American Journal of Food Technology

ISSN 1557-4571

Academic Journals Inc.

www.academicjournals.com

American Journal of Food Technology 6 (11): 974-984, 2011
ISSN 1557-4571 / DOI: 10.3923/ajft.2011.974.984

Assessment of a Maturity Index in Jabuticaba Fruit by the Evaluation of Phenolic Compounds, Essential Oil Components, Sugar Content and Total Acidity

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ABSTRACT

Jabuticaba is a Brazilian tree with edible grape like fruits which are consumed fresh or used to prepare jams, liqueurs and wines. The aim of this study was to identify the optimal harvest period for winemaking by monitoring chemical parameters during jabuticaba's development. Jabuticaba fruit was analysed at four developmental stages for total acidity, reducing sugars, anthocyanins, tannins, total phenols and essential oil composition. Total acidity significantly decreased (15.46-4.56 g citric acid 100 g^{-1}), whereas reducing sugars increased about threefold in pulps up until the full ripe stage (32.52-88.92 g glucose equivalent 100 g^{-1}). Anthocyanin levels underwent a sharp increase in the skins reaching 22.0 mg cyanidin 3-glucoside equivalent g^{-1} at complete maturation. Tannins and total phenols showed no significant differences in their levels between ripe and full ripe stages. The essential oil contains 88.1% of sesquiterpene compounds, being γ-eudesmol (33.9%) and α-eudesmol (15.5%) the major constituents. Some minor terpenoids changed significantly in their contents during jabuticaba's maturation. The monoterpenes α-pinene, β-pinene, (E)-β-ocimene, linalool and α-terpineol increased while the sesquiterpenes amorpha-4,7(11)-diene, δ-amorphene, δ-cadinene and α-cadinene decreased from green to ripe stages. However the major terpenoids showed no significant changes. Results demonstrated that the use of sugar/acid ratio and anthocyanin concentration seemed to be the most effective parameters to reveal the optimal harvest period for winemaking.

Key words: *Myrciaria cauliflora*, Myrtaceae, fruit ripening, terpenoids, tannins, anthocyanins

INTRODUCTION

Jabuticaba (*Myrciaria cauliflora* Berg.) is a short multi-stemmed Brazilian tree; its flowers and fruits grow directly on the main truck and stems (Reynertson *et al*., 2006). Jabuticaba berries have a slightly acid to sweet taste as well as thick and tough purple to blackish skin (Lima *et al*., 2011). In the popular medicine, the decoction of the skins is used as a treatment for hemoptysis, cough, bronchitis, asthma, diarrhea and dysentery, as well as a rinse for chronic throat inflammation (Reynertson *et al*., 2006; Stefanello *et al*., 2011). Two depsides were isolated from deseeded fruits and exhibited antiradical activity in DPPH assay, colon cancer cell cytotoxicity and inhibition of interleukin IL-8 production, suggesting anti-inflammatory activity (Reynertson *et al*., 2006). The fruit ethanol extract also showed inhibitory effect against *K. pneumoniae* (Haminiuk *et al*., 2011),

while leaf extracts were effective against several oral bacteria and *Candida* species (Macedo-Costa *et al*., 2009; Souza-Moreira *et al*., 2010).

Several reports have described their antioxidant activity which is mainly attributed to the high anthocyanin and flavonoid content in their skins (Einbond *et al*., 2004; Reynertson *et al*., 2008; De Assis *et al*., 2009; Romero *et al*., 2010; Santos *et al*., 2010; Rufino *et al*., 2010; Leite *et al*., 2011; Haminiuk *et al*., 2011). Recently, optimization of anthocyanin extraction from jabuticaba skins was performed using high pressure CO_2, ultrasound treatment and combination of different solvents and pH (Santos and Meireles, 2011; Santos *et al*., 2010; Montes *et al*., 2005).

Besides its biological activities, jabuticaba berries have been used to produce jams, liqueurs, distillates and wines, as an alternative to prevent post-harvesting losses (Reynertson *et al*., 2006; Agostini *et al*., 2009). In the last decade jabuticaba wine production has grown and the success among consumers has increased. The target now is to improve its quality (Da Silva *et al*., 2008; Duarte *et al*., 2010b), through the optimization of fermentation conditions (Duarte *et al*., 2011) or by managing the attributes of jabuticaba berries (Danner *et al*., 2011). The wine quality is affected directly by fruit composition which is influenced by many extrinsic factors, such as climatic conditions, soil and harvesting date (Gómez-Míguez, *et al*., 2007; Pérez-Magariño and González-San José, 2006). It is well known that phenolic and terpenoid compounds which are responsible for some of wine's organoleptic properties, such as colour, flavour, body and structure of red wines (Gómez-Míguez *et al*., 2007) alter during fruit development and ripening (Fadda and Mulas, 2010; Coelho *et al*., 2006). However, there is no information on the concentration of tannins, total phenols and terpenoid compounds during jabuticaba growth and maturation. In addition, the composition of jabuticaba's essential oil has not been previously reported.

Therefore, the main objective of this paper was to assess the optimal harvest period for winemaking, through the analysis of the evolution of phenolic and essential oil compounds, reducing sugar and total acidity during jabuticaba's development and ripening.

MATERIALS AND METHODS

Plants, materials and chemicals: Cultivated *M. cauliflora* fruits var. Pingo de mel were collected in September and October 2008 at Jabuticabal Winery (S 16° 55′ 25.9″, W 49° 21′ 41.0″), located in the outskirts of Hidrolândia, Goiás State, Brazil. About 2 kg of each fruit sample were obtained from 10 to 20 year-old trees. Fruit harvesting was performed at four developmental stages: 16-, 23-, 30- and 37-days after anthesis (DAA). After harvesting, part of the berries was washed with running water and separated manually into its components (skin, pulp and seeds) and 100 g samples of berries and each separated part were blended with 50 mL distilled water and freeze-dried. Dried samples were stored at -18°C until analysis. Tannic acid and iron (III) chloride were purchased from Merck (Darmstadt, Germany). Dinitrosalicylic acid, Folin-Ciocalteu's phenol reagent and Bovine Serum Albumin were purchased from Sigma-Aldrich (St. Louis, MO, USA). All other chemicals used were of analytical grade. After each harvest, three groups of 50 fresh berries were averaged for fresh weight measurement.

Extracts for reducing sugar and total acidity assay preparation: Freeze-dried berries or pulp (0.2 g) were extracted at 50°C with 10 mL of distilled water in an ultrasonic bath for 30 min. The extract was separated from the solid residue by centrifuging at 4,000 rpm for 15 min and transferred to a 25 mL volumetric flask. The same procedure was repeated twice with 10 and 5 mL of water for 15 min each. The extracts were combined in a final 25 mL volume and were prepared in triplicate.

Extracts for phenol assay preparation: One gram of freeze-dried berries or separated parts (skin, pulp and seeds) was homogenized with 10 mL of MeOH-formic acid (9:1) in a test tube and sonicated for 30 min. The extract was centrifuged, filtered and the marc extracted three more times for 15 min. Extracts were combined and concentrated under reduced pressure at 35°C and brought up to 25 mL in a volumetric flask. Extracts were prepared in triplicate.

Extraction of fruit essential oil: Frozen berries (ca. 500 g) were crushed in small pieces and submitted to hydrodistillation (2 h) by means of a modified Clevenger-type apparatus. At the end of each distillation essential oils were collected, dried with anhydrous Na_2SO_4, transferred to glass flasks and kept at a temperature of -18°C until analysis. Oil yields (%) were based on the fresh weight of fruit samples.

Determination of reducing sugar: Reducing sugar content was determined by the 3,5-dinitrosalicylic acid (DNSA) method (Miller, 1959). Results were expressed as glucose equivalent 100 g^{-1} dried fruit. Measurements were performed in triplicate.

Determination of total acidity: Total acidity was measured by titrating an aliquot (5.0 mL) of the extract with 0.01 mol L^{-1} of NaOH to pH 8.2. The results were expressed as g citric acid 100 g^{-1} dried fruit. Measurements were performed in triplicate.

Determination of phenolic compounds: Total phenols were quantified by the Folin-Ciocalteu method described by Escarpa and Gonzalez (2001). Results were expressed as mg tannic acid equivalent g^{-1} dried fruit or its parts. Measurements were performed in triplicate.

Tannins were measured by protein precipitation assay with the use of Bovine Serum Albumin (Hagerman-Butler method described by Waterman and Mole (1994). Results were expressed as mg tannic acid equivalent g^{-1} dried fruit or its parts. Measurements were performed intriplicate.

Anthocyanin content was determined by the pH-differential method (Wrolstad *et al*., 2005; Reynertson *et al*., 2008). Pigment concentration is expressed as cyanidin 3-glucoside equivalents per g of dry weight (DW). Measurements were performed in triplicate.

Fruit essential oil analyses: Oil sample analyses were performed on a GC-MS Shimadzu QP5050A instrument under the following conditions: A CBP-5 (Shimadzu) fused silica capillary column (30 m×0.25 mm i.d., 0.25 μm film thickness) connected to a quadrupole detector operating in the EI mode at 70 eV with a scan mass range of 40-400 m/z at a sampling rate of 1.0 scan/s; carrier gas: He (1 mL min^{-1}); injector and interface temperatures of 220 and 240°C, respectively, with a split ratio of 1:20. The injection volume was 0.4 μL (ca. 20% in hexane) and the oven temperature was raised from 60 to 246°C with an increase of 3°C/min, then 10/min to 270°C and the final temperature was held for 5 min. Individual components were identified by comparing their linear retention indices, which were determined by a co-injection with a C_8-C_{32} n-alkanes series (Sigma, USA) and mass spectra with those of the literature (Adams, 2007) and a computerized MS-database using NIST libraries (NIST, 1998).

Statistical analysis: Experimental data were assessed by one-way ANOVA using SAS GLM analyses (Statistical Analysis System, SAS Institute Inc., Cary, NC, 1996). All data was checked for homoscedasticity with the use of Hartley's test (Kanji, 2006). Whenever a difference was

established, a post-hoc Tukey test was performed. Results are shown as mean values and are joined by the standard deviation of independent measurements in some cases. The p-values below 0.05 were regarded as significant.

RESULTS AND DISCUSSION

The complete development of jabuticaba fruits usually takes 45-60 days after anthesis (Magallhães *et al*., 1996); however, fruits may complete maturation in 35 days during a hot dry season with proper irrigation and soil conditions (Andersen and Andersen, 1989). This is the case at Jabuticabal Winery's orchard where berries grow rapidly, therefore, four development stages were chosen: green (16 DAA), turning to purple (23 DAA), ripe (30 DAA) and full ripe (37 DAA).

Fruit fresh weight, reducing sugars and titratable acidity: Results for fruit weight, reducing sugars content and total acidity of jabuticaba fruits are presented in Table 1. The fresh fruit weight increased significantly until 30 DAA (2.51-7.67 g), from this date occurred a substantial reduction to 5.02 g. The reduction in the fruit's fresh weight could be due to water loss. A similar growth pattern was observed in berries such as myrtles, plums and grapes (Fadda and Mulas, 2010) and this is the opposite for pomegranate fruit which has a continuous weight increase during maturation (Shwartz *et al*., 2009).

Reducing sugars content in pulp had significant growth in all stages, while for the whole fruit considerable differences were observed mainly from green to full ripe stages (33.51-48.06 g 100 g^{-1}). Non-structural jabuticaba carbohydrates are composed mainly of reducing sugars, which make up about 90% of total soluble sugars and accumulate mostly in the pulps, even though pulps account to only 36 to 49% of the fruit (Barros *et al*., 1996; Lima *et al*., 2011). Comparable to results at full ripe stage (Table 1) when the reducing sugars content in the pulps was 88.92 g 100 g^{-1}, while the whole fruit contained 48.06 g 100 g^{-1} (ca. 50%). Maximum sugar content in the pulp at 37 DAA was very similar to 80 g 100 g^{-1} DW obtained for *M. jaboticaba* at 60 days (Barros *et al*., 1996).

Throughout jabuticaba's development, total acidity presented a constant and significant decrease in all stages. The acidity values dropped from 15.46 to 4.56 g citric acid 100 g^{-1} which is a reduction of about 70% (Table 1). Similar results were observed in two jabuticaba varieties (Sabará and Açú Paulista), in which succinic and citric acids reduced 71.3% on average in the pulp during maturation (Jham *et al*., 2007); the same occurred with myrtle berries, which showed a 70% decrease in titrable acidity (Fadda and Mulas, 2010). The most abundant organic acids detected and quantified in jabuticaba fruits were succinic and citric acids (Jham *et al*., 2007). Malic, oxalic and acetic acids were also detected although in lower amounts (Lima *et al*., 2011).

Changes in sugar and total acidity levels mean that sugar/acid ratio increased during fruit development from 2.17 (16 DAA) to 10.5 (37 DAA). This ratio can be used as a maturity index, which measures fruit quality for industrial purposes; nevertheless, there are limitations as regards variability between fruits, cultivars and year of observation (Kingston, 1992).

Total phenols, tannins and anthocyanins: Table 2 displays the results from anthocyanins, tannins and total phenols assays in parts and the whole jabuticaba fruit at four developmental stages.

A comparison of anthocyanin amounts during maturation (Table 2) showed a significant increase in the skins and also in the whole fruit levels, while seeds and pulps did not change.

Table 1: Average concentrations[a] of fresh weight (g), reducing sugars and total acidity (g 100 g^{-1}, dry weight basis) in jabuticaba fruit/parts during development

		Reducing sugars		
DAA[b]	Fresh weight fruit	Pulp	Fruit	Total acidity fruit
16	2.51±0.14C	32.52±0.55aD	33.51±0.19aC	15.46±0.22A
23	4.77±0.14B	56.57±2.48aC	42.24±0.83bB	13.36±0.11B
30	7.67±0.09A	66.21±0.08aB	43.55±0.80bB	7.14±0.11C
37	5.02±0.35B	88.92±0.13aA	48.06±0.07bA	4.56±0.03D

[a]Values are Means±RSD (n = 3). [b]Days after anthesis. Means followed by the same capital letter in the columns and by the same small letter in the rows (for reducing sugars) did not share significant differences at 5% probability by Tukey's test

Table 2: Average concentrations[a] of anthocyanins, tannins and total phenols (mg g^{-1}, dry weight basis) in jabuticaba fruit/parts during development

	Anthocyanins				Tannins				Total phenols			
DAA	Seed	Pulp	Skin	Fruit	Seed	Pulp	Skin	Fruit	Seed	Pulp	Skin	Fruit
16	0.84aA	0.42aA	0.43aD	0.56aD	7.75aA	4.02bA	7.27aA	8.16aA	18.0cA	13.7dA	34.2aA	24.2bA
23	0.70cA	0.58cA	2.16aC	1.34bC	6.61aB	3.90bB	6.78aB	7.20aB	16.3cC	12.3dB	33.2aA	18.5bB
30	0.57cA	0.45cA	12.5aB	5.94bB	6.97aB	3.15bB	7.21aB	7.06aB	16.6bAB	6.8 dC	32.9aA	14.9cC
37	0.31cA	0.63cA	22.0aA	12.0bA	7.33aAB	3.12bAB	7.44aAB	6.88aAB	17.0bAB	6.3dC	33.4aA	12.7cC

[a]Values are means (n = 3). Means followed by the same capital letter in the columns and by the same small letter in the rows did not share significant differences at 5% probability by Tukey's test

Anthocyanins are phenolic compounds responsible for fruit colour (Abyari *et al*., 2006). They accumulate in jabuticaba's skin (22.0 mg g^{-1}), whereas the fruit's pulp is whitish (0.63 mg g^{-1}). Their steep rise of about fiftyfold in the skins (0.43-22.0 mg g^{-1}) between 16 and 37 days is due to the production of cyanidin-3-glucose (Reynertson *et al*., 2008) and petunidin-3-glucose (Montes *et al*., 2005). A previous study on *M. jaboticaba* showed a similar increase of about 48-fold in anthocyanidin contents from green to ripe fruit (Magalhães *et al*., 1996). Other fruits also reveal the same drastic increase in pigment levels during ripening, such as myrtle, American cramberry and pomegranate arils (Fadda and Mulas, 2010; Vvedenskaya and Vorsa, 2004; Kulkarni and Aradhya, 2005).

Total anthocyanin values for ripe jabuticabas (5.94 and 12.0 mg g^{-1}) were higher than those for *M. cauliflora* fruits grown in the USA (2.78 mg g^{-1}) (Reynertson *et al*., 2008). Climate parameters such as temperature could be responsible for this difference. Anthocyanin accumulation is influenced by air temperature, particularly by temperature variation between day and night (Treutter, 2010). As has been reported for strawberries, hot days and cool nights increase the production of pelargonidin and cyanidin glycosides (Wang and Zheng, 2001).

Anthocyanin levels in ripe jabuticabas are of crucial importance for wine production, since higher quantities of anthocyanins extracted from fruits will enhance the colour and antioxidant properties of the wine (Tuberoso *et al*., 2007). Anthocyanin concentration may be yet another parameter for maturity index, as its level grows until full ripeness.

Total tannin (condensed and hydrolysable) accumulated mainly in seeds and skins, where they were about twofold higher than in pulps at all stages (Table 2). During ripening, tannin contents had a significant variation in all parts and the whole fruit only from green to turning purple stages (seed 7.75-6.61, pulp 4.02-3.90, skin 7.27-6.78 and fruit 8.16-7.20 mg g^{-1}); in the following stages

its levels remain constant. This tendency is opposite from skin and seed grape tannins that generally decline during all stages of maturation (Kennedy *et al*., 2000; Obreque-Slier *et al*., 2010). A continuous reduction in the tannin contents is also usual for other fruits, such as myrtle and persimmon (Fadda and Mulas, 2010; Del Bubba *et al*., 2009).

For the production of red wine, in which seeds and skins remain in contact with must, it is necessary to become aware of the adequate amounts of tannins in seeds and skins to avoid deficiency or excess of these compounds (Busse-Valverde *et al*., 2010; Mercurio *et al*., 2010).

Total phenol (phenolic acids, depsides, flavonoids, anthocyanins and tannins) levels of seeds, pulps and the whole fruit changed significantly from 16 to 23 DAA (seed 18.0-16.3, pulp 13.7-12.3 and fruit 24.2-18.5 mg g^{-1}) and from 23 to 30 DAA (seed 16.3-16.6, pulp 12.3-6.8 and fruit 18.5-14.9 mg g^{-1}). The phenols concentrations were significant different comparing seed, pulp, skin and the whole jabuticaba fruit in all stages (see rows in Table 2); and they were about twofold and fivefold higher in the skins (32.9 mg g^{-1}) when compared to seeds (16.6 mg g^{-1}) and pulp (6.8 mg g^{-1}) in a full ripe fruit, respectively. The opposite trend was observed for Carménère and Cabernet Sauvignon grapes which total phenolics contents were about fifteenfold higher in seeds compared to skins (Obreque-Slier *et al*., 2010).

During fruit maturation, total phenol amounts in skins did not alter significantly (Table 2), whereas pulps reduced 7.4 mg g^{-1} from green to ripe stages and the whole fruit had 11.5 mg g^{-1} less phenols in the full ripe stage. This tendency to total phenol reduction during maturation has also been observed in other fruits, such as myrtle, American cranberry and apple (Fadda and Mulas, 2010; Vvedenskaya and Vorsa, 2004; Renard *et al*., 2007).

Essential oils: Essential oils were analysed in three stages: green (16 DAA), turning purple (23 DAA) and ripe (30 DAA). A total of 27 compounds were identified, accounting for 96.0-97.3% of volatile constituents, with average yields of 0.0009, 0.0020 and 0.0028 wt% (Table 3). Essential oils from the three maturation stages were composed mainly of cyclic sesquiterpenes (average of 88.1%); the same tendency was observed in the leaf oil from *M. cauliflora* (Duarte *et al*., 2010a) and in other species from *Myrcia*, *Eugenia* and *Psidium* genera of the Myrtaceae family (Stefanello *et al*., 2011). Major jabuticaba components were γ-eudesmol (33.9%) and α-eudesmol (15.5%) which is an unusual essential oil composition for Myrtaceae, as (E)-caryophyllene is generally the predominant sesquiterpene (Stefanello *et al*., 2011). The leaf oil is also different from the fruit, as germacrene D (24.1%) and β-eudesmol (16.5%) are the major compounds (Duarte *et al*., 2010a). Among the monoterpenes, limonene, (Z)-, (E)-β-ocimene and α-terpineol were the main compounds in all three stages; usually they provide citric, green and flowery aromas (Bicas *et al*., 2011).

During jabuticaba's maturation stage, the monoterpene and sesquiterpene groups (hydrocarbons and oxygenated) did not altered significantly. Only minor compounds had a significant variation during fruit ripening, such as the most odoriferous monoterpene alcohols α-terpineol and linalool that varied from 0.37 to 1.79% and from 0.01 to 1.01%, respectively (Table 3). These monoterpenols are important aromatic components of muscat grape varieties (Oliveira *et al*., 2004). The lack of variation in terpenoids groups differs from results reported for mango var. "Kensington Pride" and *Vitis vinifera* L. cv. Baga (Lalel *et al*., 2003; Coelho *et al*., 2006), in which sesquiterpenes increased sharply in ripe fruits. Sesquiterpenoids were detected in several *V. vinifera* varieties; they are related to spicy and wood aromas, which may contribute positively to the quality of wine aromas (Coelho *et al*., 2006; Jiang *et al*., 2007).

Table 3: Average percentages[a] of essential oil constituents of jabuticaba fruits during development

		Days after anthesis (DAA)		
Constituent	RI	16	23	30
α-pinene	933	0.01b	0.40a	0.54a
β-pinene	976	0.01b	0.41a	0.74a
β-myrcene	990	0.25a	0.01b	0.01b
Limonene	1028	1.98a	2.03a	2.63a
(Z)-β-ocimene	1035	0.83a	0.88a	1.11a
(E)-β-ocimene	1046	1.07a	1.45ab	2.29b
Linalool	1100	0.01b	0.01b	1.01a
α-terpineol	1191	0.37b	1.19a	1.79a
δ-elemene	1338	0.01a	0.71a	0.38a
α-copaene	1377	0.80a	1.25a	1.03a
(E)-caryophyllene	1421	4.37a	6.09a	5.73a
α-humuleno	1455	0.39a	1.04a	1.07a
Amorpha-4,7(11)-diene	1477	0.57a	0.66a	0.01b
Germacrene D	1483	6.93a	6.06a	6.02a
δ-selinene	1492	1.84a	0.83a	0.85a
Bicyclogermacrene	1498	3.68a	3.62a	3.98a
δ-amorphene	1503	0.67a	0.48a	0.01b
δ-cadinene	1525	3.98a	3.39ab	2.34b
α-cadinene	1541	0.37a	0.41a	0.01b
Elemol	1552	0.01b	0.01b	1.23a
Globulol	1586	1.22a	2.13a	1.61a
5-epi-7-epi-α-eudesmol	1604	1.16a	1.19a	0.44a
10-epi-γ-eudesmol	1622	3.14a	2.68a	1.59a
γ-eudesmol	1637	34.4a	32.5a	34.7a
Cubenol	1645	3.46a	2.63a	1.13a
β-eudesmol	1654	7.80a	5.22a	10.1a
α-eudesmol	1658	16.8a	14.6a	15.1a
Monoterpene hydrocarbons		4.13a	5.16a	7.28a
Oxygenated monoterpenes		0.38a	1.20a	2.80a
Sesquiterpene hydrocarbons		23.6a	24.5a	21.4a
Oxygenated sesquiterpenes		67.9a	61.0a	65.8a
Total identified		96.0a	91.9a	97.3a
Yield (%, w/w)		0.0009a	0.0020a	0.0028a

RI: Retention Index. [a]Values are means (n = 3). Means followed by the same small letter in the rows did not share significant differences at 5% probability by Tukey's test

CONCLUSIONS

Jabuticaba fruits showed some chemical changes during development and ripening. The use of sugar/acid ratio and anthocyanin concentration seemed to be the most effective parameters to reveal the optimal harvest period for winemaking. Tannins and total phenols were not useful as parameters for a maturity index, since their levels failed to show significant differences between ripe and full ripe stages. Anthocyanin levels and sugar/acid ratio reached their maximum in the full ripe stage which suggests that harvesting for wine production should occur after 35 DAA. In addition, jabuticaba volatile compounds, mainly sesquiterpene γ-, α- and β-eudesmols, may add an exotic feature to the wine aroma.

ACKNOWLEDGMENTS

The authors are indebted to CNPq, PADCT III and FUNAPE/UFG for their financial support, as well as to CAPES for granting fellowships to G.A.C.F. and A.R.D. We also thank Dr. Paulo Antônio Silva from Jabuticabal Winery for providing valuable plant information and for helping with fruit collection.

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