpiration. In the current study, the highest weight loss was observed with basil treated with AA 0.5% (1 and 5 min) (up to 6.70 %). The process of transpiration for most vegetables can result in more than 3–10% loss of their weight, thus losing their freshness (Changsawake et al. 2017). It is important to note that increased weight loss (>3%) is associated with leafy vegetables of low quality showing leaf wilting and shrinkage (Sánchez-García et al. 2021). In addition, higher respiration rate was observed with AA 0.5%-5 min suggesting that this AA dose caused a stress to the plant tissue increasing its metabolic rate (Changsawake et al. 2017). High respiration rate in leafy vegetables like spinach and lettuce is associated with higher metabolic processes and rapid senescence that can manifest as yellowing and wilting of the leaves due to their pigment degradation (Poimenidou et al. 2016). However, basil appearance, including visual quality and green color as well as decay absence were maintained through 6 days of storage to chilled conditions (Figure 3 and Figure S1, Supporting Information).

The loss of volatile constituents is the most important characteristic to consider when assessing fresh herbs' postharvest quality (Brindisi and Simon 2023). Maintaining the quality of basil is of great essence since it assists in keeping its volatile compounds, which give its distinct fragrance (Hassan et al. 2021). The trichomes of basil leaves and flowers produce and store their EOs. A variety of aromatic compounds (mainly monoterpenes

TABLE 5 | Effects of basil EO and ascorbic acid (AA) application on fresh basil's microbial load- aerobic plate count (APC) and yeasts and molds after storage at 4°C for 6 days.

	Time (min)	Concentration	APC (log CFU/g)	Yeasts and filamentous fungi (log CFU/g)
Day 0	0	0.00%	6.11 ± 0.05A	5.79 ± 0.08A
Day 6	0	0.00%	5.22 ± 0.03aB	5.38 ± 0.14aA
EO	1	0.001%	4.91 ± 0.03c	3.78 ± 0.52c
		0.01%	4.86 ± 0.01c	4.14 ± 0.59bc
	5	0.001%	4.89 ± 0.06c	4.12 ± 0.13bc
		0.01%	4.91 ± 0.02c	4.09 ± 0.18bc
AA	1	0.50%	4.78 ± 0.06c	4.68 ± 0.09abc
		1.00%	4.63 ± 0.04d	5.04 ± 0.04ab
	5	0.50%	5.07 ± 0.06b	5.01 ± 0.16ab
		1.00%	4.80 ± 0.05c	4.77 ± 0.17abc

Values are the mean ± standard error ($n = 4$). The values for day 0 allude to the control (non-treated, 0.00%). Significant differences ($p < 0.05$) are highlighted with different lowercase Latin letters on each column. Different capital Latin letters highlight significant differences between the control on day 0 and 6.

and phenylpropenes) are released when the plant tissue is cut or otherwise injured (Patel et al. 2021). With the current work, it was found that major compounds such as eucalyptol (monoterpene with fresh/eucalyptus-like aroma) and linalool (monoterpene with sweet/floral aroma) were found to increase at the end of storage, while eugenol (phenylpropene with clove-like aroma) and *trans*-α-bergamotene were found in lower levels. Basil's EO was also found to be affected by the applied treatments with lower yield observed by all applied treatments (except basil EO 0.001%-1 min and AA 0.5%-5 min that did not differ from the control). As it happens with other herbs and vegetables, their aroma is influenced by the storage temperature among other parameters. When exposed at chilling temperatures the volatile constituents of a chilling-sensitive commodity tend to decrease. Chilling injury of fresh basil could be perceived by visual symptoms that can become obvious after 6–9 days of storage at temperatures below 4°C, however the levels of eucalyptol (1,8-cineole) have been reported to be reduced after 3–6 days while no clear signs of chilling injury were visual yet (Cozzolino et al. 2016). Thus, eucalyptol levels could be considered as an indicator for basil aroma degradation. As it seems from the current study, the applied treatments (especially basil EO) were able to preserve the quality of basil EO even though lower EO yields were found while protecting fresh basil plants from decay. Browning on basil leaves observed on the AA treated bundles at fourth and sixth day of storage might have been caused by the oxidation of possible AA residues on the leaf surfaces (Chung et al. 2021; Xylia et al. 2021).

The set of properties that render a commodity appealing for human consumption is known as quality (Al-Dairi et al. 2022). Mechanical damage during harvesting and processing as well as increased accumulation of CO_2 and ethylene in packaging and storage can accelerate the postharvest senescence process and ultimately contribute to the appearance of deteriorating symptoms such as leaf yellowing/browning, decomposing/water-soaked lesions, and/or superficial scald (Iakimova et al. 2024). For fresh basil to continue to be attractive to consumers after transportation and storage, it is essential that its volatile compounds are preserved throughout these steps. Aroma and taste are key determinants of consumer acceptance, and these attributes are strongly related to basil's major volatile compounds, such as linalool, eucalyptol, and eugenol (Patel et al. 2021). In general, volatile constituents decrease over storage time and especially at temperatures below 4°C (Cozzolino et al. 2016). In the present work, the main compounds of basil EO including eucalyptol and linalool were preserved on basil treated with the EO and AA as opposed to the control contributing to the preservation of the sweet and pleasant aroma of basil. The preservation of basil's aroma was also observed in basil treated with 2% lactic acid and storage at 4°C for 4 days (Valiolahi et al. 2019). The preservation of the fresh basil-like aroma and the leaf's bright green color (due to the chlorophyll content) in the current study could be attributed to the protective and antioxidant activity that the basil EO and AA present.

Gene expression linked to oxidative damage and senescence along with protein degradation, alterations in the metabolism of phospholipids, and activation of hydrolytic enzymes activity are some of the processes that can cause tissue disintegration (Iakimova et al. 2024). The breakdown of the membrane integrity, which causes the release of many nutrients into the surrounding area, is another primary symptom of fresh produce senescence. Leaf senescence due to elevated nutrients and electrolyte leakages has been shown in lemon basil and spinach (Changsawake et al. 2017). In order to minimize leaf decay and preserve the quality of sweet basil, it is essential to regulate the chlorophyll and protein degradation metabolism (Hassan et al. 2021). It is important to note that respiration continues over the postharvest stage and at elevated rates, respiration has been linked with rapid senescence and greater chlorophyll degradation in leafy greens (Solouki et al. 2023; Shezi et al. 2024). As was already indicated, one of the alterations that occurs during the senescence process of leafy vegetables is the breakdown of chlorophylls and other chloroplast components like thylakoids and stroma (Changsawake et al. 2017). This could lead to the loss of leaf's green color and its discoloration such as yellowing and/or browning.

The antioxidant activity of basil EO and AA in combination with the low temperature and high humidity could have prevented the oxidation and degradation of chlorophylls of fresh basil throughout the 6 days of storage. This could also have been the effect of the lower respiration rate observed in the present work, which also indicated a slower metabolic rate (Shezi et al. 2024). By lowering the metabolism of a plant tissue the process of senescing slows down, thus the extension of the commodity's shelf life. A prior research indicated that after 10 days of storage, basil's leaf quality was preserved by a combination of high humidity (90–95%) and low temperature (5°C) due to the reduced enzymatic activity as well as the lower water loss (Brindisi and Simon 2023). In addition, the protective effect of various EOs including oregano and rosemary on the chlorophyll content from different medicinal/aromatic plants such as basil and rosemary has been previously reported (Xylia et al. 2022; Xylia et al. 2024).

Basil is a rich source of phenolics and anthocyanins, which are compounds with great antioxidant activity. However, a decline in the antioxidant capacity of fresh produce is reported during senescence. The plant's defensive mechanism against senescence include antioxidants and antioxidant enzymes (Rodeo and Mitcham 2024; Changsawake et al. 2017). Non-enzymatic antioxidants like phenols, phenolic acids, and flavonoids can scavenge and eradicate free radicals that are responsible for the oxidative stress (Solouki et al. 2023). In the present research, the use of basil EO for 1 min, AA 1%-1 min, and AA 0.5%-5 min were found to increase the antioxidant activity and flavonoids content of fresh basil during storage. A previous study showed that the application of rosemary EO (0.06 and 0.2%) and AA (1 and 2%) for 10 min on fresh rosemary also resulted in increased phenolic content and antioxidant activity of fresh rosemary stored at 4°C for 12 days (Xylia et al. 2022). One possible explanation for this is the antioxidant activity that these natural products possess, causing a "light" stress (also called "eustress") on the plant tissue and the activation of plants' antioxidant mechanisms as a response. In addition, the increase in phenolic compounds and AA content of fresh basil observed by the AA 1%-1 min treatment might be attributed to the possible AA residues on basil's leaves and/or its detection via the methodologies followed for the determination of phenols and AA (Sánchez-Rangel et al. 2013).

The damage of the plant's cell wall membrane due to lipid peroxidation and the oxidation of various intracellular components are associated with the production of H_2O_2 and other ROS (Solouki et al. 2023). Oxidative stress can be caused by the surplus production of ROS and the increased metabolic rate of fresh produce (Hassan et al. 2021; Rodeo and Mitcham 2024). Extensive oxidative stress will eventually result into rapid senescence and cell death. A decrease in the H_2O_2 and MDA levels was observed with AA 1 min treatments and basil EO 0.001%-5 min. On the other hand, treatment of fresh rosemary with 0.2% rosemary EO for 10 min resulted into lower lipid peroxidation whilst AA treatment (1 and 2%) presented similar levels to the control (Xylia et al. 2022). This may be explained by the protective effects of the antioxidant activity of both investigated products (AA and basil EO). At the same time, increases in the damage indexes might have been attributed to prolonged exposure to stress. Moreover, increases in the TSS value by AA applied treatments suggesting the presence of oxidative stress as indicated by increased H_2O_2 and MDA levels caused by these treatments. The TSS has also been previously used as a reference and damage index as well (Keunen et al. 2013).

The microbial load of fresh produce is also important and is linked to the duration of its shelf life. A higher microbial load during storage under high RH will increase the perishability of a product, shortening dramatically its shelf life (Iakimova et al. 2024). Washing of fresh produce is an effective way for the removal of chemical and biological contaminants, especially when combined with antimicrobial substances (Changsawake et al. 2017). In the current work, a significant reduction of basil's APC was observed with both investigated means (basil EO and AA), whilst yeast and filamentous fungi numbers were decreased when basil was treated with basil EO. Similarly, lower microbial load was observed on rosemary treated for 10 min with AA (1% and 2%), highlighting its antimicrobial properties, as previously reported (Xylia et al. 2022). This could be explained by the slightly lower pH of the washing water as a result of the mild organic acids used as previously reported against bacteria like *S*. Enteritidis and molds like *Aspergillus* spp. (Sangcharoen et al. 2017; Hernandez-Patlan et al. 2018; Jackson-Davis et al. 2023).

Previous studies have shown the versatility and efficiency of basil's EO antimicrobial activity against *Fusarium solani* in asparagus (Grata 2016), *Escherichia coli* and *S. typhimurium* in lettuce and purslane (Karagözlü et al. 2011), and *Listeria monocytogenes* in radish sprouts (Lee et al. 2018). The antimicrobial activity of basil EO is actually attributed to its main compounds, like linalool, eucalyptol, and eugenol. These EO compounds can interfere with the microorganisms' cell wall and disrupt the phospholipid membrane, leading to the membrane's disruption and increased permeability, which eventually can cause the leakage of essential cell components and cell lysis (Dhifi et al. 2016; Jackson-Davis et al. 2023). Linalool is the most commonly found and utilized monoterpene in food and can be found in over 23 different foods. (Mączka et al. 2022). Linalool has a wide range antimicrobial activity towards pathogenic bacteria like *Staphylococcus aureus* and *Pseudomonas aeruginosa* and fungi including *Candida albicans* and *Aspergillus brasiliensis* (Herman et al. 2016). Eucalyptol is a bicyclic terpenoid with reported antimicrobial activity against various microorganisms such as *Enterococcus faecalis*, *S. aureus*, *E. coli*, *S*. Typhimurium, *Acinetobacter baumannii*, and *C. albicans* (Aldoghaim et al. 2018). Eugenol is also known to present great bacteriostatic (*Klebsiella pneumoniae, Proteus mirabilis*), bactericidal (*S. aureus*), and fungicidal (*C. glabrata*, *C. krusei*) activities (Rehab and Zeinab 2016). Overall, the synergistic activity of these compounds found in basil EO could have resulted to the lowering of fresh basil's microbial load.

5 | Conclusion

The effects of basil EO and AA, at varying concentrations and times of submersion, on the quality characteristics of fresh basil stored at chilled temperature (4°C) for 6 days were investigated with the current study. From the results, it was found that an increase in basil's respiration rate was caused by the AA application (0.5%-5 min). Increased total flavonoids and antioxidants were found on basil treated with basil EO 1 min (0.001% and

0.01%), AA 1%-1 min, and AA 0.5%-5 min. This observation highlighted the increase in basil's nutritional value at the end of storage compared to the control (initial and end day). Furthermore, both investigated products (basil EO and AA) lowered fresh basil's microbial load (aerobic plate count, yeast and filamentous fungi), contributing to the product's storability. In summary, the natural products investigated with the present study (basil EO and AA) could be considered as possible alternative preservative mean for fresh basil because they preserved the sensory qualities of basil and increased its nutritious content. It seems that the lower concentrations and shorter time of application gave more encouraging results for this product. Nonetheless, additional research is necessary for the commercialization and broadening these means for fresh basil and other herbs.

Author Contributions

Panayiota Xylia: investigation, validation, formal analysis, software, data curation, writing – original draft, methodology. **Antonios Chrysargyris**: conceptualization, methodology, software, data curation, writing – original draft, writing – review and editing, investigation, validation, supervision, visualization. **Gokhan Zengin**: conceptualization, methodology, visualization, writing – review and editing, formal analysis, data curation, validation, software. **Nikolaos Tzortzakis**: writing – original draft, writing – review and editing, project administration, resources, funding acquisition, conceptualization, methodology, data curation, investigation, supervision.

Conflicts of Interest

The authors declare no conflicts of interest.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.

Supplementary Figure: jfds70467-sup-0001-FigureS1.docx