Characteristics and composition of hackberries (*Celtis australis* L.) from Mediterranean forests

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**ABSTRACT**

The characteristics and composition of hackberries (*Celtis australis* L.) from Mediterranean forests were established and compared to other fruits. Fresh hackberries were fractionated in peel (19.8%), flesh (49.7%) and stone (29.4%). A very high value of soluble solids (53.6 °Brix) was found in the flesh. Chromatographic analysis indicated that the flesh soluble solids were mainly sucrose (12.8%), glucose (17.5%) and fructose (21.8%). An average antioxidant activity (FRAP) of 4000 μmol eq. Fe²⁺/100 g and a DPPH scavenging capacity (IC₅₀) of 7 were found. The antioxidant properties are due to the content of phenolic compounds and flavonoids, 249 and 28 mg/100 g, respectively, in the flesh. The dietary fibre in the flesh was 18 g/100 g. The hackberries flesh cell wall is constituted by pectins (55.7%) and hemicelluloses (44.4%). According to these results, hackberry would be considered of great interest for its applications as sweeting agent with antioxidant, thickener and dietary properties, in the food industry.

**Keywords:** *Celtis australis*; Characteristics; Composition; Hackberries; Pectin

**INTRODUCTION**

Hackberry is the fruit from a tree growing mainly in the forests of the Mediterranean basin (South Europe, North Africa, Minor Asia) with scientific name *Celtis australis* L. (Magni and Caudullo, 2016). That growing in North America is *Celtis occidentalis* L. (Demir et al., 2002). Several parts of *Celtis* sp. (fruits, leaves, etc.) have bioactive compounds against human diseases (Demir et al., 2002; Magni and Caudullo, 2016; Ota et al., 2017). Concerning food uses, at maturity, the dark purple fruits are small, with a hard peel and a dark yellow and very sweet flesh. The hackberry is constituted by the peel, the edible flesh and the stone, with a seed inside (El-Alfy et al., 2011a). Hackberries may be a useful ingredient in natural products manufacture due to its high content of sugars, fibre and its antioxidant capacity, due to the phenolic compounds content (Ota et al., 2017), including flavonoids. This would be considered of great interest for its applications as sweeting agent with antioxidant properties in the food industry.

The literature on the characteristics and composition of the fruits of *Celtis australis* L. is scarce (Demir et al., 2002; Ota et al., 2017). The article of Demir et al. (2002) reported on the content in ash, oil, fibre, proteins and minerals, while the article of Ota et al. (2017) reported on the content in water, fibre, protein, vitamins, minerals, and phenolic compounds. Other articles deal on the *Celtis*-leaves composition (Spitaler et al., 2009; Zehrmann et al., 2010; El-Alfy et al., 2011b; Sommavilla et al., 2012).

The aim of this research is to analyse the physical (hackberries fractionation), physicochemical (moisture content, water activity, pH, soluble solids) and nutritional characteristics (vitamin C and E, citric acid, sucrose, glucose and fructose, total phenolic compounds, total flavonoids, antioxidant activity, free radical scavenging capacity, soluble, insoluble and dietary fibre) of the Mediterranean hackberry (*Celtis australis* L.), focussing also in some traits (hackberries flesh cell walls, their fractions and their sugar composition), which has been no previously reported, as a basis for new industrial food applications development.
MATERIALS AND METHODS

Plant material
The Mediterranean hackberry fruits (Fig. 1) were harvested in October 2017, being pick up from wild trees growing in the forests of Moratalla (38° 11’ N, 1° 53’ W, altitude: 700-800 m), Spain. There were no cultivation practices. The hackberry trees grow in the forest. Consequently, they receive rainwater, when it was available, and were nourished by the soil, without irrigation and without fertilizer. The hackberries were sampled at the same developmental stage, when the fruit peel had turned dark purple, indicating maturity. The collection was made manually. Two people with baskets reach for the branches of the trees containing the hackberry and pick them up and place them in the basket. When the basket is full, it is emptied into a 20 kg box, collecting about 200 kg. Finally, all the boxes were put together and mixed.

Hackberries fractionation
Hackberries (aprox. 75 g, 100 fruits) were manually fractionated with a scalpel into peel, flesh (Fig. 2) and the stone, in triplicate. The weight of each fraction was measured. Results are expressed as % (w/w).

Moisture content
The moisture content was determined by dehydration in an oven according to Nielsen (2010) and procedural details according to Vidal Cascales and Ros García (2020).

Water activity
The water activity was measured in a Novasina water activity meter according to Kitic et al. (1986) and procedural details according to Vidal Cascales and Ros García (2020).

pH and soluble solids
To determine the pH and the soluble solids of the hackberries peel and flesh, 5 g of sample plus 15 ml of water were crushed with a Moulinex blender to obtain like a juice. The pH of the juice was measured with a pHmeter according to Ros et al. (2004). The soluble solids (“Brix) of the juice were measured with an Atago N-1E refractometer according to Ros et al. (2004).

Vitamin C
Vitamin C was determined by the method of AOAC (2000) 967.21, titration with 2,6-dichlorophenolindophenol and procedural details according to Vidal Cascales and Ros García (2020).

Previous extraction to HPLC analysis
Hackberry matter (1 g of peel or flesh) was ground with 5 mL of the corresponding HPLC eluent as extractant, using an Ultraturrax type blender. The solution was centrifuged at 16500 x g for 10 min. The supernatant was filtered through 0.22 µm prior to HPLC analysis (González Hidalgo et al., 2019).

Vitamin E
Vitamin E was determined by HPLC (González-Hidalgo et al., 2012) using a Waters Resolve Spherical Silica column.

Citric acid
Citric acid was determined by HPLC (Hellín et al., 2001) using an ORH-801 column from Interaction.

Sucrose, glucose and fructose
Sucrose, glucose and fructose were analysed by HPLC (Hellín et al., 2001) using a CHO-682 column from Interaction.

Total phenolic compounds
The total phenolic compounds content was determined using the Folin-Gioacalteu reagent (Zhou and Yu, 2004) and procedural details according to Vidal Cascales and Ros García (2020).

Total flavonoids
The total flavonoid content was determined according to Chang et al. (2002) and Lin and Tang (2007) and procedural details according to Vidal Cascales and Ros García (2020).
Antioxidant activity (FRAP)
The antioxidant activity was determined by the method described by Benzie and Strain (1996) with changes (González-Hidalgo et al., 2019) and procedural details according to Vidal Cascales and Ros García (2020).

Free radical scavenging capacity (DPPH)
The determination of the free radical scavenging capacity was carried out as described by Vega-Gálvez et al. (2009) with changes (González-Hidalgo et al., 2019) and procedural details according to Vidal Cascales and Ros García (2020).

Soluble, insoluble and dietary fibre
The insoluble dietary fibre, soluble dietary fibre, and total dietary fibre content was determined according to Prosky et al. (1988).

Isolation of hackberries flesh cell wall
The isolation of the hackberries flesh cell wall was carried-out following the procedure of de Vries et al. (1981), as described by Apolinar-Valiente et al. (2010). Briefly, the hackberry flesh was suspended in boiling water and then homogenized. The homogenized material was mixed with two parts of 96% ethanol and extracted for 30 min at 40°C. The raw alcohol insoluble solids were separated by filtration and extracted again with fresh 70% ethanol. The extractive and washing treatment with fresh 70% ethanol was repeated several times until no sugars were found in the 70% ethanol phase. Then, the alcohol insoluble solids (AIS) were washed twice with 96% ethanol and once with acetone, and finally dried overnight under an air stream (r.t.).

Fractionation of hackberries flesh cell wall
The fractionation of the hackberries flesh cell wall (AIS) into pectins and hemicelluloses was carried-out by sequential extraction (Coll-Almela et al., 2015) using a 50 mM EDTA/50 mM ammonium oxalate solution in a 50 mM sodium acetate buffer pH 5.2, a 50 mM NaOH, a 1 M NaOH and a 4 M NaOH solutions. Briefly, the fractions obtained were neutralised by pH adjusted to 5.0 (when necessary), dialysed (5000 mwco) against pure water with daily water changes and lyophilised to yield (i) pectin soluble in chelating agents (ChSS), (ii) pectin soluble in dilute alkali (DASS), (iii) hemicellulose soluble in one molar sodium hydroxide (1MASS), and (iv) hemicellulose soluble in four molar sodium hydroxide (4MASS). Results are expressed in g amounts from AIS and in % respect to AIS.

Sugar composition of hackberries flesh cell wall and polysaccharides fractions
Sugars were determined by GLC after pre-treatment (30°C, 1 h) withaq 72% sulphuric acid followed by hydrolysis with 1 M sulphuric acid (100°C, 3 h) and conversion of the products into alditol acetates (Ros et al., 1996). Uronic acids were determined in the sulphuric acid hydrolysate by the colorimetric 3,5-dimethylphenol assay (Scott, 1979).

Statistical data treatment
All extractions and analyses were made in triplicate. The values shown in the tables correspond to the average value of the analysis of each parameter in the different samples. The average values and coefficients of variation (CV, %) were calculated with the Excel (Microsoft Office 2019) software.

RESULTS AND DISCUSSION

Physical characteristics of the hackberries
Table 1 provides the physical characteristics of the hackberries (average moisture content of 42.7% from Tables 2 and 3). We found a higher equatorial diameter (11.1 mm) to that (9.1-9.5 mm) reported by Demir et al. (2002) in hackberries from Turkey, with moistures between 33 and 50%. As a consequence, the weight of 100 fruits (50 g) reported by Demir et al. (2002) is lower than the 74 g that we found (Table 1). The same occurs with the volume, 0.5 cm$^3$ (Demir et al., 2002) and 0.7 cm$^3$ (Table 1). Boudraa et al. (2010) measured the longitude of fresh hackberries from Algeria and found 9.8 mm, with a moisture content of 31%, and 0.39 g as the weight of a fruit, which is lower than the 0.77 g (Table 1) that we found in hackberries from Spain.

| Table 1: Physical characteristics of the hackberries |
|-----------------|-------|
| Hackberries     | Value* |
| Fruit weight (g)| 0.77  |
| Weight of 100 fruits (g)| 74.2  |
| Number of fruit units in 100 g| 132   |
| Equatorial diameter (mm) | 11.1  |
| Spheric volume (cm$^3$) | 0.71  |

*For all values CV <5%. Each value is the average of n≥100 fruits

| Table 2: Hackberries fractionation |
|-----------------|--------|
| Fraction            | Amount (%)* |
| Peel              | 19.8   |
| Flesh             | 49.7   |
| Stone             | 29.4   |

*For all values CV <5%. Each value is the average of n=3 x 100 fruits

| Table 3: Physicochemical characteristics of the peel and the flesh of the hackberries |
|-----------------|-------|-------|
| Fraction         | Peel* | Flesh* |
| Moisture (%)     | 39.7  | 43.9  |
| Water activity   | 0.80  | 0.80  |
| pH               | 6.6   | 6.4   |
| Soluble solids (°Brix) | 50.2  | 53.6  |

*For all values CV <5%. Each value is the average of n=6 samples
Fractionation of the hackberries in peel, flesh and stone

Fresh hackberries were manually fractionated in clean peel, edible pulp (flesh) and the stone (Table 2). To the best of our knowledge, this fractionation has not been reported before. The pulp fraction represents the 49.7% of the fresh weight of the fruit. The fact that the pulp content in mature hackberries account near the 50% of the fruit fresh weight is a good result as a basis for applications development as food, including industrial uses. The stone is the 29.4% and the peel the 19.8%. The percentage in which the stone is present also indicates the possibility of its exploitation, even at industrial level. Boudraa et al. (2010) reported that the Algerian hackberries pulp represents the 55.6% of the fruit fresh weight, quite similar to the Spanish (49.7%, Table 2).

Physicochemical characteristics of the peel and the flesh of hackberries

Table 3 provides the physicochemical characteristics of the hackberries peel and flesh. The moisture content of the flesh (43.9%) is a bit higher than the peel (39.7%). These data are of similar order of magnitude to the moisture content reported previously by Demir et al. (2002), who carried-out a dehydration of the Turkish hackberries (till 15.33 and 50% moisture content), by Boudraa et al. (2010), who measured the moisture content of fresh hackberries from Algeria (31%), and by Ota et al. (2017), who found in Croatian fresh hackberries a moisture content of 30%. Chokeberry (Aronia melanocarpa) has a moisture content of 73% (Nawirska-Olszańska et al., 2020), higher than in hackberry.

Concerning the soluble solids in the peel (50.2 °Brix) and in the flesh (53.6 °Brix), it is really a very high value. This result is in full agreement with the 53.4 °Brix reported by Ota et al. (2017). The content of soluble solids (°Brix) in common fruits (Food Data Central (FDC), 2020) is: strawberries (7.7), peach (9.5), apricot (11.1), orange (11.8), pineapple (13.1), apple (13.8), grapes (14.8), and pear (15.2), and in other berries (FDC, 2020) is: blackberries (9.6), raspberries (11.9), currants (13.8), and blueberries (14.5). The high value of soluble solids (sugars) in the hackberries is explained through the sugar analysis (see below).

It is not possible to compare the value of pH and water activity in hackberries peel and flesh, since no data are available in the scientific literature specifically on hackberries. The no acidic pH is in agreement with the amount of citric acid (see below). The water activity (0.80, Table 3), similar to the water activity of a berry fruit jam (Figuerola, 2007), agrees with the soluble solids (53.6 °Brix, Table 3).

Nutritional characteristics of the peel and the flesh of hackberries

Table 4 provides the nutritional characteristics of the hackberries peel and flesh. The content in vitamin C (3.7 and 2.2 mg/100 g, respectively) is of the same order of magnitude to that (3.9 mg/100 g) reported by Boudraa et al. (2010) in Algerian hackberries. Considering that the Daily Recommended Intake of vitamin C is between 70 and 90 mg for an adult, hackberries can no contribute significatively to this intake. The oranges (FDC, 2020) contain 53 mg/100 g, blueberries (10), blackberries (21), raspberries (26) and currants (41).

Hackberries have a low amount of vitamin E. The content in the flesh (1.0 mg/100 g, Table 4) is similar to that (0.56 mg/100 g) reported by Boudraa et al. (2010) in the edible part of Algerian hackberries. The peel of hackberries is very hard. It can no be eaten, although the content of vitamin E (13.0 mg/100 g) is higher than in the edible part (Table 4). Only the α-tocopherol isomer was found. The content of vitamin E in other berries (FDC, 2020) is up to 1.3 mg/100 g. Ota et al. (2017) found tocopherol in Croatian hackberries mesocarp, being α-tocopherol the highest (10.5 mg/100 g), while γ- and δ-tocopherol are in lower amounts (0.3 and 0.2 mg/100 g, respectively).

The content of citric acid in the edible part (0.52 g/100 g, Table 4), responsible of the pH value of 6.4 (Table 3), is similar to apple juice (0.2-0.7 g/100 g) and lower than orange (0.5-3.5 g/100 g) and lemon (5.0-9.0 g/100 g) juices (Ros et al., 2004).

The very high value of soluble solids (Table 3) for the fresh hackberries is explained by the results of the chromatographic analysis of sugars (Table 4). There is an unusually high content of sucrose (12.8 g/100 g), glucose (17.5 g/100 g) and fructose (21.8 g/100 g). These sugars
account a total of 52.1 g/100 g, which is very close to the 53.6 °Brix measured in the flesh (Table 3). The same occurs in the peel. To the best of our knowledge, this result on sugars composition has not been reported before. In peach, apple, watermelon and cherry fruits (Ma et al., 2014), the content of the sugars varied among fruits. Fructose (0.58-10.4 g/100 g) and glucose (0.93-9.96 g/100 g) are in the four fruits, while sucrose (1.58-10.6 g/100 g) was in peach, apple and watermelon. These sugars are presents in higher amounts in the Spanish hackberries (Table 4). chokeberry (Aronia melanocarpa) has a fructose content of 5.7 g/100 g and a glucose content of 7.1 g/100 g (Nawirska-Olszańska et al., 2020), which is lower than in hackberry.

The amount (249.1 mg eq. gallic acid/100 g, Table 4) found for the total phenolic compounds in the Spanish hackberries flesh is of the same order of magnitude to that (239.1 mg gallic acid/100 g) reported by Ota et al. (2017) in Croatian hackberries mesocarp, being reported that these phenolic compounds are gallic acid, 3,5-dihydroxybenzaldehyde, delphinidin-3,5-di-O-glucoside, cyanidin-3,5-di-O-glucoside and pelargonidin-3,5-di-O-glucoside (Ota et al., 2017). Dróżdż et al. (2018) report in lingonberry (Vaccinium vitis-idaea) a content of phenolic compounds between 470 and 660 mg eq. gallic acid/100 g, which is higher than in hackberry. The amount of total flavonoids (28.2 mg eq. quercetin per 100 g of flesh, Table 4) when compared to the content of total phenolic compounds in the flesh (249.1 mg eq. gallic acid/100 g, Table 4) was lower. Dróżdż et al. (2018) report in lingonberry (Vaccinium vitis-idaea) a content of flavonoids between 1 and 1.8 μg eq. catechin/100 g, which is lower than in hackberry. Spitaler et al. (2009) found the following flavonoids in Celtis australis leaves: acacetin 7-O-glucoside, apigenin 6-C-glucoside and acacetin 8-C-glucoside, and Zehrmann et al. (2010) found the following flavonoids also in Celtis australis leaves: isovitexin, cytisoside, 2’-α-L-rhamnopyranosyl-7-O-methylvitexin and 2’-α-L-rhamnopyranosyl-vitexin. Sommavilla et al. (2012) found in Celtis australis leaves phenolic compounds (chlorogenic acid and caffeic acid derivatives) and flavonoids (2’-O-α-L-rhamnopyranosylvitexin, vitexin, isovitexin, 2’-O-α-L-rhamnopyranosyl-7-O-methylvitexin, cytisoside and acacetin-7-O-glucoside). Phenolic compounds and flavonoids in the edible part of Celtis australis is still an interesting open research via chromatography.

Table 4 provides the antioxidant activity of the Spanish hackberries peel and flesh, measured by two different methods. Both FRAP and DPPH method indicate a similar antioxidant activity (approx. 4000 μmol eq. Fe²⁺/100 g) in the peel and in the flesh, and a similar scavenging capacity to the free radical DPPH (IC₅₀ of 7.0) as well. Although Ota et al. (2017) also reported the antioxidant potential as DPPH scavenging capacity of a water extract from Croatian hackberries mesocarp, the direct comparison is difficult due to that scavenging capacity (0.35) is expressed in mg gallic acid per gram of sample. In any case, the antioxidant activity of the hackberries flesh or mesocarp is considerable and it is due to the phenolic compounds and flavonoids content, as was also observed by Zehrmann et al. (2010). Chokeberry (Aronia melanocarpa) has a FRAP activity of 59 μmol eq. Fe²⁺/100 g (Nawirska-Olszańska et al., 2020), which is lower than in hackberry.

Concerning the content of fibre (Table 4), the insoluble fibre is present in a higher amount than the soluble fibre in both peel and flesh fractions and, as a consequence, the content of dietary fibre in the flesh is higher than in the peel. In this sense, the flesh contains a higher amount of insoluble fibre (15.6%), soluble fibre (2.4%) and dietary fibre (18.0%) than the peel (11.1, 2.0, 13.1%, respectively). Demir et al. (2002) reported a crude fibre content of 4.4% (90% dry matter), being our results (43.9% water content) more similar to those of Ota et al. (2017), who found that the fruit contained 10.2% total dietary fibre, 8.2% insoluble fibre, and 2.0% soluble fibre (30.0% water content). In apple, the total dietary fibre content (FDA, 2020) is 2.4% (85.6% water content). Apple is much juicy that hackberry. The high content of dietary fibre of the hackberries flesh makes very interesting a study of the flesh cell wall and the involved structural polysaccharides.

Isolation of the hackberries flesh cell wall, cell wall fractionation into polysaccharides, and sugar composition of these materials

The peel of the hackberries and the stone were removed and the flesh fraction (49.7 g per 100 g of fresh hackberry) was processed to obtain the alcohol insoluble solids (AIS): the hackberries flesh cell-wall. Table 5 provides the fractionation of the hackberries flesh cell wall (AIS) into pectin extracted with chelating agents, pectin extracted with 50 mM NaOH, hemicellulose extracted with 1 M NaOH, and hemicellulose extracted with 4 M NaOH. Table 5 provides also the sugar composition of the flesh cell wall and the flesh cell wall fractions. In this sense, the composition of the hackberries AIS indicates that main sugars are glucose (58 mol%) and galacturonic acid (23 mol%), followed by galactose (6 mol%) and arabinose (5 mol%). This sugar composition suggests that hackberries AIS, like other fruits cell walls, are composed by pectins and hemicelluloses. We follow the method of de Vries et al. (1981), proved to be an adequate method for structural polysaccharides isolation in gram amounts, as applied by Apolinar-Valiente et al. (2010). Fractionation of the hackberries AIS was carried out using non-destructive extractants (Coll-Almela et al., 2015). All buffer-soluble
pectins and Ca\textsuperscript{2+}-complexed pectins were isolated in one single fraction (ChSS), representing 45.6% of the AIS. Extraction with 50 mM sodium hydroxide at 0 °C, which triggered ester removal and detachment of hydrogen bonds, resulted in the solubilisation of 10.1% of the AIS. Pectins represented 55.7% of the hackberries AIS. The ChSS fraction represented 82% of the total pectin, while DASS accounted for 18% of the total extracted pectin. ChSS and DASS from lemon albedo represented 71% and 29%, respectively, of the total pectin (Ros et al., 1996). Extraction with 1 M and 4 M sodium hydroxide resulted in the solubilization of 30.0% and 14.4% of the AIS, respectively, as hemicellulosic fractions. Hemicelluloses represented 44.4% of the hackberries AIS. The 1MASS fraction represented 68% of the total hemicelluloses, while 4MASS is the 32%. Total extracted pectins plus total extracted hemicelluloses account 100% of AIS. There were no cellulosic neither lignocellulosic fractions, since finally nothing remained as a residue.

Table 5 shows also the sugar composition of the polysaccharidic fractions. The potential polysaccharides in each fraction based on its sugar composition, considering also the type of fractionation carried out, are the non-covalently linked to the cell-wall pectins (ChSS) and the covalently linked to the cell-wall pectins (DASS). The composition of the ChSS fraction suggests that it consists mainly of homogalacturonan regions (63 mol% of galacturonic acid). The most abundant neutral sugar was glucose (13 mol%). Other sugars such as arabinose and galactose were present only in small amounts (7 mol%). The DASS fraction contained some more neutral sugars than the ChSS fraction. The DASS fraction was relatively rich in arabinose (11 mol%), galactose (7 mol%) and glucose (14 mol%), which suggests a higher proportion of hairy regions, as has been reported for DASS pectin fractions from apple pulp (Schols et al., 1995) and lemon peel (Ros et al., 1998). The main sugars in the 1MASS fraction are glucose (28 mol%), galacturonic acid (20 mol%), galactose (19 mol%), arabinose (16 mol%) and xylose (11 mol%). The composition of the 1MASS fraction suggests that it is a very complex hemicellulose constituted by chains of arabinogalactans, arabinoxylans and xyloglucans, connected to a galacturonan region, such as occurs in the 1MASS fraction of the mandarin segment membrane (Coll-Almela et al., 2015). The main sugars in the fraction 4MASS are glucose (39 mol%) and xylose (24 mol%), followed by arabinose (11 mol%), galactose (11 mol%) and galacturonic acid (9 mol%), indicating that fraction 4MASS is a hemicellulose constituted mainly by a xyloglucan, with chains of arabinogalactans and still some galacturonans. Koh et al. (2020) reported in blueberry two pectin fractions (water soluble fraction and chelator soluble fraction), being the main sugars galacturonic acid (55 and 57%, respectively), arabinose (21 and 3%, respectively) and galactose (8 and 2%, respectively). This composition of the blueberry pectins is similar, but no equal, to hackberry.

CONCLUSIONS

The characteristics and composition of hackberries (Celtis australis L.) from Mediterranean forests suggest that, like other berry fruits, hackberries can be recognized as a natural functional product. The content in sugars, dietary fibre, pectins, phenolic compounds and flavonoids, confer sweet, dietary, thick and antioxidant properties, which would be considered of great interest for its applications as sweetening agent with antioxidant, thickener and dietary properties, in the food industry.

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Authors’ contributions
The research was designed by J.M.R.G. and R.L.S. E.V.C. made the physical, physicochemical and nutritional characterization. C.N. made the isolation of the alcohol insoluble solids. D.P. made the extraction and fractionation of the structural polysaccharides. Finally, the manuscript has been prepared by J.M.R.G. with the approval of all authors.

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