Extraction and *In Vitro* Antioxidant Activity of Mopan Persimmon Polysaccharide

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ABSTRACT: The optimal extraction parameters of Mopan persimmon polysaccharide (MPP) were ultrasonic power 900 W, ultrasonic treatment time 3 min, and water addition 2000%. Four subfractions (MPP-1, MPP-2b, MPP-3, and MPP-4b) were separated by DEAE–52 cellulose anion-exchange column chromatography and then Sephadex G-200 gel column chromatography. The *in vitro* scavenging effects of MPP and MPP-4b at a dosage of 10 g/L on hydroxyl radical were 94.15 ± 8.27% and 96.61 ± 8.06%, respectively. The 1,1-diphenyl-2-picrylhydrazyl inhibition percentages of two polysaccharides fractions were 93.37 ± 7.66% and 70.79 ± 6.15%, respectively. The reducing power (absorbance at 700 nm) of MPP and MPP-4b were 4.31 ± 0.26 and 5.09 ± 0.33, respectively. © 2011 Wiley Periodicals, Inc. J Appl Polym Sci 124: 1751–1756, 2012

Key words: antioxidants; polysaccharides; ion exchangers; biopolymers

INTRODUCTION

Persimmon (*Diospyros kaki*), cultivated as a fruit in China, South Korea and Japan, is a very important nutrient source.[1] It contains many biological active materials, such as protein, trace elements, dietary fiber, carotenoids, vitamins and carbonhydrates, etc.[2] Persimmons have the functions of antitumor,[3] lowing blood pressure,[4] prevention of apoplexy,[5] and antioxidation.[6]

Interest in natural antioxidants has increased in recent years[7] and polysaccharides obtained from plants or fungi have potential antioxidative activity.[8–10] Senji and Yuuya[11] compared the antioxidant properties of persimmon vinegar with some other commercial vinegar in radical-scavenging assays and lipid oxidation. Ahn et al.[12] analyzed the antioxidative activity of persimmon extract *in vitro* and *in vivo*. Gu et al.[13] reported the structural features and antioxidant activity of tannin from persimmon pulp. Senji et al.[14] separated the extracts of Japanese persimmon leaf tea. However, the extraction and purification of Mopan persimmon polysaccharide (MPP) and its antioxidant properties *in vitro* have not been studied.

In this work, the extraction conditions of MPP were optimized by single factor tests and orthogonal experiments, and four subfractions of MPP were obtained by DEAE-52 cellulose anion-exchange column chromatography and fractionated using Sephadex G-200 gel column chromatography. In addition, the *in vitro* antioxidant activities of MPP and its subfractions were evaluated.

EXPERIMENTAL

Materials

Mopan persimmon (2 kg) was obtained from Zibo Evergreen Food Development Co. (Shandong, China). The fruit of Mopan persimmon was air dried and milled to a particle size of < 0.5 mm in a mill Retsch ZM 200 (Kangtai, Shanghai, China), and then stored in tightly closed glass jars (1 L) at 4°C until used.

Chemicals

DEAE–52 cellulose and Sephadex G–200 were purchased from Pharmacia Co. (New Jersey, USA). 1,1-diphenyl-2-picrylhydrazyl (DPPH) was obtained from Sigma Chemicals Co. (St. Louis, USA). All other chemicals used in this experiment were analytical reagent grade and purchased from local chemical suppliers in China.

Single-factor tests for MPP extraction

Eight factors including the ultrasonic treatment power (600, 700, 800, and 900 W) with ultrasonic

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processor (Bingyang, Beijing, China), ultrasonic time (1, 2, 3, and 4 min), extraction temperature (65°C, 75°C, 85°C, and 95°C), extraction time (1, 2, 3, and 4 h), water addition (1500%, 2000%, 2500%, and 3000%), ethanol concentration (60%, 70%, 80%, and 90%), precipitation time (12, 24, 36, and 48 h), and pH (6, 7, 8, and 9) were investigated by single factor experiments for MPP extraction. The extraction rate of MPP was expressed as a percentage of MPP to Mopan persimmon fruit (w/w).

Orthogonal experiments for MPP extraction

Three parameters affecting the MPP extraction, ultrasonic power, ultrasonic treatment time, and water multiple, were selected by single factor tests. A three-factor-three-level orthogonal experiment was subsequently applied to optimize the conditions of MPP extraction.

Separation of MPP

The dried powder of Mopan persimmon (2 g) was dissolved in 50 mL NaCl (2*M*) and treated with ultrasonic processor for 3 min. Protein was removed from the Mopan persimmon by the method of Sevag.[15] After centrifugation (3000 rpm, 15 min), the supernatant liquid was mixed with 5 mL of 95% ethanol, stirred vigorously and kept at 4°C for 24 h. The precipitated MPP was lyophilized.

Purification of MPP

The lyophilized powder of MPP (25 mg) was dissolved in distilled water (5 mL), and fractionated on DEAE-52 cellulose anion-exchange column (1.6 × 20 cm). The column was eluted with distilled water, and then with gradient solutions (0.1*M*–1*M* NaCl), at a flow rate of 2 mL/min. The polysaccharide subfractions were collected with a fraction collector, detected at 490 nm by a spectrophotometer (Shandong, China) and concentrated using a rotary evaporator at 55°C, respectively. The concentrated subfractions (4 mL) were loaded onto a Sephadex G-200 gel column (1.6 × 60 cm), respectively.[16] The column was eluted with distilled water at a flow rate of 0.5 mL/min. The major fraction was collected and then freeze dried with vacuum freezing drying apparatus (Christ, Osterode, Germany).

Determination of MPP

The dried MPP and its subfractions (0.6 g) were dissolved in distilled water (1 mL), and then mixed with 5 mL of 95% ethanol at 4°C for 24 h, respectively. By centrifugation (12,000 rpm, 10 min), the precipitated polysaccharide was dissolved in distilled water (60°C), and the polysaccharide content was determined by the phenol–sulfuric acid method, using glucose as the standard.[17]

Hydroxyl radical scavenging assay

Hydroxyl radical scavenging activity was measured according to the method of Winterbourn and Sutton.[18] The reaction mixture contained 1 mL of 0.15*M* phosphate buffer saline (pH7.4), 1 mL of 40 μg/mL safranin, 1 mL of 0.945 m*M* EDTA–Fe (II), 1 mL of 3% (v/v) H_2O_2, and 0.5 mL of the MPP or its subfractions (0.5–10 g/L). After incubating at 37°C for 30 min, the absorbance of MPP or its subfractions was measured at 560 nm. The EC_{50} value (mg/L) of MPP is the effective concentration at which the hydroxyl radicals were scavenged by 50%. The hydroxyl radical scavenging activity was expressed as:

$$\text{Scavenging rate}\,(\%) = [(A_0 - A_1)/A_0] \times 100$$

where A_0 is the absorbance of the blank and A_1 is the absorbance of MPP/its subfractions.

DPPH scavenging assay

The DPPH scavenging activity of MPP and its subfractions was measured according to the method of Liu and Zhao.[19] The reaction mixture contained 2 mL of 95% ethanol, 0.1 μM DPPH, and 2 mL of the MPP or its subfractions (0.5–10 g/L). The solution was incubated at 25°C for 15 min, and the absorbance of MPP and its subfractions was determined at 517 nm. The antioxidant activity was evaluated according to the following formula:

$$\text{Scavenging rate}\,(\%) = (1 - A/A_0) \times 100$$

where A is absorbance of MPP/its subfractions and A_0 is the absorbance of the DPPH solution.

Determination of reduction power

The reducing power of MPP and its subfractions was evaluated according to the method of Oyaizu[20] with slight modification. The reaction mixtures contained 2.5 mL phosphate buffer (pH 6.6, 0.2*M*), 2.5 mL potassium ferricyanide (1%, w/v) and the MPP or its subfractions (0.5–10 g/L). After incubation at 50°C for 20 min, 2.5 mL of trichloroacetic acid (10%, w/v) was added to the mixture to terminate the reaction, and then the mixture was centrifuged at 1200 rpm for 10 min. An aliquot of 2.5 mL supernatant was collected and mixed with 2.5 mL deionized water and 0.5 mL $FeCl_3$ (0.1%, w/v). After incubation

TABLE I
Effect of Extraction Conditions on MPP Production

Extraction conditions	MPP yield (%)
Ultrasonic power (W)**	
600	9.68 ± 0.21
700	10.48 ± 0.32
800	10.89 ± 0.45
900	10.16 ± 0.36
Ultrasonic treatment time (min)**	
1	8.44 ± 0.24
2	9.86 ± 0.28
3	9.36 ± 0.31
4	8.67 ± 0.27
Extraction temperature (°C)	
65	7.16 ± 0.38
75	8.03 ± 0.29
85	8.25 ± 0.25
95	8.31 ± 0.24
Extraction time (h)	
1	6.43 ± 0.25
2	7.06 ± 0.39
3	7.93 ± 0.21
4	8.15 ± 0.39
Water addition**	
15	9.87 ± 0.33
20	10.07 ± 0.42
25	10.78 ± 0.39
30	10.05 ± 0.36
Ethanol concentration (%)*	
60	8.39 ± 0.27
70	9.18 ± 0.35
80	10.03 ± 0.38
90	9.25 ± 0.36
Precipitation time (h)*	
12	8.74 ± 0.21
24	9.39 ± 0.33
36	9.62 ± 0.38
48	9.24 ± 0.27
pH	
4	3.37 ± 0.16
6	5.81 ± 0.19
8	6.55 ± 0.13
10	6.28 ± 0.18

* $P < 0.05$, ** $P < 0.01$.

at room temperature for 15 min, the absorbance of the MPP and its subfractions was measured at 700 nm.

Statistical analysis

All experiments were performed in triplicate, and all the data were expressed as mean ± S.D. The statistics significance was evaluated using Student's *t*-test and $P < 0.05$ was taken as significant difference.

RESULTS AND DISCUSSION

Determination of parameters of MPP extraction

When ethanol concentration and precipitation time were 80% and 36 h, respectively, the maximum values of MPP extraction reached 10.03 ± 0.38% ($P < 0.05$) and 9.62 ± 0.38% ($P < 0.05$), respectively (Table I). Ultrasonic power, ultrasonic treatment time, and water addition all had significant influence on MPP extraction ($P < 0.01$). Table I showed that the optimal extraction temperature, extraction time, and pH were 95°C, 4 h, and 8, respectively. Three parameters, ultrasonic power, ultrasonic treatment time, and water addition, were finally chosen and applied to optimize the MPP extraction by orthogonal experiments.

Orthogonal optimization of MPP extraction

The three-factor-three-level design of orthogonal experiment and the results are shown in Table II. The optimum extraction conditions of MPP were ultrasonic power 900 W, ultrasonic treatment time 3 min, and water addition 2000%, while the extraction rate of MPP was 13.35 ± 0.27% ($P < 0.01$), much higher than that of single factor tests. The yield of MPP was also higher than 4.34% of pear,[21] 2.25% of peach,[22] 5.21% of pineapple,[23] and 1.42% of pitaya.[24]

Triplicate experiments were performed under the determined conditions, and the MPP extraction rate of Mopan persimmon was 13.18 ± 0.23% ($P < 0.01$), indicating that the models are adequate for MPP extraction process.

Purification of MPP subfractions

The chromatography results of MPP are shown in Figure 1. Four subfractions, named MPP-1, MPP-2, MPP-3, and MPP-4, were obtained from distilled water, and NaCl gradient of 0.1*M*, 0.2*M*, and 0.3*M*, respectively, and the yields of four subfractions were 27.80 ± 0.33%, 12.40 ± 0.21%, 31.6 ± 0.34%,

TABLE II
Results of Orthogonal Experiments for MPP Production

No.	*A* (W)	*B* (min)	*C* (%)	MPP yield (%)
1	700	2	2000	9.50 ± 0.35
2	700	3	2500	10.09 ± 0.21
3	700	4	3000	11.31 ± 0.27
4	800	2	2500	10.02 ± 0.34
5	800	3	3000	10.89 ± 0.29
6	800	4	2000	9.52 ± 0.43
7	900	2	3000	10.64 ± 0.38
8	900	3	2000	13.35 ± 0.27
9	900	4	2500	11.47 ± 0.45
K_1	10.30	10.05	10.79	
K_2	10.14	11.44	10.53	
K_3	11.82	10.77	10.95	
P	0.0066**	0.0084**	0.0137*	

* $P < 0.05$, ** $P < 0.01$.
A, ultrasonic power; *B*, ultrasonic treatment time; *C*, water addition.

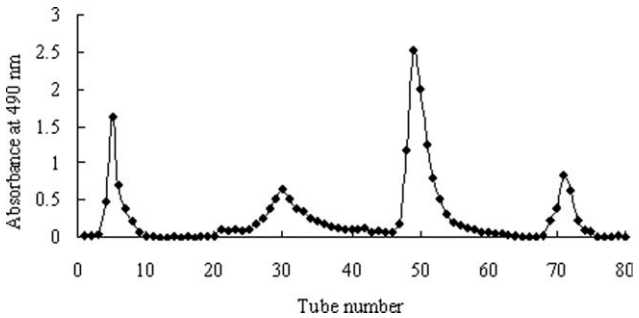

Figure 1 DEAE-52 cellulose anion-exchange column chromatogram of the MPP extracted from Mopan persimmon. The subfractions of MPP (MPP-1, -2, -3, and -4) were obtained from distilled water, and NaCl gradient of 0.1*M*, 0.2*M*, and 0.3*M*.

and 4.8 ± 0.06%, respectively. The molecular weights of MPP-1, MPP-2, MPP-3, and MPP-4 were 5.1×10^4 Da, 6.8×10^5 Da, 7.9×10^4 Da, and 1.6×10^4 Da, respectively. These four fractions were purified by a Sephadex G-200 column, and the results are described in Figure 2. Both MPP-1 and MPP-3 showed one symmetrical peak, and two other subfractions had two peaks which were MPP-2a, MPP-2b and MPP-4a, MPP-4b. Due to the lower yield of MPP-2b and MPP-4a, MPP-2a and MPP-4b along with MPP-1 and MPP-3 were selected for the evaluation of antioxidant activities *in vitro*.

Antioxidant activity of MPP and its subfractions *in vitro*

Antioxidant activities have been attributed to various reactions and mechanisms, such as radical scavenging, reductive capacity, prevention of chain initiation, binding of transition metal ion catalysts, etc.[25] In this experiment, the *in vitro* antioxidant capacities of MPP and its subfractions were evaluated using different biochemical methods of hydroxyl and DPPH radical scavenging assay, and reducing power analysis.

The hydroxyl radical scavenging activities of MPP and four subfractions were concentration-dependent at a dosage range of 0.5–10 g/L [Fig. 3(A)]. The inhibition percentage of MPP-4b at 10 g/L was 96.61 ± 8.06% ($P < 0.01$), which was 2.61 ± 0.11%, 25.66 ± 2.18%, 242.83 ± 21.63%, and 116.86 ± 10.41% higher than that of MPP, MPP-1, MPP-2a,

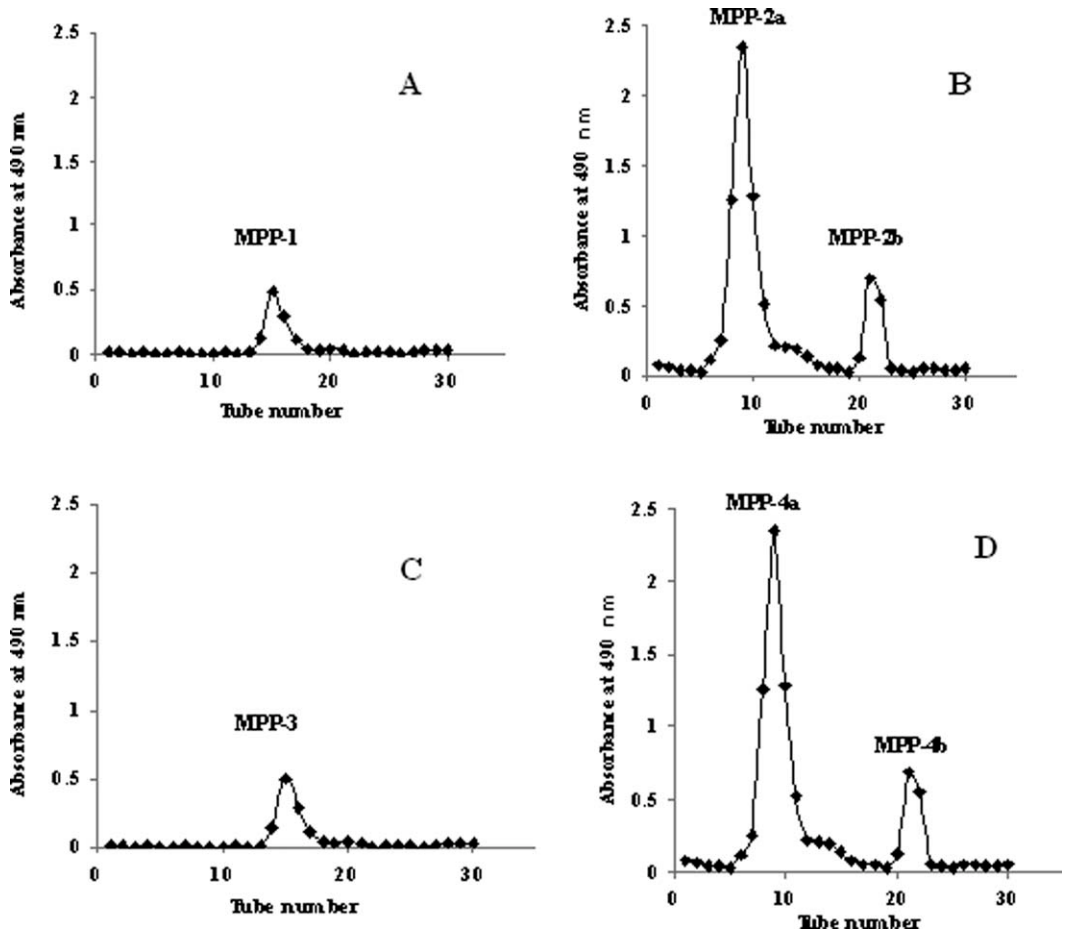

Figure 2 Sephadex G-200 column chromatogram of MPP. (A) MPP-1, (B) MPP-2a and MPP-2b, (C) MPP-3, and (D) MPP-4a and MPP-4b.

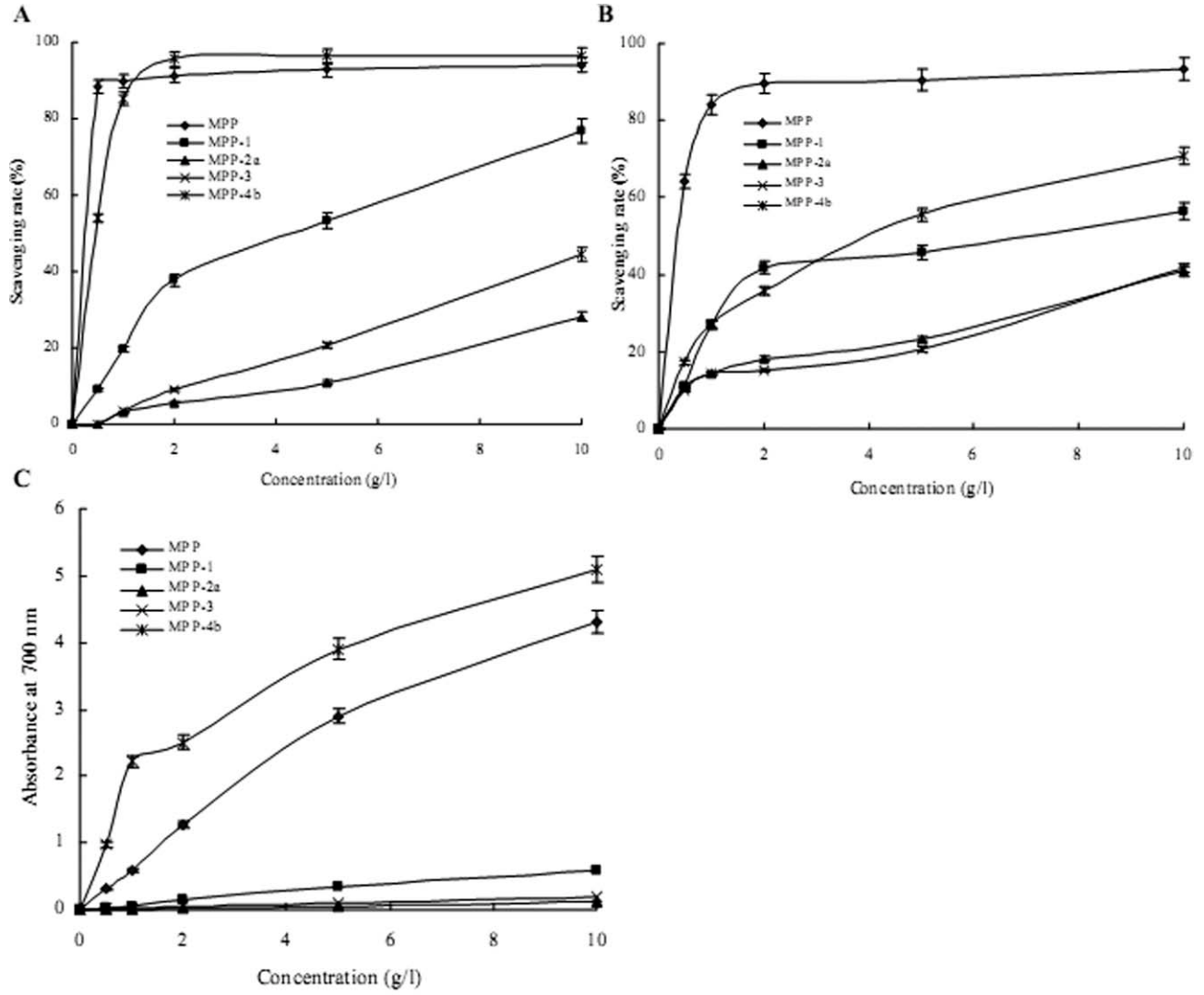

Figure 3 Antioxidant activities of MPP and its subfractions *in vitro*. (A) Scavenging effect on hydroxyl radical, (B) scavenging effect on DPPH, and (C) reducing power.

and MPP-3, respectively. The scavenging values of MPP-4b and MPP at 5 g/L reached 96.44 ± 8.64% and 92.89 ± 8.11%, respectively. The EC_{50} values of MPP and MPP-4b were 0.21 ± 0.01 g/L and 0.37 ± 0.02 g/L, indicating that the MPP and MPP-4b significantly affects the scavenging of hydroxyl radical. As shown in Figure 3(B), the DPPH scavenging rate of MPP at 10 g/L reached 93.37 ± 7.66% ($P < 0.01$), which was 65.02 ± 5.14%, 128.90 ± 11.25%, 125.15 ± 11.04%, and 31.90 ± 2.63% higher than that of MPP-1, MPP-2a, MPP-3, and MPP-4b, respectively. The EC_{50} values of MPP and MPP-4b were 0.43 g/L ($P < 0.01$) and 4.45 g/L ($P < 0.05$), respectively.

Figure 3(C) showed that the reducing power (absorbance at 700 nm) of MPP and MPP-4b of Mopan persimmon at a dosage of 10 g/L were 4.31 ± 0.26 and 5.09 ± 0.33, respectively, which were not only higher than 0.60 ± 0.03 of MPP-1, 0.13 ± 0.01 ($P < 0.05$) of MPP-2a, and 0.20 ± 0.01 ($P < 0.05$) of MPP-3, respectively, but also higher than 1.15 of nonastringent persimmon.[26] These results indicated that the MPP and MPP-4b of Mopan persimmon in this study has potential antioxidant capacities.

CONCLUSION

The MPP and its subfractions showed antioxidant activities *in vitro*, suggesting that they can be used as potential antioxidant which enhances adaptive immune responses. However, the interrelation between the biological activities and antioxidant mechanism is an area for future studies.

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