Advances in Bioresearch
Adv. Biores., Vol 8 (5) September 2017:175-181

Print ISSN 0976-4585; Online ISSN 2277-1573
Journal's URL:http://www.soeagra.com/abr.html
CODEN: ABRDC3
DOI: 10.15515/abr.0976-4585.8.5.175181

Advances in Bioresearch

ORIGINAL ARTICLE

Physicochemical, Antioxidant, Nutritional and Genomic DNA Characterization of Ajwa dates at different location

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ABSTRACT
Ajwa dates exhibit an outstanding role as nutritional and medicinal fruit. It is used as fruit as well as a source of different types of food, nutritional and bio-medicinal products. Of consequence results, ajwa dates contain a form of sugar that reveal the high level of mobility and heat energy which can easily be broken down in the body. Ajwa dates contain vitamins and minerals. The study was carried out to investigate the ajwa date fruit physio-biochemical and nutritional quality like biochemical contents (fructose, glucose and inverted sugar, pH, TSS), mineral (K+, Ca++, Na+), antioxidant, flavonoids and DNA quantification as affected at different locaties as cultivars. Fruit weight, length and diameter were higher in ajwa alqasim than in ajwa almadina large, ajwa hail and ajwa almadina small. However, inverted sugar, glucose and fructose were found higher in ajwa-Hail than in azwa-Madinah (large and small) and Azwa alqaseem fruit. Flavonoid and total antioxidant were found higher in Ajwa Hail than in Ajwa Madina and Ajwa Alqasim. In addition to that, mineral content like potassium, calcium, sodium was higher in ajwa-Madinah and Ajwa alqaseem fruit compared to others. Moreover, DNA band (segment) was wider in ajwa hail fruit and ajwa alqasim than in ajwa Almadina small and ajwa Almadina large. The results conclude that ajwa Almadina contains better nutrient, antioxidant and flavonoid than Ajwa hail and Ajwa Alqasim.
Keywords: *date fruit, fructose and glucose, minerals, DNA band*

Received 16/04/2017 Revised 11/06/2017 Accepted 12/08/2017

How to cite this article:
A.B.M Sharif Hossain, Abdelmuhsin Abdelgadir, Ahmed Ali Alghamdi and Nasir A. Ibrahim. Physicochemical, Antioxidant, Nutritional and Genomic DNA Characterization of Ajwa dates at different location. Adv. Biores., Vol 8 [5] September 2017: 175-181

INTRODUCTION

Dates are notably one of the high calorie containing fruits. They are greater sources of minerals and vitamins and compose health benefit having flavonoid, polyphenolic, antioxidants known as tannins. These possess anti-infective, anti-inflammatory, and anti-hemorrhagic (prevent easy bleeding tendencies) properties [13]. Dates nutrient is augmenting in combination with the food scene as the healthiest alternative [14]. Dates exhibit a form of sugar that gives the body high levels of mobility and heat energy and which can be easily broken down in the body. Dates contain a great many vitamins and minerals. They also contain sodium, potassium, calcium, magnesium, iron, sulphur, phosphorus and chlorine, as well as vitamins A, beta-carotene, B1, B2, B3 and B6 [13]. Ajwa dates are the best as medicinal and nutritional fruit compared to other dates fruit varieties [8]. It contains potassium, calcium, iron, carbohydrate, sugars and dietary fiber [8]. Miller *at al*., [10] reported that nutrient content was affected by environmental factors like water, sun light and temperature in Kiwi fruit. They are very rich in fibre, fat and proteins. Gropper *et al.,* [2] reported that fruit polysaccharide (cellulose and hemicellulose), lignin and pectin were varied from different location. Kulkarni *et al*., [9] 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, etc. They also suggested that molecular mechanism regulated by the biosynthesis was varied at different localities.

Hassan *et al.,* [3] stated that fruit quality (juice/sap, flavor and texture) has been varied from different localities [4]. Hossian and Boyce [6] 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 [11, 10]. They also recommended that environmental factors can affect any fruit quality and development. However, No literature found directly regarding this current research only ajwa variety except few literatures of comparative studies of ajwa and other dates varieties which related to the current research. Therefore, the study was undertaken with the following objectives
To investigate the effect of localities as cultivar on the ajwa dates fruit growth and quality. In addition to introduce innovative information on the biochemical content like sugar, fructose and antioxidant, nutrient and DNA quantification of Ajwa-Almadina, Ajwa-Hail and AjwaAlqasim dates.

MATERIALS AND METHODS

Materials

Postharvest Ajwa dates fruit (Tamar stage) were collected from the dates palm garden in Almadinah, Hail and Alqasim region, Saudi Arabia.

Methods

Total of 9 kg dates fruit were collected 3 kg for each location. From Almadina 2 types of ajwa were collected. One was ajwa large size and other was ajwa small size. Twenty five fruit were randomly selected for one treatment. Total of100 fruits were used in the experiment for analysis (Fig. 1).

Data Collection

Per fruit weight, diameter and length were measured. Fruits were ground and finally juice was extracted using centrifuge at 5000rpm and extracted juice was kept for short time in the freeze for analysis.

Sample analysis

Biochemical content analysis

Juice preparation or extraction

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

Data analysis

Biochemical (glucose, inverted sugar and fructose) content was determined. Finally DNA isolation and quantification were done by gel electrophoresis.

Glucose content test

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

Inverted sugar investigation

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

Fructose content investigation

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

Total antioxidant investigation

1mM Trolox Standard Solution was used. Water was poured to each well to make the volume to 100 µL. Samples were directly added to the wells. For small molecule TAC, samples were diluted at 1:1ratio with Protein Mask. 20 µL of sample was used into wells. 17Distilled water was put in making the volume of 100 µL. 100 µL of Cu2+ Working Solution was added to all standard and sample wells and mixed properly using a horizontal shaker and the reaction was incubated for 90minutes at room temperature. The plate was protected from light at the time of incubation and finally made the measurement of the absorbance at 570 nm (A570).

TSS and pH test

Total soluble solid (%brix) was determined by Refractometer. pH was determined by pH meter.

Flavonoid investigation

Total flavonoid content (FC) was investigated with aluminum chloride colorimetric assay, using catechin as a standard.

Nutrient content investigation

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

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 having liquid nitrogen. Sample (1.0 g tissue/5ml CTAB) was stored for 2 days at –20 0C liquid Nitrogen. Fruit tissue was crumbled in cold pestle of liquid nitrogen. Ground fruit samples were added 0.5 spatula of PVPP powder using one spatula of fine sand. Powder was scraped into dry tube and poured heated buffer and mixed smoothly. CTAB volume was adjusted to get a slurry-assembled consistency 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. Supernatant was taken out by using wide-bore paste to clean tube and repeated chloroform extraction. DNA was precipitated having 0.66 vol. of cold isopropanol and kept overnight. DNA was spooled out for 1min at 10,000rpm. 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 added 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 eppendrop 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 was undiluted and in a 1 in 10 dilution with 3µl loading buffer. Incubated for 2 hours at 38 0C then loaded loading dye (31 ul) into each sample. Micropipet was adjusted to 11 ul and load the samples in lanes 2-6. In lane 1, DNA standard added the (1 ug of DNA) standard (Lambda/HindIII digestion [10 ul sample]) plus 1 ul of loading dye. It was run at 100 volts for 1.5 hour. The gels were stained for 5 minutes in ethidium bromide and de-stain having water for 2 min. DNA fragments were migrated rapidly in the gel matrix based on size.

Statistical Analysis

Data were analyzed statistically. Standard error (SE) and Least Significant difference Test (LSDT) were employed.

RESULTS

Figure 1 shows the ajwa dates fruit physical structure and color. Table1 exhibits the fruit diameter, length and weight measurement.

Figure 1. Photograph shows the date fruit (tamar) structure, color and size

The highest fruit weight was found in ajwa-alqasim and the lowest was in ajwa-almadina (small) (Table 1). Moreover, the highest fruit diameter and length was found in in ajwa-alqasim and the lowest was in ajwa-almadina (small) (Table 1). Fructose (%) were higher in the ajwa-Hail and ajwa-Alqasim than in the ajwa-almadina large and ajwa-Almadina small varieties (Figure 2). In addition to that fruit glucose and inverted sugar content were higher in the ajwa-Hail and ajwa-Alqasim than in the ajwa-almadina large

and ajwa-Almadina small varieties (Figure 3, 4). There was a statistically significantly differences found between ajwa-Hail, ajwa-Alqasim and ajwa-almadina large and ajwa-Almadina small varieties. In Table 2, it has been seen that nutrient content K+ content was found higher in the ajwa-Alqasim and ajwa almadina than in ajwa-Hail. However, Ca++ and Na+ was higher in the in the ajwa almadina large and ajwa almadina small than in ajwa-Hail and ajwa-Alqasim (Table 2). Table 3 shows the flavonoid content was higher in ajwa almadina small and ajwa-Hail than ajwa-almadina large and ajwa-Alqasim. Total antioxidant was found higher in ajwa almadina large and ajwa-Alqasim than in ajwa almadina small and ajwa-Hail (Table 3). There was no significantly difference in pH of all varieties (Table 3). Total soluble solids were higher in ajwa-Hail than in ajwa almadina large, ajwa almadina small and ajwa-Alqasim. In Table 4, it has been seen that DNA yield was higher in ajwa-Hail than in jwa-Alqasim and ajwa-Almadina (small and large). DNA ladder or probe measurement was done by gel electrophoresis method shown in the Figure 5. It was very remarkable and distinct that DNA band or fragment was found wider and bigger in ajwa hail fruit and in ajwa alqasim than in ajwa Almadina small and ajwa Almadina large.

Table 1: Weight and size measurement of different azwa varieties in different location. Means followed by the common letters are not significantly different at the 5%level by Least Significant different test (LSDT). Mean ± SE (n= 10).

Varieties	Weight (g)	Length (cm)	Diameter (cm)
Azwa Madina Small	5.12±0.3a	2.4±0.03a	1.8±0.01a
Azwa Madina Large	8.73±0.2b	3.08±0.05ab	2.0 ±0a
Azwa Alqasim	8.87±0.1b	3.46±0.02b	2.5±0.02a
Azwa Hail	8.31±0.1b	2.95±0.04a	2.4±0a

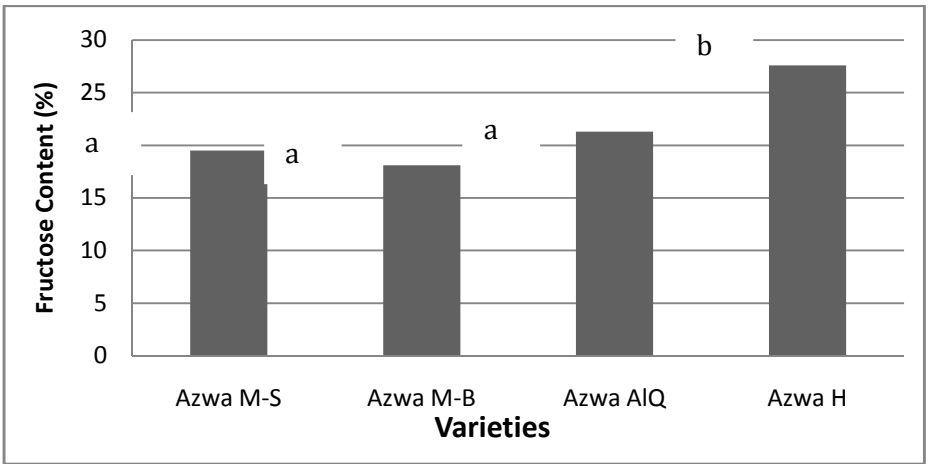


Figure 2. Fructose content determination. Means followed by the common letters are not significantly different at the 5%level by Least Significant different test (LSDT). Mean ± SE (n= 10).

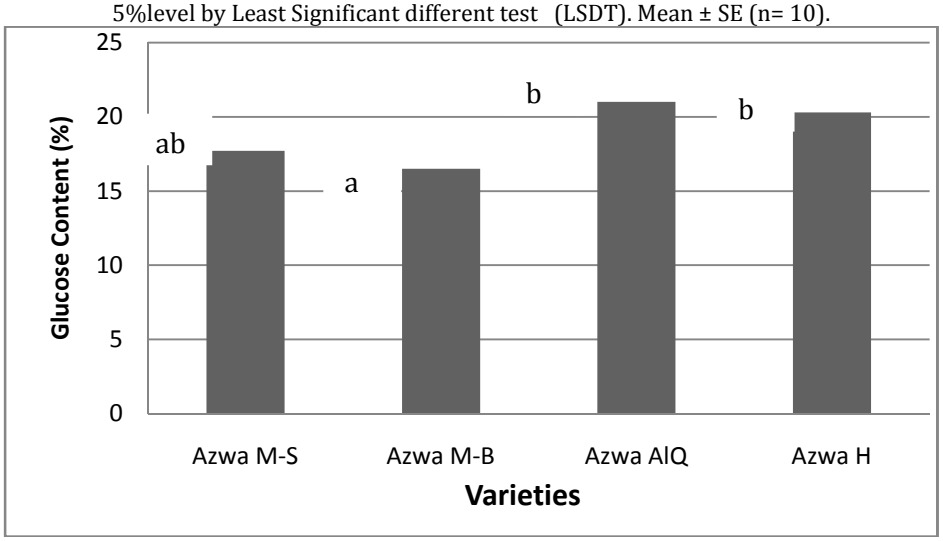


Figure 3. Glucose content determination. Means followed by the common letters are not significantly different at the 5%level by Least Significant different test (LSDT). Mean ± SE (n= 10).

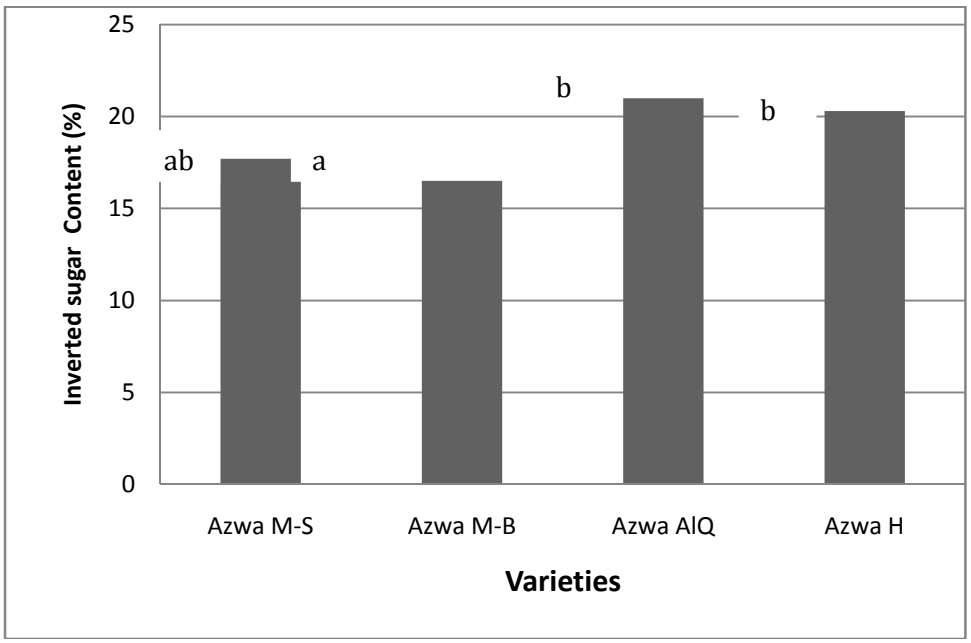


Figure 4. Inverted sugar determination. Means followed by the common letters are not significantly different at the 5%level by Least Significant different test (LSDT). Mean ± SE (n= 10).

Table 2. Nutrient content determination from different azwa varieties. Means followed by the common letters are not significantly different at the 5%level by Least Significant different test (LSDT). Mean ± SE (n= 10).

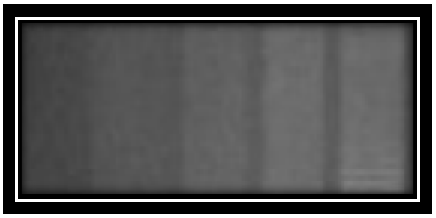
Varieties	K+ (PPM)	Ca++ (PPM)	Na+ (PPM)
Azwa Madina Small	2200±4.1b	1500±4.6bc	66±0.6b
Azwa Madina Large	2300±5.1b	1700±5.9c	41±0.6a
Azwa Alqasim	1700±4.2a	1300±3.9b	39±0.5a
Azwa Hail	2400±5.3b	950±1.9a	37±0.4a

Table 3. Flavonoid and antioxidant determination from different azwa varieties. Means followed by the common letters are not significantly different at the 5%level by Least Significant different test (LSDT). Mean ± SE (n= 10).

Varieties	Flavonoid (mg/100g)	Total antioxidant (mg/100g)	pH	Total Soluble Solids (TSS)/Brix (%)
Azwa Madina Small	392±0.5c	140±0.6c	7.2±0.001a	17±0.05a
Azwa Madina Large	131±0.3b	394±0.8b	7.0±0.001a	17±0.03a
Azwa Alqasim	127±0.7b	274±0.7b	7.1±0.002a	16.50.05a
Azwa Hail	148±0.1a	100.4±0.5d	7.1±0.003a	23±0.02b

Table 4. DNA yield ng/ul of Ajwa

Cultivars	DNA yield ng/ul
Ajwa Hail	112±0.6
Ajwa Alquasim	77±0.5
Ajwa Almadinh small	77±0.9
Ajwa Almadinh large	73±0.7



1 2 3 4 5

Figure 5. Photograph shows the DNA segment (band) of different Ajwa date fruit (tamar)
1: ajwa Madina small, 2: ajwa Madina large, 3: : Ajwa-Alqasim, 4: Ajwa-Hail, 5: DNA standard primer

DISCUSSION

From our results it has been found that fruit weight, length and diameter were higher in ajwa alqasim than in ajwa almadina large, ajwa hail and ajwa almadina small. It might be due to the different temperature, light intensity and soil profile at different localities. It has been found that fruit size, firmness [1]. Hossain *et al.,* [5, 7] suggested that fruit yield, weigh, length and diameter were found at different light intensity of date fruits. It has been seen from the result that Inverted sugar, glucose and fructose were higher in ajwa-Hail than in azwa-Madinah and Azwa alqaseem fruit. It might be due to the affecting of biochemical content by environmental factors. It has been found that soluble solids, anthocyanin and starch content, pH, and acidity were all affected by environmental factors in apple and peach [1]. It has been observed in the results that flavonoid and total antioxidant were higher in the Ajwa Hail than in Ajwa Madina and Ajwa Alqasim. Hossain [7] reported that environmental factors affected the carbohydrate and total soluble solids (TSS) of peach fruit. It was standard compared to other researcher's results. In the results, mineral content like potassium, calcium, sodium was higher in ajwa-Madinah and Ajwa alqaseem fruit compared to others. Our results show that all varieties of ajwa contained higher nutrient content like Ca, K and Na. It has been described that ajwa contains potassium (580mg), calcium (67.4mg), iron (19.4mg), carbohydrate (75.3mg), sugars (10.6mg) and dietary fiber (57.1mg) (IH, 2016). In addition to that DNA band (segment) was wider in Ajwa-Hail fruit than in Ajwa-Almadina and Ajwa Alqasim. It might be due to the environmental factors affected during the many growing season for long time and finally it might become an individual cultivar. It has been shown that light and other environmental factors induce the expression of carotenogenic *genes* during leaf and flower development and during fruit ripening [12].

CONCLUSION

It can be concluded from our results that ajwa madina contained better nutrient, antioxidant and flavonoid than ajwa hail and ajwa-alqasim. Ajwa-alqasim showed bigger size compared to the other cultivated race. However, ajwa hail exhibits better fructose, glucose and inverted sugar compared to the other cultivated races.

ACKNOWLEDGEMENT

The authors are thankful to the Research Deanship, University of Hail, Hail, KSA for providing funding facilities from the project number of 0150134/SCI which made us enabled to publish this manuscript.

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