Food and Bioprocess Technology (2024) 17:722–735
https://doi.org/10.1007/s11947-023-03165-3

RESEARCH

The Antifungal Efficacy of Flavonoids from *Sedum aizoon* L. on Grapes

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Received: 6 February 2023 / Accepted: 3 July 2023 / Published online: 8 July 2023

Abstract

The occurrence of gray mold is the main cause of rot and spoilage in grapes, and *Botrytis cinerea* is the main causative agent of gray mold. The aim of this study is to clarify the inhibitory effect of flavonoids from *Sedum aizoon* L. (FSAL) on gray mold of grapes and to provide some basis for the development of new natural plant-derived antifungal agents. The effect of FSAL on the disease resistance of grapes was investigated by measuring the disease incidence and lesion diameter. The effect of FSAL on fruit quality was studied by measuring pondus hydrogenii (pH), total soluble solid (TSS), ascorbic acid (AA) and soluble sugar content. The activities of catalase (CAT), peroxidase (POD), phenylalanine ammonia lyase (PAL), and superoxide dismutase (SOD) were used to explore the effect of FSAL on the antioxidant capacities in grapes. The effects of FSAL on the ethylene synthesis in mitogen-activated protein kinase (MAPK) signaling pathway in grapes were investigated by measuring the levels of reactive oxygen species (ROS) and the relative expression of *VvACS1*, *VvACO1*, *VvACO2*, and *VvACO3* genes. The results showed that FSAL treatment reduced disease incidence and lesion diameter, increased AA content in fruit and thus maintained fruit quality. FSAL treatment significantly increased CAT, POD, PAL, and SOD activities in fruit, and reduced the relative expression of the genes. In conclusion, FSAL has a certain inhibitory effect on gray mold while not affecting grape quality, and delays the ripening and aging of fruit.

Keywords Flavonoids from *Sedum aizoon* L. · Gray mold · Grapes · Antifungal agents · Mitogen-activated protein kinase signaling pathway · Ethylene synthesis

Introduction

Grapes (*Vitis vinifera* L.) are one of the most common fruits worldwide, which belong to deciduous vine species and are rich in nutrients such as vitamins and minerals (Magalhães Brandão et al., 2023; Sun et al., 2018). However, grapes and other fruits are susceptible to *Botrytis cinerea*, which can lead to gray mold, and can cause fruit rot and affect the storage and transportation of grapes (Xu et al., 2019). The extensive use of chemical and other antifungal agents can cause many shortcomings, such as the gradual deterioration of environmental pollution, the gradual increase of fungal resistance, and the increasing problems of human health; there is an urgent need for an antifungal agent of natural plant origin to achieve the antifungal purpose (Bu et al., 2021; Nunes, 2011). Flavonoids are the most common and natural plant-derived antimicrobial agents, and they are found in large quantities in grains, fruit, vegetables, and seeds (Alsharairi, 2021; Routray & Orsat, 2012). Some studies found that flavonoids extracted from honey have antifungal effects on *Candida albicans* (Candiracci et al., 2012), it affected the growth of fungal hyphae and the production of reactive oxygen species (ROS), and provided a basis for further research on the antifungal mechanism of flavonoids. *Sedum aizoon* L. is widely distributed in China and commonly used as a Chinese herbal medicine, it is rich in flavonoids, alkaloids, and polysaccharides and other bioactive components (Xu et al., 2015). The main components of flavonoids in the leaves and stems of flavonoids from *S. aizoon* L. (FSAL) were identified as quercetin and kaempferol by using high-performance liquid chromatography (Waters Alliance E2695, Milford, MA, USA) (Luo et al., 2020). Interestingly, our previous study has demonstrated the

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antibacterial activity of FSAL against *Pseudomonas fragi* on pork, which disrupted the intracellular sulfate assimilation pathway in bacterial bodies and led to disruption of glutathione redox homeostasis (Wang et al., 2022a). However, it is not clear whether FSAL has antifungal effect on *B. cinerea* in grapes.

In this study, FSAL was chosen as the raw material to study its effect on the antifungal activities in grapes, and to lay key foundation for the development of natural plant-derived antifungal agents for fruit.

Materials and Methods

Preparation of Flavonoids from *Sedum aizoon* L. (FSAL)

The FSAL was made in accordance with Wang et al. (2022b). Flavonoids were mostly extracted from the leaves and stems of *S. aizoon* L. (Peixian County, Xuzhou, China). They were then dried and ground into a powder. Using rotary evaporation (Hangzhou Genyu Instrument Co., Ltd., Hangzhou, China), the material was briefly extracted with ethanol (Sinopharm Chemical Reagent Co., Ltd., Shanghai, China). The crude flavonoid extract was purified by AB-8 macroporous resin (Beijing Solarbio Science & Technology Co., Ltd., Beijing, China). After rotary evaporation, the main components of the purified flavonoid sample include 235.10 mg/kg quercetin and 1328.38 mg/kg kaempferol (Luo et al., 2020), which were then lyophilized. Prior to usage, the purified sample was kept at – 80 °C.

Preparation of Fungal Suspensions

B. cinerea (ACCC 36028; Agricultural Culture Collection of China, Beijing, China) was inoculated onto potato dextrose agar (PDA) plates (Hangzhou Microbial Reagent Co., Ltd., Hangzhou, China), and cultivated for a week at 25 °C (Fan et al., 2023; Han et al., 2020). After being scraped with a coating rod, the spores of fungus were then rinsed with sterile pure water (Shuoguang Electronics Technology Co., Ltd., Shanghai, China), collected, and filtered through four layers of gauze (Shanghai Yongchuan Co., Ltd., Shanghai, China) to get rid of the hyphae. The spore count was adjusted to 10^4, 10^5, and 10^6 spores/mL by the hematocytometer (Shanghai Yongchuan Co., Ltd., Shanghai, China).

Determination of Disease Incidence and Lesion Diameter

With reference to Zhao et al. (2022a) and Wang et al. (2015), washing the purchased red grapes (Ningbo Qionge Ecological Farm Orchard, Ningbo, China) with sterile pure water and drying them in the air. The peel surface was wiped with 75% ethanol, the wounds were made by sterile pipette tips at the fruit equator 2 mm and at a depth of about 3 mm. Then a 20 µL of FSAL at a concentration of 0.5 mg/mL was injected into the wounds, and sterile pure water was used as the control group. After they were allowed to dry naturally, 10 µL of the suspension of *B. cinerea* spores at concentrations of 10^4, 10^5, and 10^6 spores/mL were injected into the wounds. After the fruit was left to air dry naturally, they was moved into sterile plastic wrap boxes and stored in a constant temperature incubator (Ningbo Jiangnan Instrument Factory, Ningbo, China) at approximately 25 °C. The disease incidence and lesion diameter were counted and recorded within sixth days, respectively. The number of diseased fruits as a percentage of the total number of fruits was defined as the disease incidence, and the diameters of the disease spots were determined by the crossover method, and the average value was calculated. The whole experiment was repeated three times (24 fruits per group).

Determination of Storage Quality of Grapes

Grapes with uniform size and undamaged surface were selected. FSAL at a concentration of 20 µL of 0.5 mg/mL was injected into the fruit wounds according to the fruit pretreatment described above, and sterile pure water treatment was used as the control group, which was left to dry naturally and then stored in the incubator, and fruit quality was measured on the first to the sixth days. Each treatment had 24 fruits, and the whole experiment was repeated three times.

The pondus hydrogenii (pH) and total soluble solid (TSS) of the fruit were measured by the pH meter (Shanghai Mettler Toledo Instrument Co., Ltd., Shanghai, China) and saccharometer (Zhejiang Tongyun Nong Technology Co., Ltd., Hangzhou, China), respectively. The determination of AA was performed by adding a certain amount of 2% oxalic acid (Sinopharm Chemical Reagent Co., Ltd., Shanghai, China) into 5 g of grapes and grinded them. After homogenized and centrifugated at 10, 000 g for 10 min, then took the supernatant for further use. It was determined by 2,6-dichloroindophenol method, made it titrate to reddish color and not fade within 30 s, and reached the titration end point. The soluble sugar content was determined by referring to the method (Das et al., 2013).

Measurement of Antioxidant Enzyme Activities in Grapes

The catalase (CAT) and phenylalanine ammonia lyase (PAL) activities were measured by the instructions of the Plant CAT activity elisa kit and Plant PAL activity elisa kit (Ningbo Hangjing Biotechnology Co., Ltd., Ningbo,

China), respectively. The principle of the kits is that during the assay, the specimen under test (to determine the antibody or antigen in it) reacts with the antigen or antibody on the surface of the solid phase carrier. The antigen–antibody complex formed on the solid-phase carrier is separated from the rest of the liquid by washing. The enzyme-labeled antigen or antibody is added and then bound to the solid-phase carrier by reaction. At this point, the amount of enzyme on the solid phase is in proportion to the amount of the substance under test in the specimen. After adding the substrate of the enzyme reaction, the substrate is catalyzed by the enzyme to become a colored product, and the amount of the product is directly related to the amount of the substance examined in the specimen, so qualitative or quantitative analysis can be performed according to the shade of the color presentation. The peroxidase (POD) activity was determined by the method of Wang et al. (2021); took 2.0 g of fruit and added 10 mL of 0.1 M sodium phosphate buffer (pH 7.0), then added a small amount of quartz sand, grinded into a homogenate in an ice bath, centrifuged at 4 °C and 12, 000 × g for 20 min, then took the supernatant and reserve, 0.1 mL of crude enzyme solution was added to 0.2 mL of 0.05 mM guaiacol, 2.5 mL of 50 mM sodium phosphate buffer (pH 5.5) followed by 0.2 mL of 0.65 M H_2O_2, and the change in absorbance value at 470 nm was measured immediately and continuously. The increase of absorbance value by 0.01 within 1 min was defined as one POD viability unit (U), and the results were expressed as U/mg protein. The activity of superoxide dismutase (SOD) was determined by the Plant SOD activity test kit (Shanghai Yongchuan Co., Ltd., Shanghai, China). According to the method of the instructions, the sample volume used for the determination was 50 μL.

Determination of Reactive Oxygen Species (ROS) Content in Grapes

The content of ROS was determined according to the instruction of the ROS content determination kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) with DCFH-DA probe. It was performed by weighing 1.25 μg of Murashige and Skoog (MS) medium (Hangzhou Microbial Reagent Co., Ltd., Hangzhou, China), adding 0.02 μg of 2,4-dichlorophenoxyacetic acid (Shanghai Yongchuan Co., Ltd., Shanghai, China) and 0.02 μg of 6-benzylaminopurine (Yinzhou Baishun Experimental Instrument Co., Ltd., Ningbo, China) as well as 30 mL of pure water, then mixed it, and sterilized. After it cooled, 0.6 g pectinase (Shanghai Yongchuan Co., Ltd., Shanghai, China) was added, and an additional 0.2 g sample was added to incubate the fruit in the dark using a thermostatic shaker (Jintan Keyu Instrument Co., Ltd., Changzhou, China) with shaking for 24 h at 25 °C. 1 mL of the cultured samples was aspirated and centrifuged at 1000 g for 6 min to remove the supernatant, followed by another wash with 1 mL of PBS buffer, repeating this step one time by adding 10 μM DCFH-DA probe with a water bath (Shanghai Jinghong Experimental Equipment Co., Ltd., Shanghai, China) at 37 °C for 1 h. The supernatant was then removed by centrifugation at 1000 g for 6 min, 1 mL of PBS buffer was added for washing, this step was repeated for two times. Finally, added 1 mL of PBS buffer and measured it with fluorescence microplate reader (Meigu molecular devices Co., Ltd., Shanghai, China). The fluorescence intensity is used to express the content of ROS.

Determination of the Relative Expression of Genes Related to the Ethylene Synthesis in Mitogen-Activated Protein Kinase (MAPK) Signaling Pathway

Extraction of RNAs: the grapes were treated as described above, while the concentration of spore suspension was chosen to be 10^4 spores/mL and uniformly sized grapes around the wound were selected for sampling. The grapes were ground by liquid nitrogen, and RNAs from the grapes were extracted by a plant RNA Mini extraction kit (Shanghai Yongchuan Co., Ltd., Shanghai, China). The eligible RNAs were stored at − 80 °C for further use. The qualified RNAs were reverse transcribed using Vazyme HiScript II Q RT SuperMix for qPCR kit (Vazyme Biotechnology Co., Ltd., Nanjing, China) to obtain cDNA, which were stored at − 80 °C for further use.

The cDNA was appropriately diluted and the reaction assay was performed using the ABI Prism7500 Rapid qPCR system (Thermo Fisher Scientific, Waltham, MA, USA) according to the ChamQ Universal SYBR qPCR Master Mix kit (Vazyme Biotechnology Co., Ltd., Nanjing, China) instructions. The 20 μL of reaction system was as follows: 0.4 μL of upstream, 0.4 μL of downstream primers, 1.7 μL of ddH_2O (Shanghai Yongchuan Co., Ltd., Shanghai, China), 10 μL of Mix staining solution (Vazyme Biotechnology Co., Ltd., Nanjing, China), and 7.5 μL of cDNA template. The reaction procedure for qPCR was: initial denaturation at 95 ℃ for 30 s, followed by 10 s at 95 ℃ and 30 s at 60 ℃ for a total of 40 cycles, followed by 10 s at 95 ℃, 60 s at 60 ℃, and 15 s at 95 ℃. After the qPCR was completed, the relative expression of the gene was calculated by the $2^{-\Delta\Delta CT}$ method using *EF1α-1* as the internal reference gene (Low et al., 2011). The sequences of the genes related to the ethylene synthesis pathway in MAPK signaling pathway (*VvACS1*, *VvACO1*, *VvACO2*, and *VvACO3*) and the internal reference genes studied in this experiment were obtained from the NCBI database. The designed primers are shown in Table 1. The gene IDs of *VvACS1*, *VvACO1*, *VvACO2*, *VvACO3*, and *EF1α-1* are GSVIVT00002708001, GSVIVT01015220001,

GSVIVT00025952001, GSVIVT00016583001, and AT5G60390.1 respectively.

Statistical Analysis

All experiments were performed in triplicate and replicated three times. Statistical analyses were performed by the SAS software package program version 9.4 (SAS Institute, Cary, NC, USA). Comparisons between groups were analyzed using one-way ANOVA, and Duncan's multiple range test was used to determine the significance of differences ($P < 0.05$). Plotting was performed using origin 2018 software (Origin Lab Inc., USA), and graph data were presented as mean ± standard deviation.

Results and Discussion

Effects of Flavonoids from *Sedum aizoon* L. (FSAL) on Disease Incidence and Lesion Diameter in Grapes

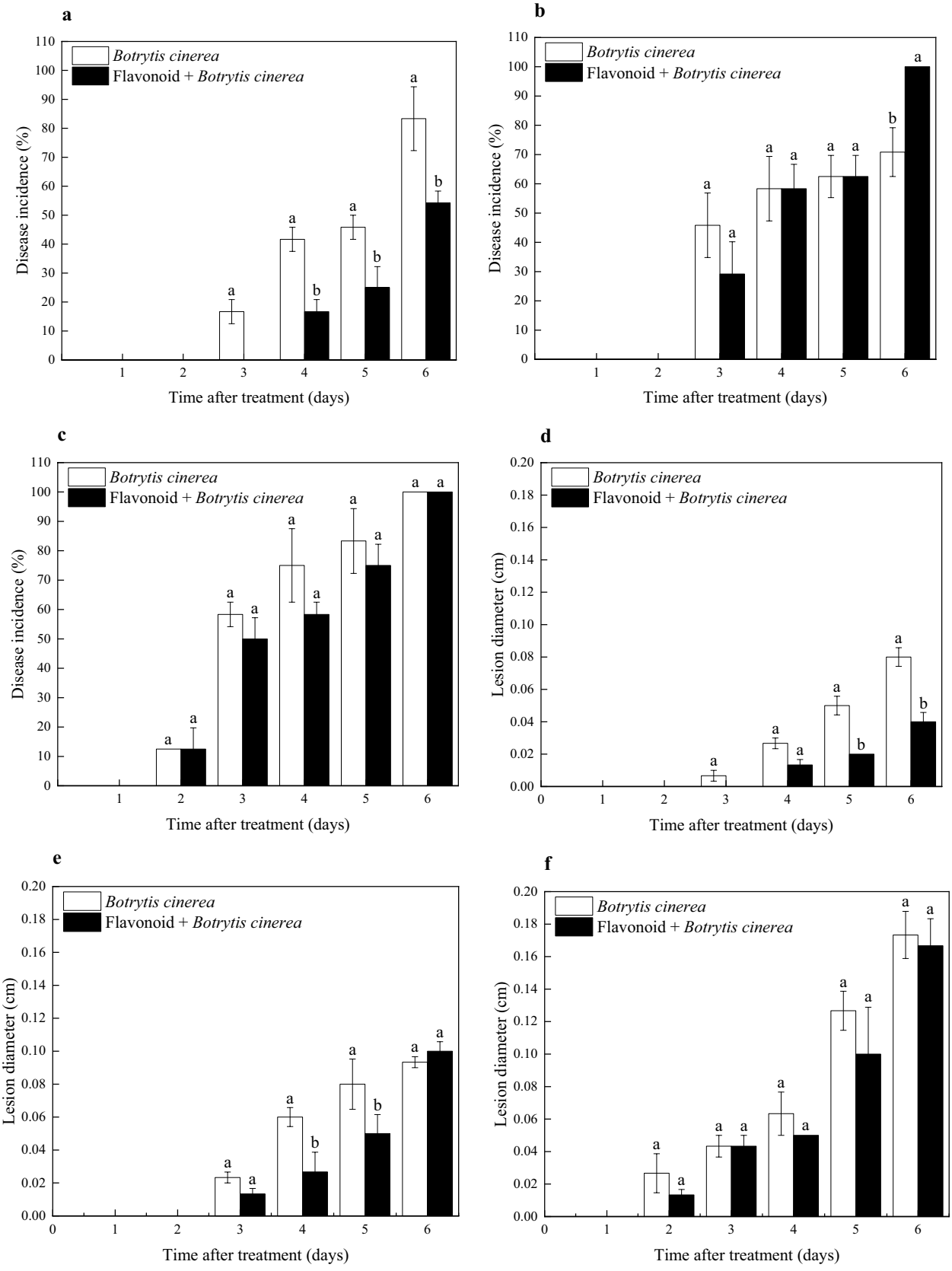
As can be seen from Fig. 1a, with increasing treatment time, FSAL had a certain inhibitory effect on *B. cinerea* at a spore concentration of 10^4 spores/mL, and the disease incidence was lower than that of the control groups. In Fig. 1b and c, the spore concentration was 10^5 and 10^6 spores/mL, FSAL had no inhibitory effect on *B. cinerea*, so the spore suspension concentration of 10^4 spores/mL was most suitable. As shown in Fig. 1d, fruit lesion diameter was lower in almost all FSAL-treated groups than in the control groups when the spore concentration was 10^4 spores/mL ($P < 0.05$). And in Fig. 1e, it was lower than in the control groups at the fourth and fifth days when the spore concentration was 10^5 spores/mL ($P < 0.05$). In Fig. 1f, with no difference between the treated and control groups at the spore concentration of 10^6 spores/mL. Besides, as shown in Fig. 2, FSAL (0.50 mg/mL) was resistant to the development of gray mold of grapes when the spore concentration of *B. cinerea* was 10^4 spores/mL. Grapes are very susceptible to *B. cinerea* in the processes of procurement, transportation and long-term storage, triggering the development of gray mold (Zimdars et al., 2017). Therefore, the control of fruit and plant rots should receive attention. *Eucalyptus* essential oil can control gray mold disease in strawberries by causing dehydration and rupture of *B. cinerea* hyphae (da Silva et al., 2020), and fennel can reduce the production of gray mold disease in apples by inhibiting the germination of conidia of *B. cinerea* and the growth of mycelia (Guigón-López et al., 2021). In the study of Zheng et al. (2017), when the spore concentration was 10^5 spores/mL, chitosan reduced the decay incidence of gray mold in kiwifruit. Radi et al. (2022) reported that cinnamon essential oil and cinnamon essential oil-loaded nanostructured lipid carriers significantly reduced the postharvest decay percentage of tangerine inoculated with *Penicillium citrinum* and *Penicillium expansum* swelling, and that 0.6 mg/mL of cinnamon essential oil treatment was more effective in extending fruit shelf life and reducing fruit decay. Li et al. (2022c) found that melatonin was effective in reducing the decay rate of tomato fruit during storage and in reducing the incidence of gray mold. According to Fernández et al. (2022), natamycin and an *Allium* extract were effective in reducing the incidence of lemon sour rot. Besides, Ali et al. (2014) reported that both propolis and cinnamon oil coating were effective in reducing the incidence of chilli anthracnose and that the mixture of the two was more useful for postharvest storage of chillis. Sempere-Ferre et al. (2022) found that *aloe vera* gel was able to reduce the decay rate of blueberries and had some preservation effect on blueberries. Similarly, in this research, the inhibition effect of FSAL on three different spore concentrations of *B. cinerea* was observed. In screening spore concentrations, FSAL was found to be the most effective in reducing the disease incidence of gray mold in grapes when the spore

Fig. 1 The disease incidence and lesion diameter of grapes in different treatment groups. *Botrytis cinerea* means grapes treated with sterile pure water (control group). Flavonoid + *Botrytis cinerea* means grapes treated with flavonoids from *Sedum aizoon* L. (0.50 mg/mL). The spore concentration of *Botrytis cinerea* in (**a**) and (**d**) was 10^4 spores/mL, the spore concentration of *B. cinerea* in (**b**) and (**e**) was 10^5 spores/mL, the spore concentration of *B. cinerea* in (**c**) and (**f**) was $10.^6$ spores/mL. Data are presented as the mean ± standard deviation of three replicates. Different letters indicate significant differences among the six different treatment groups in the same day ($P < 0.05$)

Table 1 Primer sequences of genes associated with mitogen-activated protein kinase signaling pathway of grapes

Gene	Forward primer sequence (5′-3′)	Reverse primer sequence (5′-3′)
EF1α-1	GAACTGGGTGCTTGATAGGC	AACCAAAATATCCGGAGTAAAAGA
VvACS1	CGTGGCAGGGTTGAAACAATTAGGA	CCCTTTCTCACTGTAAGAGCGGATT
VvACO1	CGCCCAAACTGATGGAAACAGAATG	GGGTACACTTCGCTTGTCTCCTTT
VvACO2	GTAGCCCAAACAGACGGCAACA	CGGGTACACTTGATCTTTCTCTGCT
VvACO3	GGCAGACAAGAATGGAAGCAGACT	TGCGAACTTTGTTGTGGCATAAAGC

Vv means, *Vitis vinifera* L., *ACO* means ACC oxidase, *ACS* means ACC synthase, *ACC* means immediate precursor substance of ethylene (1-aminocyclopropane-1-carboxylic acid)


a
Botrytis cinerea
Flavonoid + Botrytis cinerea
Disease incidence (%)
Time after treatment (days)
b
Botrytis cinerea
Flavonoid + Botrytis cinerea
Disease incidence (%)
Time after treatment (days)
c
Botrytis cinerea
Flavonoid + Botrytis cinerea
Disease incidence (%)
Time after treatment (days)
d
Botrytis cinerea
Flavonoid + Botrytis cinerea
Lesion diameter (cm)
Time after treatment (days)
e
Botrytis cinerea
Flavonoid + Botrytis cinerea
Lesion diameter (cm)
Time after treatment (days)
f
Botrytis cinerea
Flavonoid + Botrytis cinerea
Lesion diameter (cm)
Time after treatment (days)

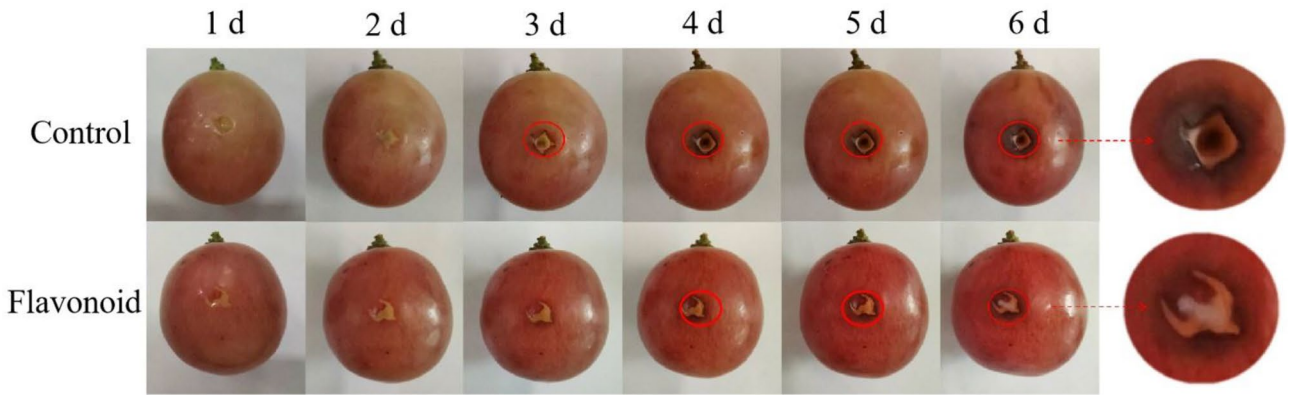


Fig. 2 Research on disease resistance of grapes under different treatments. Control indicates grapes treated with sterile pure water + 10^4 spores/mL *Botrytis cinerea* spores. Flavonoid indicates grapes treated with 0.50 mg/mL flavonoids from *Sedum aizoon* L. + 10^4 spores/mL *B. cinerea* spores. The red circle indicates the general location of the fruit disease

concentration was 10^4 spores/mL. At the same time, when the spore concentration is 10^4 or 10^5 spores/mL, FSAL can also reduce the lesion diameter of grapes.

Effects of Flavonoids from *Sedum aizoon* L. (FSAL) on Qualities in Grapes

As shown in Fig. 3, FSAL had no effect on pH, TSS, and soluble sugar content of the grapes, while the AA content of the fruit in the control groups showed a decreasing trend, and the consumption of AA in the FSAL treatment groups was less than that in the control groups ($P < 0.05$). This demonstrated the ability of FSAL to maintain AA in the fruit without affecting the qualities of the grapes. AA is an abundant antioxidant metabolite in plants and is able to function as a signal transduction molecule to transmit information on the redox status of cells (Foyer, 2007). The results indicated that FSAL had no effect on the other qualities of grapes and reduced the rate of AA oxidative damage, which in turn strengthened the protective capacities on fruit quality. Relative study has found that mushroom alcohol can effectively reduce the disease incidence of cherry fruit without adversely affecting the quality such as titratable acidity of the fruit and soluble solids (Li et al., 2022a). Ayón Reyna et al. (2022) found that chitosan could effectively control the occurrence of anthracnose in papaya, however, chitosan and mint essential oil treatment resulted in a lower TSS content of papaya fruit than the untreated fruit. It may be due to the decreased fungal infection rate in the treated fruit, which slowed the rate of fruit ripening, thereby reduced the monosaccharide content of carbohydrate hydrolysis in fruit (Ali et al., 2016; Barreto et al., 2016). Similarly, Araújo et al. (2018) reported that the use of an edible tapioca-chitosan coating made with essential oil and pomegranate peel extract reduced the TSS content in tomatoes, this may be due to the more mature fruit in the untreated group. In addition, antioxidants can also reduce the fungal infection rate of plants. It has been reported in the application of bell pepper under the condition of low ozone treatment (Alwi & Ali, 2015), it has no effect on the firmness and titratable acidity of pepper and may increase the content of ascorbic acid, and it can reduce the anthracnose incidence in pepper. Owolabi et al. (2021) investigated that the treatment by peppermint oil and lime oil in a 1:3 ratio was effective in reducing the decay rate of mangosteen fruit and maintained the pH, TSS, total phenols, hardness and titratable acidity content of the fruit. Therefore, FSAL, as a natural non-toxic antifungal agent, has similar effects in the application of fruit, which can effectively reduce the incidence rate of grape fruit, but also has differences. FSAL has no distinct impact on the quality of fruit, and can be used as a substitute for harmful antifungal agents in the future.

Effects of Flavonoids from *Sedum aizoon* L. (FSAL) on Antioxidant Enzyme Activities in Grapes

As seen in Fig. 4a, the CAT activities of the fruit in the FSAL-treated groups were higher than that of the control groups on the second, third, and fifth days of the treatment ($P < 0.05$). In Fig. 4b, the activities of POD were higher than that of the control groups from the second to the sixth days of the treatment ($P < 0.05$). As shown in Fig. 4c, the PAL activities of the FSAL treatment groups were higher than that of the control groups on the fourth and fifth days ($P < 0.05$). In Fig. 4d, it may be due to the instability of enzyme activities, the activities of SOD were lower than the control groups on the third day of the treatment ($P < 0.05$); however, they were higher than the control groups on the second, fifth, and sixth days ($P < 0.05$). Antioxidant enzymes and other redox molecules in the cell can be used

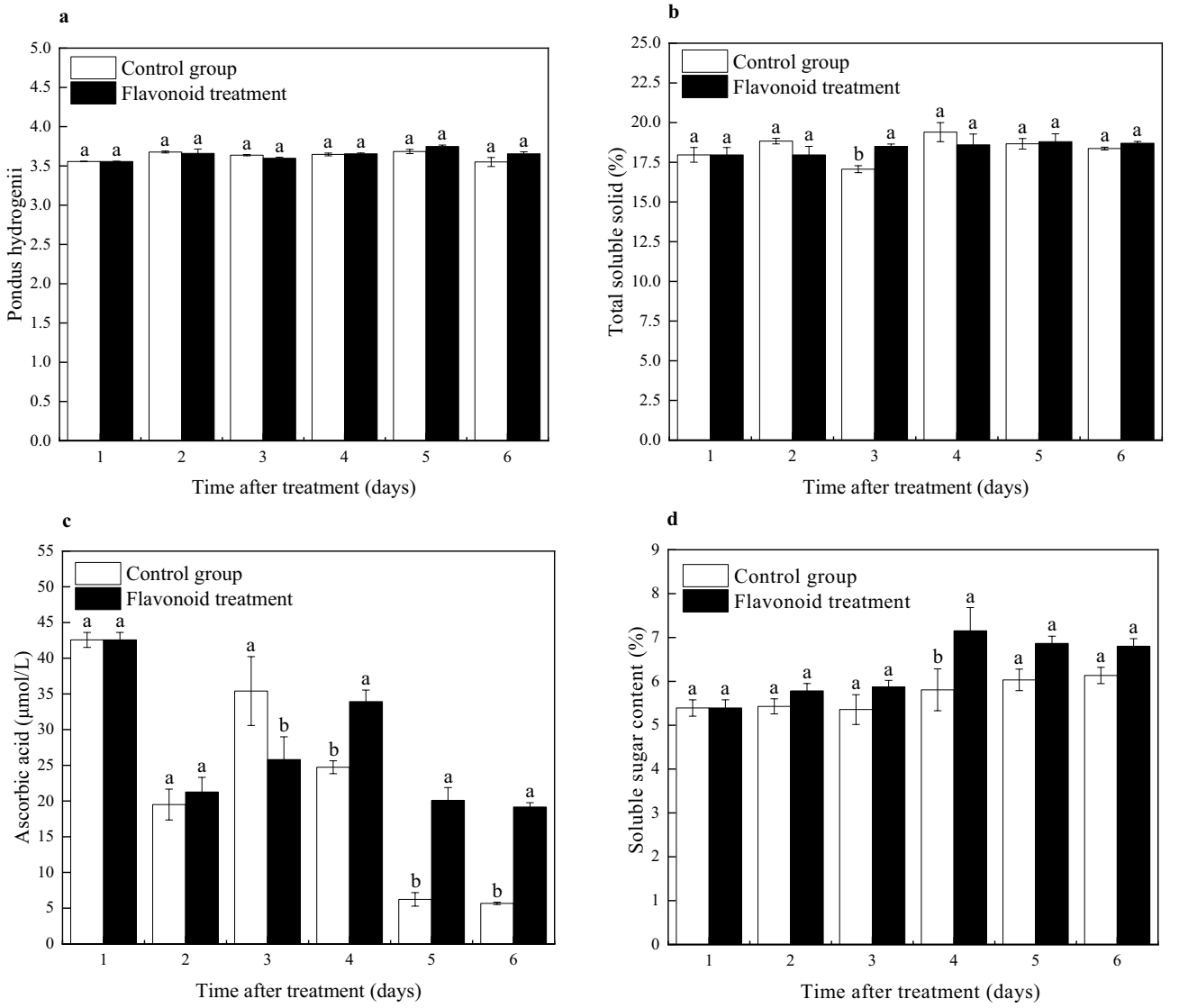


Fig. 3 Fruit qualities (pondus hydrogenii, total soluble solid, ascorbic acid and soluble sugar content) of grapes in different treatment groups. Control group indicates grapes treated with sterile pure water. Flavonoid treatment means grapes treated with 0.50 mg/mL flavonoids from *Sedum aizoon* L. Data are presented as the mean ± standard deviation of three replicates. The different letters (**a**, **b**) indicate a significant difference between the two groups at the same day ($P < 0.05$)

to balance the ROS production in the cell (Esteban et al., 2014; Khademi et al., 2013). ROS will be produced when plants are infected by pathogens (Chung, 2012). However, plants have antioxidant defense systems, such as antioxidant enzymes (Kaya et al., 2022; Piechowiak et al., 2020), which can eliminate reactive oxygen species. In the research, FSAL increased the activities of CAT, POD, PAL, and SOD, indicating that FSAL improved the antioxidant capacity of grapes. Similarly, there is a related report that chitosan coating treatment with thyme essential oil can improve the activities of antioxidant enzymes such as PAL in fresh cut carrot slices while effectively reducing the microbial counts in carrots, and maintain better sensory qualities and antioxidant capacities of carrots (Viacava et al., 2022). This may be due to the existence of phenolic monoterpenes with antioxidant properties in thyme essential oil (Ansorena et al., 2016). Fang et al. (2022) found that the combination of chitosan and tea polyphenol could reduce harvested broccoli spoilage while improving the antioxidant capacities of broccoli, thus maintaining its nutritional value. Liu et al. (2020) reported that bamboo pyroligneous acid could increase the activities of SOD, POD, and glutathione reductase in apple fruit, and improve the antioxidant capacities of fruit by reducing the content of hydrogen peroxide and malondialdehyde, and had

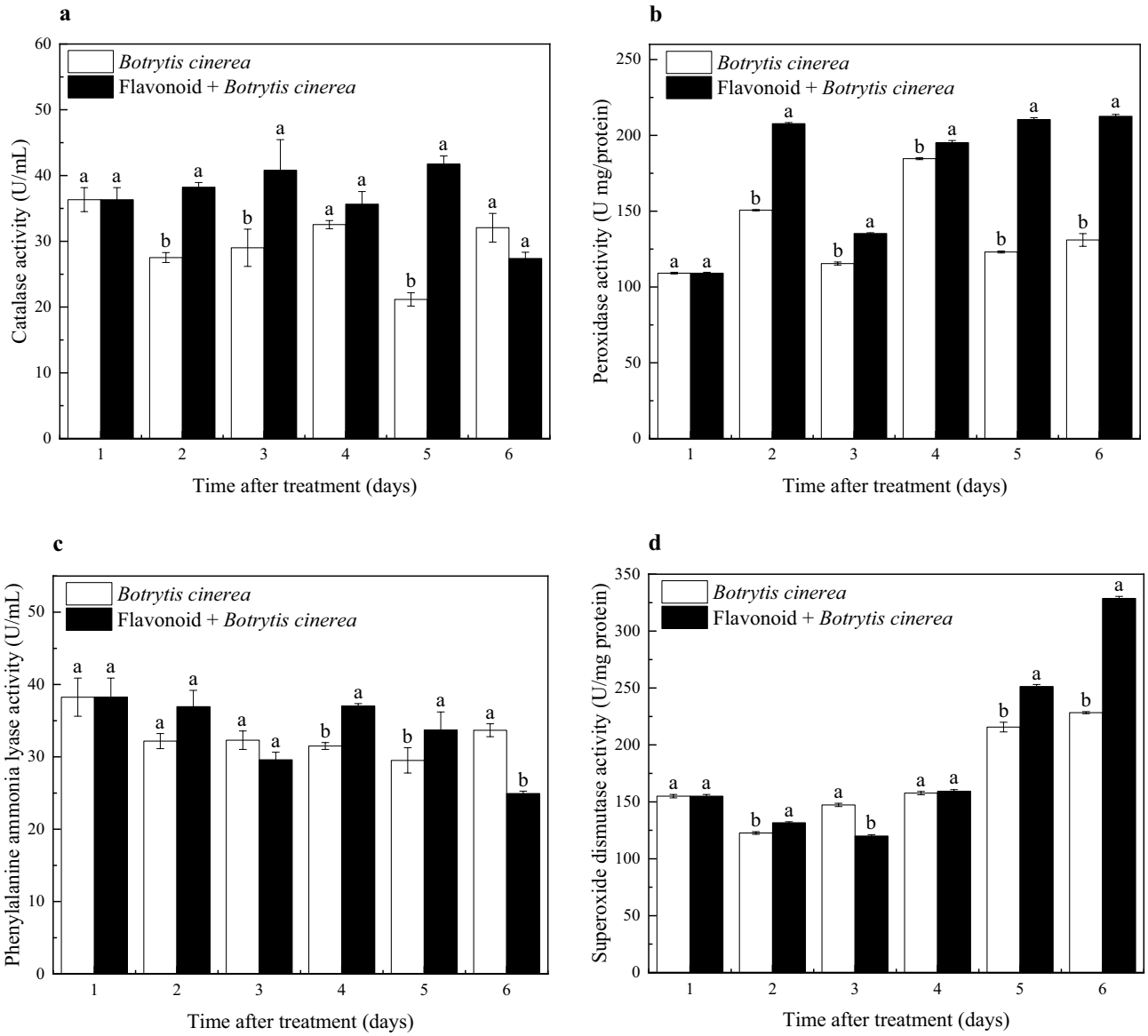


Fig. 4 Activities of antioxidant enzymes (catalase, peroxidase, phenylalanine ammonia lyase and superoxide dismutase) in grapes. *Botrytis cinerea* means grapes treated with sterile pure water + 10^4 spores/mL *Botrytis cinerea* spores (control group). Flavonoid + *Botrytis cinerea* means grapes treated with 0.50 mg/mL flavonoids from *Sedum aizoon* L. + 10^4 spores/mL *B. cinerea* spores. Different letters in the same day indicate significant differences between the control and treatment groups ($P < 0.05$)

inhibitory effects on *B. cinerea*. According to Sellamuthu et al. (2013), essential oil vapor could enhance the activities of antioxidant enzymes such as SOD, CAT, and PAL in avocado fruit and inhibit the development of anthracnose. Similarly, Zhong et al. (2022) found that chitosan-coated composite microporous packaging could effectively inhibit the weight loss of passion fruit, increase the activities of SOD, POD, and CAT in fruit, and effectively improve the antioxidant level of the fruit. These studies indicate that natural extracts may have the effect of improving the antioxidant capacities of fruits and vegetables.

Effects of Flavonoids from *Sedum aizoon* L. (FSAL) on Reactive Oxygen Species (ROS) Content in Grapes

As seen in Fig. 5, the ROS content of both the control and FSAL treatment groups showed an increasing trend. However, on the third, fourth, and fifth days of the treatment, the ROS content of the FSAL-treated groups was lower than that of the control groups ($P < 0.05$). In which the production of ROS is a defensive activity that plants make in response to adverse environmental cues (Rossi et al., 2017), and it is also a strong activator of MAPK and has a great impact on

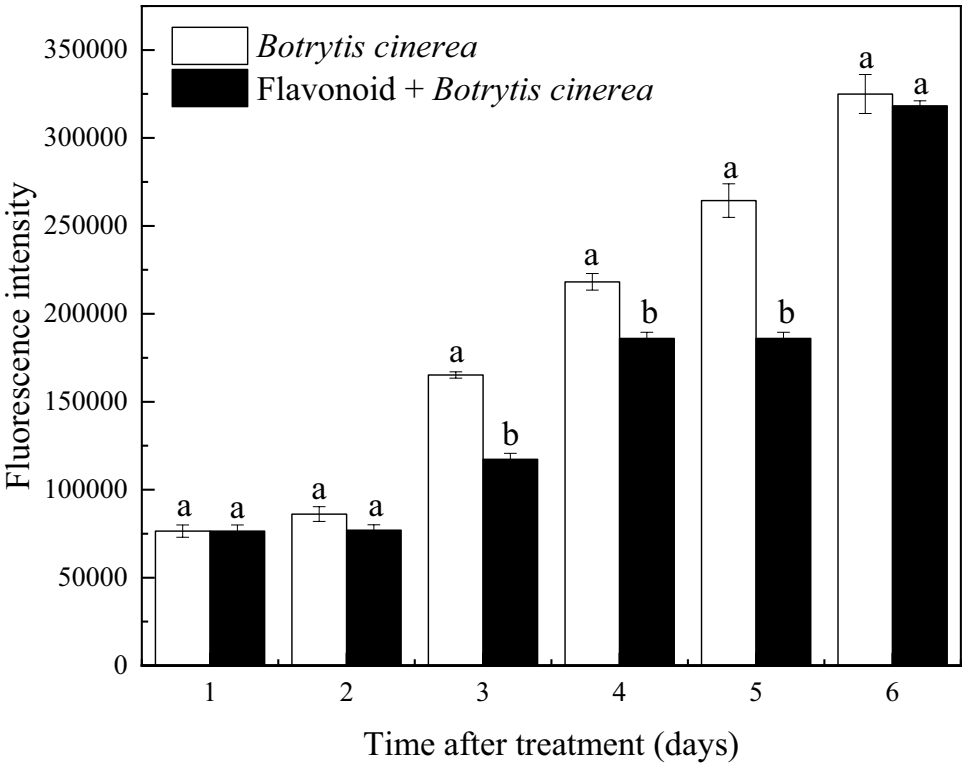


Fig. 5 Reactive oxygen species content of grapes in different treatment groups. Fluorescence intensity means reactive oxygen species content. *Botrytis cinerea* means grapes treated with sterile pure water + 10^4 spores/mL *Botrytis cinerea* spores (control group). Flavonoid + *Botrytis cinerea* means grapes treated with 0.50 mg/mL flavonoids from *Sedum aizoon* L. + 10^4 spores/mL *B. cinerea* spores. Data are presented as mean ± standard deviation of three replicates. Different letters (a, b) indicate significant differences among different treatments on the same day ($P < 0.05$)

signaling in plants (Smékalová et al., 2014). MAPK regulates a variety of cellular and physiological functions (Yu et al., 2022), and is also very important in plant signaling (Wu et al., 2021). It was shown that acibenzolar-S-methyl treatment induced the accumulation of hydrogen peroxide in apple fruit and up-regulated the expression of genes related to ROS scavenging system, and activated the expression of *MdMAPKs* (Cheng et al., 2020), which affected the MAPK signaling pathway in fruit. ROS as free radicals, it plays a very critical role in maintaining homeostasis in biological systems (Inupakutika et al., 2016). ROS is mainly found in the mitochondria and chloroplasts of plants, and the production of ROS can affect the antioxidant enzyme activity of plant cells and affect the life of plants (Zhao et al., 2022b). FSAL treatment decreased ROS production in grapes in the present study, indicating that the infestation of *B. cinerea* on grapes was reduced, and the improved protection and antioxidant capacity of fruit. There are some similar studies on plants. In tomatoes, the defense against gray mold was strengthened by maintaining the homeostasis of ROS to protect them from oxidative stress (Li et al., 2021). Eugenol treatment reduced the content of ROS in yams, scavenged ROS, and slowed membrane lipid metabolism, thereby inhibiting yam browning (Bai et al., 2022). Chen et al. (2020) found that melatonin balanced ROS production with the antioxidant system by increasing the antioxidant capacities of the fruit and reducing the accumulation of excess ROS in the fruit, thereby reducing fruit decay. These indicate that ROS production is closely related to the defense system of fruit and vegetables. At the same time, these show that the reduction of ROS accumulation is an important means to improve the freshness of fruit and vegetables.

The Relative Expression of Genes Involved in Ethylene Synthesis in Mitogen-Activated Protein Kinase (MAPK) Signaling Pathway

It can be seen from Fig. 6 that the relative expression of *VvACO1* and *VvACO2* in FSAL treatment groups is lower than that in control groups on the second day of the treatment ($P < 0.05$), and there is no difference in the relative expression of other genes. On the third and fourth days, the relative expression of *VvACS1*, *VvACO1*, and *VvACO2* genes in FSAL treatment groups was lower than that in control groups ($P < 0.05$), and there was no difference in *VvACO3* genes. On the fifth day of the treatment, the relative expression of *VvACS1*, *VvACO1*, and *VvACO3* in FSAL treatment groups was lower than that in control groups ($P < 0.05$). On the sixth day of the treatment, the relative expression of *VvACO2* and *VvACO3* in FSAL treatment groups was lower than that in control groups ($P < 0.05$), and there was no difference in other genes. A transcriptomic study of tomato seedlings under salt stress by Wei et al. (2022) showed that the MAPK signaling pathway was a relatively important signaling pathway in tomato fruit and differential gene expression was mainly enriched in the MAPK signaling pathway. MAPK signaling pathways are involved not only in

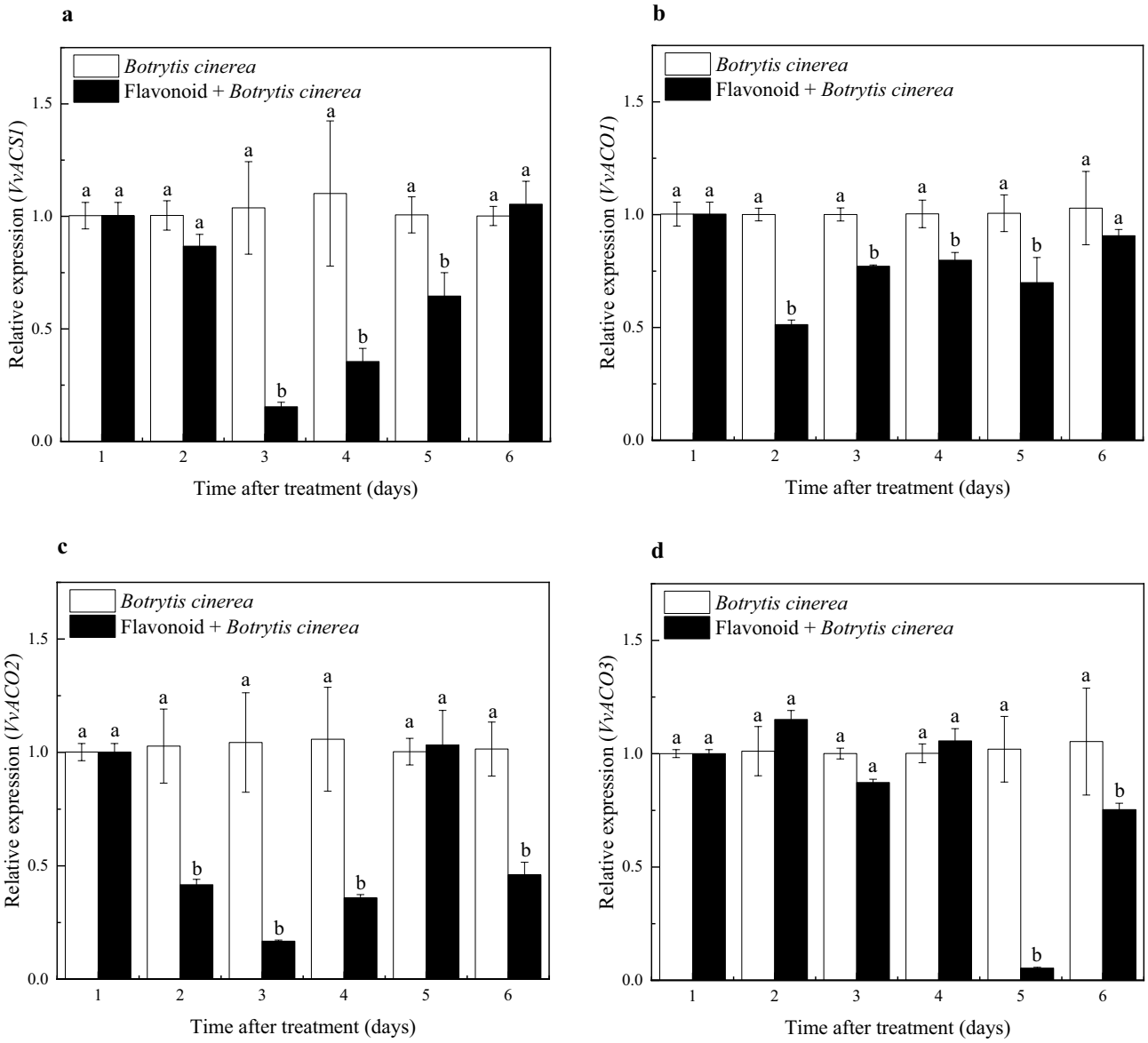


Fig. 6 The relative expression of *VvCS1*, *VvACO1*, *VvACO2*, and *VvACO3* in grapes. *Botrytis cinerea* means grapes treated with sterile pure water + 10^4 spores/mL *Botrytis cinerea* spores (control group). Flavonoid + *Botrytis cinerea* means grapes treated with 0.50 mg/mL flavonoids from *Sedum aizoon* L. + $10.^4$ spores/mL *B. cinerea* spores. Error bars show the mean ± standard deviation of three independent biological experiments. Different letters on the same day indicate significant differences between the two groups ($P < 0.05$)

the generation of ROS but also in the synthesis process of ethylene (Zwerger & Hirt, 2001). It has been shown that injury-induced ethylene biosynthesis in *Arabidopsis* is regulated by the MAPK cascade, and plant injury causes the activation of key regulators in ethylene signaling (Li et al., 2018). Ethylene is involved in important metabolic processes in plants, including flowering and fruit ripening in plants (Cai et al., 2019), and several studies have found that the MAPK signaling pathway is involved in ethylene signaling in *Arabidopsis* (Ouaked et al., 2003). The immediate precursor substance of ethylene is 1-aminocyclopropane-1-carboxylic acid (ACC), while ACC oxidase (ACO) and ACC synthase (ACS) are rate-limiting enzymes that occupy a key position in the synthesis of ethylene in plants (Liu et al., 2009). Relevant study has shown that the relative expression of *ACS1* gene encoding ACS and *ACO1*, *ACO2*, *ACO3* genes encoding ACO is positively correlated with ethylene release in general (Pun et al., 2016). At the same time, Zhao et al. (2020) found that up-regulation of ethylene biosynthesis gene expression in fruit was synchronized with ethylene emission from fruit. Ma et al. showed a decrease in ACS/ACO gene expression and a simultaneous decrease in ethylene production (Ma et al., 2021). In the present study,

the relative expression of these genes was mostly lower in the FSAL-treated group than in the control group, MAPK gene may be involved in fruit ripening (Li et al., 2022b), suggesting that FSAL treatment may have reduced the rate of ethylene release and thus the rate of fruit ripening and senescence. In a similar vein, Shen et al. (2023) found that proanthocyanidin could reduce the activities of ACS and ACO in litchi fruit and inhibit the expression of key genes for ACS and ACO synthesis to retard fruit browning and ripening.

Conclusions

In conclusion, the results showed that FSAL had a certain inhibitory effect on *B. cinerea* in grapes, especially when the concentration of the antifungal suspension was 10^4 spores/mL. It reduced the disease incidence of gray mold and lesion diameter, enhanced the antioxidant defense system, reduced the content of ROS in the fruit, and reduced the relative expression of ethylene synthesis key genes in the MAPK signaling pathway. These results provide a basis for the future development of natural plant-derived antifungal agents for fruit preservation.

Acknowledgements We thank the Key Laboratory of Animal Protein Food Processing Technology of Zhejiang Province.

Author Contribution Kaiyue Wang: investigation, formal analysis, and writing—original draft. Qingqing Ge: data curation. Xingfeng Shao: writing—review and editing. Yingying Wei: data curation. Xin Zhang: methodology. Hongfei Wang: conceptualization, supervision, and project administration. Feng Xu: resources, validation, and visualization.

Funding The study was supported by the Natural Science Foundation of Zhejiang Province [LY16C200003] and the Student Research and Innovation Program of Ningbo University (2021SRIP3607).

Data Availability The data sets generated and/or analyzed during this study can be reasonably requested from the corresponding authors.

Declarations

Competing Interests The authors declare no competing interests.

References

Ali, A., Chow, W. L., Zahid, N., & Ong, M. K. (2014). Efficacy of propolis and cinnamon oil coating in controlling post-harvest anthracnose and quality of chilli (*Capsicum annuum* L.) during cold storage. *Food and Bioprocess Technology, 7*(9), 2742–2748. https://doi.org/10.1007/s11947-013-1237-y

Ali, A., Hei, G. K., & Keat, Y. W. (2016). Efficacy of ginger oil and extract combined with gum arabic on anthracnose and quality of papaya fruit during cold storage. *Journal of Food Science and Technology, 53*(3), 1435–1444. https://doi.org/10.1007/s13197-015-2124-5

Alsharairi, N. A. (2021). *Scutellaria baicalensis* and their natural flavone compounds as potential medicinal drugs for the treatment of nicotine-induced non-small-cell lung cancer and asthma. *International Journal of Environmental Research and Public Health, 18*(10), 5243. https://doi.org/10.3390/ijerph18105243

Alwi, N. A., & Ali, A. (2015). Dose-dependent effect of ozone fumigation on physiological characteristics, ascorbic acid content and disease development on bell pepper (*Capsicum annuum* L.) during storage. *Food and Bioprocess Technology, 8*(3), 558–566. https://doi.org/10.1007/s11947-014-1419-2

Ansorena, M. R., Zubeldía, F., & Marcovich, N. E. (2016). Active wheat gluten films obtained by thermoplastic processing. *LWT-Food Science and Technology, 69*, 47–54. https://doi.org/10.1016/j.lwt.2016.01.020

Araújo, J. M. S., de Siqueira, A. C. P., Blank, A. F., Narain, N., & de Aquino Santana, L. C. L. (2018). A cassava starch-chitosan edible coating enriched with *Lippia sidoides* Cham. essential oil and pomegranate peel extract for preservation of Italian tomatoes (*Lycopersicon esculentum* Mill.) stored at room temperature. *Food and Bioprocess Technology, 11*(9), 1750–1760. https://doi.org/10.1007/s11947-018-2139-9

Ayón Reyna, L. E., Uriarte Gastelum, Y. G., Camacho Díaz, B. H., Tapia Maruri, D., López López, M. E., López Velázquez, J. G., & Vega García, M. O. (2022). Antifungal activity of a chitosan and mint essential oil coating on the development of *Colletotrichum Gloeosporioides* in papaya using macroscopic and microscopic analysis. *Food and Bioprocess Technology, 15*(2), 368–378. https://doi.org/10.1007/s11947-022-02764-w

Bai, T., Li, J., Murtaza, A., Iqbal, A., Zhu, L., Zhang, J., Zhang, B., Xu, X., Pan, S., & Hu, W. (2022). Scavenging of ROS after eugenol treatment as mechanism of slowing down membrane lipid metabolism to maintain the surface color of fresh-cut yam. *Food and Bioprocess Technology, 15*(8), 1821–1835. https://doi.org/10.1007/s11947-022-02833-0

Barreto, T. A., Andrade, S. C., Maciel, J. F., Arcanjo, N. M., Madruga, M. S., Meireles, B., Cordeiro, Â. M., Souza, E. L, & Magnani, M. (2016). A chitosan coating containing essential oil from *Origanum vulgare* L. to control postharvest mold infections and keep the quality of cherry tomato fruit. *Frontiers in Microbiology, 7*, 1724. https://doi.org/10.3389/fmicb.2016.01724

Bu, S., Munir, S., He, P., Li, Y., Wu, Y., Li, X., Kong, B., He, P., & He, Y. (2021). *Bacillus subtilis* L1–21 as a biocontrol agent for postharvest gray mold of tomato caused by *Botrytis cinerea*. *Biological Control, 157*, 104568. https://doi.org/10.1016/j.biocontrol.2021.104568

Cai, J., Cheng, S., Luo, F., Zhao, Y., Wei, B., Zhou, Q., Zhou, X., & Ji, S. (2019). Influence of ethylene on morphology and pigment changes in harvested broccoli. *Food and Bioprocess Technology, 12*(5), 883–897. https://doi.org/10.1007/s11947-019-02267-1

Candiracci, M., Citterio, B., & Piatti, E. (2012). Antifungal activity of the honey flavonoid extract against *Candida albicans*. *Food Chemistry, 131*(2), 493–499. https://doi.org/10.1016/j.foodchem.2011.09.012

Chen, Y., Zhang, Y., Nawaz, G., Zhao, C., Li, Y., Dong, T., Zhu, M., Du, X., Zhang, L., Li, Z., & Xu, T. (2020). Exogenous melatonin attenuates post-harvest decay by increasing antioxidant activity in wax apple (*Syzygium samarangense*). *Frontiers in Plant Science, 11*(569779), 2020. https://doi.org/10.3389/fpls.2020.569779.eCollection

Cheng, Y., Li, C., Hou, J., Li, Y., Jiang, C., & Ge, Y. (2020). Mitogen-activated protein kinase cascade and reactive oxygen species metabolism are involved in acibenzolar-S-methyl-induced disease resistance in apples. *Journal of Agricultural and Food Chemistry, 68*(39), 10928–10936. https://doi.org/10.1021/acs.jafc.0c04257

Chung, K. R. (2012). Stress response and pathogenicity of the necrotrophic fungal pathogen *Alternaria alternata*. *Scientifica (Cairo), 2012*, 635431. https://doi.org/10.6064/2012/635431

da Silva, P. P. M., de Oliveira, J., Biazotto, A. D. M., Parisi, M. M., da Glória, E. M., & Spoto, M. H. F. (2020). Essential oils from *Eucalyptus staigeriana* F. Muell. ex Bailey and *Eucalyptus urograndis* W. Hill ex Maiden associated to carboxymethylcellulose coating for the control of *Botrytis cinerea* Pers. Fr. and *Rhizopus stolonifer* (Ehrenb.:Fr.) Vuill. in strawberries. *Industrial Crops and Products, 156*, 112884. https://doi.org/10.1016/j.indcrop.2020.112884

Das, M. R., Sarma, R. K., Borah, S., Kumari, R., Saikia, R., Deshmukh, A. B., Shelke, M. V., Sengupta, P., Szunerits, S., & Boukherroub, R. (2013). The synthesis of citrate-modified silver nanoparticles in an aqueous suspension of graphene oxide nanosheets and their antibacterial activity. *Colloids and Surfaces. b, Biointerfaces, 105*, 128–136. https://doi.org/10.1016/j.colsurfb.2012.12.033

Esteban, MA., Cordero, H., Martínez-Tomé, M., Jiménez-Monreal, A. M., Bakhrouf, A., & Mahdhi, A. (2014). Effect of dietary supplementation of probiotics and palm fruits extracts on the antioxidant enzyme gene expression in the mucosae of gilthead seabream (*Sparus aurata* L.). *Fish & Shellfish Immunology, 39*(2), 532–540. https://doi.org/10.1016/j.fsi.2014.06.012

Fan, L., Wei, Y., Chen, Y., Jiang, S., Xu, F., Zhang, C., Wang, H., & Shao, X. (2023). Epinecidin-1, a marine antifungal peptide, inhibits *Botrytis cinerea* and delays gray mold in postharvest peaches. *Food Chemistry, 403*, 134419. https://doi.org/10.1016/j.foodchem.2022.134419

Fang, H., Zhou, Q., Yang, Q., Zhou, X., Cheng, S., Wei, B., Li, J., & Ji, S. (2022). Influence of combined edible coating with chitosan and tea polyphenol on the quality deterioration and health-promoting compounds in harvested broccoli. *Food and Bioprocess Technology, 15*(2), 407–420.

Fernández, G., Sbres, M., Lado, J., & Pérez-Faggiani, E. (2022). Postharvest sour rot control in lemon fruit by natamycin and an *Allium* extract. *International Journal of Food Microbiology, 368*, 109605. https://doi.org/10.1016/j.ijfoodmicro.2022.109605

Foyer, C. (2007). Ascorbic acid homeostasis and its effects on plant growth and defence. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology, 146*(4, Supplement), S250. https://doi.org/10.1016/j.cbpa.2007.01.583

Guigón-López, C., Holguín-Ibarra, P. D., Torres-Zapien, J. H., García-Cruz, I., Villapando, I., & Salas-Salazar, N. A. (2021). *Metarhizium anisopliae* reduces conidial germination and mycelium growth of the apple gray mold *Botrytis cinerea*. *Biological Control, 160*, 104660. https://doi.org/10.1016/j.biocontrol.2021.104660

Han, S. H., Song, M. H., & Keum, Y. S. (2020). Effects of azole fungicides on secreted metabolomes of *Botrytis cinerea*. *Journal of Agricultural and Food Chemistry, 68*(19), 5309–5317. https://doi.org/10.1021/acs.jafc.0c00696

Inupakutika, M. A., Sengupta, S., Devireddy, A. R., Azad, R. K., & Mittler, R. (2016). The evolution of reactive oxygen species metabolism. *Journal of Experimental Botany, 67*(21), 5933–5943. https://doi.org/10.1093/jxb/erw382

Kaya, C., Ugurlar, F., Ashraf, M., Alyemeni, M. N., Bajguz, A., & Ahmad, P. (2022). The involvement of hydrogen sulphide in melatonin-induced tolerance to arsenic toxicity in pepper (*Capsicum annuum* L.) plants by regulating sequestration and subcellular distribution of arsenic, and antioxidant defense system. *Chemosphere, 309*, 136678. https://doi.org/10.1016/j.chemosphere.2022.136678

Khademi, O., Salvador, A., Zamani, Z., & Besada, C. (2013). Effects of hot water treatments on antioxidant enzymatic system in reducing flesh browning of persimmon. *Food and Bioprocess Technology, 6*(11), 3038–3046. https://doi.org/10.1007/s11947-012-0959-6

Li. G., Wang, Y., Zhang, Z., Chen, Y., & Tian, S. (2022a). Mushroom alcohol controls gray mold caused by *Botrytis cinerea* in harvested fruit via activating the genes involved in jasmonic acid signaling pathway. *Postharvest Biology and Technology, 186*, 111843. https://doi.org/10.1016/j.postharvbio.2022.111843

Li, M., Li, B., Yang, M., Wang, L., Hou, G., Lin, Y., Zhang, Y., Zhang, Y., Chen, Q., Wang, Y., He, W., Wang, X., Tang, H., Yang, G., & Luo, Y. (2022b). Genome-wide identification and expression of MAPK gene family in cultivated strawberry and their involvement in fruit developing and ripening. *International of Journal of Molecular Sciences, 23*(9), 5201. https://doi.org/10.3390/ijms23095201

Li, R., Li, Y., Zhang, Y., Sheng, J., Zhu, H., & Shen, L. (2021). Transcriptome analysis reveals that *SlNPR1* mediates tomato fruit resistance against *Botrytis cinerea* by modulating phenylpropanoid metabolism and balancing ROS homeostasis. *Postharvest Biology and Technology, 172*, 111382. https://doi.org/10.1016/j.postharvbio.2020.111382

Li, S., Cheng, Y., Yan, R., Liu, Y., Huan, C., & Zheng, X. (2022c). Preharvest spray with melatonin improves postharvest disease resistance in cherry tomato fruit. *Postharvest Biology and Technology, 193*, 112055. https://doi.org/10.1016/j.postharvbio.2022.112055

Li, S., Han, X., Yang, L., Deng, X., Wu, H., Zhang, M., Liu, Y., Zhang, S., & Xu, J. (2018). Mitogen-activated protein kinases and calcium-dependent protein kinases are involved in wounding-induced ethylene biosynthesis in *Arabidopsis thaliana*. *Plant, Cell & Environment, 41*(1), 134–147. https://doi.org/10.1111/pce.12984

Liu, L., Rao, J., Chang, X., & Yi, S. (2009). Regulation of propylene and 1-methylcyclopropene on expressions of *ACS* and *ACO* genes in persimmon fruits. *Agricultural Sciences in China, 8*(10), 1187–1192. https://doi.org/10.1016/S1671-2927(08)60328-4

Liu, X., Li, J., Cui, X., Ji, D., Xu, Y., Chen, T., & Tian, S. (2020). Exogenous bamboo pyroligneous acid improves antioxidant capacity and primes defense responses of harvested apple fruit. *LWT, 134*, 110191. https://doi.org/10.1016/j.lwt.2020.110191

Low, W. L., Martin, C., Hill, D. J., & Kenward, M. A. (2011). Antimicrobial efficacy of silver ions in combination with tea tree oil against *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Candida albicans*. *International Journal of Antimicrobial Agents, 37*(2), 162–165. https://doi.org/10.1016/j.ijantimicag.2010.10.015

Luo, J., Xu, F., Zhang, X., Shao, X., Wei, Y., & Wang, H. (2020). Transcriptome analysis of *Penicillium italicum* in response to the flavonoids from *Sedum aizoon* L. *World Journal of Microbiology and Biotechnology, 36*(5), 62. https://doi.org/10.1007/s11274-020-02836-z

Ma, X., Yuan, Y., Li, C., Wu, Q., He, Z., Li, J., & Zhao, M. (2021). Brassinosteroids suppress ethylene-induced fruitlet abscission through *LcBZR1/2*-mediated transcriptional repression of *LcACS1/4* and *LcACO2/3* in litchi. *Horticulture Research, 8*(1), 105. https://doi.org/10.1038/s41438-021-00540-z

Magalhães Brandão, R., Roberto Batista, L., Elvis de Oliveira, J., Bispo Barbosa, R., Lee Nelson, D., & Graças Cardoso, M. (2023). *In vitro* and *in vivo* efficacy of poly(lactic acid) nanofiber packaging containing essential oils from *Ocimum basilicum* L. and *Ocimum gratissimum* L. against *Aspergillus carbonarius* and *Aspergillus niger* in table grapes. *Food Chemistry, 400*, 134087. https://doi.org/10.1016/j.foodchem.2022.134087

Nunes, C. A. (2011). Biological control of postharvest diseases of fruit. *European Journal of Plant Pathology, 133*(1), 181–196. https://doi.org/10.1007/s10658-011-9919-7

Ouaked, F., Rozhon, W., Lecourieux, D., & Hirt, H. (2003). A MAPK pathway mediates ethylene signaling in plants. *Embo Journal, 22*(6), 1282–1288. https://doi.org/10.1093/emboj/cdg131

Owolabi, I. O., Songsamoe, S., & Matan, N. (2021). Combined impact of peppermint oil and lime oil on Mangosteen (*Garcinia Mangostana*) fruit ripening and mold growth using closed system. *Postharvest Biology and Technology, 175*, 111488. https://doi.org/10.1016/j.postharvbio.2021.111488

Piechowiak, T., Skóra, B., & Balawejder, M. (2020). Ozone treatment induces changes in antioxidative defense system in blueberry fruit during storage. *Food and Bioprocess Technology, 13*(7), 1240–1245. https://doi.org/10.1007/s11947-020-02450-9

Pun, U. K., Yamada, T., Azuma, M., Tanase, K., Yoshioka, S., Shimizu-Yumoto, H., Satoh, S., & Ichimura, K. (2016). Effect of sucrose on sensitivity to ethylene and enzyme activities and gene expression involved in ethylene biosynthesis in cut carnations. *Postharvest Biology and Technology, 121*, 151–158. https://doi.org/10.1016/j.postharvbio.2016.08.001

Radi, M., Ahmadi, H., & Amiri, S. (2022). Effect of cinnamon essential oil-loaded nanostructured lipid carriers (NLC) against *Penicillium Citrinum* and *Penicillium Expansum* involved in tangerine decay. *Food and Bioprocess Technology, 15*(2), 306–318. https://doi.org/10.1007/s11947-021-02737-5

Rossi, F. R., Krapp, A. R., Bisaro, F., Maiale, S. J., Pieckenstain, F. L., & Carrillo, N. (2017). Reactive oxygen species generated in chloroplasts contribute to tobacco leaf infection by the necrotrophic fungus *Botrytis cinerea*. *Plant Journal, 92*(5), 761–773. https://doi.org/10.1111/tpj.13718

Routray, W., & Orsat, V. (2012). Microwave-assisted extraction of flavonoids: A review. *Food and Bioprocess Technology, 5*(2), 409–424. https://doi.org/10.1007/s11947-011-0573-z

Sellamuthu, P. S., Sivakumar, D., Soundy, P., & Korsten, L. (2013). Essential oil vapours suppress the development of anthracnose and enhance defence related and antioxidant enzyme activities in avocado fruit. *Postharvest Biology and Technology, 81*, 66–72. https://doi.org/10.1016/j.postharvbio.2013.02.007

Sempere-Ferre, F., Giménez-Santamarina, S., Roselló, J., & Santamarina, M. P. (2022). Antifungal *in vitro* potential of *Aloe vera* gel as postharvest treatment to maintain blueberry quality during storage. *LWT, 163*, 113512. https://doi.org/10.1016/j.lwt.2022.113512

Shen, W., Li, W., Shao, Y., & Zeng, J. (2023). Proanthocyanidin delays litchi peel browning by inhibiting ethylene biosynthesis, respiratory metabolism, and phenol oxidase activities. *Scientia Horticulturae, 309*, 111677. https://doi.org/10.1016/j.scienta.2022.111677

Smékalová, V., Doskočilová, A., Komis, G., & Šamaj, J. (2014). Crosstalk between secondary messengers, hormones and MAPK modules during abiotic stress signalling in plants. *Biotechnology Advances, 32*(1), 2–11. https://doi.org/10.1016/j.biotechadv.2013.07.009

Sun, Z., Hao, J., Yang, H., & Chen, H. (2018). Effect of chitosan coatings enriched with lauroyl arginate ethyl and montmorillonite on microbial growth and quality maintenance of minimally processed table grapes (*Vitis vinifera* L. Kyoho) during cold storage. *Food and Bioprocess Technology, 11*(10), 1853–1862. https://doi.org/10.1007/s11947-018-2146-x

Viacava, G. E., Cenci, M. P., & Ansorena, M. R. (2022). Effect of chitosan edible coatings incorporated with free or microencapsulated thyme essential oil on quality characteristics of fresh-cut carrot slices. *Food and Bioprocess Technology, 15*(4), 768–784. https://doi.org/10.1007/s11947-022-02783-7

Wang, D., Li, W., Li, D., Li, L., & Luo, Z. (2021). Effect of high carbon dioxide treatment on reactive oxygen species accumulation and antioxidant capacity in fresh-cut pear fruit during storage. *Scientia Horticulturae, 281*, 109925. https://doi.org/10.1016/j.scienta.2021.109925

Wang, H., Xu, F., Zhang, X., Shao, X., Wei, Y., & Wang, H. (2022a). Transcriptomic analysis reveals antibacterial mechanism of flavonoids from *Sedum aizoon* L. against *Pseudomonas fragi*. *Food Control, 134*, 108755. https://doi.org/10.1016/j.foodcont.2021.108755

Wang, K., Zhang, X., Shao, X., Wei, Y., Xu, F., & Wang, H. (2022b). Flavonoids from *Sedum aizoon* L. inhibit *Botrytis cinerea* by negatively affecting cell membrane lipid metabolism. *Applied Microbiology and Biotechnology, 106*(21), 7139–7151. https://doi.org/10.1007/s00253-022-12196-3

Wang, L., Jin, P., Wang, J., Jiang, L., Shan, T., & Zheng, Y. (2015). Methyl jasmonate primed defense responses against *Penicillium expansum* in sweet cherry fruit. *Plant Molecular Biology Reporter, 33*(5), 1464–1471. https://doi.org/10.1007/s11105-014-0844-8

Wei, L., Feng, L., Liu, Y., & Liao, W. (2022). Mitogen-activated protein kinase is involved in salt stress response in tomato (*Solanum lycopersicum*) seedlings. *International Journal of Molecular Sciences, 23*(14), 7645. https://doi.org/10.3390/ijms23147645

Wu, X., Liu, Z., & Liao, W. (2021). The involvement of gaseous signaling molecules in plant MAPK cascades: Function and signal transduction. *Planta, 254*(6), 127. https://doi.org/10.1007/s00425-021-03792-0

Xu, D., Deng, Y., Xi, P., Yu, G., Wang, Q., Zeng, Q., Jiang, Z., & Gao, L. (2019). Fulvic acid-induced disease resistance to *Botrytis cinerea* in table grapes may be mediated by regulating phenylpropanoid metabolism. *Food Chemistry, 286*, 226–233. https://doi.org/10.1016/j.foodchem.2019.02.015

Xu, T., Wang, Z., Lei, T., Lv, C., Wang, J., & Lu, J. (2015). New flavonoid glycosides from *Sedum aizoon* L. *Fitoterapia, 101*, 125–132. https://doi.org/10.1016/j.fitote.2014.12.014

Yu, Q., Zhang, L., Sun, D., Hu, Y., Li, P., Zhang, X., Ding, L., Zhou, L., Guan, Z., Fang, W., Chen, F., & Song, A. (2022). Interaction between MAPKs and MKPs phosphatases in hexaploid chrysanthemum illuminates functional paralogue diversification in polyploids. *Horticultural Plant Journal*. https://doi.org/10.1016/j.hpj.2022.01.003

Zhao, L., He, F., Li, B., Gu, X., Zhang, X., Dhanasekaran, S., & Zhang, H. (2022a). Transcriptomic analysis of the mechanisms involved in enhanced antagonistic efficacy of *Meyerozyma guilliermondii* by methyl jasmonate and disease resistance of postharvest apples. *LWT-Food Science and Technology, 160*, 113323. https://doi.org/10.1016/j.lwt.2022.113323

Zhao, M., Li, C., Ma, X., Xia, R., Chen, J., Liu, X., Ying, P., Peng, M., Wang, J., Shi, C. L., & Li, J. (2020). KNOX protein KNAT1 regulates fruitlet abscission in litchi by repressing ethylene biosynthetic genes. *Journal of Experimental Botany, 71*(14), 4069–4082. https://doi.org/10.1093/jxb/eraa162

Zhao, Y., Zhang, Y., Bai, X., Lin, R., Shi, G., Du, P., & Xiao, K. (2022b). *TaNF-YB11*, a gene of NF-Y transcription factor family in *Triticumaestivum*, confers drought tolerance on plants *via* modulating osmolyte accumulation and reactive oxygen species homeostasis. *Journal of Integrative Agriculture, 21*(11), 3114–3130. https://doi.org/10.1016/j.jia.2022.07.058

Zheng, F., Zheng, W., Li, L., Pan, S., Liu, M., Zhang, W., Liu, H., & Zhu, C. (2017). Chitosan controls postharvest decay and elicits defense response in kiwifruit. *Food and Bioprocess Technology, 10*(11), 1937–1945. https://doi.org/10.1007/s11947-017-1957-5

Zhong, Z., Zhou, L., Yu, K., Jiang, F., Xu, J., Zou, L., Du, L., & Liu, W. (2022). Effects of microporous packaging combined with chitosan coating on the quality and physiological metabolism of passion fruit after harvest. *Food and Bioprocess Technology, 15*(8), 1836–1850. https://doi.org/10.1007/s11947-022-02845-w

Zimdars, S., Hitschler, J., Schieber, A., & Weber, F. (2017). Oxidation of wine polyphenols by secretomes of wild *Botrytis cinerea* strains from white and red grape varieties and determination of their specific laccase activity. *Journal of Agricultural and Food Chemistry, 65*(48), 10582–10590. https://doi.org/10.1021/acs.jafc.7b04375

Zwerger, K., & Hirt, H. (2001). Recent advances in plant MAP kinase signalling. *Biological Chemistry, 382*(8), 1123–1131. https://doi.org/10.1515/BC.2001.142