

Oregano Oil Fumigation Suppresses Postharvest Yellowing in Broccoli by Enhancing Antioxidant Capacity

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Abstract

The present study utilized integrated physiological, transcriptomic, and metabolomic analyses to comprehensively investigate the molecular mechanisms through which oregano oil (OO) fumigation delays postharvest yellowing of broccoli during storage. Results indicated that the OO treatment significantly suppressed respiration and ethylene biosynthesis, reduced reactive oxygen species (ROS) accumulation, and membrane lipid peroxidation, while activating the antioxidant enzyme system in broccoli. OO fumigation was found to activate the MAPK signaling pathway that regulates a downstream network of transcription factors (TFs), including NAC, MYB, and WRKY TFs. This enhances both the ASA-GSH cycle and phenylpropanoid metabolism. This multi-level regulation promoted the accumulation of antioxidant metabolites (e.g., ascorbic acid and anthocyanins) and also contributed to the maintenance of cellular energy homeostasis. Our study provides the first evidence that plant essential oils modulate the antioxidant defense system in postharvest crops through the MAPK signaling pathway and provides a new potential strategy for the development of signal pathway-based green preservation technologies for harvested produce.

Highlights

Oregano oil fumigation delays broccoli yellowing postharvest.

MAPK signaling pathway activation enhances antioxidant capacity.

Multi-omics reveals coordinated ASA-GSH and phenylpropanoid pathways.

Key transcription factors *MYB75*, *WRKY22*, and *NAC1* are upregulated.

Oregano oil induces anthocyanin and ascorbate accumulation for preservation.

Introduction

Broccoli (*Brassica oleracea* var. *italica*) is a highly nutritious cruciferous vegetable, rich in glucosinolates, flavonoids, vitamins, and antioxidant compounds. These metabolites impart the recognized health benefits of broccoli and other cruciferous vegetables, that include anti-inflammatory and anticancer properties (Li et al., 2022). However, broccoli has a relatively short shelf-life and is highly susceptible to postharvest deterioration. In this regard, yellowing represents the most visible and critical morphological manifestation of senescence (Fang et al., 2020; Guo et al., 2024). This undesirable phenomenon is primarily due to the degradation of chlorophyll, membrane lipid peroxidation, and the accumulation of ROS, which are exacerbated by high respiration rates and ethylene sensitivity after harvest (Xu et al., 2021). Postharvest yellowing reduces marketability by negatively impacting consumer acceptance and also leads to significant nutritional losses, especially in antioxidant metabolites such as ascorbic acid and phenolic compounds (Wang et al., 2025a). The utilization of current preservation techniques, such as low-temperature storage and chemical treatments, are often problematic due to cost, environmental

impact, and food safety concerns. Therefore, developing natural, safe, and effective alternative to delay yellowing are needed to extend broccoli shelf life and nutritional quality, that do not have the concerns or problems of current technologies.

Plants maintain redox homeostasis through coordinated enzymatic and non-enzymatic antioxidant defense systems. The ascorbate-glutathione (ASA-GSH) cycle, comprising several non-enzymatic antioxidants, consists of three interconnected redox pairs: glutathione/glutathione disulfide (GSH/GSSG), ascorbate/dehydroascorbic acid (ASA/DHA), and NADP⁺/NADPH (Zheng et al., 2022; Yu et al., 2024). The ASA-GSH cycle regulates cellular redox potential, stabilizes membrane integrity, enhances stress tolerance, and acts as a central method of ROS scavenging to mitigate oxidative damage. Moreover, to enhance their tolerance to abiotic stresses, such as nutrient deficiency and oxidative stress, plants biosynthesize phenolic compounds (e.g., flavonoids and anthocyanins) that scavenge free radicals, quench ROS, and inhibit oxidative chain reactions thereby (Chen et al., 2023; Tan et al., 2025a). Notably, these processes mitigate oxidative injury. In this regard, previous studies have reported that increases in ROS scavenging capacity in harvested broccoli reduces oxidative damage and delays yellowing (Guo et al., 2021; Zhao et al., 2024; Shi et al., 2023).

The fumigation of harvested produce with different essential oils (EOs) has been reported to be a promising alternative to current chemical methods of postharvest preservation, with selected EOs such as lemon and cinnamon oils having Generally Recognized as Safe (GRAS) status from the U.S. Food and Drug Administration (FDA), making them suitable for application in organic postharvest management systems (Malekipoor et al., 2022). The use of EOs has multiple advantages, including their broad-spectrum antimicrobial activity, antioxidant properties, and approved use in organic postharvest systems (Dhanasekaran et al., 2025; Zhang et al., 2023). Combining EO fumigation with complementary technologies such as modified atmosphere packaging provides an ecofriendly alternative to synthetic fungicides for managing postharvest diseases and maintaining quality (Luo et al., 2024). Oregano oil (OO) is an EO that is primarily composed of carvacrol and thymol. These phenolic compounds have potent bioactivity, such as anti-inflammatory, antibacterial, and antioxidant properties (Govaris et al., 2010; Goncalves et al., 2013; Asensio et al., 2012). These properties suggest that OO has considerable potential for use in the postharvest preservation of agricultural products. Pizzo et al. (2023) reported EO as an effective alternative to chemical disinfectants for washing produce. Notably OO has been shown have the ability to suppress mold diseases and extend the shelf life of various fruits, including strawberries (Yang et al., 2024), apples (Buonsenso et al., 2023), mangoes (Sanchez-Tamayo et al., 2024), and grapes (Almasaudi et al., 2022). OO can be applied by fumigation or incorporated into edible coatings and microcapsules using polysaccharides such as chitosan (Guo et al., 2022; Shi et al., 2022). However, current research has predominantly focused on the the use of EOs in postharvest disease prevention of fruits and studies on the impact of EOs on quality deterioration and postharvest yellowing in vegetables, particularly green vegetables, remain limited. Existing studies have also been largely confined to phenotypic observations and physiological assessments. However, the molecular mechanisms underlying the preservation effect have not been investigated using advanced transcriptomic and metabolomic tools. Therefore, systematic and comprehensive studies are lacking.

In recent years, fumigation with plant essential oils, such as oregano oil, has been demonstrated to have significant efficacy in delaying broccoli senescence. However, the signal transduction mechanisms responsible for inducing host defense responses that result in improved preservation are not fully understood. In this regard, the mitogen-activated protein kinase (MAPK) cascade, a central module transducing a wide range of external stresses, represents a prime candidate for mediating host response to EO fumigation (Pitzschke et al., 2009; Rodriguez et al., 2010). Extensive studies have the function of the MAPK pathway in postharvest fruit ripening (Liang et al., 2020; Zhou et al., 2022), softening (Zhang and Chen, 2024), and disease resistance (Wang et al., 2023; Li et al., 2025). Nevertheless, its role in the regulation of postharvest yellowing in green vegetables, particularly broccoli, and specifically its role in the antioxidant and senescence-delaying signaling network activated by essential oil fumigation, remains poorly understood and warrants further investigation.

Therefore, the objective of the present study was to investigate the relationship between OO fumigation, antioxidant metabolism, and the delay in the postharvest yellowing of broccoli using transcriptomic and metabolomic tools. The overall objective was to provide a theoretical basis for the development of novel, safe, and effective preservation technologies that could serve as an alternative to current strategies.

Results

OO fumigation delays the postharvest yellowing of broccoli

Notable visual differences in quality were observed between broccoli heads in the OO treatment group and the CK group after 4 days of storage at 20°C. Broccoli heads in the control group exhibited complete yellowing while broccoli heads in the OO treatment group largely retained their green pigmentation with only a slight yellowing, (**Fig. 1A**). The OO fumigation treatment effectively delayed changes in coloration as measured by the different color parameters. Although L^* values of both groups increased significantly on day 3 and day 4 of storage, the OO-treated group had a significantly lower L^* value than the control on day 4 of storage (**Fig. 1B**). Notably, the increase in b^* values was markedly lower in the OO treatment group, on both days 3 and 4 of storage, relative to the control (**Fig. 1C**). During the period of marked color decline (days 3 and 4 of storage), broccoli heads in the OO treatment group consistently had a significantly lower yellowing index (a comprehensive indicator of color deterioration) compared to broccoli heads in the control. Notably, the OO treatment group exhibited a 30% lower index value relative to the control by day 4 of storage (**Fig. 1D**). Changes in chlorophyll content further confirmed the preservation effect of the OO treatment in that it significantly reduced the rate of chlorophyll degradation starting on day 2 of storage. By day 4 of storage, chlorophyll content was at $0.22 \text{ g}\cdot\text{kg}^{-1}$ and approximately $0.12 \text{ g}\cdot\text{kg}^{-1}$ in the control group (**Fig. 1E**).

Both the CK and OO treatment groups exhibited a continuous increase in the level of respiration over the course of 4 days of storage. The maximum respiration rate in the CK group was $351.01 \text{ mg CO}_2\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$, which was significantly higher than the peak respiration rate in the treatment group which peaked at $255.58 \text{ mg CO}_2\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$ (**Fig. 1F**). Ethylene generation in the OO treatment group remained relatively

stable during storage, while a notable increase from $0.119 \mu\text{L}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$ to $0.247 \mu\text{L}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$ was observed in the CK treatment group (**Fig. 1G**). These results collectively indicate that the fumigation of broccoli heads with oregano oil effectively delayed the postharvest senescence of broccoli by reducing the respiratory metabolism and ethylene generation. This conclusion is further supported by the consistently lower percentage weight loss observed in the OO treatment group compared to the CK group throughout the storage period (**Fig. 1H**). TSS, which include vitamins, organic acids, sugars, and other compounds, are key indicators of flavor quality and the nutritional value of fruits and vegetables. In this regard, the OO treatment group exhibited a significantly higher level of TSS during storage, compared to the CK group. In particular, the TSS content in the OO treatment group was significantly 13.7% higher on day 4 of storage than the TSS level in the CK treatment group (**Fig. 1I**).

OO treatment reduces ROS content and enhances the antioxidant capacity of harvested broccoli in storage

OO fumigation significantly alleviated oxidative injury in harvested broccoli during storage (**Fig. 2**). This evidenced by the observation that MDA content in the OO treatment group was significantly lower than it was in the control group beginning at 3 days of storage and was 18% lower than the control at day 4 of storage (**Fig. 2A**), indicating a significant lower amount of membrane lipid peroxidation had occurred in the OO treatment group. Relative electrolyte leakage, which reflects the integrity of cell membranes, was also significantly lower in the OO treatment group starting at day 3 of storage. Electrolyte leakage on day 4 of storage was 16.55% in the control group and only 8% in the OO treatment group (**Fig. 2B**), indicating that membrane structural integrity was better maintained in the OO treatment group. Additionally, ROS accumulation was effectively mitigated by the OO fumigation treatment. Specifically, H_2O_2 content decreased significantly on days 2–3 in the OO treatment group compared to the control group, and the O_2^- level remained consistently lower in the OO treatment group than the control group throughout the duration of storage (**Fig. 2C, D**). In fact, the O_2^- level in the OO treatment group remained relatively low and did not exceed $38 \mu\text{mol}\cdot\text{min}^{-1}\cdot\text{kg}^{-1}$, while the O_2^- level in the control on day 4 of storage exceeded $70 \mu\text{mol}\cdot\text{min}^{-1}\cdot\text{kg}^{-1}$. The consistent comparative trends in these four parameters clearly indicate that OO fumigation enhances the antioxidant capacity of broccoli, mitigates membrane lipid peroxidation, maintains membrane stability, and reduces oxidative stress.

Analysis of DEGs and DAMs

Transcriptomic analysis of broccoli heads in the CK and OO fumigation treatment groups exhibited high reproducibility within treatment groups with significant statistical differences exhibited between the treatment groups sample on day 0 and day 4 of storage as indicated by the Pearson correlation heatmaps and the principal component analysis (PCA) (**Fig. S2A and B**). A total of 13,897 DEGs were identified in the CK0d vs. OO4d comparison (6,032 up-regulated, 7,865 down-regulated), 14,824 DEGs in the CK0d vs. CK4d comparison (6,243 up-regulated, 8,581 down-regulated), and 8,364 DEGs in the CK4d vs. OO4d comparison (3,922 up-regulated, 4,442 down-regulated), with 2,763 DEGs shared among the three comparisons (**Fig. S2C, D**). KEGG enrichment analysis of the DEGs in the CK4d vs. OO4d

comparison revealed the top 20 significantly enriched pathways. These included cutin, suberin and wax biosynthesis (ko00073), steroid biosynthesis (ko00100), glycine, serine and threonine metabolism (ko00260), porphyrin and chlorophyll metabolism (ko00860), carotenoid biosynthesis (ko00906), flavonoid biosynthesis (ko00941), isoflavonoid biosynthesis (ko00943), glucosinolate biosynthesis (ko00966), ribosome (ko03010), and the MAPK signaling pathway (ko04016), among others (**Fig. S7**). Gene Ontology (GO) enrichment analysis of the DEGs in the CK4d vs. OO4d comparison was also conducted (**Fig. S2E**), as well as a gene set enrichment analysis (GSEA) of functionally defined gene sets ranked by log₂ fold change values. These analyses revealed that the response to oxygen-containing compounds (GO:1901700) was significantly enriched in CK samples, indicating that there was a coordinated response to oxidative stress. In contrast, "cell wall organization" (GO:0005618) and "dioxygenase activity" (GO:0016702) were enriched in the OO treatment group, suggesting enhanced cell wall integrity and antioxidant capacity (**Fig. S2E**). Collectively the data indicate that the OO treatment impacts flavonoid biosynthesis, antioxidant capacity, and cell wall functions in broccoli heads.

Metabolomic analysis revealed an evident difference between the CK and OO treatment groups, with the Pearson correlation and OPLS-DA confirming high model validity (R^2Y close to 1, $Q^2Y > 0.5$), supporting the presence of DAMs (**Fig. S3A, B**). Comparative analysis identified 4,386 DAMs in the CK0d vs. CK4d treatment group comparison, 3,717 DAMs in CK0d vs. OO4d comparison, and 4,174 DAMs in CK4d vs. OO4d (**Fig. S3C**). Volcano plot analysis indicated that 2,270 metabolites increased in abundance in the CK4d vs. OO4d comparison, while 1,904 decreased (**Fig. S3D**). KEGG pathway analysis indicated that secondary metabolite biosynthesis pathways were the most significantly enriched DAMs, with anthocyanin biosynthesis and phenylpropanoid biosynthesis both ranked among the top 20 significantly enriched pathways (**Fig. S3E**).

Integrated analysis of co-enriched DEGs and DAMs from the CK0d vs. OO4d and CK4d vs. OO4 treatment group comparisons revealed specific metabolic processes that were differentially affected by the OO fumigation treatment, confirming the fumigation treatment preferentially impacted phenolic/flavonoid metabolism and antioxidant metabolic networks (**Fig. S3F**).

Changes in gene expression profiles and metabolite abundance linked to the ascorbic acid metabolic pathway

Physiological data indicated that OO fumigation significantly improved the retention of ASA, a key metabolite in the AsA-GSH cycle, relative to the control, during the first four days of storage (**Fig. 3A**). Concomitantly, APX activity, a central enzyme in the AsA-GSH cycle, was markedly enhanced (**Fig. 3B**), indicating an increased flux through the cycle that facilitated hydrogen peroxide scavenging. Notably, PPO (**Fig. 3C**), SOD (**Fig. 3D**), CAT (**Fig. 3E**), and POD (**Fig. 3F**) were all substantially upregulated in OO fumigated broccoli heads indicating that the fumigation treatment induced a coordinated response that synergistically enhanced ROS scavenging capacity. This synergistic modulation established an effective oxidative defense response and mitigated oxidative injury in broccoli during storage.

The OO treatment significantly altered the expression patterns of key genes involved in multiple ASA biosynthesis pathways, including the D-galacturonate pathway, L-galactose pathway, myo-inositol pathway, and L-gulose pathway, as well as genes associated with the AsA-GSH cycle (**Fig. 3G**). These genes are functionally involved in ascorbate regeneration, glutathione metabolism, and redox homeostasis. Notably, genes encoding key enzymes involved in ASA biosynthesis, such as *PMI* (BoIC8t47774H), *APX* (BoIC7t46363H), *G6PD* (BoIC8t48021H), and *MIOX* (BoIC7t40612H), exhibited expression trends that were supported increased biosynthesis ASA, with significantly higher FPKM values in samples from the OO4d treatment group than samples from the CK4d treatment group (**Fig. 3H-K**).

A comparative metabolomic analysis of CK4d vs. OO4d samples identified eight DAMs related to ASA metabolism (**Fig. S4**). The data indicate that OO fumigation promoted the accumulation of ASA-synthetic metabolites within the L-galactose pathway and induced moderate changes in metabolite abundance in the L-gulose and myo-inositol pathways (**Fig. S4**). Specifically, L-ascorbic acid, dehydroascorbate (DHA), and glutathione (GSH) exhibited significantly higher abundance in OO4d samples compared to CK4d samples (**Fig. 3L**). In contrast, levels of downstream components in the AsA-GSH cycle, namely NADPH and NADP⁺, remained unchanged (**Fig. S6**).

Variations in gene expression patterns and metabolite accumulation related to the anthocyanin synthetic pathway

Transcriptomic analysis identified a total of 33 DEGs distributed across four interconnected flavonoid biosynthesis pathways (flavonoid biosynthesis: ko00941; anthocyanin biosynthesis: ko00942; isoflavonoid biosynthesis: ko00943; flavone and flavonol biosynthesis: ko00944), encoding 12 key enzymes involved in these synthetic pathways. Among them, 10 DEGs in CK0d, CK4d, and OO4d samples were annotated to flavonoid and anthocyanin metabolism (**Fig. S5, Fig. 4D**). The OO fumigation treatment significantly altered the expression level of the identified DEGs, with notable upregulation observed in genes encoding Flavone Synthase (*FLS*), Anthocyanidin Synthase (*ANS*), and *PAL* (**Fig. 4D**).

Consistent with the observed transcriptional changes, the level of both total phenolic and flavonoid metabolites were higher in the OO treatment group than they were in the control. Total phenolic content displayed a gradual increasing trend in both treatment groups during storage. However, the OO treatment group exhibited significantly higher total phenolic content (0.58 g·kg⁻¹) than the level present in the CK group (0.46 g·kg⁻¹) on day 4 when broccoli yellowing was readily evident (**Fig. 4A**). Flavonoid content in both groups increased initially and then decreased, with the levels in the OO treatment group peaking on day 1 at 15.54 g·kg⁻¹, a value that was 1.7 g·kg⁻¹ higher than the flavonoid content in the CK group (**Fig. 4B**). Notably, the OO treatment group maintained significantly higher flavonoid content than the CK group even at the end of storage on day 4 (**Fig. 4B**). Similarly, the *PAL* activity, a key enzyme in the phenylpropanoid pathway, was consistently higher in the OO treatment group than it was in the control throughout the 4 days of storage (**Fig. 4C**). Furthermore, the FPKM values of *PAL* (BoIC4t22683H), 4-Coumarate-CoA Ligase (*4CL*, BoIC6t36477H), and *ANS* (BoIC4t28155H) were significantly elevated in the

OO4d treatment group compared to the CK4d treatment, with values that were higher by 640.07%, 254.45%, and 285.71%, respectively (**Fig. 4E–G**).

Metabolomic analysis identified a total of 15 DAMs associated with anthocyanin biosynthesis in the comparison between CK4d and OO4d groups. Among these metabolites, eight anthocyanin derivatives showed significantly higher accumulation in the OO-treated group, with relative abundances predominantly exceeding 500. The key metabolites identified as the most prominent DAMs in the OO-treated group included Malvidin 3-O- β -D-galactoside, Cyanidin 3-O- β -D-sambubioside, Pelargonidin 3-O- β -D-sambubioside, Delphinidin 3-glucoside-5-caffeoyl-glucoside, Cyanidin-3,5-O-diglucoside, Pelargonidin-3-glucoside-5-glucoside, Malonylshisonin, and Petunidin-3-O-rutinoside (**Fig. 4H–O**).

Alterations in gene expression and metabolite abundances related to the MAPK signaling pathway

The MAPK signaling pathway transmits signals through a three-tier phosphorylation cascade. When membrane receptors are activated by extracellular signals, MAPKKK $\rightarrow$ MAPKK $\rightarrow$ MAPK are sequentially phosphorylated. The phosphorylated (active) MAPK is translocated to the nucleus and phosphorylates downstream transcription factors that regulate the expression of defense-related genes, thus, mediating plant immune responses, stress adaptation, and growth (**Fig. 5A**). KEGG enrichment analysis revealed significant enrichment of DEGs in the MAPK signaling pathway (ko04016) (**Fig. S7**). More detailed analysis indicated that multiple key signaling components within the MAPK cascade exhibited differential expression. In this regard, the expression levels of several kinases in the MAPK cascade in the CK4d vs. OO4d treatment groups were significantly upregulated, including *MEKK1* (BoIC3t15705H), *MKK1* (BoIC7t45958H), *MKK2* (BoIC8t47878H), *MKK2-2* (BoIC1t00927H), *MKK5* (BoIC3t17439H), *MKK4* (BoIC6t36476H), *MPK3* (BoIC3t19268H), and *MPK6* (BoIC3t15268H) (**Fig. 5A**). The observed changes in kinase expression may directly or indirectly regulate the activity of downstream transcription factors through phosphorylation.

Numerous members of the NAC, MYB, and WRKY transcription factor families exhibited altered expression levels in OO4d vs. CK4d treatment group comparison (**Fig. 5B**). A total of 25 members of the NAC family were upregulated and 12 downregulated; 37 members of the MYB family were upregulated and 20 downregulated; 32 members of the WRKY family were up-regulated and 22 down-regulated (**Fig. 5B**). Among the DEGs, the expression of *NAC055* (BoIC1t04859H) was significantly downregulated in the OO4d treatment group, while *NAC1* (BoIC3t18434H), *NAC035* (BoIC7t43835H), *WRKY22* (BoIC9t53005H), *WRKY53* (BoIC1t01828H), *MYB75* (BoIC2t08331H), *MYB12* (BoIC9t57583H), and *PR1* (BoIC9t55943H) were significantly upregulated (**Fig. 5C**). These transcription factors are known to be involved in the regulation of antioxidant response.

Correlation analysis provided insight into the relationship between changes in the expression of signaling components in the MAPK cascade and key physiological indicators in broccoli (**Fig. 5D**). *MEKK4* and *MKK2-2* were positively correlated with ASA content, chlorophyll content, and APX/SOD enzyme activity but negatively correlated with O_2^- generation and the yellowing index. In contrast, *MKK5* was negatively correlated with ASA, flavonoid, and chlorophyll content, as well as APX/SOD/ANS

enzyme activity but positively correlated with O_2^- generation and CAT activity. These correlations suggest that key components of the MAPK signaling pathway may play a crucial role in the yellowing process of broccoli by modulating a network of antioxidant transcription factors.

Discussion

Similar to other plant essential oils, oregano oil exhibits a significant ability to inhibit postharvest pathogens and extend the shelf life of horticultural crops (Khetabi et al., 2022). However, compared to other natural preservatives, such as leucine and salicylic acid, the mechanisms by which essential oil-based preservatives maintain the postharvest quality of vegetables, particularly in suppressing postharvest yellowing, have not been fully investigated. Although preliminary studies have shown that OO can extend the postharvest shelf life of blueberries (Chen et al., 2022) and onions (Zhang et al., 2022a) by inhibiting fungal growth, its regulatory effect on key signaling pathways governing chlorophyll degradation and carotenoid accumulation in plant tissues has not been fully documented. In this regard, the findings of the present study corroborate previous observations and confirm that OO fumigation significantly delays the yellowing process in broccoli, maintains the quality of broccoli during storage (**Fig. 1A–E**) and suppresses the physiological processes associated with postharvest senescence. Specifically, OO fumigation significantly reduced the respiration rate and ethylene generation (**Fig. 1F, G**), indicating that it delays quality deterioration by inhibiting key drivers of ripening and senescence. Additionally, the higher TSS content in OO-treated broccoli, relative to the control (**Fig. 1I**) suggests a potential promotion of osmolyte accumulation, while a lower rate of weight loss (**Fig. 1H**) indicates reduced water loss. These findings provide evidence that support the efficacy of OO fumigation in delaying the postharvest senescence of broccoli by regulating multiple targets.

The enhancement of the antioxidant capacity of broccoli induced by OO fumigation was directly associated with several of the measured physiological indicators. OO fumigation significantly reduced the accumulation of O_2^- and H_2O_2 in broccoli heads during storage, relative to the untreated control (**Fig. 2C, D**), indicating effective mitigation of oxidative stress. Importantly, the level of MDA, a terminal product of membrane lipid peroxidation, and the percentage electrolyte leakage, which reflects cell membrane integrity, were also significantly lower in the OO treatment group, relative to the control (**Fig. 2A, B**). These results collectively support the premise that OO fumigation mitigates oxidative stress and preserves the structural integrity of cell membranes, thereby delaying yellowing and senescence. Consistent with the established link between antioxidant capacity and the delay of yellowing reported by Zheng et al. (2019), we can infer from our physiological data that the delay in yellowing by OO fumigation is mediated, at least in part, by the induced enhancement of the capacity of the antioxidant system in broccoli during storage. This hypothesis provided the basis for our further transcriptomic and metabolomic investigation into the underlying molecular mechanisms related to the enhanced antioxidant capacity.

The AsA-GSH cycle can play a central role in delaying broccoli yellowing by maintaining intracellular redox homeostasis. The AsA-GSH cycle utilizes APX to directly scavenge ROS such as H_2O_2 using ASA,

effectively preventing lipid peroxidation in chloroplast membranes and providing structural protection for chlorophyll molecules. Simultaneously, GSH-dependent DHA reduction ensures continuous regeneration of ASA, creating an antioxidant buffering system that also inhibits chlorophyll-degrading enzyme activity (Jiao et al., 2025a; Zhang et al., 2022b). Previous studies have reported that the application of hydrogen peroxide (Zhang et al., 2023) or exogenous GSH (Jiao et al., 2025b) can enhance the antioxidant capacity of broccoli by their impact on the AsA-GSH cycle. Our earlier research demonstrated that the increased activity of key enzymes in the AsA-GSH cycle, such as APX and CAT, significantly enhance the postharvest quality of broccoli (Wang et al., 2025b; Guo et al., 2024). In the present study, physiological data indicated that the OO fumigation treatment significantly maintained the level of ASA, a core metabolite in the AsA-GSH cycle (**Fig. 3A**) but also increased the activity of APX and other antioxidant enzymes (SOD, CAT, POD) (**Fig. 3B–F**), collectively establishing an efficient ROS-scavenging system. The synergistic effect of the increased activity of the AsA-GSH cycle and antioxidant enzyme activity effectively mitigated the degree of membrane lipid peroxidation, which helped to maintain the structural integrity of cells.

Transcriptomic analysis revealed that OO fumigation had a systemic regulatory effect on the entire ascorbic acid biosynthesis network. OO fumigation simultaneously activated the expression of key genes in four alternative ASA biosynthesis pathways, namely, the D-galacturonate, L-galactose, myo-inositol, and L-gulose pathways (**Fig. 3G**). The concurrent transcriptional upregulation across multiple pathways ensures the adequate supply of ASA precursors and is an example of the redundancy and flexibility of metabolic pathways in plants related to stress response. Importantly, our analysis of the expression of genes encoding rate-limiting enzymes revealed that *PMI*, *APX*, *G6PD*, and *MIOX* all exhibited coordinated levels of high expression (**Fig. 3H–K**). The upregulation of *PMI* and *G6PD*, key regulatory points in the L-galactose pathway, would directly promote the synthesis of GDP-D-mannose, while the activation of *MIOX* would enhance the flux through the myo-inositol pathway. Increased expression of these rate-limiting enzymes are consistent with the observed changes in H_2O_2 and O_2^- levels and with the findings of Yao et al. (2021). We suggest that OO fumigation does not simply stimulate a single pathway but rather activates multiple targets in the ASA metabolic network, thereby enhancing ASA biosynthetic capacity. Metabolomic data obtained in our study provides direct functional evidence of the transcriptional regulation described above. The significant accumulation of ASA and its oxidized form DHA in broccoli in the OO treatment group (**Fig. 3L**), along with the concurrent increase in the abundance of reduced GSH, collectively indicates an overall increase in the activity of the AsA-GSH cycle. Notably, despite the significant enhancement of antioxidant metabolism, the NADPH/NADP⁺ ratio remained stable (**Fig. S6**) which has important physiological implications. It suggests that the boost in antioxidant capacity induced by OO fumigation did not come at the cost of excessive consumption of cellular reducing power reserves. This would ensure normal basal energy metabolism and avoid any potential exacerbation of oxidative stress due to NADPH depletion. This premise is supported by a study on kiwifruit in which treatment with lemongrass oil increased POD, SOD, and CAT activity, reduced H_2O_2 and O_2^- content, and maintained the antioxidant defense network (Bajaj et al., 2024).

Anthocyanin pigments can accumulate in epidermal tissue of broccoli florets, resulting in broccoli heads having a purple-green appearance (Liu et al., 2022). Anthocyanins are potent antioxidants that directly scavenge ROS with the phenolic hydroxyl groups present in their molecular structure. This property effectively reduces postharvest oxidative damage in fruits and vegetables (Sadowska-Bartosz et al., 2024; Tena et al., 2020; Dong et al., 2023). Anthocyanin accumulation is also closely related to the activation of the phenylpropanoid pathway. This process not only competitively reduces phenolic substrates that might otherwise participate in chlorophyll degradation but also indirectly inhibits chlorophyllase activity by maintaining cellular redox homeostasis. Collectively, these activities can delay chlorophyll degradation and yellowing, and reflect an antioxidant defense network that is complementary to the AsA-GSH cycle (Wang et al., 2025c; Huang et al., 2023). The integrated multi-omic analysis conducted in the present study revealed the important role of OO fumigation-induced anthocyanin accumulation in delaying broccoli yellowing. Transcriptomic data revealed that OO fumigation specifically activated genes that encode proteins that function in the flavonoid-anthocyanin biosynthesis pathway, including PAL and 4CL in the upstream phenylpropanoid pathway, as well as other enzymes that function in anthocyanin synthesis, such as ANS and FLS (**Fig. 4D, E–G**). The observed upregulation of the indicated genes would directly promote the accumulation of total phenolics and flavonoids (**Fig. 4A–B**) and channel metabolic flux towards the anthocyanin branch pathway due to the significant expression of the ANS gene. The observed upregulation of phenylpropanoid pathway genes and subsequent accumulation of phenolic/flavonoid compounds align with the transcriptomic and metabolic trends reported by Shahkoomahally (2025) in their study on broccoli shelf-life extension, with both studies demonstrating comparable patterns in key biomarker indicators. Metabolomic analysis further confirmed the significant accumulation of various anthocyanin derivatives in the OO treatment group (**Fig. 4H–O**). Notably, anthocyanin accumulation exhibited a significant positive correlation with the sustained increased activity of PAL, a key enzyme in the phenylpropanoid pathway (**Fig. 4C**). This indicates that the OO fumigation treatment enhanced the flux of the phenylpropanoid pathway, thereby boosting the synthesis capacity of polyphenolic compounds, including anthocyanins.

Anthocyanin molecules possess strong free radical-scavenging ability where the phenolic hydroxyl groups in their structure directly neutralize ROS. The synergy between anthocyanin accumulation and the antioxidant system in broccoli cells has physiological significance. On one hand, anthocyanins can act as secondary antioxidants directly enhancing the tolerance of broccoli to oxidative stress, while on the other hand, anthocyanin synthesis consumes phenylpropanoid pathway intermediates which indirectly reduces the level of phenolic substrates that could be utilized by PPO to produce browning compounds. This multi-layered antioxidant network enables the maintenance of the cellular redox balance, thereby effectively delaying chlorophyll degradation and yellowing. It appears that the increased activity of the anthocyanin pathway is not only a metabolic marker of the impact of OO fumigation on broccoli tissues but also a key mechanism by which OO regulates secondary metabolism to enhance the preservation of broccoli quality during storage. However, it is important to note that while excessive anthocyanin accumulation enhances antioxidant capacity, it may also cause phenotypic changes, leading to the purple coloration of broccoli heads. Yu et al. (2023) reported that knocking out the flavanone 3-

hydroxylase (*F3H*) gene effectively inhibited anthocyanin accumulation in broccoli during low-temperature storage, thereby maintaining its green appearance. Although no significant phenotypic abnormality induced by OO fumigation was observed in our study, determining if the regulation of anthocyanin metabolism would cause color changes in broccoli heads during longer periods of storage or in different genotypes requires further research.

Our study provides the first evidence that oregano oil fumigation regulates the antioxidant defense system in harvested broccoli during storage through activation of the MAPK signaling pathway. OO fumigation induced differential expression of multiple key components within the MAPK cascade (including MEKKs, MKKs, and MPKs), indicating significant pathway activation (**Fig. 5A**). Notably, correlation analysis revealed distinct relationships between specific MAPK components and antioxidant indicators. Specifically, *MEKK4* and *MKK2-2* exhibit a positive correlation with ASA content, chlorophyll retention, and APX/SOD enzyme activity, while a negative correlation was observed with indicators of oxidative damage, such as MDAS content and percentage electrolyte leakage (**Fig. 5D**). These data indicate that kinases may function as positive regulators in the OO-induced antioxidant response. Importantly, our study identified a downstream transcriptional factor cascade mediated by MAPK signaling. OO fumigation significantly altered the expression several NAC, MYB, and WRKY transcription factor family members (**Fig. 5B**). Specifically, key antioxidant-related transcription factors, such as *NAC035*, *WRKY35*, and *MYB75* were significantly upregulated (**Fig. 5C**). These transcription factors can serve as substrates for MAPK phosphorylation, thereby transmitting upstream signals to downstream antioxidant-related functional genes. Previous studies in model plants have reported similar regulatory mechanisms. Li et al. (2016) demonstrated that *Arabidopsis MYB75* interacts with *MPK4* and is phosphorylated, which enhances its stability and positively regulates light-induced anthocyanin accumulation. Zhao et al. (2023) reported that *MYB75* modulates light and temperature stress-regulated anthocyanin and catechin biosynthesis in tea plants (*Camelia sinensis*). These studies establish *MYB75* as a key, positively regulated hub. Complementing this paradigm, Zhou et al., (2025) reported that the R2R3-MYB transcription factor *MYB30* negatively regulates sucrose-induced anthocyanin biosynthesis in *Arabidopsis* by directly repressing *MYB75* expression and disrupting the assembly of the MBW complex. Additionally, Salami et al. (2023) demonstrated that *NAC035* is involved in regulating flavonoid and phenolic acid synthesis, and Liu et al. (2019) found that *PbrWRKY53* from *Pyrus betulaefolia* plays a role in drought tolerance and ascorbic acid accumulation. Importantly, Miao et al. (2007) and Guo et al. (2017) demonstrated that *WRKY53* interacts with *MEKK1* and undergoes phosphorylation, indicating that the MAPK pathway directly participates in redox balance by modulating *WRKY53* activity. These findings strongly align with the enhanced flavonoid accumulation and improved antioxidant capacity observed in our study and indicate that OO fumigation regulates antioxidant metabolism through a MAPK-transcription factor module. Collectively, our findings reveal a potential signaling network in which OO fumigation acts as an exogenous elicitor that activates the MAPK signaling pathway which serves as a key transduction pathway in broccoli. Activation of the MAPK signaling pathway subsequently regulates a complex network of downstream transcription factors that include NAC, MYB, and WRKY family members. This multi-level regulation is ultimately manifested at the physiological level through the

coordinated activity of multiple antioxidant pathways, such as the ASA-GSH cycle (**Fig. 3**) and phenylpropanoid metabolism (**Fig. 4**), which systemically enhance oxidative stress tolerance in broccoli during storage.

This "signal perception-pathway transduction-transcriptional regulation-metabolic reprogramming-physiological response" cascade provides a theoretical perspective for understanding the molecular mechanisms by which plant essential oils regulate postharvest senescence. Unlike conventional chemical preservatives that directly target metabolic endpoints, OO fumigation modulates the endogenous signaling pathways in plants, activating the innate defense system of plants. Depending on the antioxidant capacity of a plant species or genotype, this preservation strategy may offer more sustained effect and greater safety.

In summary, this multi-omic study demonstrated that oregano oil fumigation activates the MAPK signaling cascade in harvested broccoli heads during storage that regulate the expression of *MYB75*, *WRKY22*, and *NAC1* transcription factors, thereby synergistically enhancing two core antioxidant pathways; the ASA-GSH cycle and phenylpropanoid metabolism. Specifically, OO fumigation upregulates *PMI* in the D-galacturonate pathway, *G6PD* in the L-galactose pathway, and *MIOX* in the myo-inositol pathway to promote ascorbate biosynthesis, while also upregulating the expression of genes encoding rate-limiting enzymes (PAL, 4CL, ANS) in phenylpropanoid metabolism, to facilitate anthocyanin accumulation. This multi-level regulatory network systematically enhances cellular antioxidant defense capacity, effectively delaying the postharvest yellowing process in broccoli. Our findings provide a theoretical foundation and innovative perspective for the use of natural essential oils for the preservation of horticultural produce (**Fig. 6**).

Methods

Plant material and oregano oil fumigation

Broccoli (*Brassica oleracea* var. *Italica*, cv. *Ao-han*) heads were harvested at their optimal harvesting date (100 days after planting) in May 2025 in Pinggu District, Beijing, and transported to the laboratory under conditions of $20^{\circ}\text{C} \pm 1^{\circ}\text{C}$. Upon arrival, the broccoli heads were immediately placed in sealed bags and stored at 0°C for 1 hour to eliminate field heat. Heads of uniform size and color, and without signs of mechanical or insect damage, were selected for the study. The broccoli heads were randomly divided into two groups prior to the fumigation experiment, each group containing 54 heads. The fumigation treatment and control groups were both placed in sealed 0.03 mm polyethylene bags within a container (40 cm (L) $\times$ 30 cm (W) $\times$ 12 cm (H); 14.4 L volume) for 4 days at $20 \pm 1^{\circ}\text{C}$ and 90% relative humidity (RH). The optimal concentration for oregano oil fumigation was established through preliminary screening experiments. In the preliminary trial, oregano oil (O823242, Macklin) was applied at different concentrations (1.6, 2.0, and 2.4 mL) and their effect on broccoli yellowing (as determined by a yellowing index) and sensory quality during storage (**Fig. S1**). Results indicated that a concentration of 2.4 mL OO provided superior performance in delaying yellowing while maintaining visual integrity. Therefore, this

concentration was used in all subsequent experiments. The OO fumigation treatment group was conducted for a 24-hour period beginning immediately after the bags containing the broccoli heads were sealed. Briefly, 2.4 mL of oregano oil was placed in a sterile Petri dish inside the bag to allow uniform vapor distribution in the enclosed space. The dish containing the OO was removed after 24 h, and the broccoli was briefly ventilated and then transferred to a new polyethylene bag to eliminate residual OO vapor before being resealed for the remaining duration of the study. The control group was treated in the same manner as the OO treatment group but without any exposure to oregano oil.

Broccoli samples were collected daily for 5 days. Tissues from the sampled heads were immediately frozen in liquid nitrogen and stored at -80°C for subsequent analysis. The treatment and control groups included three biological replicates at each sampled timepoints, with each replicate comprising two broccoli heads.

Color, yellowing index and Chlorophyll

A digital colorimeter (CR-400, Konica Minolta, Japan) was used to quantify the color of the broccoli. Daily L^* , a^* , and b^* measurements were made in a fixed, marker-outlined region of each broccoli head. Five points on the broccoli head were randomly selected, and three broccoli heads were used for each replicate. Data from each replicate was first averaged and then used to determine an overall average for each experimental group. The data was used to calculate a yellowing index using the following formula: Yellowing Index = $100 \cdot (2 \cdot a^* - 0.81 \cdot b^*) / L^*$ (Zou et al., 2024).

Chlorophyll content was determined spectrophotometrically using an organic solvent extraction method. Briefly, 1.0 g of freshly harvested broccoli head sample was ground in liquid nitrogen to ensure cell lysis. The resulting powder was then placed in 10 mL of an extraction solution (acetone:ethanol = 2:1, v/v) and incubated in darkness at 4°C for 4 hours to prevent pigment degradation. Following incubation, the mixture was centrifuged for 10 minutes at $13,000 \times g$ and 4°C . Subsequently, the absorbance of the obtained supernatant was measured at 645 nm and 663 nm using a spectrophotometer. Chlorophyll a, chlorophyll b, and total chlorophyll concentrations (mg/g fresh weight) were calculated using the equations in Lichtenthaler (1987).

Weight loss, respiration intensity, and ethylene generation

Weight loss was determined by measuring the mass of individual broccoli heads using an analytical balance (Jiang et al., 2019). The initial mass (W_0) of the selected broccoli heads was recorded immediately after preparation. Subsequent measurements (W_t) were taken at 24-hour intervals. Percentage weight loss was calculated using the following formula: Weight loss (%) = $[(W_0 - W_t) / W_0] \times 100$.

Respiration intensity and ethylene generation were measured using the methodology of Guo et al. (2024). Briefly, about 750 g (2 broccoli heads) were placed in an airtight container for 20 minutes after which the level of CO_2 in the headspace of the container was measured using a GHX-3051 respirometer.

(Jun Fang Li Hua Technology-Research Institute, Beijing, China). Respiration values are presented as $\text{mg CO}_2 \cdot \text{kg}^{-1} \text{h}^{-1}$. Ethylene generation was assessed by placing about 750 g (2 broccoli heads) in a 500 mL airtight container for 1 h, after which 1 mL of headspace gas was extracted and injected into a 7820A gas chromatograph (Agilent Technologies, Inc., Santa Clara, CA, USA). A standard curve was constructed to translate peak area into ethylene concentration. Results are reported as $\mu\text{L} \cdot \text{kg}^{-1} \text{h}^{-1}$. Each data point was derived from three biological replicates, with each biological replicate consisting of three technical replicates.

Total soluble solids (TSS), ascorbic acid (ASA), and total phenolics and flavonoids

TSS was determined as described in Tan et al. (2025b) with minor adjustments. A total of 2.0 g of broccoli florets and small stems were randomly sampled and ground in with a mortar and pestle. The tissue homogenate was filtered through triple-layered cheesecloth, and the resulting cell sap was used to measure TSS with a portable refractometer. Each sampled group (control and OO fumigation) comprised three biological replicates.

Commercial assay kits (Suzhou Keming Biological Co., Ltd) were used to quantify the concentration of AsA in the sampled tissues. AsA levels are reported as milligrams per gram fresh weight (g/Kg FW). Each sampled group (control and OO fumigation) comprised three biological replicates.

The total content of phenolic and flavonoid compounds was assessed using the protocol of Sun et al. (2022) with some modifications. Briefly, 1.0 g of frozen powdered broccoli sample was mixed with 6 mL 0.5 % acetic acid in 70 % acetone. The resulting mixture was kept at 4 °C in complete darkness for 24 h and then centrifuged at 8000×g for 30 min. The resulting supernatant was used to determine total phenolic and flavonoid content. Phenolic compound content was assessed by mixing 0.5 mL of the supernatant with 2.5 mL of 10 % Folin - Ciocalteu reagent and 2 mL of Na_2CO_3 (7.5 % w/v) and then incubating the mixture at 50 °C for 5 min. Absorbance readings of the samples at 760 nm were then measured and referenced against methanol controls and total phenolic content was calculated using gallic acid standards. For flavonoid analysis, a 0.5 mL aliquot of the supernatant was reacted with 0.15 mL NaNO_2 (5 %) and 0.15 mL $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$ (10 %) at 50 °C for 5 min. Then, 1 mL of NaOH was added to stop the reaction. Absorbance of the samples was measured at 510 nm referenced to methanol blanks and quantified based on catechin equivalents. The results for total phenolic compounds are presented in gallic acid equivalents (GAE) mg/100 g FW, while flavonoid levels are expressed as catechin equivalents (CE) mg/100 g FW.

Relative electrical conductivity, MDA content, O_2^- generation, and hydrogen peroxide (H_2O_2) accumulation

The relative electrical conductivity of sample extracts was measured with a conductivity meter (DDS-11A, Shanghai Instrument and Electronic Scientific Instruments, Ltd., China) as described by Fan et al. (2016). Approximately 2.0 g broccoli floret tissue was placed in 20 mL of distilled water and thoroughly

mixed, after which electrical conductivity was measured to obtain the first value (r_1). Electrical conductivity was measured again after 10 min to obtain the second value (r_2). Each sample was then boiled for 10 minutes, cooled to room temperature, after which electrical conductivity was measured a third time (r_3). The three data point were used to calculate relative electrical conductivity using the formula: Relative electrical conductivity (%) = $(r_2 - r_1) / r_3 \times 100$.

Malondialdehyde (MDA) content was measured using the protocol of Meng, et al. (2012). Briefly, 2.0 g of broccoli florets were ground in 8% trichloroacetic acid (10 mL) and then centrifuged for 15 min at 6000 g. Then, 3.0 mL of 0.67% thiobarbituric acid was added to 2.0 mL of supernatant. The resulting solution was then incubated at 100 °C for 20 min. Absorbance of the sample solution was measured at 600, 532 and 450 nm and these data were used to calculate MDA content. MDA content is expressed as mg kg⁻¹.

H₂O₂ and O²⁻ content were measured using a commercial assay kit (Suzhou Keming Biological) Briefly, 0.1 g of lyophilized powdered broccoli was accurately measured and used in the assay kit following the manufacturer's instructions. After the addition of the supplied reagents, absorbance values of the sample solution was measured at 415 nm (H₂O₂) and 540 nm (O²⁻). H₂O₂ content is expressed as μmol/g FW and O²⁻ is expressed as nmol/g FW.

Antioxidant enzyme activity

Enzyme assays for ascorbate peroxidase (APX), polyphenol oxidase (PPO), pheophytin pheophorbide hydrolase (PPH), superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), and phenylalanine ammonia lyase (PAL) were performed using commercial assay kits (Suzhou Keming Biological) according to the manufacturer's instructions for each specific enzyme. Enzyme activity is as units per gram of fresh weight (U/g FW).

Transcriptomic and Metabolomic analyses

RNA sequencing libraries were constructed using broccoli floret tissues collected from both control and oregano oil-treated groups on day 0 and day 4 of storage. For each treatment at each time point, three independent biological replicate libraries were prepared. Total RNA extraction, cDNA library construction (including reverse transcription and PCR amplification), and subsequent RNA sequencing were conducted by Beijing Biomarker Technologies (Beijing, China). The resulting sequence data were analyzed using the BMKCloud (www.biocloud.net) platform to identify differentially expressed genes (DEGs).

Metabolomic profiling was performed by Beijing Biomarker Technologies (Beijing, China). A 50 mg aliquot of each sample was homogenized in a centrifuge tube containing 1000 μL of extraction solution (acetonitrile: methanol: water = 2:2:1, v/v) spiked with an internal standard (L-2-chlorophenylalanine, 2 μg mL⁻¹). The mixture was vortexed for 30 seconds to ensure homogeneity, followed by sonication at 35 Hz for 5 minutes in an ice-cold water bath. After incubation at -40 °C for 1 h, the samples were centrifuged.

Subsequently, 250 μL of the supernatant was transferred to a new centrifuge tube and dried under vacuum concentration. The residue was reconstituted in 300 μL of 50% acetonitrile via ice-bath sonication for 10 minutes. Finally, 1 μL of the reconstituted solution was injected for LC-MS analysis in both positive (POS) and negative (NEG) ion modes.

Acquired data were subjected to principal component analysis (PCA) to assess overall metabolic variability. Orthogonal projections to latent structures-discriminant analysis (OPLS-DA) were applied to enhance group separation and extract metabolically significant features based on PCA clustering trends. Variable importance in projection (VIP) scores were used to rank differential metabolites. Differentially abundant metabolites (DAMs) were identified using the thresholds: $|\log_2(\text{fold change})| \geq 2$, false discovery rate (FDR) < 0.01 , and adjusted p-value < 0.05 (Love et al., 2014). In our data, $\log_2(\text{fold change})$ denotes the ratio of metabolite abundance between comparative groups, while FDR represents the multiple-testing-corrected significance value.

Correlation analysis of transcriptome and metabolome data

The correlation between the expression of genes and the abundance of metabolites was determined by calculating the Pearson correlation coefficient. The data were preprocessed using the Z-value transformation method before calculating the correlation. The correlation between transcript levels and metabolite levels was determined using a correlation coefficient (CC) and coefficient p-value (CCP). The threshold used to define a significant correlation was $|\text{CC}| > 0.80$ and $\text{CCP} < 0.05$.

Statistical analysis

The selection of broccoli tissue samples was completely randomized, and each sample comprised three biological replicates at each sampled timepoint. Quantitative data are expressed as a mean $\pm$ standard deviation (SD). Statistical analyses were performed using Microsoft Excel 2024 and SPSS 27. Data were subjected to one-way analysis of variance (ANOVA) followed by post-hoc tests for mean separation, with statistical significance defined at $P < 0.05$. Graphical representations were created using Origin (2022).

Declarations

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Authorship contributions

Lin Yu, Conceptualization, Investigation, Formal analysis, Writing-original draft. **Xiaozhen Yue**, Writing-review and editing, Writing-original draft, Methodology, Funding acquisition, Conceptualization. **Bangjin Yan**, Methodology, Investigation, Data curation. **Xiaodi Xu**, Investigation. **Shuzhi Yuan**, Data curation, Software. **Guiqin Qu**, Resources, Writing -review and editing, Methodology, Formal analysis. **Qing Wang**, Writing-review and editing, Validation, Supervision, Resources, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Figures

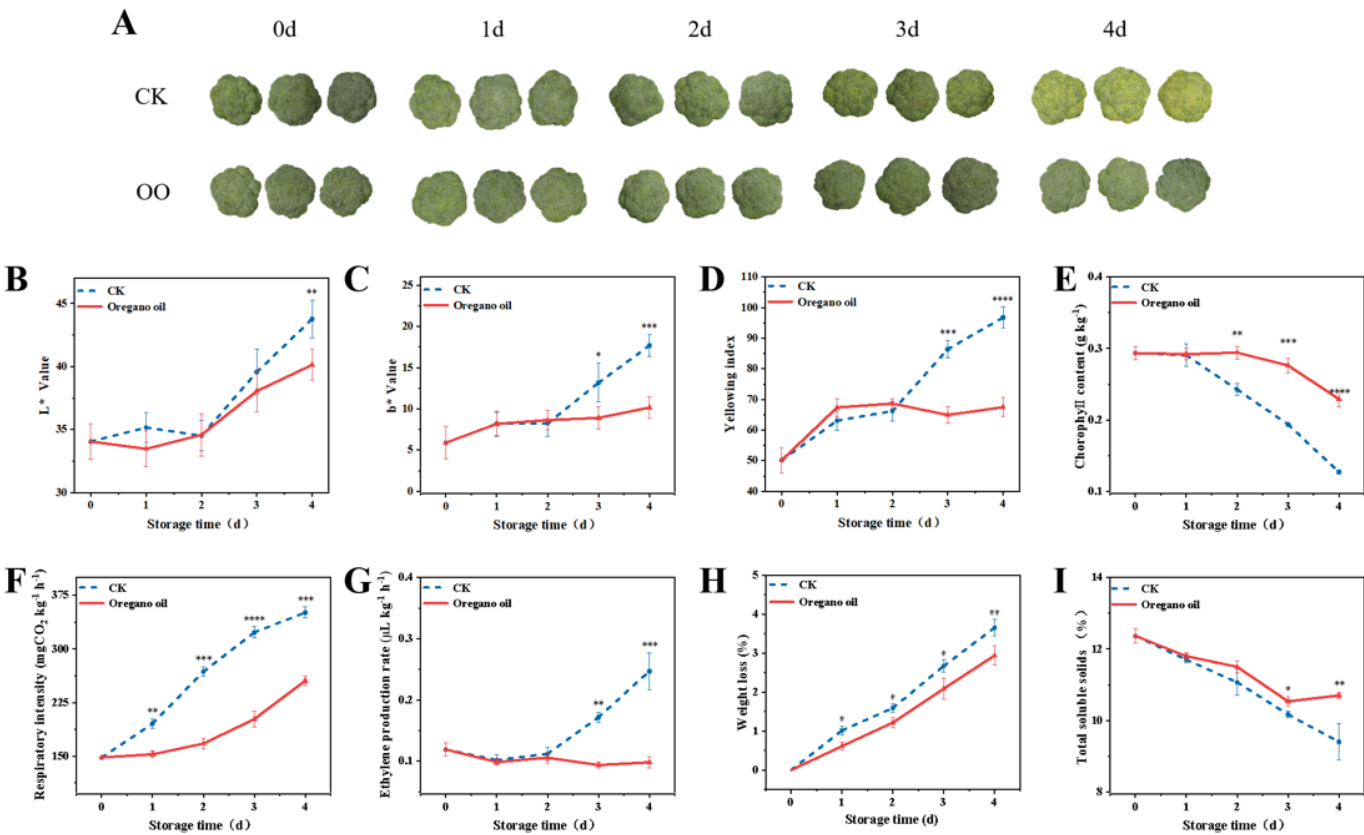


Figure 1

Effect of OO fumigation on postharvest quality of broccoli heads during storage. (A) Representative photographs of CK and OO fumigated broccoli heads. Changes in color values (B) L* and (C) b*, (D) yellowing index, (E) chlorophyll content, (F) respiration rate, (G) ethylene generation, (H) percentage weight loss, and (I) total soluble solids content. Data are the mean ± SD (n = 3 independent biological replicates).

replicates). Asterisks indicate significant differences between treatments at each time point as determined by a Student's t-test (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$).

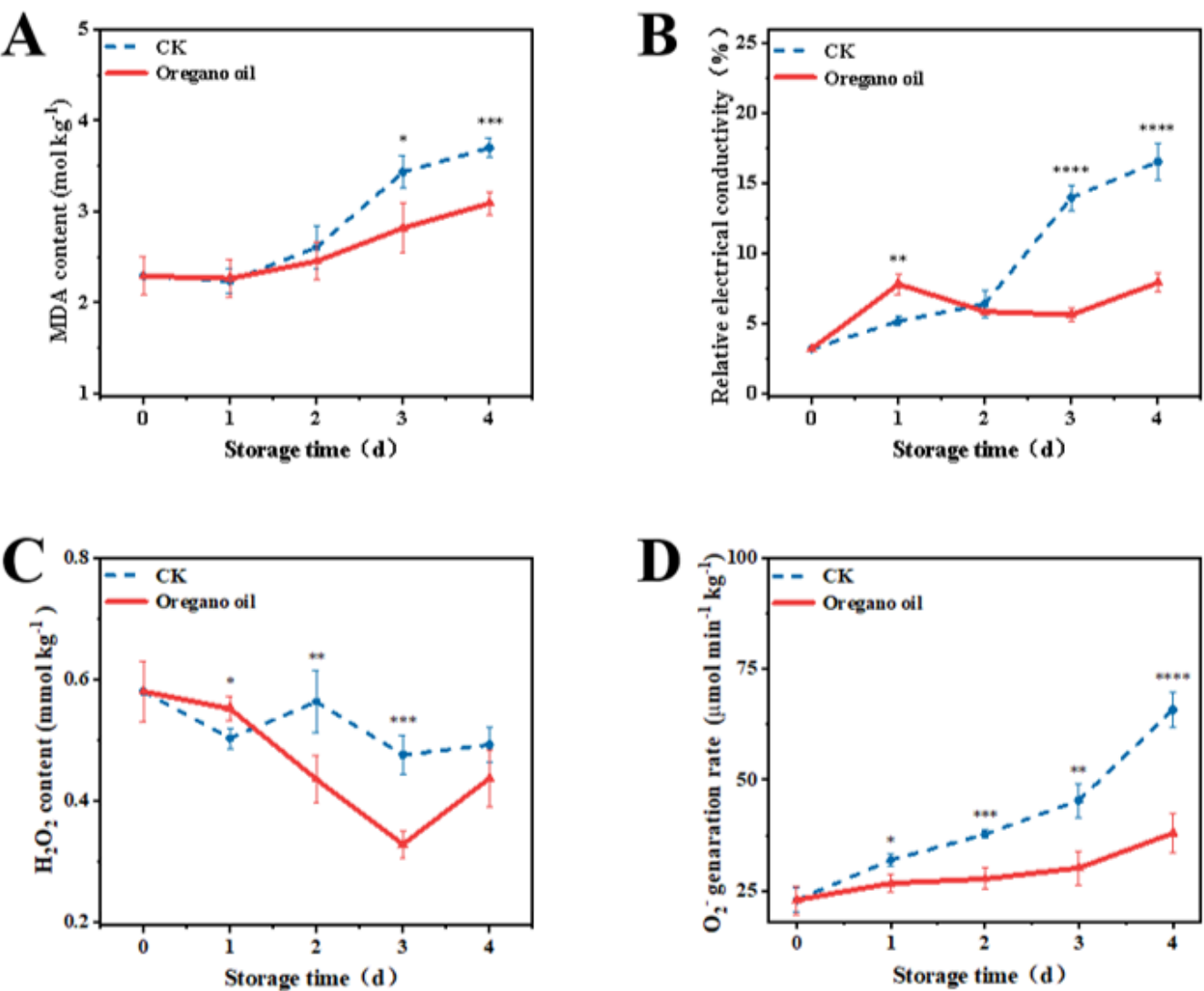


Figure 2

Effect of OO fumigation on oxidative stress-related parameters in broccoli heads during storage. (A) MDA content, (B) relative electrolyte leakage, (C) H₂O₂ level, and (D) O₂⁻ level. Data are the mean ± SD (n=3 independent biological replicates). Asterisks indicate significant differences between the control and OO fumigation treatment groups at each time point as determined by a Student's t-test (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$).

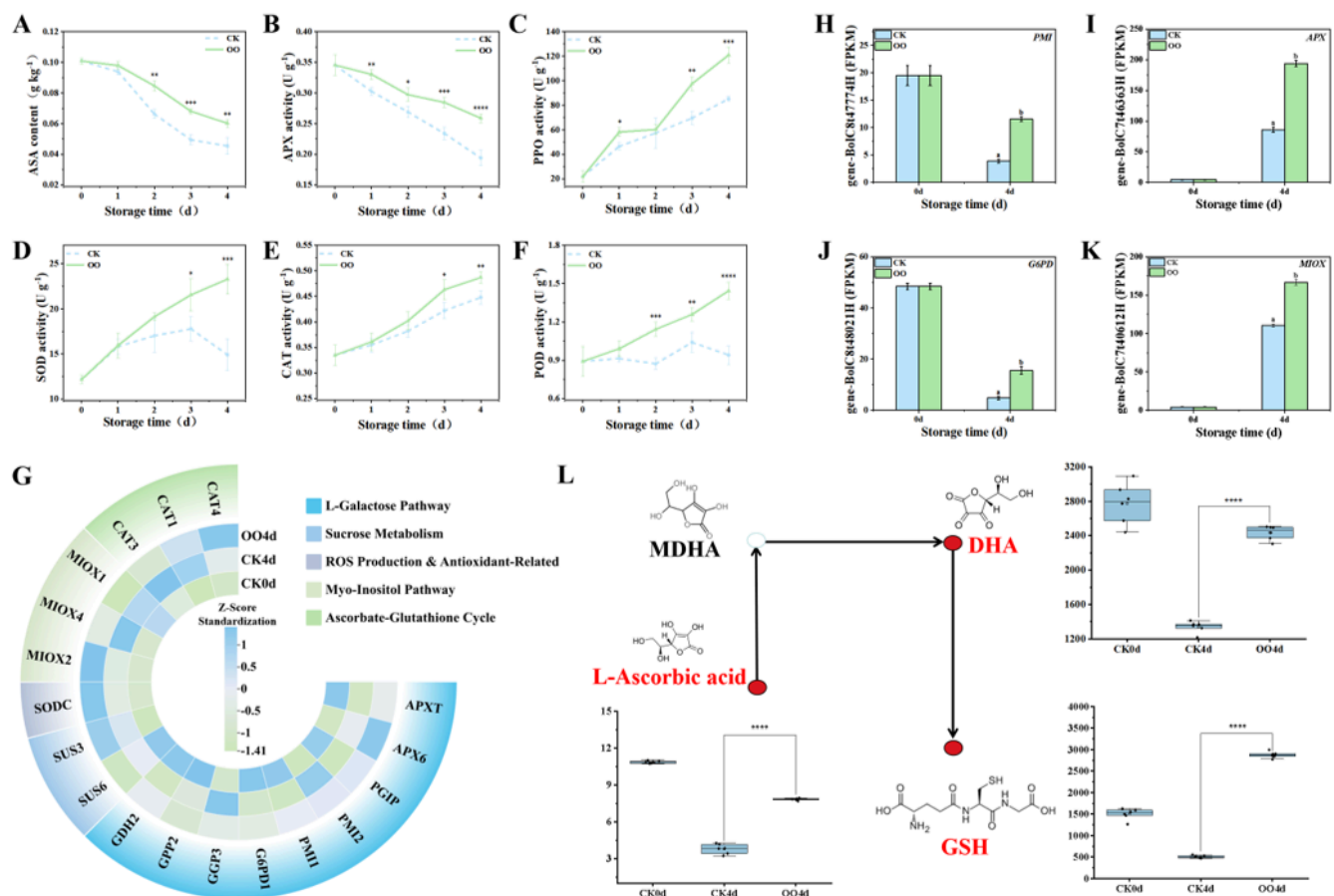


Figure 3

Effect of OO fumigation on the ascorbic acid metabolic pathway in broccoli heads in the CK0d, OO4d, and CK4 treatment groups. (A-F) Changes in metabolite levels and antioxidant enzyme activity: (A) AsA content, (B) APX activity, (C) PPO activity, (D) SOD activity, (E) CAT activity, and (F) POD activity. (G) Heatmap of DEGs in the ascorbic acid metabolic pathway identified in the CK0 vs. CK4d, CK4d vs. OO4d, and CK0d vs. OO4d treatment group comparisons. (H-K) Expression levels (FPKM values) of key genes: (H, J) *PMI*, (I) *APX*, and (K) *G6PD* in the CK0d, CK4d, OO-0d, and OO4d treatment groups (L) Major DAMs in the ASA-GSH pathway identified in the CK4d vs. OO4d treatment group comparison. Data are the mean $\pm$ SD ($n = 3$ independent biological replicates). Different lowercase letters indicate a significant difference ($P < 0.05$) between treatment groups at the same time point as determined by a Student's t-test. Asterisks indicate significant differences (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$) as determined by a Student's t-test.

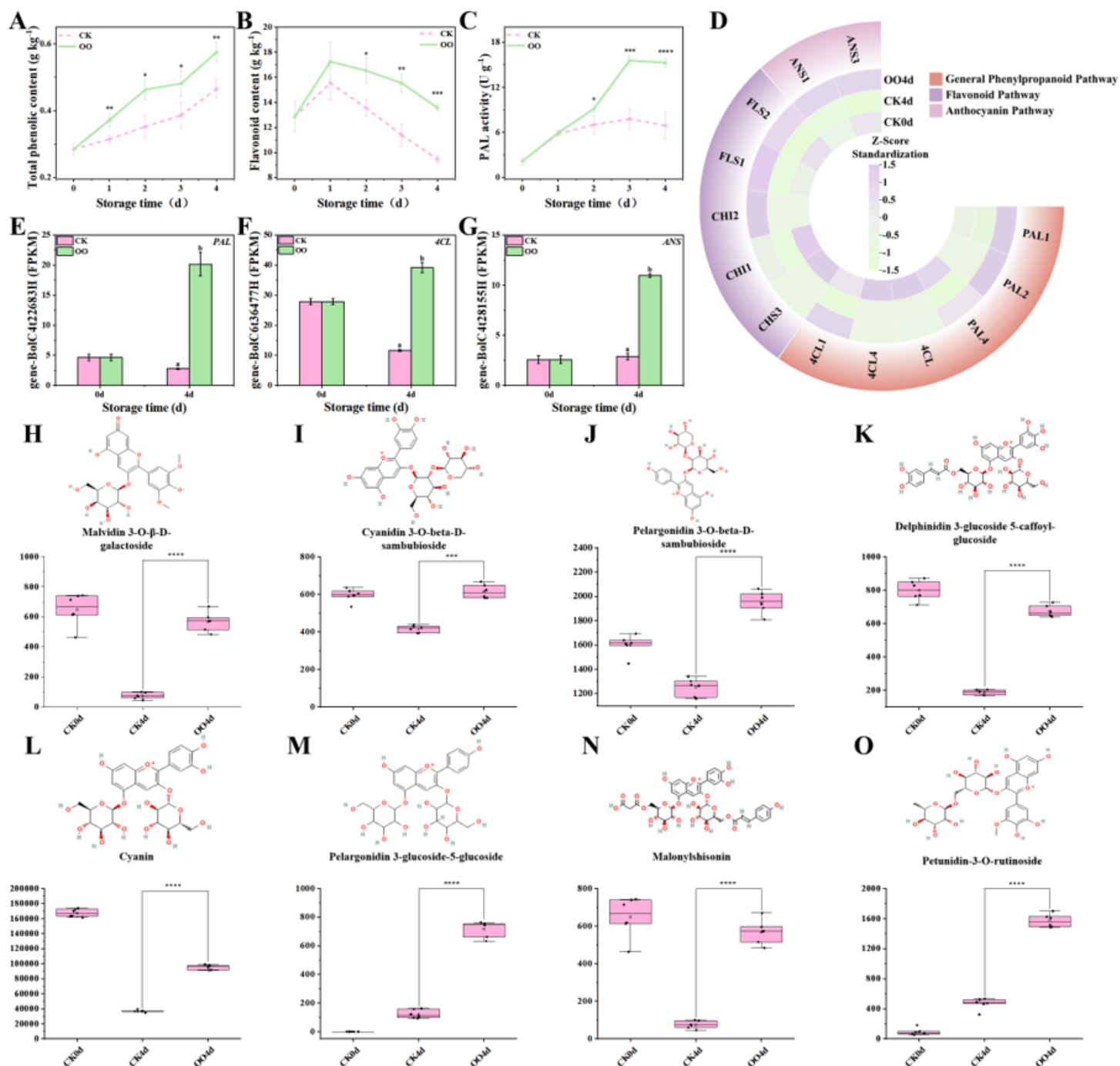


Figure 4

Effect of OO fumigation on the anthocyanin synthesis pathway in broccoli heads in the different treatment groups sampled during storage. (A) Total phenolic content, (B) total flavonoid content, and (C) PAL activity. (D) Heatmap of DEGs in the anthocyanin and phenylpropanoid pathways identified in the CK0 vs. CK4d, CK4d vs. OO4d, and CK0d vs. OO 4d treatment group comparisons. (E-G) Expression level (FPKM) of key genes: (E) *PAL*, (F) *4CL*, and (G) *ANS*. (H-O) in the CK0d, CK4d, and OO4d treatment groups. Relative abundance of differential anthocyanin metabolites identified in the CK4d vs. OO4d treatment group comparison. Data are the mean $\pm$ SD (n = 3 independent biological replicates). Different

lowercase letters indicate a significant difference ($P < 0.05$) between treatment groups at the same time point as determined by a Student's t-test. Asterisks indicate significant differences between the CKK and OO treatment groups as determined by a Student's t-test (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$).

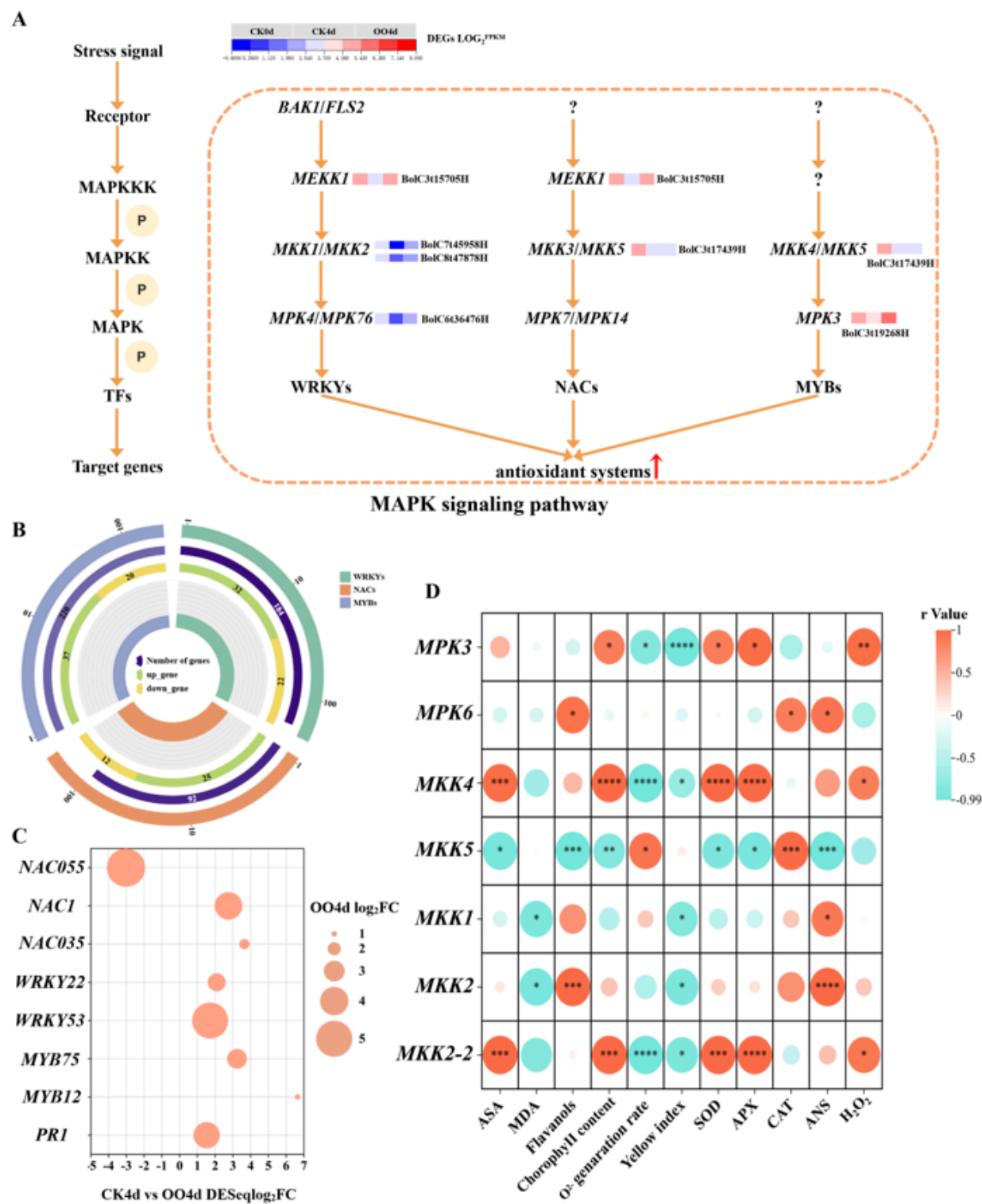


Figure 5

Comprehensive analysis of the MAPK signaling pathway and its downstream transcriptional network in broccoli in the CK0d, OO4d, and CK4d treatment groups. (A) Diagram of the MAPK cascade with along with the expression level of the DEGs identified in the CK4d vs. OO4d treatment group comparison. The diagram was modified from Pitzschke et al. (2009) and Ding et al. (2023). (B) Distribution of up- and down-regulated NAC, WRKY, and MYB transcription factor family members. (C) Expression bubble plot for pivotal transcription factors. (D) Heatmap showing correlations between FPKM values of genes encoding MAPK-related metabolic genes and major antioxidant physiological/omics traits. Data are the mean $\pm$ SD (n = 3 independent biological replicates). Asterisks denote significant differences at *P < 0.05, **P < 0.01, ***P < 0.001, and ****P < 0.0001 as determined by a Student's t-test.

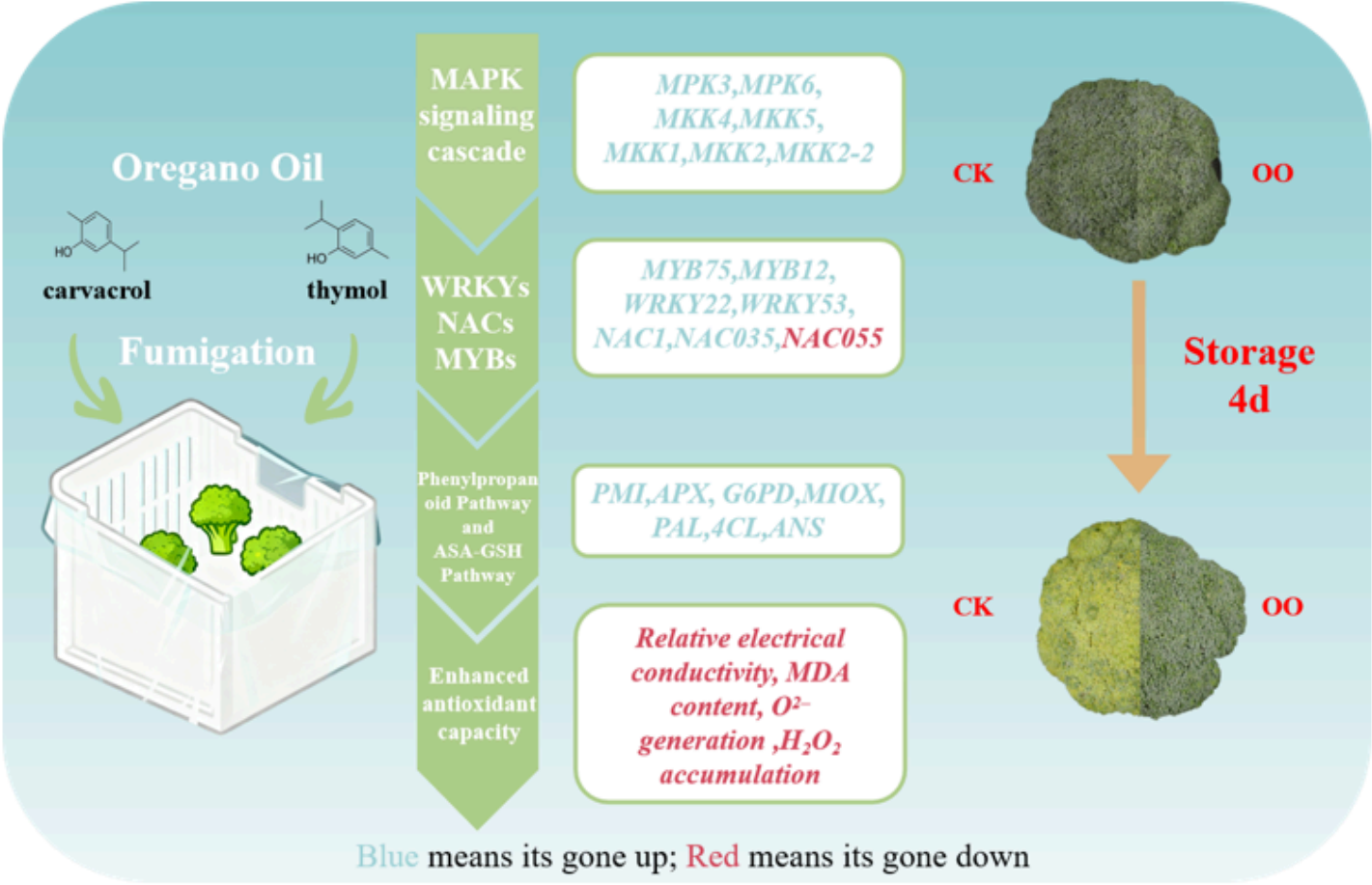


Figure 6

Diagram illustrating the integrative mechanisms through which OO fumigation delays postharvest yellowing in broccoli. The response of broccoli is initiated by the activation of the MAPK signaling pathway, which upregulates downstream transcription factors resulting in transcriptional reprogramming and subsequent changes in metabolite abundance. Changes in metabolism include an increase in the activity of key enzymes and the abundance of critical metabolites within the ASA-GSH and phenylpropanoid pathways, resulting in enhanced antioxidant capacity. Collectively, the indicated molecular events delay the postharvest yellowing of broccoli during storage.

Supplementary Files

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