

EFFECT OF ESSENTIAL OILS ON QUALITY AND SHELF LIFE OF STRAWBERRIES DURING COLD STORAGE

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Abstract

Strawberries hold significant economic value in many countries. However, due to their delicate texture and thin skin, they are prone to rapid spoilage and susceptible to various pathogens. Currently, chemicals are the primary method used to prevent pathogen infections and degradation of fresh fruits. Therefore, this research focuses on identifying organic, eco-friendly treatments that can extend the shelf life of these fruits. Essential oils (EOs) are potential candidates for prolonging strawberry shelf life and protecting against post-harvest fungal pathogens. Current study evaluates the *in vitro* activity of several EOs against *Botrytis cinerea* and the *in vivo* effects of EO treatments on the quality and physicochemical properties of strawberries (*Fragaria × ananassa* cv. Alba) stored at $4 \pm 1^\circ\text{C}$ for 15 days. Parameters such as weight loss, decay percentage, fruit firmness, soluble solid content, and total polyphenols were measured at three intervals over 15 days of cold storage. Cinnamon EO was particularly effective in reducing rot by inhibiting fungal growth, aligning with *in vitro* results. Notably, EO treatments extended the shelf life and delayed decay of strawberries up to 15 days under cold storage.

Key words: *Botrytis cinerea*, essential oils, fruit quality, shelf life, strawberries.

INTRODUCTION

The use of essential oils (EOs) as antimicrobial additives within food industry is garnering heightened attention, attributed to their abundance of biologically active compounds that possess well-documented antimicrobial and antioxidant properties (Manso et al., 2014; Calo et al., 2015; Llana-Ruiz-Cabello et al., 2015; Wrona et al., 2015; Atarés and Chiralt, 2016). EOs are complex volatile substances, comprising mainly terpenes and other aromatic compounds, found in various plant parts (Bakkali et al., 2008). They play an important role in plant protection against pest and diseases (Tzortzakakis & Economakis, 2007). Microbial spoilage and enzymatic activity are two major factors affecting products' shelf-life and inducing organoleptic qualities loss. Therefore, using efficient storage methods, including biologic solutions to prevent microbial spoilage and modulate enzymatic inactivation, can improve the products quality during storage and prolong food shelf-life.

Studies carried out in this pathway have shown that EOs exhibit strong antimicrobial activity against post-harvest fungal pathogens, mainly on *Botrytis* and *Colletotrichum*.

Thus, EOs can be used as preservatives to extend the shelf-life of small fruits, such as strawberries (*Fragaria × ananassa* Duch.), raspberries (*Rubus* spp.), blueberries (*Vaccinium* spp.) and grapes (*Vitis vinifera* L.), as well as to prevent the development of microorganisms that degrade other fresh products (Vital et al., 2016; Nazari et al., 2019; Simionato et al., 2019).

There are several ways of using EOs to protect perishable fruits from spoilage, such as volatiles, fruit coating or packaging coating (Figure 1).

The aim of this study was to evaluate *in vitro* activity of essential oils against fungal phytopathogen *Botrytis cinerea*, the causal agent of grey rot. Also, *in vivo* EOs activity on the physical qualities of strawberries (*Fragaria × ananassa* cv. Alba), in storage conditions at $4 \pm 1^\circ\text{C}$, for 15 days were assessed.

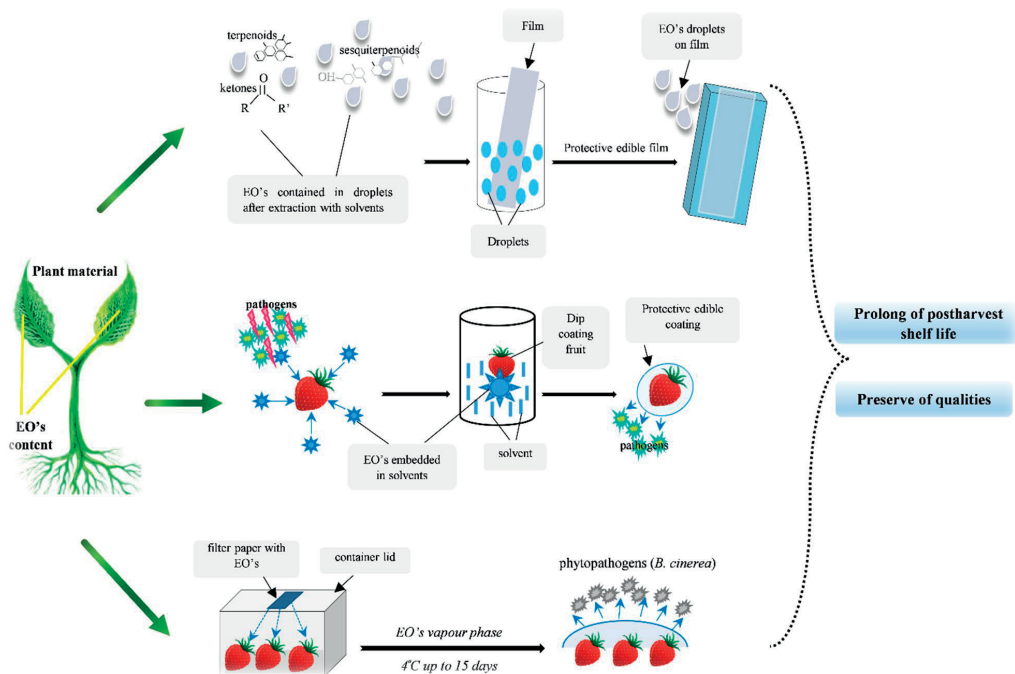


Figure 1. Potential usage of EOs in fruits protection against pathogens during storage (after Herman et al., 2019, amended by Dinu)

MATERIALS AND METODS

Experimental conditions

All experiments were conducted at the Research-Development Institute for Plant Protection, within the Laboratory of Plant Protection Bioproducts. The *in vitro* studies were performed during spring, for March to April 2024, while the *in vivo* trials and related analysis were performed during Mai to June 2024.

Commercial EOs

Commercial EOs purchased from a Romanian brand, providing natural biologic products, were used in this study. These EOs were obtained by steam distillation from different plant parts, such as bark, leaves, flowers, and flower buds of cinnamon (*Cinnamomum verum*), peppermint (*Menta piperita*) and thyme (*Thymus vulgaris*), respectively.

Fungal pathogen

A wild strain of *Botrytis cinerea* was used in both *in vitro* and *in vivo* trials. This strain was

isolated from naturally contaminated strawberry fruits expressing grey mould infection.

In vitro assessment of EOs antimicrobial activity

The antifungal activity of EO's against *B. cinerea* was determined by disk diffusion technique. The phytopathogenic fungus was refreshed on solid Potato Dextrose Agar medium (PDA medium; Carl Roth GmbH+Co.KG, Karlsruhe, Germany) to obtain an abundant growth. For the *in vitro* tests, calibrated mycelium plugs (7 mm Ø), collected from fresh fungal cultures, were inoculated on PDA, in the centre of 90 mm Ø plates. At 30 mm from the fungal inoculum, sterile paper discs, 5 mm Ø filter, were placed on medium' surface, 1 disc per plate, and impregnated with 20 µL of EO. Three replicates were prepared for each EO. Control plates only inoculated with the fungal phytopathogen were also prepared. All plates were sealed with 2 layers of parafilm M foil and alimentary foil, then incubated at 25°C, for five days. Antimicrobial activity of the EOs was assessed by measuring the fungal growth in the

test and control plates, for calculating the inhibition rate, according to the equation:

$$\% \text{ inhibition} = R_M - R_{UE} / R_M \times 100$$

where R_M is the radius of fungal mycelium growth in control plate, and R_{UE} is the radius of fungal mycelium growth into the direction of EO's impregnated disc.

***In vivo* testing of EOs on stored strawberries**

Strawberries (*Fragaria × ananassa*) of the Alba variety were harvested at the commercial ripening stage (at least 75% of the fruit surface red) from a private farm located in Giurgiu County (latitude 44.1779084° N, longitude 26.3579245° E). For the *in vivo* trials, fruits with uniform appearance (colour and shape), and no signs of fungal contamination or physical damage were selected. The fruits were then placed transparent polyethylene containers (L x l x h = 120 × 100 × 50 mm), 5 strawberries / box. Adhesive sponges of 5 mm squares were taped on the containers' lids and impregnated with 20 µL EOs of peppermint (UEM), thyme (UEC) or cinnamon (UES). The containers were sealed with parafilm to avoid vapor leaks. A control variant was also prepared by adding 20 µL of distilled water to the square sponge (Figure 2).



Figure 2. Strawberry fruits prepared for the *in vivo* trial with EOs treatments

To determine the physicochemical parameters of the stored fruits, strawberries from each experimental variant, including control, were analysed after 5, 10 and 15 days of storage at 4±0.5°C. The performed analyses included weight loss, decay percentage, fruit firmness, soluble solid content and total phenolic content.

Fruits weight loss quantification

Each strawberry fruit was separately weighed and labelled at the beginning of the experiment and at each sampling date of 5, 10, and 15 days of storage. To determine weight loss (WL), the following equation was used:

$$WL\% = (IW - FW) / IW \times 100$$

where IW is initial weight of the fruits, and FW is their final weight.

Decay percentage evaluation

The percentage of fruits degradation was determined according to Rokaya et al. (2016) method, with some modifications. The decay percentage (D %) was calculated with the following formula:

$$D\% = WIF / WAF \times 100$$

where WAF is total weight of fruits before storage and WIF is the weight of decayed strawberries after 5, 10 and 15 days of storage. Decayed fruits were considered those exhibiting brown spots, pulp softening, lesions, spoilage or mycelial coverage.

Fruit firmness analysis

The strawberries firmness was measured using a digital penetrometer equipped with a 6 mm Ø cylindrical probe (Velickova et al., 2013). Firmness was expressed as kg/cm² strength and was determined after 5, 10 and 15 days of storage at 4°C.

Measurement of the soluble solid content

A portable refractometer with automatic temperature compensation was used to measure the total solid content (SSC). Fruit samples were crushed, and 2-3 drops were distributed on the prismatic element of the measuring equipment, ensuring that the juice covered the entire reading surface. The SSC values were expressed as degree Brix and converted into g of sugars/L, considering 1°Bx is 10.04 g/L at 20°C.

Determination of total phenolic content

The total phenolic content (TPC) in strawberries was evaluated using Folin-Ciocalteu reagent, according to Singleton et al. (1999) method. Extracts are diluted with distilled water in a 1:4 (v/v) ratio, and 20 µL of the diluted extract were added to the reaction mixture. To this, 1.7 mL of distilled water and 100 µL of Folin-Ciocalteu

reagent are added. The mixture was incubated for 5 minutes at room temperature to allow the reaction to develop. Following incubation, 0.3 mL of a 20% sodium carbonate solution was added to stabilize the reaction. A control sample was prepared by replacing the extract with distilled water. The optical density of the samples was measured at 765 nm, and the results expressed as equivalent gallic acid per mL (EAG/mL) using a standard curve.

Statistical analysis

The data were statistically analysed using a one-way ANOVA, performed with GraphPad Prism software. Prior to analysis, the data were examined to verify compliance with the assumptions of normality and homogeneity. Normality was assessed using the Shapiro-Wilk test, while homogeneity of variances was evaluated through Levene's test. Post-hoc comparisons of all possible treatment pairs within each of the three storage periods (5, 10 and 15 days) were conducted using Tukey's test. Additionally, the interaction between the two variable factors, storage duration and applied treatments, was analysed using a two-way ANOVA test.

RESULTS AND DISCUSSIONS

In vitro EOs antimicrobial activity

According to the *in vitro* analysis, all three tested EOs, of peppermint (UEM), thyme (UEC) and cinnamon (UES), have completely inhibited *B. cinerea* growth during the 5 days of incubation at 25°C. Mine wile, within the control plates, the pathogen has completely colonised the agar surface (Figure 3).

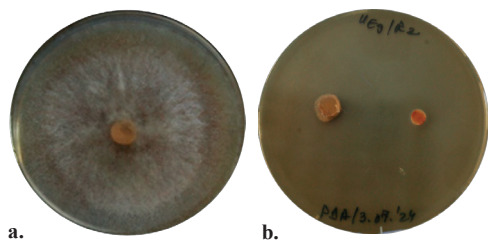


Figure 3. EOs antifungal activity against *B. cinerea* (after 5 days of incubation at 25°C):

a. Fungal control plate, b. Complete fungal growth inhibition using cinnamon EO

Currently, there are many research findings on the effectiveness of plant volatiles to control *in*

vitro microbial growth and extend the shelf life of fresh products (Rojas-Graü et al., 2007; Rokaya et al., 2016). Therefore, EOs with bioactive potential can be used as alternative method to protect food and vegetable from important pathogens, due to their antibacterial, antifungal and antioxidant properties. Moreover, plant volatile bioproducts tend to have a low mammalian toxicity and broad-spectrum antimicrobial activity, being less dangerous than synthetic compounds and generally more acceptable to the consumer (Kizil et al, 2010; Hyldgaard et al., 2012; Calo et al., 2015; Aloui & Khwaldia, 2016; Rahmawati et al., 2017; Torres-Alvarez et al, 2017).

In vivo effect of EOs on stored strawberries

Fresh healthy strawberries preserved at $4 \pm 0.5^\circ\text{C}$ in the presence, and absence of EOs were analysed during 15 days of storage. The physicochemical parameters of the fruits were examined after 5, 10 and 15 days of storage. Fruits weight loss was the first analysed parameter, as rapid water loss of fruits is considered one of the important factors that contributing to their perishability (Bakkali et al, 2008).

As expected, strawberries weight loss increased during storage (Figure 4). However, the EO treatments did not statistically influenced the measured results ($F_{(3, 24)} = 2.157$, $P = 0.1194$). Fruits respiration and moisture evaporation through the fruit skin most probably contributed to this aspect.

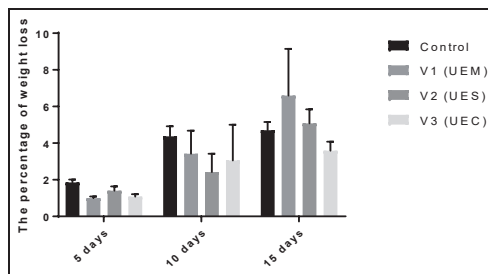


Figure 4. The diagram of the weight loss percentage of strawberries during storage at 4°C

After the first 5 days of storage at 4°C , the weight loss was relatively low across all treatments, while the control group showed significantly higher weight loss compared to the EOs treated variants (p -values < 0.05).

The reduction in weight loss, due to the EO treatments, during the first five days of fruits storage, is attributed to the antioxidant activity of EOs that acted as a protective barrier (Bakkali et al, 2008; Aloui & Khwaldia, 2016; Martínez et al., 2018).

The EOs most probably decreased the gas and water exchange between the fruits and the environment.

On the 10th days, a significant increase in weight loss was observed across all treatments, although UES treatments remain more effective in preserving fruit weight compared to the control. Despite these differences in weight loss among treatments, the variation was not statistically significant (p -values > 0.05). A similar trend was recorded on the 15th days of storage, the fruits continued to lose weight, with no statistically significant differences among the experimental variants, control and the EOs treatments.

Beside weight loss, fruit depreciation was also analysed during storage.

Signs of fungal degradation were observed in the control group (untreated fruits) from the 5th day of storage.

Fruit degradation was accentuated while extending the storage period.

In the EOs treated variants, first signs of fungal degradation were observed in fruit treated with UEM, on the 10th day of storage, however considerably lower than in the untreated control. In the 15th day of storage, the strawberry fruits treated with thyme and cinnamon EOs also started to show decaying signs, while the untreated fruits, followed by those treated with UEM revealed high percentage of decomposition. The decay percentage of cold stored strawberries is graphical presented below (Figure 5).

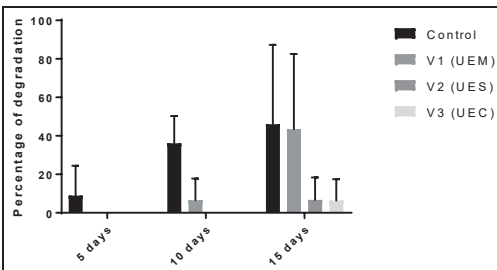


Figure 5. Strawberries decaying percentage during 15 days of storage at 4°C

Strawberries cold storage, in the presence of thyme and cinnamon EOs significantly reduced fruits degradation and inhibited fungal growth on the fruit surface. Correlating these results with those from the in vitro tests, it could be concluded that the antimicrobial effects of the EOs contributed to fruit preservation. Similar aspects being also presented in other similar studies (Kizil et al, 2010; Aloui & Khwaldia, 2016; Torres-Alvarez et al, 2017).

Firmness is considered an important quality attribute for strawberries, indicating their quality and predicting their shelf life throughout the market chain. Thus, a higher strawberry firmness is correlated with a lower fruit decay score (<https://edepot.wur.nl/503212>).

Within the EOs tests, fruits firmness was evaluated in the 5th, 10th and 15th day of storage. Results showed that the pulp firmness decreased during storage in all experimental variants (Figure 6).

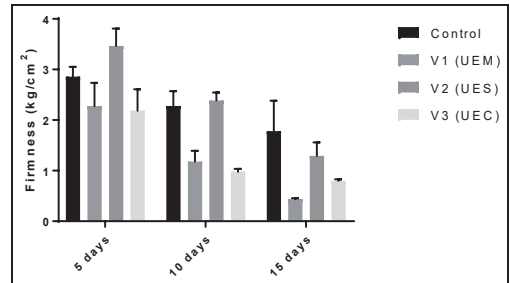


Figure 6. The effect of EOs on strawberries firmness during storage at 4°C

After five days of storage, strawberries from the UES experimental variant revealed the highest firmness parameter, significantly different from the other treatments (UEM $p = 0.0183$; UEC $p = 0.0123$). However, by the end of the 15 days of cold storage, strawberries' firmness decreased considerably, especially when peppermint and thyme EOs were used. Severino et al. (2015) reported fruit firmness maintenance for 15 days in cold storage conditions, as well as other quality attributes of fruits treated with peppermint, mandarin and lemongrass EOs. This was probably due to the selective gas and water permeability of the coating material, thus reducing respiration rate, enzymatic activities and most metabolic changes, thus delaying ripening and over-softening of strawberries (Janjarasskul & Krochta, 2010). Several other

authors also reported that strawberry fruit tissue becomes soft due to the loss of cell wall structure by increasing enzymatic activity, which leads to decreased fruit resistance during the shelf life (Aloui & Khwaldia, 2016).

Regarding the solid soluble content (SSC) the registered values remained at similar levels in samples treated with cinnamon and thyme EOs, throughout the cold storage period of 15 days (Figure 7).

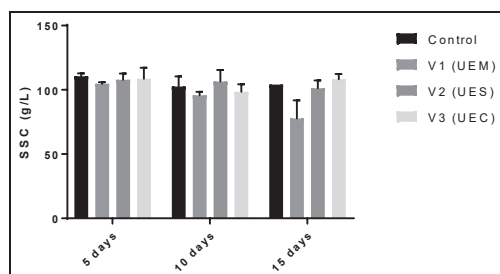


Figure 7. The effect of EO's on solid soluble content (SSC) of strawberries during storage at 4°C

Regarding the UEM treatment, SSC values were significantly different from both the control ($p = 0.0156$) and the other treatment variants (V2: $p = 0.0278$; V3: $p = 0.0068$), revealing a lower SSC concentration.

The reduction of SSC values in fruits treated with EOs could be associated with the reduced water loss from the fruit surface due to EO's ability to decrease the migration of water from fruit surface to the environment (Aloui & Khwaldia, 2016).

Evaluating the total phenolic content (TPC) in EO's treated and untreated strawberry fruits a wide values fluctuation was retrieved among treatments and storage time (Figure 8).

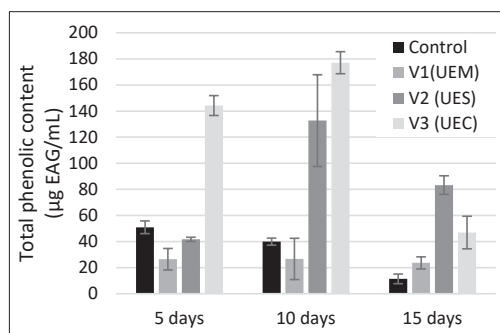


Figure 8. The effect of EO's on total phenolic content (TPC) of strawberries during storage at 4°C

TPC was significantly higher in UEC treated fruits compared the untreated ones, during the cold storage period of 15 days. After the first 5 days of storage at 4°C, the TPC in UEC treated strawberries was $144.22 \pm 7.63 \mu\text{g EAG/mL}$, meaning 2.8 times higher in thyme EO treated fruits compared to the control. After 10 days of storage, the TPC increased up to $177.08 \pm 8.46 \mu\text{g EAG/mL}$, being 4.4 times higher in UEC treated fruits compared to the untreated ones. At this storage time (10 days at 4°C), the UES treatment also revealed an increased TPC in the fruits ($132.71 \pm 35.15 \mu\text{g EAG/mL}$), 3.3 times higher than in the untreated control ($39.93 \pm 2.71 \mu\text{g EAG/mL}$). After the 15 days of cold storage, the TPC values decreased. However, both UES and UEC treatments induced a significantly higher TPC in the stored fruits, 7.3 and 4.1 times higher, respectively, than in the untreated control ($11.39 \pm 3.68 \mu\text{g EAG/mL}$).

Thyme EO is known to significantly enhance strawberry preservation, especially due to its antimicrobial and antioxidant properties. Studies have shown that thyme EO encapsulated in zein nanofiber films to protect strawberry quality (Ansarifar & Moradinezhad, 2021). Moreover, thyme oil is recommended also for soil application in strawberry fields to improve yield and reduce disease abundance (Cato et al., 2024).

CONCLUSIONS

These *in vitro* and *in vivo* studies showed the perspective of using certain plant EO's for strawberry fruits preservation. The antimicrobial activity of peppermint, thyme and cinnamon EO's against *Botrytis cinerea* phytopathogen revealed a complete inhibition of the grey mould mycelium growth. Moreover, the cinnamon and thyme EO's treatments applied to the strawberry fruits, stored at 4°C, were effective in reducing fruits rot, by inhibiting the fungal growth on strawberries surface. Several quality parameters, such as fruit firmness, SSC and TPC were also increased in cinnamon and thyme EO's treated fruits. Therefore, the application of such plant extracts based on EO's may serve as alternative to conventional treatments, offering potential advantages in limiting damage caused by grey mould in organic farming systems.

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