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SUGAR, PHENOL, FLAVONOID, ANTHOCYANIN, AND CAROTENE AS BIOACTIVE AND ANTIOXIDANT COMPOUNDS IN FICUS FRUIT FOR HUMAN HEALTH BENEFITS

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ABSTRACT

Fig (*Ficus* sp) fruit exhibits an outstanding role as a nutritional and medicinal fruit. It is utilized as a fruit as well as a source of different types of food, nutritional and bio-medicinal products. Ficus fruit contains a form of sugar that reveals a high level of mobility and heat energy which can easily be broken down in the body. Fig fruit contains vitamins, nutrient contents and minerals. The study was carried out to investigate the carbohydrate (sucrose, glucose and fructose), total soluble solids (TSS) nutrient, flavonoid and antioxidant content of fig fruit. The treatments were subjected to control (distilled water), 10% sugar solution and 15 ppm indole acetic acid (IAA). The per cent fruit set and weight were greater in 15ppm IAA than in the control and sugar solution. However, the result showed that all three treated organic solutions had a significantly higher percentage of fruit set than that of the control one. Fruit length and diameter were higher in the organic treated solution than in the control (distilled water). However, inverted sugar (sucrose), glucose, fructose, carbohydrate and total soluble solids (TSS) were found higher in 15ppm IAA than in 10% sugar solution and control. Flavonoid and total antioxidants were found higher in 15ppm IAA than in 10% sugar and control solution. Titratable acidity (TA) was the lowest in the 15ppm IAA. In addition to that, mineral content like potassium, calcium, sodium was higher in 10% sugars and 15ppm IAA compared to the control. Moreover, the DNA band (segment) was wider in 15ppm IAA and 10% sugar than in the control. The results conclude that 15ppm IAA contained better nutrients, antioxidants and flavonoids than 10% sugar and water control.

KEYWORDS: Fig fruit, sugars, nutrient, flavonoid and antioxidant.

INTRODUCTION

Figs (*Ficus carica* L.) are usually cultivated in warm, dry climates. The fig fruit is a highly perishable climacteric fruit and the oldest species of the fruit tree having been cultivated by humans for over 5000 years.[1,2] The common fig (*Ficus carica* L.) is a tree indigenous to southwest Asia and the eastern Mediterranean region; belongs to the family *Moracea*.[3] The world production of figs is about one million tons.[4] This fruit is an important crop worldwide for dry and fresh consumption.[3, 5] Figs are one of the highest calorie-containing fruits. They are greater sources of minerals and vitamins and compose health benefit having flavonoid, polyphenolic, antioxidants.[6,7] These possess have anti-inflammatory and anti-hemorrhagic properties (7 Umesh, 2009). Carotene, particularly beta-carotene and lycopene have been shown to influence the hormone level and activity to estrogen and thyroid hormones (Umesh, 2009). In addition, some researchers reported that carotinoid could interfere with estrogen signaling and potentially regulate hormone dependent cancer cell growth. Fruits nutrients are augmenting in combination with the food scene as the healthiest alternative.[8] Fig fruit contains a lot of vitamins and minerals. They also contain sodium, potassium, calcium, magnesium, iron, sulphur, phosphorus and chlorine, as well as beta-carotene, B1, B2, B3 and B6.[8] Fig fruits are the best as medicinal and nutritional fruit compared to other fruit varieties.[9] It contains potassium, calcium, iron, carbohydrate, sugars and dietary fiber.[9]

[10] It was reported that nutrient content was affected by environmental factors and they are very rich in fiber, fat and proteins. [11] It was suggested that fruit polysaccharide (cellulose and hemicellulose), lignin and pectin were varied from different location. [12] It was carried out an extensive experiment at different localities of alphonso fruit. They observed that fruit physiological (firmness, fiber) and phenotypic change has been differently occurred. They also reported that chemical composition like flavor and aroma volatiles compounds were varied at different locations. They stated that these change have been occurred due to the varied abiotic factors like light, temperature, soil pH, humidity and growth regulated hormones.[6] They also suggested that molecular mechanism regulated by the biosynthesis was varied at different localities.[13] It was stated that fruit quality has been varied from different localities.[14,15] It was stated that different temperature, water and light intensity were affected the fig fruit quality. It has been found that hydrocarbon and volatile compounds of alphonso fruit were different at different localities.[16] They also recommended that environmental factors can affect any fruit quality and development. However, little literature found regarding this current research. Therefore, the study was undertaken with the following objectives.

To investigate the carbohydrate as sugars (sucrose, glucose and fructose), total soluble solids (TSS) nutrient, beta carotene, flavonoid and antioxidant content of fig fruit.

MATERIALS AND METHODS

The experiment was conducted in the farmer's garden, located in northern region, KSA.

Plant material

Nine-year-old fig trees (*Ficus carica* L.) were used in the experiment. Total of nine trees were selected. Intercultural operation like irrigation and pesticide were done as needed. Fertilizers were applied in the 1st year when tree was planted at the rate of N, P and K (10%, 10% and 10%) 20 g respectively. The same procedures were done every year. The soil was fertile and loamy.

Treatment setting

The treatments were control (distilled water), 10% sugar solution and 15% IAA solution. The replicates were three branches for each tree. A total of nine trees were selected. Three trees were used for each treatment. The tree consisted of 3 main branches. Branch spacing was 0.05m approximately. Treatments were applied for 2 weeks at one-week intervals using the swabbing method at the beginning of initiation of the fruit set.

Data collection

Fruit set per cent was measured after 15 days starting from the following week of treatment setting.

Fruit harvesting: Fruit were harvested and recorded immediately after harvest. Fruit weight was measured after 2 months of the treatment application. Maturity index (represented by colour) was measured after the harvest. Fruit weight was measured.

Maturity index: Maturity index was determined by scoring 1–5. Green fruits were scored 1, half ripened was 3, and full ripened fruits were scored 5.

Sample analysis

Biochemical content analysis.

Juice preparation or extraction

The samples were ground with a motor and pestle and filtered the extract finally extracted fig juice was separated and stored in the freezer.

Total Soluble solids (TSS) and titratable acidity (TA): Five fruits per tree were randomly selected and used to determine soluble solids content (TSS) and titratable acidity (TA). The soluble solids content was measured with a refractometer (Atago PR-1) and TA was determined by titration with 0.1N NaOH using phenolphthalein as an indicator.

Determination of different sugars

Total carbohydrate was determined using the sum of the data of glucose, sucrose and fructose.

Glucose content determination

Glucose was checked by using a glucose refractometer. Three drops of fig juice sample were placed on the disc of the meter and data were observed and documented.

Inverted sugar (sucrose) investigation

Inverted sugar was investigated by using an inverted sugar refractometer. Three drops of fig juice sample were placed on the disc of the meter and data were observed and recorded.

Fructose content investigation

Fructose was tested by using a fructose refractometer. Three drops of fig juice sample were placed on the disc of the meter and data were investigated and analyzed.

TSS and Titratable acidity (TA) determination

The total soluble solid (% brix) was determined by Refractometer. pH was determined by a pH meter.

Flavonoid investigation

Total flavonoid content (FC) was investigated with an aluminium chloride colourimetric assay, using catechin as a standard.

Nutrient content investigation

Nutrient content (K, Na and Ca) was investigated using Horiba NO3, K, Na and Ca meters (USA). 1 drop of juice sample was placed on the disc sensor of the meter using a small dropper and data were observed and listed.

Carotenoid as beta carotene determination

Carotenoids (beta carotene) were determined according to the methods of Lichtenthaler and Buschmann (1985). The method considered repeated acetone extraction until colourless residues with a pestle and mortar and filtered using filter paper (Whatman no. 1 grade). The extracts were made up to 50 ml with acetone. The concentration of carotinoid was measured at 470nm, chlorophyll a at 666nm and chlorophyll b 53 nm in a Shimadzu UV 160A Spectrophotometer. The amount of carotenoid was calculated according to the formula.[17]

DNA isolation

5ml CTAB was heated (1210µl mercaptoethanol was added to each 5ml CTAB) in a centrifuge tube (blue-topped of 50ml) at 60-65oC. Fruit skin was separated and wrapped with aluminium foil and stored in freeze-liquid nitrogen. Sample (1.0 g tissue/5ml CTAB) was stored for 2 days at –20 0C liquid Nitrogen. Fruit tissue was crumbled in a cold pestle of liquid nitrogen. Ground fruit samples were added 0.5 spatula of PVPP powder using one spatula of fine sand. The powder was scraped into a dry tube poured heated buffer and mixed smoothly. CTAB volume was adjusted to get a slurry-assembled consistency and then incubated for 60 min at 60 o C. The same volume of chloroform/iso-amyl alcohol (24:1) was poured and mixed well 2for 3min, then transferred to the centrifuge tubes. The rotation was 5,000rpm in spin. The supernatant was taken out by using the wide-bore paste to clean the tube and repeated chloroform extraction. DNA was precipitated having 0.66 vol. of cold isopropanol and kept overnight. DNA was spooled out for 1 minute at 10,000 rpm. The DNA sample was transferred to the 5ml buffer for 20min for washing then dried briefly. 1µl 10mg/ml of RNAse enzyme was added to each 1ml T.E./DNA mixture and stored for 60min at 37 o C. It was diluted in TE, then 0.3vol 3M sodium acetate. Spooled DNA was removed, dried and stored in freeze until required.

DNA Quantification and characterization

DNA weight was measured by electric balance using Eppendorf tubes.

Materials: Electrophoresis, micropipette, Gel tray and comb, 3loading dye, ethidium bromide, agarose, 1X TBE buffer, 1.5 ml Eppendorf tubes.

Method of DNA characterization

A 0.8% agarose gel was prepared using 99.2% 1x TAE and 0.1µl of Ethidium bromide (10mg/ml)/ 10ml solution. Load samples were undiluted and in a 1 in 10 dilution with 3µl loading buffer. It was incubated for 2 hours at 38 0C then loaded loading dye (31 ul) into each sample. The micro-pipette was adjusted to 11ul and loaded the samples in lanes 2-6. In lane 1, DNA standard added the (1 ug of DNA) standard (Lambda/Hind III digestion [10 ul sample]) plus 1ul of loading dye. It was run at 100 volts for 1.5 hours. The gels were stained for 5min in ethidium bromide and de-stain having water for 2 min. DNA fragments were migrated rapidly in the gel matrix based on size.

Design of Experiment and Statistical Analysis

The experimental design was completely randomized design. There were 3 replications and 3 treatments (including control) used in the experiment. Treatments were set randomly. A total of 3 branches were used in the experiment for each tree and three trees were used for each treatment. Standard errors were calculated. The least significant difference test (LSD test) was done.

RESULTS

Table 1 exhibits the fruit set, fruit diameter, length and weight measurement. The fruit set was found higher in the 15ppm IAA than in the 10% sugar solution and water control. The highest fruit weight was found in 15ppm IAA and the lowest was in control (Table 1). Moreover, the highest fruit diameter and length was found in 15ppm IAA and the lowest was in water control (Table 1). Fruit maturity, total soluble solids and titratable acidity (TA) are shown in Table 2. Fruit maturity and TSS were higher in 15ppm IAA than in 10% sugar and control. However, TA was found lower in 15ppm IAA than in 10% sugar and control. Glucose (%) was higher in the 15ppm IAA and 10% sugar than in the water control (Table 3). In addition to that fruit glucose and inverted sugar (sucrose) content were higher in the 15ppm IAA and 10% sugar than in the water control (Table 3). Similarly, total carbohydrate was higher in the 15ppm IAA and 10% sugar than in the water control. There were statistically significant differences found among all treatments. Moreover, flavonoid, antioxidant and carotenoid content (β-carotene) were exhibited higher in the organic solution treated fruit than the water control fruit (Fig. 4). In Table 5, it has been seen that nutrient content K+, Ca++ and Na+ content were found higher in the 15ppm IAA and 10% sugar than in the water control. In Table 6, it has been seen that DNA yield was the highest in the 15ppm IAA and the lowest was in water control. DNA ladder or probe measurement was done by gel electrophoresis method shown in Figure 1. It was very remarkable and distinct that the DNA band or fragment was found wider and bigger in wider in the 15ppm IAA and 10% sugar than in the water control. Figure 2 shows the fig fruit's physical structure, maturity and colour.

Table 1: Fruit set and weight of fig fruit as affected by different treatments. Mean ±SE (n = 5).

Treatment	*Fruit set (%)*	*Fruit weight (g)*	*Diameter (cm)*	*Length (cm)*
Control (distilled water)	50.5± 1.30c	4.7±1.20b	1.7±0.01b	2.3±0.01c
10% sugar	67.5± 1.10b	5.2±1.12b	2.0±0.02b	2.7±0.01b
15ppm IAA	80.4± 0.86a	6.6±1.30a	2.5± 0.01a	3.3±0.01a

Table 2: Maturity index, TSS and TA of fig fruit as affected by different treatments. Mean ±SE (n = 5).

Treatment	Maturity index	TSS (% brix)	TA(%)
Control (distilled water)	3.5±0.89	5.4± 0.86	0.30±0
10% sugar	4.5± 0.10	7.0± 0.86	0.15±0.01
15ppm IAA	5.0± 0	8.0± 0.86	0.10±0

Table 3: Glucose, fructose and sucrose determination of fig fruit as affected by different treatments. Mean ±SE (n = 5).

Treatment	Glucose (%)	Fructose (%)	Sucrose (%)	Total carbohydrate (g/100g)
Control	9.5±0.7c	10.0±0.1c	9.0±0.2c	28.5±0.5c
10% sugar	10.6±0.6b	11.2±0.3b	9.8±0.1b	31.6±0.3b
15ppm IAA	12.1±0.5a	13.5±0.4a	11.2±0.2a	36.8±0.4a

Table 4: Flavonoid and antioxidant determinationain fig fruit. Means followed by the common letters are not significantly different at the 5%level by Least Significant different test (LSDT). Mean ± SE (n= 5).

Varieties	Flavonoid (mg/100g)	Total antioxidant (mg/100g)	β-carotene (µg/g)
Control	127±0.7c	140±0.6c	0.9±0.01b
10% sugar	148±0.1b	274±0.7b	1.1±0.02b
15ppm IAA	392±0.5a	394±0.8a	1.8±0.02a

Table 5: Nutrient content determination in fig fruit. Means followed by the common letters are not significantly different at the 5%level by Least Significant different test (LSDT). Mean ± SE (n= 5).

Treatment	K+ (PPM)	Ca++ (PPM)	Na+ (PPM)
Control	220.1±4.1c	150.2±4.6b	37.1±0.6b
10% sugar	250.5±5.1b	170.3±5.9b	41.1±0.6b
15ppm IAA	340.0±5.3a	230.4±3.9a	69.3±0.5a

Table 6: DNA yield ng/ul of fig fruit.

Treatment	DNA yield ng/ul
Control	67±0.4
10% sugar	72±0.6
15ppm IAA	110±0.5

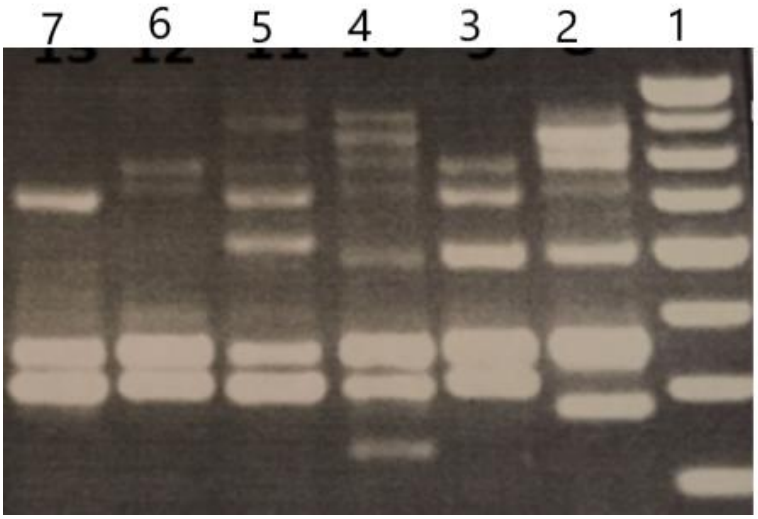
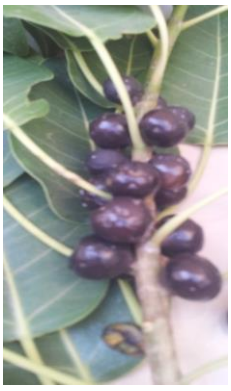


Figure 1: Photograph shows the DNA segment (band) in fig fruit at different treatment.

7: 15ppm IAA, 6: 10% sugar5:, control 4: 15ppm IAA 3: 10% sugar, 2: Control, 1: DNA standard primer

Control

Sugar (Sucrose) solution



IAA solution

Control **Sugar (Sucrose) solution** **15ppm IAA solution**

Figure 2: Photograph shows the fig fruit maturity at different treatment.

DISCUSSION

From our results, it has been found that fruit weight, length and diameter were higher in 15ppm and 10% sugar than in water control. It might be due to the plant growth regulators like 15ppm IAA and 10% sugar influencing the cell division and differentiation for the enlargement of fruits.[18-19] It was suggested that fruit yield, weight, length and diameter were found at different concentrations of growth regulators and light intensity of date and peach fruits. It has been seen from the result that inverted sugar, glucose and fructose were higher in 15ppm and 10% sugar than in water control. It might be due to the affecting of biochemical content by growth regulator and sucrose concentration. It has been found that soluble solids, pigments and carbohydrate content, pH, and acidity were all affected by different growth factors in apples and peaches.[20] It has been observed in the results that flavonoids and total antioxidants were higher in the in15ppm than in the 10% sugar and water control.[21] It was reported that growth regulators affected the carbohydrate and total soluble solids (TSS) of okra fruit (pod).[22] It was reported that the highest antioxidant and flavonoid contents were produced by the organic (compost tea) samples applied to the ginger. It was standard compared to other researcher's results. In the results, mineral contents like potassium, calcium, and sodium were higher at 15 ppm and 10% sugar compared to others. Our results show that fig contained higher nutrient content like Ca, K and Na. It has been described that sugar-bearing fruit contains potassium (580mg), calcium (67.4mg), iron (19.4mg), carbohydrate (75.3mg), sugars (10.6mg) and dietary fiber (57.1mg) [9]. In addition to that DNA band (segment) was wider in 15ppm and 10% sugar than in water control. It might be due to the growth regulator, IAA affected during the growing season for the time being and finally, it might change the band. It has been shown that gibberellic acid, ABA and other growth regulators and other environmental factors induce the expression of carotenogenic *genes* during leaf and flower development and during fruit ripening.[23] Therefore, our current results show the similarity to the other researcher's work.

CONCLUSION

It can be concluded from our results that 15ppm IAA contained better nutrient, antioxidants and flavonoids than 10% sugar and control. 15ppm IAA and 10% sugar showed bigger size fruit compared to the water control. However, 15ppm IAA exhibited the highest fructose, glucose, inverted sugar and bêta carotene in fig fruit.

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