

## Antioxidant Activity and Polyphenol and Procyanidin Contents of Selected Commercially Available Cocoa-Containing and Chocolate Products in the United States

KENNETH B. MILLER,<sup>†</sup> DAVID A. STUART,<sup>†</sup> NANCY L. SMITH,<sup>‡</sup> CHANG Y. LEE,<sup>‡</sup>  
NANCY L. MCHALE,<sup>§</sup> JUDITH A. FLANAGAN,<sup>§</sup> BOXIN OU,<sup>§</sup> AND  
W. JEFFREY HURST<sup>\*,†</sup>

The Hershey Company, P.O. Box 805, Hershey, Pennsylvania 17033-0805; Department of Food Science and Technology, Cornell University, Geneva, New York 14456; and Brunswick Laboratories, 6 Thacher Lane, Wareham, Massachusetts 02571

In the United States, commercially available foods, including cocoa and chocolate, are being marketed with statements referring to the level of antioxidant activity and polyphenols. For cocoa-containing foods, there has been no comprehensive survey of the content of these and other chemistries. A survey of cocoa and chocolate-containing products marketed in the United States was conducted to determine antioxidant activity and polyphenol and procyanidin contents. Commercially available samples consisted of the top market share products in each of the following six categories: natural cocoa, unsweetened baking chocolate, dark chocolate, semisweet baking chips, milk chocolate, and chocolate syrup. Composite samples were characterized using four different methods: oxygen radical absorbance capacity (ORAC), vitamin C equivalence antioxidant capacity (VCEAC), total polyphenols, and procyanidins. All composite lots were further characterized for percent nonfat cocoa solids (NFCS) and percent fat. Natural cocoas had the highest levels of antioxidant activities, total polyphenols, and procyanidins followed by baking chocolates, dark chocolates and baking chips, and finally milk chocolate and syrups. The results showed a strong linear correlation between NFCS and ORAC ( $R^2 = 0.9849$ ), total polyphenols ( $R^2 = 0.9793$ ), and procyanidins ( $R^2 = 0.946$ ), respectively. On the basis of principal component analysis, 81.4% of the sample set was associated with NFCS, antioxidant activity, total polyphenols, and procyanidins. The results indicated that, regardless of the product category, NFCS were the primary factor contributing to the level of cocoa antioxidants in the products tested. Results further suggested that differences in cocoa bean blends and processing, with the possible exception of Dutching, are minor factors in determining the level of antioxidants in commercially available cocoa-containing products in the United States.

**KEYWORDS:** Flavan-3-ol; antioxidant; polyphenol; cocoa; chocolate; procyanidins

### INTRODUCTION

There has recently been a great deal of interest in polyphenolic compounds, particularly flavonoids, as antioxidants. Flavonoids are synthesized by all vascular plants. As a result, fruits, vegetables, nuts, seeds, herbs, spices, and whole grains are sources of flavonoids in our diet (1). Cocoa beans are a concentrated source of antioxidants (2) and flavonoids, with the flavan-3-ols and their derivatives being present in high concentrations (3).

The discovery of flavan-3-ols and their procyanidin polymeric forms in cocoa can be traced back as early as 1909 (4). These

flavan-3-ol compounds were later identified as catechins (5, 6). In 1939, leucoanthocyanin phenolic compounds were identified (7) and, in 1955, fractionation and characterization of these compounds were reported (8). The procyanidins in cocoa have more recently been fractionated into monomers through decamers with even higher forms existing (9). Some of the most abundant polyphenols present in cocoa are the flavan-3-ol monomers, epicatechin and catechin (10), which also serve as building blocks for the polymeric procyanidin forms. The makeup of the polymeric forms is determined by the structure of the flavan-3-ol starter unit and its companion compound. Two primary forms of procyanidins occur in plants: A-type and B-type, which differ by the linkage between the individual compounds. The A-type procyanidins form 4–8 and 2–7 cross-links and can be found in cranberries. The B-type procyanidins form 4–8 cross-links. The B-1–B-4-types differ only in the

\* Author to whom correspondence should be addressed [telephone (717) 534-5145; fax (717) 534-6132; email whurst@hersheys.com].

<sup>†</sup> The Hershey Co.

<sup>‡</sup> Cornell University.

<sup>§</sup> Brunswick Laboratories.

arrangement of their catechin and epicatechin units, with the predominant forms being procyanidin B-1 found in grape, sorghum, cocoa, and cranberry, type B-2 found in apple, cocoa, and cherry, type B-3 found in strawberry and hops, and type B-4 found in raspberry and blackberry (11).

As cocoa makes its way from fresh beans to finished products, the concentration of flavan-3-ols and procyanidins can be affected by a variety of biological and processing conditions. Genetics can cause as much as a 4-fold difference in flavan-3-ol content of fresh cocoa beans (12, 13). Fermentation of fresh cocoa beans, although critical for full cocoa flavor, also tends to decrease the flavan-3-ol content (10, 14–16). Roasting of cocoa beans and treatment of cocoa powder with alkali can also affect the final content of flavan-3-ols and procyanidins (17, 18).

Cocoa-containing and chocolate products have always been favorite foods among consumers. There is some confusion about the terms cocoa and cacao because they have been used interchangeably. For this discussion, cocoa refers to the processed material remaining after partially defatting the cacao beans; cacao refers to the sum of ingredients derived from the cacao bean to include chocolate liquor, cocoa, and cocoa butter. Numerous media reports have focused attention on various health benefits associated with cocoa and chocolate consumption. The evidence increasingly suggests that the flavanol class of polyphenols found abundantly in the cocoa bean is associated with the emerging health benefits. A number of studies have implied that the flavanol content is dependent primarily upon cocoa-processing techniques. This has led to confusion among consumers. To our knowledge, there has been no comprehensive market survey to assess the variation of flavanols in commercially available cocoa and chocolate products. The purpose of this study was to compare antioxidant activity, total polyphenols, and total procyanidins for the leading U.S. market-share products in six cocoa-containing and chocolate product categories.

## MATERIALS AND METHODS

**Sample Collection and Preparation.** Products selected for this study comprised the top three selling products in each of the following product categories: natural cocoa powder, unsweetened baking chocolate, dark chocolate, semisweet baking chips, milk chocolate, and chocolate syrup (Table 1). Market share was determined from sales obtained from Information Resources Inc. from August 2004 (21). Due to a tie in the baking chocolate category, four products were collected. Nineteen different products were sampled, representing seven major manufacturers.

A statistical sampling design was chosen to obtain a composite sample of each product type that represented the average product available at retail to consumers of these products. Efforts to achieve a random sampling included (1) collection of independent production codes, (2) obtaining equal representation of the product samples from three U.S. geographic regions, and (3) blending 6–12 independent samples to make three composite analytical samples for product brand. A minimum of 18 and a maximum of 36 samples of each product listed in Table 1 were purchased at retail. A total of 450 product purchases contributed to the sample set. Products were purchased in the cities in the following three regions: (1) east (Fairfield, NJ; Hershey, PA); (2) midwest (Dillsboro, IN; Plainfield, IL; Minneapolis, MN); and (3) west (Huntington Beach, CA; Phoenix, AZ; Los Angeles CA; Port Orchard, WA; and San Francisco, CA). Products in a composite sample were thoroughly mixed by dry-blending cocoa powder and liquid-blending syrups. In the case of solid chocolates, samples were melted at 50 °C and liquid-blended. All composite samples were then stored frozen at 0 °C before extraction and analysis. All samples were coded before submission for analysis.

**Table 1.** Top-Selling Cocoa/Chocolate Products in Six Categories

product category	manufacturer/brand <sup>a</sup>	lots collected <sup>b</sup>
natural cocoa powder	Ghirardelli	18
	Hershey's	36
	Nestle Toll House	18
unsweetened baking chocolate	Baker's	18
	Ghirardelli Premium bar	18
	Hershey's	36
	Nestle	18
dark chocolate	Hershey's Special Dark	36
	Lindt Excellence (70% cocoa)	18
	Dove Promises	18
semisweet chocolate baking chips	Ghirardelli Premium	18
	Hershey's semisweet	36
	Nestle Toll House Morsels	18
milk chocolate	Hershey's	36
	Dove Promises	18
	Lindt Excellence extra creamy	18
chocolate syrup	Hershey's	36
	Kroger	18
	Nestle Nesquik	18

<sup>a</sup> Manufacturers are listed alphabetically within product category. Product order in this table has no relationship to the order shown in Table 2 or Figure 1. <sup>b</sup> Total lots collected were used to create three equal subgroups that were combined to yield three composite samples for each product type.

**Sample Analysis.** All composite samples were analyzed in duplicate for fat, nonfat cocoa solids (NFCS), oxygen radical absorbance capacity (ORAC), total polyphenols (as gallic acid equivalents, GAE), and total procyanidins. A subset of composite samples was also analyzed for vitamin C equivalence antioxidant capacity (VCEAC). NFCS determination is a gravimetric method based on an exhaustive extraction of the sample with a variety of lipophilic solvents to eliminate the lipid content (22). Total fat was determined by Soxhlet extraction (23). The ORAC is a widely used fluorescence 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 (24, 25). The total polyphenol colorimetric assay was initially developed as a method for the measurement of proteins focusing on the reagent's ability to react with hydroxyl substituents and later adapted by Singleton to measure phenolic compounds in wine (26). It is widely used and is a measure of reducing capacity. Total polyphenols were expressed using gallic acid as the standard. Procyanidins were measured by an HPLC method based on the separation of the flavan-3-ol oligomers (27). The final assay, VCEAC, uses the reaction of free radicals with blue-green ABTS using vitamin C as the standard with the resulting antioxidant activity being equivalent to a specific vitamin C concentration (28).

**Statistical Analysis.** Linear correlation of NFCS with ORAC, total polyphenols, and procyanidins, respectively, was analyzed using a force fit through the origin (NFCS = 0, variable = 0). Analysis of the combined data set was performed using standardized principal component analysis (PCA) extracting all components. PCA reduces the dimensionality of a set of variables by constructing uncorrelated linear combinations of data. The combinations are computed so that the first component, PC-1, accounts for the largest amount of the data set variance and is the major axis of points in a *p*-dimensional space. The successive components, PC-2, PC-3, PC-4, etc., account for decreasing amounts of the remaining variance in the data set. The Statgraphics Principal Components Analysis program was purchased from Manugistics, Inc., Rockville, MD.

## RESULTS

**Antioxidant Activity, Total Polyphenols, and Procyanidins.** Analytical data for the 19 product samples tested are

**Table 2.** Analysis of Selected U.S. Cocoa-Containing and Chocolate Products<sup>a</sup>

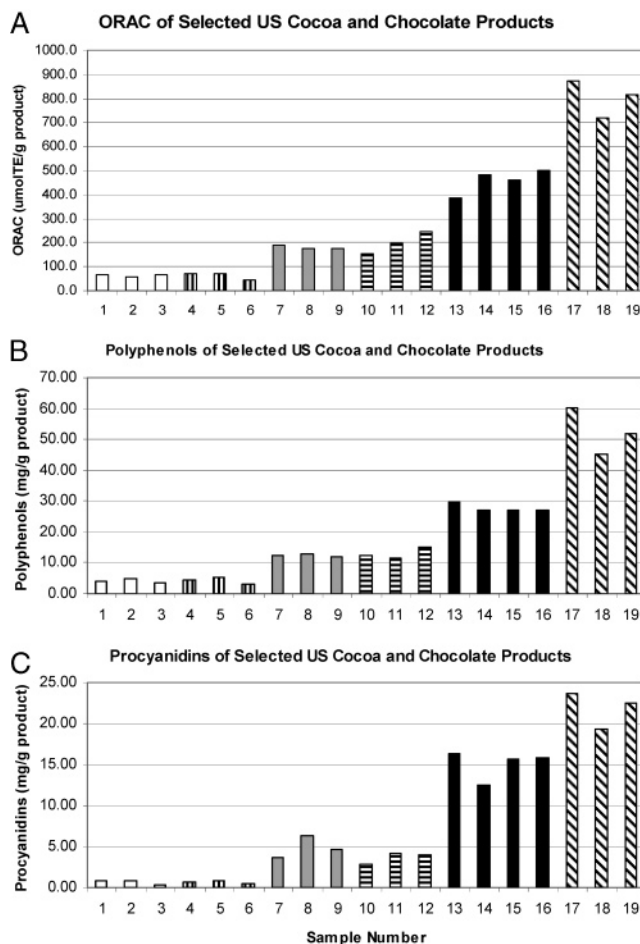
no.	code <sup>b</sup>	% NFCS	% fat	ORAC ( $\mu\text{mol}$ of TE/g)	polyphenols (mg/g of GAE <sup>c</sup> )	procyanidins (mg/g)
1	CS-1	7.3	1.2	66.7 $\pm$ 8.7	4.05 $\pm$ 0.05	0.91 $\pm$ 0.16
2	CS-2	6.6	0.8	57.5 $\pm$ 6.0	4.79 $\pm$ 0.21	0.89 $\pm$ 0.36
3	CS-3	4.8	0.7	65.7 $\pm$ 3.1	3.66 $\pm$ 0.06	0.37 $\pm$ 0.06
4	MC-1	7.2	29.30	72.0 $\pm$ 2.6	4.50 $\pm$ 0.08	0.71 $\pm$ 0.06
5	MC-2	6.4	31.36	72.3 $\pm$ 5.5	5.38 $\pm$ 0.12	0.90 $\pm$ 0.39
6	MC-3	4.9	36.98	41.7 $\pm$ 1.2	3.25 $\pm$ 0.10	0.43 $\pm$ 0.46
7	SC-1	17.0	29.00	190.3 $\pm$ 16.5	12.50 $\pm$ 0.47	3.70 $\pm$ 0.56
8	SC-2	15.2	29.84	174.0 $\pm$ 23.6	12.88 $\pm$ 0.12	6.29 $\pm$ 0.22
9	SC-3	18.6	27.80	177.3 $\pm$ 16.8	11.76 $\pm$ 0.52	4.69 $\pm$ 0.27
10	DC-1	20.0	29.97	151.7 $\pm$ 33.3	12.30 $\pm$ 0.10	2.78 $\pm$ 0.50
11	DC-2	20.7	33.37	195.7 $\pm$ 3.1	11.73 $\pm$ 0.35	4.10 $\pm$ 0.23
12	DC-3	29.5	40.70	246.0 $\pm$ 32.7	14.88 $\pm$ 0.37	4.06 $\pm$ 0.47
13	BC-1	49.1	52.40	384.0 $\pm$ 14.1	29.70 $\pm$ 0.43	16.33 $\pm$ 1.38
14	BC-2	46.6	53.08	481.0 $\pm$ 32.2	27.18 $\pm$ 0.21	12.57 $\pm$ 0.88
15	BC-3	44.8	53.09	462.7 $\pm$ 64.7	27.06 $\pm$ 2.33	15.62 $\pm$ 3.62
16	BC-4	49.4	51.55	499.0 $\pm$ 35.5	26.91 $\pm$ 2.20	15.84 $\pm$ 1.68
17	CP-1	85.2	14.80	875.0 $\pm$ 93.0	60.20 $\pm$ 4.54	23.71 $\pm$ 4.04
18	CP-2	72.2	27.80	720.0 $\pm$ 33.6	45.30 $\pm$ 1.16	19.28 $\pm$ 0.93
19	CP-3	87.3	12.70	816.3 $\pm$ 37.6	51.70 $\pm$ 0.57	22.44 $\pm$ 2.41

<sup>a</sup> Results expressed mean  $\pm$  SD per gram of product as consumed. <sup>b</sup> Product order or coding bears no relationship to the order of presentation of products in **Table 1**. CS, chocolate syrup; MC, milk chocolate; SC, semisweet chocolate chips; DC, dark chocolate; BC, baking chocolate; CP, cocoa powder. <sup>c</sup> Expressed as gallic acid equivalents.

shown in **Table 2**. Panels **A**, **B**, and **C** of **Figure 1** show the antioxidant activity (ORAC), total polyphenols, and procyanidins, respectively. Of the products tested, the natural cocoa powder samples had the highest ORAC levels and total polyphenol and procyanidin contents. Baking chocolates contained the second highest levels, followed by dark chocolates, semisweet chocolate baking chips, milk chocolates, and chocolate syrups.

**Nonfat Cocoa Solids.** The consistent pattern of the findings within product category led to further investigation of the product composition. Flavanol compounds tend to be hydrophilic. Therefore, they are found primarily in the nonfat fraction of cocoa and chocolate. As a result, the most obvious compositional factor to examine was the level of NFCS in each product category. Commercially, cocoa powder is obtained from roasted and finely ground cocoa cotyledons, which have had most of the cocoa butter pressed out. This results in a powder that contains  $\approx 88$ – $86\%$  NFCS and  $\approx 12$ – $14\%$  cocoa butter (29). Two of the cocoa powders contained 85 and 87% NFCS (**Table 2**). A third sample contained only 72.2% NFCS and 27.8% fat. This was reflected in lower ORAC, total polyphenol, and procyanidin levels for this sample.

Baking chocolates are typically made from roasted and finely ground cocoa beans with no further additions or fat removal. Closest in composition to intact cocoa beans, baking chocolates typically contain 47–49% NFCS. The sample set examined in this study ranged in NFCS from 44.8 to 49.4%. Dark chocolates and semisweet baking chips can vary widely in formulas; therefore, the NFCS is expected to vary more in this product category (29). The U.S. regulations for these products require only that sweet chocolate contains at least 15% chocolate liquor and that semisweet chocolate contains at least 35% chocolate liquor. Both sweet chocolate and semisweet chocolate can commonly be referred to as “dark chocolate”. The NFCS levels in this study ranged from 20 to 29.5% in dark chocolates and from 15.2 to 18.6% in the semisweet chocolate baking chips. The variation in the level of ORAC, polyphenols, and procyanidins was attributed to differences in formulations of dark and



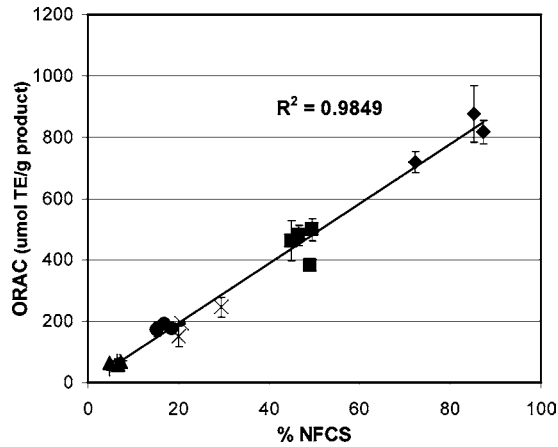
**Figure 1.** ORAC and polyphenol and procyanidin contents of U.S. products: **(A)** data for ORAC expressed as  $\mu\text{mol}$  of TE per g of product; **(B)** data for polyphenols expressed as mg of GAE per g of product; **(C)** data for procyanidins expressed as mg of procyanidins per g of product. Products are numbered as in **Table 2** with chocolate syrups (open bar), milk chocolates (vertical lines), baking chips (gray shading), dark chocolates (horizontal lines), baking chocolates (black), and cocoas (diagonal lines).

semisweet chocolate baking chips in the sample set. Finally, the level of NFCS ranged from 4.9 to 7.2% in milk chocolates and from 4.8 to 7.3% in chocolate syrups.

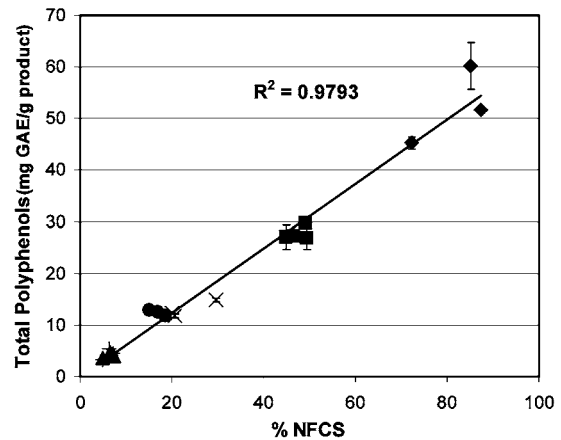
**Relationship of NFCS and ORAC Activity, Total Polyphenols, and Procyanidins.** Plots were developed to show the relationships between NFCS and ORAC, VCEAC, and TP, respectively. **Figure 2** shows a high degree of correlation between percent NFCS and ORAC ( $R^2 = 0.9849$ ). The three cocoa powders presented a clear grouping at the highest level of antioxidant activity followed by the baking chocolates. The three dark chocolates and the three semisweet chocolate baking chips tended to group together. The three milk chocolates and the three syrups also grouped together and had the lowest level of antioxidant activity.

A similar linear correlation is seen in **Figure 3** for percent NFCS and VCEAC ( $R^2 = 0.9824$ ). Again, the cocoa powder category had the highest level of antioxidant activity followed by baking chocolates, dark chocolates, semisweet chocolate baking chips, milk chocolates, and chocolate syrups. The inset of **Figure 3** also shows the correlation of ORAC and VCEAC for the same products ( $R^2 = 0.9844$ ).

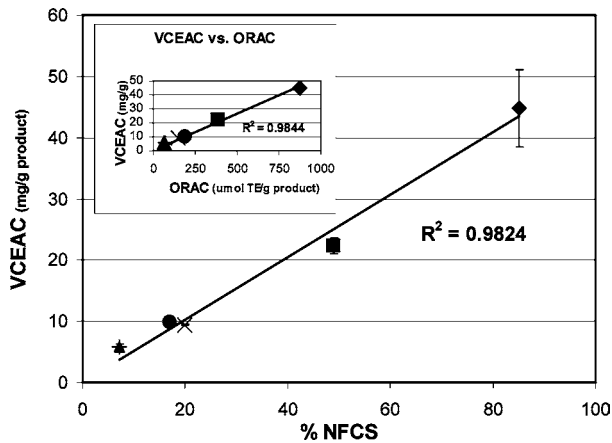
**Figure 4** shows the linear correlation of NFCS and total polyphenols ( $R^2 = 0.9793$ ). **Figure 5** shows the linear plot of NFCS and procyanidins ( $R^2 = 0.9460$ ). In both cases, natural



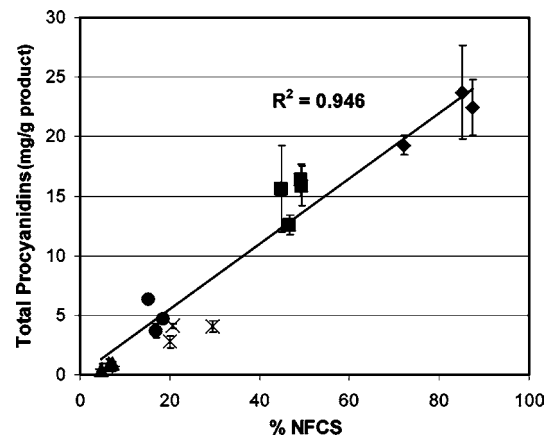
**Figure 2.** Relationship between ORAC activity and NFCS as illustrated by the line describing the best linear fit of the data, with force fitting through the origin, between ORAC activity expressed as  $\mu\text{mol}$  of TE per g of product and the percent of NFCS in the product for cocoas (◆), baking chocolates (■), dark chocolate (×), baking chips (●), milk chocolate (+), and syrups (▲). Symbols are the mean of three independently analyzed composite samples plus or minus the standard deviation (vertical bar, which may be no larger than the symbol).



**Figure 4.** Relationship between polyphenol content and NFCS as illustrated by the line describing the best linear fit of the data, with force fitting through the origin, between polyphenol content on a mg per g of product basis, and the percent of NFCS in the product for cocoas (◆), baking chocolates (■), dark chocolate (×), baking chips (●), milk chocolate (+) and syrups (▲). Symbols are the mean of three independently analyzed composite samples plus or minus the standard deviation (vertical bar, which may be no larger than the symbol).



**Figure 3.** Relationship between VCEAC and NFCS content as illustrated by the line describing the best linear fit of the data, with force fitting through the origin, between ORAC activity, expressed as  $\mu\text{g}$  of vitamin C equivalents per g of product, and the percent of NFCS in the product for a representative product sample of cocoa (◆), baking chocolate (■), dark chocolate (×), baking chips (●), milk chocolate (+), and syrup (▲). Horizontal bars show the standard deviation of the mean of the three composite samples. (Inset) Correlation of VCEAC and ORAC for a subset.



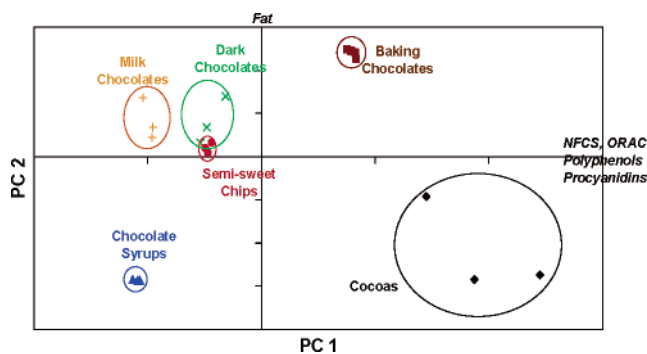
**Figure 5.** Relationship between procyanidin content and NFCS as illustrated by the line describing the best linear fit of the data, with force fitting through the origin, between procyanidins content on a mg per g of product and the percent of NFCS in the product for cocoas (◆), baking chocolates (■), dark chocolate (×), baking chips (●), milk chocolate (+), and syrups (▲). Symbols are the mean of three independently analyzed composite samples plus or minus the standard deviation (vertical bar, which may be no larger than the symbol).

cocoas had the highest total polyphenols and procyanidins followed by baking chocolates. Dark chocolates and semisweet chocolate baking chips grouped together. Milk chocolates and syrups had the lowest levels of polyphenols and procyanidins.

**Principal Component Analysis.** PCA divides the data into distinct sets that best describe relationships. PC-1 describes the statistical relationship that accounts for the greatest amount of sample variation, followed by PC-2, PC-3, PC-4, etc., which each describe decreasingly less variation in the sample set. Principal component weighting of the complete data set was done for the six attributes that were measured (Table 3A). Higher weighting scores indicate a tighter association with that principal component. Weighting on PC-1 was high and almost equal for percent NFCS, ORAC, total polyphenols, flavan-3-ol monomers, and procyanidins. The fat content of the products was heavily weighted on PC-2. Table 3B shows the level of

variation described by each of the calculated principal components. PC-1 describes 81.4% of the variation in the sample set, whereas PC-2 describes an additional 16.0%. Figure 6 graphically depicts the distribution of data for PC-1 and PC-2. This plot describes 97.5% of the sample set variation. There is a tight grouping of all products within the six product categories tested. Each product category groups independently from the other categories with the exception of an overlap of dark chocolate and semisweet chocolate baking chips. These products generally have similar formulas and, therefore, overlap in terms of percent fat and percent NFCS (Table 2). The distribution of weighted attributes is shown at the right and at the top of Figure 6. For PC-1, the attributes strongly associated with the  $x$ -axis are NFCS, ORAC activity, total polyphenols, and procyanidins. The level of fat is associated with PC-2 at the top of the  $y$ -axis. These data show a high degree of separation for product category





**Figure 6.** Principal component plot of PC-1 and PC-2 showing the distribution of cocoas (◆), baking chocolates (■), dark chocolates (x), baking chips (●), milk chocolates (+), and syrups (▲) across PC-1 and PC-2. The amount of variation described by PC-1 represents 81.4% of the data set variation; PC-2 represents 16.0%. The PC-1 axis is correlated at the right with the level of NFCS, ORAC activity, total polyphenols, and procyanidin content. The PC-2 axis is correlated at the top with the level of fat content in the product.

**Table 3.** Principal Component Analysis of Data Set

A. Component Weighting			
measure	component 1	component 2	
% NFCS	0.447174	-0.0643330	
fat	0.113507	0.9865940	
ORAC	0.447650	-0.1228980	
polyphenols	0.447494	-0.0834084	
monomers	0.431963	-0.0004086	
total procyanidins	0.447121	0.0207851	
B. Principal Component Analysis			
component no.	eigenvalue	% of variance	cumulative %
1	4.8851500	81.419	81.419
2	0.9621340	16.036	97.455
3	0.1132110	1.887	99.342
4	0.0266650	0.444	99.786
5	0.0080160	0.134	99.920
6	0.0048187	0.080	100.000

using the six measures included in the PCA. Any of the measures associated with the principal components can be inferred to be related to the other factors associated with that component. For example, baking cocoas and baking chocolates, which are higher (to the right) in PC-1 accounting for 81.4% of the sample set variation, can be inferred to be also higher in NFCS, ORAC, polyphenols, and procyanidins.

## DISCUSSION

Recent research has focused on flavan-3-ols as bioactive compounds, particularly with respect to beneficial effects on cardiovascular function. Cocoa or chocolate consumption has been associated with short-term improvements in delayed oxidation of low-density lipoprotein (LDL) cholesterol (30), improved endothelial function (31, 32), lowered blood pressure (33), and improved platelet function (19, 20, 32). A recent 15-year epidemiological study of elderly Dutch men showed that blood pressure was significantly lowered in the group consuming the highest cocoa/chocolate. The highest consuming group also had a lower incidence of death due to cardiovascular disease compared to men who did not eat cocoa or chocolate (34). To strengthen the clinical evidence, it is important to characterize the flavanol composition and antioxidant activity of various cocoa and chocolate foods available to the consumer.

Most notable in this study was the strong association between the percent NFCS and ORAC activity, polyphenols, and procyanidins, respectively, across six cocoa-containing product categories. This suggested that the most important factor in controlling the level of antioxidant activity and procyanidins was the amount of NFCS in the product formulation. This observation was supported by the linear correlation of NFCS with antioxidant activity, total polyphenols, and procyanidins. In addition, the PCA showed that the level of NFCS was closely associated with PC-1, the component that described 81.4% of the sample set variation. This relationship was consistent despite sampling products from seven different food manufacturers and across six product categories. A preliminary market basket study of U.S. cocoa-containing products also showed a similar linear relationship between product classes and procyanidin antioxidants (35).

Although manufacturing parameters including sources of beans, bean specifications, bean blends, bean roasting, grinding, conching, and recipes differ between products and manufacturers, the levels of antioxidants and flavanols in these products were primarily associated with the level of NFCS in the products tested. Substantial differences in antioxidant or procyanidin levels due to bean formulations and/or processing would have resulted in separation of a product brand from its category. This was not observed.

The relatively small differences in antioxidant capacity, total polyphenols, and flavanols within these six major categories of cocoa-containing products may be attributed to several factors. First, most large chocolate manufacturers typically rely on a small number of major cocoa-growing countries (Ivory Coast, Ghana, Indonesia, Brazil, Nigeria, and Cameroon) to supply their manufacturing needs. In 2004, these countries produced 87.9% of the world's cocoa (36). There is little country-to-country variation in the level of procyanidins in the larger origins (10, 37). In addition, chocolate manufacturers typically blend two or more bean types to satisfy consumer tastes and for product uniformity (29, 38). Blending would be expected to also reduce the variability between branded products within category. Some specific bean origins are inherently higher in procyanidins. Bean origins such as Madagascar and Java can have double the level of flavan-3-ol antioxidants, but quantities are too small to meet the production needs of large chocolate manufacturers (15, 38). Beans from smaller producing countries often make their way into the local chocolate/cocoa economy or are sold to small specialty chocolate manufacturers in the northern temperate countries. It is also possible to enhance the level of polyphenol antioxidants by adding extracts from grape seed, tea, or cocoa or by resorting to special processes to preserve antioxidants found in raw cacao seeds.

Another reason the levels of antioxidant activity and procyanidins may be so close within these product categories is that the flavanols are thought to impart astringency or bitterness to cocoa-containing products (13, 15). One needs only to compare the tastes of cocoa powder, baking chocolate, dark chocolate, and milk chocolate to see the range of bitterness imparted by cocoa. Fermentation lessens the bitter/astringent properties of the bean, an effect that has been attributed to the loss of polyphenols and flavan-3-ols during fermentation (10, 13–16, 38). Fermentation of beans also helps to produce more cocoa flavor. Longer bean fermentation time is a valued trait that commands a premium in cocoa markets. Ghana cocoa beans are a good example of a well-fermented, flavorful cocoa compared to Dominican or Indonesian beans, which are considered to be less fermented, lower quality beans because

of their bitterness and low cacao flavor. Thus, chocolate manufacturers have gravitated to cocoa blends that contain well-balanced cocoa flavor and appropriate bitterness, without being objectionable to consumers.

These observations also suggested that product-processing parameters have a relatively small effect on the final levels of antioxidant activity and procyanidins across the six product categories tested in this study. The product categories included syrups, which are aqueous suspensions; dry cocoa powders, as well as the fat- and sugar-based chocolates; and milk, dark, baking, and semisweet chips. Despite significant chemical and physical differences between product categories, little deviation in linear correlation analysis or by PCA was observed in the association of percent NFCS with the other analytical parameters. These data suggest that, among leading commercially available chocolate and cocoa brands, the biggest contributor to antioxidant and procyanidin content is the percent NFCS in the product formulation and that processing has a much smaller impact. As mentioned previously cocoas or chocolates could be "enriched" through the use of specific bean origins, under-fermented beans, or appropriate roasting conditions to preserve flavanols. However, this survey found little difference within product category and across different manufacturers for ORAC activity, polyphenols, and procyanidins in the top-selling products commercially available in the United States at the time of sampling.

One major exception to processing effects is alkalization or "Dutching" of cocoa powder. Previous studies have shown that loss of flavanols and antioxidant activity can vary with the degree of Dutching (e.g., "light" versus "heavy" Dutching) (17, 18). The current study did not include Dutched cocoas, although some of the products used Dutched chocolate liquor or Dutched cocoa powder as an ingredient. The Dutched liquor and cocoa powder were apparently minor ingredients as judged by their position in the declared ingredients statements. It was not possible to determine the extent of Dutching that occurred for these ingredients, but Dutching appeared to have a minor influence on antioxidant activity and flavanol level in the products tested.

As consumers become more aware of the potential health benefits associated with flavan-3-ols, they will also want more information about the flavanol content of products commonly available in the marketplace. This product survey compared the antioxidant activity and flavanol content of leading brands of chocolate and cocoa products. The results showed that nonfat cocoa solids were the primary factor influencing antioxidant level and polyphenol and procyanidin contents of a very broad array of products. This study showed that cocoa powders ranked the highest in antioxidants and polyphenols on an equal weight basis among the product categories tested. Next highest were unsweetened baking chocolates, which are composed solely of roasted and finely ground cocoa beans. The dark chocolates and semisweet chocolate chips had the next highest level of flavanols. Milk chocolates and chocolate syrups had the lowest flavanol content, primarily because they contain the least amount of NFCS. This relationship held across six market categories and across seven major chocolate manufacturers.

#### ABBREVIATIONS USED

NFCS, nonfat cocoa solids; ORAC, oxygen radical absorbance capacity; VCEAC, vitamin C equivalence antioxidant capacity; PC-1, principal component 1; GAE, gallic acid equivalents; TE, Trolox equivalents.

#### ACKNOWLEDGMENT

We thank Lori Sweger for coordinating the collection of the product samples, Larry Hainly for performing the principal component analysis, Dr. Donald Dahlberg for statistical sampling design and advice on the principal component analysis, Dr. Debra Miller for reviewing the manuscript, and the Analytical Research and Services Group for their continuing support.

#### LITERATURE CITED

- (1) USDA. Database for the Flavanoid Content of Selected Foods, 2003; <http://www.nal.usda.gov/fnic/foodcomp/Data/Flav/flav.pdf>.
- (2) Wu, X.; Beecher, G. R.; Holden, J. M.; Haytowitz, D. B.; Gebhardt, S. E.; Prior, R. Lipophilic and hydrophilic antioxidant capacities of common foods in the United States. *J. Agric. Food Chem.* **2004**, *52*, 4026–4037.
- (3) Gu, L.; Kelm, M. A.; Hammerstone, J. F.; Beecher, G.; Holden, J.; Haytowitz, D.; Gebhardt, S.; Prior, R. L. Concentrations of proanthocyanidins in common foods and estimations of normal consumption. *J. Am. Clin. Nutr.* **2004**, *20*, 613–617.
- (4) Ultee, A. J.; van Dorsen, J. Bijdrage tot de kennis der op Java gecultiveerde cacaosooten. In *Java Agriculture Station Report* 33; 1909.
- (5) Adam, W. B.; Hardy, P.; Nierenstein, M. The catechin of the cocoa bean. *J. Am. Chem. Soc.* **1931**, *53*, 727–728.
- (6) Freudenberg, K.; Cox, R. F. B.; Braun, E. The catechin of the cacao bean. *J. Am. Chem. Soc.* **1932**, *54*, 1913–1917.
- (7) Knapp, A. W.; Hearne, J. F. The presence of leuco-anthocyanidins in Criollo cacao. *Analyst* **1939**, *64*, 475–480.
- (8) Forsyth, W. G. C. Cacao polyphenolic substances. 3. Separation and estimation on paper chromatograms. *Biochem. J.* **1955**, *60*, 108–111.
- (9) Hammerstone, J. F.; Lazarus, S. A.; Mitchell, A. E.; Rucker, R.; Schmitz, H. H. Identification of procyanidins in cocoa (*Theobroma cacao*) and chocolate using high-performance liquid chromatography/mass spectrometry. *J. Agric. Food Chem.* **1999**, *47*, 490–496.
- (10) Kim, H.; Keeney, P. G. (–)-Epicatechin content in fermented and unfermented cocoa beans. *J. Food Sci.* **1984**, *49*, 1090–1092.
- (11) Whiting, D. Natural phenolic compounds 1900–2000: a bird's eye view of a centuries' chemistry. *Nat. Prod. Rep.* **2001**, *18*, 583–606.
- (12) Clapperton, J.; Hammerstone, J. F.; Romanczyk, R.; Yow, S.; Chan, J.; Lin, D.; Lockwood, R. *Proceedings, 16th International Conference of Groupe Polyphenols*, Lisbon, Portugal; Groupe Polyphenols: Norbonne, France, 1992; pp 112–115.
- (13) Clapperton, J.; Lockwood, R.; Romanczyk, L.; Hammerstone, J. F. Contribution of genotype to cocoa (*Theobroma cacao* L.) flavour. *Trop. Agric. (Trinidad)* **1994**, *71*, 303–308.
- (14) Forsyth, W. G. C.; Rombouts, J. E. Our approach to the study of cocoa fermentation. *Report Cocoa Conference*; Cocoa, Chocolate and Confectionary Alliance: London, U.K., 1951; pp 73–81.
- (15) Robinson, T.; Ranalli, A. W.; Phillips, A. W. Changes in cocoa tannins during processing. *J. Agric. Food Chem.* **1961**, *9*, 295–298.
- (16) Cros, E.; Villeneuve, F.; Vincent, J.-C. Evolution des composés phénoliques du cacao au cours de la fermentation en relation avec la qualité. *9th Conference Internationale sur la Recherche Cacaoyere*, Feb 12–18, 1984, Lome, Togo; Cocoa Producers Alliance: Lagos, Nigeria, 1984; pp 651–655.
- (17) Adamson, G. E.; Lazarus, S. A.; Mitchell, A. E.; Prior, R. L.; Cao, G.; Jacobs, P. H.; Kremers, B. G.; Hammerstone, J. F.; Rucker, R. B.; Schmitz, H. H. HPLC method for the quantitation of procyanidins in cocoa and chocolate samples and correlation to total antioxidant capacity. *J. Agric. Food Chem.* **1999**, *47*, 4184–4188.

- (18) Personal observations that Dutched cocoa loses as much as 80% of its flavanols.
- (19) Steinburg, F. M.; Bearden, M. M.; Keen, C. L. Cocoa and chocolate flavonoids: implications for cardiovascular health. *J. Am. Diet. Assoc.* **2003**, *103*, 2125–2223.
- (20) Lamuela-Raventos, R. M.; Romero-Perez, A. I.; Andres-Lacueva, C.; Tornero, A. Review: health effects of cocoa flavonoids. *Food Sci. Technol. Int.* **2005**, *11*, 159–176.
- (21) Information Resources Inc. Report dated Aug 25, 2004; www.infores.com.
- (22) Association of Analytical Chemists, International. Cacao mass (fatfree) of chocolate liquor. *Official Methods of the AOAC International*, 16th ed.; AOAC: Arlington, VA, 1995; Official Method 931.05.
- (23) Association of Analytical Chemists, International. Fat in cacao products; Soxhlet extraction method. *Official Methods of the AOAC International*, 16th ed.; AOAC: Arlington, VA, 1995; Official Method 963.15.
- (24) Ou, B.; Hampsch-Woodill, M.; Prior, R. Development and validation of an improved oxygen radical absorbance capacity assay using fluorescein as the fluorescent probe. *J. Agric. Food Chem.* **2001**, *49*, 4619–4626.
- (25) Huang, D.; Ou, B.; Hampsch-Woodill, M.; Flanagan, J.; Deemer, E. K. Development and validation of oxygen radical absorbance capacity assay for lipophilic antioxidants using randomly methylated-cyclodextrin as a solubility enhancer. *J. Agric. Food Chem.* **2002**, *50*, 1815–1821.
- (26) Singleton, V.; Rossi, J. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *Am. J. Enol. Vitic.* **1965**, *16*, 144–158.
- (27) Gu, L.; Kelm, M.; Hammerstone, J. F.; Beecher, G.; Cunningham, D.; Vannozzi, D.; Prior, R. Fractionation of polymeric procyanidins from low-bush blueberry and quantification of procyanidins in selected foods with an optimized normal phase HPLC-MS fluorescence detection method. *J. Agric. Food Chem.* **2002**, *50*, 4852–4860.
- (28) Kim, D.; Lee, C. Y. Comprehensive study on vitamin C equivalent antioxidant capacity (VCEAC) of various polyphenolics in scavenging a free radical and its structural relationship. *Crit. Rev. Food Sci. Nutr.* **2004**, *44*, 253–273.
- (29) Minifie, B. Cocoa processes. In *Chocolate, Cocoa, and Confectionery: Science and Technology*, 3rd ed.; Aspen Publishers: Gaithersburg, MD, 1999; pp 35–84.
- (30) Wan, Y.; Vinson, J. A.; Etherton, T. D.; Proch, J.; Lazarus, S. A.; Kris-Etherton, P. M. Effects of cocoa powder and dark chocolate on LDL oxidative susceptibility and prostaglandin concentration in humans. *Am. J. Clin. Nutr.* **2001**, *74*, 596–602.
- (31) Engler, M. B.; Engler, M. M.; Chen, C. Y.; Malloy, M. J.; Browne, A.; Chiu, E. Y.; Kwak, H. K.; Milbury, P.; Paul, S. M.; Blumberg, J.; Mietus-Snyder, M. L. Flavonoid-rich dark chocolate improves endothelial function and increases plasma epicatechin concentrations in healthy adults. *J. Am. Coll. Nutr.* **2004**, *23*, 197–204.
- (32) Hermann, F.; Spieker, L. E.; Ruschitzka, R.; Sudano, I.; Hermann, M.; Binggeli, C.; Luscher, T. F.; Riesen, W.; Noll, G.; Corti, R. Dark chocolate improves endothelial and platelet function. *Heart* **2006**, *92*, 19–120.
- (33) Grassi, D.; Lippi, C.; Necozione, S.; Desideri, G.; Ferri, C. Short-term administration of dark chocolate is followed by a significant increase in insulin sensitivity and a decrease in blood pressure in healthy persons. *Am. J. Clin. Nutr.* **2005**, *81*, 611–614.
- (34) Buijsse, B.; Feskens, E. J. M.; Kok, F. J.; Kromhout, D. Cocoa intake, blood pressure, and cardiovascular mortality. *Arch. Intern. Med.* **2006**, *166*, 411–417.
- (35) Gu, L.; Wu, X.; Ou, B.; Harnly, J.; Prior, R. L. Procyanidin (PC) content and total antioxidant capacity (TAC) of chocolate and cocoa products. *FASEB J.* **2005**, 598.20 (Abstract).
- (36) International Cocoa Organization (ICCO). Annual Report; ICCO: London, U.K., 2003/04; p 11, <http://www.icco.org/anrep/anrep0304english.pdf>.
- (37) Counet, M. C.; Ouwerck, C.; Rosoux, D.; Collin, S. Relationship between procyanidins and flavor contents of cocoa liquors from different origins. *J. Agric. Food Chem.* **2004**, *52*, 6243–6249.
- (38) Jackson, K. Recipes. In *Industrial Chocolate Manufacture and Use*; Beckett, S. T., Ed.; Blackie: London, U.K., 1988; pp 236–258.

---

Received for review January 31, 2006. Revised manuscript received April 17, 2006. Accepted April 20, 2006.

JF0602900