Chemical and morphological characterization of *Mammillaria uncinata* (Cactaceae) fruits

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Abstract

Cacti diversity is an important feature of Mexican landscapes. Mammillaria is the largest genus and most morphologically diverse from Cactaceae family. *Mammillaria uncinata* is especially abundant in highlands, and its fruits are commonly consumed among local people; however there are no studies about their chemical composition and nutraceutical properties. The aim of this work was to characterize, morphologically and chemically, the fruits of Mammillaria uncinata (Cactaceae), to study their postharvest characters, as well as, quantify some of their components with nutraceutical potential. Proximate analysis and postharvest characters were determined through AOAC methods; phytochemical profile analysis was carried out through qualitative tests, betalain pigments and total phenolic compounds were quantified spectrophotometrically; and antiradical capacity was determined by DPPH method. Mammillaria uncinata fruits presented the characteristic clavate shape and size; proximate composition was similar to other cactus fruits, except for ashes (1.23%), lipids (1.79%) and crude fiber (7.03%), which were higher than those reported for *Opuntia* fruits. Fruit juice presented high sugar content, low acidity and a sugar-acid ratio which gives it a harmonic sensorial quality. Diverse secondary metabolites were detected in different polarity extracts (alkaloids, sterols, flavonoids, saponins, coumarins and free amino acids). Nutraceutical components were quantified, such as betalain pigments (31.61 \pm 0.80 mg/100g and 16.34 ± 2.63 mg/100g, for betacyanins and betaxanthins, respectively), total phenolics $(0.921 \pm 0.07 \text{ mg gallic acid/g})$ and flavonoids $(0.490 \pm 0.05 \text{ mg catechin/g})$. The results show the chemical composition of Mammillaria uncinata fruits as well as their potential as a source of bioactive compounds for food industry use besides fresh consumption.

Keywords: Mammillaria uncinata, Cactaceae, proximal composition, nutraceutical analysis, betalains.

Introduction

Mammillaria is the largest genus and most morphologically diverse from Cactaceae family, with around 200 species distributed mainly in North and Central America (Butterworth and Wallace, 2004). Mammillaria uncinata Zuccarini ex Pfeiffer is a small to medium sized cactus, individual plants average 3 to 8 cm height with 5 to 12 cm diameter, and they are sub-globose and tubercled. Tubercles are present in 8 and 13 spiral series, with green-bluish color, and provided with latex (Bravo-Hollis and Sánchez-Mejorada, 1991; Zadnik, 1995). Mammillaria uncinata is a very common species in arid and semiarid regions from Mexico; it is distributed specially in the Highlands (Hidalgo, Queretaro, Guanajuato, San Luis Potosí, Aguascalientes and Jalisco). It frequently grows buried among different plant species such as mesquite, prickly bushes and cacti. The species is abundant in several regions of the country, especially in the highlands of Jalisco, in the region denominated "Altos Norte" (Bravo-Hollis and Sánchez-Mejorada, 1991; Arreola-Nava, 1996). Species from Mammillaria genus, known as "biznaguitas", produce small, sweet, red to violet, clavate fruits, generally edible which are commonly called "chilitos de biznaga" (chili-peppers from cacti) (Bravo-Hollis and Sánchez-Mejorada, 1991; Zadnik, 1995). Fruits from Mammillaria species are locally consumed fresh or used to prepare jam; however,

there is no information available on the chemical composition of the fruits other than the presence of betalain pigments (Wybraniec and Nowak-Wydra, 2007), even though some Cactaceae fruits have been described as potential sources of nutraceuticals (Zampini et al., 2011); hence, the aim of this research work was to analyze the morphological characters, proximal composition, pigments content, total phenolic concentration, phytochemical profile, and antiradical capacity of Mammillaria uncinata fruits from the highlands of Mexico, in order to contribute to the knowledge, conservation and, possibly, to the industrial use of this phylogenetic resource.

Materials and methods

Plant material

Fruits of *Mammillaria uncinata* Zuccarini ex Pfeiffer were collected in natural areas in Lagos de Moreno, Jalisco (Mexico) (21° 53′ North, 21° 10′ South, 101°34′ East, 102°10′ West; altitude 1,930 masl). All the samples were collected from August to September, 2010. Only ripe and healthy fruits were selected, cleaned with distilled water and stored at -80 °C until use for analysis.

Morphological characterization

Fruit size: diameter and length were determined to 20 individual fruits, using a Mitutoyo digital caliper. Fresh total weight was determined using an analytical scale (Precisa Gravimetrics, AG. XT220A).

Proximate analysis

Fruits from different individual plants were pooled, pureed and analyzed according to the procedures of Association of Official Analytical Chemists (AOAC, 1990); each analysis was performed by triplicate. Free nitrogen extract was calculated by subtraction of the five main constituents (moisture, ashes, fat, protein and fiber) to 100%.

Postharvest characters

Soluble solids, an estimate of sugars present in the juice, were determined using a portable refractometer (ATAGO, Japan) and were expressed in °Brix (AOAC, 1990). Total acidity was determined in the juice by titration using NaOH 0.1*N*; results were expressed as citric acid percentage (AOAC, 1990). Vitamin C was determined by the Tillman's method through the reduction of 2, 6 dichlorophenol-indophenol (Kirk *et al.*, 2006).

Phytochemical profile analysis

The secondary metabolites (alkaloids, sterols, flavonoids, saponins, coumarins, tannins and quinones) and free amino acids were determined in a qualitative way, according to Dominguez (1973). Analyses were carried out on four different polarity extracts. Prior to extracts preparation, *M. uncinata* fruits were dehydrated under environmental conditions (14–28 °C; 44–58 % RH) during seven days until loss of 85 to 95% of moisture. Dry samples were ground in a mortar and the obtained powder was used for the preparation of extracts according to Del Castillo-Ochoa *et al.*, (2004). Fruit powder was subjected to sequential extractions, using reflux during 30 min; with n–hexane, ethyl acetate, 80% aqueous ethanol and water. All extracts were stored protected from light at 4°C until analysis. Chemical reactions with change of color or precipitates formation indicated the presence of secondary metabolites.

The presence of alkaloids was determined using Dragendorff's, Mayer's, Warner's and Hager's reagents. Turbidity or precipitation in at least three of the four reagents was taken as evidence of the presence of alkaloids. The presence of sterols (triterpens) was determined using Libermann–Burchard's and Salkowski's reagents added to each plant extracts. Flavonoids identification was done using Shinoda's test. The saponins were determined by the foam test and Rosenthaler's reaction. For coumarins qualitative detection, KOH 0.5*M*, and UV light (366nm) were used. For tannins presumptive detection FeCl₃ solution was used. The presence of tannins was confirmed by the formation of precipitate in 1% gelatin solution, and 1% gelatin solution in 1% NaCl but not precipitate formation in 1% NaCl solution. Quinones presence was detected using toluene and alkalis after the extracts were treated with H_2O_2 , and H_2SO_4 . Free amino acids were detected in fruit extracts using ninhydrin solution. All the techniques employed are described by Sanchez-Herrera *et al.* (2011).

Nutraceutical components

Pigments extraction and quantification

Fresh sample (500 mg) was homogenized and extracted twice with 5 mL of Mc Ilvaine buffer (pH 6.5, citratephosphate) at room temperature, using a mortar and pestle. Extracts were pooled and centrifuged (Sigma 2-16K116172, Germany) at 10,000 \times g, during 20 min. Pigments concentration was determined spectrophotometrically (Jenway 6305, England) at 535 and 483 nm; the results were expressed as betanin and indicaxanthin equivalents, respectively. For calculation purposes betanin (molecular weight 550 g/mol, molar extinction coefficient 60,000 L/mol cm) and indicaxanthin (molecular weight 308 g/mol; molar extinction coefficient 48 000 L/mol cm) data were employed (Stintzing *et al.*, 2005).

Phenolic compounds quantification

Content of total phenolic compounds was determined in the extract by Folin-Ciocalteu's reagent, according to Singleton and Rossi (1965). A 0.1-mL aliquot of the extract was mixed with 0.1 mL of distilled water, 1.0 mL of 1*N* Folin-Ciocalteu's reagent and 0.8 mL of 7.5 % Na₂CO₃. The mixture was allowed to stand in the dark for 30 min at room temperature. Absorbance was measured at 765 nm. The total phenolic content was expressed as gallic acid equivalents (GAE per gram) according to a calibration curve from 100 to 500 mg/L of gallic acid. Total flavonoid concentration was determined by a colorimetric method (Liu *et al.*, 2002). Briefly, 0.25 mL of the fruit extract was diluted with 1.25 mL of distilled water. Then 0.075 mL of a 5% NaNO₂ solution was added to the mixture. After 6 min, 0.150 mL of a 10% AlCl₃·6H₂O solution was added, and the mixture was allowed to stand for another 5 min. Half of a milliliter of 1 *M* NaOH was added, and the total was made up to 2.5 mL with distilled water. The solution was mixed, and the absorbance was measured immediately against the prepared blank at 510 nm. Flavonoids content was expressed as (+)-catechin equivalents according to a calibration curve from 25 to 200 mg/mL of the standard. The results were expressed as milligrams of catechin equivalents per gram of fresh sample.

Antiradical capacity

Antiradical capacity (ARC) of methanolic extracts of *M. uncinata* fruits was determined by the 1,1-diphenyl-2picrylhydrazyl (DPPH) method (Fukumoto and Mazza, 2000), modified by Méndez-Robles *et al.* (2006). Briefly, 300 μ L of extract were placed in test tubes, and 3 mL of methanolic DPPH solution (150 μ M) were added. Tubes were mixed and incubated in the dark at 25 °C. After 60 min, the absorption was measured in 1 cm cuvettes at 520 nm. ARC was calculated according to the equation of Burda and Oleszek (2001): ARC % = 100 × [1 - (absorbance sample / absorbance of control)].

Statistical analysis

All data were reported as means \pm standard deviations. A completely random experimental design was used considering fruits from at least ten different plants, with two replicates of each experiment (SAS Institute, 1989).

Results and discussion

Morphological characters

Mammillaria uncinata fruits presented the characteristic clavate shape reported for fruits from other *Mammillaria* species (Figure 1) (Bravo-Hollis and Sánchez-Mejorada, 1991; Zadnik, 1995); they were 12.63 mm length mean and had a mean weight of 0.2 g/fruit; the end next to the flower was wider (5.03 mm diameter) than the opposite end (Table 1). *Mammillaria uncinata* fruits size and weight are smaller compared to other cacti fruits such as *Opuntia* (lengths from 4 to 11 cm, and diameters from 3 to 7 cm) (Sáenz *et al.*, 2006); *Lemaireocereus griseus* (Haw) fruits (Terán *et al.*, 2008); and *Stenocereus griseus* (Emaldi *et al.*, 2006). Differences in fruits size and shape depend, mainly, on the species analyzed.

Proximal composition

To our knowledge, this is the first time that *Mammillaria uncinata* fruits proximal composition is described (Table 2). Whole fruits including seeds and peel were analyzed. Moisture content is elevated in *M. uncinata* fruits (83.4%), similar to other cacti fruits. In their review, Sáenz *et al.* (2006) reported moisture contents

between 83.8 and 91 % in *Opuntia* spp. fruits; Emaldi *et al.*, (2006), found moisture values of 81.64% and 85.87% in the edible part of *Stenocereus griseus*, white and red varieties, respectively. Protein content (0.95%) was similar to data reported for *Opuntia* spp. fruits from 0.21 to 1.6% (Moreno-Álvarez *et al.*, 2008; Sáenz *et al.*, 2006). Contents of ashes (1.23%), lipids (1.79%) and crude fiber (7.03%) were higher than those reported in the edible part of *Opuntia* fruits; in the intervals from 0.31 to 0.51%, 0.09 to 0.7% and 0.2 to 3.16%, for ashes, lipids and crude fiber, respectively (Moreno-Álvarez *et al.*, 2008; Sáenz *et al.*, 2008; Sáenz *et al.*, 2008; or ashes, lipids and crude fiber obtained for *M. uncinata* fruits could be due to the inclusion of seeds and peel in the fruits analyses; usually those parts contain higher contents of these nutrients (Moreno-Álvarez *et al.*, 2008; Tliti *et al.*, 2011).



Figure 1. Fruits of Mammillaria uncinata.

Parameter	Mean values		
Fruit length (mm)	12.63 ± 1.54		
Fruit diameter (mm)	5.03 ± 0.540		
Fruit weight (g)	0.20 ± 0.045		

^aMeans ± standard deviation based on measures 20 fruits from at least ten plants.

Table 2. Proxi	mal composition	and caloric valu	e of <i>Mammillaria</i>	uncinata whole fruits ^a .
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Component	Content (%)
Moisture	83.40 ± 1.39
Ashes	1.23 ± 0.35
Lipids	1.79 ± 0.16
Protein (N \times 6.25)	0.95 ± 0.06
Crude fiber	7.03 ± 0.57
Nitrogen free extract	5.58 ± 1.84
Caloric value (Kcal/100g)	42.23

^aData represent mean \pm standard deviation from two independent experiments with three replications.

Postharvest characters

Mammillaria uncinata fruits presented a mean content of 14.5 °Brix (Table 3), similar to data reported for some *Opuntia* fruits in the range of 8.1 to 14.73 °Brix (Sáenz *et al.*, 2006; Moreno-Álvarez *et al.*, 2008; Chávez-Santoscoy *et al.*, 2009); and higher to data reported for *S. griseus* fruit, 11.33 °Brix (Emaldi *et al.*, 2006); and for *S. stellatus* fruits (9.33 to 9.67 °Brix) (Beltrán-Orozco *et al.*, 2009). Total acidity of *M. uncinata*

fruits (0.76 % citric acid) was higher than data reported for *Opuntia* spp. fruits in the range of 0.02 to 0.22% of citric acid (Chávez-Santoscoy *et al.*, 2009); and to the information reported by Emaldi *et al.* (2006) for *S. griseus* fruit, 0.08 to 0.15 % citric acid. According to Stintzing *et al.* (2003) the sugar-acid ratio is an important parameter characterizing the sensorial qualities of fruits products; the ratio which is known to be harmonic is from 10:1 to 18:1; for *M. uncinata* fruits, the calculated sugar-acid ratio was 19 to 1 (Table 3), which means the fruits have a pleasant sweet-sour taste. Ascorbic acid (vitamin C) content in *M. uncinata* fruits was 1.395 g/100g. It is well known that vitamin C content in cactus fruits is variable; for instance, in their review Sáenz *et al.* (2006) reported concentrations from 4.6 to 41 mg/100g in fruits of *Opuntia*; while other cactus fruits, such as *Stenocereus stellatus* (Beltrán-Orozco *et al.*, 2009), presented concentrations between 8 and 14 mg/100g. In the other hand, Stintzing *et al.* (2003) did not detect ascorbic acid in *Hylocereus* cultivars. It has been described that the variability in postharvest and quality characteristics of cactus fruits is influenced by genotype-environmental interactions (De Wit *et al.*, 2010).

Parameter	Content		
Soluble solids (°Brix)	14.515 ± 1.965		
Total acidity (Citric acid%)	0.765 ± 0.055		
Ascorbic Acid (g/ 100 g)	1.395 ± 0.27		
Sugar-acid ratio ^b	19:1		

Table 3. Postharvest characters of Mammillaria uncinata fruits^a.

^aData represent mean ± standard deviation from two independent experiments with three replications ^bCalculated as soluble solids/total acidity

Phytochemical profile

Table 4 shows the results of secondary metabolites detected in four different polarity extracts from *Mammillaria uncinata* fruits. Reaction to alkaloid identification reagents was moderate in n-hexane and 80% ethanol extracts, slight in aqueous extract, and absent in ethyl acetate extract, which indicates that alkaloids in *M. uncinata* fruits probably are in the form of organic acid salts and there is a low quantity in soluble form (Domínguez, 1973). Alkaloids are phytochemical considered to protect plants from predators because of their effects on the insects and other herbivores nerve system (Domínguez, 1973). Presence of bioactive alkaloids in stems and roots of Cactaceae has been widely described, including those with hallucinogenic properties, such as mescaline from "peyote" (*Lophophora williamsii*), and other with weaker activity, such as those present in *Coryphantha macromeris* var. *runyonii*, and *Mammillaria longimamma*; however, the presence of alkaloids in cactus fruits has not been reported (Gibson and Nobel, 1986) and no reports were found about psychotropic effects from consumption of *M. uncinata* fruits.

Table 4. Phytochemical	profile of different r	polarity extracts from	<i>Mammillaria uncinata</i> fruits ^a .

Secondary metabolites	Extracts			
	n-Hexane	Ethylacetate	80% Ethanol	Water
Alkaloids	++	-	++	+
Sterols	+++	+++	++	-
Flavonoids	-	-	++	+++
Saponins	-	-	++	++
Coumarins	-	+	++	++
Free aminoacids	-	-	+++	+++

^a Presence: (+), (+slight; ++moderate, +++ abundant). Absence: (-).

Because of their lipidic characteristics, sterols occurred in abundant quantities in n-hexane and ethyl acetate extracts; moderate quantities were present in 80% ethanol extract. Sterols are common metabolites in different tribes of Cactoideae subfamily, they are being isolated from Mexican columnar cacti such as *Peniocereus fosterianus* and organ pipe cactus *Stenocereus thurberi* (Gibson and Nobel, 1986); and from other cacti (Lim *et al.*, 2010; Ramadan and Morsel, 2003; Sánchez-Herrera *et al.*, 2011). Sterols play an important role in plant cell

membranes, different factors such as genotype and environmental conditions influence their synthesis in plants. Because of their solubility, flavonoids were abundant in aqueous extract, moderate in 80% ethanol extract and absent in non-polar extracts. Flavonoids have been found in stem, fruits and other plant portions of cacti. For example, Chuquimia et al. (2008) reported the presence of flavonoids with antioxidant capacity in Neowerdermannia vorwerckii: Vázquez-Cruz et al. (2008) studied phenolic compounds in fruits of Myrtillocactus geometrizans; Almaraz-Abarca et al. (2007) identified some flavonoids in pollen of Stenocactus multicostatus. Saponins from M. uncinata fruits were moderate in polar extracts and absent in non-polar extracts, which suggests that probably contain saccharide residues and other polar groups. The occurrence of saponins has been described widely in several cacti, such as Cereus deficiens (Zapata et al., 2003), Epiphyllum phyllantus (Padrón-Pereira et al., 2008) and Coryphantha spp. (Sánchez-Herrera et al., 2011). Coumarins were detected in polar extracts in moderate quantities: their presence in *M. uncinata* fruits contributes to make this species an interesting potential nutraceutical source since coumarins have been described to present different biochemical actions; such as antioxidant, hepatoprotective, anticoagulant, antifungal, reduction of total cholesterol and triglycerides, depending on their structure and substitution pattern (Bahadir et al., 2011; Thuong et al., 2010). Free amino acids were detected abundantly in polar extracts. A high content of free aminoacids, including taurine, has been detected in other Cactaceae fruits, such as prickly pear (Stintzing et al., 2001). Notably, tannins and quinones, widely distributed in plants, were no detected in M. uncinata fruits, as well as mucilage, which had been described in other Cactaceae fruits. A similar result was reported by Sánchez-Herrera et al. (2011) in stems of Coryphantha species. Tannins have been found in other Cactaceae such as Cereus deficiens (Zapata et al., 2003), but their absence in other Cactaceae has been reported too (Padrón-Pereira et al., 2008). More research is needed in order to quantify and study the chemical structure of different phytochemicals in Mammillaria uncinata fruits that could be used as taxonomic markers, besides their potential biological activities.

Nutraceutical components

After phytochemical profile analysis, it was decided to quantify some components of nutraceutical importance, such as total phenolic compounds, flavonoids, betalain pigments and antiradical capacity.

Betalains are nitrogenous plant pigments with yellow (480 nm, betaxanthins) or violet (536 nm, betacyanins) coloration (Strack et al., 2003). Betalain pigments were extracted from whole Mammillaria uncinata fruits, and quantified spectrophotometrically; 31.61 and 16.34 mg/100g fresh fruit of betacyanins and betaxanthins, respectively, were determined (Table 5). In Cactaceae, the ratio and concentration of betalain pigments are responsible for the color of fruits (flesh and peel) such as in *Hylocereus* spp., *Opuntia* spp., *Myrtillocactus* spp. and Selenicereus spp. (Castellanos-Santiago and Yahia, 2008; Esquivel et al., 2007; Moreno et al., 2008). According to Wybraniec and Nowak-Wydra (2007), mammillarinin is the main betalain pigment in Mammillaria species such as M. roseo-alba, M. donattii, M. coronata, among others; in the present research, 16.34 mg/100g of betaxanthins were detected in *M. uncinata* fruits, additionally to betaxanthins. The contents of betaxanthins and betacyanins determined in *M. uncinata* fruits is similar to the highest concentrations reported for other cacti fruits (Chávez-Santoscoy et al., 2009; Moreno-Álvarez et al., 2008; García-Cruz et al., 2012). There is a growing interest in the use of natural red pigments as substitutes for synthetic dyes for food coloring, since synthetic dyes are being more and more critically assessed. Moreover, interest on betalains has grown since their antioxidant and radical scavenging properties were characterized (Lu et al., 2009; Moreno-Álvarez et al., 2008) and they are widely used as additives in the food industry because of their natural colorant properties and the absence of toxicity, even at high concentrations (Moreno et al., 2008; Schwartz et al., 1983; Stintzing et al., 2003). Phenolic compounds, including flavonoids have been detected in fruits of different Cactaceae species. Presence of flavonoids in *M. uncinata* fruits was evidenced by qualitative tests in the phytochemical profile; total phenolic compounds and flavonoids from M. uncinata fruits were quantified through spectrophotometric methods. Content of total phenolics quantified in fruits of *M. uncinata* was 0.921 mg gallic acid equivalents/g (GAE), 53% from which corresponded to flavonoids (Table 5); the values detected were higher than those reported for other Cactaceae fruits; for example Chávez-Santoscoy et al. (2009) reported concentrations of total phenols between 0.022 to 0.226 mg/g GAE and flavonoids contents from 0.096 to 0.374 mg catechin equivalents/g in juices extracted from nine Mexican prickly pears (Opuntia spp.). Other cactus fruits, such as Stenocereus stellatus, analyzed by Beltrán-Orozco et al. (2009), presented total phenolics concentrations between 1.38-2.35 mg GAE/g of sample in fruits of four different colors. García-Cruz *et al.* (2012) determined 0.1665 mg GAE/g of red cultivar of *Stenocereus griseus* H., and 0.0528 mg GAE/g of orange cultivar. Phenolic compounds, including flavonoids, have been recognized as antioxidants with capacity to retard aging and to prevent degenerative diseases, such as cancer, cardiovascular diseases and cerebral dysfunctions (Ames *et al.*, 1993). Methanolic extract of *M. uncinata* fruits presented 16.77 \pm 0.024% of antiradical capacity (ARC) which is equivalent to the ARC of a butylated hydroxytoluene solution at concentration of 35.42 µM. There is a lot of information about the antioxidant capacity of prickly pear (*Opuntia* spp.) (Chávez-Santoscoy *et al.*, 2009; Fernández-López *et al.*, 2010; Piga, 2004); as well as other cactus fruits such as *Hylocereus* spp. (Esquivel *et al.*, 2007; Rebecca *et al.*, 2010; Vaillant *et al.*, 2005), *Myrtillocactus geometrizans* (Herrera-Hernández *et al.*, 2011) and *Stenocereus stellatus* (Beltrán-Orozco *et al.*, 2009); all of them agree on the presence of components with antioxidant activity in cactus fruits; however, comparison between different results is difficult to carry out, since the results come from different techniques; and comparisons among results of antioxidant capacity can only be made for the same method and for samples obtained with the same solvents.

Table 5. Betalain pigments, phenolic content and antiradical capacity of Mammillaria uncinata fruits^a.

Component	Concentration	
Betacyanins (mg/100g)	31.61 ± 0.80	
Betaxanthins (mg/100g)	16.34 ± 2.63	
Total phenolic (mg gallic acid/g)	0.921 ± 0.07	
Flavonoids (mg catechin/g)	0.490 ± 0.05	
Antiradical capacity (%)	16.77 ± 0.024	

^aData represent mean \pm standard deviation from two independent experiments with three replications.

Conclusions

This is the first time that nutritional composition and chemical profile of *Mammillaria uncinata* fruits is published. The present study shows the potential of fruits from *M. uncinata* as food and as a source of bioactive compounds, including ascorbic acid and flavonoids among others, which together with betalains, make the species a promising source of phytochemicals for their use in food and pharmaceutical industries. Further studies are needed in order to find out the exact quantities and types of the secondary metabolites present in fruits, as well as, their potential biological activities.

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