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Cacao seeds are a "Super Fruit": A comparative analysis of various fruit powders and products

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Abstract

Background: Numerous popular media sources have developed lists of "Super Foods" and, more recently, "Super Fruits". Such distinctions often are based on the antioxidant capacity and content of naturally occurring compounds such as polyphenols within those whole fruits or juices of the fruit which may be linked to potential health benefits. Cocoa powder and chocolate are made from an extract of the seeds of the fruit of *the Theobroma cacao* tree. In this study, we compared cocoa powder and cocoa products to powders and juices derived from fruits commonly considered "Super Fruits".

Results: Various fruit powders and retail fruit products were obtained and analyzed for antioxidant capacity (ORAC (μM TE/g)), total polyphenol content (TP (mg/g)), and total flavanol content (TF (mg/g)). Among the various powders that were tested, cocoa powder was the most concentrated source of ORAC and TF. Similarly, dark chocolate was a significantly more concentrated source of ORAC and TF than the fruit juices.

Conclusions: Cocoa powder and dark chocolate had equivalent or significantly greater ORAC, TP, and TF values compared to the other fruit powders and juices tested, respectively. Cacao seeds thus provide nutritive value beyond that derived from their macronutrient composition and appear to meet the popular media's definition of a "Super Fruit".

Background

Many popular press articles have deemed certain foods as "Super Foods" due to a high purported nutritive value. This trend has crossed over into numerous fruits, fruit juices, and other fruit products wherein the high nutritive value is often based on the antioxidant capacity of these foods. Such fruits are commonly referred to as "Super Fruits" in popular media [1].

Cacao (or cocoa) beans are technically not beans or legumes, but rather the seeds of the fruit of the *Theobroma cacao* tree. The pod shaped fruit is botanically classified as baccate-like (berry-like) and each pod produces approximately 35-50 seeds surrounded by a sweet pulp [2]. The pod and the pulp surrounding the cacao seed in this case constitute the fruit of cacao. After harvest, cacao seeds and their surrounding fruit pulp are typically placed in heaps or boxes and fermented under the influence of naturally occurring microbes that multiply using the sugar from the pulp as an energy source. The seeds are then dried in the sun or in wood fired ovens and shipped to cacao processors. Cacao seeds next have their thin coats removed from the embryonic tissue, which is then roasted, and milled into what is referred to as chocolate liquor. Cocoa powder is produced by mechanically pressing most of the fat (cocoa butter) from the chocolate liquor and thus represents an extract of the cacao fruit's seeds.

Cocoa powder and dark chocolate contain relatively high concentrations of certain polyphenolic compounds, most notably flavanols. Flavanols, especially the monomer epicatechin and oligomers and polymers of flavanols called proanthocyanins, can act as strong antioxidants in food systems [3]. Notably, cocoa flavanols have been associated with numerous health benefits including the stimulation of nitric oxide synthase, improving blood flow and arterial elasticity, decreasing blood pressure and platelet aggregation, and having anti-inflammatory actions [4-6].

Generally, a fruit is thought of as the edible portion of a plant that surrounds its seeds. The fruit pulp of the *Theobroma cacao* pod that surrounds its seeds can be

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consumed; however the vast majority of people have only consumed the seed-derived portion of cacao in the form of cocoa powder or chocolate. For this reason, in the current study we compared cocoa powder and chocolate, products representing the commonly eaten portion of the cacao fruit, with powders and juices from so-called "Super Fruits" on measures of antioxidant activity, as measured by oxygen radical absorbance capacity (ORAC), total polyphenol content (TP), and total flavanol content (TF).

Results and Discussion

Analysis of the fruit powders demonstrated that the antioxidant capacity (Figure 1A) of cocoa powder (634 ± 33 μMTE/g) was significantly greater than blueberry, cranberry, and pomegranate powder on a per gram basis. The total polyphenol content (Figure 1B) of cocoa powder (48.2 ± 2.1 mg/g) appeared to be greater than acai, blueberry, and cranberry powder; however these differences did not reach statistical significance. The total flavanol content (Figure 1C) of cocoa powder (30.1 ± 2.8 mg/g) was significantly greater than all of the other fruit powders tested. There were no other statistically significant differences in antioxidant capacity, total polyphenol, or total flavanol content between any of the other fruit powders tested.

Analysis of fruit products demonstrated that the antioxidant capacity (Figure 2A) of dark chocolate (9911 ± 1079 μMTE/serving) was not significantly greater, on a per serving basis, than pomegranate juice but was greater than that of all other products tested. In contrast, hot cocoa mix had significantly less antioxidant capacity (1232 ± 159 μMTE/serving) than all of the other products tested. The total polyphenol content (Figure 2B) of dark chocolate (991.1 mg/serving) was significantly greater than all of the other products tested, aside from pomegranate juice, on a per serving basis. The total polyphenol content of pomegranate juice was significantly greater than that of cranberry juice; all products tested had higher TP values than hot cocoa mix (57.6 ± 4.9 mg/serving). Finally, the total flavanol content (Figure 2C) of dark chocolate (535.6 ± mg/serving) was significantly greater than cocoa beverage (400 ± 39.5 mg/serving) on a per serving basis and both dark chocolate and cocoa beverage had significantly greater total flavanol content than hot cocoa mix, acai, blueberry, cranberry, and pomegranate juice.

To limit sampling error with each analysis method, each brand of fruit powder and product was prepared and analyzed in triplicate. There was excellent agreement in ORAC, TP, and TF values within replicates of all brands tested. The largest coefficient of variation in ORAC was 0.095 (data not shown) for replicated tests of cocoa powder. The largest coefficient of variation in TP content was 0.085 (data not shown) for replicated tests of blueberry powder. The largest coefficient of variation in TF content was 0.088 (data not shown) for replicated tests of pomegranate powder. In contrast to the lack of methodological variability, there were significant differences in ORAC, TP, and TF values between brands of some fruit powders and, in particular, fruit products. As an example, statistically significant differences in ORAC, TP, and TF between brands of acai juice are demonstrated in Figure 3A, B and 3C.

The results of the current study demonstrate that cocoa powder has equivalent or significantly higher in vitro antioxidant activity, as measured by ORAC values, compared to the tested fruit powders. Similarly, the TP content of cocoa powder was equivalent to that of the fruit powders and its TF content was significantly higher than that of all the fruit powders tested. In addition, on a per serving basis, dark chocolate had a significantly higher ORAC value and TP content than all of the fruit juices other than pomegranate juice. The ORAC value and TP content reported for dark chocolate in the current study are consistent with previously published results [7]. These results are also in keeping with a prior study which demonstrated greater ORAC values and TP content in pomegranate juice compared to the other fruit juices examined in the current study [8]. Moreover, dark chocolate and water-based beverages made with natural cocoa had greater TF content than the tested fruit juices. In contrast, hot cocoa mix had significantly lower ORAC and TP values than all of the fruit juices.

It is important to note that the cocoa powders, cocoa beverages, and dark chocolates used in this study all contained natural (or non-alkalized) cocoa. In contrast, the hot cocoa mixes were made with alkalized or so called dutched, cocoa. Alkalinization is used to mellow the flavor of cocoa [9], however the process has been shown to destroy polyphenolic compounds [10] and is likely responsible for the significant differences in ORAC, TP, and TC values observed between hot cocoa mix and the other cocoa products. The extent of polyphenol destruction is proportional to the degree of alkalization and change in the water extractable pH of the resulting powder [10]. Therefore, consumers should be aware that antioxidant capacity and polyphenol content may be severely diminished in alkalized cocoa powder and products made with alkalized cocoa.

While all of the fruit powders and fruit juices analyzed in this study have been previously referred to as Super Fruits in the popular media, the results of this study clearly demonstrate that not all brands of powders or juices from any particular fruit have the same antioxidant capacity or chemical composition. Current FDA

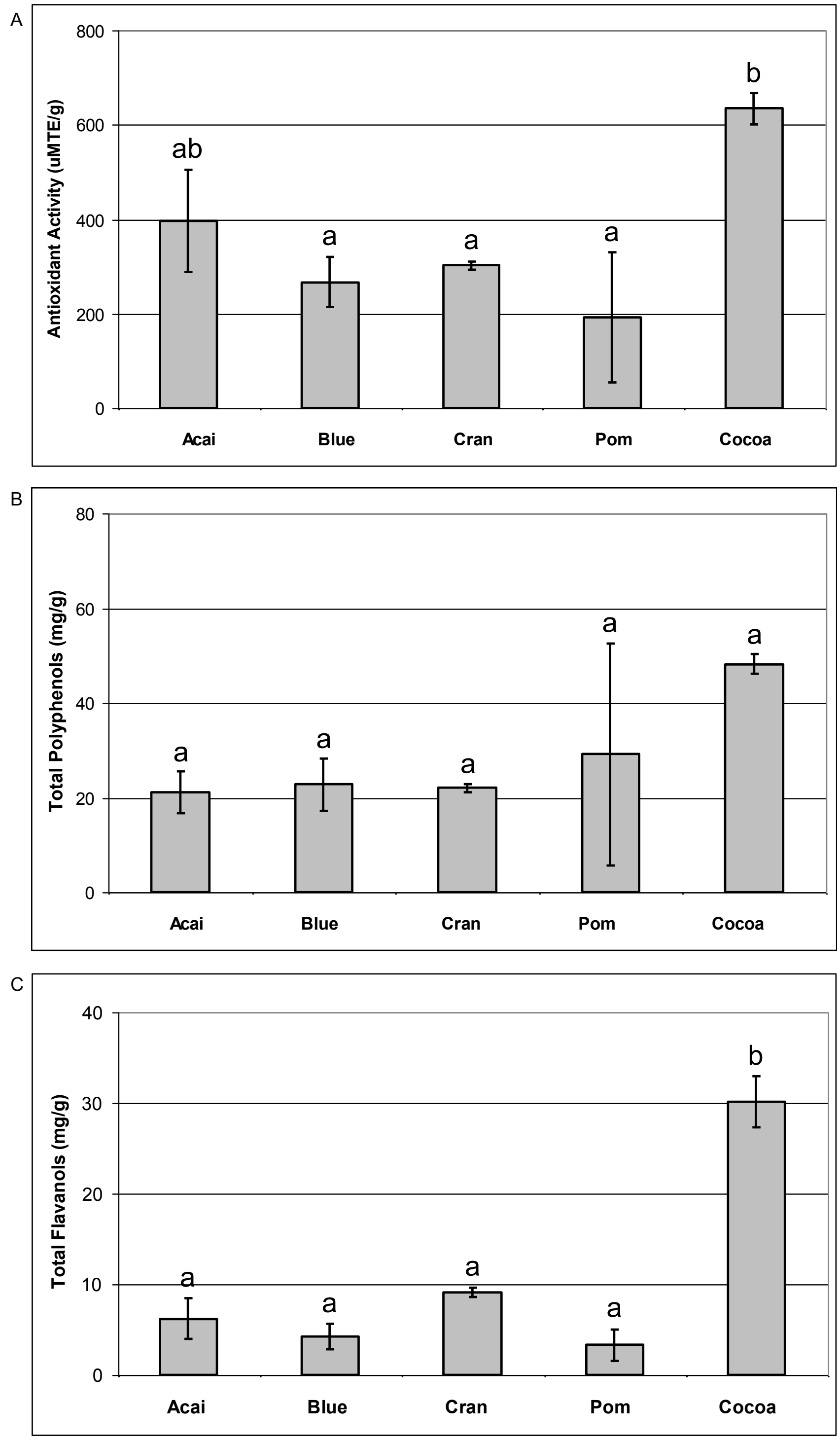

Figure 1 Antioxidant capacity and polyphenol and flavanol content of various fruit powders. A. ORAC antioxidant capacity per gram of various fruit powders. Acai, acai powder; Blue, blueberry powder; Cran, cranberry powder; Pom, pomegranate powder; Cocoa, cocoa powder. Data are expressed as the mean ± SEM. Columns not sharing superscripts are significantly different, $p > 0.05$. B. Total polyphenol content measured in gallic acid equivalents per gram of various fruit powders. Acai, acai powder; Blue, blueberry powder; Cran, cranberry powder; Pom, pomegranate powder; Cocoa, cocoa powder. Data are expressed as the mean ± SEM. Columns not sharing superscripts are significantly different, $p > 0.05$. C. Total flavanol content per gram of various fruit powders by the DMAC method. Acai, acai powder; Blue, blueberry powder; Cran, cranberry powder; Pom, pomegranate powder; Cocoa, cocoa powder. Data are expressed as the mean ± SEM. Columns not sharing superscripts are significantly different, $p > 0.05$.

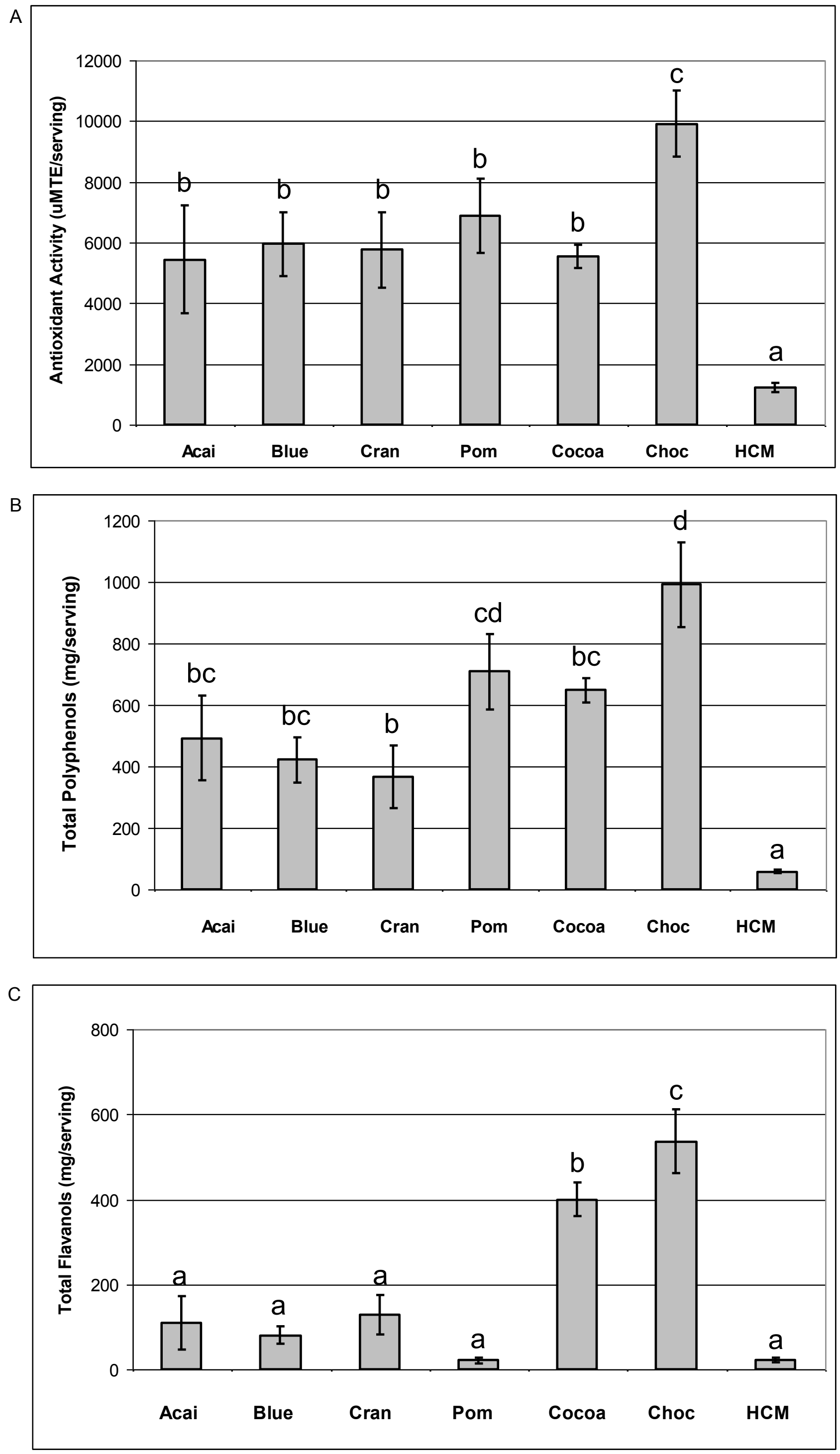

Figure 2 Antioxidant capacity and polyphenol and flavanol content of various fruit products. A. ORAC antioxidant capacity per serving of various fruit products. Acai, acai powder; Blue, blueberry powder; Cran, cranberry powder; Pom, pomegranate powder; Cocoa, cocoa beverage; Choc, dark chocolate; HCM, hot cocoa mix. Data are expressed as the mean ± SEM. Columns not sharing superscripts are significantly different, $p > 0.05$. B. Total polyphenol content measured in gallic acid equivalents per serving of various fruit products. Acai, acai powder; Blue, blueberry powder; Cran, cranberry powder; Pom, pomegranate powder; Cocoa, cocoa beverage; Choc, dark chocolate; HCM, hot cocoa mix. Data are expressed as the mean ± SEM. Columns not sharing superscripts are significantly different, $p > 0.05$. C. Total flavanol content per serving of various fruit products by the DMAC method. Acai, acai powder; Blue, blueberry powder; Cran, cranberry powder; Pom, pomegranate powder; Cocoa, cocoa beverage; Choc, dark chocolate; HCM, hot cocoa mix. Data are expressed as the mean ± SEM. Columns not sharing superscripts are significantly different, $p > 0.05$.

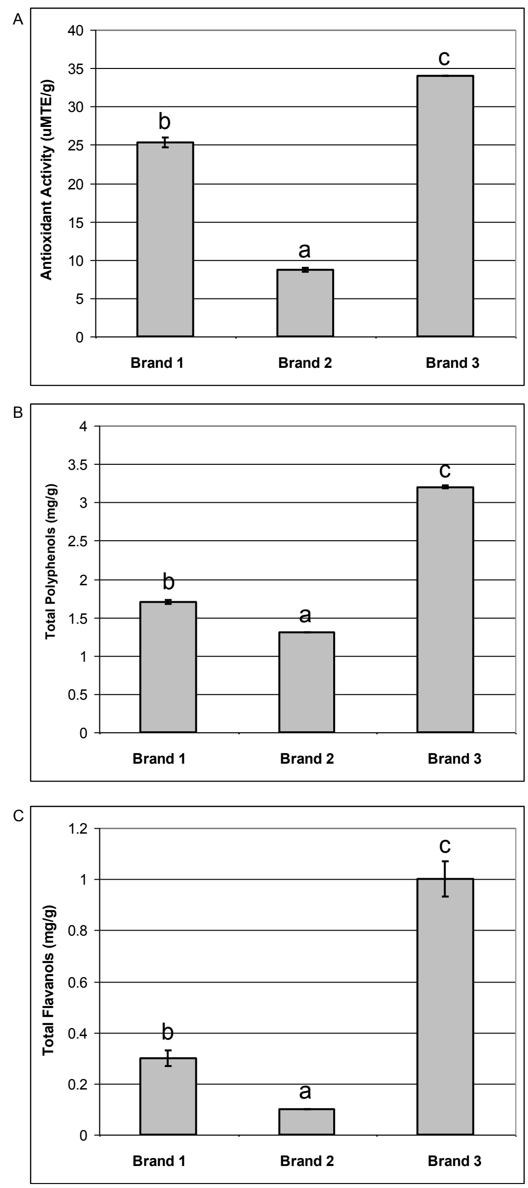

Figure 3 Differences in antioxidant capacity and polyphenol and flavanol content between brands of acai juice. A. ORAC antioxidant capacity per gram of individual acai juice brands. Data are expressed as the mean ± SEM. Columns not sharing superscripts are significantly different, $p > 0.05$. B. Total polyphenol content measured in gallic acid equivalents per gram of individual acai juice brands. Data are expressed as the mean ± SEM. Columns not sharing superscripts are significantly different, $p > 0.05$. C. Total flavanol content per gram of individual acai juice brands by the DMAC method. Data are expressed as the mean ± SEM. Columns not sharing superscripts are significantly different, $p > 0.05$.

regulations do not require that antioxidant capacity and/or polyphenol content be provided on food labels. Inclusion of this information, as has been suggested previously [10], could assist consumers in differentiating between brands and in making healthier food choices.

Conclusions

In summary, natural cocoa powder and dark chocolate have significantly greater TF values than the other fruit powders and juices tested, respectively. The high concentration of flavanols, as well as other flavonoid compounds not tested in this study, contributes directly to the observed TP and ORAC values of natural cocoa and dark chocolate. Cocoa powder thus provides nutritive value beyond that derived from its macronutrient composition. Based on this criterion, and borrowing terminology from popular media, cacao seeds should be considered a "Super Fruit" and products derived from cacao seed extracts, such as natural cocoa powder and dark chocolate, as "Super Foods". In contrast, products made with alkalized cocoa have relatively low ORAC, TP and TF values. It is important to note that ORAC, TP and TF values varied significantly between some brands of fruit powders and fruit juices. Therefore, consumers should be aware that brands of cocoa and fruit powders and products may differ substantially in their nutritive value.

Methods

Sample Collection and Preparation

Materials selected for this study included commercially available fruit powders [acai, blueberry, cranberry, pomegranate], natural (non-alkalized) cocoa, 100% non blended fruit juices [acai, blueberry, cranberry, pomegranate], natural cocoa beverage, solid dark chocolate (60-63% cacao), and hot cocoa mix. All powders were obtained via food ingredient vendors. All juices, solid dark chocolate, and hot cocoa mix were obtained at retail and analyzed in the form sold to consumers, ie. hot cocoa mixes were not combined with milk or water prior to analysis. Cocoa beverage was produced by combining 240 ml water, 25 g sugar, 1 g salt, and 12 g of natural cocoa powder and was designed to have a composition similar to that of the other fruit juices. One cup (240 ml) was considered a typical single serving of fruit juice or cocoa beverage, 40 g was considered a typical single serving of dark chocolate, and 28 g was considered a typical single serving of hot cocoa mix. Three brands were selected per fruit powder and fruit product. Each brand was analyzed for ORAC, TP, and TF in triplicate.

Antioxidant Capacity Analysis

Sample ORAC values were quantified by measuring the fluorescence of fluorescein in the presence of 2,2'-azobis (2-amidinopropane) dihydrochloride (AAPH) [11,12]. Quantitation was based on a trolox (6-hydroxy,2,5,7,8-tetramethylchroman-2-carboxylic acid) standard calibration curve, and results are reported as μm trolox equivalents per gram of sample for fruit powders and μm trolox equivalents per serving for fruit products.

Total Polyphenol Assay

Sample total polyphenol content was determined colorimetrically at 755 nm using Folin-Ciocalteu reagent as described by Singleton et al. [13]. Quantitation was based on a gallic acid standard calibration curve. Total polyphenols are reported as gallic acid equivalents per gram of sample for fruit powders and gallic acid equivalents per serving for fruit products.

Total Flavanol Assay

Sample total flavanol content was determined colorimetrically at 640 nm using 4-dimethylaminocinnamaldehyde (DMAC) as described by Payne et al. [14]. Quantitation was based on a procyanidin B-2 standard calibration curve, and results are reported as mg/g of total flavanols per gram of sample for fruit powders and mg per serving for fruit products.

Statistical Analysis

Fruit powders and products were compared using a one-way ANOVA with brand or type of fruit powder or product as the independent variable and ORAC, TP, and TF as dependent variables. The least square mean for each type of fruit powder or product was calculated from the mean value of the three brands used. Differences between brands or types of fruit powders or products were identified by a post hoc analysis conducted using Fisher's LSD. Analyses were conducted using The Statgraphics Centurion software purchased from Manugistics, Inc., Rockville, MD, 20852, USA.

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Authors' contributions

AGP, JM, and DLM conceived and designed the study. WJH and MJP carried out the chemical assays. SJC performed the data analysis and drafted the manuscript. LH performed the statistical analysis. All authors read and approved the final manuscript.

Competing interests

SJC is a paid contractor for The Hershey Company. AGP, WJH, MJP, JM, LH, and DLM are employees of The Hershey Company.

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