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Antioxidant Content and Capacity of Jordanian Date Palm Fruit at two Maturity Stages

[1]Hiba F. Al-Sayyed, [2]Refa't Al-Kurd, [3]Marwan Mwalla and [4]Salma Abdel Qader

[1]Department of Nutrition /Faculty of Pharmacy and Medical Sciences/University of Petra/Amman/Jordan
[2,3,4]Department of Nutrition /Faculty of Pharmacy and Medical Sciences/University of Petra

Correspondence Author: Hiba F. Al-Sayyed, Department of Nutrition /Faculty of Pharmacy and Medical Sciences/University of Petra/Amman/Jordan.
E-mal: halsayyed@uop.edu.jo

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Abstract

Introduction: Date palm (*Phoenix dactylifera L.*) is cultivated in Jordan for long known time. One of the most commonly grown date palm fruit varieties in Jordan is Barhi. The date palm fruits get matured due to the enzymatic action upon fruit components triggered by climatic conditions. This maturity can be felt through changes in fruit color, size, weight, and taste. Date palm fruit has three edible maturity stages i.e. Khalal, Rutab, and Tamr. Tamr has the least amount of moisture. This study is aimed at comparing the antioxidant content and the capacity of Jordanian Barhi variety of date palm fruit at two maturity stages such as Rutab and Tamr on fresh matter basis. Methods: Two methods were used to study the antioxidant content namely Folin-Ciocalteau method and total flavonoid method whereas 2,2-diphenyl-picrylhydrazyl (DPPH) and cupric antioxidant reducing capacity (CUPRAC) assays were used to study the antioxidant capacity. Three solvents were used for the fruit extraction (ethanol, methanol, and water). Results: From the results, it was inferred that different extracts of Rutab and Tamr showed significant ($P<0.05$) differences in terms of antioxidant content as well as antioxidant capacity. Rutab showed significantly ($P<0.05$) high total polyphenol content (measured by Folin-Ciocalteau method as M catechin/100 g) than Tamr which reflected in its high antioxidant capacity (measured as M trolox/100 g by CUPRAC assay) in Rutab. On the other hand, Tamr exhibited higher total flavonoid content (measured as M rutin/100 g by total flavonoid method) than Rutab. This result has been reflected by higher antioxidant capacity (measured as % DPPH scavenging capacity and as vitamin C equivalent by DPPH assay) in Tamr. Conclusion: Upon maturation from Rutab to Tamr, antioxidant content decreases while total flavonoid content increases. The antioxidant capacity increases while the %DPPH scavenging diminishes upon maturation from Rutab to Tamr. Further, some antioxidants diminish while others get concentrated upon maturation from Rutab to Tamr. The use of different solvents for extraction allowed the extraction and quantification of different polarity antioxidants of the fruit.

Key words: Date palm fruit (Phoenix dactylifera L.), Rutab, Tamr, antioxidant content, antioxidant capacity.

INTRODUCTION

Date palm (*Phoenix dactylifera* L.) is a perennial monocotyledon that belongs to *Palmae* family (Food and Agricultural Organization, FAO, 1993). Date palm has been cultivated as early as 4000 B.C. (Chao and Krueger, 2007; El-Juhany, 2010) in the Middle Eastern countries (FAO, 1993) though it is considered as a recent habit in Jordan. The most common date palm varieties that are cultivated in Jordan are *Hiani*, Red *Talal* and *Barhi* (Ministry of Agriculture (MOA), 2008). Date palm fruit has been proved as a source of many functional constituents such as antioxidant vitamins, flavonoids, and polyphenols (Al-Farsi and Lee, 2008) and used in medical applications (Al-Shahib and Marshall, 2003).

Several studies examined the antioxidant capacity of various extracts of different date palm fruit varieties (Ahmed et al., 1995; Qusti et al.,2010). According to the available literature, no study compared the antioxidant content and capacity of date palm fruit of Jordanian *Barhi* variety at two maturity stages i.e., *Rutab* and *Tamr*on in terms of fresh matter. Thus, this study is aimed at comparing the antioxidant content and capacity of date palm fruit of Jordanian *Barhi* variety at two maturity stages i.e., *Rutab* and *Tamr* on fresh matter basis. Two methods were deployed to study the antioxidant content; namely: Folin-Ciocalteau method and total flavonoid method. Two methods were used to study the antioxidant capacity; namely: 2, 2-diphenyl-picrylhydrazyl (DPPH) and cupric antioxidant reducing capacity (CUPRAC) assays. Three solvents (ethanol, methanol, and water) were used for the fruit extraction.

2. Literature review:

The maturity observed in date palm fruits is due to the enzymatic action upon fruit components induced by climatic conditions. This can be observed through the changes in the fruit color, size, weight, and taste (Allaith, 2008; Biglari et al., 2008). Before consumption, the fruit should pass through the maturity stages such as *Hababouk* and *Kimri*. Then, the fruit passes through some chemical changes related to water and sugar content in order to be edible at three different maturity stages: *Khalal*, *Rutab*, and *Tamr* (Lobo et al., 2013). To reach the *Khalal* stage, slow paced processes such as weight gain of the fruit, increase in sucrose content, decrease in moisture content, and tannin precipitation occurs which finally makes the fruit edible (Bacha, 1987; FAO, 1993). Upon ripening from *Khalal* to *Rutab*, the water content of the fruit gets reduced, and during *Tamr* stage, the water is further reduced and accordingly, the fruit becomes self-preserved (Bacha et al., 1987; FAO, 1993; Ahmed et al., 1995).

2. Methodology:

The fruits at the maturity stages *Rutab* and *Tamr*were purchased from local market and analyzed. As reported by the seller, the fruit at the *Rutab*stage was collected in the same day of purchasing (morning time), neither stored nor treated. The fruit at the maturity stage *Rutab* was prepared by washing with tap water and gentle drying by towel paper. Samples were then cut finely by knife or food chopper (Ariete®, China). About 1-3 g representative samples were conventionally

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extracted by 10 ml of the one of three extraction solvents (water, methanol, ethanol) at 90°C, 50°C, and 50°C respectively for 2 hours with intermittent shaking. The extracts were then centrifuged at 3000 rpm for 10-15 minutes (HuMax®, Germany) and filtered (Wattman filter paper No.4), purged with liquid nitrogen, and stored at -20°C (for not more than two months) until analyzed. Deionized water was used for the preparation of all standard solutions and to complete the reactions (Apak et al., 2007).

2.A. Determination of antioxidant content:

Chemicals were purchased from GCC® (UK), Fischer® (China), Labscan® (Thaihland), LabChem® (USA) and Sigma® (China). Standard curves were prepared to have r^2 value of 0.96-0.99. Samples were analyzed in duplicate with an accuracy of not less than 95% (Luterotti et al., 2006) and coefficient of variation of not more than 15%. Absorbance values were measured using UV-visible spectrophotometer (Sco Tech, Model SPUV®) at the specified wavelength values against standard concentrations of certain antioxidants and blank solutions.

2.A.1.Folin-Ciocalteaumethod:

Folin-Cioculteau method was used for the determination of antioxidant content according to Agbor et al. (2014). Sample volume (10-100 µl) was completed to 1000 µl by 10x freshly prepared Folin-Ciocalteau reagent to complete the reaction within 15- minutes. Sample concentration for antioxidants was measured against freshly prepared catechin standard (catechin standard was dissolved in methanol) at 750 nm wavelength.

2.A.2. Total flavonoid method:

Total flavonoids were analyzed as described previously (Pękal and Pyrzynka, 2014). One milliliter sample was added to methanolic solution (2% w/v) of $AlCl_3$ (0.5 ml). Then, 0.5 ml of deionized water and 0.5 ml of 1M HCl were added respectively, the mixture was shaken vigorously to complete the reaction within 10 minutes. The absorbance was measured at 400 nm wavelength against different concentrations of rutin standard solutions (rutin was dissolved either in ethanol or in methanol).

2.B. Determination of antioxidant capacity:

2.B.1. CUPRAC Assay:

CUPRAC assay was performed as described previouslyby Apak et al., (2007)..To a suitable amount of sample (0.5-5 ml), 10.21 ml concentrated (36%) HCl was added, reaction volume was then completed to 100 ml by 50% methanol, refluxed at 80°C for 2 hours, and cooled down to room temperature. Sample mixture was then neutralized to pH 7 by 1M NaOH. Then, 1 ml $CuCl_2$, 1 ml neucoprine, 1 ml acetate buffer, and suitable sample volume (500-1100 µl) were added respectively to complete the reaction volume to 4.1 ml. The reaction mixture was then incubated at 50°C for 20 minutes, cooled to room temperature and centrifuged at 3000 rpm for about 7 minutes. Sample absorbance was measured using a spectrophotometer at 450 nm (Apak et al., 2007) against different concentrations of trolox standard solutions (trolox was dissolved either in ethanol or methanol).

2.B.2. DPPH assay:

The DPPH assay procedure was performed according to Molyneux (2003). The free radical 2,2-diphenyl-picrylhydrazyl (DPPH) (2.95 ml of 0.1 mM, prepared in 80% ethanol) was added to 50 µl sample. The mixture was incubated at room temperature for 30 minutes in dark place. The absorbance was then measured at 517 nm wavelength against ascorbic acid as a standard. The scavenging percentage was calculated according to the following equation:

$$\text{Scavenging effect (\%)} = \frac{A0-A1}{A0} * 100\%$$

Where:

A_0: is the absorbance of the control

A_1: is the absorbance of the sample

2.C.Statistical analysis

The statistical analysis of data was performed using the software package for social sciences (SPSS, version 23). To detect the differences between the 2maturity stages of the fruitand the solvent of extraction, data were analyzed by factorial mixed (effect of type of fruit and extraction solvent) analysis of variance (ANOVA) (Laerd, 2018). Significant differences were considered at $P<0.05$. Data are expressed in the tables as mean ± standard deviation.

3Results:

Table 1 shows the antioxidant content (M catechin/ 100g) of the methanolic, ethanolic, and water extracts of date palm fruit (*Phoenix dactlifera* L.) at the two maturity stages *Rutab* and *Tamr* determined by Folin-Ciocalteau method. The fruit at the *Rutab* stage contained higher ($P<0.001^{**}$) concentration of antioxidants. Ethanol and ($P>0.05$) water extracted antioxidants more ($P<0.001^{**}$) than methanol did from *Rutab*. On the other hand, water followed ($P<0.05$) by methanol extracted antioxidants more ($P<0.05$) than ethanol did from *Tamr*.

Table 1: The antioxidant content (Mcatechin/ 100 g) of the methanolic, ethanolic, and water extracts of date palm fruit (*Phoenix dactylifera* L.) at the two maturity stages *Rutab* and *Tamr*determined by Folin-Ciocalteaumethod.

Maturity stage	Antioxidant content (Mcatechin/100g) as determined by Folin-Ciocalteaumethod			
	Extract			
	Ethanol	Methanol	Water	P-value
Rutab	4.9030±0.3766	2.3459±0.0357	3.7452±1.1798	0.000^{**}
Tamr	0.6269±0.0541	1.7263±0.0698	2.7589±0.1096	

[1]Values of the tables are average of duplicates ± SEM with c.v. of not more than 15%

[2]P values are used to express significant differences between *Rutab* and *Tamr*extracts at $P<0.05$.

Table 2: The antioxidant content (Mrutin/100g) of the methanolic, ethanolic, and water extracts of date palm fruit (*Phoenix dactylifera* L.) at the two maturity stages *Rutab* and *Tamr*determined by total flavonoid method[1,2,3,4.]

Maturity stage	Antioxidant content (M/100g)as determined by total flavonoids method			
	Extract			
	Ethanol	Methanol	Water	P-value
Rutab	32.2065±1.4925	9.0094±0.2760	9.0607±0.1085	0.000^{**}

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Tamr	45.2914±3.807	95.3060±3.2339	34.8237±1.2204	

[1] Values of the tables are average of duplicates ± SEM with c.v. of not more than 15%
[2] P values are used to express significant differences between *Rutab* and *Tamr*extracts at P<0.05.

Table 3: The antioxidant activity (M trolox/100g) of the methanolic, ethanolic, and water extracts of date palm fruit (*Phoenix dactylifera* L.) at *Rutab* and *Tamr*determined by CUPRAC assay[1,2,3,4.]

Maturity stage	Antioxidant activity (Mtrolox/100g)as determined by total CUPRAC assay			
	Extract			
	Ethanol	Methanol	Water	P-value
Rutab	1.5612±0.0080	4.3823±0.0793	1.9141±0.0301	0.000**
Tamr	1.5005±0.0647	4.9531±0.0989	7.1669±0.1856	

[1] Values of the tables are average of duplicates ± SEM with c.v. of not more than 15%
[2] P values are used to express significant differences between *Rutab* and *Tamr* extracts at P<0.05.

Table 4: The antioxidant activity (expressed as % of DPPH radical scavenging) of the methanolic, ethanolic, and water extracts of date palm fruit (*Phoenix dactylifera* L.) at *Rutab* and *Tamr* maturity stages determined by DPPH assay[1,2,3,4.]

Maturity stage	Antioxidant activity (expressed as % of DPPH radical scavenging) as determined by total DPPH assay			
	Extract			
	Ethanol	Methanol	Water	P-value
Rutab	35.2654±2.5656	35.8998±1.6359	45.3208±0.8192	0.000**
Tamr	11.4602±0.0626	31.5130±0.5064	50.0000±1.0083	

[1] Values of the tables are average of duplicates ± SEM with c.v. of not more than 15%
[2] P values are used to express significant differences between *Rutab* and *Tamr*extracts at P<0.05.

Table 5: The antioxidant activity (expressed as g/ml vitamin C equivalent) of the methanolic, ethanolic, and water extracts of date palm fruit (*Phoenix dactylifera* L.) at *Rutab* and *Tamr* maturity stages determined by DPPH assay[1,2,3,4.]

Maturity stage	Antioxidant activity (expressed as g/ml vitamin C equivalent) as determined by total DPPH assay			
	Extract			
	Ethanol	Methanol	Water	P-value
Rutab	232.6220±16.9235	458.4896±21.0205	382.4545±6.9138	0.000**
Tamr	75.5949±0.4127	402.4646±6.4678	421.9409±8.5093	

[1] Values of the tables are average of duplicates ± SEM with c.v. of not more than 15%
[2] P values are used to express significant differences between *Rutab* and *Tamr*extracts at P<0.05.

Table 2 shows the antioxidant content (Mrutin/100g) of the methanolic, ethanolic, and water extracts of date palm fruit (*Phoenix dactlifera* L.) at the two maturity stages *Rutab* and *Tamr* determined by total flavonoid method. The fruit at the *Tamr* stage contained significantly (P<0.01**) higher amounts of antioxidants than *Rutab*. In the same context, ethanol followed (P<0.01**) by water extracted more (P<0.01**) antioxidants than methanol did in the *Rutab* stage. In *Tamr* stage of maturity, methanol followed (P<0.01**) by ethanol extracted antioxidants more than (P<0.01**) water did.

Table 3 shows the antioxidant activity (Mtrolox/100g) of the methanolic, ethanolic, and water extracts of date palm fruit (*Phoenix dactlifera* L.) at the two maturity stages *Rutab* and *Tamr* determined by CUPRAC assay. *Tamr* exhibited higher (P<0.01**) antioxidant capacity than *Rutab*. Water followed (P<0.0**) by methanol had extracted antioxidants more (P<0.0**) powerfully than ethanol did from *Tamr*. In *Rutab*, methanol followed (P<0.01**) by water extracted antioxidants more (P<0.01**) powerfully than ethanol did.

Table 4 shows the antioxidant activity (expressed as % of DPPH radical scavenging) of the methanolic, ethanolic, and water extracts of date palm fruit (*Phoenix dactlifera* L.) at the two maturity stages *Rutab* and *Tamr* determined by DPPH assay. *Rutab* had higher (P<0.01**) antioxidant capacity than *Tamr*. Within the same context, at the two maturity stages, water followed (P<0.01**) by methanol extracted antioxidants more (P<0.01**) powerfully than ethanol did.

Table 5 shows the antioxidant activity (expressed as g vitamin C /ml) of the methanolic, ethanolic, and water extracts of date palm fruit (*Phoenix dactylifera* L.) determined by DPPH assay. *Rutab*exhibited higher (P<0.01**) antioxidant capacity than *Tamr*. Methanol followed (P<0.01**) by water extracted antioxidants more (P<0.01**) powerfully than ethanol from *Rutab*. From *Tamr*, water followed (P<0.01**) by methanol extracted antioxidants more (P<0.01**) powerfully than ethanol did.

Discussion:

The results for total polyphenols retrieved in this research (determined by Folin-Ciocalteau method) are in alignment with that of El Sohaimy et al. (2015) who found 1.10 µg catechin/g *Tamr* in Egyptian dates (through HPLC analysis). The values (i.e., the average of the three extracts) obtained in this research correspond to 1.06 µg catechin/g *Rutab* and 1.81 µg catechin/g *Tamr*. On the other hand, Saleh et al. (2011) found values much higher than the current study values (7.3, 7.5 and 5 µg/kg *Tamr*of *Ajwa*, *Sukkari*, and *Khalas* varieties).

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The figures of total flavonoids found in this research for *Rutab*are are in alignment with that of *Tamr* investigated by Saleh et al. (2011). These researchers found 6.5, 8.10, and 3.60 mg rutin/kg *Tamr* in *Ajwa*, *Sukkari* and *Khalas* varieties respectively. The values found in this research correspond to 10.2 and 35.7 mg rutin/kg *Rutab* and *Tamr* (i.e., the average of the three extraction solvents) respectively.

The CUPRAC assay results are in alignment with that of the values found in other reports (Lemine et al., 2014; Gökçen, 2016) who obtained much higher values (75.6 - 99.3 µmoltrolox activity equivalent in six different varietiesand 1.40 µmol/g Turkish *Tamr*). Our values correspond to 18.33 µmoltrolox activity equivalent/100 g *Rutab* and 9.08 µmoltrolox activity equivalent/ 100 g *Tamr*. These differences are quite natural since the current study used CUPRAC assay whereas the other researchers used DPPH assay. The most important point to be noted is that the study by Lemini et al. (2014) found a percentage loss of antioxidant activity (measured as trolox equivalent) by 0.4-39.1% upon maturation from *Khalal* stage to *Tamr* stage (range is due to different varieties). In this research, the percentage of difference of antioxidant activity between *Rutab* and *Tamr* (upon ethanol extraction only) is 50.4%.

DPPH values reported in other studies (72.915%, average of two extracts) were double the values obtained in this study (30% for the *Tamr* stage, average of the three solvents) (El Sohaimy et al., 2015). The critical information to be noted here is that similar to the current study results, water was found to be more powerful in antioxidant extraction than ethanol (El Sohaimy et al., 2015). In accordance to the DPPH% results tabulated in table 4, the current study results for antioxidant capacity (expressed as vitamin C equivalent, Table 5) are much higher than the values reported in literature (Saleh et al., 2011; Lemine et al., 2014; El Sohaimy et al., 2015).

The differences between the current study results and the results of other reports might be due to the difference between the maturity stages analyzed, methods of analysis, experimental standardization conditions, variety (Biglari et al., 2008; Qusti et al., 2010) studied and geographical area where the samples were collected (Halvorsen et al., 2002; Boundries et al., 2007; Qusti et al., 2010; Saleh et al. 2011).

The increased antioxidant content (measured by total flavonoid method, Table 2) upon maturation from *Rutab* to *Tamr* might be due to the reduction in moisture content that might have led to the concentration of antioxidants. This got reflected in the increase in antioxidant capacity (measured by CUPRAC assay, Table 3). Similar to the current study results, at *Tamr* stage, Qusti et al. (2010) found a significant (r^2 =0.605, P<0.05) correlation between the antioxidant activity (assayed by IC50) and phenolic content (mg GAE / g edible fruit).

The reduction in antioxidant content (measured by Folin-Ciocalteau method, Table 1) upon maturation from *Rutab* to *Tamr* probably reflects some decomposition of antioxidants which might have occurred either due to heat or storage (Thompson et al., 2006; Kuhnle et al., 2009). This proposed decomposition was reflected by the reduction in antioxidant capacity (measured by DPPH assay, Tables 4 and 5). Similar to the current study results, Allaith (2008) analyzed the relationship among the content of different functional components, characteristics of the date palm fruit and the antioxidant activity (evaluated by FRAP assay). Upon maturation from *Khalal* to *Rutab*, he found a significant negative correlation ($r = -0.267$, $P<0.01^{**}$) between the antioxidant activity and ripening. At the *Rutab* stage, a significant negative correlation was found between the antioxidant activity and color ($r = -0.318$, $P<0.01^{**}$). Unexpectedly, there were insignificant correlations found between the antioxidant activity whereas neither the phenol content (r =0.162, p>0.05) nor the ascorbic acid content (r = −0.275, P>0.05) were found.

Using different solvents of different polarities in this research allowed the extraction of different antioxidants thus providing a scientific value to the results found in this investigation. A close review of literature regarding the bioactive components of the date palm fruit shows that the fruit contains phenols (Qusti et al., 2008; Chaira et al., 2007), carotenoids such as lycopene, violaxanthin, leukoxanthin (Vayalil, 2012), α and β-carotenes, zeaxanthin, neoxanthin and lutein (Al-Farsi and Lee, 2010;Boudries et al., 2007; United States Department of Agriculture (USDA), 2010). Furthermore, the fruit contains polyphenols (Saleh et al., 2011) flavonoids (with their different classes i.e. flavones such as luteolin and apigenein, flvonols such as quercetin and isorhamnetin, flavononeS) (Boudries et al., 2007;Vayalil, 2012) anthocyanins (Chaira et al., 2007; Al-Farsi and Lee, 2008), phenolic acids such as *p*-hydroxbenzoic, syringic, vanillic, caffieic, *p*-coumaric, ferulic (Allaith, 2008), snapic acids (El-Rayes, 2009), protocatechuic, *p*-hydroxybenzoic, chlorogenic, isochlorogenic, and datylifericacid (Vayalil, 2012), metals such as Se, Cu, Zn, and Mn, enzymes such as: phytase, invertase, peroxidase (Qusti et al., 2010) and antioxidant vitamins such as the vitamins C and E (Saafi et al., 2011). The presence of such diverse compounds in the date palm fruit make it capable of possessing antioxidant capacity as shown in tables 3, 4, and 5.

The date palm fruit at the *Tamr* stage is assumed to be moderate in its antioxidant content and activity (Qusti et al., 2010) and similar to those present in lemon and sweet cherry (Halvorsen et al., 2002) in terms of amounts, but higher than those found in tomatoes (Zujko and Witkowska, 2011), mango (Pellegrini et al., 2006), spinach, garlic, broccoli, kiwi fruit, figs (Halvorsen et al., 2006), radish, carrots, potatoes, fennel, cabbage, oats, and rice (Zujko and Witkowska, 2011).

The date palm fruit at the *Rutab* and *Tamr* stages contains high amounts of free and total phenols (on fresh weight basis) than those in apricots, cranberry, figs, green grapes, and plums (Vinson et al., 2005). This characteristic was proved during *in vitro* (Allaith, 2008;Khanavi et al., 2009;Qusti, 2010; Saleh et al., 2011) and *in vivo* investigations (Abo-El-Soaud et al., 2004; Khanavi et al., 2009,Saafi et al., 2011; Vayalil, 2012).

CONCLUSION

Date palm fruit (*Phoenix dactylifera* L.) at *Rutab* and *Tamr* maturity stages contains different types of antioxidants and exhibits antioxidant capacity. Upon maturation from *Rutab* to *Tamr*, the antioxidant content and the capacity of the fruit change.

5. Limitations and recommendations:

Limitations of this research include the analysis of only two maturity stages and only one variety of the fruit for antioxidant content and capacity. It is recommended -thus- to analyze different varieties of the fruit and include the edible maturity stage of *Khalal* in the analysis. The use of experimental methods that identify the antioxidant compounds of the fruit at the three edible maturity stages is recommended to add greater value for scientific antioxidant database.

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7. Author contribution:

Dr. Hiba Al-Sayyed and Miss Salma AbdelQader did the experimental procedures, statistical analysis of data, and wrote the manuscript. Dr. Refat Al-Kurd and Prof. Marwan Mwalla revised the manuscript.

8. Conflict of interest:

The authors declare that there is no conflict of interest among them.

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