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Effect of Sunning as Post Harvest Treatment for Insect Pests on Antioxidants and Physicochemical Properties of Date Fruit

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ABSTRACT

Date fruit from three date palm (*Phoenix dactylifera*) cultivars namely Khalas, Shiashy and Rizez were selected for study. Freshly harvested date fruit is prone to insect-pest infestation. It is, therefore, important to preserve it properly to maintain fruit quality and enhance shelf life for human consumption. The main aim of this study was to evaluate the effect of sunning as insect-pest post-harvest control treatment on the quality characteristics and the antioxidants of mature date fruit before and after sunning treatment. Dates treatments of exposure to direct sun light for two days (48 h) significantly increased the level of total phenolic compounds to more than 175% in all cultivars. The trend was found in both assays for total phenolics and total flavonoids with the same percentage in Khalas, Rizez than Shiashy. The FRAP value of fresh (control) Shiashy (151.14 mg/100 g) was significantly higher than fresh Rizez and Khalas dates (145.31 mg/100 g; 79.33 mg/100 g, respectively). Khalas cultivar showed highest antiradical activity (240%) than Shiashy (178%) and Rizez (171%), respectively. The results suggested that treatment of date fruit by exposing to direct sunlight is an excellent substitute to chemical fumigation for safety and to maintain quality characteristics for human consumption.

Key words: Antioxidants, phenolic compounds, flavonoids, FRAP assay, sunning treatment, date characteristics

INTRODUCTION

Antioxidants are defined as those substances which reduce damage due to oxygen, such as that caused by free radicals. Well-known antioxidants include enzymes and other substances, such as vitamin C, vitamin E and beta carotene, which are capable of counteracting the damaging effects of oxidation to prevent certain diseases (Shahidi, 1997; Silva *et al*., 2007).

Kingdom of Saudi Arabia is one of the major date producing countries in the world (FAO, 2004). Date palm can grow under severe arid climatic conditions (Alkhateeb and Ali-Dinar, 2002). Presently, many date palm varieties are commercially grown in some countries such as Algeria, China, Egypt, Iran, Iraq, Pakistan, Saudi Arabia, Sudan and the United Arab Emirates (UAE) (Ibrahim and Khalif, 1998). Although, Vayalil (2002) reported that date fruit contains antioxidant and antimutagenic characteristics, but still little is known about the phenolic profile of the ripe date fruit. Many researchers have presented a general view of the main phenolic compounds of date fruit from Tunisia and Spain, respectively (Lorente and Ferreres, 1988; Regnault-Roger *et al*., 1987). However, presence of phenolics, vitamin C and E, carotenoids and Flavonoids control the antioxidant properties of dates (Saura-Calixto and Goni, 2006).

Major chemical constituents of date fruit are carbohydrates, sugars, fat, protein, fiber, minerals and vitamins which vary with the types of cultivars and growing conditions of date palm tree (Al-Hooti *et al*., 1995; Al-Shahib and Marshall, 2003; Sawaya *et al*., 1982). In another study, Mansouri *et al*. (2005) reported the Total Phenolic Contents (TPC) and antiradical efficiency of Algerian dates as 2.49-8.36 mg gallic acid (GAE) per 100 g on fresh weight basis and 0.08-0.22, respectively. Native sun-dried dates from Oman were studied by Al-Farsi *et al*. (2005a, b, 2007).

Generally, date fruit is harvested at Tamar stage with Total Soluble Solids (TSSs) of 60-84° Brix (Pareek, 1985). The moisture contents of date fruit vary from 60% at maturity to about 25% at this stage (Barreveld, 1993). The range of optimum moisture contents of dates for safe storage was reported between 24 and 25% (Falade and Abbo, 2007).

Fresh date fruit is mostly consumed and dehydrated to remove moisture from date fruit to maintain its quality and extend shelf life (Barreveld, 1993; Cheng *et al*., 2006; Kulkarni *et al*., 2008). Dehydrated and mature date fruits are also used for the preparation of sweets, snacks, confectionery, bakery products, health foods, date bars and date syrup (Al-Hooti *et al*., 1997; Abd El-Mohsen and El-Din, 1995; Riedel, 1986). Heat treatment is reported to induce changes in structure and composition of plant tissues (Lewicki, 1998) and prevent sugar spotting and change the color of date fruit at the early stage of maturity (Kulkarni *et al*., 2008). Moreover, problems associated with sun drying of dates are also reported (Doymaz, 2004, 2005; Guine and Castro, 2002).

The main objective of this study was to investigate the effect of sunning as insect-pest post harvest control treatment on the quality characteristics and the antioxidant of mature dates.

MATERIALS AND METHODS

The study was carried during 2011 cropping season in Al-Ahsa, Eastern Region of Saudi Arabia.

Sunning treatment of dates: Date fruit from three date palm cultivars (Khalas, Shiashy and Rizez) was collected for experiment. About 240 kg of dates from each cultivar were spread in 12 plastic baskets with a size of 55×35×21 cm^3, then covered with a thin polyethylene sheet (0.5 mm thickness) and the edges of cover were scoured by sand. A HOPO 08 data logger was placed in each treatment to record temperature and humidity during the study period. The dates were exposed to direct sun light for four periods i.e., half day, one day, one day and half and two days. After terminating the experiment, dates were analyzed for different chemical constituents to test the effect of sunning on physicochemical proprieties of dates.

Chemicals and reagents: The chemicals and reagents used for analyzing the antioxidant compounds in dates were: Gallic acid, catechin, sodium nitrate, sodium carbonate, Folin-Ciocalteu's phenol reagent, ascorbic acid, trichloro-acetic acid, sodium nitrite, aluminium chloride, methanol were purchased from Merck (Darmstadt, Germany). Chemicals such as 2, 20-azinobis (3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt (ABTS), 2,4,6-tripyridyl-S-triazine (TPTZ) were supplied by Sigma-Aldrich, USA, $FeCl_3$-$3H_2O$, potassium persulphate, sodium acetate, Trolox (6-hydroxy-2,5,7,8 tetramethylchroman-2-carboxylic acid) and sodium carbonate were obtained from Sigma-aldrich. All chemicals and reagents used were of analytical grade for synthetic antioxidant flavonoids and polyphenolic compounds, Organic solvents for isolation and separation.

Sample extraction and preparation: The edible part of dates was pitted, dried, crushed and blended for 10 min in a blender. Date sample of 3 g from each treatment was extracted with 100 mL methanol, at room temperature (20°C) for 5 h using an orbital shaker. The extracts were then filtered and centrifuged at 4000 rpm, for 10 min. The supernatant was concentrated under reduced pressure at 40°C for 3 h using a rotary evaporator (Heidolph-Laborota, Germany) to obtain the dates methanolic crude extract. The crude extract was stored in dark glass bottles for three days in a freezer until use.

Total phenolic contents Folin-Ciocalteu assay: Total phenolics were determined using Folin-Ciocalteu reagents (Singleton and Rossi Jr., 1965). Date extract (40 mL) or gallic acid standard was mixed with 1.8 mL of Folin-Ciocalteu reagent (pre-diluted 10-fold with distilled water) and allow to stand for 5 min at room temperature, then 1.2 mL of sodium bicarbonate (7.5%) was added to the mixture. After standing for 60 min at room temperature, absorbance was measured at 765 nm. The results were expressed as mg gallic acid equivalents (GAE)/100 g sample according to the procedure of Shui and Leong (2006).

Total flavonoids contents: Flavonoids were determined according to the colorimetric assay of Kim *et al.* (2003). Four milliliter of distilled water, 0.3 mL of 5% sodium nitrite solution and 0.3 mL of 10% aluminum chloride solution were added to 1 mL of date extract. The test tubes were incubated at ambient temperature for 5 min followed by the addition of 2 mL of 1 M sodium hydroxide to the mixture. Immediately, the volume of reaction mixture was made up to 10 mL with distilled water. The mixtures were thoroughly vortexed and the absorbance of the pink color developed was determined at 510 nm. A calibration curve was also prepared with catechin. The results were expressed as mg catechin equivalents (CEQ)/100 g date sample on dry-weight basis.

Measurement of antioxidant contents using FRAP assay: Antioxidant Activity (AA) of date palm fruit extracts was determined using a modified method of the assay of Ferric Reducing Antioxidant Power (FRAP) of Benzie and Strain (1999). The FRAP reagent contained 2.5 mL of 10 mM tripydyltriazine (TPTZ) solution in 40 mM HCl plus 2.5 mL of 20 mM $FeCl_3.6H_2O$ and 25 mL of 0.3 M acetate buffer at pH 3.6. Freshly prepared FRAP reagent (3.0 mL) was warmed at 37°C and mixed with 50 μL of dates extract. The reaction mixtures were later incubated at 37°C. Later on, the absorbance was recorded at 593 nm with reference to a reagent blank containing distilled water which was also incubated at 37°C for up to 1 h instead of 4 min, which was the original time applied in FRAP assay. A standard curve was prepared by using ascorbic acid standard solution at various concentrations.

Antioxidant activity measurement using ABTS assay: Antioxidant Activity (AA) was measured using an improved ABTS method as described by Cai *et al.* (2004) and Re *et al.* (1999). The ABTS radical cation ($ABTS^+$) solution was prepared through the reaction of 7 mM ABTS and 2.45 mM potassium per-sulphate after incubation at 23°C in dark for 16 h. The $ABTS^+$solution was then diluted with 80% ethanol to obtain an absorbance of 0.700±0.005 at 734 nm. $ABTS^+$ solution (3.9 mL; absorbance of 0.700±0.005) was added to 0.1 mL of the test sample and mixed vigorously. The mixture was allowed to stand for 6 min at room temperature and the absorbance was recorded at 734 nm. A standard curve was obtained by using Trolox standard solution at various concentrations (ranging from 0-15 mM) in 80%

ethanol. The absorbance of the reaction samples was compared to that of the Trolox standard and the results were expressed in terms of Trolox Equivalent Antioxidant Capacity (TEAC).

Data analysis: Data were analyzed by ANOVA and regression techniques for treatment evaluation at 5% level of significance according to SAS Institute (2001).

RESULTS AND DISCUSSION

Three date palm cultivars namely Khalas, Shiashy and Rizez were selected for study in the East region of Arabian peninsula for their premium date eating quality. Because, these three cultivars are considered to produce soft dates according to the date fruit classification scheme in which the dates are classified into soft, hard or semi-soft depending on the type and amount of reducing and non-reducing sugars (Sawaya *et al*., 1983; Hussein *et al*., 1976).

Fresh dates (control): Fresh dates of the cultivars differed significantly in total phenolics and total flavonoids. Also, the cultivars were significantly different in the two assays used to estimate the antioxidant activities i.e., FRAP and ABTS (Table 1).

Effect of various treatments on phenolic and flavonoid constituents: Levels of the various phenolic constituents of the cultivars after various treatments are presented in Table 1. The direct sunlight exposure treatments showed differential effects on the phenolic constituents of all the cultivars. However, the response of three cultivars was similar to various direct sunning treatments on total phenolics and total flavonoids (Table 1).

Treatments of exposure to direct sun light for two days (48 h) significantly increased the level of total phenolic compounds to more than 175% in all cultivars. The trend was same in both assays (FRAP and ABTS) for total phenolics and total flavonoids in Khalas, Rizez than Shiashy.

Table 1: Interaction effect of direct sun light exposure and treatment on total, total flavonoids, FRAP and ABTS assays of date fruit

Cultivar	Treatment	Total phenolic content (mg/100 g)	Total flavonoids (mg/100 g)	FRAP (mg/100 g)	ABTS inhibition (%)	ABTS (Trolox meq mM/100 g)
Khalas	Control	201.69±2.76^{fg}	27.444±5.09^{i}	79.33±1.85^{def}	0.385±0.004^{ef}	41.43±1.43^{j}
Khalas	12 h	241.73±2.59^{efg}	30.355±3.67^{h}	70.93±1.24^{ef}	0.381±0.006^{cdef}	43.71±0.52^{h}
Khalas	24 h	169.17±0.95^{g}	23.986±3.34^{k}	66.48±0.56^{f}	0.357±0.007^{ef}	41.52±0.79^{i}
Khalas	36 h	250.94±1.56^{ef}	31.585±3.62^{g}	112.69±3.42^{def}	0.267±0.23^{f}	45.52±1.46^{g}
Khalas	48 h	707.50±1.14^{a}	96.333±6.67^{a}	293.99±3.65^{a}	1.044±0.005^{a}	99.81±0.08^{a}
Shiashy	Control	304.58±2.38^{de}	51.667±6.67^{c}	151.14±3.98^{cd}	0.514±0.002^{bcde}	54.95±0.22^{e}
Shiashy	12 h	332.25±3.19^{d}	51.074±6.79^{c}	111.52±2.83^{def}	0.549±0.007^{bc}	57.57±1.07^{d}
Shiashy	24 h	328.33±1.72^{d}	45.122±5.06^{d}	151.36±3.69^{cd}	0.531±0.008^{bcd}	55.81±1.29^{e}
Shiashy	36 h	265.63±0.94^{def}	37.222±3.47^{f}	101.60±0.98^{def}	0.463±0.008^{bcde}	50.62±0.73^{f}
Shiashy	48 h	535.94±2.26^{c}	92.244±7.06^{b}	281.44±4.41^{a}	1.031±0.001^{a}	98.16±0.07^{a}
Rizez	Control	197.71±1.71^{fg}	22.611±2.55^{l}	145.31±1.61^{cd}	0.375±0.03^{def}	57.95±1.95^{cd}
Rizez	12 h	267.71±1.72^{def}	18.956±2.69^{m}	141.86±2.29^{bcd}	0.527±0.007^{bcde}	55.19±1.57^{e}
Rizez	24 h	315.56±1.57^{de}	26.433±2.03^{j}	125.58±2.46^{def}	0.358±0.009^{b}	61.09±1.23^{b}
Rizez	36 h	250.75±4.22^{ef}	18.889±1.93^{n}	118.74±2.29^{cdef}	0.566±0.007^{b}	59.33±0.68^{c}
Rizez	48 h	625.31±5.96^{b}	44.333±3.34^{e}	268.75±1.69^{a}	1.04±0.001^{a}	99.52±0.09^{a}
LSD at 0.05%		7.38	0.77	3.73	0.1712	1.76

Means in each column followed by the same letter(s) did not differ at $p<0.50$

Effect of various treatments on antioxidant activities

Ferric reducing antioxidant power (FRAP): The FRAP value of fresh (control) Shiashy (151.14 mg/100 g) was significantly higher than fresh Rizez and Khalas dates (145.31 mg/100 g and 79.33 mg/100 g, respectively) (Table 1). With the exposure to direct sun light for two days (48 h), both the Shaishy and Rizez cultivars showed an increase in FRAP value but it was highest in Khalas cultivar. The order of increasing trend was Khalas (370%)>Shiashy (186%)>Rizez (185%).

Trolox equivalent antioxidant capacity (TEAC): Fresh dates of all cultivars differed significantly in their capacities to quench ABTS radicals (Table 1). On the basis of values of Trolox Equivalents in control and 48 h treatments, the antiradical activity was highest in Khalas cultivar (99.81/41.43 = 240%) than Shiashy (98.16/54.95= 178%) and Rizez (99.52/57.95 = 171%). The amount of antioxidant required to produce inhibition of ABTS radical was 0.385, 0.514 and 0.375 for Khalas, Shiashy and Rizez, respectively. Almost twice the amount of fresh Khalas and Rizez dates were required to produce the same inhibition effect as that of fresh Shiashy.

The effect of different treatments on % ABTS radical inhibition, expressed as fresh weight (fw) needed to cause inhibition, is shown in Table 1. However, two cultivars (Khalas and Rizez) with exposure to direct sun light for two days (48 h) significantly ($p<0.05$) increased the capacity to quench ABTS radicals in the range of 36% as compared to the fresh sample. Simultaneously, the capacity to quench ABTS radicals was in the range of 49% with Shiashy.

DISCUSSION

Effect of mild and severe heat treatment on antioxidant constituents: Food subjected to processing may undergo intentional, inevitable and accidental nutritional losses, but it also improves the nutritional quality of some foods. This study revealed varying degrees of impact on the antioxidant activity and constituents of dates subjected to various heat treatments and direct sun light for four periods of exposures (half day, one day, one day and half and two days). One overview is the relatively high increase in total phenolics and antioxidant in most treatments. Moreover, all the tree cultivars responded similarly to most direct sun light periods.

Opposing conclusions for the effect of heating treatments and direct sun light periods on phenolic antioxidant in fruit, vegetable and other materials of plant origin can be found in the literature. A decrease in phenolic content in dates after thermal treatment was reported by many workers (Crozier *et al*., 1997; Ismail *et al*., 2004; Peleg *et al*., 1991; Sahlin *et al*., 2004; Shahdadi *et al*., 2011). Reduction of phenolics compounds in dates after 24 h treatments of Khalas and Shiashy under direct sunlight is in agreement with these studies. The loss or decrease in free phenolics, procyanidins and flavan-3-ols except flavanols was reported in sun-dried raisins (Karadeniz *et al*., 2000). Influence of heating on total polyphenol compounds depends on exposure duration and the stability of individual phenolic compounds. At high temperatures, certain phenolics decompose or combine with other plant components. Phenolic reduction in processed products is also influenced by the nature of the raw materials, variety and the degree of individual phenolic compounds stability (Peleg *et al*., 1991).

On the other hand, other workers reported increase in phenolic content of some processed plant materials (Turkmen *et al*., 2005; Gahler *et al*., 2003; Halvorsen *et al*., 2006; Randhir *et al*., 2004). Manzocco *et al*. (1998) reported that some polyphenols increased their stability during processing as a result of chain-breaking efficiency. Similar contradictive conclusions were also reported for the effect of drying on polyphenols (Larrauri *et al*., 1997; Piga *et al*., 2003).

Several explanations for the increase in total phenolic content were put forward. Heat treatments lead to the breakdown of cellular constituents and cell wall and the release of phenolic acids (Cheng *et al*., 2006). Polymerisation and oxidation of the thermally dissociated phenolics may increase the level of phenolics (Randhir *et al*., 2004). Moreover, heat processing may free some of the compartmentalized phenolics thus, causing an increase in their levels in dates (Allaith *et al*., 2012). It is known that phenolic compounds are essentially located in large cells under the skin during early stage of maturation and tend to aggregate at late stage.

Results of this study showed that the effects of heating and durations on antioxidant constituency of dates dependent on treatment and cultivar. Generally, short periods heat treatments (less than 36 h) stabilized the level of total phenolics in the all the cultivars, whereas, 48 h heat treatments significantly increased the level of total phenolics in the thee cultivars. The study findings are in agreement with similar observations reported by (Allaith *et al*., 2012). Vinson *et al*. (2005) reported that extreme temperature and greater exposure to sunlight increased the level of polyphenolics in dates. Al-Farsi *et al*. (2005a) found significant increase in total and free phenolics in dates after sun-drying. Increase in bound phenolics after sun-drying of grapes was also reported by (Karadeniz *et al*., 2000). They attributed this increase to structural changes caused by drying which lead to cell rupturing that brings polyphenolases into contact with their substrates resulting in the formation of colored polymers.

Although, dates from all the cultivars exposed for long periods (48 h) in the sun and heat treatments had identical levels of total phenolics, but with different levels of total flavonoids. As the treatment of two days involves exposing the date fruits to direct sunlight which rises the internal temperature to more than 50°C, then it is reasonable to assume that 48 h treatment actually combines both thermal heating and exposure to sunlight. Moreover, these two days treatment might induce the biosynthetic pathway of both the free phenolics and the total flavonoids as indicated. These findings suggest the involvement of more than one mechanism.

Al-Farsi *et al*. (2005a), who found that sun-drying significantly increased the total, free and bound phenolics in all date varieties studied. This suggested that an increase in free phenolic acids in sun-dried dates might be due to hydrolysis of linkages between bound acid and lignin or arabinoxylans (called as Glucorono extracted from wheat bran by barium hydroxide). However, this study results, although, do not dispute this hypothesis, point to another mechanism. The large increase in total phenolics of dates over the baseline, which is the control treatments, is an indicator of the involvement of de novo synthesis. Severe heat treatment and direct sunlight for two days might result in denaturation of some enzymes involved in the synthesis of some phenolics, but apparently not those involved in biosynthesis of flavonoids. In addition, it is well documented that synthesis of anthocyanins in some plants is influenced by UV radiation (Guo *et al*., 2008).

Effect of various treatments on antioxidant and antiradical activities: Many antioxidant compounds are known to exist in date palm fruit Mansouri *et al*. (2005), Vayalil (2002), Al-Farsi *et al*. (2005a, b), Biglari *et al*. (2008), Allaith (2008), Vinson *et al*. (2005). The interaction of these compounds contributes to overall antioxidant activity and it is difficult to measure total antioxidant activity on the basis of individual components (Pinelo *et al*., 2004). Different assays used to estimate the antioxidant capacity of complex systems generally gave varying results. Furthermore, different antioxidant components have been shown to behave differently (Prior *et al*., 2005). Therefore, the antioxidant activity expressed in this study was in the form of total activity. Two potential methods, ABTS and FRAP, based on different principles were selected for

measurement of antioxidant activity of the test extracts. ABTS measures the ability of the natural antioxidants to scavenge the free radicals whereas the FRAP measures the total reducing capacity of the test compounds. Therefore, different mechanisms of antioxidant actions can be expected from these two methods. The values of ABTS and FRAP methods indicate the power of activity: i.e., the higher the values, the higher the antioxidant activity.

In this study, the ranking of the antioxidant capacity of treatments using both assays was almost the same. Treatments that included exposure to sunlight ranked higher using FRAP. Similar ranking order was found in all cultivars using ABTS assay.

Many researchers used the Ferric Reducing Antioxidant Power assay (FRAP mg Ascorbic acid equivalent/100 g) to assess the antioxidant activity of dates. Halvorsen *et al.* (2002) reported a FRAP value of 1.01 mmol/100 g Fresh Weight (FW), which is lower than the study results which might be due to different maturity stages of dates and different cultivars. Chinese date was having a FRAP value of 6.98 mmol/100 g wet weight (Guo *et al.*, 2003). Allaith (2008) showed that FRAP value of date cultivars varied with maturity stage and ranged from 1.23-14.1 mmol/100 g FW at fully colored unripe dates. This same stage was used in the current study. Biglari *et al.* (2008) reported FRAP values between 11.65 and 20 mol/100 g Dry Weight (DW) for several Iranian soft dates, which is in strong agreement with the current study.

Trolox Equivalent Antioxidant Capacity (TEAC) using ABTS assay provides an indication of hydrogen/electron-donating capacity of plant materials and is widely used for the determination of their radical scavenging abilities. The mechanism of the reaction between antioxidant and ABTS depends on the structural conformation of the antioxidant. Some compounds react very quickly with ABTS, while weak antioxidants may react slowly and the reaction may not be completed in a short assay time (Bondet *et al.*, 1997).

Mansouri *et al.* (2005) studied the percentage radical inhibition of seven Algerian date fruits and reported that the antiradical activity ranged from 0.08-0.22. These authors expressed their findings as antiradical efficiency which does not allow for direct quantitative comparison. Miller *et al.* (2000) ranked the date fruits as the second highest dried fruit with antioxidant activity. The antioxidant activity of date fruits was also assessed using other methods (Vayalil, 2002; Al-Farsi *et al.*, 2005a; Vinson *et al.*, 2005). It should be noted, however, that change in antioxidant activity during different treating methods is not only because of the gain or loss of naturally occurring antioxidant constituents. It can also be due to the presence of highly heat stable natural antioxidants, synergetic action between antioxidant compounds and formation of novel compounds having pro-oxidant or antioxidant activity (Nicoli *et al.*, 1999). Thus, the antioxidant activities of processed date fruit may be enhanced, lost or remain stable. The variation in antiradical activity of the investigated sample between Ferric Reducing Antioxidant Power assay and Trolox Equivalent Antioxidant Capacity assay is not surprising because the antiradical activity of date sample depends upon which free radical is used in the assay.

CONCLUSIONS

In conclusion, certain heating methods used to preserve date fruits may either stabilize or improve their antioxidant activity. The length of exposure period of date fruits to direct sun light treatments significantly increased the level of total phenolics in the three cultivars (Shaishy, Khlas and Rizez). Also, the heat and exposure to direct sun light increased the level of total phenolics and total flavonoids in all the cultivars. Among the studied chemical compounds contributing to the antioxidant activity, the most affected constituent by these treatments were total phenolic, total

flavonoids, FRAP and ABTS assays of date fruit. The study provided a great potential for future investigations on date fruits from other regions of Saudi Arabia with varying climatic conditions and different date palm cultivars.

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