



OPEN Variation in antioxidant capacity, antioxidant activity and mineral composition during flower development of oil-bearing rose (*Rosa damascena* Mill.)

Damla Önder

Oil-bearing rose is an economically important rose species with a wide range of uses such as cosmetics, perfumery, food and health, but the changes in antioxidant capacity and antioxidant activity during flower development are not well understood. The antioxidant content and free radical scavenging properties of oil-bearing rose flowers are used in the cosmetic industry to modulate skin damage, and in the food industry as a source of antioxidants and sweeteners. The present investigation was carried out to explore the antioxidant capacity, antioxidant enzyme activity, and the composition and distribution of minerals in petals of oil-bearing rose at five flower development stages. The total antioxidant capacity of petals was determined using CUPRAC, DPPH, FRAP, FIC and ABTS methods. The antioxidant capacity of petals decreased during the flower development, suggesting that flowers in stage I and II are valuable sources of antioxidants. CUPRAC, DPPH, FRAP, FIC and ABTS scavenging activity of the petals at various developmental stages are strongly and positively correlated with each other. The activity of the antioxidant enzymes; superoxide dismutase, catalase, glutathione reductase, ascorbate peroxidase was highest at the bud stage (stage I), whereas the fully opened flowers (stage V) exhibited the lowest activity in oil-bearing rose petals. During the development of flower, malondialdehyde (MDA) content increased significantly from stage I to stage III and decreased at stage IV. Here we detected the contents of 15 elements in petals, some of them, especially calcium, magnesium, potassium and phosphorus showed significant changes during rose flowering. Generally, the highest mineral content was observed in stage I while the lowest content observed in stage V of flower development. These results showed a close link between flower development, antioxidant capacity, enzymatic antioxidant activity and mineral content, with stage I exhibiting the best antioxidant activity, mineral content and free radical scavenging potential. This work will serve as a baseline for understanding the possible roles of antioxidant capacity, antioxidant enzymes, mineral content and their interactions in the regulation of flower development.

The oil-bearing rose (*Rosa damascena* Miller) is a hybrid between *R. gallica* and *R. phoenicia* and is an important member of Rosaceae family because its flowers are used in essential oil production. The flowering period of oil-bearing rose extends from late April to early June, and flower development from bud stage to full bloom takes approximately 11–13 days¹. Oil-bearing rose flowers are collected by hand in the early hours (4:00–10:00 A.M.) of the morning and used for industrial purposes immediately after harvest. Approximately 55–60% of the flowers of the oil-bearing rose produced are used for rose oil and rose water production, 40–45% for rose concrete and rose absolute and 1–2% for food purposes².

In traditional medicine, oil-bearing rose flowers have been used as for chest and abdominal pains, menstruation and digestive ailments (mild laxative for constipation), and as a cardio-tonic agent to strengthen the heart^{3,4}. Rose flowers are used traditionally as food and medicinal purposes, not only for their visual appeal, sweet taste and flavor, but also for their high antioxidant capacity and antioxidant enzyme activity. There are numerous known sources of natural antioxidants, including edible flowers. Essential oil⁵, decoction⁶, absolute⁷, aqueous⁸,

Department of Biology, Faculty of Arts and Sciences, Süleyman Demirel University, Isparta 32260, Turkey. email: damlaguvercin@sdu.edu.tr

methanol¹ and ethanol⁵ extracts of oil-bearing rose are used because of their antioxidant activity. Oil-bearing rose flowers possess one of the highest antioxidant activity and have been tested as a caffeine-free tea making source⁹. Therefore, the high antioxidant capacity and activity of oil-bearing rose flowers are used as a source of antioxidants and sweeteners in the food industry and for therapeutic purposes in traditional medicine, as well as their beneficial effects in scavenging free radicals³. Furthermore, the mineral compositions found in roses are among the alternative sources of nutrition that are vital to healthy functioning of an organism.

Since petals are flower organs that primarily determine the commercial importance of flowers, emphasis has been given to the cultural, physiological and biochemical processes that occur during flower development^{10,11}. Flower development is a progressive oxidative degradation process and developmental processes have been shown to be significantly influenced by reactive oxygen species (ROS), which are produced by metabolic processes in organelles¹². A synergistic antioxidant defensive system, which enzymes like catalase (CAT), peroxidase (POX), superoxide dismutase (SOD), and glutathione reductase (GR) has been theorized to protect cells from damage caused by ROS^{13,14}. Antioxidant enzymes typically have the ability to delay or prevent the initiation and spread of oxidative chain reactions¹⁵. Additionally, these compounds function as metal chelators to inhibit the production of hydroxyl radicals¹⁶. Notably, enzymatic defense systems involving superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR) and ascorbate peroxidase (APX) also contribute to ROS scavenging along with antioxidant compounds. The production of ROS such as superoxide (O_2^-) and hydrogen peroxidase (H_2O_2) by cells is an indication of plants development^{17,18}. SOD, CAT, GR and APX are important enzymes playing crucial roles in antioxidant defense. SOD catalyzes the dismutation of O_2^- to O_2 and H_2O_2 , and CAT, APX and GR then catabolize H_2O_2 into H_2O and O_2 , thereby limiting the additional radical production from H_2O_2 ^{19,20}. H_2O_2 is a potent oxidizing agent that can activate the signaling pathway to stimulate cellular proliferation or differentiation²¹. It is produced in the biological system by antioxidant enzymes such as SOD²². The increase in H_2O_2 content causes the formation of hydroxyl radical ($\cdot OH$), which initiates lipid peroxidation and damage to cellular components²³. Therefore, the regulation of ROS production by enzymatic antioxidants is of great interest in biological research.

The ability of redox compounds in food and biological systems to scavenge free radicals is known as antioxidant capacity. The capacity of antioxidant enzymes have been reported to play an important role in the regulation of flower development²⁴. However, there is no agreement on an ideal method for estimating the antioxidant capacity²⁵. The cupric reducing antioxidant capacity (CUPRAC) assay uses copper (II)-neocuproine reagent as the oxidizing agent and is a very fast way to evaluate the antioxidant activity of extracts in a short time. In this method, the increase in absorbance was measured at 450 nm based on the reduction of copper(II)-neocuproin to the highly colored copper(I)-neocuproin chelate as a result of the color change from light blue to orange yellow. Over other chromogenic reagents (such as 2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH)), CUPRAC has the advantages of being more stable and accessible²⁶. Using an assay for the reduction of Fe^{3+} to Fe^{2+} , the ferric reducing antioxidant power (FRAP) technique determined the reducing capacity of petal samples. The Fe^{3+} /ferricyanide complex is converted to the ferric form in the presence of reducing agents that also serve as antioxidants in the samples²⁷. FRAP acts as a reducing agent with its ability to donate a single electron or hydrogen for the reduction of antioxidants²⁸. FRAP capacity is due to the presence of antioxidant compounds involved in electron transfer. These antioxidant compounds have the ability to neutralize free radicals, convert them into stable compounds and terminate the reactions initiated by free radicals²⁹. The most common method for assessing the radical-scavenging capacity of plant extracts is the DPPH assay³⁰. The antioxidants are the parts of plants that can physically quench the stable purple DPPH radical, turning it into a yellow DPPH radical, which can be seen^{21,31}. The DPPH test measures the radical scavenging ability of extracts. Iron causes the formation of $\cdot OH$ by stimulating lipid peroxidation³². Iron ions are also considered the most effective pro-oxidants widely used in the food industry. The antioxidant capacity in ethanolic extracts of *R. damascena* showed a high antioxidant capacity when compared with the standard antioxidant L-ascorbic acid³³. The antioxidant capacity of the fresh flower extract of *R. damascena* flowers was higher than the spent flower extract³⁴. The antioxidant effect of oil-bearing rose and its inhibitory effect on lipid oxidation were evaluated in an in vivo study. The results suggested that oil-bearing rose showed a potent antioxidant and lipid peroxidation inhibitory effect comparable to tocopherol, and the rose could be considered as a medicinal source for the treatment and prevention of many free radical diseases⁴.

Since antioxidant compounds and antioxidant activity of enzymes are important part of flower development, it is necessary to investigate antioxidant content and antioxidant enzyme activities to better understand their changes during flower development to determine a suitable period for harvesting. Though, there are some reports on antioxidant capacity and antioxidant activity of rose petals^{1,35,36}, to the best of our knowledge, there is no available information on antioxidant enzyme activity and antioxidant capacity at the different developmental stages of oil-bearing rose. Mineral distribution and metabolic changes in rose flowers that occur during the growth of oil-bearing rose petals are yet to be studied. The present study was conducted to characterize the mineral content, antioxidant capacity and enzymatic antioxidant activity at five distinct development stages of oil-bearing rose, which is traditionally used as cosmetic, pharmaceutical and food. Thus, flower development periods with high ratios of these components can be determined and flowers in this period can be preferentially harvested at a suitable development period.

Results

Total antioxidant capacity of oil-bearing rose petals at different flower development stages

Different in vitro antioxidant test methods could be used to determine the total antioxidant capacity (TAC), a crucial quality parameter for medicinal and edible plants. Each assay has its advantages and disadvantages, but not a single assay could measure antioxidant capacity accurately. Thus, it was necessary to determine antioxidant

capacity of oil-bearing rose petals by CUPRAC, DPPH, FRAP, ferrous ion-chelating (FIC) and ABTS assays in the present study (Fig. 1).

CUPRAC assay showed a range of 0.13 ± 0.001 – 0.65 ± 0.007 mmol Trolox g^{-1} for oil-bearing rose in this study. In general, the highest CUPRAC value was found in stage I (0.65 ± 0.007) and it decreased significantly to 0.13 ± 0.001 at stage V of flower development (Fig. 1a). There was no significant statistical difference between the last stages (stages IV and V) of flower development. The DPPH· scavenging activity decreased from 0.68 ± 0.002 to 0.55 ± 0.004 mmol Trolox g^{-1} throughout the flower development of oil-bearing rose. While DPPH· anti-oxidant capacity measurement results were statistically same for the first two stages, it decreased from 0.68 to 0.58 mmol Trolox g^{-1} from stage II to stage III and continued to decline from stage III to IV and did not change in stage V of flower development (Fig. 1b). The antioxidant capacity measured by FRAP assay showed a similar pattern to CUPRAC assay (Fig. 1c). FRAP values were between 0.22 ± 0.009 and 0.83 ± 0.05 mmol Trolox g^{-1} . As shown in Fig. 1c, the FRAP value was 0.83 mmol Trolox g^{-1} at stage I, then decreased significantly until stage III (0.24 ± 0.01) and remained unchanged between stage III to V. The FIC scavenging activity of oil-bearing rose petals at different developmental stages was found to be ranging from 0.35 ± 0.01 to 0.42 ± 0.02 mmol EDTA g^{-1} (Fig. 1d). The highest FIC value was detected in stage I and II, and dropped significantly to 0.37 ± 0.001 at stage III and did not change the stages III to V. The highest ABTS activity among the five developmental stages was observed in Stage I (Fig. 1e) similar to the other methods. The ABTS values continuously decreased from

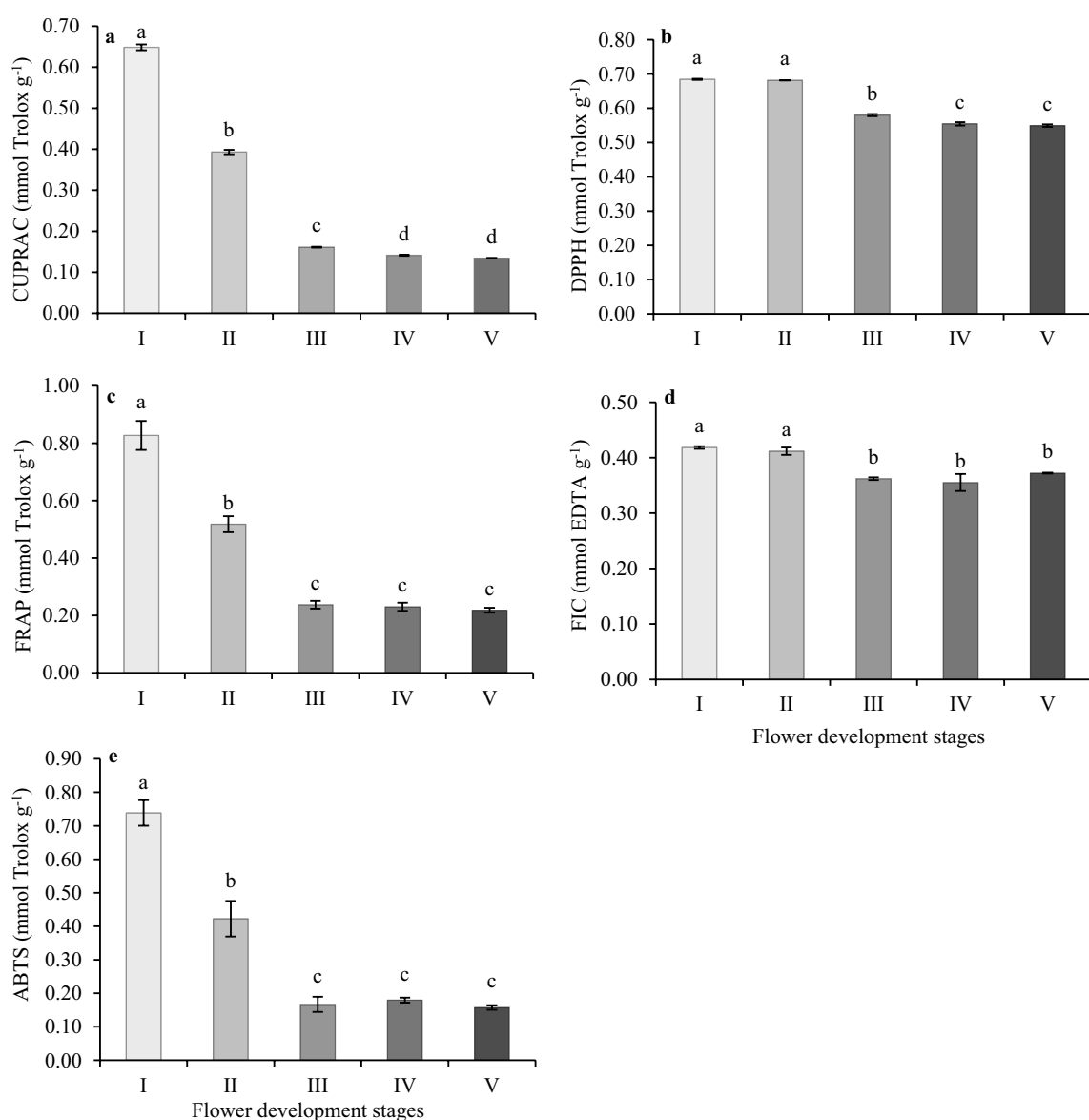


Figure 1. Total antioxidant capacities of oil-bearing rose petals during the flower development. (a) CUPRAC radical scavenging assay, (b) DPPH radical scavenging assay, (c) FRAP radical scavenging assay, (d) FIC radical scavenging assay, (e) ABTS radical scavenging assay. Data are represented as mean \pm standard deviation ($n = 3$). Mean values with a different letters were significantly different ($p \leq 0.05$).

0.74 ± 0.04 to 0.16 ± 0.007 mmol Trolox g^{-1} from stage I to V. It reduced by 43% in stage II and 77% in stage III as compared to stage I.

Antioxidant enzyme activities of oil-bearing rose petals at different stages of flower development

SOD activity decreased significantly from stages I to II and III, thereafter, the SOD activity decreased further at stage IV and remained the same at stage V (Fig. 2a). SOD activity was 35.5, 55.5, and 58.5% less at II, III and V stages, respectively, compared to stage I. The highest CAT activity was observed at stage I (9.06 U mg/protein) and III (7.05 U mg/protein), and the CAT activity was not significantly different at stages IV and V (Fig. 2b). The lowest CAT activity was observed at the stage II (5.73 ± 0.6 U mg protein $^{-1}$) of flower development. GR activity showed a similar pattern to CAT activity during the flower development of oil-bearing rose, hence the GR activity was highest in stage I (15.84 ± 1.67 U mg protein $^{-1}$) and was at the lowest in stage II (9.75 ± 0.24 U mg protein $^{-1}$), and significantly increased at stage III (15.29 ± 0.834 U mg protein $^{-1}$) (Fig. 2c). GR activity was not significantly different between the stage IV and V. APX activity also showed a similar activity pattern to SOD activity during flower development of oil-bearing rose plants (Fig. 2d). A significant decrease from stage I (94.53 ± 2.24) to stage II (47.15 ± 1.3) and a significant increase at stage III (55.6 ± 0.46) was observed. APX activity later dropped to 18.303 U mg protein $^{-1}$ at stage IV and its activity did not change at stage V. The H_2O_2 content was significantly higher during the stage II (439.85 ± 5.9 $\mu\text{mol } g^{-1}$) and stage III (444.54 ± 5.3 $\mu\text{mol } g^{-1}$) compared to stage I. In

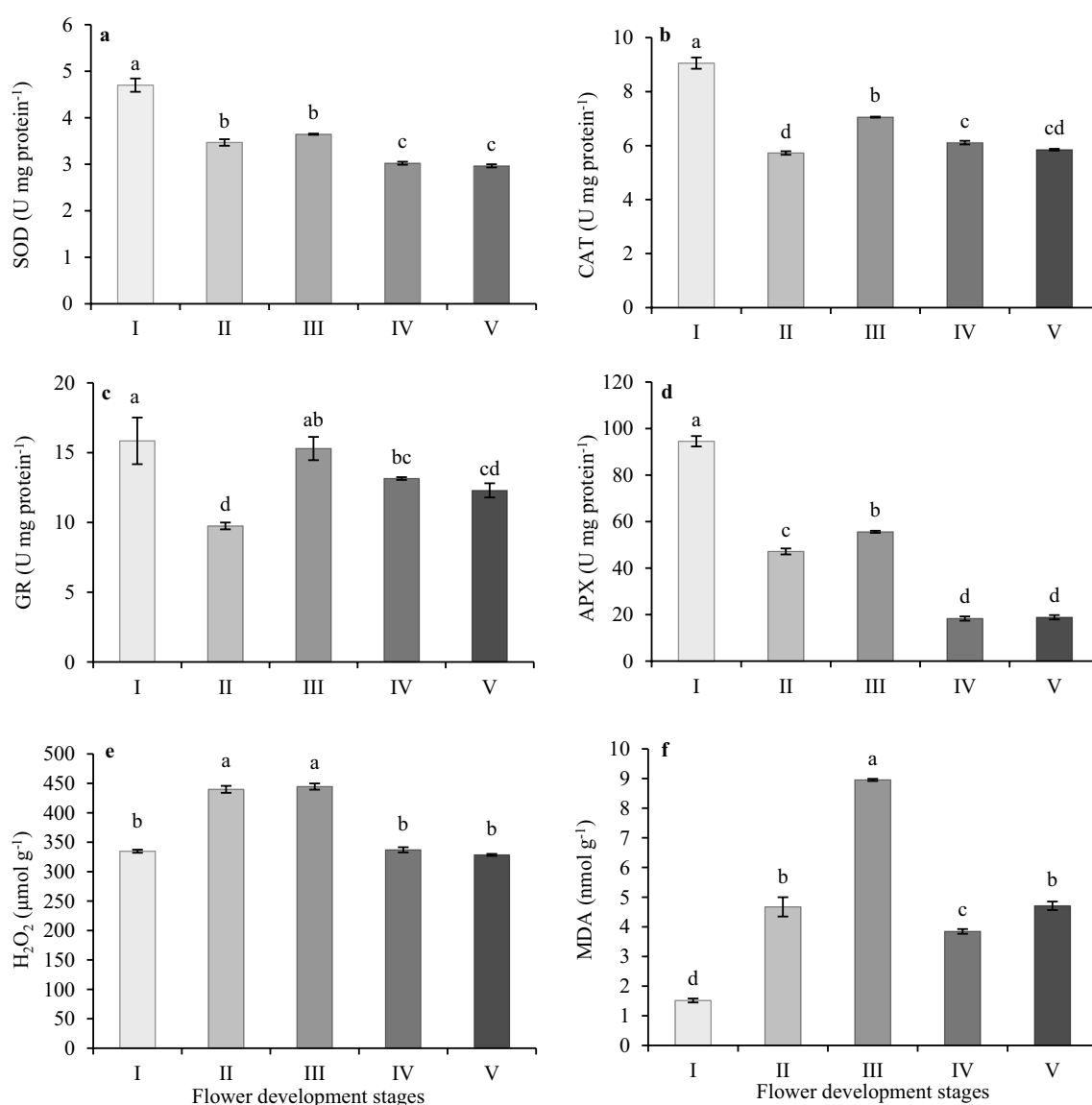


Figure 2. Antioxidant enzyme activities of oil-bearing rose petals during the flower development. (a) superoxide dismutase activity (SOD), (b) catalase (CAT), (c) glutathione reductase (GR), (d) Ascorbate peroxidase (APX), (e) hydrogen peroxide (H_2O_2), (f) malondialdehyde (MDA). Data are represented as mean \pm standard deviation ($n = 3$). Mean values with a different letters were significantly different ($p \leq 0.05$).

contrast, a significant decrease in H_2O_2 ($337.12 \mu\text{mol g}^{-1}$) was recorded in stage IV and stage V. However, no significant differences in H_2O_2 content were observed among the stages I, IV, and V (Fig. 2e). There was a regular increase in MDA content from stage I to III and the highest MDA content was detected at stage III (8.95 nmol g^{-1}) compared to all other flower development stages. MDA content showed more than five-fold increase at stage III compared to stage I (Fig. 2f). While a significant decrease was detected from stage III to stage IV, on the contrary, a slight increase was observed from stage IV to stage V.

Mineral composition of oil-bearing rose petals at different flowering stages

In order to determine the amount of mineral nutrients, we used high-throughput elemental analysis technology (ICP-OES), and found significant variations throughout the flower development stages (Fig. 5). Al and Se contents could only be quantitatively determined at stage II, and Hg, Cd, Cr, Cu and Fe were at such low levels that they could not be determined at any flower development stage (Table 1). Ca, K, Mg, Mn, P and Si had the highest accumulation at stage I and they had the lowest accumulation at stage V. Conversely, Zn was at the highest level at stage III and lowest at stage V. While the amount of Na was too low to be determined at stages I and III, Na was detected at stages II, IV and V, and the highest level of Na was observed at stage IV (0.042 mg g^{-1}) of flower development. K was the most abundant mineral in all developmental stages, followed by P, Mg and Ca, respectively.

The correlation analysis

Correlation analysis was carried out to determine the relationships between antioxidant enzyme activities and antioxidant capacity during flower development of oil-bearing rose. The result of correlation analysis for five different flower development stages of oil-bearing rose is shown in Fig. 3. Of the 66 coefficients, 16 were significant at the $P \leq 0.05$, $P \leq 0.01$, and $P \leq 0.001$ levels. ABTS, FIC, FRAP, DPPH and CUPRAC, which are predictors of antioxidant capacity, were significantly and positively correlated with each other. APX was only positively correlated with CAT and SOD activity. SOD activity showed significant positive correlation with CUPRAC, FRAP, ABTS and CAT activity. MDA content was negatively correlated with antioxidant capacity, but it was positively correlated with H_2O_2 content.

Changes in antioxidant capacity and antioxidant enzyme activities were further analyzed with PCA analysis during the flower development and the results were shown in Fig. 4. The two principal components (PC) represented 84.19% of the total variation (66.91% and 17.28% for PC1 and PC2, respectively). The flower development stages were divided into four clusters. PCA analysis showed that stages III, IV and V were closely related and associated with MDA accumulation. The effects of flower development stages on antioxidant capacity and enzymes were distributed along the PC1 axis in the order $V > IV > III > II > I$. GR, CAT, SOD and APX were significantly changed at stage I of flower development and clustered on the right side of the PC1 axis. Antioxidant capacity was significantly affected in stages I and II and clustered on the right side of the PC1 axis.

Elemental composition	Flower development stages				
	I	II	III	IV	V
Al	tr*	0.053 ± 0.02 a	tr	tr	tr
Ca	1.219 ± 0.02 a	0.752 ± 0.03 b	0.495 ± 0.04 c	0.573 ± 0.01 c	0.316 ± 0.06 d
Hg	tr	tr	tr	tr	tr
Cd	tr	tr	tr	tr	tr
Cr	tr	tr	tr	tr	tr
Cu	tr	tr	tr	tr	tr
Fe	tr	tr	tr	tr	tr
K	18.410 ± 0.19 a	15.790 ± 0.19 b	13.140 ± 0.23 c	15.840 ± 0.24 b	11.300 ± 1.52 c
Mg	1.978 ± 0.12 a	1.494 ± 0.04 b	1.209 ± 0.082 c	1.451 ± 0.04 b	0.729 ± 0.09 d
Mn	0.030 ± 0.01 a	0.021 ± 0.02 a	0.020 ± 0.001 a	0.019 ± 0.02 a	0.011 ± 0.001 a
Na	tr	0.019 ± 0.02 ab	tr	0.042 ± 0.005 a	0.009 ± 0.001 b
P	2.540 ± 0.02 a	2.240 ± 0.07 ab	1.586 ± 0.064 bc	1.730 ± 0.28 bc	1.184 ± 0.46 c
Se	tr	0.007 ± 0.001 a	tr	tr	tr
Si	0.030 ± 0.02 a	0.020 ± 0.02 a	0.015 ± 0.003 a	0.024 ± 0.02 a	tr
Zn	0.026 ± 0.002 ab	0.014 ± 0.001 ab	0.083 ± 0.06 a	0.017 ± 0.001 ab	0.008 ± 0.002 b

Table 1. Mineral composition (mg g^{-1} DW) of oil-bearing rose petals during the flower development determined by ICP-OES. Data are represented as mean \pm standard deviation ($n = 3$). Mean separation within each row by least significant differences (LSD) at $P \leq 0.05$. Means followed by the same letters are not significantly different from one another. *tr = trace amount, less than $< 0.005 \text{ mg g}^{-1}$.

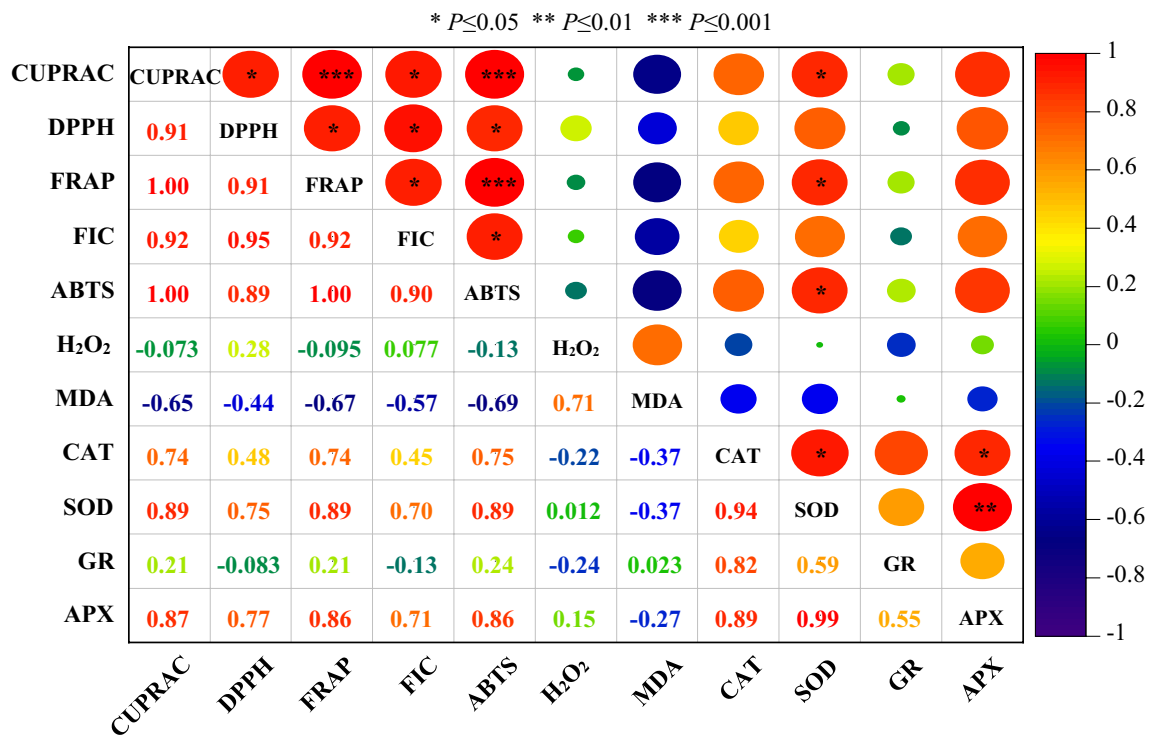


Figure 3. Correlations between antioxidant capacity and antioxidant enzyme activities generated by a heat map using mean values obtained from five flower development stages of oil-bearing rose. The color scale displays the intensity of the correlation coefficient values of the measured parameters. (*H₂O₂* hydrogen peroxidase, *MDA* malondialdehyde, *CAT* catalase, *SOD* superoxide dismutase, *GR* glutathione reductase, *APX* ascorbate peroxidase).

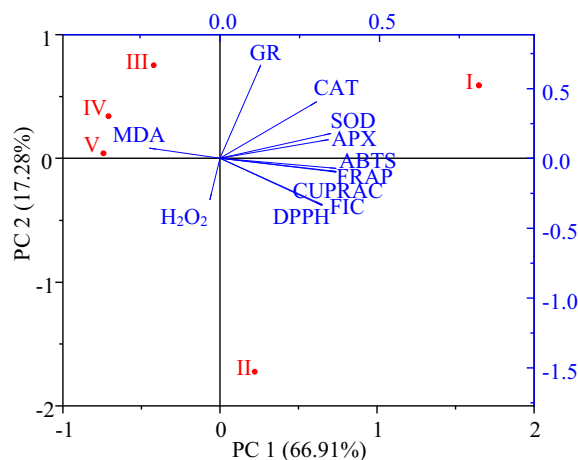


Figure 4. Principle component analysis (PCA) of antioxidant capacity and antioxidant enzyme activities during flower development of oil-bearing rose. (*H₂O₂* hydrogen peroxidase, *MDA* malondialdehyde, *CAT* catalase, *SOD* superoxide dismutase, *GR* glutathione reductase, *APX* ascorbate peroxidase).

Discussion

The antioxidant content and free radical scavenging properties of plants are used in the cosmetic industry to modulate skin damage³⁷. Therefore, antioxidant-rich plants are widely used to protect against oxidative degradation. Recent decades have seen a rise in interest in nutrition and food science thanks to natural diets with antioxidant activity³⁸. In general, the human health benefits of edible flowers are attributed to their antioxidant activity, namely their pronounced inhibitory effects on free radicals^{39,40}. Since antioxidants in flowers are known as natural antioxidants, they are used as an alternative to prevent oxidative degradation of foods, thus minimizing the harm caused by these oxidative compounds in humans⁴¹. The important antioxidant activity in edible flower extracts, such as *Begonia*, *Tropeolum* and *Rosa* were reported^{42–44}.



Figure 5. Five developmental stages of oil-bearing rose flowers used in the study.

Plants, due to differences in flower development, experience biochemical changes and ROS generation resulting in a decrease in antioxidant activity⁴⁵. As flower development progressed in oil-bearing rose, SOD and CAT activity decreased, leading to $O_2^{\cdot-}$ accumulation, resulting in increased MDA content at stage III. Contrary to our results, SOD activity increased in gladiolus (*Gladiolus grandiflora*) from early stages to stage IV of flower development, and then decreased after the blooming of flowers¹⁷. The activities of CAT and GR in oil-bearing rose showed a marginal decrease from the stage I to stage II, and showed a slight increase in stage III. Gladiolus flowers showed a continuous decline in CAT activity from bud stage through the full bloom stage¹⁷. In apples, CAT, SOD and peroxidase enzymes had higher activity during early stages of flower development⁴⁶. It was possible that the increases in CAT activity at bud stage could have resulted from the accumulation of H_2O_2 due to a higher rate of respiration during the pre-flowering stage. While CAT, GR and APX activity was the highest at bud stage, H_2O_2 content was lower, which suggests that antioxidant enzyme activities were sufficiently high to scavenge excess H_2O_2 accumulation. Furthermore, radical scavenging activity showed an increased trend from bud to full flowering stages in safflower (*Carthamus tinctorius*) flowers⁴⁷. The scavenging power of free radicals produced in the samples is correlated with the measured antioxidant capacity. The key enzyme, APX, is used by plants to detoxify H_2O_2 using the ascorbate glutathione cycle⁴⁸. Shamsi et al.⁴⁹ and Cheruth et al.⁵⁰ reported that APX activity was significantly high at the pre-flowering stage and declined during the flowering and post-flowering stages in date palm. The decrease in APX activity with development progression is due to decreased SOD activity⁴⁸. Throughout flower development, the scavenging capacity decreased because of an increase in ROS accumulation and a decrease in the activity of antioxidant enzymes. The behavior of antioxidant enzymes at different stages of flower development in oil-bearing rose reflects their active role in managing during the developmental processes.

Loss of membrane permeability during blooming and developmental processes may be caused by the oxidation of already-existing membrane components, such as increased lipid peroxidation during flowering. Accordingly, the combined reduction of antioxidant capacity and free radical scavenging system as antioxidant enzyme activities during the open flowering stage may result in a higher accumulation of reactive oxygen species (ROS)^{46,51}. However, the balance between H_2O_2 accumulation and neutralization changed hence antioxidant enzyme activities were statistically significantly reduced at stage II, and a significant increase in MDA level was also observed at stages II and III, which prompted an overall increase of antioxidant enzyme activities at stage III of flower development.

There are different methods to evaluate the antioxidant capacities in extracts, therefore five different methods were selected for the evaluation of the antioxidant activity in oil-bearing rose, which differs from each other in terms of substrates, probes, reaction conditions, and quantification methods. All five different methods showed that stage I of oil-bearing rose flowers showed the strongest antioxidant capacity, and flowers in stages IV and V had the lowest antioxidant capacity. Therefore, the best harvest time for oil-bearing rose flowers are stage I and II for the capture of the highest antioxidant capacity. Similar results have also been reported for apple cultivars (*Malus* spp.) and *R. hybridia* petals^{52,53}. ABTS, DPPH and FRAP results revealed that apple petals at the bud stage exhibited much higher antioxidant activity compared to the other four developmental stages. However, the antioxidant capacity of daylily flowers (*Hemerocallis fulva* Linn.) showed an increasing trend during flower development⁴². Concerning reducing power, chelating power and lipoperoxidation inhibition capacities, borage flower (*Borago officinalis* L.) extracts showed an increase in antioxidant levels during flower development and reached their maximum at full bloom⁵⁴. The strong antioxidant capacity of oil-bearing rose flowers at bud stages suggests that they can be used as a source of bioactive substances, and they could have great potential to improve human health. However, due to the fact that the DPPH radical is unstable and can only respond to lipophilic antioxidants⁵⁵, it showed a different tendency to decrease compared to other antioxidant capacities during the development of oil-bearing rose. Therefore, it is recommended to perform two or more antioxidant capacity tests in studies.

In addition, since phenolic compounds are important plant components with redox properties responsible for antioxidant capacity, there is an important linear relationship between total phenolic components and antioxidant capacities^{21,56}. In our previous study⁵⁷, it was determined that the polyphenols content of the oil rose decreased during the development of the oil-bearing rose, and in the current study, the CUPRAC, FRAP and ABTS contents decreased during the development of the oil-bearing rose. When the results of these two studies are examined together, the hypothesis that there is a linear relationship between antioxidant capacity (CUPRAC, FRAP and ABTS) and polyphenol content in oil-bearing rose is supported.

Mineral concentrations in the petals of oil-bearing rose varied throughout by the developmental stages of flowers. The mineral composition shows that K was the most abundant mineral at all flower developmental stages, followed by P, Mg, Ca, Mn, Si and Zn. For the main effects of flowering, the maximum amount of Ca, K, Mg, Mn, P and Si concentrations were at bud stage, while the minimum was at fully opened flowers stage. Stage I of oil-bearing rose flower development had the highest concentrations for the mineral compounds, which have various potential health benefits as they affect homeostasis and metabolism⁵⁸. Such high mineral content at the bud stage may be linked to a better accumulation of these nutrients in the early days of flower development and potentially a lower vegetative-generative growth rate. Ca plays an important role in many plant signal transduction pathways⁵⁹, cell division and cell wall formation⁶⁰. Therefore, higher Ca concentration may be associated with petal development and expansion from bud stage to full bloom in oil-bearing rose flowers. In *Opuntia* flowers, it was determined that the K content decreased at full flowering stage compared to the initial flowering, while Ca, Mg and Na contents increased⁶¹. As petals contain less than 0.005 mg g⁻¹ of Cd and Cr, the levels are within acceptable limits when compared to other edible flowers⁶². These toxic elements in the petals at all stages of development showed concentrations below the maximum tolerable limit, which is not harmful to human health in terms of toxicity.

Multivariate analysis methods such as heat-mapped correlation and PCA can show the effects of developmental stages of oil-bearing rose flowers on antioxidant activity and antioxidant capacity. A strongly significant correlation ($r^2 = 0.94$) was observed between the DPPH and FRAP tests in different Zhongyuan tree flowers, concluding that the two methods for Zhongyuan tree flowers were comparable⁶³. A positive correlation ($0.874 \leq r^2 \leq 0.968$) was found between the antioxidant capacities determined by FRAP, DPPH and ABTS methods during the development of loquat (*Eriobotrya japonica*) flowers⁶⁴. Similarly, Wong et al.⁶⁵ determined a strong correlation between DPPH and FRAP methods. Positive correlations were observed between the antioxidant capacity of the oil-bearing rose petals measured by various methods (CUPRAC, DPPH, FRAP and ABTS). However, a strong correlation was observed between FIC activity and all antioxidant capacities, which may indicate that antioxidants may be the main chelators of ferrous ions. So far, a clear relationship between antioxidant activity and ferrous ion chelating activities has not been reported. PCA revealed the accumulation of antioxidant capacity and antioxidant enzyme activities, as well as decreased capacity and activity of antioxidants from stage III of flower development.

Conclusion

Antioxidant capacity, antioxidant enzyme activities and mineral compositions were associated with the flowering stages of oil-bearing rose. Our results demonstrated that the antioxidant capacity, as measured by CUPRAC, DPPH, FRAP, FIC and ABTS methods, was at the maximum level at bud stage and then significantly decreased throughout flower development. Our results also showed that the levels of antioxidant capacity are different due to different stages of flower development. The activities of SOD, CAT, GR and APX were the highest at stage I and the lowest at stage IV and V. Mineral composition of petals changed depending on the development stage in oil-bearing rose flowers. The antioxidants found in oil-bearing rose flowers at different stages of development have strong development potential for market applications in the nutraceutical or cosmetic industries. These results can provide a theoretical basis for further use of oil-bearing rose flower sources. The antioxidant abilities of oil-bearing rose flowers may have great potential in preventing diseases associated with free radicals. Further research on oil-bearing rose flowers is appropriate to determine whether it has any effect on oxidative stress-related diseases.

Materials and methods

Oil-bearing rose (*Rosa damascena* Mill. var. *trigintipetale*) flowers were hand-picked in a plantation grown for commercial purposes in Ardıçlı village (latitude 37° 48' 19.9" (N), longitude 30° 12' 34.7" (E), 906 m altitude) in Isparta, Türkiye. To minimize the effects of environmental conditions, rose flowers were collected in the early morning hours (06:00–08:00 AM). Five developmental stages were differentiated based on opening state of sepals and petals as described by Önder et al.⁵⁷ (Fig. 5). All samples were collected in three replicates, and taken to the laboratory within an hour after the harvest, and stored at – 80 °C until analysis.

Measuring the total antioxidant capacity of oil-bearing rose petals

For measuring antioxidant capacity with five different methods were used (CUPRAC, DPPH, FRAP, ABTS and FIC). Petals (1 g) were ground in liquid nitrogen, and homogenized in 10 mL of ice-cold 80% methanol. The homogenate was shaken on a rotary shaker for 10 min, then centrifuged at 15,000×g for 20 min at 4 °C. This procedure was repeated twice and the supernatants were pooled, brought to 25 mL volume with 80% methanol. The pooled extracts were stored at – 20 °C until the determination of the antioxidant capacity. All antioxidant capacity results were calculated on a fresh weight (FW) basis.

Cupric reducing antioxidant capacity (CUPRAC) assay

The CUPRAC test was performed according to the method of Apak et al.⁶⁶. CUPRAC reactions were set up as follows: 1 mL of 0.01 M copper (II) chloride, 1 mL of 0.0075 M neocuproine solution and 1.0 mL of 1 M ammonium acetate buffer solution (pH 7.0) were added successively into a glass tube. Subsequently, 0.2 mL of the 40-fold diluted extract solution was taken and 0.9 mL of deionized water was added to obtain a total volume of 4.1 mL and mixed well. Absorbance against a reagent solution without extract was measured at 450 nm (Shimadzu UV-1280, Kyoto, Japan) after 30 min. The antioxidant activity was calculated as Trolox equivalents per g of fresh weight (mmol TR g⁻¹ FW) and calculated according to the following equation:

$$\text{CUPRAC}(\text{mmolTRg}^{-1}) = \frac{A}{\epsilon_{TR}} \times \frac{V_m}{V_s} \times D_f \times \frac{V_E}{m}$$

where; A : sample absorbance measured at 450 nm; ϵ_{TR} : molar absorption coefficient of TR compound in the CUPRAC method ($1.67 \times 10^4 \text{ L mol}^{-1} \text{ cm}^{-1}$); V_m : total volume (mL) of CUPRAC method measuring solution; V_s : sample volume (mL); D_f : dilution factor (if needed); V_E : volume of the prepared extract (mL); m : the amount of sample taken in the extraction process (g).

2,2-diphenyl-1-picrylhydrazyl (DPPH) assay

DPPH radical-scavenging activity was measured according to Bener et al.⁶⁷. DPPH method was applied as follows: 1 mL of 40-fold diluted extract solution, 1 mL 99% ethanol and 2 mL of 0.2 mM of DPPH[•] solution were added to a glass tube and mixed well. The reaction mixture was incubated at room temperature in the dark for 30 min. The absorbance of the samples was measured at 515 nm against ethanol with a UV-vis spectrophotometer (Shimadzu UV-1280, Kyoto, Japan). Free radical-scavenging activity was expressed as Trolox equivalents per g of fresh weight (mmol TR g⁻¹ FW) and calculated according to the following equation:

$$\text{DPPH}(\text{mmolTRg}^{-1}) = \frac{\Delta A}{\epsilon_{TR}} \times \frac{V_m}{V_s} \times D_f \times \frac{V_E}{m}$$

where ϵ_{TR} : molar absorption coefficient of TR compound in the DPPH method ($2.17 \times 10^4 \text{ L mol}^{-1} \text{ cm}^{-1}$); V_s : sample volume (mL); V_m : total volume (mL) of DPPH method measuring solution; D_f : dilution factor (if needed); V_E : volume of the prepared extract (mL); m : the amount of sample taken in the extraction process (g).

Ferric reducing antioxidant power (FRAP) assay

The ferric reducing antioxidant power was measured according to the method of Berker et al.⁶⁸. The following reactions were set up to measure FRAP activity: 1 mL of 50-fold diluted extract solution, 2.5 mL of 0.2 M phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferricyanide solution were added to a glass tube and incubated for 20 min in a water bath at 50 °C. After incubation, 2.5 mL of 10% trichloroacetic acid (TCA) was added, and thoroughly mixed. An aliquot of 2.5 mL solution was mixed with 2.5 mL of distilled water and 0.5 mL of 0.1% iron (II) chloride solution; then the absorbance of the resulting Prussian blue solution was measured at 700 nm (Shimadzu UV-1280, Kyoto, Japan) after 2 min against a reagent blank. Ferric reducing antioxidant power activity was expressed as Trolox equivalents per g of fresh weight (mmol TR g⁻¹ FW) and calculated according to the following equation:

$$\text{FRAP}(\text{mmolTRg}^{-1}) = \frac{A}{\epsilon_{TR}} \times \frac{V_m}{V_s} \times D_f \times \frac{V_E}{m}$$

where; A : sample absorbance measured at 700 nm; ϵ_{TR} : molar absorption coefficient of TR compound in the FRAP method ($1.77 \times 10^4 \text{ L mol}^{-1} \text{ cm}^{-1}$); V_m : total volume (mL) of FRAP method measuring solution; V_s : sample volume (mL); D_f : dilution factor (if needed); V_E : volume of the prepared extract (mL); m : the amount of sample taken in the extraction process (g).

2,2'-Azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assay

The ABTS was measured according to the method of Re et al.⁶⁹. ABTS^{•+} (7 mM) was dissolved in water and subsequently reacted with 2.45 mM potassium persulfate at a ratio of 1:0.5 to produce a radical cation. It was then incubated for 12–16 h in the dark at room temperature for the oxidation of ABTS. Before starting the analysis, ABTS^{•+} radical solution was diluted in ethanol until an absorbance of 0.7 ± 0.02 at 734 nm was obtained. ABTS method was applied as follows: 1 mL of 100-fold diluted extract solution, 3 mL deionized water and 1 mL of 7 mM of ABTS^{•+} solution were added to a glass tube and mixed well. Absorbance against a reagent solution without a sample was measured at 734 nm with a UV-Vis spectrophotometer (Shimadzu UV-1280, Kyoto, Japan) after 6 min. Trolox equivalents per g of fresh weight (mmol TR g⁻¹ FW) and calculated according to the following equation:

$$\text{ABTS}(\text{mmolTRg}^{-1}) = \frac{\Delta A}{\epsilon_{TR}} \times \frac{V_m}{V_s} \times D_f \times \frac{V_E}{m}$$

where ϵ_{TR} : molar absorption coefficient of TR compound in the ABTS method ($2.6 \times 10^4 \text{ L mol}^{-1} \text{ cm}^{-1}$); V_s : sample volume (mL); V_m : total volume (mL) of ABTS method measuring solution; D_f : dilution factor (if needed); V_E : volume of the prepared extract (mL); m : the amount of sample taken in the extraction process (g).

Ferrous ion-chelating (FIC) assay

The ferrous ion-chelating ability was determined in accordance with a method described by Decker and Welch⁷⁰ with minor modifications. FIC reactions contained 1 mL of extract solution, 3.7 mL of distilled water and 100 µL of 2 mM iron (II) chloride. The reaction was initiated by the addition of 200 µL of 5 mM ferrozine. The reaction was well mixed and incubated for 20 min at room temperature. After incubation, absorbance was determined at 562 nm (Shimadzu UV-1280, Kyoto, Japan) against a blank. Distilled water (1 mL) was used as a blank instead of the ferrozine solution, which helped with error correction due to the uneven color of the sample solutions. FIC was calculated with the following formulae:

$$FIC(\text{mmolEDTA g}^{-1}) = \frac{\Delta A}{\varepsilon_{EDTA}} \times \frac{V_m}{V_s} \times D_f \times \frac{V_E}{m}$$

where ε_{EDTA} : molar absorption coefficient of EDTA compound in the ABTS method ($13.5 \times 10^4 \text{ L mol}^{-1} \text{ cm}^{-1}$); V_s : sample volume (mL); V_m : total volume (mL) of FIC method measuring solution; D_f : dilution factor (if needed); V_E : volume of the prepared extract (mL); m the amount of sample taken in the extraction process (g).

Determination of antioxidant enzyme activities in oil-bearing rose petals

For SOD, CAT, GR and APX extraction, petals (10 g) were ground in liquid nitrogen and homogenized in 25 mL of 100 mM ice-cold 50 mM sodium phosphate (pH 6.4) containing 0.5 g of polyvinylpyrrolidone. The APX extract contained 1 mM ascorbic acid in addition to the other components of the extraction buffer. The homogenate was centrifuged at $15,000 \times g$ for 20 min at 4 °C. The resulting supernatants were filtered through Miracloth (Merck, Germany) and stored in the dark at -20 °C until antioxidant enzyme activities were determined in filtered aliquots.

SOD (EC 1.15.1.1) activity was measured by using the method of Giannopolitis and Ries⁷¹. The reaction mixture contained 2.8 mL reaction buffer (50 mM sodium phosphate buffer (pH 7.8), 13 mM methionine, 75 μM nitroblue tetrazolium (NBT), 0.1 mM EDTA, 50 mM sodium carbonate), 0.1 mL of 2 mM riboflavin, and 0.1 mL of the enzyme extract. The test tubes were irradiated under two fluorescent lamp ($2 \times 15 \text{ W}$) for 15 min, and absorbance was noted at 560 nm with a spectrophotometer (Shimadzu UV-1280, Kyoto, Japan). One unit of SOD activity was defined as the level of enzyme activity that caused a 50% decrease in SOD-inhibitable NBT reduction.

CAT (EC 1.11.1.6) activity was measured by following the method of Chance and Maehly⁷². The 3 mL reaction mixture contained 100 μL of enzyme extract and 2.9 mL of sodium phosphate buffer (50 mM, pH 7.0) containing 40 mM hydrogen peroxide (H_2O_2). Decreasing absorbance for H_2O_2 ($\varepsilon_{240} = 36 \text{ M}^{-1} \text{ cm}^{-1}$) was recorded at 240 nm (Shimadzu UV-1280, Kyoto, Japan) for 3 min. One unit of CAT activity was defined as H_2O_2 decomposition per minute.

GR (EC 1.6.4.2) activity was determined following the method of Murshed et al.⁷³ with minor modifications. The 1 mL reaction mixture contained 100 μL of enzyme extract, 350 μL of sodium phosphate buffer (100 mM, pH 7.8) and 500 μL of 2 mM oxidized glutathione (GSSG). The reaction was initiated by adding 50 μL of 2.4 mM NADPH solution. Decreasing absorbance for NADPH ($\varepsilon_{340} = 6.22 \text{ mM}^{-1} \text{ cm}^{-1}$) was recorded at 340 nm (Shimadzu UV-1280, Kyoto, Japan) for 3 min. One unit of GR activity was defined as the amount of enzyme that caused the oxidation of 1 mmol NADPH per minute at pH 7.8 at 25 °C.

APX (EC 1.11.1.11) activity was measured by using the method of Nakano and Asada⁷⁴. The 1 mL reaction mixture contained 100 μL of enzyme extract and 700 μL reaction buffer (sodium phosphate buffer (50 mM, pH 7.0), 0.5 mM ascorbate and 0.1 mM EDTA Na_2). The reaction was initiated by adding 200 μL of 1.2 mM H_2O_2 solution. Decreasing absorbance for H_2O_2 ($\varepsilon_{290} = 2.8 \text{ mM}^{-1} \text{ cm}^{-1}$) was recorded at 290 nm (Shimadzu UV-1280, Kyoto, Japan) for 3 min. One unit of APX was defined as the amount of enzyme that caused the decomposition of 1 mmol H_2O_2 per minute at pH 7.0 at 25 °C.

All of the antioxidant enzyme activity was based on protein concentration; each activity result represents the average of three replications. The protein content was determined following Bradford method⁷⁵. All specific antioxidant enzyme activities were expressed as U mg protein⁻¹.

Determination of H_2O_2 and malondialdehyde in oil-bearing rose petals

For H_2O_2 and malondialdehyde (MDA) extraction, petals (1 g) were ground in liquid nitrogen, homogenized in 10 mL of 0.1% trichloroacetic acid (TCA) and centrifuged at $13,000 \times g$ for 20 min at 4 °C. The resulting supernatants were used directly for the assays. H_2O_2 content was determined according to the method of Velikova et al.⁷⁶. The reaction mixture contained 0.5 mL of 10 mM phosphate buffer (pH 7.0), 1 mL potassium iodide (1 M) and 0.5 mL of the extract. The reaction was developed for 1 h in dark. The absorption of the mixture was measured at 390 nm (Shimadzu UV-1280, Kyoto, Japan), and water was used as blank. A standard curve was used to determine the amount of H_2O_2 from known H_2O_2 concentrations. The H_2O_2 content was expressed as $\mu\text{mol g}^{-1} \text{ FW}$.

MDA was determined according to the method of Heath and Packer⁷⁷. The 1 mL of supernatant was added to 4.5 mL 0.5% 2-thiobarbituric acid (TBA) in 20% TCA. The mixture was boiled in a water bath for 30 min, and the reaction was stopped by cooling the reaction tubes on ice. Then the samples were centrifuged at $10,000 \times g$ for 10 min, the absorbance at 532 nm was measured and subtracted from the non-specific absorbance at 600 nm. The amount of MDA-TBA complex was calculated from the extinction coefficient $155 \text{ mM}^{-1} \text{ cm}^{-1}$.

Determination of mineral compositions in oil-bearing rose petals

Mineral composition of petals were determined using Inductively coupled plasma-optical emission spectroscopy (ICP-OES). Samples were prepared by wet burning by adding 8 mL nitric acid + 2 mL H_2O_2 to 0.5 g of a sample using ETHOS ONE (Milestone, Italy) microwave sample preparation according to EPA 3015 method, and the final volume was completed to 20 mL with distilled water. Measurements in ICP-OES were performed using Optima 5300 DV Spectrometer (Perkin Elmer, USA) according to EPA 6010 method. In the petals, each element was measured at a specific wavelength (Al: 396.1, Ca: 317.9, Hg: 253.6, Cd: 228.8, Cr: 267.7, Cu: 327.4, Fe: 238.2, K: 766.5, Mg: 285.2, Mn: 257.6, Na: 589.6, P: 213.6, Se: 196.0, Si: 251.6 and Zn: 206.2 nm).

Statistical analysis

All analyzes were performed with three replicates. Results were subjected to analysis of variance (ANOVA) using SPSS Statistics 22.0 software (IBM, Armonk, NY, USA). Least significant differences (LSD, $P \leq 0.05$) were used to distinguish differences between the means. To show the relationship between the measured parameters,

Pearson's linear correlation analysis (Heatmap correlation) was calculated using OriginPro software (version 2021, OriginLab, Northampton, MA). The mean values of antioxidant capacity and antioxidant enzymes at different stages of flower development were used for multivariate principal component analysis (PCA). All values were expressed as mean \pm standard deviation.

Ethics declarations

The collection of plant material and the performance of experimental research on such plants complied with the national guidelines of (Türkiye).

Data availability

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Received: 4 February 2023; Accepted: 9 October 2023

Published online: 12 October 2023

References

1. Baydar, N. G. & Baydar, H. Phenolic compounds, antiradical activity and antioxidant capacity of oil-bearing rose (*Rosa damascena* Mill.) extracts. *Ind. Crops Prod.* **41**, 375–380 (2013).
2. Erbaş, S., & Mutlucan, M. Oil-bearing rose (*Rosa damascena* Miller) farming and production of rose-based products in Turkey. *International Flower Conference*, May 11–12 162–176 (2023).
3. Mahboubi, M. *Rosa damascena* as holy ancient herb with novel applications. *J. Tradit. Complement. Med.* **6**, 10–16 (2016).
4. Shahriari, S. *et al.* In vivo antioxidant potentials of *rosa damascena* petal extract from Guilan, Iran comparable to α -tocopherol. *Int. J. Pharmacol.* **3**, 187–190 (2007).
5. Yassa, N., Masoomi, F., Rankouhi, S. R. & Hadjiakhoondi, A. Chemical composition and antioxidant activity of the extract and essential oil of *Rosa damascena* from Iran, population of Guilan. *Daru* **17**, 175–180 (2009).
6. Vinokur, Y., Rodov, V. & Reznick, N. Rose petal Tea as an antioxidant rich beverage: Cultivar effects. *J. Food Sci.* **71**, S42–S47 (2006).
7. Ulusoy, S., Boşgelmez-Tınaz, G. & Seçilmiş-Canbay, H. Tocopherol, carotene, phenolic contents and antibacterial properties of rose essential oil, hydrosol and absolute. *Curr. Microbiol.* **59**, 554–558 (2009).
8. Cho, E. *et al.* Study on the inhibitory effects of Korean medicinal plants and their main compounds on the 1, 1-diphenyl-2-picrylhydrazyl radical. *Phytomedicine* **10**, 544–551 (2003).
9. Vinokur, Y. *et al.* Rose petal tea as an antioxidant-rich beverage: Cultivar effects. *J. Food Sci.* **71**, 42–47 (2006).
10. Eason, J. R. & Webster, D. Development and senescence of *Sandersonia aurantiaca* (Hook.) flowers. *Sci. Hortic.* **63**, 113–121 (1995).
11. Önder, S., Tonguç, M., Önder, D., Erbaş, S. & Mutlucan, M. Flower color and carbohydrate metabolism changes during the floral development of *Rosa damascena*. *South Afr. J. Bot.* **156**, 234–243 (2023).
12. Kumar, N., Bhandari, P., Singh, B., Gupta, A. P. & Kaul, V. K. Reversed phase-HPLC for rapid determination of polyphenols in flowers of rose species. *J. Sep. Sci.* **31**, 262–267 (2008).
13. Fridovich, I. The biology of oxygen radicals: General concepts. In *Oxygen Radicals and Tissue Injury. Proceedings of Upjohn Symposium* (ed. Halliwell, B.) 1–5 (The Upjohn, 1988).
14. Agarwal, S. & Pandey, V. Antioxidant enzyme responses to NaCl stress in *Cassia angustifolia*. *Biol. Plant* **48**, 555–560 (2004).
15. Castejón, M. L., Montoya, T., Alarcón de la Lastra, C. & Sánchez-Hidalgo, M. Potential protective role exerted by secoiridoids from *Olea europaea* L. in cancer, cardiovascular, neurodegenerative, aging-related, and immunoinflammatory diseases. *Antioxidants* **9**, 149 (2020).
16. Gouvinhas, I. *et al.* Critical review on the significance of olive phytochemicals in plant physiology and human health. *Molecules* **22**, 1986 (2017).
17. Ezhilmathi, K., Singh, V. P., Arora, A. & Sairam, R. K. Effect of 5-sulfosalicylic acid on antioxidant activity in relation to vase life of *Gladiolus* cut flowers. *Plant Growth Regul.* **51**, 99–108 (2007).
18. Saeed, T., Hassan, I., Abbasi, N. A. & Jilani, G. Effect of gibberellic acid on the vase life and oxidative activities in senescing cut *gladiolus* flowers. *Plant Growth Regul.* **72**, 89–95 (2014).
19. Scandalios, J. G. Oxygen stress and superoxides dismutase. *Plant Physiol.* **101**, 7–12 (1993).
20. Yanik, F., Aytürk, Ö., Çetinbaş-Genç, A. & Vardar, F. Salicylic acid-induced germination, biochemical and developmental alterations in rye (*Secale cereale* L.). *Acta Bot Croat.* **77**, 45–50 (2018).
21. Aryal, S. *et al.* Total phenolic content, flavonoid content and antioxidant potential of wild vegetables from Western Nepal. *Plants* **8**, 96 (2019).
22. Buettner, G. R. Superoxide dismutase in redox biology: The roles of superoxide and hydrogen peroxide. *Anti-Cancer Agents Med. Chem.* **11**, 341–346 (2011).
23. Saed-Moucheshi, A., Pakniyat, H., Pirasteh-Anosheh, H. & Azooz, M. M. Chapter 20—Role of ROS as Signaling Molecules in Plants. In *Oxidative Damage to Plants* (ed. Ahmad, P.) 585–620 (Academic Press, 2014).
24. Ahmad, S. S. & Tahir, I. Increased oxidative stress, lipid peroxidation and protein degradation trigger senescence in *Iris versicolor* L. flowers. *Physiol. Mol. Biol. Plants* **22**, 507–514 (2016).
25. Wu, X. *et al.* Lipophilic and hydrophilic antioxidant capacities of common foods in the United States. *J. Agric. Food Chem.* **52**, 4026–4037 (2004).
26. Apak, R., Güçlü, K., Özyürek, M. & Celik, S. E. Mechanism of antioxidant capacity assays and the CUPRAC (cupric ion reducing antioxidant capacity) assay. *Microchim. Acta.* **160**, 413–419 (2008).
27. Meir, S., Kanner, J., Akiri, B. & Philosoph-Hadas, S. Determination and involvement of aqueous reducing compounds in oxidative defense systems of various senescing leaves. *J. Agric. Food Chem.* **43**, 1813–1819 (1995).
28. Rabeta, M. S. & Faraniza, R. N. Total phenolic content and ferric reducing antioxidant power of the leaves and fruits of *Garcinia atrovirdis* and *Cynometra cauliflora*. *Int. Food Res. J.* **20**, 1691 (2013).
29. Muddathir, A. M., Yamauchi, K., Batubara, I., Mohieldin, E. A. M. & Mitsunaga, T. Anti-tyrosinase, total phenolic content and antioxidant activity of selected Sudanese medicinal plants. *S. Afr. J. Bot.* **109**, 9–15 (2017).
30. Dudonné, S., Vitrac, X., Coutiere, P., Woillez, M. & Mérillon, J.-M. Comparative study of antioxidant properties and total phenolic content of 30 plant extracts of industrial interest using DPPH, ABTS, FRAP, SOD, and ORAC assays. *J. Agric. Food Chem.* **57**, 1768–1774 (2009).
31. Chang, L.-W., Yen, W.-J., Huang, S. C. & Duh, P.-D. Antioxidant activity of sesame coat. *Food Chem.* **78**, 347–354 (2002).
32. Guo, T., Wei, L., Sun, J., Hou, C. L. & Fan, L. Antioxidant activities of extract and fractions from *Tuber indicum* Cooke & Masee. *Food Chem.* **127**, 1634–1640 (2011).

33. Kumar, N., Bhandari, P., Shamsher, S. & Bari, B. Antioxidant activity and ultra-performance LC-electrospray ionization-quadrupole time-of-flight mass spectrometry for phenolics-based fingerprinting of Rose species: *Rosa damascena*, *Rosa bourboniana* and *Rosa brunonii*. *Food Chem. Toxicol.* **47**, 361–367 (2009).
34. Özkan, G., Sagdiç, O. & Baydar, H. Antioxidant and antibacterial activities of *Rosa damascena* flower extracts. *Int. J. Food Sci. Technol.* **10**, 277–281 (2004).
35. Schieber, A., Mihalev, K., Berardini, N., Mollov, P. & Carle, R. Flavonol glycosides from distilled petals of *Rosa damascena* Mill. *Zeitschrift für Naturforschung* **60**, 379–384 (2005).
36. Shikov, V., Kammerer, D. R., Mihalev, K., Mollov, P. & Carle, R. Antioxidant capacity and colour stability of texture-improved canned strawberries as affected by the addition of rose (*Rosa damascena* Mill.) petal extracts. *Food Res. Int.* **46**, 552–556 (2012).
37. Michalak, M. Plant-derived antioxidants: Significance in skin health and the ageing process. *Int. J. Mol. Sci.* **23**, 585 (2022).
38. Lee, Y. H., Choo, C., Watawana, M. I., Jayawardena, N. & Waisundara, V. Y. An appraisal of eighteen commonly consumed edible plants as functional food based on their antioxidant and starch hydrolase inhibitory activities. *J. Sci. Food Agric.* **95**, 2956–2964 (2015).
39. Fu, M. & Mao, L. In vitro antioxidant activities of five cultivars of daylily flowers from China. *Nat. Prod. Res.* **22**, 584–591 (2008).
40. Mlcek, J. & Rop, O. Fresh edible flowers of ornamental plants: A new source of nutraceutical foods. *Trends Food Sci. Technol.* **22**, 561–569 (2011).
41. Franzen, F. L., Lidório, H. F. & Oliveira, M. S. R. Edible flower considerations as ingredients in food, medicine and cosmetics. *J. Anal. Pharm. Res.* **7**, 271–273 (2018).
42. Fu, M., He, Z., Zhao, Y., Yang, J. & Mao, L. Antioxidant properties and involved compounds of daylily flowers in relation to maturity. *Food Chem.* **114**, 1192–1197 (2009).
43. Friedman, H. *et al.* Characterization of yield, sensitivity to Botrytis cinerea and antioxidant content of several rose species suitable for edible flowers. *Sci. Hortic.* **123**, 395–401 (2010).
44. Friedman, H. *et al.* Edible flowers new crops with potential health benefits. *Acta Hortic.* **755**, 283–290 (2007).
45. Jajic, I., Sarna, T. & Strzalka, K. Senescence, stress, and reactive oxygen species. *Plants* **4**, 393 (2015).
46. Abassi, N. A., Kushad, M. M. & Endress, A. G. Active oxygen-scavenging enzymes activities in developing apple flowers and fruits. *Sci. Hortic.* **74**, 183–194 (1998).
47. Salem, N., Msaada, K., Hamdaoui, G., Limam, F. & Marzouk, B. Variation in phenolic composition and antioxidant activity during flower development of safflower (*Carthamus tinctorius* L.). *J. Agric. Food Chem.* **59**, 4455–4463 (2011).
48. Mittler, R., Vanderauwera, S., Gollery, M. & Van Breusegem, F. Reactive oxygen gene network of plants. *Trends Plant Sci.* **9**, 490–498 (2004).
49. Al Shamsi, S. R., Rabert, G. A., Kurup, S. S., Alyafei, M. A. M. & Jaleel, A. Biochemical changes and antioxidant variations in date palm (*Phoenix dactylifera* L.) varieties during flower induction and development. *Plants* **10**, 2550 (2021).
50. Cheruth, A. J., Kurup, S. S. & Subramaniam, S. (2015). Variations in hormones and antioxidant status in relation to flowering in early, mid, and late varieties of date palm (*Phoenix dactylifera*) of United Arab Emirates. *Sci. World J.* 2015:846104
51. Chakrabarty, D., Chatterjee, J. & Datta, S. K. Oxidative stress and antioxidant activity as the basis of senescence in chrysanthemum florets. *Plant Growth Regul.* **53**, 107–115 (2007).
52. Zhang, Y., Luo, Y., Wang, X., Chen, Q., Sun, B., Wang, Y. & Tang, H. Variation in bioactive compounds, antioxidant enzymes and radical-scavenging activity during flower development of *Rosa hybrida*. In *AIP Conference Proceedings 2018, April 1956(1)*, 020021 (AIP Publishing LLC, 2018).
53. Han, M., Zhao, Y., Meng, J., Yin, J. & Li, H. Analysis of physicochemical and antioxidant properties of *Malus* spp. petals reveals factors involved in flower color change and market value. *Sci. Hortic.* **310**, 111688 (2023).
54. Salem, N., Msaada, K., Hammami, M., Limam, F., Vasapollo, G. & Marzouk, B. (2014). Variation in anthocyanin and essential oil composition and their antioxidant potentialities during flower development of Borage (*Borago officinalis* L.). *Plant Biosyst.* **148**, 444–459
55. Bibi Sadeer, N., Montesano, D., Albrizio, S., Zengin, G. & Mahomoodally, M. F. The versatility of antioxidant assays in food science and safety: Chemistry, applications, strengths, and limitations. *Antioxidants* **9**, 709 (2020).
56. Shrestha, P. M. & Dhillion, S. S. Nepal'de yerel olarak yönetilen bir ormandaki yabani gıda türleri ile ilgili çeşitlilik ve geleneksel bilgi. *Agrofor. Sist.* **66**, 55–63 (2006).
57. Önder, S., Tonguç, M., Erbaş, S., Önder, D. & Mutlucan, M. Investigation of phenological, primary and secondary metabolites changes during flower developmental of *Rosa damascena*. *Plant Physiol. Biochem.* **192**, 20–34 (2022).
58. Kyriacou, M. C. *et al.* Functional quality in novel food sources: Genotypic variation in the nutritive and phytochemical composition of thirteen microgreens species. *Food Chem.* **277**, 107–118 (2019).
59. Vaz, A. P. A. & Kerbauy, G. B. Effects of mineral nutrients on in vitro growth and flower formation of *Psychomorhis pusilla* (Orchidaceae). In *XXV International Horticultural Congress, Part 10: Application of Biotechnology and Molecular Biology and Breeding-In Vitro* **520**, 149–156 (1998).
60. Dickens, C. W. S. & Van Staden, J. The induction and evocation of flowering in vitro. *S. Afr. J. Bot.* **54**, 325–344 (1988).
61. Ammar, I., Ennouri, M., Bali, O. & Attia, H. Characterization of two prickly pear species flowers growing in Tunisia at four flowering stages. *LWT-Food Sci. Technol.* **59**, 448–454 (2014).
62. Araújo, S., Matos, C., Correia, E. & Antunes, M. C. Evaluation of phytochemicals content, antioxidant activity and mineral composition of selected edible flowers. *Qual. Assur. Saf.* **11**, 471–478 (2019).
63. Fan, J., Zhu, W., Kang, H., Ma, H. & Tao, G. Flavonoid constituents and antioxidant capacity in flowers of different Zhongyuan tree penoy cultivars. *J. Funct. Foods.* **4**, 147–157 (2012).
64. Zhou, C., Sun, C., Chen, K. & Li, X. Flavonoids, phenolics, and antioxidant capacity in the flower of *Eriobotrya japonica* Lindl. *Int. J. Mol. Sci.* **12**, 2935–2945 (2011).
65. Wong, S. P., Leong, L. P. & Koh, J. H. W. Antioxidant activities of aqueous extracts of selected plants. *Food Chem.* **99**, 775–783 (2006).
66. Apak, R., Güçlü, K., Özyürek, M., Esin Karademir, S. & Erçağ, E. The cupric ion reducing antioxidant capacity and polyphenolic content of some herbal teas. *Int. J. Food Sci. Nutr.* **57**, 292–304 (2006).
67. Bener, M. *et al.* Microwave-assisted extraction of antioxidant compounds from by-products of Turkish hazelnut (*Corylus avellana* L.) using natural deep eutectic solvents: Modeling, optimization and phenolic characterization. *Food Chem.* **385**, 132633 (2022).
68. Berker, K. I., Güçlü, K., Tor, I. & Apak, R. Comparative evaluation of Fe (III) reducing power-based antioxidant capacity assays in the presence of phenanthroline, batho-phenanthroline, tripyridyltriazine (FRAP), and ferricyanide reagents. *Talanta* **72**, 1157–1165 (2007).
69. Re, R. *et al.* Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radic. Biol. Med.* **26**, 1231–1237 (1999).
70. Decker, E. A. & Welch, B. Role of ferritin as a lipid oxidation catalyst in muscle food. *J. Agric. Food Chem.* **38**, 674–677 (1990).
71. Giannopolitis, C. N. & Ries, S. K. Superoxide dismutases: II. Purification and quantitative relationship with water-soluble protein in seedlings. *Plant Physiol.* **59**, 315–318 (1977).
72. Chance, B. & Maehly, A. C. *Assay of Catalases and Peroxidases* 764–775 (1955).
73. Murshed, R., Lopez-Lauri, F. & Sallanon, H. Microplate quantification of enzymes of the plant ascorbate–glutathione cycle. *Anal. Biochem.* **383**, 320–322 (2008).

74. Nakano, Y. & Asada, K. Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. *Plant Cell Physiol.* **22**, 867–880 (1981).
75. Bradford, M. M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **72**, 248–254 (1976).
76. Velikova, V., Yordanov, I. & Edreva, A. J. P. S. Oxidative stress and some antioxidant systems in acid rain-treated bean plants: Protective role of exogenous polyamines. *Plant Sci.* **151**, 59–66 (2000).
77. Heath, R. L. & Packer, L. Photoperoxidation in isolated chloroplasts: I. Kinetics and stoichiometry of fatty acid peroxidation. *Arch. Biochem. Biophys.* **125**, 189–198 (1968).

Author contributions

Conceptualization, methodology, investigation, visualization writing-original draft preparation.

Competing interests

The author declares no competing interests.

Additional information

Correspondence and requests for materials should be addressed to D.Ö.

Reprints and permissions information is available at www.nature.com/reprints.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>.

© The Author(s) 2023