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Stability of Cocoa Antioxidants and Flavan-3-ols over Time

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Several recent reports have been published indicating that the antioxidant activity of olive oil and tea leaves is not stable over product shelf lives of about one year. We have measured the antioxidant activity, total polyphenols, flavan-3-ols monomers, and procyanidin levels in milk and dark chocolate, in cocoa powder, and in cocoa beans. Results show that for the cocoa products studied, antioxidant activity, and flavan-3-ol levels are stable over typical shelf lives of one year under controlled storage and over 2 years in ambient storage in the laboratory. We also show that 80 year old cocoa powder and 116 year old cocoa beans still show very high levels of antioxidant activity and flavan-3-ol content.

KEYWORDS: Flavan-3-ol; antioxidant; epicatechin; catechin; cocoa; chocolate; procyanidins

INTRODUCTION

The levels of flavanols found in cocoa powder and dark chocolate are among the highest dietary sources of these compounds in foods or beverages (*1*). Studies have shown that, on a weight basis, naturally processed, commercially available cocoa powder contains some of the highest levels of flavan-3-ols, 34.6 ± 0.68 mg/g (*2*), followed by baking chocolate, dark chocolate, and the other cocoa containing foods or beverages (*3*, *4*). It has also been shown that general indicators of antioxidant activity, such as ORAC and total polyphenols but also the flavan-3-ols are very closely correlated to the amount of cocoa solids in foods or beverages (*3*−*5*). The benefits that cocoa powder and chocolate have for cardiovascular health have been reviewed (*6*−*8*). Evidence for these benefits comes from epidemiological surveys and from interventional feeding trials in humans. The main molecules thought to be responsible for at least some of these health benefits are the low molecular weight flavan-3-ols, especially (−)-epicatechin and dimers (*9*, *10*).

There have been two recent reports that show that the antioxidant levels in liquid or dry products can change as products age (*11*, *12*). Baiano et al. (*11*) showed that the antioxidant activity of olive oil is closely associated with the level of olive oil phenolic compounds, whereas the oxygen radical scavenging activity as measured by 2,2′-azinoethylbenzthiazoline-6-sulfonic acid (ABTS) activity can change and in fact decreases with the time of storage of olive oil. In a second study, Freidman et al. (*12*) found that dry green tea leaves when stored at 20 °C lost significant amounts of epigalloepicatechin gallate (EGCG) and epicatechin gallate (ECG), but epicatechin is virtually stable during the same period. These reports have gained attention in both the popular and food nutrition press because of the widespread consumer interest in natural antioxidants, especially the polyphenols, which are potential contributors to human health and might be lost during the storage of food or beverages.

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We report here the stability of cocoa flavan-3-ols and their associated antioxidant activities in milk and dark chocolate, cocoa powders, and cocoa beans. We provide evidence that cocoa antioxidant activity, total extractable polyphenols, flavan-3-ol monomers, and procyanidins in cocoa powder and chocolate are very stable over time. Data comes from controlled shelf life studies of samples of milk chocolate stored for one year, analysis of a dark chocolate used as laboratory standards stored at room temperature for over two years, from the analysis of several cocoa powders with one over 80 years old, and from 116 year old cocoa beans.

ANALYTICAL METHODS

Samples. The 6 samples of the two milk chocolates used in the 12 month shelf life study were both commercially available products from The Hershey Company. One milk chocolate called Milk Chocolate 1 was the Antioxidant Milk Chocolate Bar and the other milk chocolate called Milk Chocolate 2 was the Whole Bean Chocolate Bar. The dark chocolate used in stability tests as a laboratory test standard was a commercially available Hershey's Special Dark Chocolate. All cocoa powders used in stability tests were commercially available Natural Hershey's Cocoa that were stored under various conditions described later in this article. The historical cocoa bean sample exhibited at the 1893 Columbia Exposition was a gift of the Department of Economic Botany at the Field Museum in Chicago, IL.

Methods. All samples were analyzed for oxygen radical absorbance capacity (ORAC), total polyphenols (as gallic acid equivalents), and the sum of the flavan-3-ol; catechin and epicatechin with some samples were also analyzed for procyanidins. ORAC is a widely used fluorescent method for assessing antioxidant capacity in biological samples. The current method allows for the determination of lipophilic and hydrophilic antioxidant capacities. It is based on the inhibition of a peroxy-radical induced oxidation initiated by the thermal based decomposition of azo compounds such as AAPH using fluorescein as a fluorescent probe and Trolox as a standard substrate (*13*). The total polyphenol colorimetric assay was initially developed as method for the measurement of proteins focusing on the reagent's ability to react with hydroxyl substituents and later adapted by Singleton and Rossi to measure phenolic compounds in wine (*14*). It is widely used and is a measure of reducing capacity. Total

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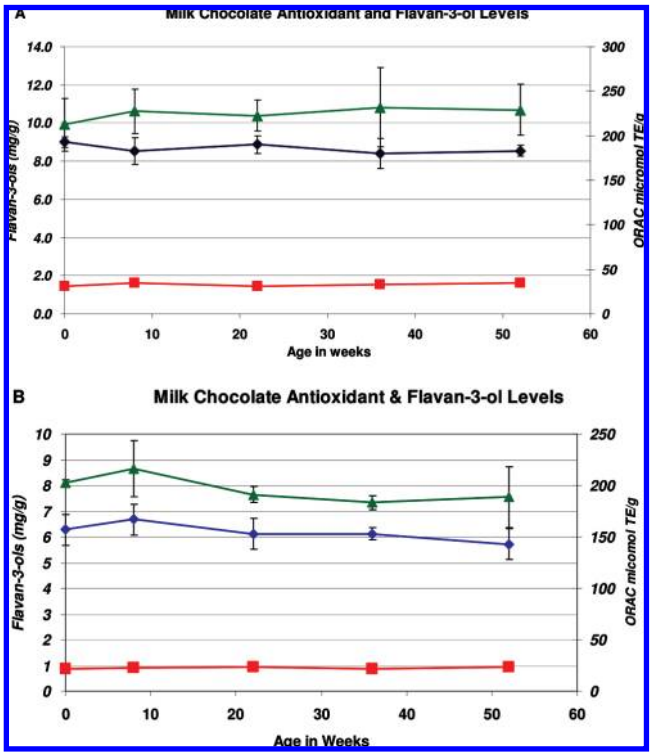

Figure 1. Level of ORAC antioxidant activity and flavan-3-ol as monomers and as total procyanidins in two milk chocolates. (**A**) Milk chocolate 1. (**B**) Milk chocolate 2.

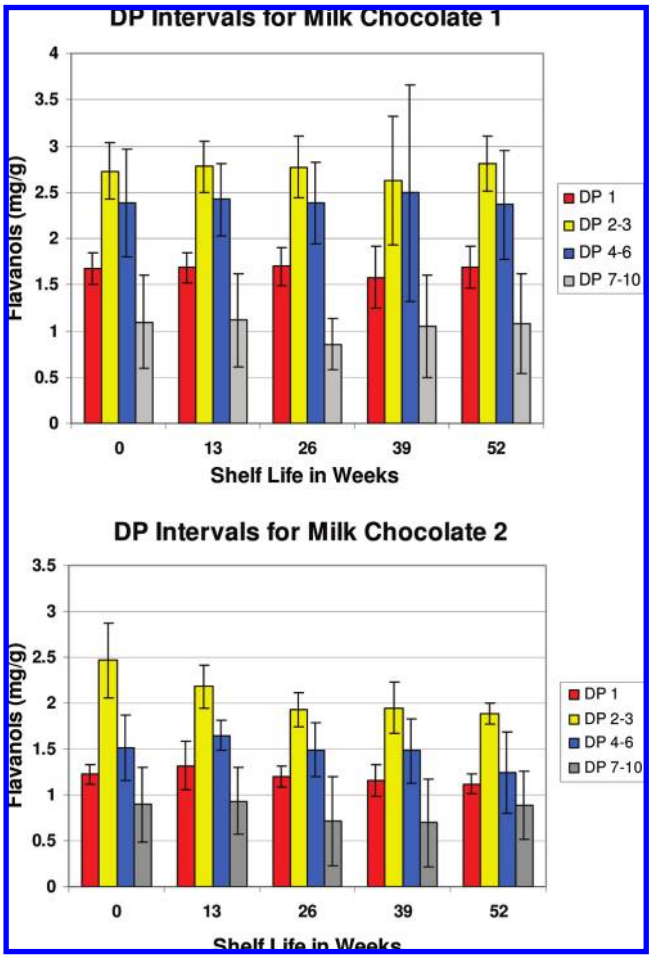

Figure 2. Intervals of degrees of polymerization for the aged milk chocolate bars shown in **Figure 1**.

polyphenols were expressed using gallic acid as the standard. The flavan-3-ol monomers, catechin and epicatechin, were determined using an HPLC method with fluorescence detection previously described by Nelson and Sharpless (*15*). The procyanidin method used for the analysis of the samples was that of Gu et al. (*16*).

RESULTS AND DISCUSSION

Controlled Shelf Life Studies with Two Milk Chocolates. The two nationally distributed milk chocolates previously described were stored for 1 year under conditions designed to replicate retail storage at temperatures ranging from 18 to 24 °C. The chocolates were measured at successive intervals for ORAC, total polyphenols, flavan-3-ol monomers (epicatechin and catechin), and total procyanidins. The data in **Figure 1** show the results of shelf life testing over 12 months for ORAC activity, for flavan-3-ol monomers, and for total procyanidins for the two milk chocolates. Error bars for each point represent the standard deviation of the mean of three measurements. **Figure 2** provides a more detailed picture of the procyanidin content of these two samples. The results indicate that the levels of antioxidant activity, of flavan-3-ol monomers, of flavanol oligomers of various sizes, and of total procyanidins found in both of the milk chocolate bars tested, are very stable over one year of typical retail conditions. In data not shown, the level of total polyphenols was also stable within standard deviations for both milk chocolates over one year of storage. The data in **Figure 2** also indicates that there is no significant polymerization of low molecular weight material into higher molecular weight material during the time of storage.

Testing of Laboratory Standard Cocoa Powder and Dark Chocolate. Figure 3 shows ORAC levels for a commercially available cocoa powder and the total polyphenol level of a commercial dark chocolate. The cocoa powder and the dark chocolate used as internal standards in our analytical laboratory were stored at ambient laboratory temperatures, which typically range between 20 to 26 °C. It is apparent that the ORAC of the cocoa powder and the total polyphenol content of the dark chocolate are essentially constant over a period in excess of 800 days, and these values are within the range of variation of the measurement.

Stability of Antioxidant Chemistry and Flavanols in Cocoa Powders and Beans. Table 1 shows the results of three commercial lots of cocoa powder purchased in 2004, with single samples of cocoa powder obtained locally. Single samples were measured from the 1982 and 1929 cocoa powders because there was limited material available. The 1982 and 1929 samples were in the continuous possession of The Hershey Company and stored under office conditions, while the 2004 and 2008 samples were purchased from local retail grocery stores in the Hershey area. All samples were measured for ORAC, total polyphenols, and flavan-3-ol monomers (epicatechin plus catechin). The values for ORAC, total polyphenols, and monomers for the 2008, 1982, and 1929 cocoa samples are within the standard deviation of the mean of the 2004 commercial cocoa samples. Also shown in **Table 1** is the analysis of fermented Guatemala cocoa beans that were displayed at the 1893 Chicago Exposition. The ORAC, TP, and the flavan-3-ol monomer levels are well within the levels expected for cocoa beans. The results with 27- and 80-year old cocoa powders and with the 116 year old cocoa beans indicate that the antioxidant activity and the levels of the small molecular weight flavan-3-ols remain essentially the same over time.

The results seen here are not at odds with the results of Friedman et al. (*12*) who observed that EGCG and ECG are both lost from tea leaves over time, whereas epicatechin is not lost. We too find that flavan-3-ol monomers are quite stable over

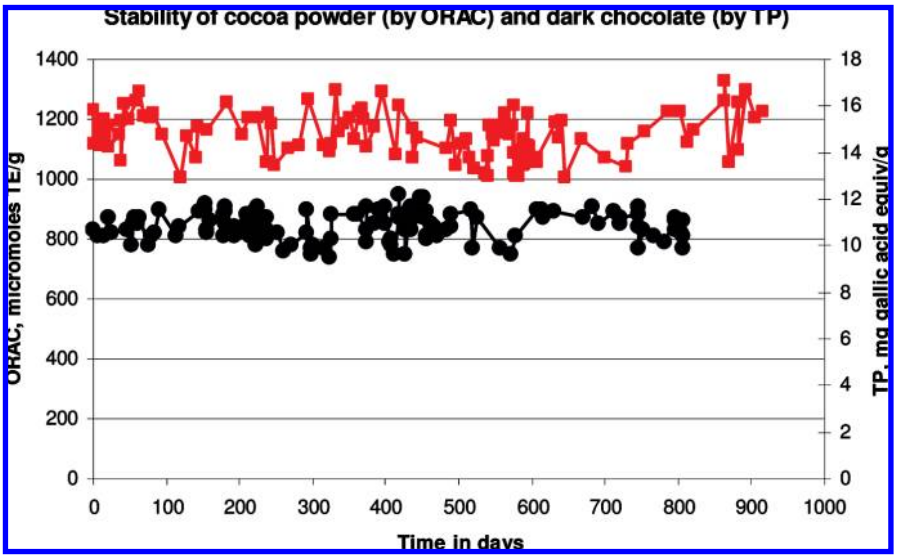

Figure 3. Antioxidant activity of cocoa powder and total polyphenols of dark chocolate measured over time.

Table 1. Stability of Antioxidant Activity, Total Polyphenols and Flavanol Content of Cocoa Powders and Beans over Time[a]

samples	year	ORAC (μmol TEeq/g)	total polyphenols (mg/g)	flavan-3-ol monomers (mg/g)
cocoa powder	2008	797 ± 31	58 ± 1	2.66
	2004[b]	875 ± 93	60.2 ± 4.5	2.79 ± 1.23
	1982	947	56.6	2.82
	1929	796	55.5	1.78
cocoa beans	1893	840	61.7	1.95

[a] All data are reported on a fat-free basis. [b] Samples purchased and analyzed in 2004 and published in ref *3* by Miller et al.

time. This suggests that the gallated flavan-3-ols may be more susceptible to oxidation reactions compared to epicatechin it self. The results also indicate that cocoa flavanols appear to be stable in milk or dark chocolate (**Figures 1** and **3**) and in cocoa powders (**Figure 3** and **Table 1**) or cocoa beans (**Table 1**). This data does not speak to the stability of liquid cocoa containing products such as chocolate syrup or chocolate milk as these measurements were beyond the scope of the present study.

In summary, the results reported here demonstrate the stability of cocoa antioxidants measured by ORAC, total polyphenols, or flavan-3-ol compounds and epicatechin, catechin, and their oligomers/polymers. These results indicate that unsubstituted flavan-3-ols (e.g., epicatechin, procyanidin oligomers, and procyanidins polymers) are very stable under representative commercial retail and room storage conditions in the chocolates, cocoa powders, and cocoa beans studied and agree with the results of Friedman et al. (*12*) who studied epicatechin in tea.

ABBREVIATIONS USED

ORAC, oxygen radical absorbance capacity; Epi, epicatechin; Cat, catechin; ABTS, 2,2′-azinobis(3-ethylbenzothiazoline-6 sulfonic acid assay; EGCG, epigallocatechingallate; ECG, epicatechingallate; TP, total polyphenols.

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