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

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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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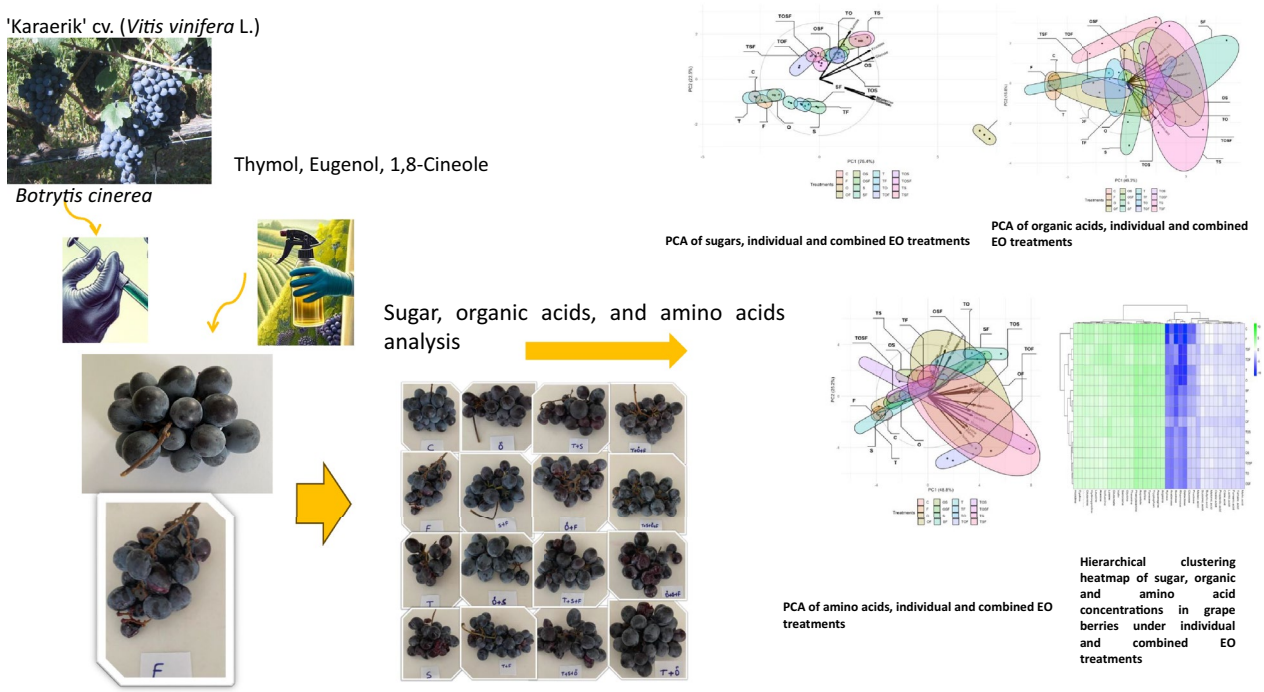
Graphical Abstract



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 detriments to postharvest 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 1×10^5 conidia/mL, as measured by a hemocytometer, following the protocol of Abdel-Rahim and Abo-Elyousr [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.

Fig. 1 The appearance of the berries after EOs treatments

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 1×10^5 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

immersed 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 1×10^5 spores/mL. Post-inoculation, the grapes were stored in transparent plastic boxes at a temperature of +4 °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, 10µL 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_2SO_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_2SO_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-phthaldialdehyde (OPA) and fluorenylmethyl-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_2PO_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/1, which was markedly higher compared to the control C, which had a negligible average of 0.00 g/1. Similarly, glucose and fructose levels were highest in the OF treatment, with values of 17.97 g/1 and 19.64 g/1, respectively, again significantly surpassing the control group's levels of 0.18 ± 0.48 mg/100 g for glucose and 0.15 g/1 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/1, galactose at 4.09 g/1, xylose at 5.64 g/1, and arabinose at 3.97 g/1. 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/1, glucose at 13.61 g/1, and fructose at 17.84 mg/100 g. Other treatments such as T, TF, and F exhibited varying but generally lower sugar levels. For

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

Treatment (Y)[x]	Sucrose	Glucose	Fructose	Rhamnose	Galactose	Xylose	Arabinose
C	0.00 ± 0.07e	0.18 ± 0.48 h	0.15 ± 0.41 h	0.05 ± 0.06e	0.01 ± 0.02 g	0.00 ± 0.15f	0.15 ± 0.04 g
F	0.01 ± 0.01e	0.30 ± 0.51 h	0.45 ± 0.34 h	0.09 ± 0.00e	0.00 ± 0.06 g	0.03 ± 0.17f	0.86 ± 0.07 cd
O	0.15 ± 0.09e	2.51 ± 0.57fgh	2.70 ± 0.34fgh	0.05 ± 0.07e	0.07 ± 0.00 g	0.80 ± 0.11de	0.55 ± 0.08ef
OF	1.33 ± 0.01d	17.97 ± 0.23a	19.64 ± 0.67a	6.17 ± 0.09a	4.09 ± 0.09a	5.64 ± 0.09a	3.97 ± 0.04a
OS	3.48 ± 0.07a	12.71 ± 0.37bc	16.66 ± 0.55b	1.63 ± 0.05bc	0.95 ± 0.01bcd	1.45 ± 0.23bc	1.05 ± 0.08bc
OSF	2.72 ± 0.05b	10.28 ± 0.40d	13.47 ± 0.69 cd	1.32 ± 0.06 cd	0.77 ± 0.07ef	1.17 ± 0.28bcd	0.85 ± 0.01 cd
S	0.36 ± 0.05e	4.81 ± 0.19f	5.24 ± 0.32f	1.65 ± 0.03bc	1.09 ± 0.06b	1.51 ± 0.15bc	1.06 ± 0.08bc
SF	0.18 ± 0.00e	2.97 ± 0.26 fg	3.19 ± 0.32 fg	1.03 ± 0.08d	0.67 ± 0.04f	0.94 ± 0.18 cd	0.65 ± 0.05def
T	0.08 ± 0.01e	1.12 ± 0.67gh	1.26 ± 0.19gh	0.05 ± 0.05e	0.03 ± 0.06 g	0.31 ± 0.08ef	0.41 ± 0.00 fg
TF	0.25 ± 0.04e	3.91 ± 0.37f	4.21 ± 0.23f	1.36 ± 0.00 cd	0.88 ± 0.07cde	1.24 ± 0.16bcd	0.85 ± 0.02 cd
TO	2.50 ± 0.09b	10.71 ± 0.43 cd	13.86 ± 0.43c	1.40 ± 0.08bcd	0.79 ± 0.00def	1.24 ± 0.21bcd	0.87 ± 0.00 cd
TOF	1.74 ± 0.05c	7.38 ± 0.47e	9.78 ± 0.60e	0.07 ± 0.01e	0.10 ± 0.03 g	0.81 ± 0.00de	0.87 ± 0.08 cd
TOS	2.56 ± 0.02b	10.76 ± 0.49 cd	13.91 ± 0.45c	1.40 ± 0.06bcd	0.79 ± 0.06def	1.24 ± 0.05bcd	0.88 ± 0.03 cd
TOSF	2.00 ± 0.09c	8.70 ± 0.32de	11.25 ± 0.41de	1.13 ± 0.04d	0.64 ± 0.09f	1.00 ± 0.17bcd	0.71 ± 0.07de
TS	3.67 ± 0.07a	13.61 ± 0.28b	17.84 ± 0.34ab	1.75 ± 0.08b	1.02 ± 0.01bc	1.55 ± 0.10b	1.12 ± 0.01bc
TSF	2.40 ± 0.04b	8.50 ± 0.60de	11.59 ± 0.19cde	0.10 ± 0.02e	0.14 ± 0.06 g	0.87 ± 0.09de	1.22 ± 0.07b
Significance	< 2e-16 ***	< 2e-16 ***	< 2e-16 ***	< 2e-16 ***	< 2e-16 ***	< 2e-16 ***	< 2e-16 ***

x, Mean seperation 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

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*

Treatment (Y)[x]	Oxalic.acid	Propionic acid	Tartaric acid	Butyric acid	Malonic acid	Malic acid	Lactic acid	Citric acid	Maleic acid	Fumaric acid	Succinic acid
C	9.71 ± 2.31d	12.61 ± 2.85b	10.84 ± 1.45b	19.78 ± 2.34b	19.96 ± 2.75b	9.45 ± 1.32b	9.83 ± 2.46b	12.30 ± 1.66c	4.26 ± 1.06b	11.51 ± 2.45b	11.00 ± 3.16c
F	9.89 ± 3.25d	13.21 ± 2.78b	10.63 ± 1.56b	19.55 ± 2.45b	19.15 ± 2.65b	9.36 ± 1.36b	9.69 ± 2.54b	12.82 ± 1.19c	4.56 ± 2.13b	10.65 ± 2.67b	10.81 ± 3.23c
O	17.06 ± 3.21abcd	19.00 ± 3.65ab	14.68 ± 2.68ab	22.96 ± 3.39ab	28.60 ± 3.15ab	13.44 ± 2.09ab	20.75 ± 3.00ab	18.26 ± 1.23abc	9.46 ± 3.32 a	21.60 ± 2.16ab	25.69 ± 3.15abc
OF	15.59 ± 2.41abcd	19.17 ± 3.19ab	11.49 ± 1.12ab	18.44 ± 2.24b	17.63 ± 2.37b	11.73 ± 1.13ab	15.82 ± 2.14ab	18.92 ± 2.43abc	8.00 ± 2.56 ab	18.03 ± 2.60ab	22.08 ± 3.43abc
OS	22.79 ± 3.15ab	19.35 ± 2.22ab	17.91 ± 3.45ab	29.43 ± 3.65ab	28.84 ± 3.45ab	11.78 ± 1.67ab	26.80 ± 3.50a	22.26 ± 2.35ab	9.12 ± 2.55 ab	24.82 ± 2.10ab	26.35 ± 3.54abc
OSF	15.53 ± 2.19abcd	20.18 ± 4.56ab	16.03 ± 2.23ab	25.90 ± 3.87ab	26.88 ± 2.87ab	11.13 ± 1.54ab	16.99 ± 2.26ab	16.52 ± 1.66abc	6.63 ± 2.27 ab	16.96 ± 2.28ab	30.05 ± 3.19ab
S	20.86 ± 4.35abcd	23.87 ± 4.34a	14.06 ± 1.59ab	21.02 ± 2.18ab	20.45 ± 2.15b	11.13 ± 1.18ab	20.96 ± 2.18ab	14.44 ± 1.67bc	11.60 ± 3.72 a	18.84 ± 2.90ab	23.01 ± 3.53abc
SF	22.59 ± 5.26abc	22.04 ± 3.67ab	20.95 ± 2.27a	27.92 ± 3.20ab	35.37 ± 4.49a	17.68 ± 2.39a	28.17 ± 3.36a	20.41 ± 2.36abc	6.28 ± 1.05 b	29.73 ± 2.58a	29.06 ± 3.44ab
T	10.77 ± 2.17 cd	14.12 ± 2.55b	12.55 ± 1.43ab	21.16 ± 2.37ab	19.14 ± 2.23b	9.33 ± 1.34b	11.49 ± 2.09b	13.66 ± 1.41c	5.05 ± 1.15 b	12.52 ± 2.41b	17.39 ± 3.30bc
TF	18.14 ± 2.24abcd	18.85 ± 2.67ab	16.12 ± 2.65ab	17.73 ± 1.37b	20.71 ± 2.18b	10.81 ± 1.54ab	17.95 ± 2.67ab	17.69 ± 1.23abc	9.45 ± 2.27ab	16.37 ± 2.65ab	28.56 ± 3.71ab
TO	21.10 ± 3.43abcd	17.73 ± 2.45ab	15.75 ± 1.98ab	25.85 ± 2.76ab	24.35 ± 3.67ab	13.86 ± 2.50ab	25.37 ± 3.06a	19.88 ± 2.19abc	10.38 ± 3.09a	22.95 ± 2.47ab	35.50 ± 3.27a
TOF	17.18 ± 2.17abcd	18.91 ± 2.33ab	14.07 ± 1.16ab	20.38 ± 2.12ab	22.46 ± 2.76ab	9.82 ± 1.87b	16.50 ± 2.16ab	18.57 ± 1.27abc	8.82 ± 2.57ab	15.81 ± 2.41ab	22.57 ± 3.45abc
TOS	18.41 ± 2.32abcd	21.29 ± 3.69ab	19.32 ± 2.42ab	32.48 ± 3.34a	26.82 ± 3.54ab	11.91 ± 1.62ab	21.75 ± 2.89ab	16.55 ± 1.98abc	6.43 ± 2.31b	21.23 ± 2.50ab	33.55 ± 3.16ab
TOSF	19.09 ± 2.15abcd	21.13 ± 2.87ab	14.39 ± 1.00ab	25.54 ± 2.39ab	31.81 ± 2.89ab	15.22 ± 2.40ab	20.33 ± 2.35ab	20.31 ± 2.00abc	10.52 ± 3.26a	24.02 ± 2.62ab	29.08 ± 3.37ab
TS	25.71 ± 4.37a	25.25 ± 3.95ab	19.14 ± 1.28ab	25.74 ± 2.24ab	26.00 ± 2.00ab	16.14 ± 2.09ab	25.27 ± 3.21a	23.20 ± 2.23a	8.55 ± 2.61ab	21.40 ± 2.00ab	26.86 ± 3.33abc
TSF	11.56 ± 2.26bcd	11.02 ± 2.17b	13.59 ± 1.54ab	19.25 ± 1.17b	29.80 ± 2.16ab	10.66 ± 1.17ab	21.11 ± 2.47ab	22.24 ± 1.87ab	5.65 ± 2.45b	20.44 ± 2.89ab	28.22 ± 3.60ab
Significance	0.0001***	0.0427*	0.0087**	0.0015**	0.0012**	0.0005***	2.86e-05***	0.0001***	0.0001***	0.0027 **	5.2e-05 ***

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/1, glucose at 1.12 g/1, and fructose at 1.26 g/1. 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 tyrocine 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 (PC_1 at 75.4% and PC_2 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 PC_1 axis. Conversely, the treatments labeled T, F, O, and S clustered closely together on both PC_1 and PC_2. 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 (PC_1 and PC_2) 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 PC_1, 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 PC_1 or PC_2 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*

Treatment (Y)[x]	Aspartate	Glutamate	Asparagine	Serine	Glutamine	Histidine	Glycine	Theonine	Arginine	Alanine	Tyrocine
C	290.25 ± 41.75c	124.93 ± 36.56b	258.83 ± 33.72bc	353.47 ± 24.95b	162.58 ± 34.88ab	85.26 ± 21.38b	211.23 ± 16.09bcd	222.38 ± 22.90ab	259.68 ± 36.30b	121.31 ± 55.27ab	352.25 ± 33.01 cd
F	306.75 ± 33.50bc	128.13 ± 36.01b	238.16 ± 36.67c	343.18 ± 40.00b	143.72 ± 19.89ab	115.52 ± 26.20ab	216.71 ± 11.77bcd	220.03 ± 23.55ab	251.52 ± 22.67b	101.61 ± 58.00b	349.47 ± 37.00 cd
O	429.12 ± 53.45bc	208.58 ± 31.08ab	341.35 ± 45.75abc	394.96 ± 35.87b	136.41 ± 55.90ab	118.43 ± 33.67ab	167.29 ± 16.00d	222.58 ± 10.77ab	276.97 ± 38.00b	95.69 ± 40.27b	355.80 ± 40.05 cd
OF	448.64 ± 47.30bc	242.48 ± 65.12ab	404.41 ± 57.60ab	422.50 ± 60.71b	148.40 ± 16.36ab	193.66 ± 45.77a	261.34 ± 11.10abc	223.24 ± 23.78ab	358.23 ± 60.56ab	285.84 ± 60.21ab	529.82 ± 45.90abc
OS	686.41 ± 37.21a	234.09 ± 23.00ab	321.20 ± 12.56abc	414.11 ± 27.62b	127.23 ± 33.67b	143.57 ± 21.36ab	219.26 ± 15.68bcd	207.00 ± 40.00ab	297.12 ± 44.87ab	130.70 ± 70.99ab	536.69 ± 20.00abc
OSF	418.55 ± 55.76bc	261.69 ± 30.08ab	435.67 ± 52.79a	499.48 ± 50.79ab	144.87 ± 40.89ab	154.75 ± 15.88ab	221.12 ± 20.00abcd	275.81 ± 28.89ab	361.20 ± 36.80ab	120.50 ± 67.24ab	473.75 ± 67.05abcd
S	441.79 ± 43.70bc	217.90 ± 56.51ab	338.89 ± 60.71abc	390.09 ± 57.90b	140.00 ± 60.43ab	124.21 ± 55.30ab	179.13 ± 55.09 cd	216.64 ± 35.76ab	288.87 ± 60.35ab	93.37 ± 56.26b	354.42 ± 33.90 cd
SF	447.96 ± 41.69bc	321.27 ± 22.67a	411.98 ± 48.60ab	608.84 ± 10.18a	139.26 ± 35.09ab	198.35 ± 45.14a	309.99 ± 34.55a	291.80 ± 21.00a	312.53 ± 11.20ab	164.23 ± 50.20ab	632.04 ± 30.06a
T	291.24 ± 55.76c	174.96 ± 39.12ab	311.32 ± 41.77abc	405.17 ± 40.79b	137.54 ± 16.55ab	128.78 ± 25.37ab	211.52 ± 11.80bcd	240.29 ± 26.90ab	290.62 ± 31.09b	116.49 ± 77.20ab	395.83 ± 37.03bcd
TF	433.85 ± 23.67bc	242.08 ± 65.06ab	382.88 ± 25.67abc	447.41 ± 59.01ab	153.85 ± 40.81ab	133.23 ± 50.67ab	187.74 ± 13.01bcd	252.54 ± 29.85ab	311.80 ± 38.00ab	108.54 ± 70.29ab	402.67 ± 66.00bcd
TO	528.22 ± 59.72ab	248.11 ± 55.16ab	400.68 ± 63.55ab	486.75 ± 79.99ab	156.52 ± 18.37ab	207.72 ± 22.50a	258.16 ± 18.03abc	271.33 ± 27.00ab	329.97 ± 34.80ab	147.46 ± 60.89ab	557.86 ± 36.05ab
TOF	327.61 ± 63.98bc	268.37 ± 33.67ab	351.31 ± 32.75abc	373.10 ± 69.20b	155.70 ± 20.01ab	172.56 ± 33.70ab	174.06 ± 11.50 cd	178.60 ± 11.78b	397.87 ± 47.34a	394.87 ± 23.26ab	309.23 ± 40.08d
TOS	520.65 ± 30.45ab	251.94 ± 29.58ab	318.86 ± 40.45abc	431.61 ± 33.67ab	164.28 ± 33.04ab	163.34 ± 25.30ab	236.39 ± 15.47abcd	197.99 ± 13.60ab	359.73 ± 80.67ab	258.31 ± 66.00ab	466.73 ± 39.67abcd
TOSF	477.18 ± 67.74abc	225.26 ± 29.98ab	380.95 ± 65.62abc	438.41 ± 65.09ab	153.19 ± 27.05ab	134.06 ± 20.38ab	191.05 ± 22.00bcd	247.50 ± 23.66ab	309.65 ± 26.07ab	106.40 ± 13.20ab	395.65 ± 23.00bcd
TS	505.41 ± 41.34abc	208.39 ± 38.10ab	332.67 ± 50.79abc	425.38 ± 40.96b	178.07 ± 47.57a	177.26 ± 24.36ab	268.78 ± 11.05ab	248.53 ± 67.89ab	261.01 ± 89.02b	137.27 ± 66.90ab	487.34 ± 38.06abcd
TSF	465.05 ± 63.50abc	247.99 ± 25.60ab	423.37 ± 28.40a	390.22 ± 66.70b	174.02 ± 50.51b	208.97 ± 27.36a	244.22 ± 18.00abcd	187.62 ± 68.09ab	377.34 ± 60.36ab	411.31 ± 56.20a	464.01 ± 37.09abcd
Significance	2.74e-05 ***	0.0078 **	0.0016 **	0.0018 **	0.0001***	0.0024 **	4.06e-05 ***	0.0224 *	0.00621 **	0.00259 **	1.57e-05 ***

Treatment (Y)[x]	Cystine	Valin	Methionine	Tryptophan	Phenylalanine	Isoluecine	Leucine	Lysine	Hydroxyproline	Sarcosine
C	122.23 ± 16.15de	149.63 ± 26.90b	149.57 ± 24.16b	278.21 ± 44.01de	440.30 ± 53.47d	160.88 ± 45.77ab	80.17 ± 33.76c	178.48 ± 87.10d	153.18 ± 10.77bc	223.51 ± 11.57c
F	109.74 ± 19.11e	145.18 ± 21.99b	145.55 ± 88.90b	273.79 ± 55.67de	428.29 ± 35.67d	138.54 ± 41.00b	76.41 ± 36.76c	174.96 ± 70.77d	147.10 ± 16.88c	213.68 ± 56.10c
O	129.04 ± 16.00cde	207.88 ± 28.77ab	146.42 ± 90.00b	291.22 ± 23.43cde	605.86 ± 55.23abcd	107.04 ± 55.67b	138.84 ± 37.70bc	174.54 ± 78.15d	128.45 ± 11.10d	214.03 ± 77.67c
OF	232.87 ± 20.15ab	256.27 ± 26.90ab	195.67 ± 26.11ab	422.95 ± 59.27abcd	812.09 ± 46.56abc	225.60 ± 66.00ab	277.83 ± 32.03ab	433.34 ± 09.00b	196.39 ± 20.09a	269.97 ± 69.90b
OS	172.77 ± 35.55abcde	212.42 ± 90.00ab	177.36 ± 60.56ab	414.81 ± 42.09abcd	753.51 ± 57.66abcd	115.40 ± 49.76b	131.02 ± 55.53bc	235.94 ± 12.16 cd	120.39 ± 35.06d	212.53 ± 33.80c
OSF	164.50 ± 82.10abcde	232.38 ± 88.05ab	195.54 ± 11.88ab	376.11 ± 32.98bcd	731.77 ± 89.56abcd	133.12 ± 96.70b	155.14 ± 70.13abc	223.61 ± 60.55 cd	93.37 ± 55.60e	281.73 ± 43.57b
S	127.45 ± 78.16cde	204.24 ± 26.97ab	154.20 ± 36.19b	300.08 ± 43.78cde	600.78 ± 44.32abcd	103.86 ± 80.00b	131.93 ± 22.89bc	182.50 ± 05.80d	125.33 ± 37.44d	223.23 ± 70.09c
SF	254.62 ± 17.55a	290.29 ± 28.99a	224.95 ± 55.07a	561.58 ± 83.54a	899.48 ± 34.56a	144.83 ± 65.87ab	220.38 ± 60.09abc	320.54 ± 56.16c	195.41 ± 22.06a	283.58 ± 87.15b
T	134.64 ± 17.16cde	185.00 ± 20.90ab	165.37 ± 43.23ab	309.85 ± 66.43cde	535.11 ± 45.57bcd	151.90 ± 23.65ab	112.46 ± 33.77bc	194.20 ± 87.16d	155.86 ± 18.06bc	237.31 ± 89.68c
TF	146.18 ± 22.10bcde	235.62 ± 11.91ab	164.64 ± 35.39ab	328.30 ± 25.52bcde	686.01 ± 87.98abcd	121.50 ± 30.70b	157.93 ± 89.00abc	196.44 ± 66.10d	145.70 ± 42.70c	240.95 ± 55.07c
TO	218.31 ± 67.00abc	293.47 ± 40.90a	201.09 ± 34.28a	491.19 ± 17.43ab	842.10 ± 56.67ab	159.58 ± 86.09ab	199.68 ± 37.76abc	274.09 ± 56.78c	155.27 ± 89.15bc	266.59 ± 50.15bc
TOF	187.58 ± 23.15abcde	259.82 ± 25.09ab	222.69 ± 65.56a	187.90 ± 75.43e	499.24 ± 87.23 cd	388.82 ± 70.60a	324.73 ± 11.00a	595.52 ± 58.10a	219.09 ± 17.34a	335.22 ± 36.67ab
TOS	205.17 ± 89.10abcd	237.05 ± 55.97ab	210.22 ± 43.54a	362.36 ± 13.23bcde	692.43 ± 45.64abcd	241.48 ± 46.94ab	232.61 ± 36.78abc	452.34 ± 70.88b	173.54 ± 58.22b	261.59 ± 16.34bc
TOSF	144.79 ± 60.55bcde	203.72 ± 09.04ab	164.57 ± 92.25ab	323.83 ± 43.55bcde	677.95 ± 55.54abcd	119.03 ± 50.66b	136.06 ± 55.80bc	194.09 ± 13.87d	142.83 ± 45.33c	242.28 ± 78.26c
TS	175.30 ± 19.10abcde	208.33 ± 56.89ab	201.79 ± 33.98a	468.75 ± 65.89abc	683.95 ± 32.43abcd	101.27 ± 43.78b	148.68 ± 12.79bc	211.40 ± 60.12d	143.30 ± 65.70c	288.72 ± 60.05b
TSF	206.52 ± 11.16abcd	259.57 ± 77.70ab	246.94 ± 43.54a	322.20 ± 55.78bcde	711.81 ± 46.55abcd	342.72 ± 56.78ab	280.06 ± 30.09ab	528.43 ± 77.17a	188.00 ± 50.78a	369.31 ± 50.45a
Significance	1.8e-05 ***	0.0046 **	0.0004 ***	6.61e-07 ***	0.0001 ***	0.0019 **	6.42e-05 ***	2.7e-05 ***	0.0344 *	0.0002***

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

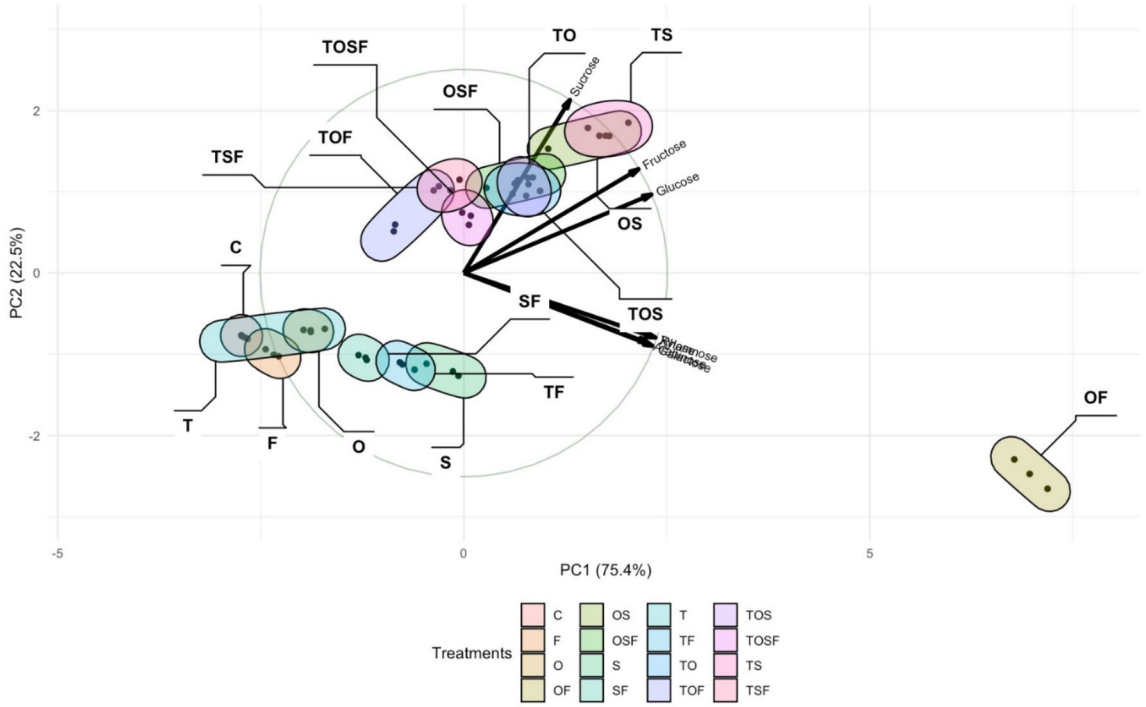


Fig. 2 Principal component analysis (PCA) of sugars, individual and combined EO treatments

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

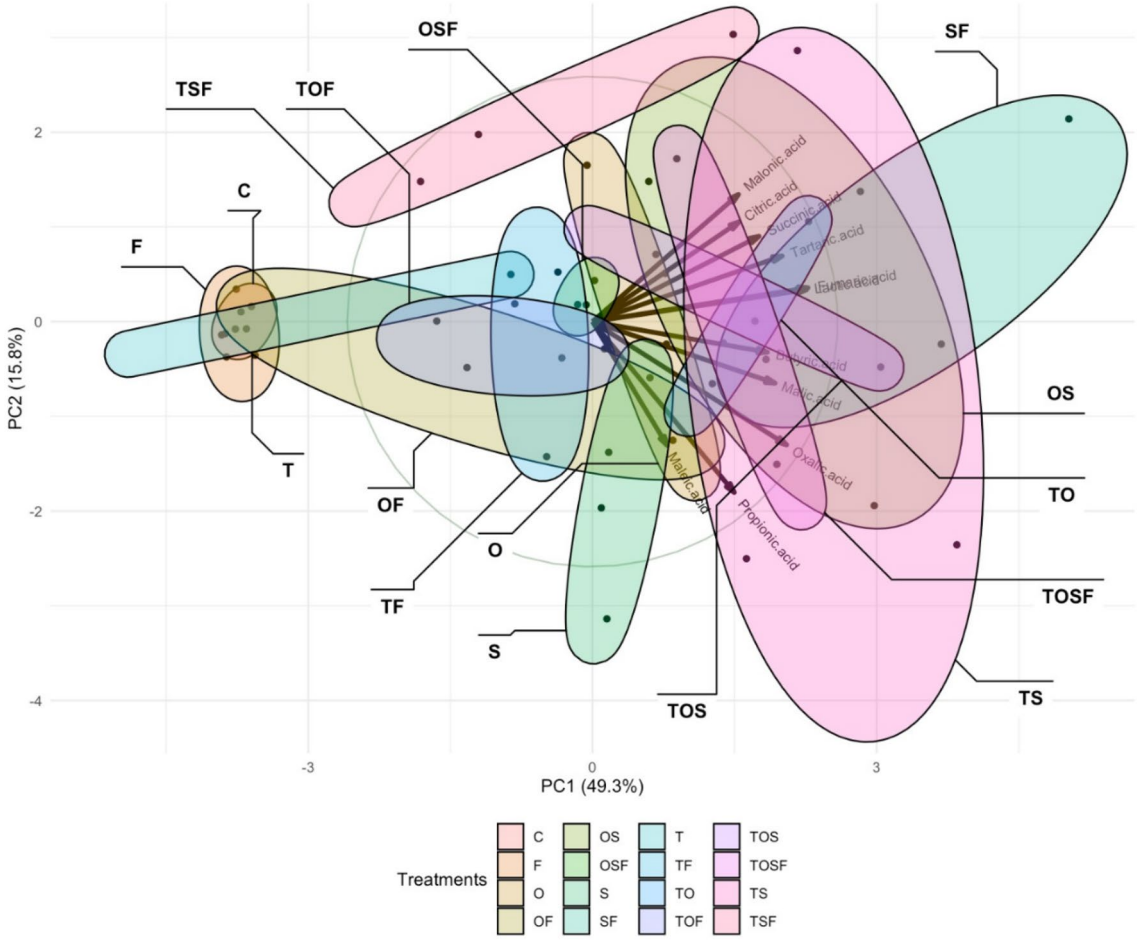


Fig. 3 Principal component analysis (PCA) of organic acids, individual and combined EO treatments

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, cystine, 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].

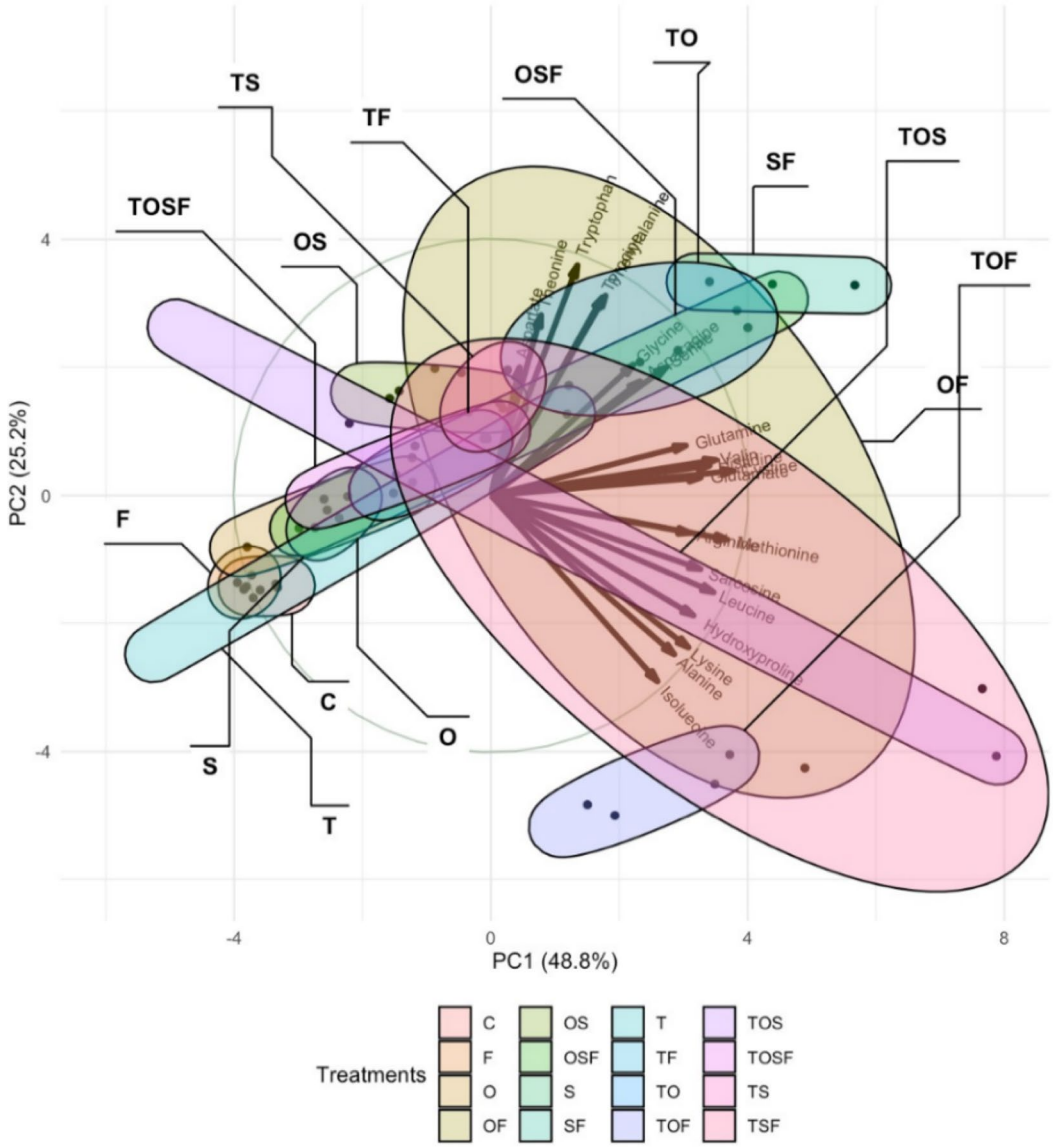


Fig. 4 Principal component analysis (PCA) of amino acids, individual and combined EO treatments

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

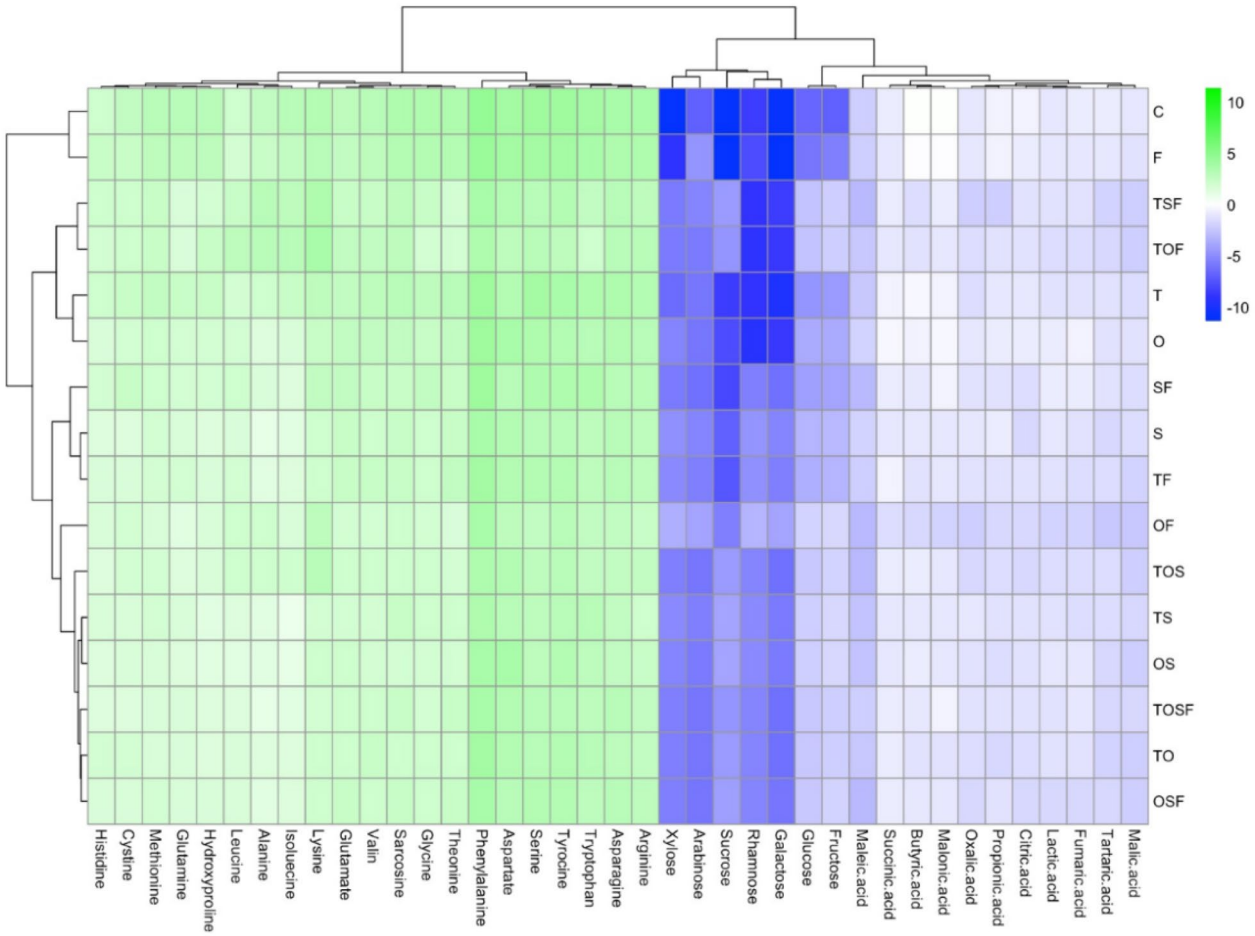


Fig. 5 Hierarchical clustering heatmap of sugar, organic and amino acid concentrations in grape berries under individual and combined EO treatments

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 postharvest 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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Declarations

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Not applicable.

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Competing interests

The authors confirm that they have no conflicts of interest with respect to the work described in this manuscript.

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