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Research Article

# Determination of the Biochemical and Antioxidant Enzyme Activities of Rose Oil (*Rosa damascena* Mill.) Collected in Different Time Periods

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**Abstract:** The aim of this study was to investigate the changes in the biochemical and antioxidant enzyme activities of oil of rose petals collected at different time intervals during the day. The results of the present study revealed that significant changes occurred in the biochemical content of the oil of rose petals due to collecting at various intervals in a day. The total phenolic content exhibited a statistically significant increase over the day, reaching a 151.57% increment by 14.00 p.m. compared to the initial level. Similarly, total flavonoid content and total antioxidant activity progressively increased. Proline, known for its versatile roles, including antioxidant defense, increased from 7.43 mg $g^{-1}$ in the morning to 24.96 mg $g^{-1}$. Significant temporal changes were observed in antioxidant enzyme activities as well. Catalase (CAT) activity, for instance, increased by 588.22% from 6.00 a.m. to 14.00 p.m., with similar alterations noted in superoxide dismutase (SOD), ascorbate peroxidase (APX), and peroxidase (POD) activities. The results highlight a correlation between flower collection time and biochemical activities, with a noticeable increase in antioxidant enzyme activities as the day progresses. The findings emphasize the importance of considering plant physiology and environmental factors when determining optimal flower collection times. In conclusion, it can be said that the collection time of flowers influences the quality of rose oil and early morning collection may be more suitable.

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## 1. Introduction

Economically valuable and also known as Damask Rose, *Rosa damascena* Mill. is considered a member of the Rosaceae family. This plant naturally grows in Syria, Morocco, Andalusia, Iran, and the Caucasus (Babaei et al., 2007; Hatipoğlu et al., 2022; Macit et al., 2023). It originates from the ancient city formerly known as Damascus and is therefore commonly referred to as Damask rose. However, in Turkey, it is also known by various names such as Pink oil rose, Oil rose, Distillation rose, Damask rose, and Isparta rose (Özçelik et al., 2013; Baydar, 2016). While Oil rose is primarily cultivated for industrial purposes in the production of rose oil, especially in Turkey and Bulgaria, its medicinal usage has been gradually increasing in recent years.

Plants undergo significant biochemical changes under stress, including accumulating reactive oxygen species (ROS), particularly $O_2$ and $H_2O_2$, in chloroplasts, mitochondria, and peroxisomes. These ROS are natural by-products of cell metabolism and play a crucial role in signaling mechanisms. However, the excessive accumulation of ROS triggers oxidative stress by initiating lipid peroxidation, protein reduction, and DNA fragmentation, ultimately leading to cell death. Simultaneously, plants have developed enzymatic (superoxide dismutase (SOD), peroxidase (POD), ascorbate peroxidase (APX), catalase (CAT)), and non-enzymatic (glutathione, β-carotene, ascorbic acid, α-tocopherol) antioxidant defense mechanisms to reduce oxidative damage. Consequently, the balance between ROS production and antioxidant enzyme activities determines whether oxidative damage will occur (Kuşvuran et al., 2011; Farajzadeh et al., 2017; Popović et al., 2017; Selmi et al., 2017; Demir and Başayiğit, 2021).

Medicinal and aromatic plants are cultivated for their bioactive substances. However, bioactive substances can vary significantly depending on the different organs of the plant, growth stages, and harvest times. Therefore, producers of medicinal and aromatic plants need to thoroughly understand the variation in bioactive substance content of source plants and harvest, cut, and collect them when the active substances are most abundant (Başyiğit and Baydar, 2017). For instance, the mutagenic substance methyl eugenol in rose oil is either undesired or within certain limits. In Isparta roses, this ratio is generally around 2%. However, in roses with delayed harvesting and distillation, this ratio exceeds 4%, which is undesirable (Baydar et al., 2007). Therefore, this research aims to determine the time-dependent changes in the biochemical contents and antioxidant enzyme activities of petals collected from commercially grown *R. Damascena* in Isparta province at different intervals (06.00 a.m., 08.00 a.m., 10.00 a.m., 12.00 p.m., 14.00 p.m.).

## 2. Material and Methods

### 2.1. Material

The plant materials (*R. damascena*) used in the study were collected from a commercial garden located in the village of Yakaören, Isparta province. Petals were collected homogeneously to represent the entire plant (from the top, middle, and bottom parts of the plant). The study was designed according to randomized block experimental design, and the analyses were conducted with 3 replications for each collection time, with each replication consisting of 12 trees. The collected petals were frozen with liquid nitrogen and stored at -80°C in the Agricultural Biotechnology Department of the Faculty of Agriculture at Isparta Applied Sciences University until the next collection time.

### 2.2. Method

#### *2.2.1. Biochemical analyses*

***Determination of total phenolic and flavonoid contents:*** Two grams (2±0.01 g) of rose petals were weighed and homogenized in 10 ml of 80% methanol. The obtained homogenate was centrifuged at 4000 g for 10 minutes, and the liquid portion was collected. The remaining pellet was re-extracted with an additional 10 ml of 80% methanol, and the above steps were repeated. The Folin-Ciocalteau method, as specified by Singleton and Rossi (1965), was used to determine the total phenolic content. The results were calculated and expressed as mg gallic acid equivalent per gram (mg GAE $g^{-1}$). The total flavonoid content was determined according to the method described by Zhishen et al. (1999) and expressed as mg catechin equivalent per gram (mg CE $g^{-1}$).

***DPPH free radical scavenging activity:*** The antioxidant activity was measured based on the ability to capture DPPH radicals, following the method of Kumaran et al. (2006). The antioxidant activity of appropriately diluted samples in a specific concentration range was compared with the Trolox standard in terms of hydrogen bonding capability. The absorbance of the resulting color at 517 nm was measured using a UV spectrophotometer.

***Total proline content:*** Six-tenths of a gram (0.6 g) of rose petals were weighed, and 3 ml of 3% sulfosalicylic acid was added. After homogenization, the mixture was centrifuged at 12 000 g for 10 minutes at room temperature. The analysis was conducted according to the method of Bates et al. (1973), and the results were expressed as mg/g and calculated relative to the D-Proline standard.

***Lipid peroxidation (MDA) content:*** Ten grams (10 g) of rose petals were weighed, and 25 ml of cold 100 mM sodium phosphate buffer containing 0.5 g polyvinyl polypyrrolidone (PVPP) was prepared. After homogenization, the samples were centrifuged at 27 000 g for 50 minutes at 4 °C. The analysis was performed according to the method reported by Jiang et al. (2010), and the MDA content was calculated in nmol $g^{-1}$.

### *2.2.2. Antioxidant enzyme analyses*

***Catalase (CAT) enzyme activity:*** Ten grams (10 g) of rose petals were weighed, and a cold 50 mM sodium phosphate buffer containing 0.5 g polyvinyl polypyrrolidone (PVPP) was prepared (pH: 7.0). After homogenization, the samples were centrifuged at 27 000 g for 50 minutes at 4 °C. The analysis was carried out according to the method described by Beers et al. (1952), and the specific activity was expressed as U mg $protein^{-1}$.

***Superoxide dismutase (SOD) enzyme activity:*** Ten grams (10 g) of rose petals were weighed, and a cold 100 mM sodium phosphate buffer containing 0.5 g polyvinyl polypyrrolidone (PVPP) was prepared. After homogenization, the samples were centrifuged at 27 000 g for 50 minutes at 4 °C, and the supernatant was used for analysis. The analysis was performed according to the method reported by Constantine and Stanley (1977), and the results were expressed as U $mg^{-1}$ protein.

***Ascorbate peroxidase (APX) enzyme activity:*** Four grams (4 g) of rose petals were weighed, and 12 ml of 50 mM potassium phosphate buffer (pH: 7.3) containing 1 mM (EDTA), 2 mM DTT, and 1 mM ascorbic acid was added. After homogenization, the samples were centrifuged at 10 000 g for 15 minutes at 4 °C. The analysis was conducted according to the method described by Nakano et al. (1981), and the results were expressed as mol/min/g protein.

***Peroxidase (POD) enzyme activity:*** Ten grams (10 g) of rose petals were weighed, and a cold 100 mM sodium phosphate buffer containing 0.5 g polyvinyl polypyrrolidone (PVPP) was prepared. After homogenization, the samples were centrifuged at 27 000 g for 50 minutes at 4 °C, and the supernatant was used for analysis. The analysis was performed according to the method reported by Jiang et al. (2010), and the results were expressed as $\Delta A_{460}$ $min^{-1}$ mg $protein^{-1}$.

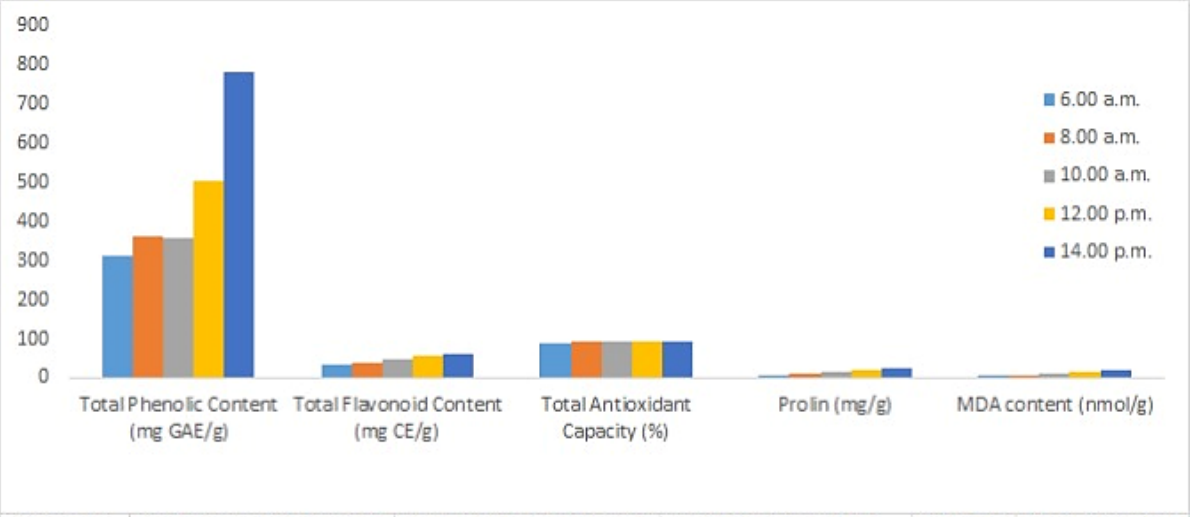
## 2.3. Statistical analysis

The experimental study was established in a factorial arrangement with 3 replications in a randomized block experimental design. The obtained data were subjected to one-way analysis of variance method using Minitab 17 statistical software. The differences that emerged were identified according to the Tukey multiple comparison test, and the differences between the means were indicated using different letters. Correlation matrix and biplot (Cos2) analyses between properties were made with the "FactoMineR, factoextra, pca3d, Methane" packages in the R package program. Principal component analysis was performed and (biplot) visuals were created for the first and second dimensions according to Cos2 values that show the importance of the variables in the components.

## 3. Results and Discussion

The results of the biochemical analyses conducted on rose petals collected at different time intervals are presented in Figure 1. Changes in the biochemical contents occurred over time, and this change was found to be statistically significant ($p<0.05$). Analysis of the rose petals collected at 6.00 a.m. in the morning (at dawn) revealed a total phenolic content of 310.32 mg GAE $g^{-1}$, with an increase in total phenolic content observed in samples collected at 08:00 a.m., 10:00 a.m., 12:00 p.m., and 14.00 p.m. hours due to the influence of sunlight and heat. The total phenolic content of rose petals collected at 14.00 p.m. was determined to be 780.70 mg GAE $g^{-1}$, indicating a 151.57% increase compared to the initial level. Similarly, an increase in total flavonoid content over time was observed. Analysis of rose petals collected at 6.00 a.m. in the morning showed a total flavonoid content of 33.90 mg CE $g^{-1}$, while in samples collected at 14.00 p.m., it was 62.33 mg CE $g^{-1}$, representing an 83.86% increase in flavonoid content. An increase in total antioxidant activity over time was also observed. Upon examination of Figure 1, it was determined that the DPPH radical scavenging activity increased by 8.87% between 6.00

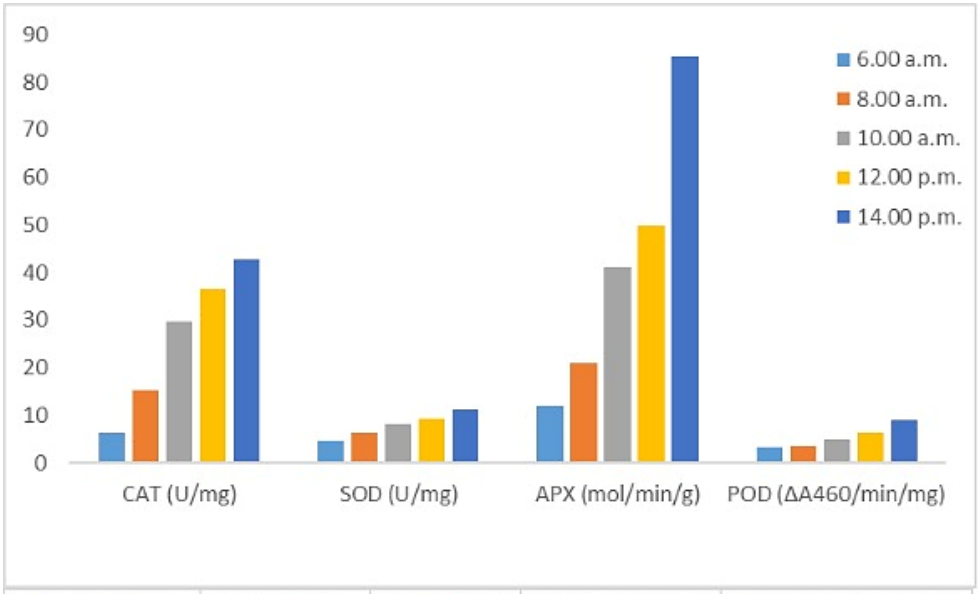
a.m. in the morning and 2:00 in the afternoon. Proline, despite acting as an osmolyte, is known as a potent antioxidant defense molecule, a metal chelator (separator), a protein stabilizer, a ROS scavenger, and an inhibitor of programmed cell death (Çelik, 2023). Research has shown a positive relationship between proline accumulation and increased stress tolerance in plants (Yildirim et al., 2021; Dindar et al., 2023; Yildirim et al., 2023). In the research, it was determined that while the proline content in the samples collected in the morning was 7.43 mg $g^{-1}$, it reached 24.96 mg $g^{-1}$ at 14.00 p.m., an increase of 236%.




| Sampling Time | Total Phenolic Content (mg GAE/g) | Total Flavonoid Content (mg CE/g) | Total Antioxidant Capacity (%) | Prolin (mg/g) | MDA content (nmol/g) |
|---|---|---|---|---|---|
| 6.00 a.m. | 310,32±5,02 c | 33,90±2,69 c | 87,50±0,57 d | 7,43±1,66 c | 5,00±1,38 c |
| 8.00 a.m. | 363,80±22,40 c | 40,67±1,64 c | 92,56±0,46 c | 11,25±1,71 bc | 8,42±1,56 bc |
| 10.00 a.m. | 356,30±18,30 c | 48,73±0,89 b | 93,85±0,12 b | 16,37±2,74 b | 13,18±0,48 b |
| 12.00 p.m. | 503,60±23,20 b | 54,77±1,65 b | 94,81±0,12 ab | 22,11±1,42 a | 14,05±0,85 ab |

Figure 1. Changes in biochemical contents of oil rose petals over time. *a,b: The difference between the means indicated by different letters in the same column is significant at the P≤0.05 level.

The results of antioxidant enzyme analyses conducted on rose petals collected at different time intervals are presented in Figure 2. Changes in antioxidant enzyme activities over time were observed, and this change was found to be statistically significant ($p<0.05$). According to the findings, the lowest CAT activity was observed in oil roses collected at 6.00 a.m. in the morning, with 6.20 U $mg^{-1}$, while the highest CAT activity was found in roses collected at 14.00 p.m., with 42.67 U $mg^{-1}$. A significant increase was particularly observed from 8:00 a.m. onwards, reaching a 588.22% increase at 14.00 p.m. p.m. Analysis of rose petals collected at 6.00 a.m. revealed an increase in SOD activity over time, with the highest activity again observed in samples collected at 14.00 p.m. The SOD activity, which was 4.67 U $mg^{-1}$ at 6.00 a.m., reached 11.33 U $mg^{-1}$ with a 142.61% increase at 14.00 p.m.. An increase in APX activity was observed continuously between 6.00 a.m. and 14.00 p.m., reaching the highest APX activity at 14.00 p.m. The APX activity in oil rose petals collected at 6.00 a.m. was 12.11 mol $min^{-1}$ $g^{-1}$, while it reached 85.55 mol $min^{-1}$ $g^{-1}$ with a 606.44% increase at 14.00 p.m. Similarly, an increase in POD activity was observed between 6.00 a.m. and 14.00 p.m. The POD activity in oil rose petals collected at 6.00 a.m. was 3.42 $\Delta A_{460}$ $min^{-1}$ $mg^{-1}$, while it reached 8.91 $\Delta A_{460}$ $min^{-1}$ $mg^{-1}$ with a 160.52% increase at 14.00 p.m.




| Sampling Time | CAT (U/mg) | SOD (U/mg) | APX (mol/min/g) | POD (ΔA460/min/mg) |
|---|---|---|---|---|
| 6.00 a.m. | 6,20±0,40 d | 4,67±0,88 d | 12,11±0,19 c | 3,42±0,26 d |
| 8.00 a.m. | 15,20±1,77 c | 6,33±0,12 cd | 20,96±0,63 c | 3,74±0,51 cd |
| 10.00 a.m. | 29,79±3,60 b | 8,20±1,62 bc | 41,11±1,92 b | 5,02±0,29 bc |
| 12.00 p.m. | 36,62±1,15 ab | 9,32±1,04 ab | 49,81±6,24 b | 6,22±0,78 b |
| 14.00 p.m. | 42,67±5,82 a | 11,33±0,49 a | 85,55±9,49 a | 8,91±0,83 a |

Figure 2. Changes in antioxidant activity of oil rose petals over time.*a,b: The difference between the means indicated by different letters in the same column is significant at the P≤0.05 level.

In the study, Pearson correlation coefficients for the collection times and biochemical as well as antioxidant enzyme activities, along with PCA variables, are presented in Figure 3. A strong positive correlation (ranging from 0.66 to 1.00) was observed between collection times and biochemical and antioxidant enzyme activities, indicating an increase in the examined features over time. It was revealed by the correlation matrix that there were statistically significant positive changes in biochemical and antioxidant enzyme activities over time. The first principal component explained 94.2% of the variance, and the second principal component explained 4.7%. The squares of the coordinates (cos2) were an indication of how successfully the relevant variable was expressed with the principal component. All features have high cos2 values. Cos2 values of the features in the same direction indicate that the correlation between features is positive.

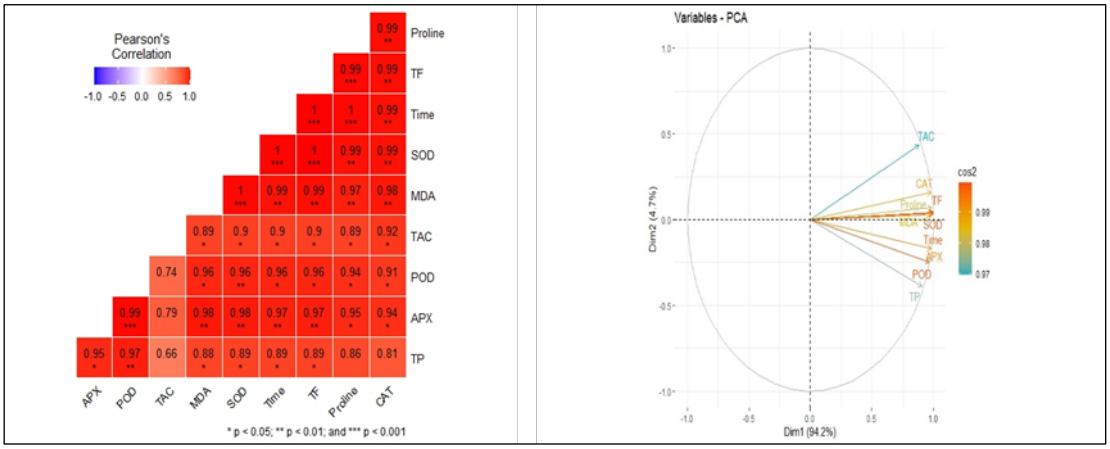



Figure 3. Pearson's correlation coefficients among biochemical and antioxidant enzyme properties used in the study. APX: Ascorbate peroxidase; POD: Peroxidase; TAC: Total antioxidant capacity; MDA: Malondialdehyde; SOD: Superoxide dismutase; TF: Total phenolic content; CAT: Catalase.

The cosmetic industry extensively utilizes plants' antioxidant properties and free radical scavenging abilities to regulate skin damage (Michalak, 2022). Therefore, plants abundant in antioxidants are commonly employed as a protective measure against oxidative degradation (Lee et al., 2015). Recent years have witnessed a growing interest in nutrition and food science, driven by diets that exhibit antioxidant activity (Fu and Mao, 2008). Generally, the health advantages of edible flowers are linked to their antioxidant activities, which exert notable inhibitory effects on free radicals. Recognized as natural antioxidants, the antioxidants in flowers serve as an alternative means to prevent the oxidative deterioration of food, thereby minimizing the harm caused by these oxidative compounds in humans (Franzen et al., 2018). In the Lakes region, a flowering season lasting approximately two months begins in the first week of May and continues until the first week of July, depending on the altitude. In the rose gardens situated at elevations between 800 and 1500 meters in the region, flowering is delayed by 2-3 days for every 100-meter increase in altitude. Throughout the flowering season, rose flowers are manually collected starting from the early hours of the day, broken individually from beneath the ovaries, and transported to rose oil factories in sacks for distillation (Baydar et al., 2013). The productivity and quality of rose products vary according to the climate and soil characteristics of the region where oil roses grow, the direction and altitude of the area, cultivation techniques, harvest timing and duration of storage, distillation, and extraction processes, as well as preservation and drying methods (Babu et al., 2002; Misra et al., 2002; Safari et al., 2004; Kazaz et al., 2009; Kazaz et al., 2010). Baydar et al. (2013) reported a decrease in the ratio of essential oil components of oil roses as the morning progressed towards the evening in their volatile oil component analysis conducted at different times of the day. Therefore, a positive relationship between the collection time and the quality of oil roses is evident. In our study, it was observed that as the collection time was delayed, there was a rapid increase in the biochemical and antioxidant enzyme activities in oil roses. It was determined that the petals, which determine the quality of rose oil, were exposed to stress throughout the day. Indeed, Çelik (2023) has reported a relationship between antioxidant enzymes, biochemical contents, and stress conditions. Upon reviewing previous studies, it is noteworthy that the biochemical and antioxidant enzyme responses of oil roses under salinity and drought stress conditions, as well as the effects of different collection times with stress-reducing applications, have not been investigated concerning antioxidant enzyme activities and some biochemical contents (Kashefi et al., 2012; Zahedi-Amiri et al., 2019; Alizadeh et al., 2021; Hamza et al., 2022; Hessini et al., 2022; Omidi et al., 2022; Demir and Başayiğit, 2022; Tiryaki et al., 2023). Therefore, our study was the first study on the subject. It is known that there are changes in plants' biochemical and enzymatic activities during the flowering development stages, and the production of reactive oxygen species (ROS) occurs in plant cells (Jajic et al., 2015). Ezhilmathi et al. (2007) reported an increase in antioxidant enzyme activities in plants at the beginning of the flowering stages. However, they noted a decrease in enzyme activities towards the end of flowering. The reason for this is associated with the accumulation of reactive oxygen species (ROS). Therefore, comparing the results obtained in our study with both Baydar et al. (2013) and Ezhilmathi et al. (2007) studies, it can be stated that as the collection time is delayed, there is an increase in ROS accumulation in rose petals, accompanied by an increase in enzyme activities.

## Conclusion

This study examined the changes in biochemical contents and antioxidant enzyme activities in rose petals collected at different time intervals. The results of the research show that there are significant changes in biochemical contents and antioxidant enzyme activities over time, and these changes are statistically significant ($p<0.05$). The total phenolic content in rose petals collected at 6.00 a.m. was 310.32 mg GAE $g^{-1}$. Due to the influence of sunlight and heat, the total phenolic content increased to 780.70 mg GAE $g^{-1}$ at 14.00 p.m., indicating a 151.57% increase. The total flavonoid content in samples collected at 6.00 a.m. was 33.90 mg CE $g^{-1}$, while in samples collected at 14.00 p.m., it was 62.33 mg CE $g^{-1}$, representing an 83.86% increase. The proline content in samples collected in the morning was 7.43 mg $g^{-1}$, reaching 24.96 mg $g^{-1}$ by 14.00 p.m., showing a 236% increase. The DPPH radical scavenging activity showed an 8.87% increase from 6.00 a.m. to 14.00 p.m. The lowest CAT activity was observed at 6.00 a.m. with 6.20 U $mg^{-1}$, while the highest CAT activity was observed at 14.00 p.m. with 42.67 U $mg^{-1}$, showing a 588.22% increase. The SOD activity at 6.00 a.m. was 4.67 U $mg^{-1}$, reaching 11.33 U $mg^{-1}$ at 14.00 p.m., showing a 142.61% increase. The APX activity at 6.00 a.m. was

12.11 mol $min^{-1}$ $g^{-1}$, reaching 85.55 mol $min^{-1}$ $g^{-1}$ at 14.00 p.m., showing a 606.44% increase. The POD activity at 6.00 a.m. was 3.42 $\Delta A_{460}$ $min^{-1}$ $mg^{-1}$, reaching 8.91 $\Delta A_{460}$ $min^{-1}$ $mg^{-1}$ at 14.00 p.m., showing a 160.52% increase.

As a result, the quality of rose oil depends on a series of factors, which can influence various stages, from the cultivation of flowers to their harvest, distillation process, and storage. Different rose species have different chemical compositions, affecting the quality of the oil. Growing conditions such as soil quality, climate, humidity levels, and sunlight directly impact the growth of the rose plant and the quality of its flowers. Additionally, the time of day when flowers are harvested also affects the quality of rose oil. Generally, the early morning hours are considered when rose petals have the most concentrated and high-quality volatile oil content. Therefore, based on the data obtained in our study, using appropriate distillation and extraction methods on rose petals collected at 6.00 in the morning would result in higher-quality rose oil. As time progresses, an increase in cellular ROS accumulation due to the stress experienced by the flowers may increase biochemical and antioxidant enzyme activities, which could also affect the oil quality.

## Ethical Statement

Ethical approval is not required for this study.

## Conflict of Interest

The authors declares that there are no conflicts of interest.

## Funding Statement

There is not funding sources in the study.

## Author Contributions

CÇ and AVP contributed to the study conception and design. CÇ and AVP performed the data analysis. CÇ and AVP wrote the first draft of the manuscript. CÇ and AVP acquired funds and supervised the analyses. CÇ and AVP supplied the seed material. All authors commented on previous versions of the manuscript and read and approved the final manuscript.

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