Effects of treatment by vapour of essential oil from *Thymus vulgaris* and *Satureja montana* on postharvest quality of sweet cherry (cv. Ferrovia)

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Summary

During storage time, postharvest diseases and senescence processes can decrease quality parameters of sweet cherries. In this study, the effects of two different concentrations (4.5 mg·ml^{-1} and 18 mg·ml^{-1}) of essential oil of *Thymus vulgaris* and *Satureja montana* (vapour treatment) on quality and sensory parameters of sweet cherries were investigated. The fruits were stored for 28 days in a cold room (1 °C) at 90–95% relative humidity. After the cold storage, the fruits were stored for 3 days at 20 °C. Concerning the results, it was observed that the use of essential oils reduced the weight loss and the vitamin C content loss. The effect of these treatments on titratable acidity and total soluble solids was more limited. Vapour phase of essential oils demonstrated the capability to control postharvest pathogens on cherries even without direct contact, reducing postharvest waste up to about 62 % compared to untreated fruit. Furthermore, the sensory analysis showed a positive effect on the visual aspect, while it pointed out a negative effect on off-flavour and aroma mainly for savory samples.

Keywords

biofumigation; essential oils; savory; sweet cherry; thyme

Sweet cherries (*Prunus avium* L.) are very important fruits for a healthy diet, in particular for their antioxidant power and vitamins. During storage time, postharvest diseases and senescence processes can decrease quality parameters of the final product. Many diseases caused by common pathogens affect the storage of sweet cherries, in particular *Monilinia laxa* (Aderh. and Ruhl.) (brown rot), *Botrytis cinerea* Pers. (grey mould) and, with a lower incidence, *Alternaria alternata* (Fr.:Fr.) Keissl. (Alternaria rot), *Penicillium expansum* Link (blue mould), *Rhizopus stolonifer* (Ehrenb.) Vuill. (Rhizopus rot) and *Cladosporium* spp. (green rot) [1, 2]. The European legislation restricts the use of synthetic fungicides to control postharvest diseases [3]. For this reason, the research is focused on developing innovative and sustainable strategy to preserve fruit quality and decrease food waste, using treatments with low environmental impact.

Several authors have recognized essential oils (EOs) as an important tool to fight postharvest diseases because of their antimicrobial properties [4–6]. Antimicrobial properties are the result of chemical composition of EOs, which are rich in phenolic compounds, mainly carvacrol and thymol mainly. The antimicrobial activity seems to be connected to damage of permeability of cytoplasmatic membrane [7–10]. Moreover, a positive correlation between the number of chemical compounds and the effectiveness on postharvest pathogen control was supposed [11, 12]. EOs of *Thymus*

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vulgaris and *Satureja montana* are mainly composed by thymol and carvacrol and their antifungal effects were investigated on many fruits [5]. In the last years, the efficacy of EOs vapour as a fumigant was investigated, the use of vapour being aimed at the decrease in phytotoxicity and organoleptic modification caused by the direct contact of EOs with the fruits [13]. In this study, we investigated the effects of *T. vulgaris* and *S. montana* EOs, by vapour treatment, on quality and sensory parameters of sweet cherries during cold storage and shelf life.

MATERIALS AND METHODS

Plant material

Sweet cherries (cv. Ferrovia) were picked by hand in the middle of June from an experimental orchard located in Pecetto (Torino, Italy, 45°01'21.4"N 7°44'44.5"E) and transported immediately to the laboratory of Department of Agricultural, Forestry and Food Sciences (University of Torino, Grugliasco, Italy) during the summer 2016. All fruits were selected to be defect-free and were randomly placed directly in commercial plastic baskets made from perforated polyethylene. Each plastic basket contained about 50 fruits and the weight was about 450 g. The plastic baskets were divided into 5 groups: control and 4 treated (by EOs of thyme, *T. vulgaris,* and savory, *S. montana).* The fruits were treated with two concentrations of EOs and were stored for 28 days in a cold room (at a tempterature of 1 °C) at 90–95% relative humidity. After the cold storage, the fruits were stored for 3 days at 20 °C (shelf life).

Essential oil treatments and storage

EOs of thyme (*T. vulgaris*) and savory (*S. montana*) used in the assays were a commercial preparation provided by Soave (Turin, Italy). EO diffusers were made by adding EO (10 % v/v) into sterilized deionized water (88 % v/v) added with 15 $g \cdot l^{-1}$ agar-agar (Merck, Darmstadt, Germany) and Tween 20 (2 % v/v) (Merck) at 60 °C. The emulsion was poured into Petri dishes and let solidify. Lower EO concentrations were obtained by serial dilutions. After agar solidification, the diffusers were installed in storage cabinets under the fruit boxes. Vapour treatments were performed at 4.5 $mg \cdot ml^{-1}$ and 18 $mg \cdot ml^{-1}$ EOs concentrations. The samples were marked as: Control – untreated fruit samples; T1 – fruit samples treated with *T. vulgaris* EO at 4.5 $mg \cdot ml^{-1}$; T2 – fruit samples treated with *T. vulgaris* EO at 18 $mg \cdot ml^{-1}$; S1 – fruit samples treated with *S. montana* EO at 4.5 $mg \cdot ml^{-1}$; S2 – fruit samples treated with *S. montana* EO at 18 $mg \cdot ml^{-1}$. The analyses were performed weekly during cold storage and after shelf life. The trial was performed twice.

Weight loss

Weight loss was determined by weighing two plastic baskets for treatment at the beginning of the trial and during storage (7, 14, 21 and 28 days of storage and after shelf life). Values were reported as a percentage of weight loss from initial basket weight.

Colour measurement

The colour parameters of the fruits were measured weekly during the cold storage, with a Minolta chromameter (CR400; Konica Minolta, Osaka, Japan), calibrated against a standard white plate and using the CIE *L*a*b** (lightness, redness, yellowness) scale. The surface of 15 fruits per treatment was evaluated, on two opposite sides of the fruit.

Fruit quality parameters

Total soluble solids (*TSS*) and titratable acidity (*TA*) were measured every 7 days using juice extracted from 100 g of cherries blended at high speed by a homogenizer Ultra-Turrax T-25 (IKA Werke, Staufen, Germany). Three replications were used for each treatment.

TSS analysis was performed using a digital refractometer (Atago refractometer model PR-32; Atago Italia, Milan, Italy), and *TA* using a Titratable acidity station (Titration workstation Titralab AT1000Series; Radiometer Analytical, Villeurbanne, France). For total soluble solids, data are given as Brix degrees. Titratable acidity was determined by titration with 0.1 $mol \cdot l^{-1}$ NaOH and it was expressed as milliequivalents per litre.

Vitamin C was extracted and expressed as sum of ascorbic (AA) and dehydroascorbic acid (DHAA). For the extraction, 10 ml of an extraction solvent (methanol/water, 5 : 95 v/v) was added with 10 g of fruit flesh from 5 fruits per treatment and homogenized with a T-25 Ultra-Turrax for 3 min. Then, pH was adjusted to 2.2–2.4 and the extract was applied to a C18 Sep-Pak cartridge (Waters, Milford, Massachusetts, USA). The eluate (0.750 ml) was added to 0.350 ml of 1,2-phenylenediamine dihydrochloride (Fluka Chemika, Buchs, Switzerland) and left to stand for 37 min. Then it was analysed by high performance liquid chromatography (HPLC) using an Agilent chromatographic system equipped with a Kinetex-C18 column (150 mm × 4.6 mm, particle size 5 μm; Phenomenex, Torrance, California, USA).

Three replicate analyses were performed on the flesh. The AA and DHAA contents were expressed as milligrams per kilogram of fresh weight.

Sensory evaluation

Fifteen trained panelists, aged 25–50 years, performed the sensory evaluation. Three samples for each treatment and for each panelist were presented in coded trays to prevent subjectivity. The evaluation was performed at room temperature (24 °C). Visual aspect, sweetness, acidity, bitterness, presence/absence of off flavour and aroma were evaluated using a five-point hedonic scale: l – poor, 2 – fair, 3 – good, 4 – very good, 5 – excellent. The sensory evaluation was performed with a ranked scale after 14 and 28 days of cold storage.

Disease incidence and plant pathogen identification

The incidence of diseased fruits (percentage of rotten fruits of the total fruits) was calculated for each treatment weekly and after shelf life. Postharvest pathogens were isolated by transferring small pieces of symptomatic fruit tissues, previously washed in 1% sodium hypochlorite in order to avoid contamination with saprophytic fungal spores or other microorganisms commonly present on fruit surfaces and rinsed in sterile deionized water, onto potato dextrose agar (PDA, Merck) plates amended with 25 $mg \cdot l^{-1}$ streptomycin sulfate (Merck). Rotting agents were allowed to grop on the synthetic media and, after 7 days, the culture was used for DNA extraction by using the EZNA Plant DNA Kit (Omega Bio-Tek, Norcross, Georgia, USA). The internal transcribed spacer (ITS) region of rDNA of isolates was amplified using the ITS1/ITS4 primers [14]. ITS amplicons were sequenced by BMR Genomics (Padua, Italy) and DNA sequences were compared with those present in the National Center for Biotechnology Information (NCBI) database (Bethesda, Maryland, USA).

Statistical analysis

Analysis of variance (ANOVA) was performed on the data using SPSS (Statistics version 24; IBM, Armonk, New York, USA). Least significant differences (*LSD*) at a significance level of 0.05 were used to compare treatment means with Tukey's test. Mean values were considered significantly different at $P \leq 0.05$. The sources of variance were the treatments and the days of storage.

RESULTS AND DISCUSSION

Weight loss

Significant differences were observed between the samples treated with EO and the control (Tab. 1). The weight loss showed statistical differences among treatments during the storage. After 7, 14, 21 and 28 days, we observed that the samples treated by fumigation with EO presented a lower weigh loss than the control samples, in particular S2 and T1 recorded the lowest values during shelf life at 20 °C. This was in accordance with the studies of Martínez-Romero et al. [15] and Serrano et al. [16] on grapes and sweet cherries treated with thymol, eugenol and menthol vapours. Similar results were reported in the review of Sivakumar and Bautista-Banos [6], in 'cherry' [16], 'Crimson Seedless' [17] and 'Autumn Royal' table grapes [18] and peaches [19]. Evaluating the storage time for each treatment, we observed the highest weight loss at the end of storage and in the control sample. The differences

Tab. 1. Weight loss of sweet cherries.

Storage	7 days (1 °C)	14 days (1 °C)	21 days (1 °C)	28 days (1 °C)	3 days (20 °C)
Weight loss [%]					
Control	0.8 ± 0.1^{aC}	0.9 ± 0.0^{aB}	1.0 ± 0.0^{aAB}	1.1 ± 0.1^{aA}	2.8 ± 0.2^{a}
T1	0.5 ± 0.0^{bC}	0.7 ± 0.0^{bB}	0.7 ± 0.0^{bB}	0.8 ± 0.0^{bA}	2.4 ± 0.1^{a}
T2	0.6 ± 0.1^{bB}	0.7 ± 0.1^{bB}	0.9 ± 0.1^{abAB}	0.9 ± 0.1^{abA}	2.5 ± 0.2^{a}
S1	0.7 ± 0.0^{abC}	0.8 ± 0.0^{abBC}	0.9 ± 0.1^{aAB}	1.0 ± 0.1^{abA}	2.7 ± 0.0^{a}
S2	0.5 ± 0.1^{bB}	0.7 ± 0.1^{bAB}	0.7 ± 0.1^{bA}	0.8 ± 0.1^{bA}	2.6 ± 0.4^{a}

Mean values followed by the same letter are not significantly different at $P \leq 0.05$ level. Uppercase letters (A, B, C) in the same row are used to compare the storage time influence. Lowercase letters (a, b) in the same column are used to compare the treatment influence.
Control – untreated fruit samples, T1 – fruit samples treated with *T. vulgaris* essential oil at 4.5 $mg \cdot ml^{-1}$, T2 – fruit samples treated with *T. vulgaris* essential oil at 18 $mg \cdot ml^{-1}$, S1 – fruit samples treated with *S. montana* essential oil at 4.5 $mg \cdot ml^{-1}$, S2 – fruit samples treated with *S. montana* essential oil at 18 $mg \cdot ml^{-1}$.

Tab. 2. Changes of colour parameters.

Storage	0 days	7 days (1 °C)	14 days (1 °C)	21 days (1 °C)	28 days (1 °C)	3 days (20 °C)
Lightness *L**						
Control	21.90 ± 3.61 aA	21.27 ± 3.46 aA	20.04 ± 2.81 aA	21.40 ± 2.54 aA	20.55 ± 2.59 aA	31.36 ± 7.96 b
T1	22.35 ± 2.92 aA	21.05 ± 2.67 aA	20.51 ± 2.48 aA	21.46 ± 2.51 aA	20.85 ± 2.32 aA	37.18 ± 3.15 a
T2	20.88 ± 2.35 aAB	20.20 ± 2.30 aB	20.37 ± 1.80 aAB	21.76 ± 1.67 aA	20.13 ± 2.00 aB	30.28 ± 8.58 b
S1	23.13 ± 4.57 aA	20.45 ± 3.22 aBC	19.64 ± 2.88 aC	22.10 ± 2.34 aAB	19.48 ± 2.47 aC	34.33 ± 6.31 ab
S2	24.15 ± 4.34 aA	20.40 ± 3.16 aB	20.68 ± 2.62 aB	21.51 ± 2.50 aB	21.18 ± 2.84 aB	35.90 ± 4.08 a
Redness *a**						
Control	30.70 ± 6.0 aA	29.31 ± 4.58 bAB	29.80 ± 4.66 bA	25.74 ± 5.43 bcB	25.52 ± 5.77 abB	10.44 ± 4.61 a
T1	33.45 ± 5.48 aA	33.55 ± 4.67 aA	32.18 ± 5.60 aA	29.76 ± 4.98 aAB	27.93 ± 6.04 aB	11.07 ± 5.37 a
T2	33.61 ± 4.53 aA	29.61 ± 3.52 bB	28.48 ± 3.73 bB	24.76 ± 3.58 bcC	25.10 ± 4.42 abC	11.71 ± 3.94 a
S1	28.31 ± 5.81 aA	28.05 ± 6.23 bAB	27.43 ± 5.53 bABC	23.76 ± 5.13 cC	24.10 ± 5.36 bBC	13.72 ± 7.12 a
S2	30.61 ± 6.33 aA	31.00 ± 4.83 abA	30.11 ± 4.54 abAB	27.37 ± 3.36 abAB	26.85 ± 4.77 abB	13.75 ± .6.46 a

Mean values followed by the same letter are not significantly different at $P \leq 0.05$ level. Uppercase letters (A, B, C) in the same row are used to compare the storage time influence. Lowercase letters (a, b) in the same column are used to compare the treatment influence.
Control – untreated fruit samples, T1 – fruit samples treated with *T. vulgaris* essential oil at 4.5 mg·ml^{-1}, T2 – fruit samples treated with *T. vulgaris* essential oil at 18 mg·ml^{-1}, S1 – fruit samples treated with *S. montana* essential oil at 4.5 mg·ml^{-1}, S2 – fruit samples treated with *S. montana* essential oil at 18 mg·ml^{-1}.

of weight loss between the samples were caused probably by different factors, as EO vapours seem to act on the dehydration process and weight loss [17]. However, the detailed mechanism involved in the reduction of weight loss is still unknown [6]. During our trial, the effect of the treatment on respiratory coefficient and transpiration was not considered. However, it is possible to assume an effect of the treatment on respiratory metabolism and transpiration.

Colour changes

The colour of the fruits depends in particular on the degradation of anthocyanins, which are phenolic compounds (flavonoids), and on the respiratory metabolism [20, 21]. The colour parameters considered were the lightness L^* and a^* (Tab. 2). During cold storage, no significant differences were observed for the parameters L^* between the treatments, while differences were observed after shelf life at 20 °C. In fact, after shelf life, the highest values of lightness were observed on T1, S1 and S2 samples. Evaluating the lightness modification during cold storage, we observed a decrease in almost all treatments (except for control, T1 and T2) and the highest decrease at the end of the trial was recorded on S1 sample.

Concerning the parameter a^*, the indicator of red, we found significant differences during cold storage. In particular, the results showed that the samples T1 and S2 had values higher than those with other treatments after 7, 14 and 21 days of storage. After 28 days of storage, T1 samples were statistically different from S1. On the contrary, after shelf life, we did not observe differences among treatments. During the storage, there was a decrease in values of a^* in all samples.

The EO treatment did not seem to have any effect on the L^* parameter during the cold storage, while an effect was observed after 3 days of shelf life. Probably, the low temperature had a greater effect in delaying the loss of lightness than that of EO treatment, as demonstrated by Bernalte, Sabio, Hernandez and Gervasini [22]. The effectiveness of EOs emerges after shelf life as a probable consequence of a slowdown of the ripening process [18].

Change of colour parameter a^* is closely linked with degradation of anthocyanins and of hydroxycinnamic acids [20]. Anthocyanin pigments are labile compounds that are subject to degradative reactions. Their structure and the matrix in which they are in, are responsible of their stability. During fruit storage, there are many factors that condition anthocyanins as pH, light and temperature. The increase of pH could be a cause of destruction of pigment and usually it is a consequence of respiratory metabolism [23].

The activity of polyphenoloxidase, peroxidase and glycosidase enzymes can also have a devastating effect on anthocyanins. These enzymes may be native to the cherry tissue or their source may be

the mould contamination. The decrease in *a** parameter found in this work could be attributed to all these causes.

VALERO et al. [18] observed on grapes similar effect on the treated samples and, in particular, an acceleration in pigment loss on control samples compared to berries treated with EOs. The authors suggested that the lower colour changes in treated samples (T1 and S2 samples) could be related to a delay in the ripening process associated with a reduction in mould contamination.

Fruit quality parameters

During the storage, we evaluated the contents of *TA*, *TSS* and vitamin C (Tab. 3). Concerning acidity, significant differences among treatments were observed after 14 days, when the lower value was recorded on T2 and S1 treatments, and after 21 days, when the treatment with highest values of *TA* was S2 treatment. On the contrary, at the end of trial, no significant differences were recorded among the samples. Regarding the storage, we observed a significant decrease of *TA* in all samples, while the lowest values were detected in control samples.

The analysis of *TSS* showed statistical differences among the treatments after 7, 14 days and after shelf life. In particular, we observed significant differences between control and S2 samples after 7 days, while after 14 days there were differences between the control and the other treatments. Finally, there were differences among control, T1 and S2 after shelf life (20 °C).

Cherries showed an increase in *TSS* during cold storage. A gradual decrease in weight, connected with increased *TSS*, is a characteristic process during storage [24, 25]. *TA* and *TSS* are measures of ripening stage of fruits and a decrease in *TA* was recognized as a consequence of the use of organic acids as substrates for respiratory metabolism [26]. In particular, it is known that the increase in *TSS* is connected with a decrease of fruit's weight caused by water loss and respiration. As the sugar content is relative to weight of the fruit, if the weight decreases, accordingly increases the sugar content [16, 24, 25].

Tab. 3. Changes of quality parameters.

Storage	0 days	7 days (1 °C)	14 days (1 °C)	21 days (1 °C)	28 days (1 °C)	3 days (20 °C)
Titratable acidity [meq·l^{-1}]						
Control	77.04 ± 8.99^{aA}	65.04 ± 4.55^{aAB}	63.41 ± 0.74^{aBC}	53.02 ± 0.14^{cBC}	51.78 ± 2.47^{aC}	41.79 ± 2.2^{a}
T1	77.04 ± 8.99^{aA}	63.05 ± 1.59^{aB}	60.07 ± 1.06^{abB}	56.26 ± 1.70^{bcB}	53.59 ± 1.42^{aB}	44.20 ± 1.34^{a}
T2	77.04 ± 8.99^{aA}	61.66 ± 3.11^{aB}	57.88 ± 3.05^{bB}	57.09 ± 2.19^{bB}	55.29 ± 3.04^{aB}	44.63 ± 3.78^{a}
S1	77.04 ± 8.99^{aA}	61.63 ± 0.99^{aB}	57.96 ± 1.53^{bB}	55.95 ± 0.91^{bcB}	53.52 ± 1.57^{aB}	43.46 ± 2.86^{a}
S2	77.04 ± 8.99^{aA}	70.29 ± 7.00^{aAB}	62.79 ± 0.18^{aAB}	63.44 ± 2.58^{aAB}	56.05 ± 2.37^{aB}	46.34 ± 1.95^{a}
Total soluble solids [°Brix]						
Control	13.40 ± 0.17^{aB}	14.70 ± 0.53^{aA}	15.20 ± 0.10^{aA}	15.23 ± 0.15^{aA}	15.50 ± 0.00^{aA}	15.90 ± 0.10^{a}
T1	13.40 ± 0.17^{aC}	13.77 ± 0.67^{abBC}	14.67 ± 0.29^{abAB}	15.00 ± 0.44^{aA}	15.30 ± 0.10^{aA}	15.73 ± 0.12^{ab}
T2	13.40 ± 0.17^{aC}	13.53 ± 0.21^{abC}	14.13 ± 0.12^{bcBC}	14.70 ± 0.53^{aAB}	15.07 ± 0.06^{aA}	15.27 ± 0.06^{b}
S1	13.40 ± 0.17^{aC}	14.33 ± 0.21^{abB}	14.40 ± 0.26^{bcB}	14.83 ± 0.06^{aAB}	15.17 ± 0.06^{aA}	15.63 ± 0.06^{ab}
S2	13.40 ± 0.17^{aB}	13.70 ± 0.29^{bB}	13.80 ± 0.36^{bB}	14.50 ± 0.17^{aA}	14.87 ± 0.15^{aA}	15.20 ± 0.44^{b}
Vitamin C [mg·kg^{-1}]						
Control	172.10 ± 24.51^{aA}		118.3 ± 15.81^{cB}		90.0 ± 8.68^{bB}	
T1	172.10 ± 24.51^{aA}		129.5 ± 9.89^{bcB}		90.5 ± 1.32^{bC}	
T2	172.10 ± 24.51^{aA}		158.2 ± 1.27^{aA}		112.6 ± 15.71^{abB}	
S1	172.10 ± 24.51^{aA}		152.9 ± 12.89^{abA}		96.7 ± 0.26^{abB}	
S2	172.10 ± 24.51^{aA}		167.1 ± 3.65^{aA}		116.8 ± 8.51^{aB}	

Mean values followed by the same letter are not significantly different at $P \leq 0.05$ level. Uppercase letters (A, B, C) in the same row are used to compare the storage time influence. Lowercase letters (a, b) in the same column are used to compare the treatment influence.

Control – untreated fruit samples, T1 – fruit samples treated with *T. vulgaris* essential oil at 4.5 mg·ml^{-1}, T2 – fruit samples treated with *T. vulgaris* essential oil at 18 mg·ml^{-1}, S1 – fruit samples treated with *S. montana* essential oil at 4.5 mg·ml^{-1}, S2 – fruit samples treated with *S. montana* essential oil at 18 mg·ml^{-1}.

Tab. 4. Sensory properties of sweet cherries after 14 and 28 days of storage.

Storage	14 days (1 °C)	28 days (1 °C)
Visual Aspect		
Control	3.80 ± 0.86	3.93 ± 0.88
T1	3.73 ± 0.80	3.73 ± 0.70
T2	3.33 ± 0.90	3.93 ± 0.88
S1	3.60 ± 0.99	3.73 ± 0.96
S2	3.47 ± 0.83	3.87 ± 0.74
Sweetness		
Control	3.40 ± 1.18	3.47 ± 0.83
T1	3.07 ± 0.59	3.73 ± 0.88
T2	2.73 ±0.96	3.13 ± 0.99
S1	2.87 ± 1.06	3.53 ± 0.99
S2	3.00 ± 1.07	2.67 ± 0.72
Acidity		
Control	2.27 ± 0.88	1.87 ± 0.74
T1	2.07 ± 0.80	1.47 ± 0.74
T2	2.47 ± 1.06	1.87 ± 0.83
S1	2.53 ± 0.83	1.87 ± 0.59
S2	1.93 ± 0.88	2.47 ± 1.06
Bitterness		
Control	1.33 ± 0.82	1.27 ± 0.59
T1	1.00 ± 0.53	1.13 ± 0.35
T2	1.20 ± 0.41	1.20 ± 0.41
S1	1.27 ± 0.70	1.27 ± 0.38
S2	1.20 ± 0.41	1.40 ± 0.91
Overall assessment		
Control	3.27 ± 1.03	3.27 ± 0.88
T1	2.87 ± 0.83	3.40 ± 0.91
T2	2.93 ± 0.70	3.33 ± 0.82
S1	2.67 ± 1.05	2.60 ± 0.83
S2	3.07 ± 0.88	2.53 ± 1.06
Off-flavours [%]		
Control	13	7
T1	40	13
T2	27	20
S1	27	33
S2	27	33
Off-aroma [%]		
Control	20	0
T1	13	7
T2	40	7
S1	40	60
S2	40	67

Visual aspect, sweetness, acidity, bitterness and overall assessment were evaluated on a ranked scale of 1–5 (1 – very low, 2 – low, 3 – medium, 4 – high, 5 – very high). Off-flavours and off-aroma were evaluated as percentage of positive answers on panel's total.
Control – untreated fruit samples, T1 – fruit samples treated with *T. vulgaris* essential oil at 4.5 mg·ml^{-1}, T2 – fruit samples treated with *T. vulgaris* essential oil at 18 mg·ml^{-1}, S1 – fruit samples treated with *S. montana* essential oil at 4.5 mg·ml^{-1}, S2 – fruit samples treated with *S. montana* essential oil at 18 mg·ml^{-1}.

In our study, we observed that, concerning *TA*, the treatment S2 maintained always the highest values while, concerning *TSS*, the values were always higher in the control samples. This result showed that the treatment with a high concentration of EO seems to act on the water loss, as observed by Valverde et al. [17] and Sivakumar and Bautista Banos [6], and on respiratory metabolism. The obtained values, in fact, were strictly correlated with the weight loss (Tab. 1) and were in agreement with the results of Martinez-Romero et al. [15] and Serrano et al. [16].

Concerning vitamin C content, after 14 and 28 days, the non-processed cherries contained 118.3 mg·kg^{-1} and 90.0 mg·kg^{-1} FW, respectively. EO vapours seemed to have caused a slower decrease of total vitamin C content in T2 and S2 than in the control samples. It is known that the content of vitamin C decreases during cold storage because AA and DHAA are important oxidants used in the natural oxidative reactions of plant cells [27]. We observed during storage a decrease of vitamin C in all samples, but T2, S1 and S2 samples revealed the lowest losses of vitamin C. The reason for this is connected with the antioxidant properties of EOs that inhibit the consumption of vitamin C and the slowdown of respiration [28, 29]. Moreover, some components of essential oils, such as thymol, showed oxidant properties more pronounced than other [30, 31] and the higher EO concentrations probably were the most effective.

Sensory properties

In the sensory evaluation, we considered the effects of EOs on different cherry parameters and the presence/absence of off-flavours and off-aroma after 14 and 28 days (Tab. 4). Regarding the visual aspect, we observed that all treatments had values higher than 3.0 (medium) and, in particular control and T2 samples after 28 days, reached values close to 4.0 (high). Regarding sweetness, the results showed medium values in all treatments except in T2 and S1 samples after 14 days and S2 samples after 4 weeks, in which lower values were recorded. The acidity and bitterness evaluation showed low values in all cherry samples. The panel found that red cherries reached medium acceptability score in control and S2 samples after 2 weeks, and in control, T1 and T2 samples after 4 weeks. Unfortunately, the presence of off-flavours and off-aroma was identified as a problem for the treated samples, mainly after 14 days of storage. Sensory analysis showed that EO treatment did not affect the visual aspect, while sweetness, acidity and bitterness could be positively influenced after 14 and 28 days of cold storage.

Tab. 5. Postharvest disease incidence.

Storage	7 days (1 °C)	14 days (1 °C)	21 days (1 °C)	28 days (1 °C)	3 days (20 °C)
Disease incidence [%]					
Control	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	2.4 ± 0.8^{a}	3.6 ± 0.8^{a}	33.6 ± 6.9^{a}
T1	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	2.0 ± 1.7^{a}	3.2 ± 1.5^{a}	12.8 ± 4.4^{b}
T2	0.0 ± 0.0^{a}	0.8 ± 0.5^{a}	2.4 ± 1.3^{a}	3.2 ± 1.1^{a}	20.4 ± 3.7^{ab}
S1	0.0 ± 0.0^{a}	0.8 ± 0.5^{a}	5.2 ± 1.3^{a}	6.0 ± 1.7^{a}	22.4 ± 5.5^{ab}
S2	0.0 ± 0.0^{a}	0.4 ± 0.4^{a}	2.4 ± 0.8^{a}	4.4 ± 2.3^{a}	19.2 ± 3.8^{a}

Means followed by the same letter are not significantly different at $P \leq 0.05$ level. Lowercase letters (a, b) in the same column are used to compare the treatment influence.
Control – untreated fruit samples, T1 – fruit samples treated with *T. vulgaris* essential oil at 4.5 mg·ml^{-1}, T2 – fruit samples treated with *T. vulgaris* essential oil at 18 mg·ml^{-1}, S1 – fruit samples treated with *S. montana* essential oil at 4.5 mg·ml^{-1}, S2 – fruit samples treated with *S. montana* essential oil at 18 mg·ml^{-1}.

Moreover, the panelists notified the presence of off-flavours and off-aroma after 14 and 28 days in S1 and S2 samples, which caused a final score of "low". Probably, prolonged exposure to EOs could have produced residues of EOs on fruits and this could have caused a modification of metabolism as well as development of compounds causing off-flavour and off-aroma [31]. A similar result was reported by BAKKALI et al. [32] who observed that the effect of EOs on fruit flavour was one of the most common problems encountered in the application of EOs in the food industry [6].

Postharvest disease incidence

The effect of EO fumigation on postharvest pathogen development was here demonstrated on sweet cherries. Thyme and savory EOs decreased disease incidence during shelf life compared to untreated fruit. In cold storage, pathogen development was very low (Tab. 5) and pathogen control was mainly achieved by low temperature. During 28 days at 1 °C, no difference between EO treatments was assessed (low rot incidence). Pathogen development occurred mainly at 20 °C during shelf life. Savory essential oil fumigation at highest concentration induced 42.9% rot reduction with respect to the untreated control, and 33.3% reduction when used at 4.5 mg·ml^{-1}. Fumigation with thyme EO at 4.5 mg·ml^{-1} was the most effective treatment, leading to a reduction of diseased fruit of 61.9 % compared to the control. According to KUMAR et al. [33], *T. vulgaris* EO can be considered an ideal plant-based preservative for the enhancement of shelf life of fruit. Nevertheless, thyme EOs concentration must be carefully monitored since high amounts of EO lead to a reduction in postharvest rots control. No evident phytotoxic effects were observed in the trial, but high amount of thyme EO (18 mg·ml^{-1}) can lead to a higher susceptibility to pathogens attacks. As reported previously [34, 35], thymol showed a slight phytotoxic effect when used at 10 mg·l^{-1} causing cherry stem browning.

As revealed by microbiological analysis and ITS-based molecular identification, cherries were affected by *M. fructicola* and *B. cinerea* (data not shown). In particular, EO fumigation reduced total rots and relative incidence of *M. fructicola*, indicating a higher effectiveness against this pathogen. *M. fructicola* was isolated from 91 % of rotten untreated fruit, while 9 % of fruit were affected by *B. cinerea.* The percentage of brown rots in fumigated fruit ranged from 77 % using thyme EO at 18 mg·ml^{-1} to 66.6 % using thyme EO at 4.5 mg·ml^{-1}.

CONCLUSIONS

The results showed that the use of EOs in the postharvest sweet cherries storage reduced the weight loss and the vitamin C loss. The effect of these treatments on titratable acidity and total soluble solids was limited. The most effective treatments were those with high concentration of EOs (savory 18 mg·ml^{-1}). Regarding the mode of action, EOs probably helped to reduce the respiratory metabolism with the result of maintaining a better red colour (by thyme EO at 4.5 mg·ml^{-1} or savory EO at 18 mg·ml^{-1}). Vapour phase of EOs demonstrated the capability to control postharvest pathogens on cherries even without direct contact, reducing postharvest waste by up to about 62 % compared to untreated fruit. Finally, the sensory analysis showed a positive effect on the visual aspect, while it had a negative effect on off-flavour and aroma mainly for savory samples. In this sense, further studies should be carried out

in order to obtain a compromise between the antimicrobial effectiveness and sensory impact of the studied treatment. In conclusion, EO treatment could be used at postharvest storage of sweet cherry to preserve the quality of the final product against the negative action of food pathogens in particular during shelf life. However, the treatment may affect sensory properties of the final product because of the interaction of aromatic profiles of fruits and essential oils. Therefore, further research needs to be done to evaluate the effect of the treatment on respiration and transpiration of samples in relationship with the inhibitory effects against microbial growth.

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Received 30 October 2017; 1st revised 11 January 2018; accepted 29 January 2018; published online 11 May 2018.