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VvPPO1 expression analysis and polyphenol oxidase activity during *Vitis vinifera* L. berry development: PPO inhibitory effects of catechin and *Rosa canina* extract

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A B S T R A C T

Purpose: Grapes are economically important fruit crops valued for their nutritional properties and diverse by-products. Maintaining postharvest quality is critical due to rising healthy food demand. Enzymatic browning, mediated by polyphenol oxidase, reduces marketability and organoleptic quality. This study characterized polyphenol oxidase activity, *VvPPO1* gene expression, and total phenolic content in grape berry flesh and peel during maturation. Concurrently, *Rosa canina* hips extract was investigated as a natural polyphenol oxidase inhibitor, providing an alternative to synthetic inhibitors. **Research Method:** Polyphenol oxidase activity in grape berry flesh and peel was measured spectrophotometrically in the absence and presence different concentrations of the *R. canina* hips extract and catechin using caffeic acid as the substrate. *VvPPO1* gene expression level was quantified by qRT-PCR. **Findings:** The results indicated that polyphenol oxidase activity varied between tissues and during berry maturation. polyphenol oxidase activity was higher in the flesh ($71.17 \pm 7.9 \mu\text{M min}^{-1} \text{g}^{-1} \text{FW}$) than in the peel, with activity decreasing by 74% in flesh and 58% in peel at 110 days after full bloom. Conversely, *VvPPO1* expression in peel (75.87 ± 0.088) was more than in flesh and showed a downward trend during ripening. *R. canina* hips extract and catechin inhibited polyphenol oxidase activity in a dose-dependent manner, with IC₅₀ values of 0.7 mg mL^{-1} and 0.035 mg mL^{-1} , respectively, demonstrating their potential to suppress grape polyphenol oxidase activity. **Research limitations:** This study did not evaluate the in vivo effects of *R. canina* hips extract on grape berry polyphenol oxidase activity or *VvPPO1* gene expression in relation to Enzymatic browning control. **Originality/Value:** This study identifies a key time during grape berry maturation for controlling browning. Furthermore, it demonstrates that *R. canina* hips extract is a promising natural polyphenol oxidase inhibitor to improve grape shelf life that meets consumer demands for clean-label food products.

Keywords:

Berry browning, Bidaneh Sefid grape cultivar, Natural inhibitor, *VvPPO1* Gene expression

INTRODUCTION

Grape (*Vitis vinifera* L.), of the family *Vitaceae*, is a rich source of bioactive phenolic compounds including flavonoids and stilbenes, exhibiting high antioxidant capacity and health-promoting properties. Grapes have been cultivated for approximately 8,000 years due to their nutritional and medicinal value (Prathiksha & Hegde, 2022). It can be consumed in various forms, such as table grapes, verjuice, raisins, sap, vinegar, juice, fermented products, jams, and seed oil. Consequently, grape cultivation plays an essential economic role in producing countries (Singh et al., 2023). Currently, grapes represent one of the most significant fruit crops globally, with an annual production of more than 75 million tons (FAO, 2022). Iran is among the most suitable countries for grape cultivation and the production of grape-based products due to its favorable climatic conditions (Mirfatah et al., 2024). Iran also possesses the world's greatest diversity of grape cultivars. Traditional production methods and their by-products techniques, along with the preservation of biodiversity and ecosystems, have led to the recognition of Iran as a Globally Important Agricultural Heritage System (GIAHS) by the FAO. A vital grape variety in Iran is *Vitis vinifera* L. cv. Bidaneh Sefid, which is widely cultivated to produce raisins and table grapes. In table grapes, commercial quality depends closely on visual appearance, which is severely compromised by browning phenomena. Consequently, browning represents a limiting factor in grape handling and processing. This undesirable phenomenon occurs through both chemical and enzymatic pathways, where alternation of phenolic compounds reduces the nutritional value, organoleptic properties, antioxidant capacity, and shelf life of grape products (Núñez-Delicado et al., 2005; Fronk et al., 2017). Enzymatic browning (EB) is estimated to be responsible for over 50% of annual losses in agricultural and horticultural products during post-harvest and storage stages. This phenomenon primarily results from oxidation of phenolic compounds by polyphenol oxidase (PPO) in the presence of oxygen or reactive oxygen species (ROS) (Moon et al., 2020). PPOs are a group of copper-containing enzymes, nearly ubiquitous in the plant kingdom, responsible for initiating EB following biotic or abiotic stress damage. This damage causes the release of phenolic compounds and PPO from their respective cellular compartments, leading to their subsequent interaction in the presence of molecular oxygen. These reactions result in the formation of dark-brown complex polymers known as melanin (Taranto et al., 2017). PPO activity is particularly high in fruits and vegetables containing significant levels of phenolic compounds. Among fruits, grapes are a major source of phenolic compounds and exhibit high PPO activity. In *Vitis vinifera* cv. Bidaneh Sefid, EB typically occurs on berry skin surfaces during maturation and accelerates in damaged berries or during crushing for juice or wine production (Núñez-Delicado et al., 2005).

The study of the browning phenomenon encompasses the entire process of melanin formation, from gene regulation to the synthesis of final macromolecular products. Gene expression studies revealed that grape PPO (*VvPPO*) transcription is upregulated in damaged fruit tissues (Suehiro et al., 2014). Research showed that *VvPPO1* is identical to *GPO1* from 'Sultana' grape (Dry & Robinson, 1994) and that a newly identified PPO gene (*VvPPO2*) from 'Shine Muscat' (*Vitis labruscana* Bailey × *V. vinifera* L.) is a paralog of *VvPPO1* (Suehiro et al., 2014). Therefore, analysis of *VvPPO1* gene expression, which is primarily involved in EB of grape berries, aims to understand the molecular basis of this process.

The principles of EB prevention can be categorized into two main approaches: inhibition of the enzyme and elimination or transformation of the substrate (Moon et al., 2020). Therefore, various physical and chemical methods have been developed to prevent EB. Previous studies indicate that inhibition of grape PPO (*PPOvv*) using chemical antibrowning

agents is one of the most effective, convenient, and economical approaches to prevent EB (Tinello & Lante, 2017). Ascorbic acid, citric acid, and sodium metabisulfite effectively inhibit PPOvv at low concentrations (Gawlik-Dziki et al., 2007). Ascorbic acid reduces quinones and directly inhibits PPO, while citric acid lowers pH and chelates histidine and copper at the enzyme's active site. Sulfites prevent browning by forming stable colorless products with o-quinones and binding PPO's active site (Moon et al., 2020). However, SO₂-based treatments may impair grape taste, cause cracking and bleaching, and are restricted by the FDA due to hypersensitivity and health concerns (Taylor et al., 1986). Therefore, chemical antibrowning agents used in foods should be harmless, non-allergenic, and should not affect the organoleptic properties of food. Recently, there has been an emphasis on consumer-oriented innovations in the food industry. Consumers increasingly prefer natural and eco-friendly PPO inhibitors for safer and healthier food products (Zhang et al., 2022). Research showed that some natural products exhibit a specific inhibitory effect on PPO activity. Quercetin (QrC) and Catechin (CtC), which are abundant in grapes, can inhibit PPOvv activity (Tinello & Lante, 2017). Likewise, plant extracts rich in phenolic compounds offer health benefits and act as PPO inhibitors, contributing to the development of value-added fruit and vegetable products (Zhang et al., 2022). Dog rose (*Rosa canina* L.), a well-known medicinal plant, has demonstrated therapeutic effects against inflammation, stomach ulcers, arthritis, rheumatism, gout, and sciatica. These effects are attributed to its high content of bioactive and antioxidant compounds. *R. canina* hips extract (RCext) is rich in phenolic compounds like CtC, QrC, and ferulic acid, enabling it to inhibit PPOvv activity and control EB effectively. Moreover, RCext can be obtained through simple and minimal processing, serving as a safe substitute for chemical inhibitors and high-risk antibrowning agents, while also exhibiting favorable sensory and nutritional properties in grape berries and fresh-cut fruits (Bakhtiar et al., 2023).

The goal of this research is to analyze the expression pattern of the *VvPPOI* gene and PPOvv activity during the development of *Vitis vinifera* L. cv. Bidaneh Sefid. Additionally, the study aims to evaluate the inhibitory effects of natural compounds, specifically CtC and RCext, on PPOvv activity as potential alternatives to synthetic antibrowning agents.

MATERIALS AND METHODS

Plant material

Vitis vinifera L. cv. Bidaneh Sefid grape were selected in July-September from a research vineyard located in Malayer, Iran (34.254 °N, 48.845 °E). Three vines with different orientation were harvested at 90 and 110 days after full bloom (DAFB) when the grapes reached approximately 13° and 22° Brix, respectively (Suehiro et al., 2014). Healthy berries were collected from each bunch, immediately frozen in liquid nitrogen, and stored at -80°C until use.

Total soluble solids (TSS)

Total soluble solids (°Brix) as an index of technological ripening for the grapes were determined using a hand refractometer (KRÜSS, HR181, Germany) (Meléndez et al., 2013).

Determination of total phenolic content (TPC)

Extraction of phenolic compounds from peel and flesh sections of grape berries was performed using a modified version of the method reported by Romero-Pérez et al. (2001). The peel and flesh samples (1 g each) were crushed with 3 mL of 70% ethanol as the extraction solvent. These samples were placed on ice and thoroughly vortexed for 20 min.

The resulting extract was centrifuged (Heraeus Biofuge Stratos, Germany) at 4°C and 10,000 rpm for 15 min, and the supernatant was collected.

TPC of extracts was determined using the Folin–Ciocalteu colorimetric method (Singleton & Rossi, 1965). Crude extract (60 µL) was mixed with 40 µL of Folin–Ciocalteu reagent (diluted 1:4) and 1600 µL of deionized water, and then incubated for 15 min at room temperature in the dark. Subsequently, 300 µL of 20% sodium carbonate solution was added, and the absorbance of the mixture was measured at 740 nm using a UV-Vis spectrophotometer (AnalytikJena, Specord 210, Germany) after incubation for 60 min at 25°C. The concentration of TPC was determined using a calibration curve prepared with gallic acid standard solutions.

PPOvv enzymatic activity determination

Peel (10 g) and flesh (40 g) from berries were ground in liquid nitrogen, then immediately homogenized in phosphate buffered saline (PBS) solution (120 mL and 140 mL, respectively; 0.05 M, pH 6.3) containing phenylmethanesulfonyl fluoride (PMSF) (1 mM) using a vortex mixer at 4°C. The crude extract was centrifuged twice at 2500 rpm for 15 min at 4°C. The supernatant, containing the soluble enzymatic extract, was collected and stored at -20°C for further analysis.

PPOvv specific activity was measured using a UV/visible spectrophotometer (AnalytikJena Specord210, Germany) by monitoring the decrease in absorbance of caffeic acid (CfA) ($\lambda_{\text{max}} = 311 \text{ nm}$, $\epsilon = 13,300 \text{ cm}^{-1} \text{ M}^{-1}$) at $22 \pm 0.1^\circ\text{C}$ for 10 min according to the method described by Haghbeen and Tan (2003). The presented results are the averages of triplicate measurements. The standard reaction mixture consisted of 1.5 mL of 0.05 M sodium phosphate buffer (pH 6.3), 0.06 mL of CfA, and 0.4 mL of PPOvv. Reaction velocities were calculated from the slope of the linear portion of the activity curves. One unit of enzymatic activity was defined as the amount of enzyme that oxidized 1 µmol of substrate per minute.

Preparation of dog rose hips extract as a PPOvv inhibitor

Iranian *R. canina* L. hips were obtained from a local market in Karaj, Iran. The mature hips were cut into small pieces, and after seed removal, the pieces were crushed using a swing mill (Retsch MM301, Germany) for 1 min; the process was repeated four times. The crushed materials were then extracted under constant agitation in distilled water at room temperature for 10 min. The plant material:water ratio was 100:1 (mg/mL). The extracts were centrifuged at 12,000 rpm for 15 min and stored at -20°C in the dark conditions.

PPOvv inhibition by CtC and RCext

Inhibitory experiments were conducted in PBS (0.05 M, pH 6.3) using a constant amount of PPOvv and CfA as substrate, in the presence of various concentrations of inhibitors, including CtC and RCext. Measurements were performed in a 1.5-mL quartz cuvette using a UV-Vis spectrophotometer (AnalytikJena Specord 210, Germany) at 311 nm and $22 \pm 0.1^\circ\text{C}$. The IC_{50} value, defined as the inhibitor concentration required to reduce enzyme activity by 50%, was determined graphically by plotting the relative activities against inhibitor concentrations for each compound using kinetic data (Burlingham & Widlanski, 2003).

RNA extraction and cDNA synthesis

Total RNA was extracted from 150 mg of frozen grape berry peel and flesh (ground in liquid nitrogen) using an RNA extraction kit (Viragen, Iran). RNA purity and integrity were analyzed using 1.5% agarose gel electrophoresis and a NanoDrop spectrophotometer (BOECO, Germany). cDNA was then synthesized in a 96-well thermal cycler (Bio-Rad

MjMini) and a cDNA synthesis kit (AddScript, Korea), with both oligo (dT)₂₀ and random hexamer primers (Resuehr & Spiess, 2003), following the manufacturer's protocol.

Information on the *VvPPO* Gene sequences and expression analysis

Gene sequence data for PPOvv (catechol oxidase, EC 1.10.3.1; tyrosinase, EC 1.14.18.1) were obtained from the Grape Genome Browser using BLAST. Eight predicted PPOvv-encoding genes (accession numbers: XM_059740140, NM_001281116, XM_010648796, AB871370, AB871371, KT381013, XM_010646789, and XM_059735497) were identified in the NCBI genome database for *Vitis vinifera* L.

Sequence alignment and assembly analyses identified two primary homologous PPO genes in grapes: *VvPPO1* (scaffolds NW_003724172 and NW_003724396) and *VvPPO2* (scaffold NW_003724396), both located on chromosome 10 in the NCBI grape genome database. The homology between *VvPPO1* and *VvPPO2* was 73% and 63% at the nucleotide and amino acid levels, respectively (Suehiro et al., 2014). Phylogenetic analysis showed that *VvPPO3* and *VvPPO4* are closely related to *VvPPOs*, with *VvPPO3* (scaffold KT381013.1, chromosome 10) clustering with *VvPPO1* (95% nucleotide identity) but showing lower similarity to *VvPPO2* (64%). *VvPPO4* exhibited reduced similarity (41–43%) to the other *VvPPOs* and is located on scaffolds NC_081807 and NC_081823 (chromosomes 3 and 19) (Katayama-Ikegami et al., 2017).

In this study, the *VvPPO1* gene was selected for its role in EB during grape berry maturation (Suehiro et al., 2014; Dry & Robinson, 1994; Velasco et al., 2007; Goes da Silva et al., 2005; Rosales et al., 2013). Its sequence entries are available in NCBI (accession numbers: Z27411, U83274, MN164611). The *Vitis vinifera* L. Elongation Factor 1- γ (*VvEF1- γ*) (Wei et al., 2021) was used as the internal reference gene. Online software Primer-BLAST (NCBI) and Oligo Analyzer 3.1 were used to design primer pairs for this experiment (Table 1).

The expression levels of the *VvPPO1* gene were quantified by qRT-PCR (MIC Thermal Cycler, Australia) following the manufacturer's instructions. Reactions (10 μ L) included 1 μ L cDNA, 5 μ L SYBR Premix Ex Taq II (Biofact), 0.5 μ L each of forward and reverse primers (10 μ M), and 3 μ L nuclease-free water. PCR conditions were 95°C for 15 min, followed by 40 cycles of 95°C (30 s), annealing (20 s), and 72°C (20 s). Reactions were performed in triplicate for each sample, and threshold cycle (Ct) values were determined using micPCR software (version 2.12.6). The specificity of PCR products was validated by dissociation curve analysis and 2% agarose gel. Gene expression levels were calculated using the Livak ($2^{-\Delta\Delta C_t}$) method following normalization to an internal reference gene (Livak & Schmittgen, 2001). Analysis of variance (ANOVA) was performed to assess significant differences in gene expression levels during grape berry development (Casassola et al., 2015).

Table 1. Nucleotide sequences and Ta (°C) of primers used in this study.

Target gene	Gene Accession	Primer name	Sequence (5'-3')	Ta (°C)	Length	Product length
<i>VvPPO1</i>	NM_001281116.1	<i>VvPPO1</i> F10	ATGCTAGTTCCCCATCATCAC	58	21	199
		<i>VvPPO1</i> R10	CGGTATGGGTAACCAAGGAAA	58	21	
<i>VvEF1-γ</i>	XM_002264364.5	<i>VvEF1-g</i> F4	TGGACGTGCTGTATACCTTC	58	20	204
		<i>VvEF1-g</i> R3	TGAGGGAAGTCCGAGGTAAAG	58	21	

VvPPO1: *Vitis vinifera* L. Polyphenol oxidase gene, *VvEF1- γ* : *Vitis vinifera* L. Elongation Factor 1- γ , Ta: Annealing temperature.

Statistical analysis

All experimental data are expressed as mean values $\pm$ standard deviation (SD) from three replicates. The comparisons between variables were performed using one-way ANOVA followed by Duncan's multiple range test ($P < 0.05$), with SAS v9.4, GenStat v12.1 software. Visualization of trait patterns was conducted as matrix plots using heatmap analysis in PAST 3 (PAleontological STatistics).

RESULT AND DISCUSSION

Total phenolic content

Comparison of TPC in peel and flesh, expressed as mg gallic acid equivalent /g of fresh weight (mg GAE/g FW) at 90 and 110 DAFB is given in Figure 1. The TPC concentration ranged from 0.21 ± 0.012 to 0.77 ± 0.008 mg GAE/g FW, with the highest concentration detected in berry peel. According to Santos et al. (2011), the phenolic compound content in peel and flesh of grape berries was 1.43 and 0.04 mg GAE g⁻¹, respectively. The TPC in both peel and flesh showed no significant change from 90 to 110 DAFB (Fig. 3a). However, the peel extract exhibited higher TPC than the flesh extract at both harvest time. These results are consistent with the findings of da Silva et al. (2022), who reported that total phenolic compounds in grape peel (0.94 to 11.05 mg kg⁻¹) were higher than in grape flesh (0.13 to 0.38 mg kg⁻¹). Most studies also reported a general decrease in phenolic compounds during berry ripening, with considerable variation in TPC among different cultivars. The TPC in different grapes may vary according to the tissue, species, cultivar, ripening stage, and environmental conditions during growth (Teixeira et al., 2013). Research indicates that TPC accumulation in grapes occurs in three phases: an initial slow accumulation, followed by a rapid increase that stabilizes, and a subsequent decrease at the end of ripening (e.g., anthocyanins). In contrast, other compounds exhibit their highest concentrations at flowering, decline between flowering and berry set, and remain constant during berry development (e.g., flavonols) (Colombo et al., 2021). These findings are in agreement with the present study and corroborate the TPC results obtained for *Vitis vinifera* L. cv. Bidaneh Sefid at 90 and 110 DAFB.

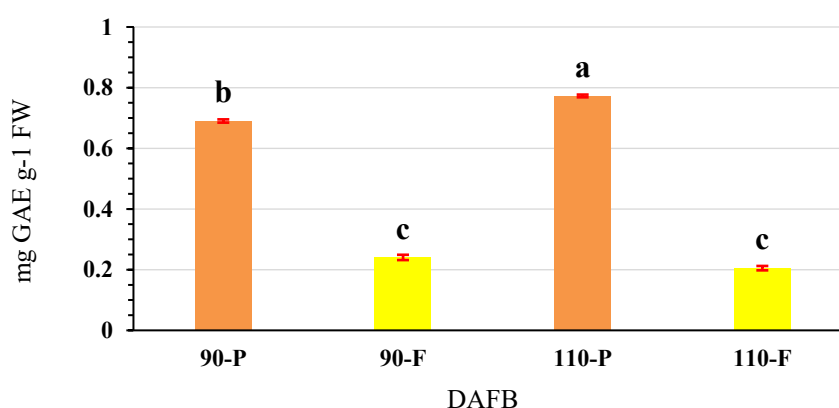


Fig. 1. Quantitative analysis of TPC (mg GAE/g FW) in peel and flesh at 90 and 110 DAFB.

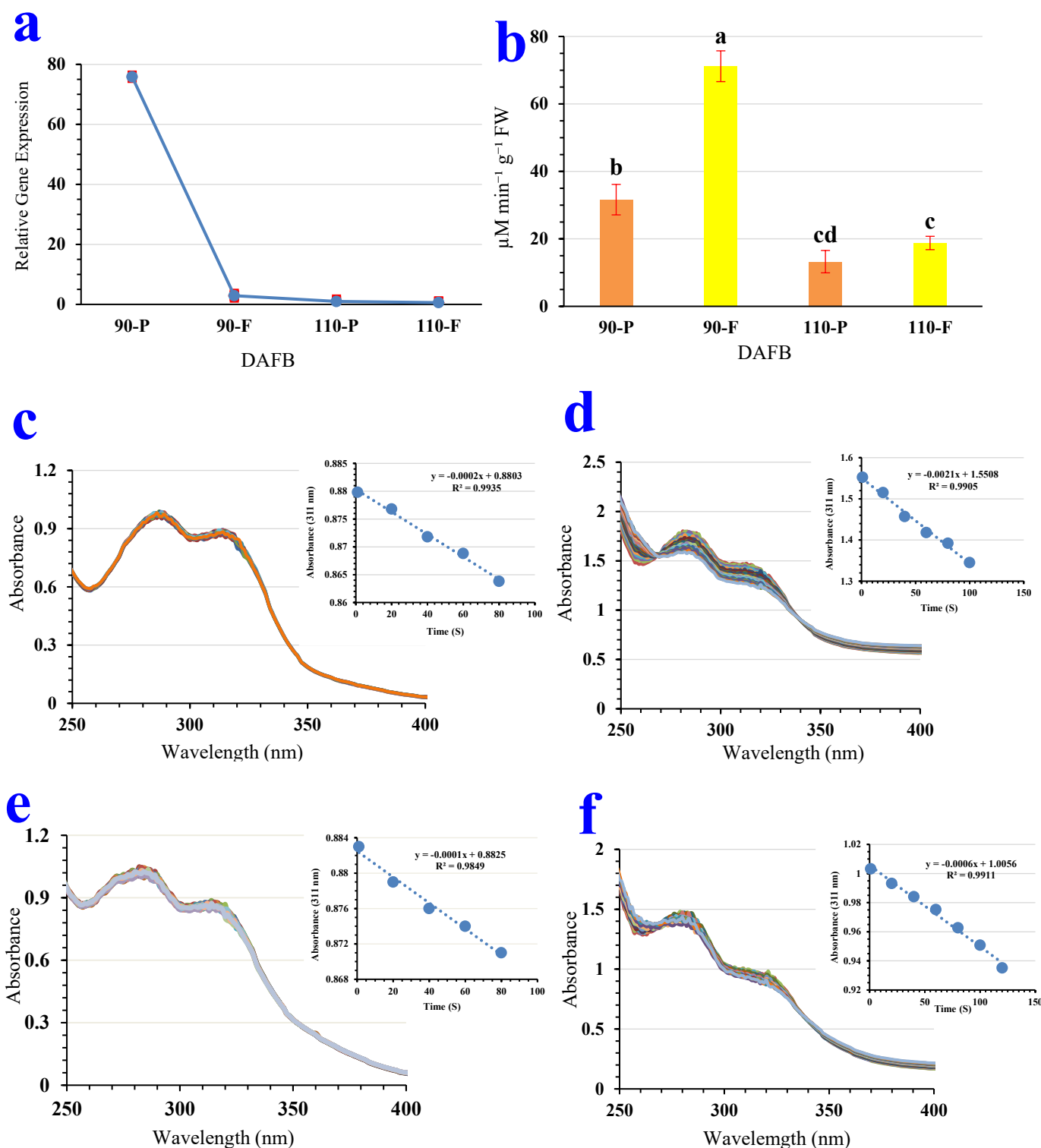
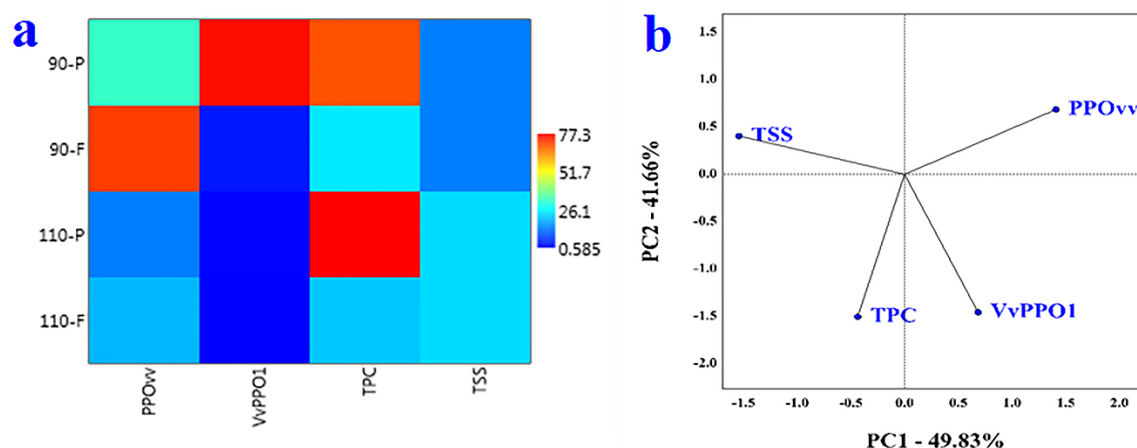


Fig. 2. Comparative analysis of (a) *VvPPOI* gene expression in grape berry peel (P) and flesh (F) at 90 and 110 DAFB and (b) Values of PPOv specific activity [activity rate in the presence of CfA in PBS (0.05 M, pH 6.3) at 22°C]; PPOv activity reactions; (c) in peel at 90 DAFB, (d) in flesh at 90 DAFB, (e) in peel at 110 DAFB and (f) in flesh at 110 DAFB, (sub-diagrams in c-f correspond to PPOv velocity curve).

Table 2. Experimental data expressed as mean $\pm$ standard deviation (SD) for peel and flesh tissues at 90 and 110 DAFB.

	Peel				Flesh			
DAFB	TSS (°Brix)	TPC (mg/g FW)	PPOvv ($\mu\text{M min}^{-1} \text{g}^{-1}$ FW)	<i>VvPPO1</i>	TSS (°Brix)	TPC (mg/g FW)	PPOvv ($\mu\text{M min}^{-1} \text{g}^{-1}$ FW)	<i>VvPPO1</i>
90	13.4 $\pm$ 1.8	0.69 $\pm$ 0.009b	31.63 $\pm$ 7.8b	75.87 $\pm$ 0.088	13.4 $\pm$ 1.8	0.24 $\pm$ 0.015c	71.17 $\pm$ 7.9a	2.894 $\pm$ 0.522
110	22.6 $\pm$ 1.7	0.77 $\pm$ 0.008a	13.26 $\pm$ 5.74cd	0.983 $\pm$ 0.516	22.6 $\pm$ 1.7	0.2 $\pm$ 0.012c	18.78 $\pm$ 3.4c	0.585 $\pm$ 0.176

PPOvv: *Vitis vinifera* L. Polyphenol oxidase enzyme, *VvPPO1*: *Vitis vinifera* L. Polyphenol oxidase gene, TPC: Total phenolic content, TSS: Total soluble solids.

**Fig. 3.** (a): Color-coded heat map revealing various values in the datasets and (b) biplot diagram showing the directions of vectors indicating the intensity of variable correlations*

* The angle between trait vectors indicates the strength and direction of the correlation between traits. Specifically, the correlation coefficient equals the cosine of the angle between vectors: when vectors are aligned (0°), the correlation is +1 (positive correlation); when vectors are perpendicular (90°), the correlation is zero (no linear relationship); and when vectors are opposite (180°), the correlation is -1 (negative correlation) (Yan & Kang, 2002).

PPOvv enzymatic activity

PPOvv specific activity was determined by utilizing CfA as a diphenolic substrate (Table 2). The crude PPOvv exhibited activity on the CfA substrate. Previous research showed that CfA has the highest binding affinity with PPOvv, making it suitable for catecholase activity assays (Unal & Sener, 2014). Figure 2 illustrates the high activity rate of PPOvv for flesh at 90 and 110 DAFB (d, f), but a slow reaction rate in the peel at both time points (c, e). The results of PPOvv activity determined for the peel and flesh are shown in the Table 2. The data indicated that PPOvv activity in both tissues varied during ripening, with significantly higher activity in flesh ($71.17 \pm 7.9 \mu\text{M min}^{-1} \text{g}^{-1} \text{FW}$) compared to peel ($31.63 \pm 7.8 \mu\text{M min}^{-1} \text{g}^{-1} \text{FW}$) (Fig. 2b). These trends are illustrated in Figure 3a for the 90 and 110 DAFB time points studied. The general pattern in both peels and flesh showed high PPOvv activity at 90 DAFB (13.4 °Brix), followed by a decline at 110 DAFB (22.6 °Brix). PPOvv activity in peel and flesh decreased by 58% and 74%, respectively, at 110 DAFB compared to 90 DAFB. Troiani et al. (2003) observed similar results to ours for the behavior of PPOvv in the peel and flesh of grape cultivars. Accordingly, PPOvv activity in the flesh was twice as high as that observed in the peel. Likewise, PPOvv activity in both tissues declined over time across all cultivars.

A significant negative correlation was observed between TSS (°Brix) and PPO_{vv} activity when PPO_{vv} was plotted against °Brix (Fig. 3b). The sugar content increased throughout grape berry maturation, aligning with findings reported by Teixeira et al. (2013). Likewise, Assefa et al. demonstrated that dark-colored grapes were produced while sugar accumulation was reduced and phenolic compounds remained unchanged (Assefa et al., 2025). In this regard, both sugar accumulation and PPO_{vv} activity are affected during berry maturation. Furthermore, research has shown that sugars act as antibrowning agents, preventing EB. Consequently, PPO_{vv} activity appears to be inversely associated with °Brix in this study. The observed decrease in activity may be attributed to several factors, including proteolytic degradation, conformational changes, reduced substrate concentrations, and transformation of phenolic compounds into other substances (Jukanti, 2017).

Expression of *VvPPOI* in the berry peel and flesh

To better understand the relationship between phenolic compound oxidation and berry browning in *Vitis vinifera* L., we quantified the expression of *VvPPOI* (NM_001281116) by qRT-PCR using gene-specific primers in the peel and flesh of *Vitis vinifera* L. cv. Bidaneh Sefid at 90 and 110 DAFB. This analysis was conducted to establish the baseline expression of the *VvPPOI* gene in berries that showed no visual signs of browning (Table 2). Regarding *VvPPOI*, the expression pattern in peel was similar to that found in flesh, with the highest induction at 90 which subsequently declined until the 110 DAFB (Fig. 2a). Heatmap analysis revealed high transcript level of *VvPPOI* in peel at 90 DAFB, which was 77-fold higher than at 110 DAFB and significantly different from other treatments (Fig. 3a, Table 2). In the flesh, a decrease in the accumulation of *VvPPOI* mRNA was also observed at 110 DAFB; however, the level was significantly lower than that in the peel (Fig. 2a). Given the higher relative gene expression of *VvPPOI* and TPC in the peel compared to the flesh, EB is likely accelerated in the peel. In this study, a positive correlation was observed between *VvPPOI* gene expression and TPC, revealing that increased gene expression in grape peel was associated with higher TPC concentrations. Likewise, elevated levels of *VvPPOI* may be attributed to both the primary localization of PPO_{vv} in peel tissue and its role in pigment formation (Thipyapong et al., 2007). Notably, the induction of visual browning in berries has been associated with the activation of defense responses in grapes exposed to stress. However, *VvPPOI* expression in both peel and flesh was induced by environmental stresses. Research has shown that manipulation of the *VvPPOI* gene expression plays a crucial role in the EB reaction of grape berries. Accordingly, suppression of *VvPPOI* expression reduces PPO_{vv} activity and decreases browning capability. Furthermore, increases in TPC strengthen the plant's defense system. Consequently, transgenic plants with reduced *VvPPOI* expression demonstrate higher susceptibility to biotic and abiotic stresses (Suehiro et al., 2014). These results are consistent with findings from previous studies. Suehiro et al. (2014) reported that expression levels significantly decreased over time from 50 to 80 DAFB. Although expression levels showed a transient increase at 90 DAFB in berries, they subsequently reduced and remained low in berry peel after 100 DAFB. Therefore, the reduction in PPO_{vv} enzyme activity during grape berry development may result from decreased *VvPPOI* expression. This finding aligns with previous studies indicating that PPO_{vv} can be detected in immature grape berries during early developmental stages and remain active from fruit set onward. Research has shown that *VvPPOI* expression occurs at high levels in young developing berries, leaves, and roots, but remains minimal in mature tissues (Fronk et al., 2017). This expression pattern likely reflects the role of the PPO_{vv} enzyme in various biosynthetic pathways during grape phenological development. Similarly, studies suggest PPOs may have functions beyond plant defense,

contributing to growth, cell differentiation, somatic embryogenesis, photosynthesis, amplification of wounding signals, and the phenylpropanoid pathway (Taranto et al., 2017).

Inhibition of PPOvv activity

Once browned, grapes experience a considerable reduction in market value. This browning is considered a physiological disorder in table grapes. Due to consumers' increasing preference for natural and eco-friendly PPO inhibitors, utilizing RCext, and CtC as natural PPO inhibitors presents a valuable strategy for controlling EB (Fig. 5).

The negative correlation between PPOvv activity and TPC, as observed in Figure 3b can be attributed to the PPO inhibitory effects of specific phenolic compounds such as CtC, QrC, Oxyresveratrol, ferulic acid, and gallic acid. As shown in this study, the reduced enzyme activity in peel compared to flesh may result from the inhibitory effects of TPC during maturation. Kamah et al. (2025) reported that CtC, QrC, polydatin and Luteolin in grape berry extract demonstrated inhibitory activity against mushroom tyrosinase. This finding is supported by the strong correlation between anti-tyrosinase activity and CtC (Kamah et al., 2025).

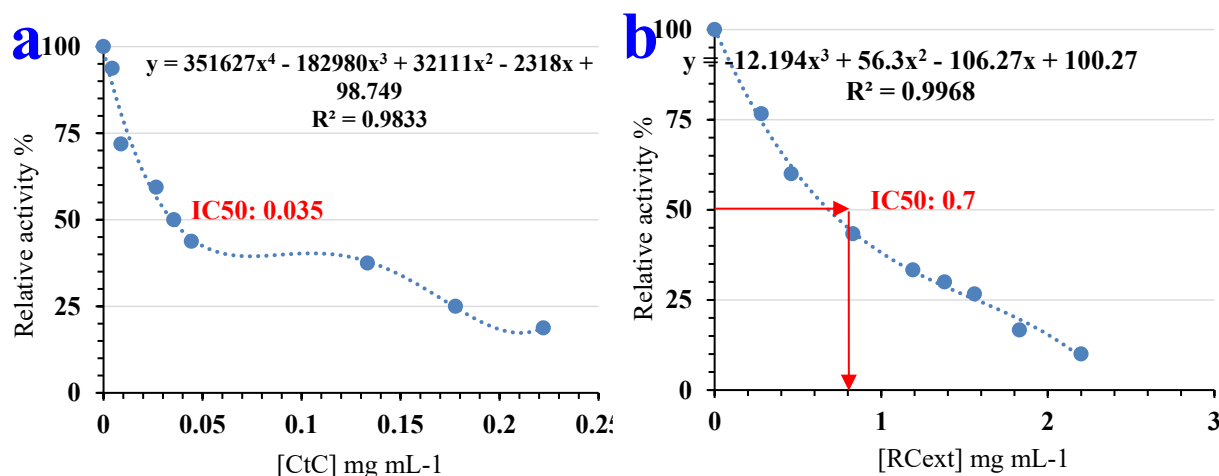


Fig. 4. Kinetics graphs of PPOvv [in the presence of various amounts of (a) CtC (0–3 mg mL⁻¹) and (b) RCext (0–3 mg mL⁻¹)] with CfA (50 µM) in PBS (0.05 M, pH 6.3) at 22°C.

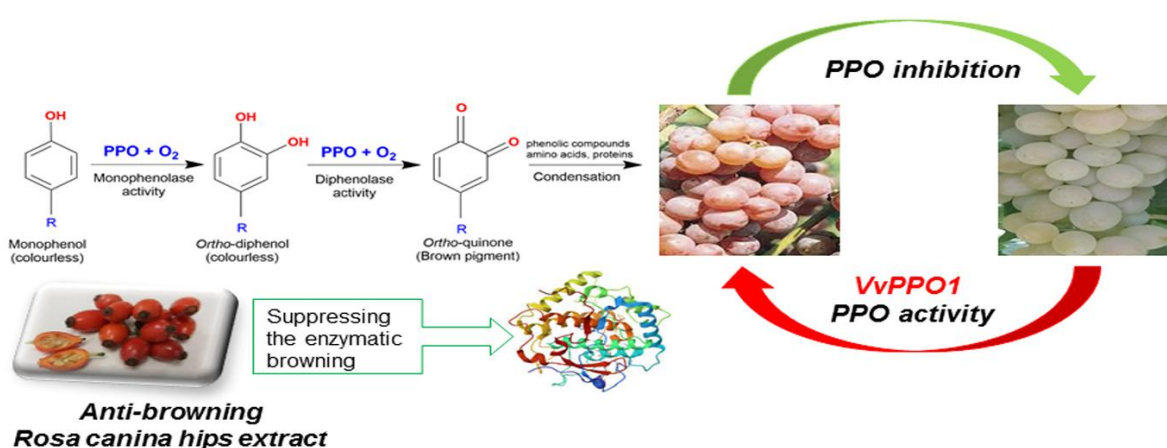


Fig. 5. The schematic of the enzymatic browning process and PPOvv inhibition of RCext.

Table 3. Variance analysis for the effects of berry maturation time points (DAFB), tissue type (PF), and their interaction (DAFB × PF) on TSS, TPC, PPOvv, and *VvPPOI* in the *Vitis vinifera* L. cv. Bidaneh Sefid.

S.O.V	df	PPOvv	<i>VvPPOI</i>	TPC	TSS
DAFB	1	3756.3**	4469.3*	0.001**	254.7**
PF	1	1523.3**	4037.9*	0.77**	0.00001**
DAFB×PF	1	867.7**	3950.7*	0.01**	0.000001**
R	2	46.63 ^{ns}	554.2 ^{ns}	0.0003 ^{ns}	0.000001 ^{ns}

S.O.V; Source of variation, df: Degree of freedom, PPOvv: *Vitis vinifera* L. Polyphenol oxidase enzyme, *VvPPOI*: *Vitis vinifera* L. Polyphenol oxidase gene, TPC: Total phenolic content, TSS: Total soluble solids.

ns, *, **: Non-significant and significant at 5% and 1% probability levels, respectively, Showing significant differences according to Duncan's test for each factor DAFB: days after full bloom, PF: Peel and Flesh, and their interaction.

IC50 values

In this study, the catecholase activity of PPOvv was measured spectrophotometrically in the absence and presence of different concentrations of CtC and RCext using CfA as the substrate. The IC50 values, indicating the concentration required to achieve 50% of PPO activity, were determined from dose-response curves and are presented in Figure 4. The kinetic curves in Figure 4a-b were well fitted to a polynomial regression model ($R^2 \geq 0.99$). The dose-dependent response demonstrated that CtC was a more effective inhibitor of PPOvv activity, with an IC50 value of 0.035 mg/mL, compared to 0.7 mg/mL for RCext. Consequently, CtC exhibited a 20-fold greater inhibitory effect than RCext. Since CtC is one of the main phenolic compounds in grapes, it may effectively prevent grape browning. Research has demonstrated that unripe grape juice, depending on the cultivar, inhibits commercial PPO enzyme activity by 56% to 68%. CtCs are recognized as depigmenting agents that may contribute to the whitening effect observed in unripe grapes (Tinello & Lante, 2017). A similar study demonstrated that quercetin glycosides are among the most important PPOvv inhibitors in grape peel (Okuda et al., 2019). RCext is rich in bioactive compounds that display both antibrowning activity and strong antioxidant potential (Zocca et al., 2011). Research has shown that CtC is the most abundant flavonoid in *R. canina*, with concentrations from 225.3 to 472.7 mg/kg, while rutin is the second, ranging between 65.3 mg/kg and 256.9 mg/kg. Moreover, rosehips serve as a rich source of anthocyanins, which have been used as natural colorants in the food industry (Zhou et al., 2023). Based on these mechanistic findings, a marked difference in browning progression is expected between RCext-treated and untreated grape berries during storage. Future studies will validate this application through a dedicated shelf-life study, with visual documentation of browning inhibition in whole fruit as a key outcome measure.

CONCLUSION

PPO catalyzes the endogenous oxidation of phenolic compounds and is responsible for EB in plant products. EB represents a major problem for various grape-based products, particularly table grapes and juices, considerably reducing their market value. Based on this result, some phenolic compounds in grapes act as inhibitors of PPOvv activity. Enzymatic studies demonstrated that authentic standards of CtC and RCext effectively inhibit PPOvv. Furthermore, RCext, as a viable and low-cost natural source of this compound, extends grape shelf-life and meets clean-label consumer preferences. These findings highlight CtC and RCext treatment at veraison may affect EB, given that both *VvPPOI* gene expression and PPOvv activity increase at 90 DAFB. Collectively, these results suggested that RCext and CtC are promising alternatives to SO₂ and can be used as antibrowning agents in fresh-cut

products via dipping treatments or incorporation into edible coating formulations. To further elucidate the effects of CtC and RCext in controlling browning reactions, a comprehensive study of qualitative parameters in grape berries should be conducted.

Conflict of interest

The authors have no conflict of interest to report.

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