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Effect of Sorbitol Spraying on Chlorophyl, Total Phenolic and Flavonoid in *Fragaria ananassa*. Duch. cv. Albion Leaves

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A R T I C L E I N F O

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A B S T R A C T

Strawberry (*Fragaria* × *ananassa* Duch.) is one of the most widely consumed and cultivated fruits worldwide. Sorbitol plays a role in plant responses to many biotic and abiotic stresses. In this research, we intended to understand the effect of sorbitol spraying on the bioactive compounds of strawberry leaves. The application of sorbitol at different concentrations (0, 25, 50 mM and 75 mM) greatly improved strawberry characteristics such as total chlorophyll, chlorophyll a and b, carotenoids, and total phenolics. As sorbitol concentrations increased, chlorophyll a and chlorophyll b values increased in the samples taken during the fruiting period and higher values were obtained. The carotenoid content increased by approximately 189.49% and the total phenolic content increased by 30.85% in strawberry plants treated with sorbitol compared with the control. Supply of sorbitol decreased flavonoid content. The results indicate that sorbitol treatment has no inhibitory influence on the overall growth of strawberries. Among the biochemical parameters analyzed, chlorophyll, phenolic, and carotenoid contents increased, whereas flavonoid content decreased with sorbitol application.

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Introduction

Strawberries, which can be produced in many climates, are a fruit preferred by everyone because of their aromatic properties, delicious, fragrant, and good view. Different types of strawberries are grown around the world, especially in temperate climates. Strawberry is botanically a berry-like fruit in the genus Fragaria of the *Rosaceae* family (Oğuz, 2021). Strawberries are important for health because of their anti-carcinogenic, anti-inflammatory, and anti-neurodegenerative properties and play a strong economic and commercial role in the fruit industry (Giamperi et al., 2012; Nile & Park., 2014). The composition of polyphenols in strawberry fruit may vary depending on the variety, growing conditions, cultivation methods, and degree of ripeness (Pradas et al., 2015). Other important compounds found in strawberries are flavanols (Aaby et al., 2005), and strawberries are an excellent source of ellagic acid (Koponen et al., 2007; Michalska et al., 2017). The achenes (Aaby et al., 2005) and inedible parts of strawberry plants, such as rhizomes and leaves, were analyzed for phenolic compounds and antioxidant capacity (Simirgiotis & Schmeda-Hirschmann, 2010). In one study, young leaves of strawberries were found to be richer in polyphenolic substances than fruit flesh or older leaves (Wang & Lin, 2000). However, the level of bioactive compounds in strawberry leaves may influence the content of bioactive compounds in strawberry plants that will subsequently bear fruit. Such information could be valuable for breeders and help them identify plants that will bear fruit rich in bioactive compounds (Michalska et al., 2017).

In addition, the leaves of plants whose fruits are harvested are often used as biomass waste. In Turkey, approximately 676.818 tons (FAO, 2023) tons of strawberries are produced annually, which results in a significant amount of leaf waste at the end of the harvest season. It has been reported by different researchers that strawberry leaves contain phenolic compounds and have antioxidant properties (Buricova et al., 2011; Raudoniūtė et al., 2011; Wang and Lin, 2000; Sato et al., 2019). Strawberry leaves are also composed of polyphenols, which are recognized for their antioxidant capacity and benefits on human health, from anti-aging properties to cancer treatment (Muthukumaran et al., 2017). Studies have shown that strawberry leaf extracts can also be used as food additives and anti-aging cosmetic ingredients (Lee et al., 2018; Silva et al., 2022).

Sorbitol, commonly known as glucitol ($C6H14O6$), is a six-carbon sugar alcohol with a sweet taste. It is mostly derived from potato starch, but it is also found in nature, for example, in apples, pears, peaches, and prunes. Sorbitol is converted to fructose by the enzyme sorbitol-6-phosphate 2-dehydrogenase (Elbatrawy et al., 2023; Li et al., 2020). One of the most widely available polyols in higher plants is sorbitol. It is directly produced by photosynthesis in mature plant leaves (Issa et al., 2020; Li et al., 2020), parallels sucrose, and both serve similar functions, such as transporting carbon skeletons and energy between sources and sink organs (AL-Taee et al., 2022., Noiraud et al., 2001). Sorbitol is a major photosynthetic end product in plants of the Rosaceae family, and its metabolism is closely associated with the yield and quality of the fruit (Teo et al., 2006; Zhou et al., 2006).

Most plants' growth rate and productivity are affected negatively by abiotic stresses such as drought, salt, and extreme temperatures. (Sharma et al., 2019). Breeding of highly resistant crop varieties and direct application of exogenous chemicals directly on the crop can be used to protect against the damage caused by stress (Jin X et al., 2019; Li, et al., 2023). Chemicals such as sorbitol, sugars, sugar alcohols, amino acids (Theerakulpisut et al., 2012), γ-aminobutyric acid (Jin et al., 2019), 6-Benzylaminopurine (Rezaei et al., 2020), methyl jasmonate (Tayyab et al., 2020), ascorbic acid (Khazaei et al., 2020), and abscisic acid (Awan et al., 2021) have been proven to play a role in increasing plant stress tolerance to abiotic stressors (Li et al., 2023).

During plant growth and development, sorbitol acts as an energy source and is responsible for the translocation of carbon skeletons. It works as a signaling molecule, helping against abiotic and biotic stresses and regulating plant growth (Khaliq et al., 2023). Both have the same functions and energy from leaves to all other organs. Salt and drought stress increase sorbitol transport in both the xylem and phloem (Noiraud et al., 2001). According to a recent study, sorbitol provided resistance to salinity stress in wheat plants, and sorbitol application to non-saline-treated plants also proved to be beneficial as it increased yields. (Khaliq et al., 2023). In addition, a study showed that under salt stress, less sorbitol-producing Japanese persimmon leaves showed a decrease in photosynthetic activity compared with the leaves of wild-type plants, which increased the tolerance of sorbitol-producing plants to salt stress (Gao et al., 2001; Penna et al., 2006).

This study was conducted to evaluate the total chlorophyll, carotenoid, total phenolic, and total flavonoid contents of sorbitol treatments by foliar spraying in strawberry leaves during two growth periods: flowering and fruit ripening.

Materials and Methods

Material and Design

The study was conducted in the greenhouse belonging to the Department of Plant and Animal Production located in Amasya University Suluova Vocational School Campus (520m, 40°50'42.8' N and 35°38'0.08' E) during the growth period in 2021. Frigo seedlings of the Albion strawberry variety, which is a day-neutral strawberry, were used in the experiment. The frigo seedlings were planted in 3-L pots filled with the peat perlite mixture at the rate of 1:1 on 20.04.2022. Three different concentrations of sorbitol (0, 25, 50 and 75 g/L) were applied to strawberry leaves in addition to the control treatment (spraying with water). One month after planting, when the plants had 3-4 leaves, the first application of sorbitol was applied on 20.05.2022 and the second application was sprayed on 10.06.2022. Leaf samples were collected at two different times to determine the effect of sorbitol. The first leaf sample was taken 2 months after planting during the strawberry flowering stage (20.06.2022) and the second sample was taken 3 months after planting during the fruiting stage (10.08.2022). Conventional fertilizer (ammonium sulfate) was applied on May 16. For biochemical analysis, the leaves that had grown to their full size during these periods were cut off and kept at 20°C until analysis. Analyses were performed at the Suluova Vocational School laboratory.

Determination of Chlorophyll a, Chlorophyll b, Total Chlorophyll and Carotenoid Contents

Total chlorophyll, chlorophyll a, chlorophyll b, and carotenoid contents in fresh leaves of Albion strawberry cultivar are indicated as mg/g fresh weight per plant and were determined according to Witham et al. (1971) and Kırgeç et al. (2023).

Extraction Method

The TPC and TFC contents were determined in extracts obtained from dried leaf samples. The extracts were made according to the method of Ay et al. (2018). 200 mL of methanol (CH_3OH) was added to the samples prepared as 5 g each and the solutions obtained were macerated on a shaker for three days. The resulting suspension was then subjected to filtration with filter paper to remove the liquid component. The obtained liquid extracts were then treated in a rotary evaporator to remove the CH_3OH solvent.

Determination of Total Phenolic Content (TPC)

The TPC content of Albion plant extracts were calculated according to the method described by Slinkard and Singleton (1977). The data obtained were presented as mg gallic acid/g (mg GAE/g) in dried leaf samples.

Determination of Total Flavonoid Content (TFC)

The amount of TFC in Albion leaf samples was determined using quercetin standard solution following the methods described by Park et al. (2008). The total flavonoids were presented as mg quercetin equivalent (mg QE/g) per g of dried fraction.

Statistical Analysis

The experiment was set up with three repeats (10 plants in each repeat), four sorbitol doses (0, 25, 50 and 75 g/L), and two growing periods (flowering and fruit ripening period) according to a randomized plot design. The results were statistically evaluated by analysis of variance (ANOVA) using statistical analysis system software (SPSS) version 22. Significant differences were evaluated using the least significant difference (Tukey) and differences were considered statistically significant when $P < 0.05$.

Results and Discussion

Chlorophyll a Content

The chlorophyll a content obtained from the Albion strawberry leaf samples treated with sorbitol is shown in Figure 1. In general, chlorophyll a content was statistically higher in leaf samples harvested during fruit ripening than in leaves harvested during flowering period. The best result was found at the 50 g/L dose of sorbitol (1.10 mg/g) during the fruit ripening period, while the 25 g/L application during the flowering period gave the highest result for the amount of chlorophyll a (1.12 mg/g). The lowest amount of chlorophyll a was discovered in the control plants (0.29 mg/g) during the flowering period. The chlorophyll content in leaves is an indicator of the physiological state of a plant. Chlorophyll is the main photosynthetic pigment in plant leaves which contain chlorophyll as chlorophyll a and chlorophyll b. Leaf chlorophyll level is directly related to plant stress and physiological status of plants (Gargın, 2011; Gitelson et al., 2003). It has been reported that chlorophyll content decreases significantly when plants are under any kind of stress. Li et al. (2023) found that the chlorophyll content in maize seedlings was significantly reduced by high sorbitol concentrations.

The results obtained showed that the highest chlorophyll b content was obtained during the flowering period from the 75 g/L treatment (0.92 ± 0.79 mg/g), followed by the 25 g/L (0.61 ± 0.54 mg/g) treatment. The lowest value of chlorophyll b content was found in the control group (0.16 ± 0.62 EU/mg) in both periods. This shows that sorbitol treatment at 75 g/L can significantly increase the chlorophyll b content in Albion leaves. In their study on spinach, Humaira Gul et al. (2017) showed a non-significant increase in chlorophyll a, chlorophyll b, and total chlorophyll in plants treated with sorbitol at different doses. In another study, sorbitol application improved chlorophyll and chlorophyll b parameters under salt stress and normal conditions in wheat leaves (Khaliq et al., 2023).

Total Chlorophyll Content (TCC)

The highest TCC was obtained from the treatment of 75 g/L (1.87 ± 0.80 mg/g), which was 85.14-fold higher than that of the control (1.01 ± 0.03 EU/mg) during the fruit ripening period. However, it was also found that the total chlorophyll content decreased with increasing doses of sorbitol applied during the flowering period. As seen in Figure 3, the TCC of strawberry cv. Albion plant increased by 159.72 % during the fruit ripening period compared with the flowering period. It has been concluded that strawberry plants may have higher chlorophyll content in their leaves, which expand as they reach a certain maturity during the fruiting period, compared to younger leaves harvested during the flowering period. Indeed, Porro et al. (2002) examined the changes in chlorophyll content in the leaves of the Chardonnay grape variety according to phenological stages, and the measurements showed that the chlorophyll content increased as the leaves matured.

The study revealed the significant impact of different growth periods on the plant's total chlorophyll content. In our study, the total chlorophyll content was between 0.554-1.878 mg/g Uzal and Yıldız (2013) obtained TCC values between 0.683 and 2.814 mg/g in nine different strawberry varieties under salt stress. Photosynthetic pigments determine the physiological state of plants (Singh et al., 2004) found a decrease in chlorophyll and carotenoid contents in seedlings of lentil genotypes due to increasing salt levels. In another study, the total chlorophyll, chlorophyll a, and chlorophyll b contents of maize leaf segments decreased with sorbitol concentration (Jain et al., 2010).

Carotenoid Content

Carotenoid content determined from 'Albion' strawberry samples treated with sorbitol is shown in Figure 4. The highest carotenoid content was obtained from the sorbitol 50 g/L (0.4509 ± 0.45 mg/g) treatment compared with the 25 g/L treatment (0.2494 ± 0.24 mg/g) during the fruit ripening period. The lowest carotenoid content was recorded in the control (0.1542 ± 0.15 mg/g) treatment during the flowering period. When Figure 4. is reviewed, it is seen that 25 g/L sorbitol treatment increased the carotenoid content 189.49-fold compared with the control. In general, the carotenoid content of leaves was higher during the fruiting period than during the flowering period. This is due to the increase in the content of carotenoids in the leaves depending on the increased light intensity during the ripening period of the fruits.

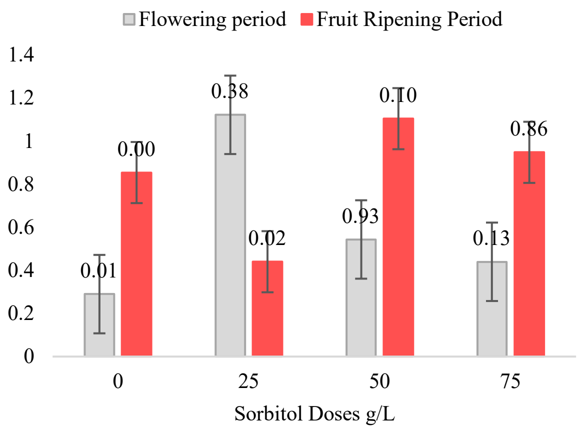

Figure 1. Effect of spraying sorbitol at different concentrations on Albion chlorophyll a (mg/g FW)

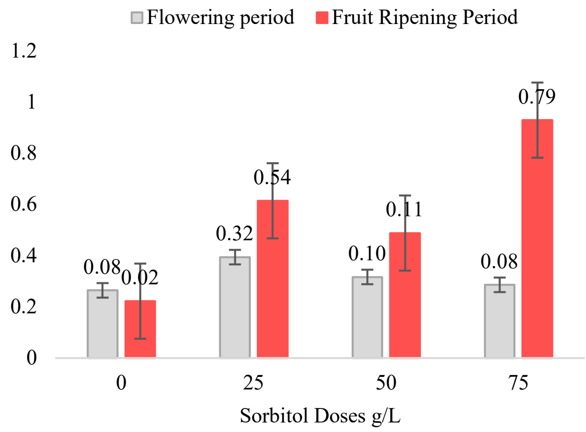

Figure 2. Effect of spraying sorbitol at different concentrations on Albion chlorophyll b (mg/g FW)

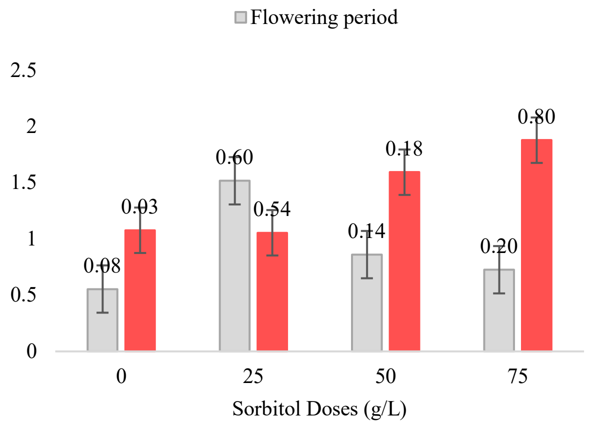

Figure 3. Effect of spraying sorbitol at different concentrations on Albion total chlorophyll (mg/g FW)

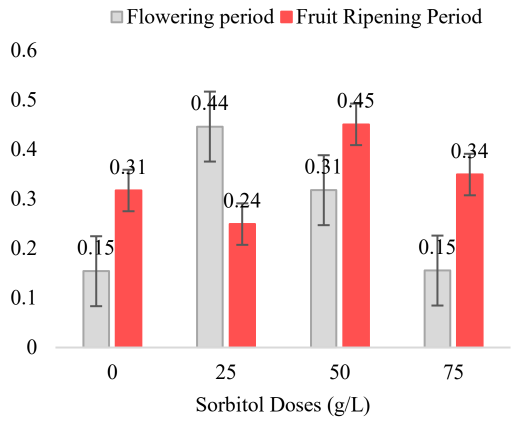

Figure 4. Effect of spraying sorbitol at different concentrations on Albion carotenoid content (mg/g FW)

Some studies have reported that more carotenoids accumulate in leaves exposed to sunlight (Demmig-Adams & Adams 1992, Demmig-Adams et al., 1996; Lauria et al., 2021). Jain et al. (2021) found that total chlorophyll and carotenoids decreased with the increase in sorbitol concentration when maize leaves were treated with sorbitol.

Total Phenolic content (TPC)

TPC formed from Albion samples treated with sorbitol are shown in Figure 5. The highest TPC during the fruit ripening period was 253.58 mg GAE/g with 75 g/L sorbitol treatment. The lowest TPC during the flowering period was found to be 173.09 mg GAE/g with the control. When the related figure is analyzed, the total phenolic content increased by 30.85 % in the 75 g/L sorbitol treatment during the fruit ripening period.

To our knowledge, there is no previous study on the effects of sorbitol treatment on total phenolic and flavonoid content in strawberries. However, there are studies on the effects of sorbitol on total phenolic and flavonoid content in some other plant species. In some of these studies, sorbitol concentrations increased or decreased total phenolic and flavonoid content. Açıkgöz (2021) determined that among the sorbitol doses applied in cell suspension cultures with *Ocimum basilicum*, 50 mg/L application was highly effective on TPC. Humaira Gul et al. (2017) studied the effect of sorbitol (15, 30, and 45 mM sorbitol) on the TPC of spinach. Plants treated with 45 mM sorbitol showed a significant decrease ($P<0.05$) compared to those treated with 30 mM sorbitol and control. According to El Far et al. (2009), high sorbitol concentrations increased TPC in all sweet potato genotypes used in the experiment. This study revealed a significant impact of different growth periods on the plant's total phenolic content. Dilek et al. (2022) investigated the effect of safflower sorbitol application and found that different periods of plant growth significantly affected phenolic compounds. Özeker and Tanrısever (1999) determined that phenolic substance compositions varied even in one-week periods in their study on strawberries. In studies examining the bioactive components of olive leaves, it was found that the amount of phenolic substances varied statistically significantly throughout the year (Doğançay, 2013; Kurtulmuş, 2016).

Total Flavonoid Content

The TFC of Albion strawberry variety samples treated with sorbitol is presented in Figure 6. The highest value of TFC was found as 199.58 mg QE/g during the flowering stage with the control. The lowest TFC during the fruit ripening period was 96.42 mg QE/g with 75 g/L sorbitol treatment. As illustrated in Figure 6, the TFC of Albion plants decreased by 51.57% during the fruit ripening stage compared to the flowering stage. In our study, it was observed that different developmental periods were effective on the amount of flavonoid substances. While the highest flavonoid content was observed during the flowering period, the flavonoid content decreased during the fruit ripening period. When compared with previous studies (Dilek et al., 2022; Steberl, Hartung, Munz & Graeff-Hönninger, 2020), it was determined that our study was in accordance with the literature in terms of the decrease in the amount of flavonoid substance as the development period progressed.

In the study conducted by Dilek et al. (2022) in safflower, the highest amount of flavonoid (24.49 mg QE/g) was obtained from the lowest sorbitol concentrations (5g/l) before the blooming period. The following conclusions were reached in the study that there was a significant difference in flavonoid contents in different growth periods as in our study. Similarly, in spinach, flavonoids increased in sorbitol treatments at concentrations of 15 and 30 mM, while a decrease was recorded at higher treatments (45 mM sorbitol). (Humaira et al., 2017). In general, the increase in flavonoid content of leaves is a favorable indicator for plant adaptation to abiotic stress conditions, which in turn helps plant protection (Dixon & Paiva, 1995; Grace & Logan, 2000; Viršilė et al., 2018). Contrary to our study, Açıkgöz (2021) determined that the highest total flavonoid content of *Ocimum basilicum* was 200 mg/L at the highest sorbitol treatment concentration according to the control. It has been determined that the flavonoid content increased with increasing sorbitol doses.

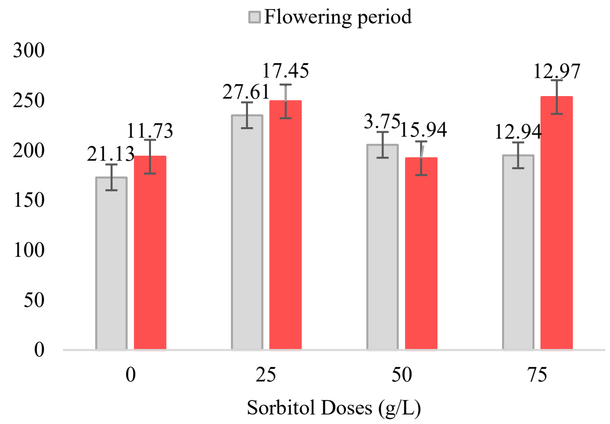

Figure 5. Effect of different doses of sorbitol spraying on Albion phenolic content (mg/g DW).

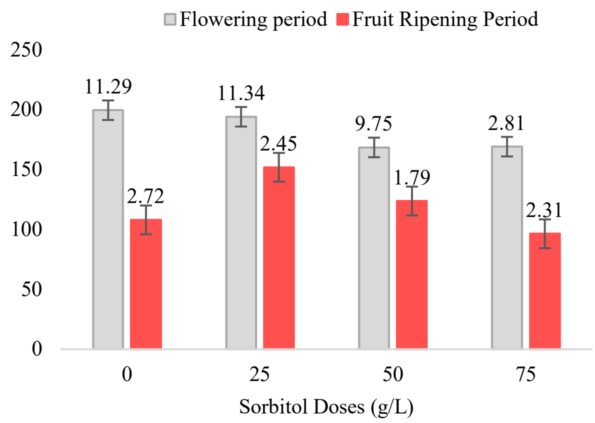

Figure 6. Effect of different doses of sorbitol spraying on Albion flavonoid content (mg/g DW).

In our study, it was determined that total phenolic and total flovanoid contents tended to increase or decrease according to the sorbitol doses and growth periods. In fact, secondary substances in plants are affected by many parameters such as variety, planting time, fertilization, irrigation, development periods, harvest time, environmental stress conditions. These parameters are quantified in the secondary substances variability (Kizil et al., 2008; Mohammadi & Tavakoli, 2015; Caliskan & Caliskan, 2018). There are also studies showing that different harvest times in safflower significantly change the secondary metabolite content of the plant (Dilek et al., 2022; Salem et al., 2011).

Conclusion

This study focused on the effect of sorbitol treatment on strawberry leaf compounds on biochemicals. Sorbitol has been studied in different species to reduce the negative effects of various stressors. To our knowledge, the effects of sorbitol treatments on chlorophyll a (mg/g), chlorophyll b (mg/g), total chlorophyll (mg/g), carotenoid (mg/g), total phenolic content (TPC) and flavonoid content (TPC) in *Fragaria* × *ananassa*, Duch. were studied for the first time. It was determined that the highest chlorophyll b, total chlorophyll and total phenolic content in strawberry was obtained from 75 g/L sorbitol application. According to the findings obtained in our study shows that the application of sorbitol to strawberry plants has a positive effect on the examined parameters. Therefore, it may be useful to use sorbitol for both stress factors and to increase yield.

Chlorophyll b, total chlorophyll and total phenolic contents of strawberries at different developmental stages were highest during the fruit ripening period. Chlorophyll a, total chlorophyll, carotenoid and total phenolic contents were found to be the lowest during the flowering period. It has been proved by studies that secondary metabolites in plants vary according to plant species, type and severity of stress applied, harvest time and developmental stages. As a result, we can say that secondary metabolite content in strawberry follows the order of flowering period < fruit ripening period.

Declarations

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