

Comparison of some biochemical characteristics of different citrus fruits

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Abstract

The goal of this investigation was to evaluate the antioxidant properties of some citrus fruits. The contents of dietary fibre, total polyphenols, essential phenolics, ascorbic acid and some trace elements of lemons, oranges and grapefruits were determined and compared with their total radical-trapping antioxidative potential (TRAP). There were no significant differences in the contents of total, soluble and insoluble dietary fibre in the studied peeled fruits or their peels. The contents of total, soluble and insoluble dietary fibre in peels were significantly higher than in peeled fruits ($P < 0.05$ in all cases). The peeled lemons, oranges and grapefruits contain 164 ± 10.3 ; 154 ± 10.2 and 135 ± 10.1 and their peels 190 ± 10.6 ; 179 ± 10.5 and 155 ± 10.3 mg/100 g of total polyphenols, respectively. The content of total polyphenols in peeled lemons and their peels was significantly higher than in peeled oranges and grapefruits and their peels, respectively. The content of total polyphenols in the peels was significantly higher than in peeled fruits ($P < 0.05$ in all cases). The same results were obtained in the investigation of essential phenolics and ascorbic acid. The content of Fe in peeled lemons and their peels was significantly higher than in peeled oranges and grapefruits and their peels, respectively. Also the TRAP was significantly higher in peeled lemons and their peels than in peeled oranges and grapefruits and their peels, respectively. In all three fruits, the TRAP was significantly higher in peels than in peeled fruits ($P < 0.05$). In conclusion, lemons possess the highest antioxidant potential among the studied citrus fruits and are preferable for dietary prevention of cardiovascular and other diseases. The peels of all citrus fruits are rich in dietary fibres and phenolic compounds and suitable for industrial processing. © 2001 Published by Elsevier Science Ltd. All rights reserved.

Keywords: Citrus fruits; Dietary fiber; Total polyphenols; Phenolic acids; Ascorbic acid; Trace elements; TRAP

1. Introduction

A considerable amount of epidemiological and clinical evidence demonstrates a significant decrease in morbidity and mortality from cardiovascular and other diseases among fruit and vegetable consumers (Gey, Stahelin, & Eichhizer, 1993; Hertog, Feskens, Hollamn, Katan, & Kromhout, 1993; Hertog et al., 1995). The positive influence of such diet is attributed to their

antioxidant effect (Longeril et al., 1994; Partiff, Rubba, Bolton, Marotta, Hertog, & Mancini, 1994; Rimm, Asherio, Giovannucci, Spiegelman, Stampfer, & Willett, 1996; Rimm, Katan, Ascherio, Stampfer, & Willett, 1996). It was shown that phenolic compounds, and particularly flavonoids possess antioxidant properties (Morel, Lescoat, Cillard, & Cillard, 1994; Morel et al., 1993). The role of dietary fibre is not clear. In our previous studies, performed on laboratory animals (Gorinstein et al., 2000; Leontowicz, Gorinstein, Bartnikowska, Leontowicz, Kulasek, & Trakhtenberg, 2001), we did not find that dietary fibre possesses antioxidant properties. But some authors claim that certain parts of dietary fibre exert antioxidant effects (Larrauri, Goni,

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Martin-Carron, Ruperez, & Saura-Calixto, 1996; Lin, 1994). That is why high dietary fibre-formulated food products are currently being developed (Grigelmo-Miguel, Gorinstein, & Martín-Belloso, 1999; Grigelmo-Miguel & Martín-Belloso, 1999). Ascorbic acid and some trace elements (iron, copper and manganese) are effective in prevention and treatment of atherosclerosis and its complications (Gey et al., 1993; Wills, 1985). Citrus fruits have a high content of phenolics, dietary fibre, ascorbic acid and trace elements (Marlett, 1992; Marlett & Vollendorf, 1994; Peleg, Naim, Romseff, & Zehavi, 1991; Stohr & Herrman, 1975). Spain is one of the major producers and exporters of various kinds of citrus fruits. Most popular with European and North American consumers are grapefruits (*Citrus paradisi*), oranges (*Citrus sinensis*) and lemons (*Citrus limons*). Which of these citrus fruits is preferable for dietary prevention of cardiovascular and other diseases? To find a proper answer, all components of grapefruits, oranges and lemons that possess antioxidant properties were studied and compared with their total radical-trapping antioxidative potential (TRAP). It was found that peels of fruits are major sources of natural antioxidant (Bocco, Cuvelier, Richard, & Berset, 1998; Gorinstein, Kulasek et al., 1998). Some even propose to use these by-products of the juice extraction industry as natural antioxidants (Bocco et al., 1998). Therefore, the peeled fruits and their peels were studied separately. As far as we know there are no other such comprehensive comparative investigations.

2. Materials and methods

2.1. Fruits and sample preparation

The studied grapefruits (*Citrus paradisi*), oranges (*Citrus sinensis*) and lemons (*Citrus limon*) were purchased from the same farmer. Twenty-four of each of the (selected at random) fruits were washed in distilled water. They were separated by hand into peeled fruits and peels. Samples of 1 kg were obtained for each part of all three fruits.

2.2. Dietary fibre content

Determination of total, soluble and insoluble dietary fibre was done according to Prosky, Asp, Schweizer, De Vries, and Furda (1992).

2.3. Total polyphenol content

The extraction of the crude polyphenols was achieved using aqueous methanol or ethanol. Ten grams of peeled grapefruits, oranges and lemons and their peels were separately homogenized. A SD-45N homogenizer

(Arthur H. Thomas Co., Philadelphia, PA, USA) was used with 125 ml of 95% ethanol for 1 min and then the homogenized samples were gently boiled in a water bath with a watch glass serving as a reflux condenser above a beaker. After this procedure, the fruit samples were cooled and filtered under vacuum using Whatman No 1 paper. The filtrates were reduced by evaporation under vacuum at 60°C to a volume of 10 ml and then made up to the volume of 100 ml with distilled water. The samples were passed through Chromobond C 8 before reading at 725 nm. Results were calculated as mg of chlorogenic acid per 100 g of fresh fruits. Total polyphenols were determined by the Folin-Ciocalteu method and measured at 675 nm (Singleton & Rossi, 1965).

2.4. Contents of individual antioxidant compounds

Determination of phenolic acids was done according to Garcia-Sanches, Carnero, and Heredis (1988), with our modifications and changes in the extraction procedure of the samples, using a combination of methanol, petroleum ether and ethyl acetate (Gorinstein et al., 1994). Fluorescence emission spectra of phenolic acids were measured using Model FP-770, Jasco-Spectrofluorometer at an excitation wavelength of 260 nm and emission wavelength of 363 nm. Determination of ascorbic acid and trace elements was done as previously described.

2.5. The TRAP determination

The TRAP measurement was performed as previously described (Slavikova et al., 1998). Extracts of peeled lemons, oranges, grapefruits and their peels were obtained. Each of the peeled fruits (0.5 g) and their peels were separately ground and shaken at room temperature with 2 ml of distilled water or acetone. After centrifugation at 2800 g, the water and acetone extracts were used for TRAP analyses.

Peroxy radicals, produced at a constant rate by thermal decomposition of 2,2-azo-bis-2-amidinopropane hydrochloride (ABAP- Polyscience, Warrington, PA), were monitored by luminol-enhanced chemiluminescence (CL). The reaction was initiated by mixing 475 µl phosphate-buffered saline, 50 µl 10 mM luminol in 100 mM borate buffer (pH 10.0), and 50 µl ABAP. This mixture was incubated (37°C) in the temperature-controlled sample carousel of the luminometer BioOrbit 1251 (BioOrbit, Finland) for 15 min. During this period of time, a steady state of the CL signal was reached. Then, 20 µl of genuine juice or water (acetone) extract were added directly into the cuvette and the samples were measured for another period of time (τ) until a 50% recovery of the original steady state CL signal. Trolox (8.0 M; Aldrich Chemical Co., Milwaukee, WI), a water-soluble analogue of tocopherol, was used as a

Table 1
Dietary fibre content in studied fresh peeled fruits and their peels^a

Samples	Fibre (g/100 g)					
	Total		Soluble		Insoluble	
	DW	FW	DW	FW	DW	FW
Peeled lemons	7.34±0.8a	1.31±0.08a	2.49±0.3b	0.43±0.04b	4.83±0.5c	0.86±0.06c
Peels of lemons	14.0±1.3x	2.49±0.22x	4.93±0.4y	0.82±0.08y	9.04±0.9z	1.60±0.10z
Peeled oranges	7.28±0.8a	1.30±0.08a	2.45±0.3b	0.43±0.04b	4.82±0.5c	0.85±0.08c
Peels of oranges	13.9±1.3x	2.49±0.22x	4.71±0.5y	0.83±0.09y	9.18±1.0z	1.65±0.10z
Peeled grapefruits	7.41±0.8a	1.32±0.10a	2.35±0.3b	0.41±0.05b	4.95±0.5c	0.90±0.07c
Peels of grapefruits	13.9±1.2x	2.47±0.22x	4.70±0.5y	0.82±0.08y	9.16±0.9z	1.63±0.10z

^a Values are means±S.D. of five measurements. Means in columns without letters (a–c for peeled fruits, x–z for peels) in common differ significantly ($P < 0.05$). DW., dry weight; FW, fresh weight.

reference inhibitor instead of sample. The stoichiometric factor of trolox (the number of peroxy radicals trapped per added molecule of antioxidant) is 2.0. The TRAP value for sample measured can be obtained from the equation: $TRAP = 2.0 \times [\text{trolox}] \times \tau_{\text{sample}} / f \times \tau_{\text{trolox}}$, where f is the dilution of sample measured. The results obtained are expressed as nmol of peroxy radicals trapped by 1 ml of sample. Solvents (water and acetone) were verified to have negligible TRAP.

2.6. Statistics

The results of this investigation are means of five measurements. To verify the statistical significance of all parameters the values of means ±S.D. were calculated. To compare several groups, analysis of variance (ANOVA) was used. In the assessment of TRAP, the Spearman correlation coefficient (R) and P -value were used to show correlations and their significance. Linear regressions were also calculated. The P values of < 0.05 were adopted as statistically significant.

3. Results

3.1. Dietary fibre

The ranges of the total dietary fibre content were 2.43–2.54 and 1.29–1.34 g/100 g fresh fruits for peels and peeled citrus fruits, respectively. The ranges of the insoluble dietary fibre content were 1.57–1.63 and 0.81–0.87 g/100 g fresh fruits for peels and peeled citrus fruits, respectively. The ranges of the soluble dietary fibre content were 0.85–0.91 and 0.43–0.47 g/100 g fresh fruits for peels and peeled citrus fruits, respectively.

The statistically evaluated results (ANOVA) of the contents of total, soluble and insoluble dietary fibre in peeled lemons, oranges and grapefruits and their peels are summarized in Table 1.

As can be seen, there were no significant differences in the contents of total, soluble and insoluble dietary fibre

in peels and peeled citrus fruits. The contents of total, soluble and insoluble dietary fibre in peels were significantly higher than in peeled fruits ($P < 0.05$).

3.2. Total polyphenols

The total polyphenol content in peeled lemons, oranges and grapefruits were 164 ± 10.3 ; 154 ± 10.2 and 135 ± 10.1 mg/100 g of fresh fruits, respectively. The total polyphenol contents in peels of lemons, oranges and grapefruits were 190 ± 10.6 ; 179 ± 10.5 and 155 ± 10.3 mg/100 g of fresh fruits, respectively. Fig. 1 shows graphically comparative contents of total polyphenol in fresh peeled lemons, oranges and grapefruits and their peels. The differences in the contents of total polyphenols in peeled lemons, oranges and grapefruits were statistically significant ($P < 0.05$). The highest content of total polyphenols was in lemons and the lowest — in grapefruits. The differences in the contents of total polyphenols in peels of fresh lemons, oranges and

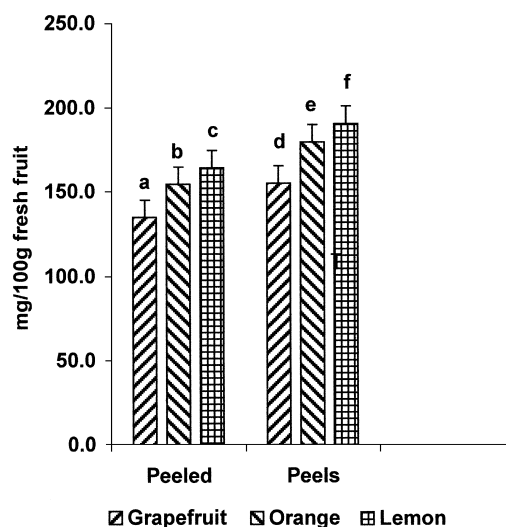


Fig. 1. Total polyphenol content in lemons, oranges and grapefruits. Means±S.D. (vertical lines). Bars with different letters are significantly different ($P < 0.05$).

grapefruits were similar to the differences in the content of total polyphenols in peeled fruits. The highest content of total polyphenols was in peels of lemons and the lowest — in peels of grapefruits. The content of total polyphenols in peels of fresh lemons, oranges and grapefruits was significantly higher than in peeled fruits (Table 2).

3.3. Phenolic acids

The contents of total (bound and free) ferulic, sinapic, *p*-coumaric and caffeic acids were significantly higher in peels of these fruits than in peeled fruits. The highest content was of ferulic and the lowest — of caffeic acid in both peeled fruits and their peels. The content of the studied phenolic acids were significantly higher in peeled lemons and their peels than in peeled oranges and grapefruits and their peels (Table 2).

3.4. Ascorbic acid

The content of ascorbic acid was significantly higher in peeled lemons and oranges and their peels than in peeled grapefruits and their peels. The content of ascorbic acid was significantly higher in peels than in peeled fruits (Table 2).

3.5. Trace elements

Fig. 2 shows graphically comparative contents of all studied trace elements in fresh peeled lemons, oranges and grapefruits and their peels. The differences in the contents of all trace elements in peels of lemons, oranges and grapefruits were statistically significant higher than in peeled fruits ($P < 0.05$). The content of Fe was significantly higher in peeled lemons and their peels than in peeled oranges and grapefruits and their peels, respectively.

3.6. Total radical-trapping antioxidative potential

The results of the measurement of TRAP in comparison with all studied antioxidant compounds are summarized in Table 2. According to this table, the

TRAP values in the peeled lemons, oranges and grapefruits were 4480 ± 398 , 2111 ± 199 and 1111 ± 102 nmol/ml, respectively, and the TRAP values in the peels of these fruits were 6720 ± 601 , 3183 ± 311 and 1667 ± 161 nmol/ml, respectively. The statistical evaluation (ANOVA) demonstrates significant differences in the TRAP. The highest TRAP was in peeled lemons and their peels and the lowest — in peeled grapefruits and their peels (P ranges < 0.01 – 0.005). The TRAP in peels

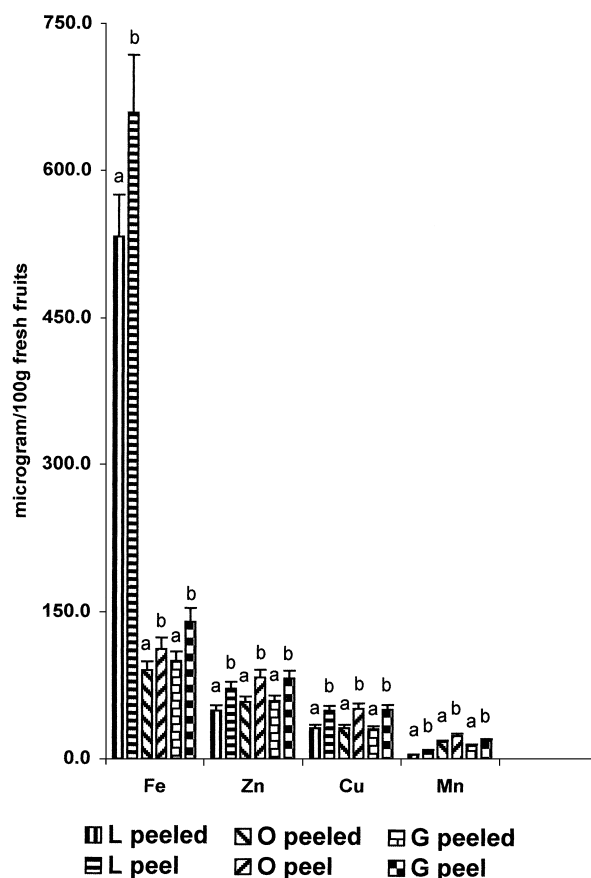


Fig. 2. Content of some trace elements in lemons (L), oranges (O) and grapefruits (G). Means \pm S.D. (vertical lines). Bars with different letters are significantly different ($P < 0.05$).

Table 2

Antioxidant compounds (mg/100 g) and total radical-trapping antioxidative potential (TRAP) (nmol/ml) in fresh lemons (L), oranges (O) and grapefruits (G)^a

Fruits	Total polyphenols	Ferulic acid	Sinapic acid	<i>p</i> -coumaric acid	Caffeic acid	Ascorbic acid	TRAP
Peeled L	164 \pm 10.3a	38.8 \pm 4.1a	36.4 \pm 3.1a	31.3 \pm 3.1a	12.1 \pm 1.1a	47.9 \pm 4.7a	4480 \pm 398a
Peel of L	190 \pm 10.6x	44.9 \pm 4.2x	42.1 \pm 4.1x	34.9 \pm 3.4x	14.2 \pm 1.3x	59.8 \pm 5.4x	6720 \pm 601x
Peeled O	154 \pm 10.2b	34.1 \pm 3.1b	30.71 \pm 3.1b	24.1 \pm 2.2b	8.1 \pm 0.8 b	47.7 \pm 4.9a	2111 \pm 199b
Peel of O	179 \pm 10.5y	39.2 \pm 4.0y	34.9 \pm 3.1y	27.9 \pm 2.5y	9.5 \pm 0.8y	59.6 \pm 5.2x	3183 \pm 311y
Peeled G	135 \pm 10.1c	27.1 \pm 3.0c	27.3 \pm 2.9c	10.8 \pm 1.1c	5.0 \pm 0.5c	35.1 \pm 3.5c	1111 \pm 102c
Peel of G	155 \pm 10.3z	32.3 \pm 3.1z	31.9 \pm 3.0z	13.1 \pm 1.3z	5.6 \pm 0.5z	43.8 \pm 4.1y	1667 \pm 161z

^a Values are means \pm S.D. of 5 measurements; means in columns without letters (a–c for peeled fruits, x–z for peels) in common differ significantly ($P < 0.05$)

was significantly higher than in peeled fruits. A very good correlation was observed between TRAP and the total polyphenols content ($R > 0.9$, $P < 0.001$). But the correlation between TRAP and individual antioxidant compounds was relatively lower ($R=0.69$, $P=0.008$; $R=0.69$, $P=0.008$; $R=0.68$, $P=0.011$; $R=0.69$, $P=0.008$ and $R=0.68$, $P=0.011$ for ferulic, sinapic, *p*-coumaric, caffeic and ascorbic acids, respectively).

4. Discussion

Atherosclerosis is still one of the most dangerous diseases in the industrial countries — the principal cause of death in Western civilization (Gaziano, 1994; Hennekens & Gaziano, 1993). Recent evidence suggests that one of the important mechanisms predisposing to development of atherosclerosis is the oxidation of cholesterol-rich LDL-C particles (Steinberg, Parathasarathy, Carew, Khoo, & Witztum, 1989). Oxidation of LDL-C enhances its atherogenicity and facilitates penetration of lipids into the arterial wall. There is a significant decrease in morbidity and mortality from cardiovascular and other diseases among fruit and vegetable consumers (Bartnikowska, 1999; Rimm & Ascherio et al., 1996). During the last 15 years, our team of cardiologists, biochemists and dietitians have been studying various kinds of nutritional products in order to improve the diet for patients with coronary atherosclerosis (Gorinstein, Barthikowska, Kulasek, Zemser, & Trakhtenberg, 1998; Gorinstein & Kusalek et al., 1998; Gorinstein, Zemser, Berliner et al., 1997; Gorinstein, Zemser, Haruenket, Chuthakorn, Martín-Belloso, & Trakhtenberg 1998; Gorinstein, Zemser, & Lichman et al., 1997; Gorinstein et al., 2000; Grigelmo-Miguel et al., 1999; Leontowicz et al., 2001). Like Davidson, Dugan, Burns, Bova, Story, & Rankin et al. (1993), Drennan (1991), Shinnick, Mathews, and Ink (1991), and Turnbull and Leeds (1987), we found that phenolic substances, ascorbic acid, certain trace elements and to a less extent dietary fibre are the biologically active components of alcohol beverages, fruits and vegetables. All these components are in citrus fruits. Spain is one of the major producers and exporters of lemons, oranges and grapefruits. It was decided to define the antioxidant potential of each fruit and to purpose the most preferable one for dietary prevention of cardiovascular and other diseases. To the best of our knowledge, there are no other such comprehensive comparative investigations.

The results of our investigation show that all citrus fruits possess a high content of dietary fibre. There were no significant differences in the contents of total, soluble and insoluble dietary fibre in the studied peeled fruits or

their peels. The contents of total, soluble and insoluble dietary fibre in peels were significantly higher than in peeled fruits ($P < 0.05$ in all cases). These results are in accordance with the results of others (Marlett, 1992; Marlett & Vollendorf, 1994).

It was found that the contents of total polyphenols in peeled lemons and their peels were significantly higher than in peeled oranges and grapefruits and their peels, respectively. The content of total polyphenols in the peels was significantly higher than in peeled fruits ($P < 0.05$ in all cases). These results are in accordance with the results of others (Belitz & Grosch, 1999).

The same results were obtained in the investigation of essential phenolics (ferulic, sinapic, *p*-coumaric and caffeic acids) and ascorbic acid. Also, these results are in accordance with the results of others (Peleg et al., 1991; Stohr & Herrman, 1975).

Like others (Peleg et al., 1991), we found that all four studied hydroxycinnamic acids (ferulic, sinapic, *p*-coumaric and caffeic) occur mainly in bound forms and mostly in peels. But we found that the concentrations of these acids were different from the concentrations reported by other investigators (Peleg et al., 1991; Stohr & Hermann, 1975), and anticipated the contents of the studied compounds are influenced by region, climate conditions, ripeness and other factors (Geyas, Young, Blankenship, & McFeeters, 1996). But the same tendency was observed; the content of ferulic, sinapic, *p*-coumaric and caffeic acids was significantly higher in peels than in peeled fruits. The highest concentrations were of ferulic, sinapic and *p*-coumaric acids and the lowest — of caffeic acid in both peeled fruits and their peels.

The total radical-trapping antioxidative potential is a relatively unspecific maker of free radical scavenging activity. There are some other much better techniques such as Elsectron pulse resonance (EPR) and spin trapping. But the measurement of TRAP also reflects the total antioxidant capacity.

Like the total polyphenols, TRAP was significantly higher in peeled lemons and their peels than in peeled oranges and grapefruits and their peels, respectively. In all three fruits the TRAP was significantly higher in peels than in peeled fruits ($P < 0.05$).

It has been suggested that, the higher the total polyphenolic content, the greater is the antioxidant activity (Abu-Amsha, Croft, Puddey, Proudfoot, & Beilin, 1996). Therefore, the total polyphenol contents of the studied fruits were compared with their total radical-trapping antioxidative potential. It was found that the highest content of total polyphenols was in peeled lemons and their peels and the significantly highest TRAP was also in peeled lemons and their peels. Therefore, this investigation confirms the suggestion of Abu-Amsha et al. (1996), that, the higher the total polyphenolic content, the greater is the antioxidant capacity.

5. Conclusion

1. There are no significant differences in the contents of dietary fibre in the studied citrus fruits. The content of dietary fibre in peels is significantly higher than in peeled fruits.
2. The total polyphenol contents in peeled lemons and their peels are significantly higher than in peeled oranges and grapefruits and their peels, respectively. The content of total polyphenols in the peels is significantly higher than in peeled fruits.
3. The contents of ferulic, sinapic, *p*-coumaric and caffeic acids, ascorbic acid and some of the studied trace elements are significantly higher in peeled lemons and their peels than in oranges and grapefruits. The highest concentration is of ferulic, sinapic and *p*-coumaric acids and the lowest — of caffeic acid in both peeled fruits and their peels.
4. The TRAP of peeled lemons and their peels is significantly higher than in peeled oranges and grapefruits and their peels, respectively. The TRAP in the peels is significantly higher than in peeled fruits.
5. The highest antioxidant potential of lemons makes this fruit preferable for dietary prevention of cardiovascular and other diseases.
6. The peels of citrus fruits are rich in dietary fibres and phenolic compounds and suitable for industrial processing.

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