Advance Journal of Food Science and Technology 6(2): 215-220, 2014
DOI:10.19026/ajfst.6.13
ISSN: 2042-4868; e-ISSN: 2042-4876

Submitted: September 24, 2013 Accepted: October 14, 2013 Published: February 10, 2014

Research Article

Synergistic Antioxidant Activity of Sweet Potato Extracts in Combination with Tea Polyphenols and Pueraria Flavonoid in Vitro

Xiaojuan Liu, Fenglin He, Lichao Zhao, Aimei Zhou and Xin Liu
College of Food Science, South China Agricultural University, Guangzhou 510642, P.R. China

Abstract: The antioxidant activity of Sweet Potato Extracts (SPE) can be enhanced by the presence of these other active antioxidants such as Tea Polyphones (TP) and Pueraria Flavonoid (PF). Since many of these natural antioxidants are consumed together in foods, the potential for synergistic interactions is high in the human diet. The aim of this study was to determine what concentrations and combinations of antioxidants among SPE, TP and PF are capable of producing synergistic antioxidant effects, based on potato-based food products. Solutions of the antioxidant activity of SPE, TP and PF, alone and in different combinations were measured using the stable free radical 2, 2-Diphenyl-1-Picrylhydrazyl (DPPH) and Ferric Reducing Anti-oxidant Power (FRAP) method. A comparison of the antioxidant activity of the combinations of antioxidants to the arithmetic sum of the antioxidant activity of the individual antioxidants was used to calculate the Synergistic Effects (SEs) between the antioxidants. The results showed that all concentrations of TP and PF combination with SPE (1 and 1.5%) could produce significant SEs ($p<0.05$) of DPPH and FRAP for the two or three component mixtures. With the concentration of 1% SPE, 1×10^{-5} g/mL TP and 5×10^{-5} g/mL PF in the three-component mixture, the highest SE of DPPH and FRAP was both detected. The results suggested that the antioxidant property of this combination was substantially superior to the sum of the individual antioxidant effects and these interactions can enhance the antioxidant effectiveness of SPE. The results could guide in the formulation and development of functional food products that have high antioxidant potential.

Keywords: Antioxidant, DPPH, FRAP, sweet potato, synergistic effect

INTRODUCTION

Sweet potato is important dietary source in developing countries. It is considered as a resource of functional foods because of its high contents of physiologically active compounds such as vitamin C, β-carotene, chlorogenic acid, caffeic acid, quercet in and rutin (Guang and Wu, 2006). Currently, antioxidant food has become a research hotspot. The contents of vitamin C and β-carotene are especially high in sweet potato and they are natural antioxidants, so the sweet potato can be made into antioxidant food. But the manufacturing procedures of sweet potato are mostly cooking and frying. Under these conditions, the acceleration of both thermal and oxidative decomposition reactions occurs (Grangdgirard *et al*., 1984), which accelerated loss of vitamin C and β-carotene (Sheehy *et al*., 1994; Liu and Lee, 1998), leading to the decline of antioxidant capacity of the final product (Smith and Ray, 2007). Many studies have shown that some natural antioxidants could be added to food for enhancing its antioxidant function because these antioxidants can protect the functional ingredient from damaged and can also synergistically interact with them (Heo *et al*., 2007). Tea polyphenols and pueraria flavonoid are commonly used in food as natural antioxidants for their good ant oxidation effect (Ma *et al*., 2000; Liu *et al*., 2008a; Cai *et al*., 2009; Guerra *et al*., 2000). It was also found that green tea polyphenols showed strong synergistic ant oxidation activity with other antioxidants, such as vitamin E and vitamin C (Zhou *et al*., 2005; Niki *et al*., 1984).

Antioxidants exist in nature in combination and a combination of different antioxidants might act additively and even synergistically (Fuhrman *et al*., 2000). The synergistic effects for lycopene-β-carotene and lycopene-α-tocopherol mixtures on the 2, 2-Diphenyl-1-Picrylhydrazyl free radical (DPPH) were observed (Liu *et al*., 2008b). Synergistic antioxidant effects of ethanol extracts of Centella asiatica (CE) and α-tocopherol at different ratios using the 2, 2'-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) (ABTS) radical-scavenging capacity was also studied. The results showed that the combined mixture of CE and α-tocopherol could exhibit synergism that subsequently increased the antioxidant activity of the mixture and the synergistic effect was depended on the ratios of the antioxidants. The antioxidant activity of a multi-component system is not only dependent on the type of compounds present and their concentrations, but also on

Corresponding Author: Xin Liu, College of Food Science, South China Agricultural University, Guangzhou 510642, P.R. China

the ratios at which they are mixed (Zanfini *et al*., 2010). Kirakosyan and Mitchell (2010) determined how polyphenolics in fruits interacted in terms of expression of their antioxidant action using the Trolox Equivalent Antioxidant Capacity (TEAC) antioxidant assay and found that the synergism was significantly affected by dose ratios (Kirakosyan and Mitchell, 2010). A recent review summarized the complex aspects of antioxidant reactions using chemical assays such as the 2, 2-Diphenyl-1-Picrylhydrazyl free radical (DPPH) and Ferric Reducing Anti-oxidant Power (FRAP) (Huang *et al*., 2005). It also found that the concentration of compounds and the ratio at which they are mixed are important variables in defining the antioxidant capacity and synergistical type.

Although there are a certain amount of the antioxidants in sweet potatoes, it is necessary that some exogenous antioxidants should be added to enhance the antioxidant ability if the sweet potatoes are made into antioxidant functional foods. The objective of this research was to study the synergistic antioxidant effects of Sweet Potato Extracts (SPE) in combination with Tea Polyphenols (TP) and Pueraria Flavonoid (PF). In this research, methods of DPPH radicals and FRAP were used for estimating the antiradical activity since the determinations of them are rapid and reliable.

MATERIALS AND METHODS

Chemicals: 2, 2-Diphenyl-1-Picrylhydrazyl (DPPH) and TPTZ (2, 4, 6-Tripyridyl-s-Triazine) were purchased from Sigma-Aldrich, USA. Tea polyphenols and pueraria flavonoid were purchased from Fu Zhou Corona Science and Technology Development Co., Ltd., China. All other chemical reagents were of analytical grade and obtained from Shanghai Chemical Reagent Co., China.

Methods: Preparation of sweet potato extracts and antioxidant solutions. The sweet potato was removed the peel and was extracted in 75% ethanol in a ratio of 1:10 by ultrasonic wave for 30 min; the extracts were subsequently filtered through filter paper and then filtered through 0.45 um sterile membrane, stored at -4°C. For preparing stock solution of Tea Polyphenols (TP) and Pueraria Flavonoid (PF), dried powder (10 mg) and dried powder (15 mg) was dissolved in ethanol (10 mL), respectively, then filtered through 0.45 um sterile membrane, stored at -4°C. All procedures were performed under dim light and all stock solutions were stirred at room temperature for 10 min before further use. The composition and concentration of the antioxidant solutions are shown in Table 1.

Table 1: Composition and concentration of individual antioxidants and their mixtures

Trial No.	SPE (%)	TP (10^{-5} g/mL)	PF (10^{-5} g/mL)
A1	0.5	-	-
A2	1.0	-	-
A3	1.5	-	-
A4	2.0	-	-
A5	2.5	-	-
A6	3.0	-	-
B1	-	0.50	-
B2	-	0.75	-
B3	-	1.00	-
B4	-	3.00	-
B5	-	5.00	-
B6	-	10.00	-
C1	-	-	1.0
C2	-	-	3.0
C3	-	-	5.0
C4	-	-	7.0
C5	-	-	9.0
C6	-	-	10.0
M1	1.0	0.50	-
M2	1.5	0.75	-
M3	1.5	1.00	-
M4	2.0	0.50	-
M5	2.0	0.75	-
M6	2.0	1.00	-
M7	1.0	-	1.0
M8	1.5	-	3.0
M9	1.5	-	5.0
M10	2.0	-	1.0
M11	2.0	-	3.0
M12	2.0	-	5.0
M13	1.0	0.50	1.0
M14	1.0	0.50	5.0
M15	1.0	1.00	1.0
M16	1.0	1.00	5.0
M17	1.5	0.75	3.0
M18	2.0	0.50	1.0
M19	2.0	0.50	5.0
M20	2.0	1.00	1.0
M21	2.0	1.00	5.0

DPPH free radical scavenging assay: The radical-scavenging capacity was determined according to the procedure of Mensor *et al*. (2001) with slight modifications. To start the reaction, 2 mL of DPPH (125 umol/L) was added to 2 mL of the antioxidant at room temperature. The mixtures were shaken quickly and then let it rest for about 30 min in the dark and then the absorbance was measured at 540 nm with a UV-1800 PC spectrophotometer (Shanghai US-spectrum Instrument Co., Ltd., China). Each cuvette was removed from the spectrophotometer and incubated at room temperature. To make sure that they were not exposed to light, the cuvettes were covered with an opaque container.

FRAP assay: The Ferric Reducing Anti-oxidant Power (FRAP) was performed as previously described by Benzie and Strain (1996) with slight modifications. The fresh FRAP reagent was prepared daily by mixing acetate buffer (300 mmol/L, pH3.6), TPTZ solution (10 mmol/L in 40 mmol/L HCl) and 20 mmol/L $FeCl_3 \cdot 6H_2O$ solution in proportions of 10:1:1. The mixture was incubated at 37°C for several minutes. 0.1 mL samples dissolved in ethanol were added directly to 3.9 mL of FRAP reagent. The absorbance of the reaction mixture was then measured at 593 nm after 10

min. The calibration curve was plotted by injecting standard solutions of ferrous sulphate at concentrations from 0.1 to 0.5 mmol/L. The results were expressed in mmol Fe^{2+} per litre sample.

Analysis methods:

Calculation of Synergistic Effects (SEs) of antioxidant mixtures: The synergistic effect of the combination of SPE with other antioxidants was calculated as the ratio between the experimental antioxidant capacity of the oxidation reaction versus the theoretical antioxidant capacity. The experimental scavenging capacity of DPPH ($\%ESC_{DPPH}$) was calculated using the following equation (Mensor *et al*., 2001):

$$\%ESC_{DPPH} = 100 - \{[(A\ sample - A\ blank) \times 100] / A\ control\} \quad (1)$$

A_{sample} = The absorbance value of the sample (antioxidant (s) plus DPPH solution)
A_{blank} = The absorbance value of the blank (antioxidant (s) plus ethanol)
$A_{control}$ = The absorbance value of control (DPPH solution plus ethanol)

The theoretical scavenging capacity of DPPH ($\%TSC_{DPPH}$) is the sum of the scavenging capacities of each antioxidant, calculated using the individual scavenging capacity in the following equation (Fuhrman *et al*., 2000; Young *et al*., 2012):

$$\%TSC_{DPPH} = 100 - \{[(100 - ESC_1) /100] \times [(100 - ESC_2) /100] \times [(100 - ESC_3) /100]\} \quad (2)$$

ESC_{1-3} = The percentage ESC of the individual antioxidant

The SE of DPPH was calculated using the following equation (Fuhrman *et al*., 2000):

$$SE\ (DPPH) = ESC_{DPPH}/TSC_{DPPH} \quad (3)$$

where synergism was shown when SE was greater than 1 (SE>1). Likewise, the theoretical ferric reducing anti-oxidant power (TSC_{FRAP}) was calculated using the following equation:

$$TSC_{FRAP} = 100 - \{[(100 - ESC_1) /100] \times [(100 - ESC_2) /100] \times [(100 - ESC_3) /100]\} \quad (4)$$

ESC_{1-3} = The percentage ESC of the individual antioxidant:

$$SE\ (FRAP) = ESC_{FRAP}/TSC_{FRAP} \quad (5)$$

where synergism was shown when SE was greater than 1 (SE>1).

Statistical analysis: All the experiments were performed at least in triplicate. Student's t-test was used for comparison between two means (ESC and TSC), using SPESS (Statistical Package for the Social Sciences, SPESS Inc., Chicago, IL, USA). Differences were considered statistically significant when $p<0.05$ and $p<0.01$.

RESULTS AND DISCUSSION

The antioxidant effect of SPE, TP and PF: The SPE, TP and PF used in this study showed antioxidant activity in a dose-dependent manner in DPPH radical scavenging capacity and FRAP (Fig. 1). As the SPE concentration increased, the DPPH scavenging capacity and FRAP increased. When the concentration of SPE was 2.5%, the DPPH radical scavenging capacity was more than 50% and the value of FRAP was more than 0.07, which indicated that the SPE was very efficient antioxidant. As previously research in our study, we found that the contents of vitamin C and β-carotene was 67 and 7.5 mg/100g in sweet potato, respectively. Vitamin C can directly and rapidly scavenge free radicals and inhibit their formation (Kawther *et al*., 2010). β-carotene quenched singlet oxygen rapidly but its role as a radical-scavenging antioxidant. Low concentrations of vitamin C and β-carotene could react immediately with DPPH (Liu *et al*., 2008b). In another studies, it was reported that there was a significant and moderately strong relationship between antioxidant capacity and β-carotene content. These may be the main reasons that SPE were shown to be antioxidant effects. The correlation between the SPE content and the DPPH radical scavenging capacity, FRAP was also estimated. The highest correlation coefficient (R^2 = 0.997; R^2 = 0.996, respectively) was found, indicating a relatively strong relationship. For the natural antioxidants, TP and PF, showed a linear growth in DPPH scavenging capacity and FRAP values in a dose-dependent manner and the linear relationship between the test concentration range was very good. The R^2 of DPPH scavenging capacity was 0.996 and 0.995, respectively and the R^2 of FRAP was 0.998 and 0.998, respectively. Taking into account of the calculation of Synergistic Effects (SEs) in which the TSC (DPPH) would surpass 100% if the TSC (DPPH) of TP and PF were all more than 40%, we chose the lowest three concentrations of TP and PF for the subsequent SE experiments. Besides, these results could be used to calculate the theoretical value for the subsequent SE experiments.

Synergistic effect of SPE in combination with TP and PF: In order to investigate if synergistic interactions occurred when SPE and TP or PF were mixed, we analyzed two and three component mixtures in which the compounds were combinedin Table 1. To examine the SEs, the DPPH radical scavenging

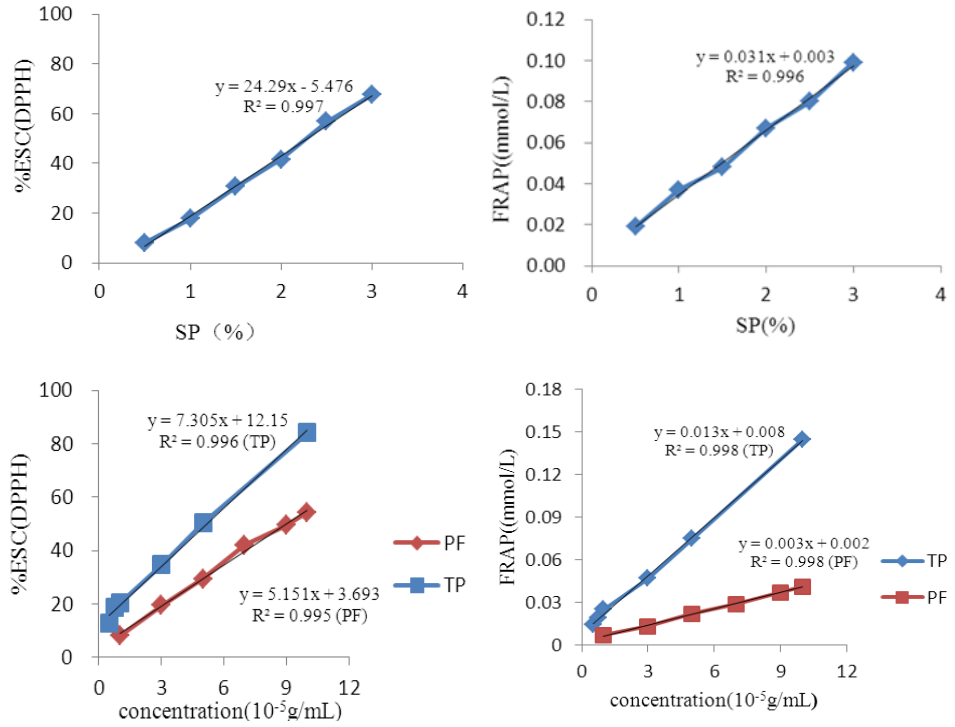

Fig. 1: DPPH radical scavenging capacity and FRAP of SPE, TP and PF at various concentrations

Table 2: The ESC and SE values for two component mixtures (SPE and TP)

Trial No.	%ESC[a] (DPPH)	SE[b] (DPPH)	ESC (FRAP)[a] (nmol/L)	SEb (FRAP)
M1	48.21±0.65	1.35**	0.081±0.001	1.72**
M2	57.77±0.79	1.17**	0.094±0.005	1.50**
M3	74.75±0.56	1.46**	0.120±0.003	1.71**
M4	66.02±4.00	1.10	0.087±0.009	1.21
M5	70.46±4.11	1.14	0.126±0.005	1.34*
M6	76.81±5.46	1.21	0.122±0.005	1.42**

Asterisks denote a significant difference compared with the respective value (*p<0.05, **p<0.01); [a]: Values are the mean (n = 3) ±S.D.; [b]: SE>1: Synergistic effect found, SE<1: No synergistic effect found

Table 3: The ESC and SE values for two component mixtures (SPE and PF)

Trial No.	%ESC [a] (DPPH)	SE[b] (DPPH)	ESC (FRAP)[a] (nmol/L)	SE[b] (FRAP)
M7	54.94±1.06	1.95**	0.069±0.002	1.78**
M8	74.27±0.99	1.48**	0.092±0.003	1.45**
M9	80.30±3.40	1.33*	0.104±0.007	1.36*
M10	65.31±1.27	1.24**	0.079±0.003	1.10
M11	80.83±3.43	1.29*	0.099±0.005	1.14*
M12	86.08±5.71	1.19	0.110±0.002	1.27**

Asterisks denote a significant difference compared with the respective value (*p<0.05, **p<0.01); [a]: Values are the mean (n = 3) ±S.D.; [b]: SE>1: Synergistic effect found, SE<1: No synergistic effect found

capacity and FRAP of SPE, TP and PF were studied. Based on the data obtained from the individual antioxidants, the theoretical values (TSC) were calculated. If the experimental value (ESC) is the same as the theoretical value, then the contribution of the individual antioxidant would be additive. If the ESC is greater than the TSC value, then an interaction happened among the antioxidants, thus displaying synergism. Mathematically speaking, when aratio of

Table 4: The ESC and SE values for three component mixtures (SPE, TP and PF)

Trial No.	%ESC [a] (DPPH)	SEb (DPPH)	ESC (FRAP)[a] (nmol/L)	SE[b] (FRAP)
M13	46.94±0.13	1.050**	0.072±0.003	1.379**
M14	74.41±2.25	1.147*	0.115±0.003	1.706**
M15	53.96±2.08	1.113*	0.083±0.005	1.437*
M16	83.33±1.14	1.217**	0.128±0.005	1.761**
M17	72.92±0.71	1.060*	0.110±0.002	1.432**
M18	77.06±1.95	1.114**	0.119±0.008	1.431*
M19	90.60±3.20	1.016	0.139±0.002	1.415**
M20	81.96±2.27	1.141*	0.126±0.003	1.434**
M21	90.16±1.53	0.936	0.151±0.004	1.454**

Asterisks denote a significant difference compared with the respective value (*p<0.05, **p<0.01); [a]: Values are the mean (n = 3) ±S.D.; [b]: SE>1: Synergistic effect found, SE<1: No synergistic effect found

ESC/TSC>1, it would indicate an SE. The SEs of the combined antioxidants with the SPE, TP and PF were shown in Table 2 to 4.

Table 2 and 3 presented the results obtained from the analysis of two-component mixtures. While the concentrations of antioxidants increased, the DPPH radical scavenging capacity and FRAP increased. In the mixtures of SPE and TP (Table 2), the SEs of DPPH radical scavenging capacity were greater than 1 for all the combinations (M1-M6). However, significant SE ($p<0.05$) was produced only by the mixture M1, M2 and M3, in which the concentration of SPE was 1 and 1.5%. When the concentration of SPE was increased to 2%, the DPPH radical scavenging capacity increased, but the significant ($p<0.05$) SE was not produced. The highest SE of DPPH was detected for M3, in which the concentration of SPE and TP was 1.5% and 1×10^{-5}

g/mL, respectively. The SEs of FRAP were a little difference. The SEs of FRAP were greater than 1 and statistically significant ($p<0.05$) for all the combinations (M1-M6) except M4 in which the concentration of SPE and TP was 2% and 0.5×10^{-5} g/mL, respectively. The highest SE of FRAP was detected for M1, in which the concentration of SPE and TP was 1% and 1×10^{-5} g/mL, respectively. In the mixtures of SPE and PF (Table 3), the SEs of DPPH radical scavenging capacitywere greater than 1 for all the combinations (M7-M12) and statistically significant ($p<0.05$) for all the combinations except M12 in which the concentration of SPE and PF was 2% and 5×10^{-5} g/mL, respectively. The highest SE was detected for M1, in which the concentration of SPE and TP were all the lowest (1%). The growth trend of FRAP values were similar like these but SEs of FRAP were different from the DPPH. The SEs of FRAP were greater than 1 and statistically significant ($p<0.05$) for all the combinations (M7-M12) except M10 in which the concentration of SPE and TP was 2% and 1×10^{-5} g/mL, respectively. The highest SE was also detected for M1. These results of two-component mixtures indicated that concentration and ratio of SPE and TP, SPE and PF could produce a large impact on the antioxidant capacity and synergistic effect.

The antioxidant capacity and SEs of the three-component mixtures were shown in Table 4. The SEs of DPPH radical scavenging capacity were all greater than 1 and statistically significant ($p<0.05$) for M13-M17, in which the concentration of SPE was 1 and 1.5% while the concentration of TP varied from 0.5 to 1×10^{-5} g/mL and the concentration of PF varied from 1 to 5×10^{-5} g/mL. When the concentration of SPE reached to 2% (M18-21), The SEs were all greater than 1 but statistically significant ($p<0.05$) only for M18 and M20, in which the concentration of PF was the lowest (1×10^{-5} g/mL). The value of DPPH radical scavenging capacity was the most and the SE was also greater than 1, but not statistically significant ($p<0.05$) for M19, in which the concentrations of SPE and PF were all the largest (2% and 5×10^{-5} g/mL, respectively) while the concentrations of TP was the 0.5×10^{-5} g/mL. Though the value of DPPH radical scavenging capacity was very high, the SE was not produced for M21, in which the the concentration of SPE, PF and TP were all the maximum. The highest SE of DPPH radical scavenging capacity was detected for M16, in which the concentration of SPE was the lowest (1%) and the concentration of TP and PF were all the highest (1×10^{-5} and 5×10^{-5} g/mL, respectively). The SEs of FRAP were a little of difference from DPPH. The SEs of FRAP were all greater than 1 and statistically significant ($p<0.05$) for all the combinations. The highest SE of FRAP was also detected for M16. These results of three-component mixtures indicated that concentration and ratio of SPE, TP and PF could produce a large impact on the antioxidant capacity and synergistic effect.

CONCLUSION

This study highlighted the importance of the combined mixture of SPE, TP and PF to exhibit synergism that subsequently increased the antioxidant activity of the mixture. There are many factors that might contribute to SEs of mixed antioxidants in a biological system. The concentration and combination ratio of mixed antioxidants are the important factors. It has been shown that specific concentrations and combinations of antioxidants in mixtures were more effective than the corresponding single antioxidants in the DPPH and FRAP. The results showed that all concentrations of TP and PF combination with SPE (1 and 1.5%) could produce significant SEs ($p<0.05$) of DPPH radical scavenging capacity and FRAP for all the two or three component mixtures. The highest SE of DPPH radical scavenging capacity was detected with the concentration of 1.5% SPE and 1.0×10^{-5} g/mL TP while the highest SE of FRAP was detected with the concentration of 1% SPE and 1.0×10^{-5} g/mL TP in the mixtures of SPE and TP. With the concentration of 1% SPE and 1.0×10^{-5} g/mL PF in the mixtures of SPE and PF, the highest SE of DPPH radical scavenging capacity and FRAP was both detected. With the concentration of 1% SPE, 1.0×10^{-5} g/mL TP and 5.0×10^{-5} g/mL PF in the three-component mixture, the highest SE of DPPH radical scavenging capacity and FRAP was both detected. The results indicate that an optimum combination of antioxidants may play a significant role in enhancement of the oxidative status of biological systems. These could guide in the formulation and development of functional food products that have high antioxidant potential.

ACKNOWLEDGMENT

The authors thank the Municipal Science and Technology Program of Guangzhou (The key technologies and industrialization of characteristic preserved fruit safely deep-processing).

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