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Enhancement of Edibility of "Barhi" and "Medjool" Date Palm Cultivars at Khalal Mature Stage

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Abstract: Date palm fruits (*Phoenix dactylfera* L.) cv. Barhi and Medjool were harvested at the khalal stage at three different harvesting dates. Fruits were treated or not with 100% CO_2 in 20 L air tight glass jars for one or two days. Results for gene expression involved in PAs biosynthesis (PAL, CHI and C4H) indicate that tannins synthesis is reduced in late stage of fruit development and at harvest. However, different CO_2 durations treatments had significantly different effects on Barhe and Medjool fruits for final color index, firmness, acetaldehyde and ethanol. Whereas, Internal Ethylene Content (IEC) and total soluble solids were not significantly affected. In contrast, tannin content in date fruit decreased significantly under CO_2 duration compared to the control which was pronounced on enhancement of Barhe fruit edibility compared to Medjool fruit, especially at the 48 h CO_2 duration.

Key words: Astringency, carbon dioxide, ethanol, gene expression, *Phoenix dactylfera* L., tannin

INTRODUCTION

The date palm cultivars "Barhi" and "Medjool" are commonly grown in the Jordan valley. The former can be marketed at the khalal stage, the stage during which the fruit reaches harvest maturity (although some astringency is still present) and color that is characteristic of the variety (Sawaya *et al*., 1982). While the later can't be marketed at that stage due to the high astringent taste. Astringency in date palm fruit is as a result of high contents of soluble tannins in the pulp (Rouhani and Bassiri, 1976; Sawaya *et al*., 1982) which declines as the fruit ripen (Rouhani and Bassiri, 1976).

Soluble tannins, responsible of astringency, are polymerized by acetaldehyde produced under anaerobic condition to form an insoluble non-astringent compound (Matsuo and Ito, 1982). However, accumulation of acetaldehyde and ethanol in the fruit flesh can occur after artificial deastringency treatment in a high CO_2 atmosphere (Matsuo and Ito, 1977). A high CO_2 concentration is known to be an efficient deastringency treatment (Gazit and Adato, 1972; Matsuo and Ito, 1977), but it usually damages the fruit by partial softening or flesh darkening (Sato *et al*., 1962). In persimmon cultivars, CO_2 treatment is significantly more effective in astringency removal than the application of ethanol (Yamada *et al*., 2002). Deastringency of persimmons fruit by high CO_2 application above 80% for 1-2 days is considered efficient while fruit firmness was retained (Matsuo *et al*., 1976; Pesis and Ben-Arie, 1984; Gazit and Levy, 1963). Tarutani and Manabe (1957) found that astringency is removed when persimmon fruit is kept 3-4 days in an atmosphere containing 78% CO_2. Esguerra *et al*. (1992) pointed out that postharvest application of ethanol vapor (25 up to 75%) on "Amas" bananas removed astringency. However, little information is available about the effectiveness of high CO_2 treatment on quality response of date palm fruit (Al-Redhaiman, 2005; Dehghan-Shoar *et al*., 2010). The aim of the present study was to evaluate the postharvest high CO_2 treatment under modified atmosphere condition on astringent date "Barhi" and "Medjool" cultivars and to investigate expression of genes encoding key enzyme involved in tannin biosynthesis.

MATERIALS AND METHODS

Fruit samples: Date palm fruits (*Phoenix dactylfera* L.) cv. "Barhi" and "Medjool" bunches were harvested at the "Khalal" stage at three different harvesting dates from a uniform group of date palm trees grown under the same cultural practices at the University Research Station in the Jordan valley. Fruits bunches were transferred to the horticulture lab at the Faculty of Agriculture, The University of Jordan, Amman. On the same day, for each cultivar, the fruits were removed from the bunch and were

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immersed directly in a container containing pure water to separate fruits by density into mature and immature according to Awad (2007). The mature fruits were left to air dried on trays for 1 h and then used in the CO_2 treatments.

CO_2 fruit treatment: For each harvesting date, ten fruits from each cultivar were placed in 20 L airtight glass jars for the CO_2 gas treatment. From the inlet septum, each jar with its contents was flushed with N_2 for 15 min in order to completely replace the normal air from the outlet septum, then purging with pure CO_2 gas at a flow rate of 0.5 l h^{-1} for 15 min then closed the outlet prior to the inlet septum. For the control treatments, the fruits were just placed inside the jars at normal air conditions. The glass jars, containing the fruits, were left for one or two days for further fruits measurements at room temperature. The treatments were repeated three times for each cultivar and harvesting date.

Measurements: For CO_2 treatment, six treated fruits from each cultivar and harvesting date were used for determining the fruit color, firmness and fruit taste score and for the measurement of Internal Ethylene Concentration (IEC), Total Soluble Solids (TSS), tannins, ethanol and acetaldehyde contents. For fruit color determination, the external skin and internal flesh fruit color were measured as an average of three equatorial reading for each fruit in the treatment using a chromometer (Minolta Corporation Instruments, Ramsey NJ, USA). The L, a, b Hunter parameters were used and were expressed as color index (Arnal and Del Rio, 2004). Fruit firmness for each treated fruit sample was tested with a puncture tester (Ametek, Large, FL, USA) using a 2 mm plug diameter; the plunger head was placed against the flesh in the peeled area at two opposite points on the equator of the fruit result were calculated as Lb. Fruit taste was scored subjectively on a scale of 1-5: 5: Completely edible, 4: Almost eating stage, 3: Partially edible, 2: Fairly inedible and 1: Inedible. For IEC measurement, 100 μL of the internal fruit core gas were withdrawn using a hypodermic needle from the central cavity of each treated fruit following Maier *et al.* (1973) procedure with some modifications. The samples were analyzed for ethylene on a PYE UNICAM, PU 4500 GC with hydrogen flame ionization detector and Porapak Q column. Temperature of the injection port, column and detector were 250, 130 and 300°C, respectively and the helium carrier gas flow rate was 8 mL min^{-1}. Ethylene gas concentrations were calculated relative to a standard gas containing ethylene. For TSS measurements, each treated fruit was cut into four longitudinal parts and the two opposite parts were placed in a pistil to extract the juice. The juice was extracted by a plastic syringe then filtered by a nylon filter 0.45 μM and the filtrate was used to determine TSS by using a hand refractometer instrument, the results were expressed as Brix°. To measure ethanol and acetaldehyde contents, half of the treated fruit was taken and placed in a glass tube and crushed by a small metal pistil (20 times) and then placed (with a closed septum) in a water path at 25°C for 5 min. Through the septum, 1 mL headspace gas sample was taken by a plastic syringe and then injected directly to gas chromatograph (PYE UNICAM, PU 4500). Temperature of the injection port, column and detector were 250, 150 and 300°C, respectively and the helium carrier gas flow rate was 8 mL min^{-1}. A standard liquid mixture containing 0.1% ethanol and 0.1% acetaldehyde was prepared in the same glass tube to calculate ethanol and acetaldehyde concentration in the sample headspace according to Alsmairat *et al.* (2011), the concentrations were reported as parts per million (ppm). Total tannins content was determined for each replicate using 5 g of fruit tissue macerated in 3 mL water. The supernatant was collected by a plastic syringe then filtered by nylon filter (0.45 μM). In a 100 mL volumetric flask; 1 mL from the sample filtrate, 75 mL of water, 5 mL folin-Denis reagent and 10 mL of Na_2CO_3 were added. Water was added until reached a final volume of 100 mL then the flask contents were mixed well. The absorbance was measured after 30 min at 760 nm spectrophotometer (PYE UNICAM, SP8-100UV/VIS) for each sample. The Folin-Denis reagent for tannic acid (AOAC, 1990) was used as a standard to determine the total tannin content of the extracts.

Gene expression analysis: Fruits from each cultivar and harvesting date were used for gene expression analysis of three selected genes (Phenylalanine Ammonia Lyase (PAL), Cinnamate 4-Hydroxylase (C4H) and Chalcone isomerase (CHI)) involved in the phenylpropanoid biosynthesis pathway. In addition, green fruits harvested one month before the first harvesting date was used as a control. For each harvesting date and cultivar, three replicates were prepared with two fruits per replicate and the samples were then stored at -80°C until total RNA extraction. For RNA extraction, the samples were ground into a fine powder using pistil and mortar in liquid nitrogen. The fine powder was used to isolate total RNA using SV Total RNA Isolation System Kit (Promega, Madison, USA) following the manufacturer's instructions. The synthesis of the first strand cDNA from the extracted RNA (1 μg) was performed using the GoScriptTM reverse transcription kit (Promega, Madison, Wisconsin) and an oligo (dT)18 primer following the manufacturer's instructions. Semi-quantitative RT-PCR analysis was

performed using the first-strand cDNA library prepared from fruit samples using gene-specific and internal reference gene primers. For this purpose, specific primer pairs for the three targeted genes and actin as a reference were designed (Table 1) based on retrieved DNA sequences from Date Palm Whole Genome Shotgun project deposited at GenBank databases (Accession No. ACYX00000000; Al-Dous *et al*., 2011) using TBLASTN search with the amino acid sequence of the corresponding homologous genes from arabidopsis described in Solfanelli *et al*. (2006) For semi-quantitative RT-PCR, the synthesized cDNAs were used as templates in a PCR to amplify DNA fragments of the corresponding genes. The PCR was performed in an Applied Biosystems 9700 thermocycler (Applied Biosystems, Carlsbad, CA, USA) using the i-MAXII PCR Master Mix solution (iNtRON Biotechnology, Seoul, Korea) and different primer combinations (Table 1). The 25 μL reactions contained 2.5 μL of the synthesized cDNA, 0.5 μM of each primer and 12.5 μL of i-MAXII solution. The PCR conditions were 94°C for 5 min; 30-35 cycles of 94°C for 45 sec, 50-60°C (depending on the primer combination) for 30 sec and 72°C for 1 min and a final extension at 72°C for 10 min. The PCR products were separated on 1.5% agarose gels and visualized using the Safe Red stain (iNtRON Biotechnology, Seoul, Korea) using Gel Doc™ XR+(BioRad, Hercules, CA).

Experimental design and data analysis: A Completely Randomized Design (CRD) with three replicates was used. For data analysis ANOVA procedure of SAS system was used for treatments, cultivars and harvesting dates means compared by LSD range test at 5% level of probability.

RESULTS AND DISCUSSION

Data in Table 1 and 2 show that CO_2 treatments on "Medjool" fruits had a significant effect on final color index that was more pronounced at the 48 h compared to the 24 h duration treatments as shown in Fig. 1. In

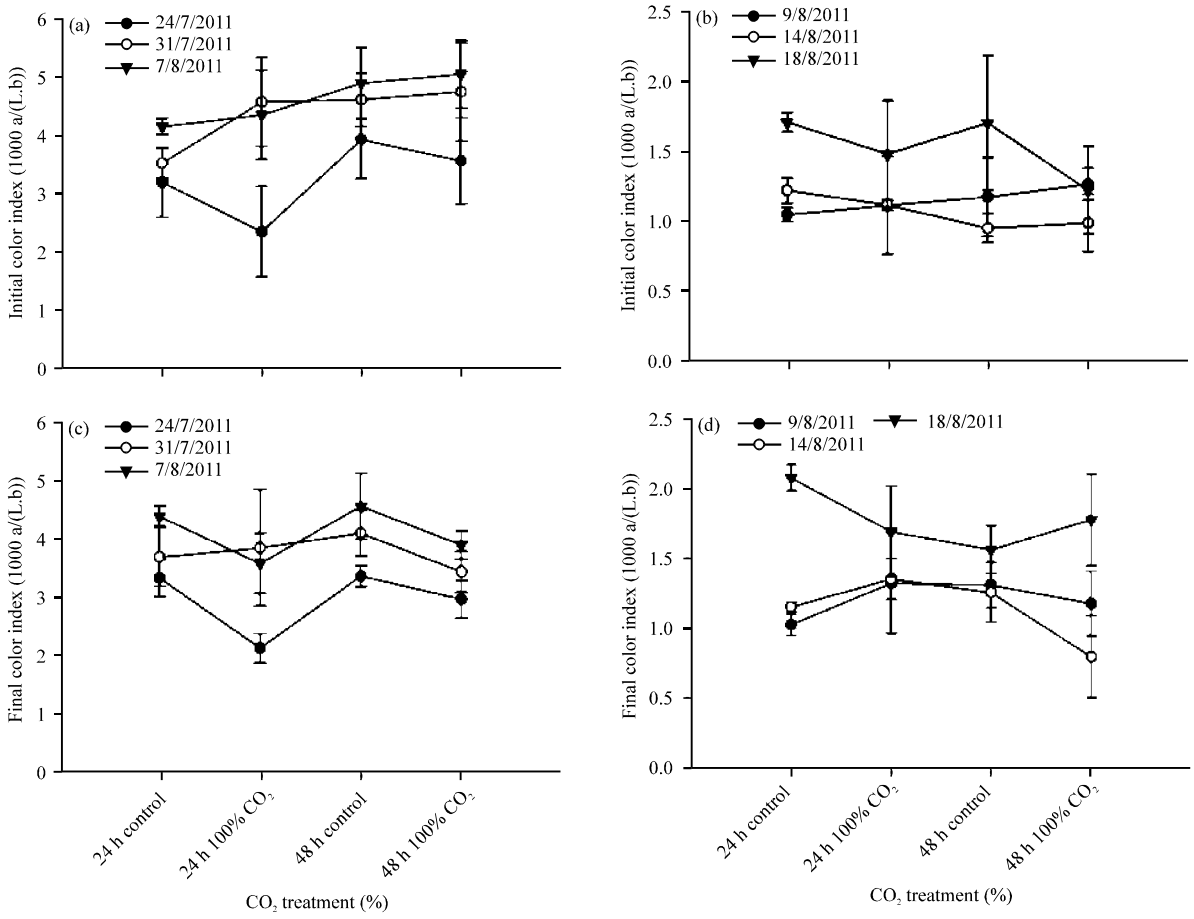

Fig. 1(a-d): Effect of carbon dioxide treatments (%) for one or two days on the initial and final color index of date palm fruits of "Medjool" and "Barhe" cultivars at different picking dates (a, c) Medjool and (b, d) Barhe

Table 1: List of primers used for gene expression analysis in date palm fruits

Gene	Forward primer (5'-3')	Reverse primer (5'-3')
Actin	TCCTTCACCACAACTGCAGA	AATTTTCATGCTGCTTGGGG
PAL	CGAGCTTGGACTATGGCTTC	CAAGTCCTTCTCGCAGAACC
C4H	AAGAAGCTGGCGAGTACCAA	GCGGCAGTAGCTCAAAGTTC
CHI	TCGGACTGCGGAAGAGTAAT	CATAACCTTGCTCCCTGGAA

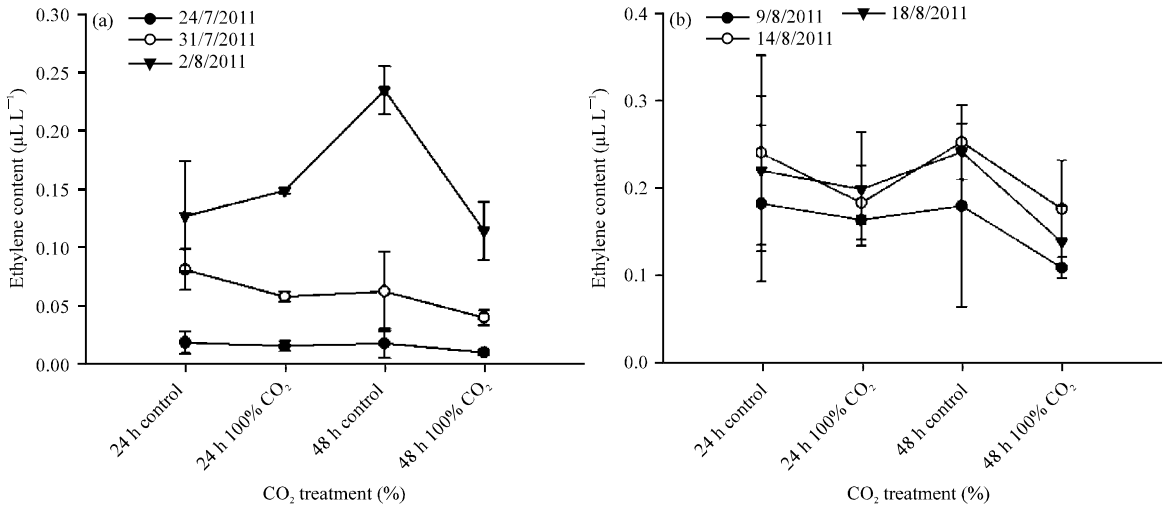

Fig. 2(a-b): Effect of carbon dioxide one or two days treatments (%) on ethylene concentration of "Medjool" and "Barhe" cultivars at different picking dates (a) Medjool and (b) Barhe

contrast, these effects were not found in "Barhi" fruit samples at the two treatments. Salari *et al.* (2008), reported that modified atmosphere enhanced color changes but these changes were more affected by temperature than by atmosphere composition. Date palm fruit cultivars that were held in CO_2 at different duration periods showed no significant difference for the internal ethylene content compared to the control (Table 2 and Fig. 2). Treatments showed different levels of acetaldehyde and ethanol accumulation in the headspace above the fruits (Table 2). The CO_2 treatments results revealed that acetaldehyde was higher than ethanol level above date palm fruit headspace in the two cultivars (Fig. 3). Pesis and Ben-Arie (1986), suggested that CO_2 atmosphere is involved in higher level of acetaldehyde production. High CO_2 concentration triggered anaerobic respiration that resulted in acetaldehyde formation (Arnal and Del Rio, 2003). Moreover, these results might indicate that the high CO_2 concentration depressed the conversion of acetaldehyde to ethanol, the end product in anaerobic metabolism, causing the intermediate acetaldehyde to accumulate (Pesis and Ben-Arie, 1984). Also, in tomato treated with 50-90% CO_2 in air, Pesis and Marinansky (1993) found that the higher the concentration of CO_2 used the greater the production of acetaldehyde and ethanol. The CO_2 duration treatments on "Medjool" date fruits did not show any significant changes of firmness (Table 2). However, the low and high duration treatment on "Barhe" fruits reduced fruit firmness significantly with higher reduction at high duration (Table 2 and Fig. 4). These results agree with Yamada *et al.* (2002), who observed a decrease in persimmon firmness after 100% CO_2 treatment for 24 h. Total soluble solids in "Medjool" and "Barhe" date were not significantly affected by the different CO_2 duration treatment as shown in Table 2 and Fig. 5. These results confirm the data reported by Al-Redhaiman (2005) for "Barhi" date fruit stored in controlled atmosphere. In the contrary, tannin content in date fruit, decreased significantly under different CO_2 duration treatments compare to control in "Medjool" and "Barhe" fruits (Table 2 and Fig. 6). These results were corroborated by the sensory evaluation (Table 2 and Fig. 7). It has been shown that CO_2 treatment enhanced significantly the fruit edibility; however, this affect was clearer in "Barhe" than in "Medjool" fruit.

"Barhe" date palm fruit responded very well to CO_2 treatment for astringency removal, however, fruit cultivars with rich tannin content such as "Medjool" need some different applications that might be investigated in future research.

Changes in expression levels of three selected genes involved in PAs biosynthesis (PAL, CHI and C4H) were analyzed at different fruit developmental stages between the two cultivars. At early stage of development, green

Table 2: Comparison of the main effect of CO2 treatments on the parameters tested

Treatment (T)	Initial color index (1000 a/(L.b))	Final color index (1000 a/(L.b))	Ethylene content (μL.L^{-1})	Acetaldehyde content (ppm)	Ethanol content (ppm)	Tannine content (%)	Total soluble solides content (Brix°)	Fruit firmness (Lb)	Taste panelist score
Medjool									
24 h control	3.6000	3.8106	0.07521	3.56	4.2511	0.7448	29.5222	13.1333	1.7556
24 h 100% CO_2	3.7348	3.1964	0.07373	20.52	82.7844	0.6124	29.7667	12.2222	2.8667
48 h control	4.4582	4.0117	0.10470	3.95	4.2611	0.7420	28.7889	12.7889	2.0222
48 h 100% CO_2	4.4317	3.4429	0.05443	23.96	303.0600	0.5488	29.5889	11.8333	3.0444
Main effects (T)	*	*	ns	*	*	*	ns	ns	*
Barhe									
24 h control	1.3321	1.4172	0.2127	4.39	2.5089	0.4460	33.0889	8.3000	2.0667
24 h 100% CO_2	1.2441	1.4543	0.1806	36.87	89.8878	0.2850	33.6667	2.8778	4.3778
48 h control	1.2827	1.3750	0.2230	5.52	3.1400	0.4335	32.0000	7.9778	2.6444
48 h 100% CO_2	1.1669	1.2499	0.1401	43.06	117.0400	0.2108	33.8889	0	4.8889
Main effects (T)	ns	ns	ns	*	*	*	ns	*	*

*Significant at probability level ($p \leq 5.0\%$). ** Values are the average of the different harvest dates

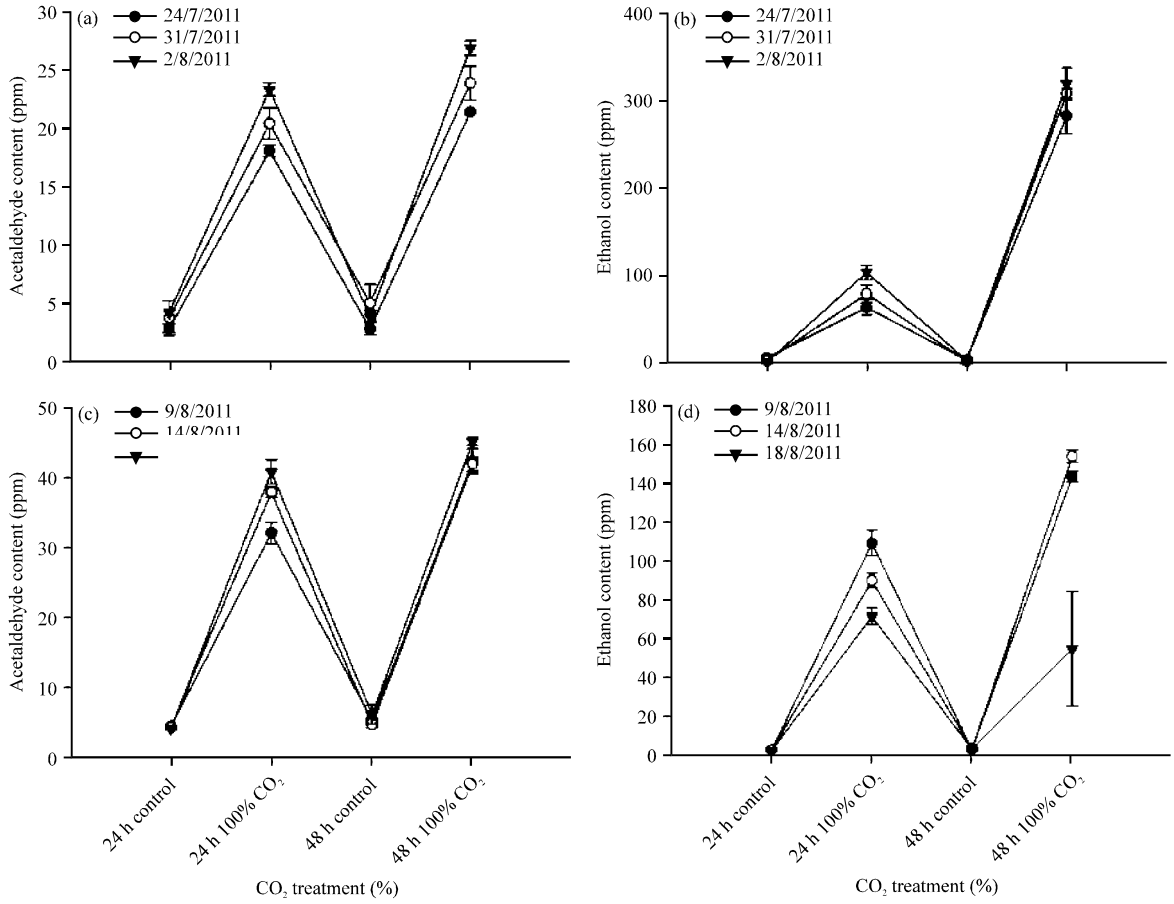

Fig. 3(a-d): Effect of carbon dioxide one or two days treatments (%) on acetaldehyde or ehtanol concentration of "Medjool" and "Barhe" cultivars at different picking dates (a-b) Medjool and (c-d) Barhe

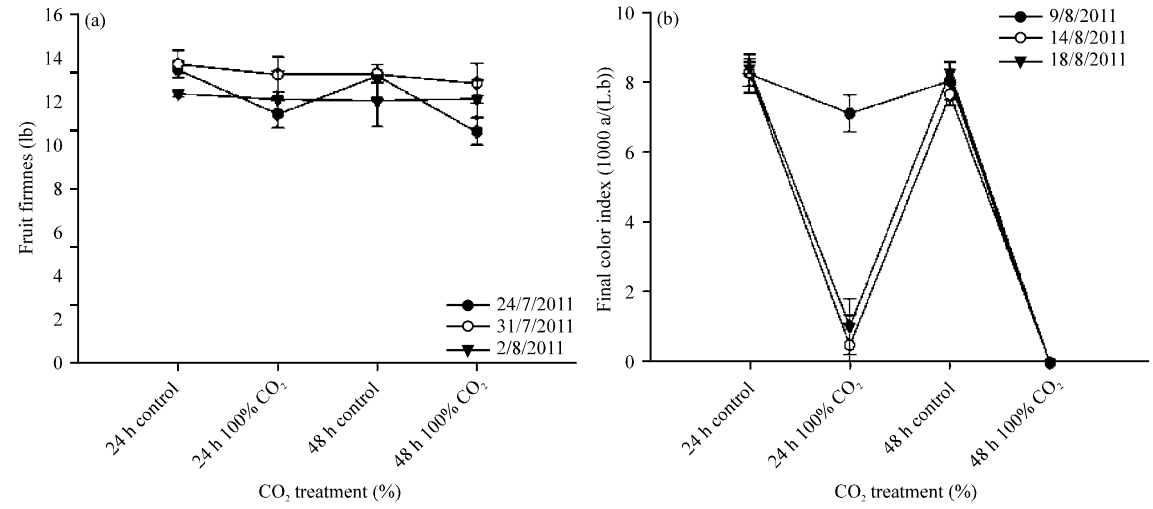

Fig. 4(a-b): Effect of carbon dioxide one or two days treatments (%) on date palm fuits firmness of "Medjool" and "Barhe" cultivars at different picking dates (a) Medjool and (b) Barhe

fruits of both cultivars showed high levels of expression in all studied genes reflecting active phenylpropanoid biosynthesis in fruit tissues which might indicate accumulation of tannins in developing (Fig. 8). However,

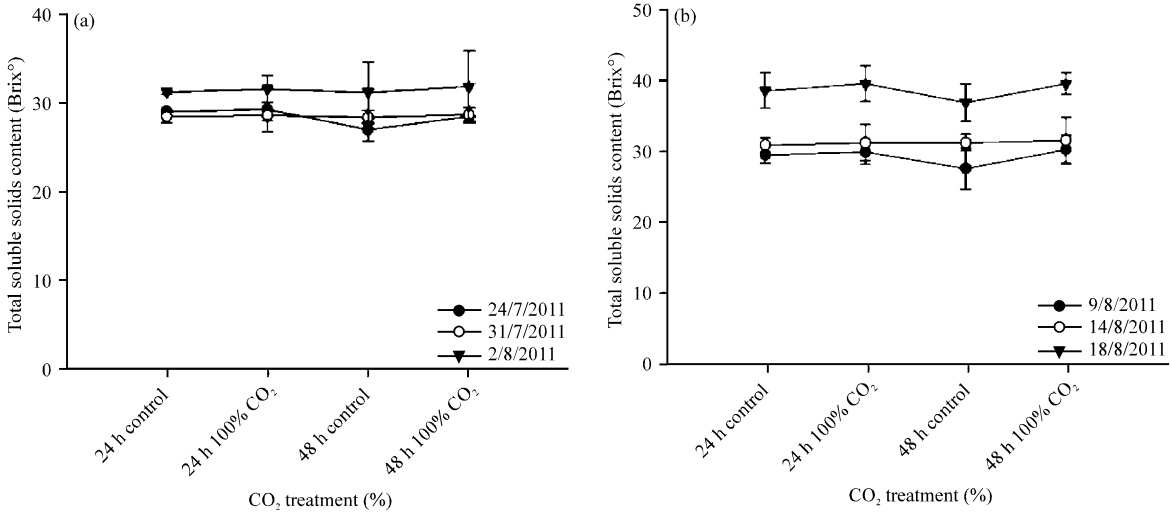

Fig. 5(a-b): Effect of carbon dioxide one or two days treatments (%) on total soluble solids content of "Medjool" and "Barhe" cultivars at different picking dates (a) Medjool and (b) Barhe

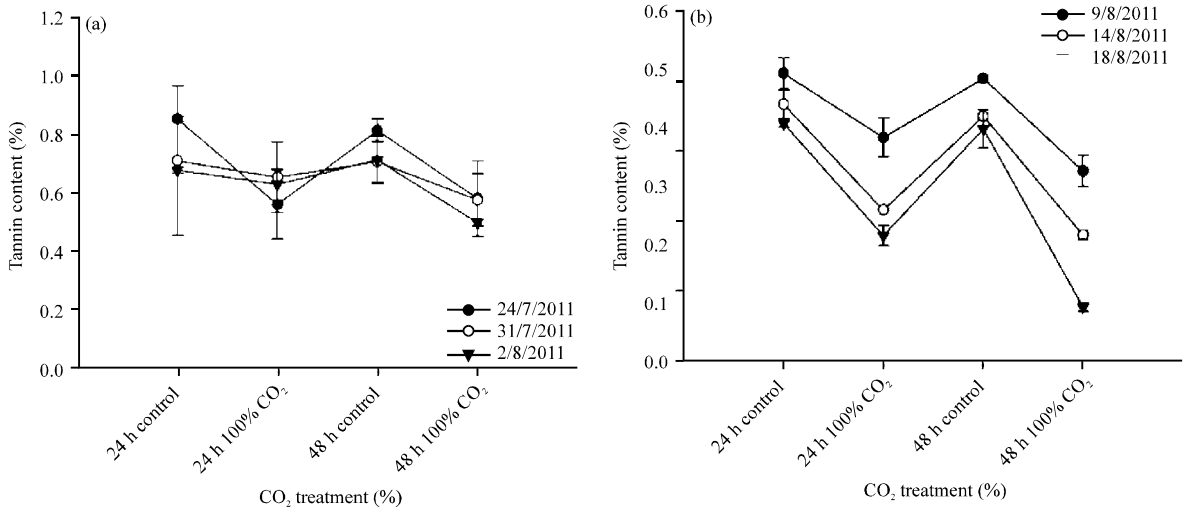

Fig. 6(a-b): Effect of carbon dioxide one or two days treatments (%) tannine contents of "Medjool" and "Barhe" cultivars at different picking dates (a) Medjool and (b) Barhe

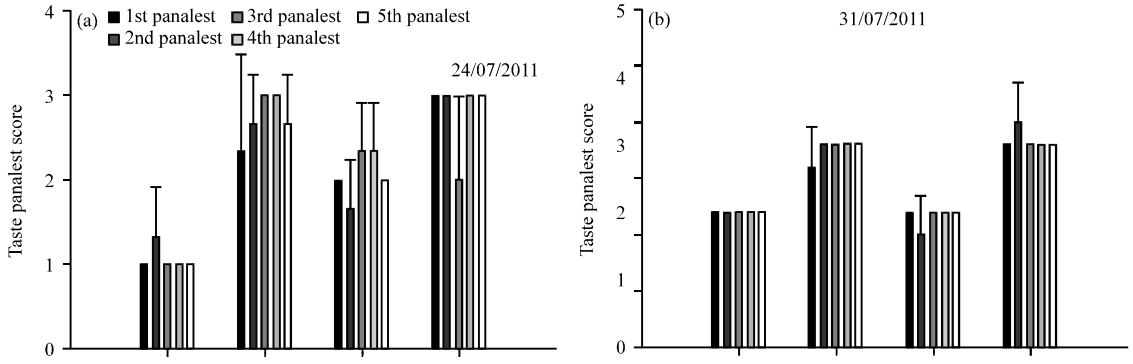

Fig. 7(a-f): Continue

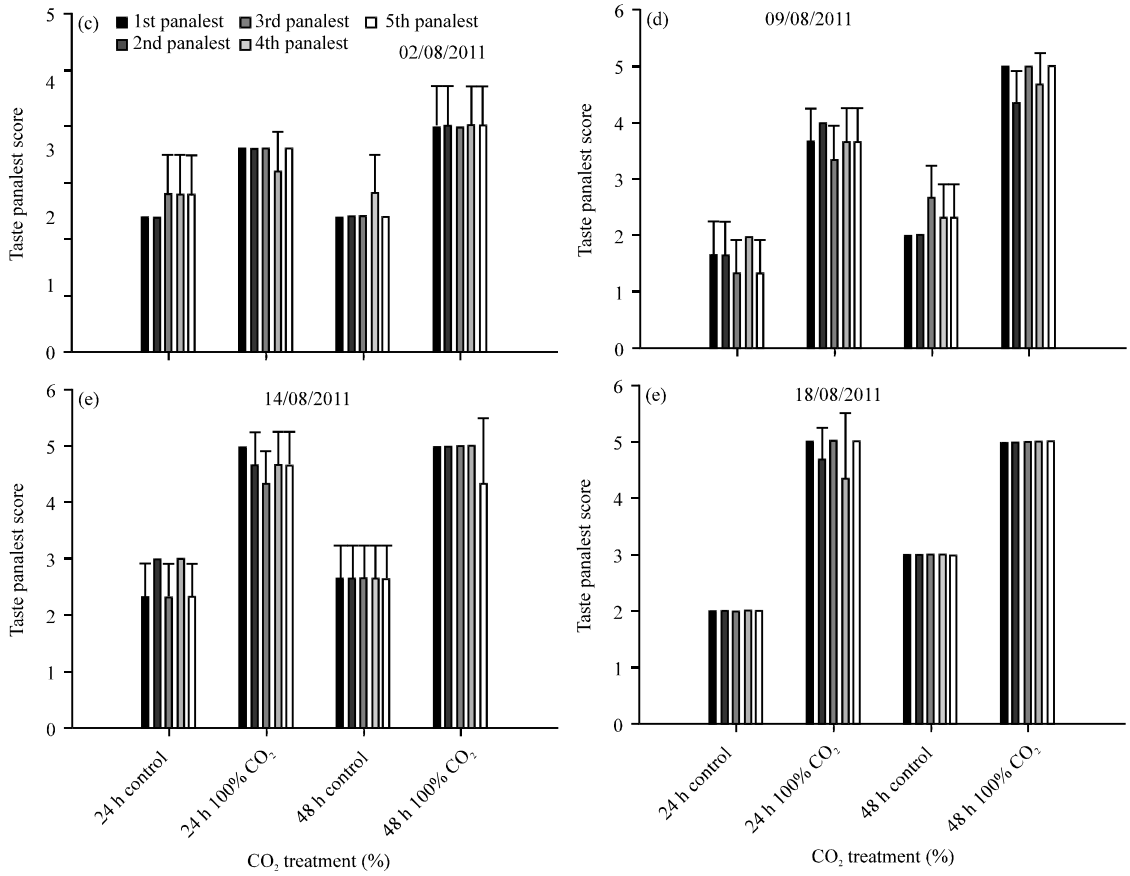

Fig. 7(a-f): Effect of carbon dioxide one or two days treatments (%) on teaste palanest score (1-5) at different picking dates palm fruit cv. (a-c) Medjool and (d-f) Barhe

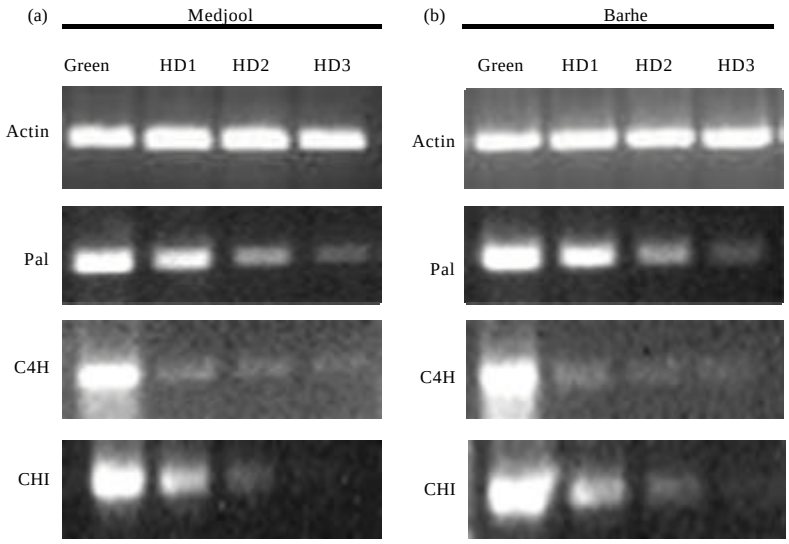

Fig. 8(a-b): Changes in expression of three selected genes in "Medjool" and "Barhe" date palm fruit at three different harvest dates (a) HD1: 24-7-2011, HD2: 31-7-2011 and HD3: 7-8-2011 and (b) HD1: 9-8-2011, HD2: 14-8-2011 and HD3: 18-8-2011

at the first harvest date lower gene expression levels of two genes (PAL and CHI) were observed in both cultivars when compared with green fruit samples harvested one month earlier. Further reduction in gene expression levels were observed in samples harvested after one or two weeks later indicating less active phenylpropanoid biosynthesis in both cultivars (Fig. 8). These results indicate that tannins synthesis is reduced in late stages of fruit development and at harvest. Similar results were observed in presimmon (Diospyros kaki Thunb.) where expression levels of PAL, Dihydroflavonol reductase (DFR), Chalcone synthase (CHS) and Dihydroflavonol reductase (F3H) genes declined into undetectable levels in late stages of fruit development in Japanese cultivars (Ikegami *et al*., 2005). Furthermore, whole gene expression profiling on multi-staged fruit development of date palm showed higher levels of expression of secondary metabolites genes in early fruit developmental stages when compared with late stages (Yin *et al*., 2012).

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