

## Quality characteristics and antioxidant activity of the mango (*Mangifera indica*) fruit under arginine treatment

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Received: March 28, 2021 Accepted: June 23, 2021

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### Abstract

Mango fruit is one of the most important fruits in terms of nutritional value. One of the important characters of mango is the presence of antioxidants such as polyphenols, carotenoids, and anthocyanins in its fruits and vegetables. These compounds are involved in preventing diseases and maintaining health in humans. Amino acids have been used to preserve and enhance fruits quality. In this study, the mango fruits of a local cultivar were sprayed with the arginine amino acid at concentrations of 0, 200, and 400  $\mu$ M at the time of fruit formation and 10 days after this stage. Results showed that total phenolics, anthocyanins, carotenoids, antioxidant capacity, total soluble solids, pH, fruit weight, and seed weight of the fruits increased with the increase in arginine concentration and 400  $\mu$ M arginine had a better effect on these characters. Comparison of the times of application indicated that the second stage of the foliar application was generally more effective than the first stage in increasing the studied characters.

**Keywords:** Antioxidant; Arginine; Mango; Quality

**Citation:** Pakkish Z and Mohammadrezakhani S, 2021. Quality characteristics and antioxidant activity of the mango (*Mangifera indica*) fruit under arginine treatment. Journal of Plant Physiology and Breeding 11(1): 63-74.

### Introduction

Mango is a popular tropical fruit because of its taste, aroma, and flavor. It is cultivated in more than 100 tropical and subtropical countries (Bally *et al.* 2020). Mango has a high nutritional value and is the main source of antioxidants including carotenoids, ascorbic acid, and phenolic compounds (Manthey and Perkins 2009). The phenolic compounds include flavonoids, phenolic acids, xanthenes, and gallotannins (Kim *et al.* 2010), although this composition varies between different mango cultivars (Manthey and Perkins 2009). Antioxidants delay or remove the oxidation of oxidizing substrates in cells (Halliwell 1996). The antioxidant enzymes protect the cells of the organisms from oxidative damage. As an example, superoxide dismutase (SOD) converts

superoxide radicals into hydrogen peroxide and oxygen (Yim *et al.* 1996). Mango contains a mixture of sugars and acids (Lebaka *et al.* 2021), which are the important constituents in the sweetness and acidity of fruit (Malundo *et al.* 2001).

Increasing yield and quality of mango fruit may be achieved through appropriate cultural methods including foliar fertilization, which contains some amino acids and plant nutrients. Amino acids synthesize many compounds that are important for production and fruit quality (do C Mouco *et al.* 2009). L-arginine is one of the most important amino acids in plants. Arginine is constituent of proteins and serves as a precursor for the biosynthesis of several molecules such as proline and polyamines (Chen *et al.* 2004). In

general, arginine is an essential metabolite for many cellular and developmental processes (Winter *et al.* 2015). It has been suggested that both endogenous and exogenous arginine play roles in the responses of plants to stresses such as drought (Nasibi *et al.* 2011). Researchers have reported the positive role of arginine in reducing the inhibition that is caused by exposure of plants to stress conditions (Hassanein *et al.* 2008; Khalil *et al.* 2009). Arginine increased growth and root weight and length, chlorophylls, and carotenoids in faba bean (Nassar *et al.* 2003). The use of chemicals to maintain the quality and marketability of fruits for post-harvest has been advocated in recent years. The purpose of this study was to evaluate the effect of arginine on maintaining quality and antioxidant compounds in mango fruits.

## Materials and Methods

### Plant materials

This research was conducted in a commercial orchard located in the south of the Kerman province of Iran. The 15-year-old trees at a spacing of 6 × 6 m with standard cultural practices were selected. The experiment was laid out as factorial based on the randomized complete block design with three replications.

Branches containing fruits of desired trees were treated with the amino acid arginine at concentrations of 0, 200, and 400 µM. The mango fruits of a local cultivar were sprayed at two stages:

Stage 1: at the time of fruit formation (fruit diameter less than one centimeter).

Stage 2: ten days after the first stage.

The branches with approximately similar lengths and the number of fruits were selected from the same age trees and 3-4 fruits were selected in each branch. The treated branches were separated 24 h after spraying and the treated fruits were immediately frozen in liquid nitrogen and stored at -80 °C for later analyses.

### Analysis of biochemical characters

The extractions of samples were prepared by homogenizing 1 g of fruit in 4 ml of ice-cold, 50 mM potassium phosphate buffer (pH 7.0) with 2 mM Na-EDTA, and 1% (w/v) polyvinyl-pyrrolidone (PVP). The homogenate was centrifuged at 10,000 × g for 10 min (Bradford 1976). Then, the following enzymes were assayed.

### Ascorbate peroxidase activity

Ascorbate peroxidase (APX) activity was determined according to Nakano and Asada (1987). The assay mixture contained 100 µg of the enzyme extract added which was added to the assay solution [50 mM potassium phosphate buffer (pH 6.6) and 2.5 mM ascorbate] and the reaction was initiated by adding 10 mM H<sub>2</sub>O<sub>2</sub>. The decrease in the absorbance of ascorbate was read at 290 nm for 3 min against assay solution ( $\epsilon = 2.8 \text{ mM}^{-1} \text{ cm}^{-1}$ ).

### Catalase

Catalase (CAT) activity was measured according to Dhindsa *et al.* (1981). The extract of the crude enzyme (50 µM) was added to the catalase assay solution [50 mM potassium phosphate buffer (pH 7.0) and 15 mM H<sub>2</sub>O<sub>2</sub>]. Absorbance was read at

240 nm at 1 min intervals.

### SOD

SOD was measured by the method of Beauchamp and Fridovich (1971). To obtain the reaction mixture, 50 µl of the crude enzyme extract was mixed with the SOD assay solution [50 mM potassium phosphate buffer (pH 7.8), 13 mM L-methionine, 2 µM riboflavin, 0.1 mM EDTA, and 75 mM nitro blue tetrazolium (NBT)]. After shaking the test tube, it was put in a lightbox for 15 min. One unit of the enzyme activity was equal to the amount required to inhibit the 50% NBT reduction by observing the absorbance at 560 nm.

### Carotenoids and photosynthetic pigments

An amount of 0.1 g fruit in 15 ml of 80% acetone was homogenized and then, the absorbance was read by a spectrophotometer at the wavelengths of 663.2, 646.8, and 470 nm (Lichtenthaler 1987). The concentration of photosynthetic pigments and carotenoids were calculated using the following formulae:

$$\text{Chl a} = 12.25 A_{663.2} - 2.79 A_{646.8}$$

$$\text{Chl b} = 21.21 A_{646.8} - 5.1 A_{663.2}$$

$$\text{Carotenoids} = (1000 A_{470} - 1.8 \text{ Chl a} - 85.02 \text{ Chl b}) / 198$$

### Total phenolic content

Total phenolic content was measured according to Zieslin and Ben (1993). A 2 ml volume of 80% methanol was added to 1 g of the plant tissue. Then, 0.5 mL of methanolic extract was mixed with 0.5 mL Folin-Ciocalteu reagent on a shaker for 15-20 sec and after 3 min, 1 ml of saturated

sodium carbonate and 1 ml of distilled water were added to this solution. This mixture was incubated in a dark room for 2 h. Then, the absorbance was recorded by a spectrophotometer at 725 nm against deionized water.

### Measurement of DPPH

To measure DPPH, 0.2 g from the plant tissue was grounded in a mortar and pestle in 2 mL of absolute ethanol at 4 °C. An amount of 1.5 milliliters from the solution was mixed with 0.25 mL of 0.5 mM DPPH and 0.5 mL of 100 mM acetate buffer (pH 5.5). The absorbance was recorded at 517 nm after 30 min according to ABE *et al.* (1998).

### Anthocyanin content

The ethanolic HCl solution (0.25 M) was added to an aliquot of the extract to reach a dilution of 1:10 based on Kim *et al.* (2003). After mixing the solution, the absorbance was read at 520 nm ( $A_{520}$ ) using the ethanolic HCl solution as blank. Total anthocyanin content was measured as cyanin equivalents per 100 g of the fresh tissue (Kim *et al.* 2003).

### Total soluble solids

The total soluble solids (TSS) content was measured using a hand-held refractometer (American Optical Co., NH, USA).

### Acidity measurement

The acidity was measured by a pH meter (Model 3320 manufactured by Jenway, UK).

### **Fruit and seed weights**

Fruit and seed weights were measured by a digital scale (ELB 1200, Shimadzu, Kyoto, Japan).

### **Statistical analysis**

The experimental design was laid out as factorial based on randomized complete blocks with three replications. The data were first subjected to analysis of variance and then the means were compared using Duncan's multiple range test at  $p \leq 0.05$ . The SAS version 9.1 was used for all analyses.

## **Results**

### **Enzyme activity**

The activity of antioxidant enzymes was presented in Table 1. The application of arginine with different concentrations at both stages enhanced the activity of APX, CAT, and SOD compared to the control fruits at  $p \leq 0.05$ . At each stage, fruits treated with 400  $\mu\text{M}$  arginine showed the highest levels of enzyme activity (Table 1).

### **Antioxidant capacity**

The results showed that the fruits treated with 400  $\mu\text{M}$  arginine had higher antioxidant capacity than the control fruits at both stages. However, the difference of 200  $\mu\text{M}$  arginine with the controls was only significant at stage 2. Also, with increasing the arginine concentration, the antioxidant capacity increased at stage 1 in the mango fruits (Figure 1).

### **Carotenoids content**

The levels of carotenoids of stages 1 and 2 were not significantly different in the control fruits. Mango fruits that were sprayed with both concentrations of arginine at both stages resulted in an increase in the carotenoids content compared to the control fruits (Figure 2). The carotenoids content related to the arginine concentration of 400  $\mu\text{M}$  at the second stage was significantly higher than other treatments.

### **Total phenolic content**

The amount of total phenolic content in the control fruits at both stages was significantly lower than the treated fruits. The highest total phenolic compound was observed in the fruits treated with 400  $\mu\text{M}$  arginine at ten days after the first stage (Figure 3).

### **Anthocyanin**

The anthocyanin content of the treated fruits significantly increased with the increase in arginine as compared to the control fruits. The highest anthocyanin content was observed in the fruits treated with 400  $\mu\text{M}$  arginine at stage 2 (Figure 4).

### **Total soluble solids and pH content**

The TSS increased significantly only in the fruits treated with the 400  $\mu\text{M}$  arginine at the second stage as compared to the control fruits. However, pH increased significantly by treating the fruits with arginine at both stages. Arginine application at the 400  $\mu\text{M}$  concentration showed the highest effect on pH at the second stage (Figures 5 and 6).

### Fruit and seed weight

Arginine pretreatment with both concentrations (200 and 400  $\mu\text{M}$ ) at stage 2 resulted in a significant increase of the fruit weight and seed weight of the mango fruit as compared to the

respected controls (Figures 7 and 8). However, at stage 1, only 400  $\mu\text{M}$  was effective in increasing the fruit weight when compared with the control fruits.

Table 1. Ascorbate peroxidase (APX), superoxide dismutase (SOD), and catalase (CAT) activity in mango fruits

Treatment	SOD		APX		CAT	
	Stage 1	Stage 2	Stage 1	Stage 2	Stage 1	Stage 2
Control	18.04c	17.98c	10.25c	10.38c	7.11c	7.08c
200 $\mu\text{M}$	21.65b	23.45b	14.11b	16.32b	9.23b	10.87b
400 $\mu\text{M}$	26.38a	31.94a	18.27a	21.38a	12.98a	14.26a

Note: Values in the same column with different superscript letters are significantly different at  $p \leq 0.5$  by Duncan's multiple range test. Stage 1: at the time of fruit setting; Stage 2: ten days after stage 1.

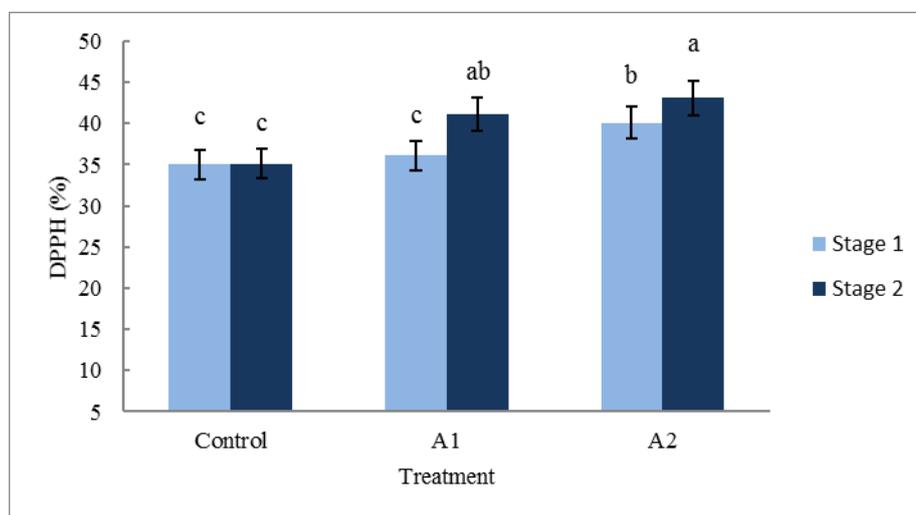


Figure 1. Effect of different concentrations of arginine on DPPH in the mango fruits at the time of fruit formation (stage 1) and ten days after stage 1 (stage 2). A<sub>1</sub>: 200  $\mu\text{M}$ , A<sub>2</sub>: 400  $\mu\text{M}$

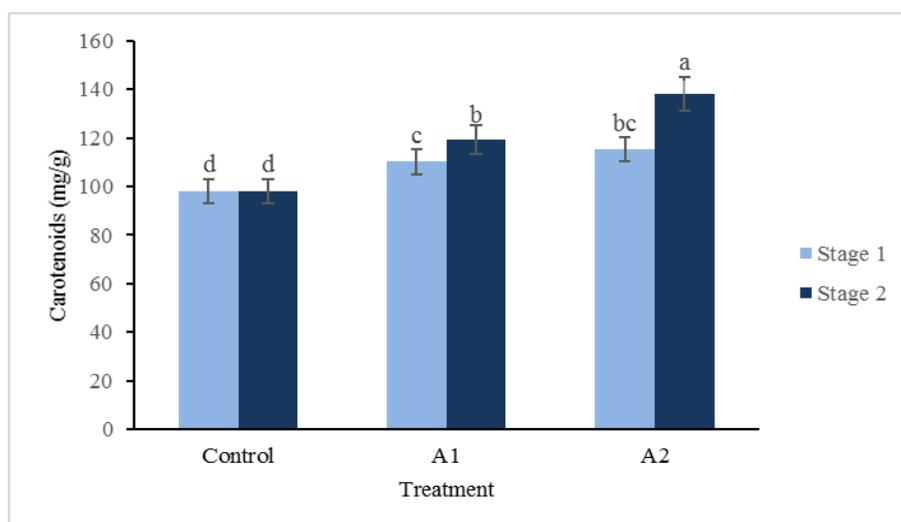


Figure 2. Effect of different concentrations of arginine on the amount of carotenoids in the mango fruits at the time of fruit formation (stage 1) and ten days after stage 1 (stage 2). A<sub>1</sub>: 200  $\mu\text{M}$ , A<sub>2</sub>: 400  $\mu\text{M}$

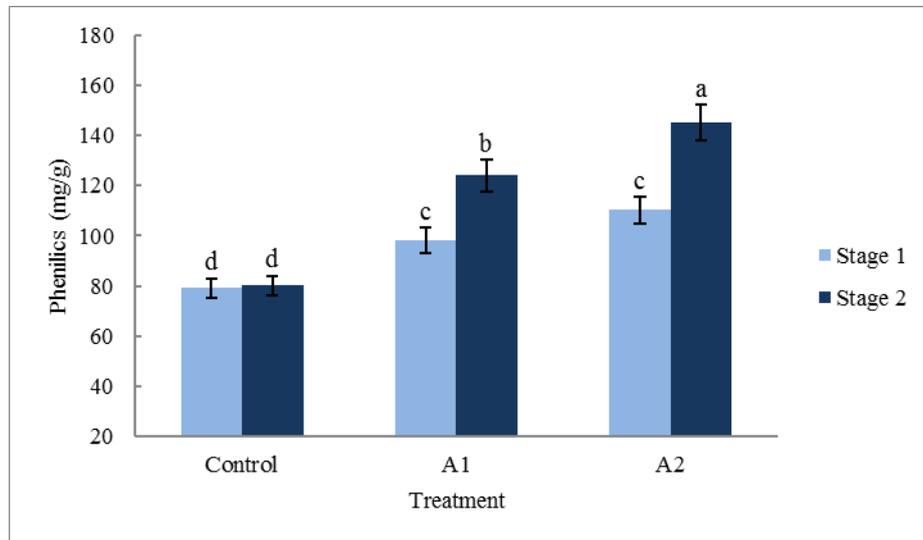


Figure 3. Effect of different concentrations of arginine on the total phenolic content in the mango fruits at the time of fruit formation (stage 1) and ten days after stage 1 (stage 2). A<sub>1</sub>: 200 μM, A<sub>2</sub>: 400 μM

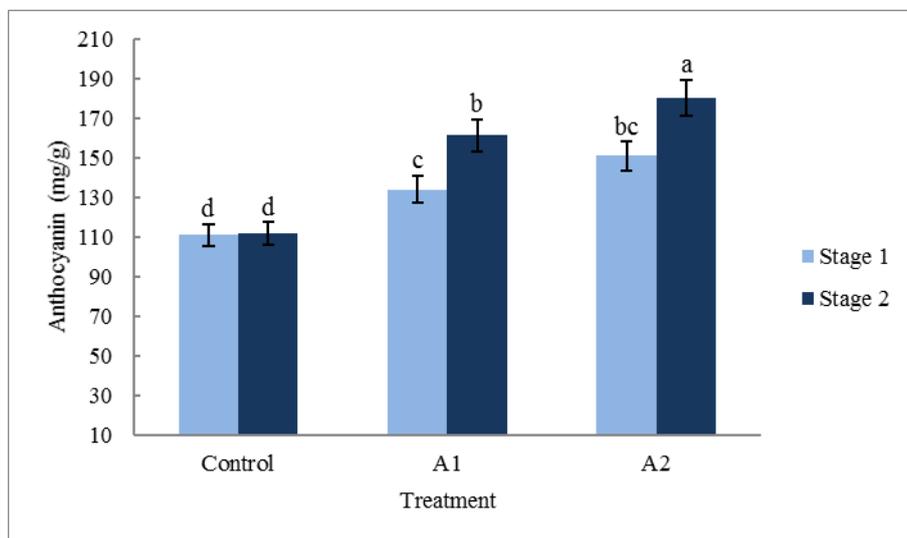


Figure 4. Effect of different concentrations of arginine on the amount of anthocyanin in the mango fruits at the time of fruit formation (stage 1) and ten days after stage 1 (stage 2). A<sub>1</sub>: 200 μM, A<sub>2</sub>: 400 μM

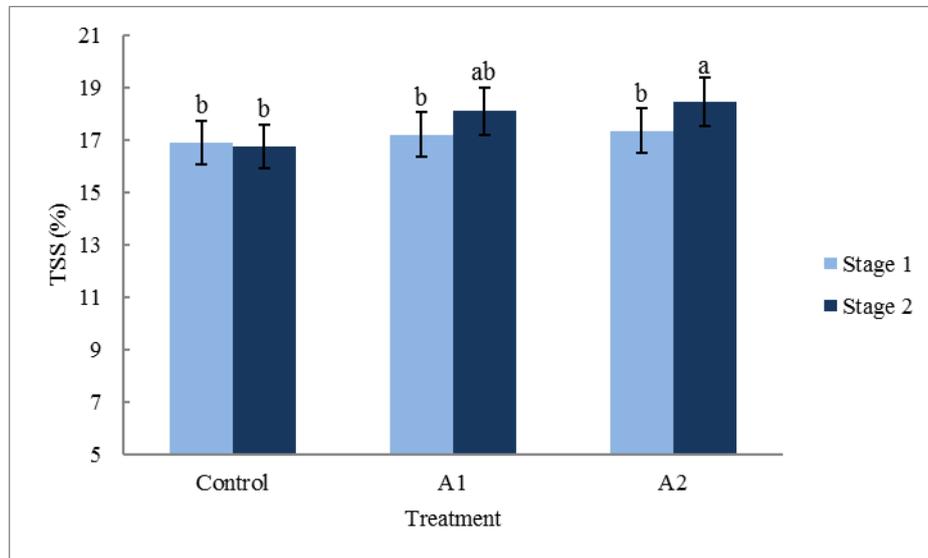


Figure 5. Effect of different concentrations of arginine on total soluble solids (TSS) in the mango fruits at the time of fruit formation (stage 1) and ten days after stage 1 (stage 2). A<sub>1</sub>: 200  $\mu$ M, A<sub>2</sub>: 400  $\mu$ M

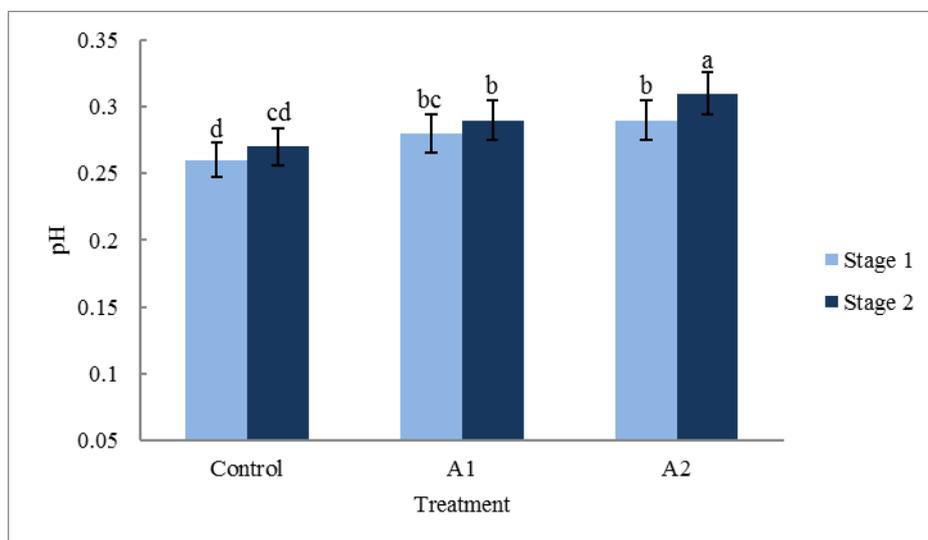


Figure 6. Effect of different concentrations of arginine on pH of the mango fruits at the time of fruit formation (stage 1) and ten days after stage 1 (stage 2). A<sub>1</sub>: 200  $\mu$ M, A<sub>2</sub>: 400  $\mu$ M

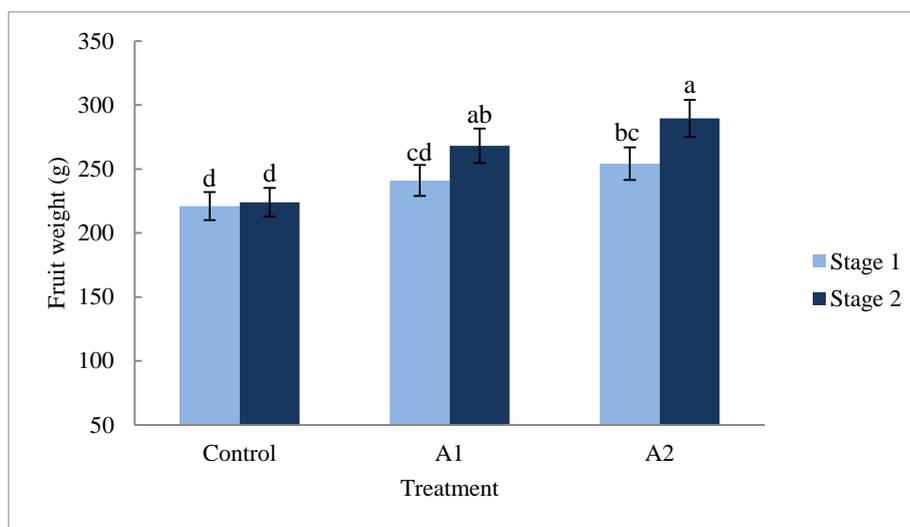


Figure 7. Effect of different concentrations of arginine on the fruit weight of the mango fruits at the time of fruit formation (stage 1) and ten days after stage 1 (stage 2). A<sub>1</sub>: 200  $\mu$ M, A<sub>2</sub>: 400  $\mu$ M

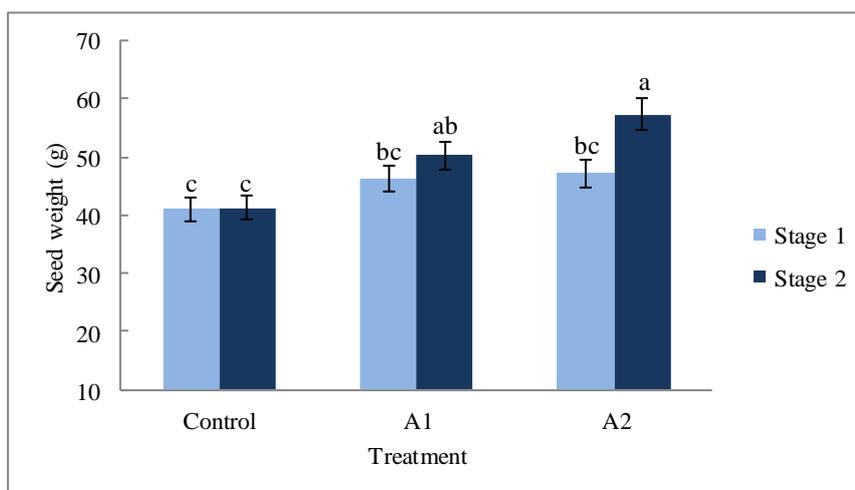


Figure 8. Effect of different concentrations of arginine on the seed weight of the mango fruits at the time of fruit formation (stage 1) and ten days after stage 1 (stage 2). A<sub>1</sub>: 200  $\mu$ M, A<sub>2</sub>: 400  $\mu$ M

## Discussion

The results of this experiment showed that the use of arginine increased the fruit weight, seed weight, and chemical properties of the mango fruit. Arginine acts as the precursor of the polyamines in plants (Winter *et al.* 2015) and polyamines play important roles in regulating

plant growth and developmental processes and fine-tuning defense mechanisms against environmental stresses (Winter *et al.* 2015; Chen *et al.* 2019). Arginine helps in the better synthesis of chlorophyll and improves photosynthesis (Yagi and Al-Abdulkareem 2006). Also, arginine stimulates the synthesis of phytohormones related

to flowers and fruits (Tarengi and Martin-Tanguy 1995). According to Mostafa *et al.* (2010), foliar application of arginine showed a significant increase in the growth and yield-related traits in wheat. Hassanein *et al.* (2008) reported that arginine was effective in improving the growth and yield in wheat at the high-temperature stress conditions. Mohseni *et al.* (2017) also reported an increase in the fruit weight of the strawberry cultivar Paros when treated with arginine. Nassar *et al.* (2003) reported that the application of arginine (as a putrescine precursor) increased plant growth, and fresh weight, dry weight, and length of roots and shoots in faba beans.

In this study, the application of arginine raised the activity of SOD, CAT, and APX antioxidants, and the application of 400  $\mu\text{M}$  arginine had the highest positive effects on the activity of these enzymes. During fruit ripening, overproduction of ROS causes oxidative damage, thereby reducing the ability of the antioxidant system to remove the free radicals such as  $\text{H}_2\text{O}_2$  and  $\text{O}^{2-}$  (Jimenez *et al.* 2002). The formation of several antioxidative enzymes, such as SOD, POX, and CAT, and non-enzymatic antioxidants like beta-carotene and ascorbic acid prevents the accumulation of ROS (Hodges *et al.* 2004). Polyamines protect the membranes from ROS injury (Verma and Mishra 2005). Polyamines act against  $\text{H}_2\text{O}_2$  and  $\text{O}^{2-}$  by the induction of APX, CAT, SOD and other enzymes. Increase in the activity of these enzymes plays a key role in controlling ROS and provide protection for the degradation of biomolecules against oxidative

damage (Verma and Mishra 2005). According to Razzaq *et al.* (2014), putrescine application on the 'Samar Bahisht Chaunsa' mango delayed fruit softening due to the suppression of the ethylene production and increasing the activities of antioxidants such as SOD, POX, and CAT.

Arginine improved total phenolics, anthocyanins, carotenoids, TSS, and pH of the mango fruits in this study that is in concordance with the results in several crops (Nassar *et al.* 2003; Nasibi *et al.* 2011; Mohseni *et al.* 2017). According to Nasibi *et al.* (2011), treating the tomato plants with arginine increased the soluble sugars. The effect of arginine on the strawberry cultivar Paros resulted in an increase in the amount of phenolic compounds, anthocyanins, and TSS (Mohseni *et al.* 2017). Arginine increased the amount of carotenoids in faba beans compared with the control plants (Nassar *et al.* 2003). The existence of an appreciable amount of total phenolic content in mango may increase the intake of the antioxidants in the diet (Scalbert and Williamson 2000).

### Conclusion

Treating fruits of mango with 200 and 400  $\mu\text{M}$  arginine at the time of fruit formation (stage 1) and ten days after stage 1 (stage 2) resulted in the improvement of fruit weight, seed weight, anthocyanins, carotenoids, pH, phenolics, TSS, and antioxidant compounds. Treatment of arginine at concentration of 400  $\mu\text{M}$  at the stage 2 was most effective in improving the quality and quantity of mango fruits.

**Conflict of Interest**

The authors declare that they have no conflict of

interest with any people or organization concerning the subject of the manuscript.

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## تأثیر کاربرد خارجی اسید آمینه آرژنین بر برخی ویژگی‌های کیفی و فعالیت آنتی‌اکسیدانی در میوه‌های انبه (*Mangifera indica*)

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### چکیده:

پلی فنول‌ها، کاروتنوئیدها و آنتوسیانین‌های موجود در میوه‌ها و سبزیجات به دلیل اثرات آنتی‌اکسیدانی بیشتر مورد توجه قرار گرفته‌اند. این ترکیبات در پیشگیری از بیماری‌ها و حفظ سلامت انسان نقش دارند. اسیدهای آمینه برای حفظ و افزایش کیفیت میوه‌ها استفاده شده‌اند. در این آزمایش، میوه‌های انبه با اسید آمینه آرژنین در غلظت‌های ۰، ۲۰۰ و ۴۰۰ میکرومولار تیمار شدند. میوه‌های رقم محلی در دو مرحله شامل زمان تشکیل میوه و ۱۰ روز پس از مرحله اول سم پاشی شدند. تأثیر آرژنین بر کل فنول‌ها، آنتوسیانین‌ها، ظرفیت اکسیدانی، کاروتنوئیدها، کل مواد جامد محلول، pH، وزن میوه و دانه در انبه مورد بررسی قرار گرفت. نتایج نشان داد که صفات اندازه‌گیری شده با افزایش غلظت آرژنین افزایش می‌یابند. آرژنین ۴۰۰ میکرومولار منجر به بهبود کل فنولیک‌ها، آنتوسیانین‌ها، ظرفیت آنتی‌اکسیدانی، کاروتنوئیدها، کل مواد جامد محلول، pH میوه و وزن دانه در میوه‌های انبه شد. مقایسه زمان کاربرد آرژنین نشان داد که ۱۰ روز پس از تشکیل میوه به طور کلی موثرتر از مرحله اول در بهبود صفات مورد مطالعه بود.

واژه‌های کلیدی: آرژنین؛ انبه؛ آنتی‌اکسیدان؛ کیفیت میوه