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Phenolic composition of *Flacourtia indica*, *Opuntia megacantha* and *Sclerocarya birrea*

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Abstract

The phenolic acid composition of the peel and pulp of the fruits of *Flacourtia indica* (Burm. f.) Merr., *Opuntia megacantha* (L.) Mill and *Sclerocarya birrea* (A. Rich.) Hochst., from Zimbabwe, were analysed using traditional colorimetric as well as HPLC methods. The total phenolics, flavanoids and condensed tannin levels varied with species. *Sclerocarya birrea* pulp had the highest total phenolics, flavanoids and condensed tannins, i.e., 2262 μg GAE/g, 202 μg catechin/g and 6.0% condensed tannins, respectively. *Flacourtia indica* pulp contained the least total phenolics, flavanoids and condensed tannins 334 μg GAE/g, 41 μg catechin/g and 1.4%, respectively. There were no significant differences in the total phenolics between the peels and the pulps of the individual fruits. However, significant differences were noted in the flavanoids and the condensed tannins between the peels and pulps of the fruits assayed. Ferulic acid, caffeic acid and vanillic acid were the dominant phenolic acids in the three fruits. There were differences between the phenolic acids in the peels and the pulps of the fruits.

Keywords: Phenolic compounds; Phenolic acids; Flavanoids; Wild fruits

1. Introduction

Besides many essential nutritional components, plants contain phenolic compounds, a large group of biologically active non-nutrients (Shahidi & Naczk, 1995). Plant phenolic compounds are hydroxylated derivatives of benzoic acid and cinnamic acids. Flavanoids, the largest group of phenolic compounds, include flavonols, flavones, catechins, proanthocyanidins, anthocyanidins and isoflavonoids. Phenolic compounds are important in plant defence mechanisms against invading bacteria and other types of environmental stress, such as wounding and excessive light or UV radiation (Harborne, 1994; Herrmann, 1989; Wallace & Fry, 1994).

Phenolic compounds are of possible pharmacological value and have been reported to have antioxidative and anticarcinogenic effects. Flavonoids have long been recognised to possess antiinflammatory, antiallergenic, antiviral and antiproliferative activities (Frankel, Kanner, German, Parks, & Kinsella, 1993; Harborne, 1994; Kuda, Tsunekawa, Goto, & Araki, 2005; Sharma, Stutzman, Kellof, & Steele, 1994).

Flacourtia indica is the *munhunguru* or Batoka plum and produces fruit that is eaten fresh and has a pleasant but rather sour taste. The fruits make a good jelly with the seeds and skin being discarded (Tredgold, 1986). *Opuntia megacantha* is the *mudorofia* or pricky pear and produces pear-shaped golden green or purple sweet and juicy fruits within a tough skin that is studded with spines (Tredgold, 1986). *Sclerocarya birrea* is the *marula* or *mupfura* tree and is a deciduous tree that produces abundant yellowish green and plum shaped fruit, that contain sour sweet pulp covered by a tough skin. The white pulp surrounds a single oil rich seed (Tredgold, 1986).

There are no reports on the phenolic acids of *F. indica*, *O. megacantha* and *S. birrea* yet they could be used as

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cheap sources of essential phenolic compounds that could be used as antioxidants. The aim of our study was to determine the amount of phenolic compounds, including tannins and flavanoids, and to study the HPLC profiles of the three wild fruits, *F. indica*, *O. megacantha* and *S. birrea* which are found in many parts of Zimbabwe and are sold at market places and roadsides in urban areas around Zimbabwe.

2. Materials and methods

2.1. Reagents

The chemical standards used were all of analytical grade. Folin–Ciocalteu, rhodanine, tannic acid, gallic acid, catechin, vanillic acid, caffeic acid, *p*-coumaric acid, protocatechuic acid, ferulic acid, *p*-hydroxybenzoic acid, *p*-hydroxybenzaldehyde were obtained from Sigma–Aldrich Chemie (Steinheim, Germany). Sodium carbonate, methanol (HPLC grade), ascorbic acid, L-butanol, HCl, ethyl acetate, diethyl ether, anhydrous Na_2SO_4, acetonitrile (super purity solvent), acetic acid and ferric ammonium sulfate were obtained locally.

Standard catechin (4 mg/ml distilled water), methanol:HCl (5:1 v/v), vanillin reagent (1 g/100 ml distilled water), Folin–Ciocalteu reagent (1 N), 20% sodium carbonate, standard tannic acid (0.5 mg/ml), 50% methanol in distilled water (1:1 v/v), butanol:HCl reagent (95:5 v/v) and ferric reagent (2% ferric ammonium sulfate in 2 M HCl) were used for analysis.

2.2. Procurement of samples

Fresh ripe samples of *F. indica*, *O. megacantha* and *S. birrea* fruits were bought at random from areas around Harare during the November 2004 rainy season when they were in abundance.

2.3. Preparation of fruit samples

Upon arrival at the laboratory, the fruits were cleaned with running water and manually separated into a peel fraction and pulp fraction.

2.4. Extraction of phenolic compounds

Total phenolic compounds were extracted from the peel and the pulp as described by Makkar (1999). The peel or pulp sample (2 g) was extracted twice with cold 50% aqueous methanol (10 ml). The two volumes were combined, made up to 20 ml, centrifuged at 3000 rpm for 10 min and transferred into small sample bottles for analysis.

2.5. Extraction of phenolic acids for HPLC analysis

Phenolic acids were extracted following the method described by Muchuweti, Ndhlala, and Kasiyamhuru (2006). Fresh samples (5 g) of the fruit portions were extracted twice with ethyl acetate (10 ml) and the organic fractions combined. After 30 min of drying with anhydrous Na_2SO_4, the extract was evaporated to dryness under vacuum. The residue was dissolved in methanol/water (2 ml; 1:1 v/v) before analysis by HPLC.

2.6. Folin C assay for total phenolics

Total phenolic compounds were determined following the method of Makkar (1999). To a sample (50 μl), distilled water was added to make up to 1 ml, followed by 1 N Folin C reagent (500 μl) and sodium carbonate (2.5 ml). After 40 min at room temperature, absorbance at 725 nm was read on a Spectronic 20® Genesys™ spectrophotometer (Thermo Electron Corporation, Waltham, MA, USA) against a blank that contained methanol instead of sample. Total phenolics were expressed in terms of equivalent amounts of gallic acid (GAE).

2.7. Vanillin assay for flavanoids

The sample (5 μl) was made up to 1 ml with distilled water in a test tube before adding methanol–HCl (2.5 ml) and finally vanillin reagent (2.5 ml). After 20 min absorbance at 500 nm was read using a Spectronic 20® Genesys™ spectrophotometer against a blank that contained methanol in place of sample. The flavanols were expressed as catechin equivalents, following Makkar (1999).

2.8. The butanol–HCl assay for condensed tannins

To the sample (500 μl), butanol–HCl (3 ml) reagent was added, followed by ferric reagent (100 μl). After mixing, the tubes were placed in a boiling water bath. After 60 min, absorbance at 550 nm was measured against a blank prepared by mixing tannin-containing extract (500 μl) with butanol reagent (3 ml) and ferric reagent (100 μl), but without heating. Condensed tannin (% in dry matter) as leucocyanidin equivalent was calculated by the formula:

$$(A_{550\ nm} \times 78.26 \times \text{dilution factor})/(\%\ \text{dry matter}),$$

as described by Porter, Hrstich, and Chan (1986).

2.9. HPLC analysis for phenolic acids

A Shimadzu HPLC system (Shimadzu, Tokyo) with a SCL-6B Shimadzu system controller, C-R AX Shimadzu Chromatopac, Shimadzu SPD-10 AV UV–Vis detector, equipped with a Dynamax 60A C18 column, was used for analysis of phenolic compounds. Five microlitres of sample were injected and the flow rate was set at 1 ml/min. All samples in duplicate were filtered through a 0.22 μm filter unit (Millex® – GV, Molsheim, France) before injection and the solvents were filtered through a 0.45 μm filter (Whatman, Maidstone, England). Two mobile phases, *A*, which contained water:acetic acid (98:2

v/v), and *B*, which contained water:acetonitrile:acetic acid (78:20:2 v/v/v), were used. The gradient profile was 0% solvent *B* at the start, rising to 80% within 55 min, remaining at 80% up to 70 min and falling back to 0% at time 80 min. Detection was carried out by measuring absorbance at 280 nm according to Pena-Neira, Strella, Garcia- Vallejo, Hernandez, and Suarez (2000). After each run, the system was reconditioned for 15 min before analysis of the next sample.

3. Results and discussion

As shown in Fig. 1, the total phenolic content of *S. birrea* was eight times higher than that of *F. indica* and *O. megacantha*, which contained similar amounts. Except for *O. megacantha*, there were no significant differences in the amount of phenolic compounds in the peels and the pulps. The level of total phenolic compounds in *S. birrea* fruit is similar to that reported for *Uapaca kirkiana* (Muchuweti et al., 2006).

In Fig. 2, it can be seen that there were more flavanoids in *S. birrea* fruit than in *F. indica* or *O. megacantha* fruit. In *S. birrea* the pulp contained twice as much flavanoids than the peel whereas in the other two fruits, the peels contained more flavanoids. In all cases, the content of flavonoids in the pulp was significantly different from that in the peels.

The distribution of phenolic compounds in fruit is important when optimising the yield of phenolic compounds in processed fruit and vegetables (Hirota, Shimoda, & Takahama, 1998). In *S. birrea*, the pulp is the most important source of flavanoids whereas in the other fruits the peel would be an important source. Fruit that have been reported to contain more flavonoids in the peel than the pulp are apple, peach and grape (Price, Prosser, Richetin, & Rhodes, 1999).

As shown in Fig. 3, *F. indica* and *O. megacantha* contained lower levels of tannins than *S. birrea* where there were slightly more tannins in the pulp than the peel.

The results of the HPLC analysis of extracts from pulp and peels of the tree fruits are shown in Figs. 4–9. A substantial proportion of the phenolic acids eluted whilst the solvent was predominantly aqueous. A small number of peaks were observed in the organic phase of the gradient (after 55 min). In all cases, there were numerous peaks that were not identified because of lack of suitable standards.

Caffeic acid, ferulic acid and *p*-coumaric acid were found in extracts of the peel and pulp of *F. indica*. As can be seen in Figs. 4 and 5, the peel contained *p*-hydroxybenzaldehyde, which was not in the pulp, while the pulp contained vanillic acid, which was not in the peel. Three intense peaks that eluted around 60 min from the pulp extracts were not detected in the peel.

Caffeic acid, protocatechuic acid, *p*-hydroxybenzaldehyde, ferulic acid and *p*-coumaric acid were common to the peel and pulp of *O. megacantha* fruit, as shown in Figs. 6 and 7. However, the peel contained *p*-hydroxybenzoic acid, which was not detected in the pulp, whilst the pulp contained vanillic acid, which was not detected in the peel. The profiles of the peel and pulp of *O. megacantha* showed a hump characteristic of polymeric phenolic compounds, as the mobile phase increased towards 80% acetonitrile.

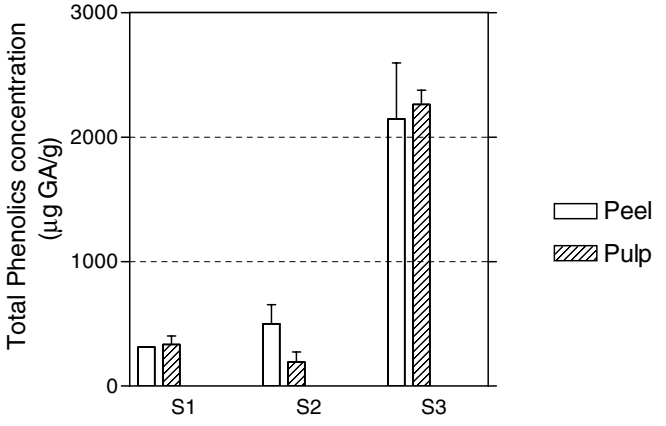

Fig. 1. Total phenolic compounds in *F. indica* (S1), *O. megacantha* (S2) and *S. birrea* (S3) fruits. Number of replicates per sample = 3.

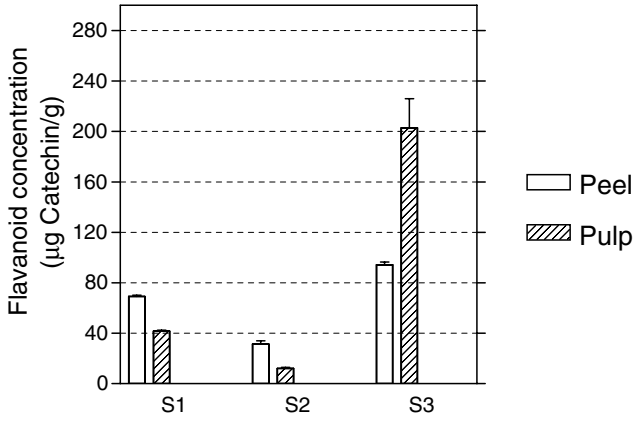

Fig. 2. Flavanoid concentration in *F. indica* (S1), *O. megacantha* (S2) and *S. birrea* (S3) fruits. Number of replicates per sample = 3.

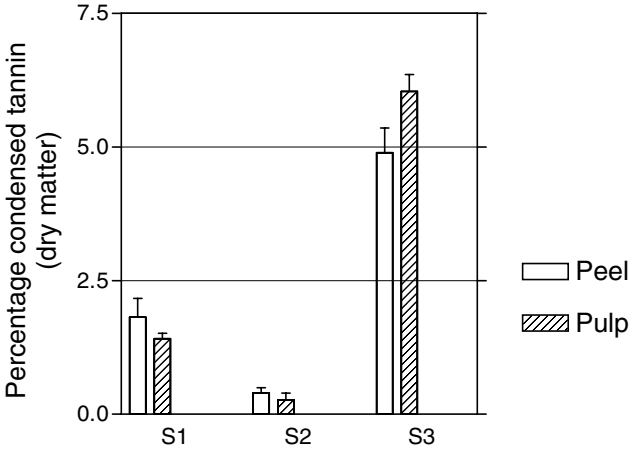

Fig. 3. Percentage condensed tannins as leucocyanidin equivalents in *F. indica* (S1), *O. megacantha* (S2) and *S. birrea* (S3) fruits. Number of replicates per sample = 3.

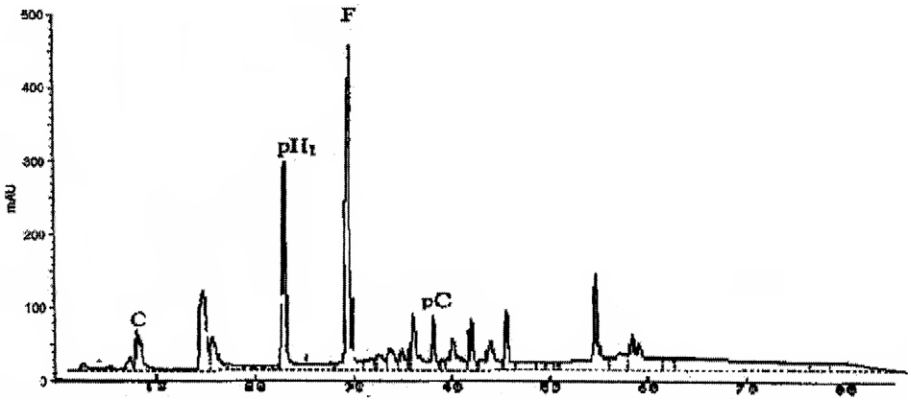

Fig. 4. HPLC chromatogram of methanol extract of *F. indica* peel. The peaks were identified as caffeic acid (C), *p*-hydroxybenzaldehyde (pH_1), ferulic acid (F) and *p*-coumaric acid (pC).

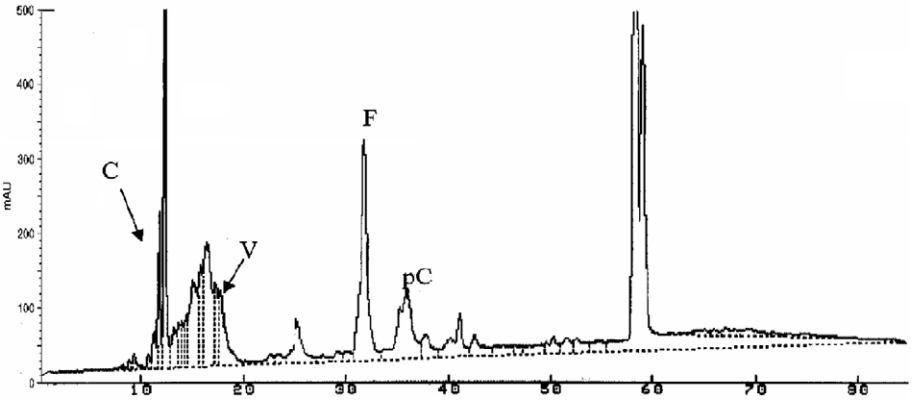

Fig. 5. HPLC chromatogram of methanol extract of *F. indica* pulp. The peaks were identified as caffeic acid (C), vanillic acid (V), ferulic acid (F) and *p*-coumaric acid (pC).

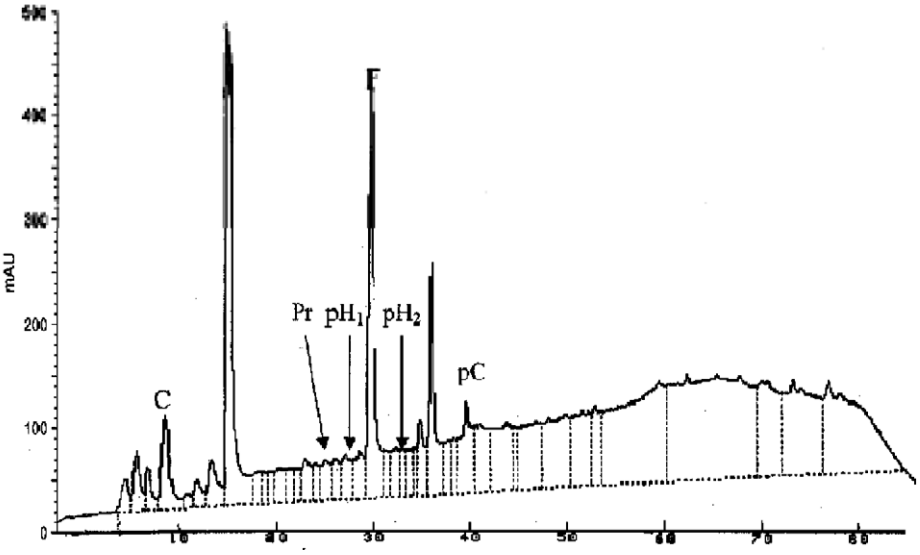

Fig. 6. HPLC chromatogram of methanol extract of *O. megacantha* peel. The peaks were identified as caffeic acid (C), protocatechuic acid (Pr), *p*-hydroxybenzaldehyde (pH_1), ferulic acid (F), *p*-hydroxybenzoic acid (pH_2) and *p*-coumaric acid (pC).

Caffeic acid, vanillic acid, *p*-hydroxybenzaldehyde, ferulic acid, *p*-hydroxybenzoic acid and *p*-coumaric acid were identified in the peel (Fig. 8) and caffeic acid, ferulic acid and *p*-coumaric acid in the pulp of *S. birrea* (Fig. 9).

The values of phenolic compounds that are presented in this report are for fruit as found in the local market. Variations in the phenolic acid content would be expected to depend on the nutritional status of the plant

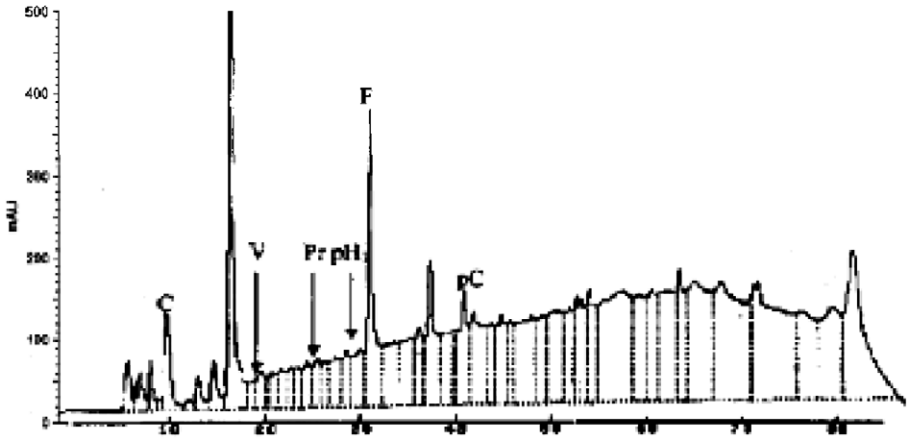

Fig. 7. HPLC chromatogram of methanol extract of *O. megacantha pulp. The peaks were identified as caffeic acid (C), vanillic acid (V), protocatechuic acid (Pr), p-hydroxybenzaldehyde (pH_1), ferulic acid (F) and p-coumaric acid (pC).*

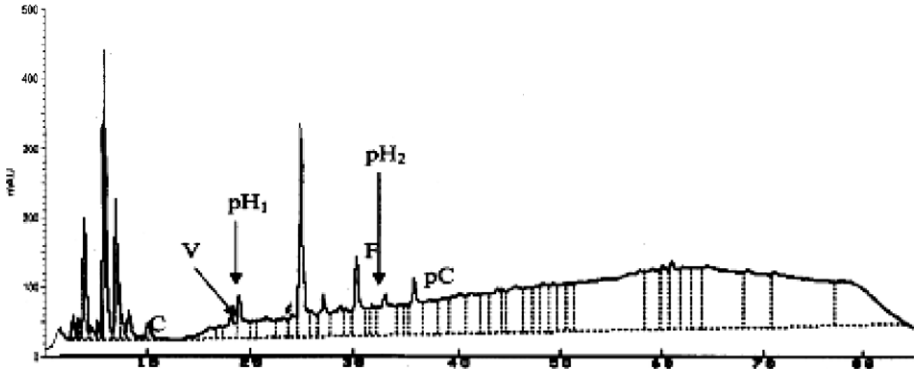

Fig. 8. HPLC chromatogram of methanol extract of *S. birrea* peel. The peaks were identified as caffeic acid (C), vanillic acid (V), *p*-hydroxybenzaldehyde (pH_1), ferulic acid (F), *p*-hydroxybenzoic acid (pH_2) and *p*-coumaric acid (pC).

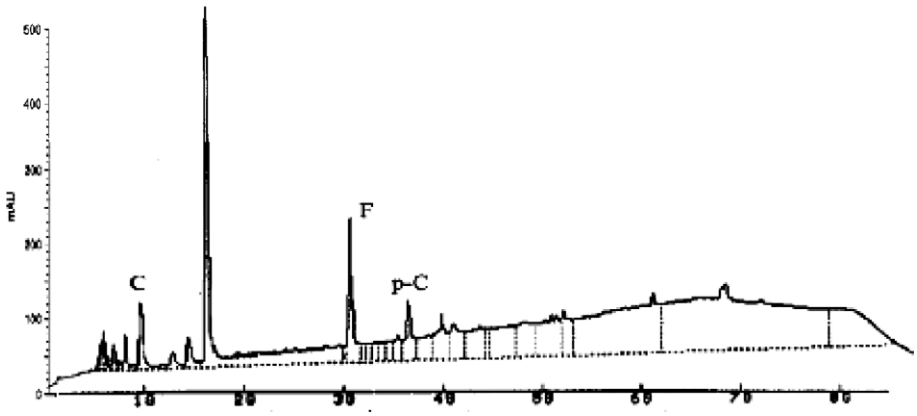

Fig. 9. HPLC chromatogram of methanol extract of *S. birrea* pulp. The peaks were identified as caffeic acid (C), ferulic acid (F) and *p*-coumaric acid (pC).

and environmental conditions. Whereas there are reports of phenolic compounds being unevenly distributed between peels and pulps (Kim, Tsao, Yang, & Cui, 2006; Price et al., 1999), most of the phenolic compounds in *F. indica*, *O. megacantha* and *S. birrea* were, more or less, evenly distributed between the peel and pulp. The exception was the flavanoids of *S. birrea*, which were predominantly in the pulp.

4. Conclusion

The phenolic content and profiles of the phenolic compounds of *F. indica*, *O. megacantha* and *S. birrea* were successfully obtained. The presence of significant amounts of phenolic compounds in the fruits could result in the fruits being used as sources of cheap natural antioxidants and radical scavengers.

Acknowledgement

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