Understanding the impact of essential oils on grape metabolism and pathogen resistance: a study with a focus on *Botrytis cinerea*

Ozkan Kaya1,2*, Sinem Karakus3,4*, Abdurrahim Bozkurt1, Turhan Yılmaz5, Hanifeh Seyed Hajizadeh6* and Metin Turan7

Abstract

**Background** The exploration of EOs and their impact on primary metabolites in agricultural products, such as grapes, holds significant importance in the post-harvest preservation and enhancement of berry quality. Therefore, our research aimed to dissect the effects of various EO treatments on the biochemical composition of grapes, specifically examining alterations in sugar, organic acid, and amino acid levels.

**Results** Our findings highlighted that the OF treatment, a combination of Eugenol and *Botrytis cinerea*, significantly escalated sugar content, with remarkable increases in sucrose, glucose, and fructose compared to the control group. The SF treatment led to the highest levels of certain organic acids, including tartaric, butyric, and malonic acids, whereas the TO treatment prominently augmented amino acids such as tyrosine and phenylalanine. Utilizing PCA, our study effectively captured the variability within the treatment dataset, revealing distinct clusters of treatments and their correlations with specific metabolites. The PCA biplot underscored the influence of treatments on metabolic profile of grape berries, with treatments like OSF, TO, and TOSF demonstrating close associations with specific amino acids, suggesting positive correlations.

**Conclusion** Our current results indicate that EOs substantial influence on the metabolic profile of grapes, resulting in enhanced fruit quality and extended shelf life. Variations in sugar, organic acid, and amino acid levels among different EO treatments highlight their potential for improving post-harvest characteristics. To sum up, these findings are valuable for the agricultural and food industries, showcasing the utility of EOs as natural preservatives and enhancers in fruit cultivation and storage.

**Keywords** Essential oils, Grape metabolism, Post-harvest preservation, Berry quality, Pathogen resistance

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Introduction
Grapes (Vitis vinifera L.), a globally prevalent fruit, are cultivated from deciduous vine species and are a rich source of essential nutrients, including various vitamins and minerals [1, 2]. The harvesting of table grapes typically occurs from late July to October, with their market price fluctuating depending on the season, often decreasing during peak harvest due to overproduction and increasing during off-harvest periods [3]. Consequently, controlling postharvest diseases is crucial for optimizing farmer income. Table grapes, being non-climacteric, are particularly susceptible to postharvest handling conditions, influencing factors such as storage, transportation, and marketing [4]. Many physiological, pathological, and physical elements contribute to the reduced shelf life of these fruits [5]. One of the primary detractors to post-harvest quality in table grapes is the fungal pathogen Botrytis cinerea, responsible for gray mold. This disease notably degrades the quality of the fruit, as uncontrolled infections lead to rapid mycelial spread to adjacent berries [6]. To mitigate this, antifungal agents such as imazalil and thiabendazole have been employed during pre- and post-harvest periods [7]. However, the persistent use of these synthetic fungicides has engendered the emergence of resistant fungal strains, raising concerns regarding chemical residues in fruits and their subsequent marketability [8, 9]. Furthermore, the use of such chemicals is prohibited in organic grape production [10], and regulatory restrictions are increasingly stringent [11]. This scenario aligns with the growing consumer demand for foods with lower levels of chemical preservatives, urging the industry and researchers to innovate sustainable and effective methods for preserving fruit quality [12, 13].

Considering these challenges, the focus has shifted towards employing microorganisms and natural products with inherent antimicrobial properties. Among these, EOs have been identified as a promising alternative, offering a reduced environmental impact, and exhibiting antifungal, antibacterial, antioxidative, and preservative properties [14–16]. The organoleptic quality of fruit, including taste, aroma, color, and texture, significantly influences consumer acceptance, with sugar and acid content playing a pivotal role [17]. Post-harvest aging in fruits is accelerated by factors such as respiration, dehydration, and fungal activity, which impact metabolic pathways responsible for the alteration of sugar, amino acid, and organic acid levels [18, 19]. Understanding these dynamics is vital for mitigating quality loss in post-harvest fruits and devising more effective strategies against
pathogens. Analyzing fruit or berry samples affected by pathogens is, therefore, crucial for the fruit industry in ensuring resilient and quality products for consumers [20, 21]. The complexity of fruit responses to postharvest storage conditions, dependent on species and cultivar, presents a significant challenge in research. The reconfiguration of fruit or berry metabolism due to abiotic and biotic stresses encountered during postharvest storage, such as cold, hypoxia, and pathogen attacks, directly influences the accumulation of metabolites responsible for taste and aroma, critical attributes for consumer preference and the fruit industry [22]. While numerous molecular mechanisms active during fruit postharvest storage and senescence are yet to be fully understood, future research is anticipated to elucidate these processes and optimize storage conditions [23].

Despite extensive research in this field, there remains a noticeable gap in studies addressing the impact of sugar, organic acid, and amino acid content in fruits on the efficacy of essential oil components against post-harvest gray mold formation in specific grape cultivar like *Vitis vinifera* L. cv. ‘Karaerik’. This study aims to bridge this gap by comprehensively investigating the interactions between sugars, organic acids, amino acids, and essential oils in combating the pathogen in this grape cultivar. This research is critical in enhancing our understanding of fruit preservation and quality maintenance, contributing to the development of more effective and sustainable post-harvest management strategies.

**Materials and methods**  
**Study site and plant material**  
This study was conducted in a commercial vineyard in Erzincan province, northeastern Türkiye, during the 2023 growing season. Erzincan is characterized by a relatively short growing season, spanning approximately 180 to 190 days, and cool climate conditions, with an accumulation of 1587 heat units (base 10 °C) [24]. In this region, grapevine yield and quality are often compromised by environmental challenges such as autumn rainfalls and winter and spring frosts [24, 25]. The experimental plant material consisted of 22-year-old, own-rooted ‘Karaerik’ cv. (*Vitis vinifera* L.) vines, cultivated at the Erzincan Horticultural Research Institute vineyard. The vines were oriented in west–east rows, with a trunk height of approximately 100 cm, and spaced at 2.5 m within rows and 2.0 m between rows. The vines were trained to a bilateral cordon shoot system with spur-pruned cordon, maintaining 28 nodes per vine. Our research design incorporated three replicates, each comprising six vines. From each vine, two bunches were selected for the applications. During both growing seasons, the shoots were hedged once in the third week of August. Standard vineyard management practices, including pest management, were adhered to throughout the study period.

**Isolation and preparation of *B. cinerea***:  
*B. cinerea*, the fungal pathogen under investigation, was isolated, molecularly identified, and employed in this study, as described by Karakus et al. [26]. Prior to experimentation, *B. cinerea* was cultured on Potato Dextrose Agar (PDA) medium for seven days at a stable temperature of 25 °C. Spores were harvested by scraping them into distilled water using a sterilized bacterial L-shape rod and subsequently filtered through a sterile muslin sheet. The concentration of the resulting conidial suspension was adjusted to 1 × 10⁵ conidia/mL, as measured by a hemocytometer, following the protocol of Abdel-Rahim and Abo-Elyour [27].

**Procurement and storage of essential oils**  
Essential oil components, specifically thymol, eugenol, and 1,8-cineole, were sourced from Sigma–Aldrich, Shanghai, China. To preserve their integrity, these compounds were stored at 4 °C in a dark environment, mitigating any potential degradation due to light or temperature variations.

**Inoculation and storage of berries**  
The study focused on assessing the impact of thymol, eugenol, and 1,8-cineole essential oils on the ‘Karaerik’ cultivar of berries. To ensure scientific rigor and reproducibility, we conducted each experiment three times. We utilized a controlled experimental setup, where treatments were applied in isolation and in combination, allowing us to parse out the effects attributable to EOs alone, the pathogen alone, and their combination. The inoculation method was standardized across all treatment groups, including control groups, to ensure that any physical damage was consistent across the experiment. This standardization allowed us to attribute observed metabolic changes to the treatments applied rather than to the inoculation process itself. To ensure consistency and accuracy in our results, samples were taken from standardized locations on the berries, equidistant from the inoculation sites. This approach mitigated the potential variability in metabolite concentrations that could arise from differing proximities to the wound sites, thereby providing a more accurate reflection of the treatment effects on the grape’s biochemical composition. Furthermore, the inclusion of control groups that underwent the wounding process without pathogen or EO application served as a baseline to assess the impact of the wounds alone on the metabolic profile of the berries.

"..."
Following the protocols established by Yousef et al. [28] with slight modifications, berries were first surface sterilized using 2% sodium hypochlorite for two minutes, then washed thrice with tap water, and air-dried at room temperature. From each grape cluster, ten berries were wounded to a depth of approximately 2 mm as per Pedrotti et al. [29]. For the inoculation solution, 5 mL of the stock solution was diluted with 395 mL of water. The treatment applications were as follows: Control (C) with distilled water. For the treatment of the control group, we specified that it consisted of uninoculated fruits which served as a baseline to compare the effects of essential oil treatments and pathogen inoculation on the grape’s biochemical composition; Pathogen (F) with a spore suspension of $1 \times 10^5$ conidia mL$^{-1}$; and various combinations of Thymol (T), Eugenol (O), and 1,8-Cineole (S) at concentrations of 1.25 µL or 1 µL. These concentrations were selected based on preliminary studies conducted by Karakus et al. [26] to avoid potential fruit peel deformation. The experiment incorporated both ‘Karaerik’ grape cultivar, totaling 32 treatments, and was conducted in a completely randomized design. Each treatment was replicated thrice, using three grape clusters per replicate. For EO Application and Fungal Inoculation, berries were

Fig. 1 The appearance of the berries after EOs treatments
immers in their respective EO solutions for 15 min and then allowed to dry at room temperature for four hours. The wound sites on the fruits were then inoculated with 125 μL of a conidial suspension of *B. cinerea* at a concentration of $1 \times 10^5$ spores/mL. Post-inoculation, the grapes were stored in transparent plastic boxes at a temperature of $+4 \, ^\circ\text{C}$ and a high humidity level of 90 ± 5%. Then, the infected berries were inspected after twenty days of incubation to assess the impact of the treatments. This protocol was adapted from Almasaudi et al. [30] to evaluate the effectiveness of the EOs in controlling the growth of *B. cinerea* on berries. The appearance of the fruits after EOs applications is presented in Fig. 1.

**Sugar analysis**

The quantification of sugars in berry samples was conducted using a modified version of the method described by Ma et al. [31]. The sugars analyzed included arabinose, fructose, galactose, glucose, rhamnose, sucrose, and xylose. High-Performance Liquid Chromatography (HPLC) coupled with Evaporative Light Scattering Detection (ELSD) was employed for this purpose. The HPLC analyses were performed on a Waters 2695 separation module, which was integrated with an Alltech 3300 ELSD detector. The separation of the extracted samples was achieved using an X-Bridge-TM Amide column, characterized by a 4.5 μm particle size and dimensions of 4.6 mm×250 mm i.d. Prior to analysis, both samples and standards were filtered through 0.45 μm Millipore filters. For the HPLC runs, 10 μL aliquots of the samples were injected into the system. The HPLC-ELSD conditions were meticulously optimized in accordance with the parameters outlined by Ma et al. [31]. The solvent system consisted of an 85% acetonitrile and 15% water (v/v) ratio. The flow rate was set at 1 mL/min. The temperatures of the drift tube and the column were maintained at 82 °C and 45 °C, respectively, and the nebulizer gas flow rate was adjusted to 2 L/min. The detection of sugar peaks was achieved by employing calibration standards of HPLC grade sugars sourced from Sigma-Aldrich, Shanghai, China. The calibration involved comparing the sample peaks with the known concentrations of the standard sugars, thereby allowing for the accurate quantification of sugars in the berry samples.

**Organic acid analysis**

The extraction of organic acids from berry samples was conducted using the method established by Bevilacqua and Califano [32]. This involved taking a 5 mL aliquot of the berry sample and mixing it with 20 mL of 0.009 N H$_2$SO$_4$. The mixture was then agitated using a shaker for one hour to ensure thorough blending. Following this, the mixture was subjected to centrifugation at a speed of 15,000 rpm for 15 min. The resulting supernatant was first passed through filter paper and subsequently filtered twice using a 0.45 μm membrane filter. To further purify the sample, it was then passed through a SEP-PAK C18 cartridge. For the analysis of the extracted organic acids, an HPLC system equipped with an Aminex column (HPX-87 H, 300 mm×7.8 mm) was employed. The system was operated and controlled using a PC equipped with Agilent software, ensuring precise control and data acquisition. The detection of organic acids in the HPLC system was facilitated using a Diode-Array Detector (DAD) set at wavelengths of 214 nm and 280 nm (Agilent, USA). This setup was chosen to optimally detect the specific absorbance of the organic acids present in the samples. The mobile phase for the analysis was 0.009 N H$_2$SO$_4$, which had been filtered through a 0.45 μm membrane filter to ensure purity and prevent any potential clogging of the HPLC system.

**Analysis of free amino acids**

To determine the free amino acid content, 1 g of the berry sample was mixed with 0.1 N HCl. This mixture was then homogenized using an Ultra Turrax (Ika, T-25) and incubated at 4 °C for 12 h to ensure complete extraction of the amino acids. Post-incubation, the samples were centrifuged at 1200 rpm for 50 min. The clear supernatant was then carefully filtered using a 0.22 μm Millex Millipore filter to remove any particulate matter. The filtered supernatants were subsequently transferred to vials for analysis via High-Performance Liquid Chromatography (HPLC), following the protocol described by Aristoy and Toldra [33]. The HPLC system utilized for this analysis was an Agilent 1200, equipped with Zorbax Eclipse-AAA columns (4.6×150 mm, 3.5 μm). Detection of amino acids was carried out at a wavelength of 254 nm. Standards for free amino acids were established through comparison with derivatization agents O-phthalaldehyde (OPA) and fluoresceinmethyl-chloroformate (FMOC) chemicals. This step is crucial for enhancing the detection and quantification of amino acids in the HPLC system. The mobile phase of the chromatographic system consisted of two solutions: mobile phase A (40 mM NaH$_2$PO$_4$, pH: 7.8) and mobile phase B (Methanol/Acetonitrile/Water in a 45/45/10, v/v/v ratio). The column temperature was maintained at 40 °C, and the flow rate was set at 2 mL/min. A diverse range of amino acids, including aspartate, glutamate, leucine, glutamine, lysine, phenylalanine, sarcosine, asparagine, tryptophan, histidine, glycine, thionine, serine, alanine, arginine, tyrosine,
valine, cysteine, methionine, hydroxyproline, isoleucine, and proline, were detected in the berry samples. The contents of these amino acids were quantified as µg/l FW (fresh weight) after a 26-min derivation process in the HPLC system.

**Statistical analysis**

For statistical evaluation, we leveraged the agricolae package in R Studio, to conduct all descriptive analyses. We assessed the influence of variables such as thymol, eugenol, and 1,8-cineole essential oils (EOs), along with their interactions, on the levels of sugar, organic acids, and free amino acids using ANOVA, adhering to the protocols established by the R Core Team. To confirm the suitability of our statistical models, we initially checked the normality of our data with the chi-square test. We then employed linear models (via the lm function in R) to closely examine the main impacts of the variables, thymol, eugenol, and 1,8-cineole EOs, on the concentrations of sugars, organic acids, and free amino acids. After identifying significant effects, we proceeded with post-hoc analyses using the HSD test, utilizing the agricolae package once more, to identify differences between treatment levels. In addition, we conducted PCA using the ggbiplot2 package in R Studio (Vq., 2021) to reduce the complex data of sugar, organic acid, and free amino acids into fewer dimensions, facilitating easier interpretation. This PCA was crucial for revealing hidden patterns and trends, shedding light on the intricate relationships between the investigated factors and the concentrations of sugar, organic acid, and free amino acids. Finally, to visually represent the data and explore potential relationships or variations, we created heatmaps with the heatmap package in R Studio.

**Results**

In our results, the treatment labeled OF consistently showed the highest levels of all sugars. Specifically, sucrose content in the OF treatment reached an average of 17.97 g/l, which was markedly higher compared to the control C, which had a negligible average of 0.00 g/l. Similarly, glucose and fructose levels were highest in the OF treatment, with values of 17.97 g/l and 19.64 g/l, respectively, again significantly surpassing the control group’s levels of 0.18 ± 0.48 mg/100 g for glucose and 0.15 g/l for fructose. Additionally, the study revealed a pronounced increase in the content of rhamnose, galactose, xylose, and arabinose in the OF treatment samples. Rhamnose content in the OF treatment was measured at 6.17 g/l, galactose at 4.09 g/l, xylose at 5.64 g/l, and arabinose at 3.97 g/l. In stark contrast, the control group demonstrated the lowest levels of these sugars. Similarly, the TS treatment showed elevated levels of certain sugars, with sucrose at 3.67 g/l, glucose at 13.61 g/l, and fructose at 17.84 mg/100 g. Other treatments such as T, TF, and F exhibited varying but generally lower sugar levels.

**Table 1** The sugar contents (g/l) of harvested ‘Karaerik’ grape berries for various individual and combined EO treatments aimed at countering B. Cinerea

<table>
<thead>
<tr>
<th>Treatment (Y)</th>
<th>Sucrose</th>
<th>Glucose</th>
<th>Fructose</th>
<th>Rhamnose</th>
<th>Galactose</th>
<th>Xylose</th>
<th>Arabinose</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>0.00 ± 0.07e</td>
<td>0.18 ± 0.48h</td>
<td>0.15 ± 0.41h</td>
<td>0.05 ± 0.06e</td>
<td>0.01 ± 0.02g</td>
<td>0.00 ± 0.15f</td>
<td>0.15 ± 0.04g</td>
</tr>
<tr>
<td>F</td>
<td>0.01 ± 0.01e</td>
<td>0.30 ± 0.51h</td>
<td>0.45 ± 0.34h</td>
<td>0.09 ± 0.00e</td>
<td>0.00 ± 0.06g</td>
<td>0.03 ± 0.17f</td>
<td>0.86 ± 0.07cd</td>
</tr>
<tr>
<td>O</td>
<td>0.15 ± 0.09e</td>
<td>2.51 ± 0.57fg</td>
<td>2.70 ± 0.34fg</td>
<td>0.05 ± 0.07e</td>
<td>0.07 ± 0.00g</td>
<td>0.80 ± 0.11de</td>
<td>0.55 ± 0.08ef</td>
</tr>
<tr>
<td>OF</td>
<td>1.33 ± 0.01d</td>
<td>17.97 ± 0.23a</td>
<td>19.64 ± 0.67a</td>
<td>6.17 ± 0.09a</td>
<td>4.09 ± 0.09a</td>
<td>5.64 ± 0.09a</td>
<td>3.97 ± 0.04a</td>
</tr>
<tr>
<td>OS</td>
<td>3.48 ± 0.07a</td>
<td>12.71 ± 0.37bc</td>
<td>16.66 ± 0.55b</td>
<td>1.63 ± 0.05bc</td>
<td>0.95 ± 0.01bcd</td>
<td>1.45 ± 0.23bc</td>
<td>1.05 ± 0.08bc</td>
</tr>
<tr>
<td>OSF</td>
<td>2.72 ± 0.05b</td>
<td>10.28 ± 0.40cd</td>
<td>13.47 ± 0.69cd</td>
<td>1.32 ± 0.06cd</td>
<td>0.77 ± 0.07ef</td>
<td>1.17 ± 0.28bcd</td>
<td>0.85 ± 0.01cd</td>
</tr>
<tr>
<td>S</td>
<td>0.36 ± 0.05e</td>
<td>4.81 ± 0.19f</td>
<td>5.24 ± 0.32f</td>
<td>1.65 ± 0.03bc</td>
<td>1.09 ± 0.06b</td>
<td>1.51 ± 0.15bc</td>
<td>1.06 ± 0.08bc</td>
</tr>
<tr>
<td>SF</td>
<td>0.18 ± 0.00e</td>
<td>2.97 ± 0.26fg</td>
<td>3.19 ± 0.32fg</td>
<td>1.03 ± 0.08d</td>
<td>0.67 ± 0.04f</td>
<td>0.94 ± 0.18cd</td>
<td>0.65 ± 0.05def</td>
</tr>
<tr>
<td>T</td>
<td>0.08 ± 0.01e</td>
<td>1.12 ± 0.67gh</td>
<td>1.26 ± 0.19gh</td>
<td>0.05 ± 0.05e</td>
<td>0.03 ± 0.06g</td>
<td>0.31 ± 0.08ef</td>
<td>0.41 ± 0.00fg</td>
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<tr>
<td>TF</td>
<td>0.25 ± 0.04e</td>
<td>3.91 ± 0.37f</td>
<td>4.21 ± 0.23f</td>
<td>1.36 ± 0.00cd</td>
<td>0.88 ± 0.07cde</td>
<td>1.24 ± 0.16bcd</td>
<td>0.85 ± 0.02cd</td>
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<tr>
<td>TO</td>
<td>2.50 ± 0.09b</td>
<td>10.71 ± 0.43cd</td>
<td>13.86 ± 0.43c</td>
<td>1.40 ± 0.08bcd</td>
<td>0.79 ± 0.00def</td>
<td>1.24 ± 0.21bcd</td>
<td>0.87 ± 0.00cd</td>
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<tr>
<td>TOF</td>
<td>1.74 ± 0.05c</td>
<td>7.38 ± 0.47e</td>
<td>9.78 ± 0.60e</td>
<td>0.07 ± 0.01e</td>
<td>0.10 ± 0.03g</td>
<td>0.81 ± 0.00de</td>
<td>0.87 ± 0.08cd</td>
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<tr>
<td>TOS</td>
<td>2.56 ± 0.02b</td>
<td>10.76 ± 0.49cd</td>
<td>13.91 ± 0.45c</td>
<td>1.40 ± 0.06bcd</td>
<td>0.79 ± 0.06def</td>
<td>1.24 ± 0.05bcd</td>
<td>0.88 ± 0.03cd</td>
</tr>
<tr>
<td>TOSF</td>
<td>2.00 ± 0.09c</td>
<td>8.70 ± 0.32de</td>
<td>11.25 ± 0.41de</td>
<td>1.13 ± 0.04d</td>
<td>0.64 ± 0.09f</td>
<td>1.00 ± 0.17bcd</td>
<td>0.71 ± 0.07de</td>
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<tr>
<td>TS</td>
<td>3.67 ± 0.07a</td>
<td>13.61 ± 0.28b</td>
<td>17.84 ± 0.34ab</td>
<td>1.75 ± 0.08b</td>
<td>1.02 ± 0.10bc</td>
<td>1.55 ± 0.10ab</td>
<td>1.12 ± 0.01bc</td>
</tr>
<tr>
<td>TSF</td>
<td>2.40 ± 0.04b</td>
<td>8.50 ± 0.60de</td>
<td>11.59 ± 0.19cde</td>
<td>0.10 ± 0.02e</td>
<td>0.14 ± 0.06g</td>
<td>0.87 ± 0.09de</td>
<td>1.22 ± 0.07b</td>
</tr>
</tbody>
</table>

Significance: **< 2e-16***, *< 2e-16***, **< 2e-16***, **< 2e-16***, **< 2e-16***, **< 2e-16***, **< 2e-16***, **< 2e-16***. For a given factor (different letters within a column represent significant differences (Tukey test, * Significant at p-value < 0.05; **, Significant at p-value < 0.01; *** Significant at p-value < 0.001). Data are stated as averages of the data and their standard errors.
Table 2 The organic acid contents (mg/l) of harvested 'Karaerik' grape berries for various individual and combined EO treatments aimed at countering B. Cinerea

<table>
<thead>
<tr>
<th>Treatment (Y)</th>
<th>Oxalic acid</th>
<th>Propionic acid</th>
<th>Tartaric acid</th>
<th>Butyric acid</th>
<th>Malonic acid</th>
<th>Malic acid</th>
<th>Lactic acid</th>
<th>Citric acid</th>
<th>Maleic acid</th>
<th>Fumaric acid</th>
<th>Succinic acid</th>
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<tbody>
<tr>
<td>C</td>
<td>9.71 ± 2.31d</td>
<td>12.61 ± 2.85b</td>
<td>1084 ± 1.45b</td>
<td>19.78 ± 2.34b</td>
<td>19.96 ± 2.75b</td>
<td>9.45 ± 1.32b</td>
<td>9.83 ± 2.46b</td>
<td>12.30 ± 1.66c</td>
<td>4.26 ± 1.06b</td>
<td>11.51 ± 2.45b</td>
<td>11.00 ± 3.16c</td>
</tr>
<tr>
<td>F</td>
<td>9.89 ± 3.25d</td>
<td>13.21 ± 2.78b</td>
<td>1063 ± 1.56b</td>
<td>19.55 ± 2.45b</td>
<td>19.15 ± 2.65b</td>
<td>9.36 ± 1.36b</td>
<td>9.69 ± 2.54b</td>
<td>12.82 ± 1.19c</td>
<td>4.56 ± 2.13b</td>
<td>10.65 ± 2.67b</td>
<td>10.81 ± 3.23c</td>
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<tr>
<td>O</td>
<td>17.06 ± 3.21abcd</td>
<td>19.00 ± 3.65ab</td>
<td>1468 ± 2.68ab</td>
<td>22.96 ± 3.39ab</td>
<td>28.60 ± 3.15ab</td>
<td>13.44 ± 2.09ab</td>
<td>20.75 ± 3.00ab</td>
<td>18.26 ± 1.23abc</td>
<td>9.46 ± 3.32a</td>
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<td>17.63 ± 2.37b</td>
<td>11.73 ± 1.13ab</td>
<td>15.82 ± 2.14ab</td>
<td>18.92 ± 2.43abc</td>
<td>8.00 ± 2.56ab</td>
<td>18.03 ± 2.60ab</td>
<td>22.08 ± 3.43abc</td>
</tr>
<tr>
<td>OS</td>
<td>22.79 ± 3.15ab</td>
<td>19.35 ± 2.22ab</td>
<td>1791 ± 3.45ab</td>
<td>29.43 ± 3.65ab</td>
<td>28.84 ± 3.45ab</td>
<td>11.78 ± 1.67ab</td>
<td>26.80 ± 3.50a</td>
<td>22.26 ± 2.35ab</td>
<td>9.12 ± 2.55ab</td>
<td>24.82 ± 2.10ab</td>
<td>26.35 ± 3.54abc</td>
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<tr>
<td>OSF</td>
<td>15.53 ± 2.19abcd</td>
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<td>1603 ± 2.23ab</td>
<td>25.90 ± 3.87ab</td>
<td>26.88 ± 2.87ab</td>
<td>11.13 ± 1.54ab</td>
<td>16.99 ± 2.26ab</td>
<td>16.52 ± 1.66abc</td>
<td>6.63 ± 2.27ab</td>
<td>16.96 ± 2.28abc</td>
<td>30.05 ± 3.19ab</td>
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<tr>
<td>S</td>
<td>20.86 ± 4.35abcd</td>
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<td>1406 ± 1.59ab</td>
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<td>11.49 ± 2.09ab</td>
<td>13.66 ± 1.41c</td>
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<td>1252 ± 2.41b</td>
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<td>20.31 ± 2.00abc</td>
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<td>26.00 ± 2.00ab</td>
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<td>19.25 ± 1.17b</td>
<td>29.80 ± 2.16ab</td>
<td>10.66 ± 1.17ab</td>
<td>21.11 ± 2.47ab</td>
<td>22.24 ± 1.87ab</td>
<td>5.65 ± 2.45b</td>
<td>2044 ± 2.89ab</td>
<td>28.22 ± 3.60ab</td>
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<tr>
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<td>0.0427*</td>
<td>0.0008**</td>
<td>0.0015**</td>
<td>0.0012***</td>
<td>0.0005***</td>
<td>2.86e-05***</td>
<td>0.0001***</td>
<td>0.0001***</td>
<td>0.0027**</td>
<td>5.2e-05***</td>
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x, Mean separation in Treatments y; For a given factor (different letters within a column represent significant differences (Tukey test, *, Significant at p-value < 0.05; **, Significant at p-value < 0.01; ***, Significant at p-value < 0.001). Data are stated as averages of the data and their standard errors.
instance, treatment T showed sucrose at 0.08 g/l, glucose at 1.12 g/l, and fructose at 1.26 g/l. This indicated a moderate increase in sugar levels compared to the control group, but these values were significantly lower than those observed in the OF, OS, or TS treatments (Table 1).

The SF treatment emerged as the best practice for achieving the highest organic acid levels, particularly notable in tartaric acid (20.95 mg/l), butyric acid (27.92 mg/l), and malonic acid (mg/l). Closely following this, the OS treatment demonstrated significant levels of organic acids, with oxalic acid at 22.79 mg/l and lactic acid at 26.80 mg/l. The application of various treatments resulted in significant differences in the oxalic acid content of the samples, with treatment TS exhibiting the highest oxalic acid concentration (25.71 mg/l) and treatment C showing the lowest (9.71 mg/l). The control represented the weak application in terms of organic acid content, showing notably low concentrations, such as oxalic acid at 9.71 mg/l and propionic acid at 12.61 mg/l. Slightly higher but still on the lower end was the F treatment, with oxalic acid at 9.89 mg/l and propionic acid at 13.21 mg/l, thus indicating a marginal increase compared to the control group. In the context of dominant organic acids, the SF treatment’s malonic acid concentration was the highest at 35.37 mg/l. This was closely followed by the TO treatment, which showed the highest level of succinic acid at 35.50 mg/l. Additionally, butyric acid in the SF treatment was also notably high at 27.92 mg/l. The OS treatment followed, with high levels of oxalic acid at 22.79 mg/l and lactic acid at 26.80 mg/l. The least common organic acid was observed in the control, with maleic acid recording the lowest concentration at 4.26 mg/l. Following this, the F treatment exhibited a slightly higher, yet still low, level of maleic acid at 4.56 mg/l. Another weaker acid was oxalic acid in the control group, with a concentration of 9.71 mg/l (Table 2).

The SF treatment exhibited statistically significant higher concentrations of several amino acids, with phenylalanine reaching 899.48 µg/l and tyrosine at 632.04 µg/l. Glutamate was also significantly elevated in this treatment, measured at 321.27 µg/l. In the TO treatment, there were statistically significant higher levels of tyrosine at 557.86 µg/l and phenylalanine at 842.10 µg/l, along with Leucine and Lysine showing higher concentrations compared to other treatments. The control demonstrated statistically significant lower concentrations of amino acids, with cystine at 122.23 µg/l and Valine at 149.63 µg/l being notably low, histidine and glycine levels in this group were also lower in a statistically significant manner. The F treatment showed slightly higher but still statistically lower levels of cystine (109.74 µg/l) and valine (145.18 µg/l). The OS and OSF treatments displayed statistically significant higher levels of asparagine, with OSF recording 435.67 µg/l. The S treatment’s serine level at 390.09 µg/l and SF’s glutamine level at 213.26 µg/l were also significantly higher. T and TF treatments showed moderate, yet statistically significant differences in amino acids like serine, asparagine, and glutamine compared to other treatments. Variations in arginine levels were statistically significant across treatments, with TO and OSF showing higher concentrations. Alanine and tyrosine also exhibited significant variations, with TO and SF treatments having higher levels of these amino acids (Table 3).

Our findings indicated that the principal component analysis (PCA) effectively captured the variability within the treatment dataset, with the first two principal components accounting for a combined total of 97.9% of the variance (PC1 at 75.4% and PC2 at 22.5%). The treatments were categorized into distinct clusters as per the PCA biplot, with each cluster color and shape-coded to correspond to specific treatments, as depicted in the legend. Our observations demonstrated that the vectors representing original variables such as glucose, fructose, and maltose were directional and of varied lengths, which directly corresponded to their contribution to the principal components. We noted that the proximity of these vectors to the treatment groups was indicative of their influence on the treatment characteristics. Our analysis revealed that the OF treatment was significantly separated along the PC1 axis. Conversely, the treatments labeled T, F, O, and S clustered closely together on both PC1 and PC2. Furthermore, our findings suggested a relationship between treatment profiles and original variables. The OSF treatment cluster was positioned nearer to the glucose and fructose vectors. In contrast, the TOS treatment cluster’s proximity to the maltose vector indicated a strong association with maltose (Fig. 2). On the other hand, our organic acid findings elucidated the underlying structure of the dataset through a PCA biplot, where the first two principal components (PC1 and PC2) captured 49.3% and 16.8% of the variance, respectively. The distribution of treatment clusters across the biplot revealed discernible patterns of similarity and differentiation among the treatments. Our results identified that treatments exhibiting proximity to each other, such as T, F, O, and S, shared similar profiles. Conversely, the treatment labeled OF was notably distanced from others along PC1, highlighting a distinct profile with potentially unique characteristics or effects. In addition, our analysis detected positive correlations between certain treatments and variables; treatments aligned with vectors pointing towards the positive axis of PC1 or PC2 were positively correlated with the corresponding variables. The OSF treatment was situated...
Table 3  The amino acid contents (µg/l) of harvested 'Karaerik' grape berries for various individual and combined EO treatments aimed at countering B. cinerea

<table>
<thead>
<tr>
<th>Treatment (Y)</th>
<th>Aspartate</th>
<th>Glutamate</th>
<th>Asparagine</th>
<th>Serine</th>
<th>Glutamine</th>
<th>Histidine</th>
<th>Glycine</th>
<th>Theanine</th>
<th>Arginine</th>
<th>Alanine</th>
<th>Tyrpine</th>
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<tr>
<td>C</td>
<td>290.25 ± 41.75c</td>
<td>124.98 ± 36.5b</td>
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<td>354.47 ± 24.9b</td>
<td>162.58 ± 21.3b</td>
<td>211.28 ± 36.2a</td>
<td>22.32 ± 24.9ab</td>
<td>29.66 ± 36.3a</td>
<td>121.31 ± 35.2b</td>
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</tr>
<tr>
<td>F</td>
<td>306.75 ± 33.00c</td>
<td>138.58 ± 16.4b</td>
<td>283.26 ± 36.76c</td>
<td>343.14 ± 40.0b</td>
<td>147.32 ± 31.2b</td>
<td>216.71 ± 11.7bc</td>
<td>22.00 ± 33.5ab</td>
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<tr>
<td>O</td>
<td>429.12 ± 34.5b</td>
<td>208.38 ± 31.6b</td>
<td>334.06 ± 35.13bc</td>
<td>394.96 ± 33.8b</td>
<td>184.38 ± 6.3b</td>
<td>229.12 ± 33.9abc</td>
<td>22.58 ± 7.07b</td>
<td>27.64 ± 8.06a</td>
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<td>351.80 ± 40.05c</td>
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<td>OF</td>
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<td>OS</td>
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<td>414.11 ± 27.62b</td>
<td>143.57 ± 5.27a</td>
<td>219.62 ± 16.58ab</td>
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<td>252.34 ± 29.16ab</td>
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<td>400.88 ± 65.15ab</td>
<td>486.75 ± 79.99ab</td>
<td>156.52 ± 18.37b</td>
<td>207.72 ± 22.95a</td>
<td>258.16 ± 18.03ab</td>
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<td>351.31 ± 32.75ab</td>
<td>373.10 ± 69.00ab</td>
<td>175.50 ± 20.14ab</td>
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x, Mean separation in Treatments y; For a given factor (different letters within a column represent significant differences (Tukey test), *, Significant at p-value < 0.05; **, Significant at p-value < 0.01; ***. Significant at p-value < 0.001). Data are stated as averages of the data and their standard errors.
close to the vector for citric acid, indicating a positive association. Similarly, the TSF treatment’s closeness to the malic acid vector suggested a positive correlation with malic acid content. Our study also inferred negative correlations where treatments were aligned with the negative direction of the vectors. These correlations suggested that as the level of a given variable decreased, the association with the respective treatment increased, possibly implicating these variables in the differentiation of treatment effects (Fig. 3).

Our findings from the PCA biplot for amino acids revealed that PC1 and PC2 explained 48.8% and 25.2% of the variance in the dataset, respectively. The treatments, discernible by distinct color coding, displayed varying degrees of correlation with the vectors representing different amino acids. The spatial distribution of the treatments across the biplot provided insights into their similarities and differences, with some treatments forming closely-knit clusters, while others were more dispersed. We also noted that treatments such as OSF, TO, and TOSF were closely associated with vectors for amino acids like glutamine, valine, and alanine, indicating positive correlations with these compounds. In contrast, the treatment labeled OF was positioned opposite the direction of most amino acid vectors. Our study demonstrated that treatments clustered together, such as F, C, O, and S, shared a degree of similarity, which could be attributed to common amino acid profiles or similar influences on metabolic pathways. Conversely, the spatial separation of treatments indicated distinct amino acid associations, which could reflect differences in their application or biological impact (Fig. 4). Our study provided a comprehensive analysis of the interactions between various treatments and a range of biochemical parameters, including sugars, organic acids, and amino acids. The heatmap illustrated a complex pattern of associations, with certain treatments showing pronounced effects on the concentration levels of these compounds. The treatments OF, OS, OSF, S, SF, T, TF, TO, TOF, TOS, TOSF, TS, and TSF were found to have elevated levels of simple sugars such as sucrose, glucose, and fructose, as evidenced by the prevalence of darker blue shades in the heatmap. Conversely, the treatments C and F were associated with lower concentrations of these sugars, as indicated by the green shades on the heatmap. The heatmap also revealed differential expressions of rhamnose, galactose, xylose, and arabinose, with treatments such as TO, TOF, and TOSF showing increased levels, denoted by blue hues. Regarding organic acids, our study found that treatments such as OSF, TSF, and TOSF exhibited higher concentrations of oxalic acid, propionic acid, tartaric acid, and other organic acids, as indicated by the blue colors. For amino acids, the heatmap showed that treatments OSF, TSF, and TOSF had higher concentrations
of a broad spectrum of amino acids, including aspartate, glutamate, and glutamine, among others (Fig. 5).

Discussion

Our current research provides a detailed examination of the influence of EOs on primary metabolites in the ‘Karaerik’ grape cultivar, specifically focusing on their role in post-harvest preservation. Our analysis encompassed a broad spectrum of metabolites, including sugars, organic acids, and amino acids, thereby uncovering significant facets of fruit metabolism affected by EOs and the pathogenic presence of *B. cinerea*. Recognizing the fundamental role of primary metabolites in fruit quality and plant development, these our results contribute to a deeper understanding of fruit metabolism, potentially aiding in the development of novel methods for its manipulation [34]. In this investigation, we identified a total of 39 primary metabolites: 7 sugars (sucrose, glucose, fructose, rhamnose, galactose, xylose, and arabinose); 11 organic acids (oxalic, propionic, tartaric, butyric, malonic, malic, lactic, citric, maleic, fumaric, and succinic acids); and 21 amino acids (tyrosine, glutamate, asparagine, serine, glutamine, histidine, glycine, threonine, arginine, alanine, aspartate, cysteine, valine, methionine, tryptophan, phenylalanine, isoleucine, leucine, lysine, hydroxyproline, and sarcosine). Our results demonstrate a pronounced effect of EO treatments on sugar metabolism in the ‘Karaerik’ grape cultivar. Notably, the OF treatment, encompassing both Eugenol and *B. cinerea*, consistently exhibited the highest sugar levels across all types, including sucrose, glucose, fructose, rhamnose, galactose, xylose, and arabinose (Table 1). We assume this significant elevation in sugar content is a result of stress-induced metabolic changes due to EO application and fungal infection, as stress conditions in plants often lead to sugar accumulation as a defensive mechanism [35]. The stark contrast in sugar content that was observed between the OF treatment and the control group, which displayed minimal sugar levels (Table 1), underscored the potential of EOs to modify fruit metabolism under pathogenic stress [36].
However, the complexity of interactions between EOs, fungal pathogens, and plant metabolism presents a challenging landscape for attributing changes to a singular cause directly. This complexity is compounded by the natural variability inherent in biological systems and the multifaceted nature of stress responses in plants. Interestingly, the TS treatment also exhibited heightened sugar levels, albeit less pronounced than the OF treatment (Table 1). This disparity in response to different EO treatments suggests a compound-specific influence on sugar metabolism [37]. Given that sugars are crucial for the organoleptic qualities of fruits, affecting sweetness and overall flavor, these findings are particularly relevant.

Pathogen infestation typically reduces postharvest berry quality, but our results, alongside recent studies, indicate that pathogen colonization correlates with changes in host carbohydrate levels, presenting new opportunities for disease control. For instance, starch accumulation in the host as an initial response to bacterial effectors or volatile organic compounds may help contain microbial spread [38, 39]. Furthermore, in advanced infestation stages, the rapid conversion of starch to sugars can provide energy and carbon for synthesizing antimicrobial compounds [40, 41]. The increased sugar levels in EO-treated grapes could, therefore, have significant implications for fruit quality, particularly in terms of taste.

Fig. 4 Principal component analysis (PCA) of amino acids, individual and combined EO treatments
and consumer preference [42]. On the other hand, the moderate rise in sugar levels in treatments such as T and TF compared to the control, though substantially lower than in OF and TS treatments, indicates a potential dose-dependent effect of EOs on sugar metabolism. These observations are somewhat different from other researchers’ findings, suggesting that EO application in managing post-harvest diseases may also trigger metabolic alterations in berries. Such changes could be leveraged not only to enhance berry quality but also to inhibit pathogen proliferation [9]. The study also highlights the significant influence of EO treatments on organic acid levels. Notably, the SF treatment emerged as the most effective in elevating the levels of key organic acids such as tartaric, butyric, and malonic acids. The SF treatment’s effectiveness in increasing organic acid content, particularly in tartaric, butyric, and malonic acids, indicates the potential of EOs in modulating fruit metabolism under post-harvest conditions. This is further supported by the significant levels of oxalic and lactic acids observed in the OS treatment. Conversely, C demonstrated notably lower concentrations of these acids, indicating the limited capability of untreated berries to resist pathogen-induced stress and maintain organic acid levels. The distinct increase in organic acid content in EO-treated grapes, compared to the C and F treatments, highlights the potential of EOs as an alternative to conventional post-harvest treatments (Table 2). Organic acids are vital for the flavor profile, nutritional value, and overall acceptability of fruits [43]. Findings suggest that the application of specific EOs, in combination with the stress of fungal infection, can lead to a notable increase in certain organic acids, which are crucial for fruit quality and shelf life. The ability of EOs to enhance organic acid content suggests their role in not only controlling fungal infections but also in positively influencing fruit metabolism, which is crucial for extending shelf life and preserving fruit quality during storage and transportation [44].

Our results revealed considerable alterations in amino acid concentrations following EO treatments. Notably,
the SF treatment displayed significantly elevated levels of several amino acids, particularly phenylalanine and tyrosine. Similarly, the TO treatment resulted in marked increases in tyrosine, phenylalanine, leucine, and lysine levels (Table 3). This suggests a robust response of the grape’s metabolic system to these treatments, potentially indicative of a stress response or a metabolic adaptation geared towards enhancing defense mechanisms against the pathogen [45]. In contrast, the control group exhibited significantly lower concentrations of essential amino acids such as cystine and valine, possibly reflecting the baseline metabolic state of the grapes in the absence of EO treatment or pathogen stress. The diminished amino acid levels in the control group might also imply a lack of activation of the grapes’ inherent defense mechanisms. Moreover, our study demonstrates that the application of various EOs leads to significant changes in the amino acid profiles of grapes. Particularly, the SF and TO treatments showed increased levels of amino acids like phenylalanine, tyrosine, and glutamate. We assume that these alterations indicate that EOs can modulate amino acid metabolism in grapes, potentially augmenting their natural defense mechanisms against B. cinerea [46]. However, this contrasts with the study on Pseudomonas fluorescens ZX-produced VOCs, where the transcriptome analysis of B. cinerea exposed to VOCs revealed significant changes in genes related to amino acid metabolism, implying a direct interference by the VOCs in the pathogen’s metabolic pathways [44]. The comparison of these studies highlights different strategies in combating B. cinerea. While our findings suggest an indirect approach where EOs enhance the grape’s defense mechanisms, including alterations in amino acid metabolism, the P. fluorescens ZX study showcases a direct antifungal action. On the other hand, the observed increase in specific amino acids in EO-treated grapes may contribute to reinforcing the grape’s biochemical pathways, potentially reducing susceptibility to fungal attacks. As noted by Mccarthy and Walsh [46], antifungal drugs typically inhibit fungal pathogen growth through three primary mechanisms: inhibiting ergosterol biosynthesis, binding to ergosterol to lyse cells, and inhibiting cell wall biosynthesis. However, Nishida et al. [47] suggested that targeting amino acid metabolism, a crucial nutrient source for fungi, could form the basis of a new class of antifungal drug targets. Mccarthy and Walsh [46] also observed that some antifungal substances significantly disrupt amino acid transport and metabolism, inhibiting the growth of pathogenic yeasts and fungi. Therefore, the specific alterations in amino acid metabolism observed in our study necessitate further investigation. Additionally, other metabolic pathways in B. cinerea that exhibited significant changes following treatments represent promising directions for future research.

Our study demonstrated the effectiveness of PCA in elucidating the variability within the treatment dataset. The substantial variance explained by the first two principal components (97.9%) highlights the robustness of PCA in capturing the essential features of complex datasets. Our findings resonate with similar research where PCA has been instrumental in reducing data dimensionality while preserving critical information [48]. The distinct clustering of treatments, as observed in the PCA biplot, provides a clear visualization of their relationships and differences. This approach of data visualization is particularly valuable in identifying patterns and correlations that might not be apparent in raw data [37, 49]. The directional vectors representing original variables such as glucose, fructose, and maltose, and their proximity to treatment groups, offer significant insights into their influence on treatment characteristics. Our findings suggest that treatments like OF, which are significantly separated along PC1, might have unique metabolic impacts or applications. This aspect of PCA analysis is crucial in identifying key variables that drive differences between groups, as noted in similar studies [44, 50]. Furthermore, the close clustering of treatments such as T, F, O, and S indicates commonality in their effects or compositions, which could be vital for applications where similar treatment outcomes are desired. Our analysis revealed interesting correlations between treatments and specific organic acids and amino acids. The proximity of treatments like OSF to the citric acid vector, for instance, suggests a strong association, which could be explored further for potential applications in metabolic studies or food science. Similarly, the observed associations with amino acids like glutamine, valine, and alanine point to specific metabolic pathways that might be influenced by these treatments [45, 51, 52]. The spatial distribution of treatments in relation to amino acid vectors offers a map of metabolic influence, which is a powerful tool for hypothesis generation in biochemical research. Our findings from the heatmap analysis provided a detailed overview of how different treatments affect the concentration of sugars, organic acids, and amino acids. The elevated levels of simple sugars in certain treatments, as indicated by darker blue shades, could have significant implications for their use in food industry applications or in studying carbohydrate metabolism [36, 37]. In contrast, the green shades indicating lower sugar concentrations in treatments like C and F might be relevant in contexts where reduced sugar levels are desirable.
Conclusion
In our results of the study, we meticulously examined the impact of different treatments, such as OF and SF, on various biochemical parameters. Based on our results, the OF treatment exhibited substantial increases in sugar content, including sucrose, glucose, and fructose, when compared to the control group. Similarly, the SF treatment demonstrated the highest levels of certain organic acids like tartaric, butyric, and malonic acids. Additionally, the SF treatment displayed significantly elevated concentrations of amino acids, particularly phenylalanine and tyrosine. Key findings from our investigation revealed that PCA effectively captured the variability within the treatment dataset, highlighting distinct clusters of treatments and their correlations with specific metabolites. The PCA biplot elucidated the influence of treatments on grape metabolic profiles, indicating unique associations between treatments and biochemical parameters. In conclusion, our study findings underscore the potential of EOs to significantly alter grape metabolic profiles, offering opportunities for enhancing fruit quality and extending shelf life. Manufacturers and stakeholders in the agricultural and food industries can benefit from these insights, as EOs emerge as valuable natural preservatives and enhancers in fruit cultivation and storage. In the future, however, further research and development efforts can focus on optimizing the application of EOs to create tailored strategies for different fruit species and explore their potential use in large-scale agricultural production, with the aim of realizing sustainable and economically viable solutions for improving post-harvest fruit quality and food safety.

Abbreviations
EOs  Essential oils
C  Control
F  Pathogen
S  1,8-cineole
O  Eugenol
T  Thymol
OF  Eugenol + pathogen
OS  Eugenol + 1,8-cineole
OSF  Eugenol + 1,8-cineole + pathogen
SF  1,8-cineole + pathogen
TF  Thymol + pathogen
TO  Thymol + eugenol
TOF  Thymol + eugenol + pathogen
TSF  Thymol + 1,8-cineole + pathogen
TOSF  Thymol + eugenol + 1,8-cineole + pathogen
TS  Thymol + 1,8-cineole
TOS  Thymol + eugenol + 1,8-cineole
EOs  Essential oils
PCA  Principal component analysis

Author contributions
S.K. A.B. and O.K. conceptualized and designed the experiments, with O.K. conducting them. H.S.H., O.K., M.T., and T.Y. analyzed the data. S.K. and O.K. drafted and reviewed the final manuscript. O.K. wrote the manuscript. All authors have read and consented to the publication of the manuscript.

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