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# Survey of Commercially Available Chocolate- and Cocoa-Containing Products in the United States. 2. Comparison of Flavan-3-ol Content with Nonfat Cocoa Solids, Total Polyphenols, and Percent Cacao

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A survey of a broad range of chocolate- and cocoa-containing products marketed in the United States was conducted to provide a more detailed analysis of flavan-3-ol monomers, oligomers, and polymers, which can be grouped into a class of compounds called procyanidins. Samples consisted of the three or four top-selling products within the following six categories: natural cocoa powder, unsweetened baking chocolate, dark chocolate, semisweet baking chips, milk chocolate, and chocolate syrup. Composite samples were characterized for percent fat (% fat), percent nonfat cocoa solids (% NFCS), antioxidant level by ORAC, total polyphenols, epicatechin, catechin, total monomers, and flavan-3-ol oligomers and polymers (procyanidins). On a gram weight basis epicatechin and catechin content of the products follow in decreasing order: cocoa powder > baking chocolate > dark chocolate = baking chips > milk chocolate > chocolate syrup. Analysis of the monomer and oligomer profiles within product categories shows there are two types of profiles: (1) products that have high monomers with decreasing levels of oligomers and (2) products in which the level of dimers is equal to or greater than the monomers. Results show a strong correlation ($R^2$ = 0.834) of epicatechin to the level of % NFCS and also very good correlations for $N$ = 2−5 oligomers to % NFCS. A weaker correlation was observed for catechin to % NFCS ($R^2$ = 0.680). Other analyses show a similar high degree of correlation with epicatechin and $N$ = 2−5 oligomers to total polyphenols, with catechin being less well correlated to total polyphenols. A lesser but still good correlation exists between the calculated percent cacao (calcd % cacao) content, a proxy for percent cacao, and these same flavanol measures, with catechin again showing a lesser degree of correlation to calcd % cacao. Principal component analysis (PCA) shows that the products group discretely into five classes: (1) cocoa powder, (2) baking chocolate, (3) dark chocolate and semisweet chips, (4) milk chocolates, and (5) syrup. PCA also shows that most factors group closely together including the antioxidant activity, total polyphenols, and the flavan-3-ol measures with the exception of catechin and % fat in the product, which group separately. Because catechin distribution appears to be different from the other flavan-3-ol measures, an analysis of the epicatechin to catechin ratio was done, indicating there is a >5-fold variation in this measure across the products studied. The cocoa-containing products tested range from cocoa powder with 227.34 ± 17.23 mg of procyanidins per serving to 25.75 ± 9.91 mg of procyanidins per serving for chocolate syrup. These results are discussed with respect to other studies on commercial products, the bioavailability of the flavanols, and the possible role of processing on the amount of catechin in products.

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

## INTRODUCTION

Several excellent reviews have recently been published (*1*, *2*) describing both short- and long-term benefits of cocoa-containing products, especially dark chocolate, on cardiovascular health. Observational studies indicate that consumption of cocoa or chocolate over extended periods is associated with a decrease in blood pressure and/or a decrease in the risk of cardiovascular disease (*3*, *4*). The health benefits of cocoa and dark chocolate include increased blood flow at the brachial artery (*5*) and the left descending coronary artery (*6*), decreased blood pressure (*7*), decreased platelet aggregation (*8*), and increased HDL-cholesterol (*9*, *10*).

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Importantly, there is an increasing body of evidence that commonly available, commercial chocolates ranging from 50 to 80% cacao (*6, 7, 9−12*) and cocoa powder (*12*) can confer these benefits.

From bioavailability studies it is known that the flavan-3-ol monomers, (−)-epicatechin and (+)-catechin, rapidly appear in the blood and have a peak concentration 2 h after consumption (*13*, *14*). There is limited evidence that dimers are also absorbed (*15*). Although it is uncertain whether the trimers and higher polymers of the flavanols are readily absorbed, it has been suggested that over prolonged exposure, bacterial breakdown and colonic absorption of smaller molecules may occur (*16*). These reports underscore the importance of knowing the composition of commercially available cocoa- and chocolate-containing products.

Several published reports and government databases show the composition of the flavanols in commercially available products (*17−20*). Much of this information lacks sufficient detail regarding the monomeric, oligomeric, and polymeric composition of the flavanols because values have been averaged or because data are reported for groups of oligomers (*17−19*). In other cases the procyanidins have been compared to the percent cacao in the product or to theobromine, a proxy for nonfat cocoa solids in the product, rather than to the (brown) cocoa solids in the product (*21*, *22*). Knowing the detailed composition of commercially available products is therefore increasingly important, especially for the low molecular weight flavan-3-ols, which are thought to be responsible for many of the heart health benefits (*14*).

In this paper, the top-selling chocolate- and cocoa-containing products from the U.S. market have been characterized in detail for their level of flavanols. These results are also compared to the nonfat cocoa solids, total polyphenol, and calculated percent cacao content in these products. The results show that most measures of flavanol chemistry, including epicatechin and the oligomeric and polymeric flavan-3-ols (procyanidins), have a predictable relationship. The exception is catechin, which varies more widely than the other flavan-3-ols. These results are discussed with respect to the importance of the bioavailability of the monomeric flavanols and to the possible effect of cocoa processing.

## 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 chocolate baking chips, milk chocolate, and chocolate syrup (**Table 1**). The list of the top-selling products and sample collection methods are described in greater detail in Miller et al. (*20*). Nineteen different products were sampled representing seven major manufacturers. Due to a tie in the baking chocolate category, four products were collected. A total of 450 product purchases contributed to the sample set, each with a different product code designation. Products were purchased in the cities in the following three regions: (1) east (Fairfield, NJ; Hershey, PA); (2) midwest (Dillsboro, IN; Plainfield, IL;

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

| product category | manufacturer/brand[a] |
|---|---|
| natural cocoa powder | Ghirardelli Cocoa<br>Hershey's Cocoa<br>Nestle Toll House Cocoa |
| unsweetened baking chocolate | Baker's Baking Chocolate<br>Ghirardelli Premium Baking Bar<br>Hershey's Baking Chocolate<br>Nestle Baking Chocolate |
| dark chocolate | Hershey's Special Dark<br>Lindt Excellence (70% Cocoa)<br>Dove Promises Dark Chocolate |
| semisweet chocolate baking chips | Ghirardelli Premium Baking Chips<br>Hershey's Semi-Sweet Chips<br>Nestle Toll House Morsels |
| milk chocolate | Hershey's Milk Chocolate<br>Dove Promises Milk Chocolate<br>Lindt Excellence Extra Creamy Milk Chocolate |
| chocolate syrup | Hershey's Syrup<br>Kroger Syrup<br>Nestle Nesquik Syrup |

[a] Manufacturers listed alphabetically within product category. Product order in this table has no relationship to the order shown in **Table 2** or **Figure 1**.

**Table 2.** Analysis of Selected U.S. Cocoa-Containing and Chocolate Products

| no. | code[a] | % NFCS | % fat | ORAC ($\mu$mol of TE) | VCEAC[b] | total polyphenols[c] | epicatechin (mg/g) | catechin (mg/g) | Epi + Cat (mg/g) |
|---|---|---|---|---|---|---|---|---|---|
| 1 | CP-1 | 85.2 | 12.4 | 875 | 42.3 | 60.2 | 2.827 | 0.896 | 3.723 |
| 2 | CP-2 | 72.2 | 21.7 | 720 | 37.4 | 45.3 | 1.263 | 0.347 | 1.610 |
| 3 | CP-3 | 87.3 | 11.0 | 816 | 40.2 | 51.7 | 1.471 | 0.492 | 1.964 |
| 4 | BC-1 | 46.6 | 53.1 | 481 | 27.8 | 27.2 | 1.148 | 0.727 | 1.875 |
| 5 | BC-2 | 44.8 | 53.1 | 463 | 23.3 | 27.1 | 1.223 | 0.255 | 1.478 |
| 6 | BC-3 | 49.4 | 51.6 | 499 | 27.6 | 26.9 | 1.202 | 0.355 | 1.557 |
| 7 | BC-4 | 49.0 | 52.4 | 384 | 23.4 | 29.7 | 0.995 | 0.625 | 1.620 |
| 8 | DC-1 | 20.7 | 33.4 | 196 | 13.6 | 11.7 | 0.326 | 0.151 | 0.478 |
| 9 | DC-2 | 29.5 | 40.7 | 246 | 16.5 | 14.9 | 0.312 | 0.107 | 0.419 |
| 10 | DC-3 | 20.0 | 30.0 | 152 | 9.5 | 12.3 | 0.371 | 0.233 | 0.605 |
| 11 | SSC-1 | 15.2 | 29.8 | 174 | 11.6 | 12.9 | 0.578 | 0.112 | 0.690 |
| 12 | SSC-2 | 18.6 | 27.8 | 177 | 11.3 | 11.8 | 0.460 | 0.233 | 0.693 |
| 13 | SSC-3 | 17.0 | 29.0 | 190 | 10.1 | 12.5 | 0.412 | 0.237 | 0.648 |
| 14 | MC-1 | 7.25 | 29.3 | 72.0 | 5.8 | 4.5 | 0.148 | 0.082 | 0.231 |
| 15 | MC-2 | 6.40 | 31.4 | 72.3 | 8.0 | 5.4 | 0.126 | 0.041 | 0.167 |
| 16 | MC-3 | 4.90 | 37.0 | 41.7 | 3.9 | 3.3 | 0.023 | 0.006 | 0.029 |
| 17 | CS-1 | 6.60 | 0.80 | 57.7 | 5.6 | 4.8 | 0.069 | 0.036 | 0.106 |
| 18 | CS-2 | 4.80 | 0.70 | 65.7 | 5.3 | 3.7 | 0.030 | 0.030 | 0.060 |
| 19 | CS-3 | 7.34 | 1.20 | 66.7 | 5.6 | 4.1 | 0.122 | 0.059 | 0.181 |

[a] Product order or coding bears no relationship to the order of presentation of products in **Table 1**. CP, cocoa powder; BC, baking chocolate; DC, dark chocolate; SSC, semisweet chocolate chips; MC, milk chocolate; CS, chocolate syrup. [b] VCEAC units expressed as vitamin C equivalents (mg/g) (*27*). [c] Total polyphenols expressed as gallic acid equivalents.

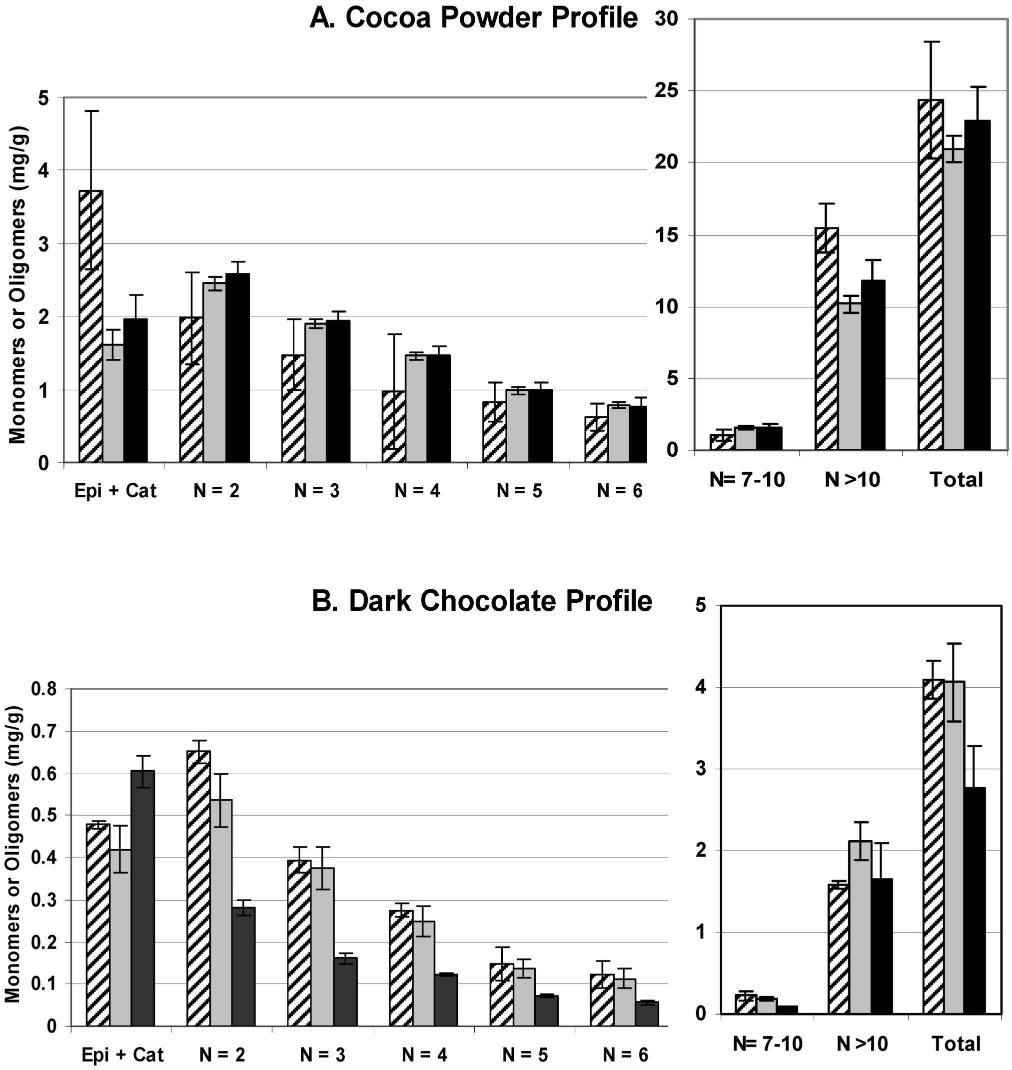


**Figure 1.** (**A**) Level of flavan-3-ol monomers, oligomers, and polymers in cocoa powders: bars represent CP-1 (cross-hatched), CP-2 (gray), and CP-3 (black). (**B**) Level of flavan-3-ol monomers, oligomers, and polymers in dark chocolates: bars represent DC-1 (cross-hatched), DC-2 (gray), and DC-3 (black).

**Table 3.** Analysis of Selected U.S. Cocoa-Containing and Chocolate Products for Flavan-3-ols According to the Method of Gu et al. (*30*)

| no. | code[b] | monomers | dimers | trimers | tetramers | pentamers | hexamers | DP 7−10 | DP > 10 | total |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | CP-1 | 2.015 | 1.980 | 1.472 | 0.973 | 0.832 | 0.622 | 1.025 | 15.46 | 24.38 |
| 2 | CP-2 | 1.642 | 2.453 | 1.900 | 1.462 | 0.989 | 0.783 | 1.553 | 10.16 | 20.94 |
| 3 | CP-3 | 1.774 | 2.590 | 1.949 | 1.466 | 1.000 | 0.774 | 1.565 | 11.76 | 22.88 |
| 4 | BC-1 | 2.199 | 2.080 | 1.221 | 0.738 | 0.364 | 0.221 | 0.460 | 5.284 | 12.57 |
| 5 | BC-2 | 1.678 | 1.919 | 1.428 | 1.110 | 0.819 | 0.658 | 1.215 | 6.810 | 15.64 |
| 6 | BC-3 | 1.911 | 2.040 | 1.520 | 1.231 | 0.930 | 0.746 | 1.407 | 6.093 | 15.88 |
| 7 | BC-4 | 0.995 | 0.850 | 0.539 | 0.250 | 0.236 | 0.175 | 0.345 | 12.93 | 16.32 |
| 8 | DC-1 | 0.700 | 0.651 | 0.393 | 0.275 | 0.146 | 0.122 | 0.226 | 1.583 | 4.098 |
| 9 | DC-2 | 0.350 | 0.536 | 0.376 | 0.249 | 0.136 | 0.113 | 0.190 | 2.113 | 4.062 |
| 10 | DC-3 | 0.324 | 0.280 | 0.161 | 0.124 | 0.072 | 0.056 | 0.100 | 1.659 | 2.778 |
| 11 | SSC-1 | 0.482 | 0.732 | 0.582 | 0.458 | 0.330 | 0.260 | 0.489 | 2.954 | 6.286 |
| 12 | SSC-2 | 0.614 | 0.698 | 0.452 | 0.308 | 0.185 | 0.137 | 0.282 | 2.017 | 4.693 |
| 13 | SSC-3 | 0.367 | 0.320 | 0.199 | 0.154 | 0.097 | 0.066 | 0.130 | 2.367 | 3.699 |
| 14 | MC-1 | 0.123 | 0.111 | 0.071 | 0.054 | 0.038 | 0.028 | 0.045 | 0.254 | 0.723 |
| 15 | MC-2 | 0.181 | 0.172 | 0.124 | 0.094 | 0.064 | 0.043 | 0.082 | 0.140 | 0.899 |
| 16 | MC-3 | 0.092 | 0.086 | 0.0630 | 0.042 | 0.027 | 0.017 | 0.050 | 0.057 | 0.434 |
| 17 | CS-1 | 0.073 | 0.106 | 0.086 | 0.076 | 0.056 | 0.060 | 0.136 | 0.172 | 0.763 |
| 18 | CS-2 | 0.046 | 0.083 | 0.060 | 0.050 | 0.033 | 0.033 | 0.066 | 0.000 | 0.370 |
| 19 | CS-3 | 0.096 | 0.086 | 0.066 | 0.056 | 0.040 | 0.033 | 0.052 | 0.418 | 0.846 |

[a] Results expressed as mg per gram of product as consumed. [b] Product order or coding bears no relationship to the order of presentation of products in **Table 1**. CP, cocoa powder; BC, baking chocolate; DC, dark chocolate; SC, semisweet chocolate chips; MC, milk chocolate; CS, chocolate syrup.

Minneapolis, MN;) and (3) west (Huntington Beach, CA; Phoenix, AZ; Los Angeles CA; Port Orchard, WA; San Francisco, CA). Products in a composite sample were thoroughly mixed by dry blending cocoas and by liquid blending syrups; chocolates were melted at 50 °C and liquid blended.

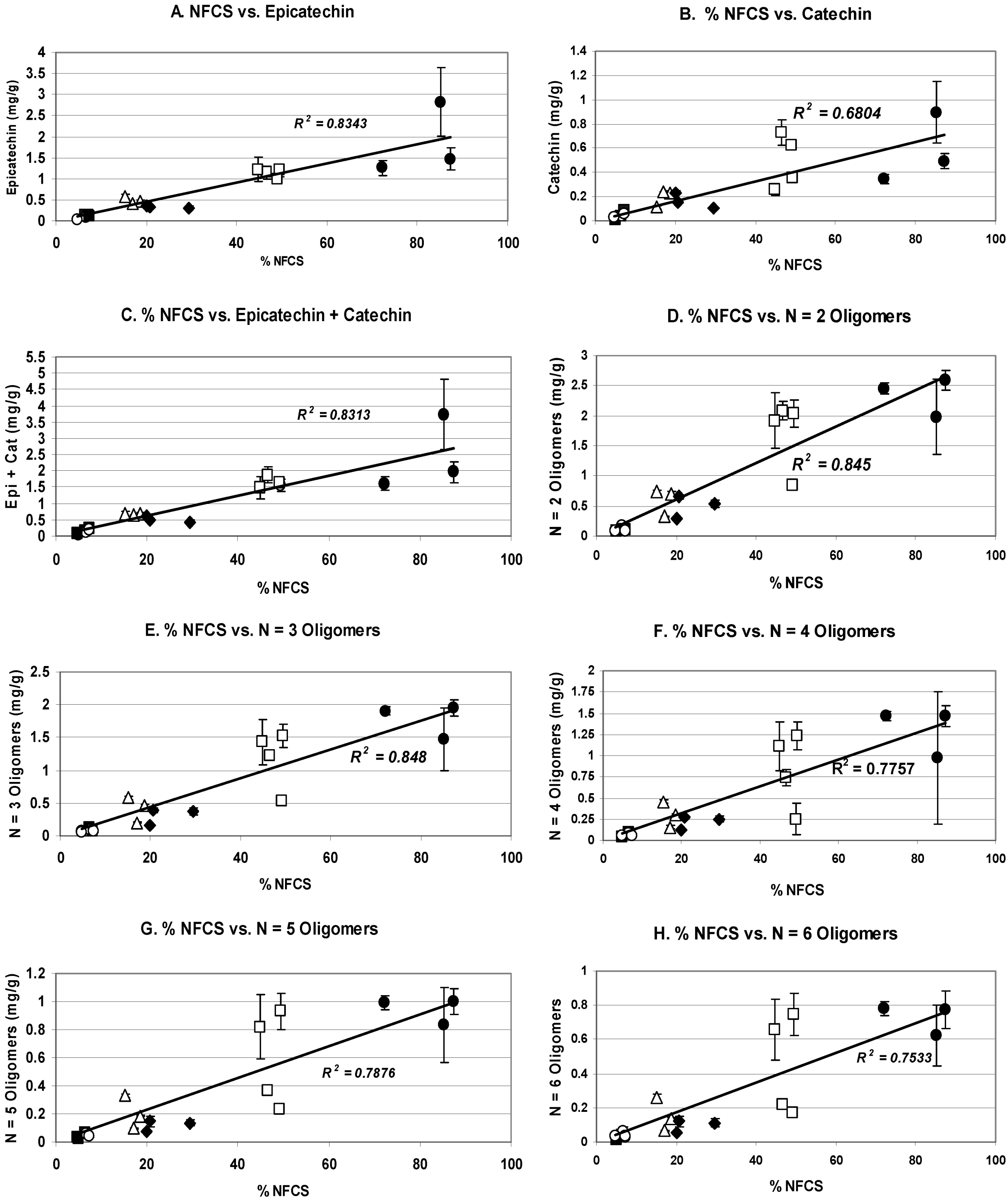


**Figure 2.** Relationship between percent nonfat cocoa solids and epicatechin (**A**), catechin (**B**), epicatechin + catechin (**C**), $N$ = 2 oligomers (**D**), $N$ = 3 oligomers (**E**), $N$ = 4 oligomers (**F**), $N$ = 5 oligomers (**G**), and $N$ = 6 oligomers (**H**). Product categories: cocoa powder (●); baking chocolate (□); dark chocolate (◆); semisweet baking chips (△); milk chocolate (■); chocolate syrup (○).

Composite samples were then stored at 0 °C before extraction and analysis. Samples were coded before submission for blind analysis. Epicatechin, catechin, and the total flavan-3-ol (procyanidins) content were measured in three independent samples; the values were averaged, and the standard deviation was calculated.

**Sample Analysis.** Composite samples were analyzed in duplicate for percent fat, percent nonfat cocoa solids (% NFCS), oxygen radical absorbance capacity (ORAC), total polyphenols (as gallic acid equivalents), and total procyanidins. A subset of composite samples was also analyzed for vitamin C equivalence antioxidant capacity (VCEAC). The % NFCS was determined by a gravimetric method based on exhaustive extraction of the sample with a variety of lipophilic solvents to eliminate the lipid content (*23*). Total fat was determined by Soxhlet extraction (*24*). 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 thermally based decomposition of azo compounds, like AAPH, using fluorescein as a fluorescent probe and Trolox as a standard substrate (*25*, *26*). The VCEAC method is a 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 (*27*). The total polyphenol colorimetric assay was initially developed as a method for the measurement of proteins focusing on the reagent's

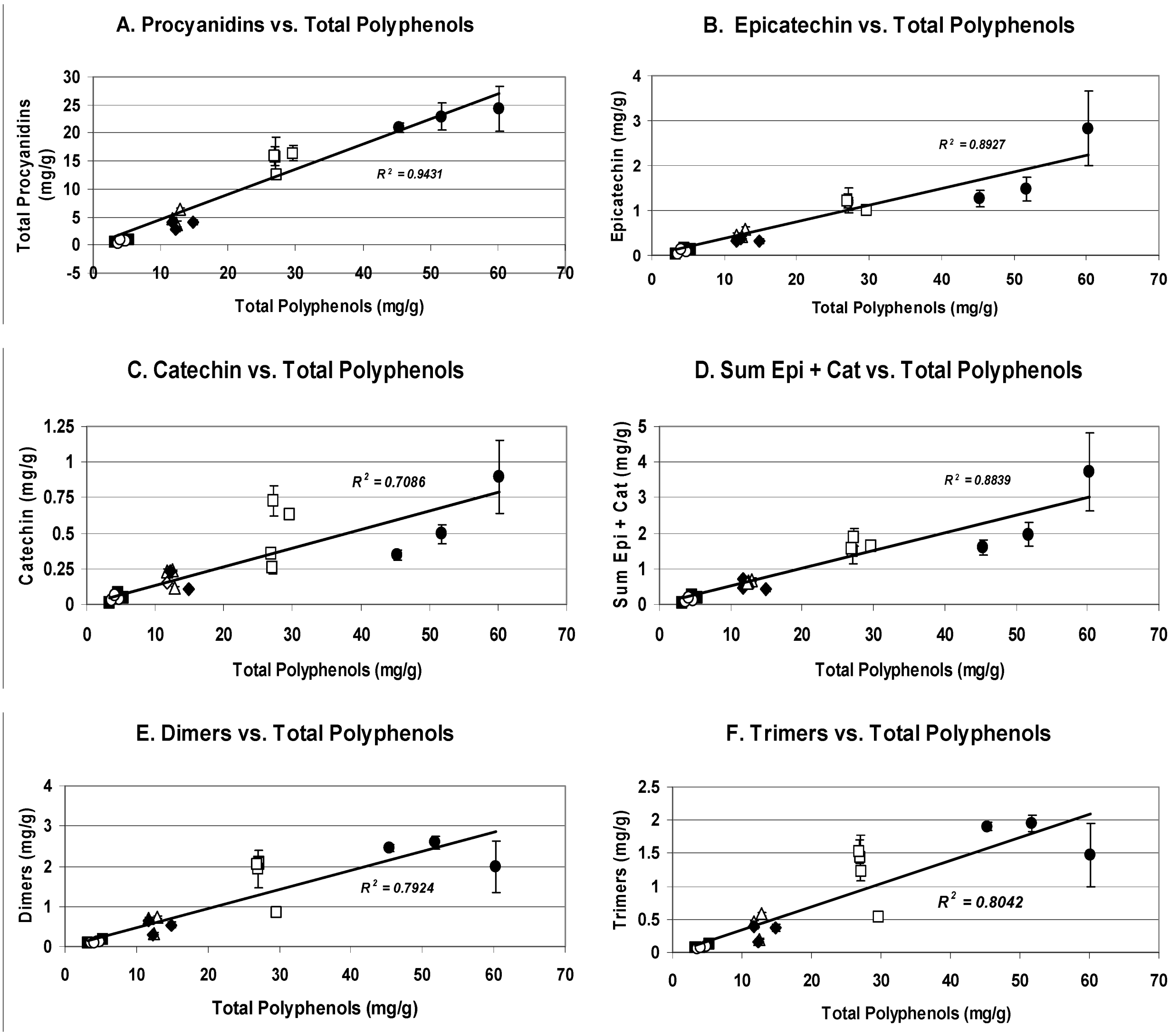


**Figure 3.** Correlation of the total polyphenols to the amount of flavan-3-ol monomers and oligomers and total (procyanidins) of commercial products. Product categories: baking chocolate (□); dark chocolate (◆); semisweet baking chips (△); milk chocolate (■); chocolate syrup (○).

ability to react with hydroxyl substituents and later adapted by Singleton and Rossi (*28*) to measure phenolic compounds in wine. It is widely used and is a measure of reducing capacity. Total polyphenols were measured using gallic acid as the standard. The flavan-3-ol monomers of (+)-catechin and (−)-epicatechin were measured by using the HPLC method of Nelson and Sharpless (*29*) standardized to (−)-epicatechin. Procyanidins were measured by using an HPLC method based on the separation of the flavan-3-ol oligomers (*30*).

**Calculation of Percent Cacao.** In commercial chocolate products, the confectionery industry has defined percent cacao as the sum of the cocoa liquor, cocoa powder, and cocoa butter in the formulation. Only one of the products tested here displayed the percent cacao designation. Lacking a percent cacao declaration, we estimated the percent cacao in products by summing the measured percent fat and percent nonfat cocoa solids of each product and reported this as the calculated percent cacao (calcd % cacao).

**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. The Statgraphics Principal Components Analysis program was purchased from Manugistics, Inc., Rockville, MD.

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.

**Table 4.** Calculated Percent Cacao for the Products

| code | % NFCS | % fat | calcd % cacao |
|---|---|---|---|
| BC-1 | 46.6 | 53.1 | 99.7 |
| BC-2 | 44.8 | 53.1 | 97.9 |
| BC-3 | 49.4 | 51.6 | 101.0 |
| BC-4 | 49.0 | 52.4 | 101.4 |
| DC-1 | 20.7 | 33.4 | 54.1 |
| DC-2 | 29.5 | 40.7 | 70.2 |
| DC-3 | 20.0 | 30.0 | 50.0 |
| SSC-1 | 15.2 | 29.8 | 45.0 |
| SSC-2 | 18.6 | 27.8 | 46.4 |
| SSC-3 | 17.0 | 29.0 | 46.0 |
| MC-1 | 7.25 | 29.3 | 36.6 |
| MC-2 | 6.40 | 31.4 | 37.8 |
| MC-3 | 4.90 | 37.0 | 41.9 |
| CS-1 | 6.60 | 0.80 | 7.4 |
| CS-2 | 4.80 | 0.70 | 5.5 |
| CS-3 | 7.34 | 1.20 | 8.5 |

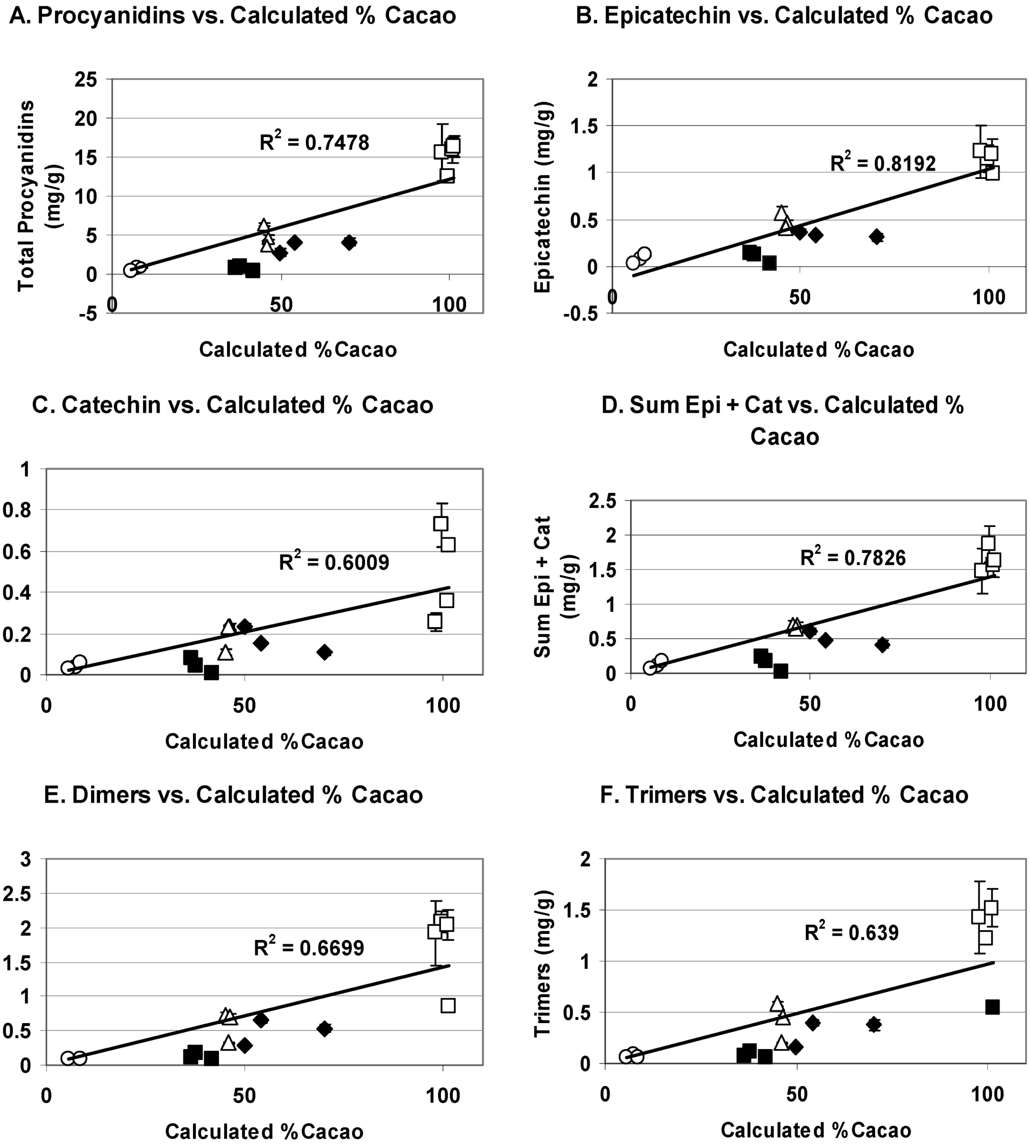


**Figure 4.** Correlation of the calculated percent cacao to the amount of flavan-3-ol monomers, oligomers, and total flavan-3-ols (procyanidins) for commercial products. Product categories: baking chocolate (□); dark chocolate (◆); semisweet baking chips (△); milk chocolate (■); chocolate syrup (○).

## RESULTS AND DISCUSSION

**General Analytical Results for Products. Table 2** shows the details of the analysis of the chocolate- and cocoa-containing products. The data includes % fat, % NFCS, ORAC, VCEAC, total polyphenols, and flavan-3-ol monomers measured according to the method of Nelson and Sharpless (*29*). The highest levels of epicatechin were found in cocoa powder, closely followed by baking chocolate, then dark chocolate and baking chips, followed by milk chocolate and syrup. The levels of catechin follow the same order but with more overlap between product categories. For example, some cocoa powders (CP-2 and CP-3) have lesser amounts of catechin compared to baking chocolates BC-1 and BC-4. Thus, there appears to be a greater variation in the level of catechin in the product samples within category compared to the level of epicatechin. This order for monomers is the same order described by Gu et al. (*19*).

**Comparison of the Monomers, Oligomers, and Total Procyanidins by Product Category. Table 3** shows the level of flavan-3-ol monomers, oligomers, and polymers and total flavan-3-ols (procyanidins) measured with the HPLC method (*19*) in the same products. The amount of monomers measured in **Table 2** closely coincides with the level of monomers measured by the sum of epicatechin and catechin (Epi + Cat) shown in **Table 1**. Throughout the remainder of this paper, we will use Epi + Cat as the measure of monomers because the latter measurement has the better calibration and standardization. The highest levels of total procyanidins are found in the cocoa powders followed by baking chocolates. There is overlap in total procyanidins between dark chocolate and semisweet chips, and the lowest levels of total procyanidins are found in milk chocolate and chocolate syrups. Similar results for the total procyanidins were found by Gu et al. (*19*) and by Miller et al. (*20*).

**Relationship of Flavanols within a Product Class. Figure 1** displays the relationship of the monomers (Epi + Cat; **Table 2**), oligomers, and polymeric procyanidins (**Table 3**) for just the cocoa powders and dark chocolates. All of the cocoa powders (**Figure 1A**) have similar levels of total procyanidins (far right). Within the cocoa powder category, there are two types of flavanol patterns; a single powder (CP-1) has high levels of monomers and lower amounts of oligomers. By comparison, two cocoa powders, CP-2 and CP-3, have lower monomers than dimers. A similar relationship exists for dark chocolates (**Figure 1B**); DC-3 has higher levels of monomers compared to dimers, whereas DC-1 and DC-2 have lower monomers compared to dimers. In graphs

**Table 5.** Principal Components Analysis of Data Set

(**A**) Component Weighting

| measure | component 1 | component 2 | component 3 |
|---|---|---|---|
| 1. % NFCS | 0.2662 | −0.0207 | 0.1401 |
| 2. % fat | **0.0526**[a] | **-0.7105**[a] | −0.6201 |
| 3. ORAC | 0.2689 | 0.0115 | 0.1229 |
| 4. VCEAC | 0.2685 | −0.0354 | 0.0941 |
| 5. total polyphenols | 0.2636 | −0.0139 | 0.2229 |
| 6. epicatechin | 0.2522 | −0.1274 | 0.2399 |
| 7. catechin | 0.2184 | **-0.4314**[a] | 0.3428 |
| 8. monomers | 0.2567 | −0.1745 | −0.1244 |
| 9. dimers | 0.2640 | 0.0309 | −0.1691 |
| 10. trimers | 0.2650 | 0.1171 | −0.1703 |
| 11. tetramers | 0.2571 | 0.2075 | −0.2398 |
| 12. pentamers | 0.2591 | 0.2108 | −0.1862 |
| 13. hexamers | 0.2546 | 0.2454 | −0.2090 |
| 14. $N$ = 7−10 | 0.2522 | 0.2649 | −0.2377 |
| 15. $N$ > 10 | 0.2471 | −0.1593 | 0.2766 |
| 16. total procyanidins | 0.2692 | −0.0484 | 0.0719 |

(**B**) Percent of Variance Described by Components

| component | eigenvalue | % of variance | cumulative percentage |
|---|---|---|---|
| 1 | 13.46 | 84.16 | 84.16 |
| 2 | 1.090 | 6.814 | 90.97 |
| 3 | 1.02 | 6.422 | 97.39 |
| 4 | 0.2158 | 1.349 | 98.74 |
| 5 | 0.1170 | 0.731 | 99.41 |
| 6 | 0.0566 | 0.354 | 99.82 |

[a] Figures in bold separate out from the rest of the values.

not shown for the other product categories, one can identify products that show the same general relationship, namely, higher levels of monomers than dimers in baking chocolate BC-4, semisweet chips (SSC-3), milk chocolate (MC-1), and syrup (CS-3) compared to baking chocolates BC-1, BC-2, and BC-3, semisweet chips SSC-1 and SSC-2, milk chocolates MC-2 and MC-3, and syrups CS-1 and CS-2, which have equal or lower monomers than dimers. Thus, there is variation within cocoa- and chocolate-containing product categories in the low molecular weight composition of products not previously recognized by Gu et al. (*19*) due to averaging of their data across the monomer and oligomer categories.

**Relationship of Monomers and Oligomers to % NFCS.** Earlier studies measuring total procyanidins over a range of cocoa-containing products showed a strikingly linear relationship to % NFCS in the formula of the products. Gu et al. (*19*) reported a linear correlation of $R^2$ = 0.99, and Miller et al. (*20*) reported a linear coefficient of correlation of $R^2$ = 0.946. **Figure 2** shows the relationship between epicatechin, catechin, total monomers, and selected oligomers up to $N$ = 6 compared to the measured level of % NFCS in each product. In **Figure 2A**, comparison of epicatechin to the % NFCS shows a linear relationship with an $R^2$ = 0.834, among the highest coefficients of correlation. By comparison, the level of catechin to % NFCS (**Figure 2B**) has a much lower correlation to a linear model ($R^2$ = 0.680). In **Figure 2C** is shown the sum of monomers, which has a linear coefficient of correlation of $R^2$ = 0.831. The relationships of dimers (**Figure 2D**) or trimers (**Figure 2E**) to the % NFCS have the highest linear correlation coefficients ($R^2$ = 0.845 and $R^2$ = 0.848, respectively). The same relationship for tetramers (**Figure 2F**), pentamers (**Figure 2G**), and hexamers (**Figure 2H**) is shown. This high degree of correlation of % NFCS to total procyanidins (*19*, *20*), to epicatechin, and to flavanol oligomers (**Figure 2**) occurs regardless of the brand, the proprietary cocoa blends, the roasting conditions, the processing conditions, or the physical composition of the product, be it a powder or fat- or water-based. Analyses of 69 chocolates, reported by Cooper et al. (*21*), found much lower coefficients of correlation ($R^2$ = 0.36) for epicatechin compared to % NFCS as estimated by theobromine as a proxy for % NFCS. The same authors found the highest coefficient of correlation of % NFCS was to the level of total polyphenols ($R^2$ = 0.73). We find a much closer linear relationship of the flavanols to % NFCS ($R^2$ ranging from 0.848 to 0.680), which suggests that direct measurement of % NFCS is more accurate than using theobromine as a proxy for % NFCS (*21*). Not taken into account in our data is the fact that we compared flavanol levels and % NFCS across a very broad spectrum of product categories and a smaller sampling of products. Additionally, as pointed out by Cooper et al. (*21*), it is not possible to measure % NFCS in products that contain other additions, such as nuts or wafers, which will interfere with direct measurement of % NFCS by the methods used herein. Cooper et al. (*21*) also found that the correlation of epicatechin to % NFCS ($R^2$ = 0.36) was greater than that for catechin to % NFCS ($R^2$ = 0.25).

**Relationship of Monomers, Oligomers, and Total Procyanidins to Total Polyphenols.** The results shown in **Figure 3** compare the flavanol measures to the level of total polyphenols in the same products. In **Figure 3A**, the relationship between total procyanidins and total polyphenols shows a very high linear coefficient of correlation of $R^2$ = 0.9431. The relationships of epicatechin, epicatechin plus catechin, dimers, and trimers all have $R^2$ between 0.89 and 0.79. The correlation of total polyphenols to catechins is much lower at $R^2$ = 0.7086, suggesting a higher amount of variation in the level of catechin in the products compared to the other flavanols.

**Relationship of Monomers, Oligomers, and Total Procyanidins to Calcd % Cacao.** It is possible to estimate the % cacao in these products by summing % fat and % NFCS. We specifically refer to this as calcd % cacao, recognizing that this is a proxy for the actual % cacao of the products. **Table 4** provides this calculation for all products except cocoa powder, in which most of the cocoa butter has been removed by pressing. For baking chocolates, the calcd % cacao is near 100% for all of the samples, which makes sense because baking chocolate is essentially unsweetened cocoa beans that have been roasted, finely ground, and solidified. The calcd % cacao was plotted against selected flavanol measures, and the coefficient of correlation was determined in **Figure 4**. The coefficients of correlation for total procyanidins (**Figure 4A**), of epicatechin (**Figure 4B**), and of epicatechin plus catechin (**Figure 4D**) are among the highest coefficients of correlation ($R^2$ = 0.7478, $R^2$ = 0.8192, and $R^2$ = 0.7826, respectively). The calcd % cacao probably has some inherent error, as seen by the displacement of the milk chocolates (black squares) to the right of the line of best linear fit. This displacement is probably due to two factors relating to the measurement of fat content. First, milk chocolate typically has cocoa butter added to the formula (*31*) to bring the level of fat up to or in excess of 30%. Because cocoa butter contains no flavan-3-ols, part of the shift to the right is likely due to the added cocoa butter. Second, the direct measurement of fat used here cannot distinguish between cocoa butter and the additional fat from milk. This error would also tend to shift the calcd % cacao to the right. If milk chocolates are removed from the correlation analysis, the coefficients of correlation of the data for total procyanidins, epicatechin, and epicatechin plus catechin compared to the calcd % cacao all increase ($R^2$ = 0.816, $R^2$ = 0.8464, and $R^2$ = 0.8502, respectively). These results indicate that over the product range tested, the calcd % cacao is a good predictor, with the possible exception of milk chocolate, of the level of the total and low molecular weight

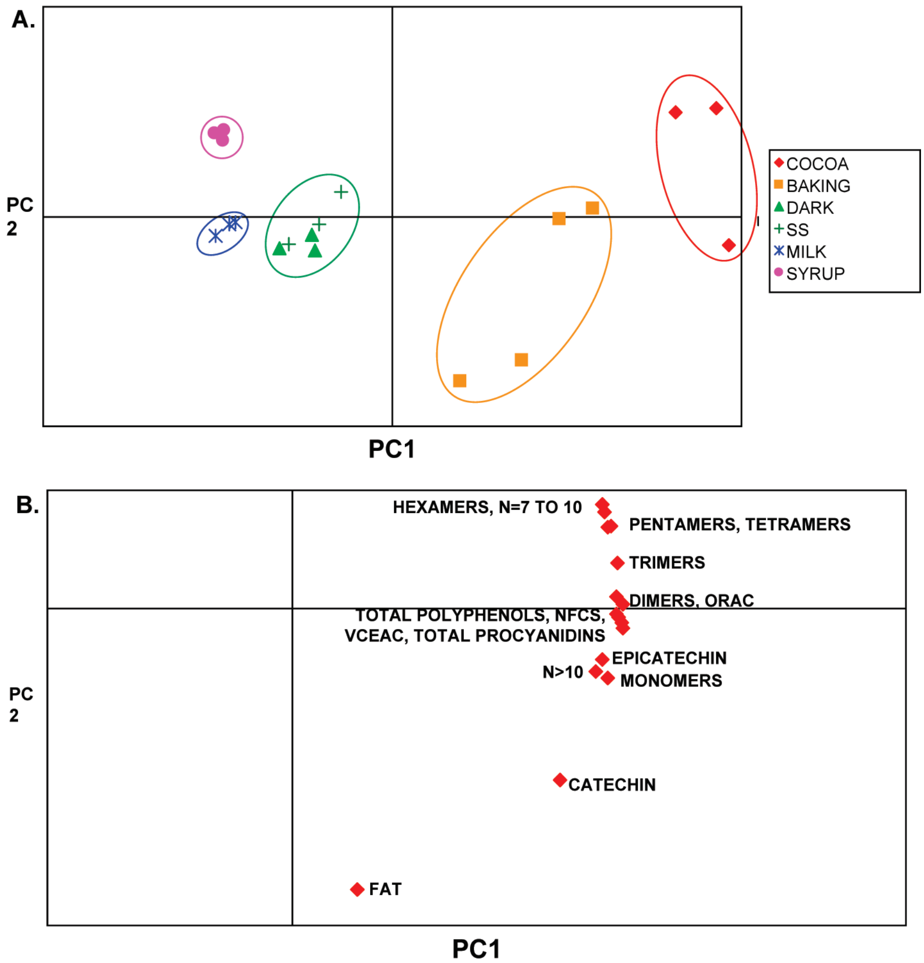


**Figure 5.** Principal component analysis of the data presented here for PC-1 and PC-2: (**A**) cocoa powder (red diamonds); baking chocolate (prange squares); dark chocolate (light green triangles); baking chips (green crosses); milk chocolate (blue stars); chocolate syrup (pink circles); (**B**) factor loading on PC-1 and PC-2.

flavanols in the product, although not nearly as good as % NFCS (*19*, *20*) (**Figure 2**) or total polyphenols (**Figure 3**) (*21*). Confectioners who are familiar with the distribution of flavanols in chocolate liquor, cocoa butter, and cocoa powder can and do make chocolates that would be expected to have more or fewer flavanols than the sample set tested. White chocolates or chocolates made with high amounts of alkali-treated chocolate liquor or cocoa powder (*32*) would be expected to be lower in flavanols. In a like manner, niche products made with unfermented cocoa beans (*33*) or beans with minimal roasting (*34*), both treatments that conserve flavanols, would have higher than typical flavanol content. However, because consumers have become increasingly familiar with % cacao labeling of dark chocolate products, our analysis of commercial products indicates that calcd % cacao is a reasonable, but not perfect, guide to the level of epicatechin, oligomeric flavanols, and total flavanols. Clearly, direct measurement and reporting of flavanols is the best and most transparent indicator of flavanol content for consumers.

**Principal Component Analysis of the Data. Table 5** shows the principal component factor analysis for the data shown in **Tables 2** and **3**. In **Table 5**, component 1, % fat has the lowest value (0.0526) compared to the other factors and for component 2, % fat has the lowest value (−0.7106), with catechin being the second lowest value (−0.43137). The principal component weighting (**Table 5**) indicates that PC-1 and PC-2 account for 90.968% of the variation in the data set. In **Figure 5** is shown the product groupings (**Figure 5A**) and the factor separation (**Figure 5B**) for

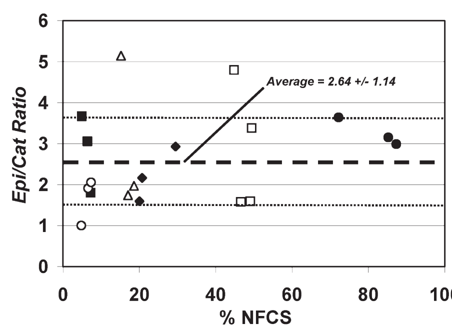


**Figure 6.** Ratio of epicatechin to catechin for the chocolate and cocoa-containing products. Product values for % NFCS (bottom) are used to separate the samples along the *X*-axis. Product categories: cocoa powder (●); baking chocolate (□); dark chocolate (◆); semisweet baking chips (△); milk chocolate (■); chocolate syrup (○).

PC-1 and PC-2. The product categories of cocoa powders, baking chocolates, milk chocolates, and syrups group into distinct areas on the PCA relationship (**Figure 5A**). The exception is the dark chocolate and semisweet chip products, which overlap one another, primarily because they have about the same formula but come in different shapes, forming different product categories. When the chemical attributes are plotted along PC-1 and PC-2 (**Figure 5B**), most of the other flavanol attributes measured as well as ORAC, VCEAC, total polyphenols, and total procyanidins group higher on the PC-2 axis. The % fat shows the most

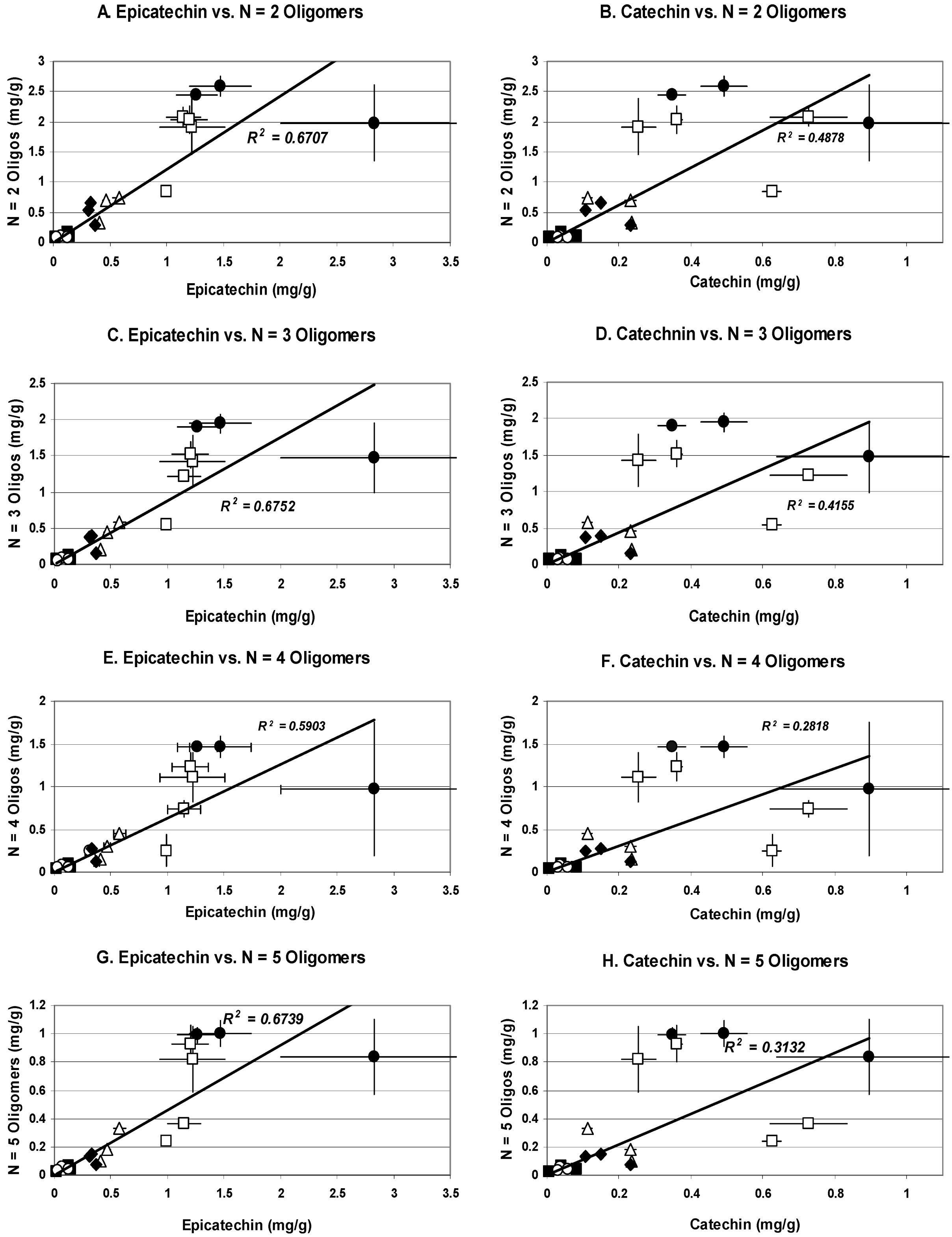


**Figure 7.** Comparison of epicatechin or catechin to dimers (**A** and **B**), trimers (**C** and **D**), tetramers (**E** and **F**), or pentamers (**G** and **H**) in the chocolate- and cocoa-containing samples.

separation from the other attributes, and this separation is along the PC-1 and PC-2 axes. Catechin also separates from the other attributes along the PC-2 axis. This analysis indicates that measures of antioxidant activity, total polyphenols, and the flavanol measures, except for catechin, all group closely together and are not major contributors to overall variation in these data. By contrast, % fat and catechin appear to be major contributors to the variation in the products tested.

**Ratio of Epicatechin to Catechin.** Because the data (**Table 2**; **Figures 2−4**) and the statistical analysis (**Table 5**; **Figure 5**) all indicate that catechin is more variable than epicatechin or the flavan-3-ol oligomers in the products tested, we compared the ratio of epicatechin to catechin in the test samples and displayed these according to the % NFCS in each product (**Figure 6**). This representation shows that the epicatechin to catechin ratio varies between 1.0 for a single syrup sample to

**Table 6.** Average Amount of Flavanols per Serving in Each of the Product Categories Tested Reported as the Mean of Products Tested (Standard Deviation)

| category | serving size (g) | Epi | Cat | Epi + Cat | *N* = 2 | *N* = 3 | *N* = 4 | *N* = 5 | *N* = 6 | *N* = 7−10 | *N* > 10 | total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| cocoa powder | 10 | 18.53 (8.48) | 5.79 (2.84) | 24.32 (11.32) | 23.41 (3.20) | 17.74 (2.62) | 13.00 (2.83) | 9.40 (0.94) | 7.26 (0.90) | 13.81 (3.08) | 124.62 (27.18) | 227.34 (17.23) |
| baking chocolate | 15 | 17.13 (1.54) | 7.36 (3.33) | 24.49 (2.58) | 25.83 (8.77) | 17.65 (6.64) | 12.48 (6.62) | 8.81 (5.08) | 6.74 (4.41) | 12.85 (7.98) | 116.71 (52.38) | 226.53 (25.69) |
| dark chocolate | 40 | 13.47 (1.23) | 6.55 (2.57) | 20.02) (3.79 | 19.55 (7.59) | 12.40 (5.17) | 8.62 (3.24) | 4.74 (1.61) | 3.88 (1.43) | 6.89 (2.59) | 71.42) (11.45 | 145.84 (30.08) |
| semisweet chips | 15 | 7.25 (1.28) | 2.91 (1.06) | 10.16 (0.37) | 8.75 (3.43) | 6.17 (2.93) | 4.60 (2.28) | 3.06 (1.76) | 2.32 (1.47) | 4.50 (2.70) | 36.69 (7.10) | 73.39 (19.58) |
| milk chocolate | 40 | 3.97 (2.67) | 1.73 (1.53) | 5.70 (4.12) | 4.91 (1.76) | 3.43 (1.32) | 2.53 (1.08) | 1.72 (0.76) | 1.18 (0.54) | 2.37 (0.81) | 6.01 (3.97) | 27.43 (9.38) |
| chocolate syrup | 39 | 2.87 (1.80) | 1.63 (0.60) | 4.50 (2.39) | 3.56 (0.49) | 2.75 (0.54) | 2.36 (0.54) | 1.67 (0.47) | 1.63 (0.60) | 3.31 (1.73) | 7.67 (8.20) | 25.75 (9.91) |

5.1 for a semisweet chip product. The average ratio of the samples is 2.64, with three products lying beyond 1 standard deviation of the mean. Thus, it is clear that there is a wide range in the ratio of monomeric composition of the products tested.

**Relationship of Epicatechin and Catechin to Flavanol Oligomers in Chocolate- and Cocoa-Containing Products.** Cooper et al. (*22*) compared the relationship between dimers and higher molecular weight procyanidins to epicatechin and catechin in 68 chocolates and found that there was better correlation to the level of epicatechin than to catechin. We have plotted similar data for the products tested and show these relationships in **Figure 7**. Comparing panels **A** and **B** of **Figure 7**, we find there is a much higher linear correlation of epicatechin to dimers ($R^2$ = 0.671) than there is for catechin to dimers ($R^2$ = 0.488). The correlations with epicatechin to trimers (**Figure 6C**; $R^2$ = 0.675), tetramers (**Figure 6E**; $R^2$ = 0.590), and pentamers (**Figure 6G**; $R^2$ = 0.674) are all much closer to linear than the catechin to trimer (**Figure 6D**; $R^2$ = 0.415), tetramer (**Figure 6F**; $R^2$ = 0.282), or pentamer (**Figure 6H**; $R^2$ = 0.313). Similar comparisons by Cooper et al. (*22*) found that the correlation coefficients were closer to unity for epicatechin (range of $R^2$ from 0.989 to 0.617). However, for catechin, their correlations are more similar to ours (range of $R^2$ from 0.643 to 0.311). One possible difference is that Cooper et al. (*22*) studied samples mostly from the dark chocolate category, whereas here we studied 19 products from 6 different cocoa-containing products covering a broad range of cacao formulations.

**Epicatechin, Catechin, Oligomers, and Procyanidins per Serving.** In **Table 6** are shown the average and standard deviation of epicatechin, catechins, total monomers by the sum of Epi + Cat method, $N = 2-6$ oligomers, $N = 7-10$ oligomers, $N > 10$, and the total flavan-3-ols in the six categories of products tested on a per-serving basis as defined. Total procyanidins are highest in a serving of cocoa powder and baking chocolate, both at 227 mg per serving. The level found in the average dark chocolate is about 146 mg/serving. Semisweet chips have 73 mg per 15 g serving. The total procyanidins per serving for the average milk chocolate is 27 mg per serving and that for chocolate syrup is 26 mg per serving, which are the lowest categories tested or about one-eighth the level of cocoa powder or baking chocolate. These results indicate that the flavan-3-ols are significant and integral components of all products tested. These data also show that all cocoa-containing products, like the ones tested here, contribute to the overall contribution of flavan-3-ols in the diet.

**Factors That May Contribute to Differences in Catechin versus Epicatechin.** Analyses reported here did not attempt to resolve the level of (+)-catechin, the native form of catechin found in fresh cocoa beans (*35*, *36*), from (−)-catechin. There is direct evidence that (−)-epicatechin can be epimerized to (−)-catechin during cocoa bean roasting, and especially after alkali processing of cocoa powder, the so-called Dutched process (*34*, *35*). We speculate that the catechin measured in this study is a combination of the (+)- and (−)-enantiomers and may reflect epimerization due to processing conditions. The high level of variation in the level of catechin reported here may reflect the different roasting conditions used by manufacturers as well as the use of alkali-treated cocoa liquor or powder used in several of the products tested in this survey. In examining the bioavailability of monomeric flavanols in rats, Donovan et al. (*37*) found that (−)-epicatechin is much more bioavailable than (+)-catechin, which in turn is much more bioavailable than (−)-catechin. Because the variation in catechin appears to be greater than that of epicatechin in this sample set, this suggests that the variation in catechin is more of an indicator of cocoa or chocolate processing than a reflection of the availability of the more biologically active epicatechin (*37*).

**Conclusions.** This detailed flavanol analysis shows that epicatechin, flavanol oligomers, and total procyanidins are an integral and essential part of commonly available chocolate- and cocoa-containing products. The concentration of the flavanols, with the possible exception of catechin, vary in a predictable way. There is a strong degree of correlation between flavanols and % NFCS in the products studied, with cocoa powder being highest and chocolate syrup being lowest in these compounds. This makes sense because we know that the flavanols are associated with the nonfat particles in these products and not with the fat component. The level of flavanols is equally well correlated with the total polyphenols measured in these products, probably due to the association of the polyphenols with the nonfat component and not with the fat component. These results are in agreement with those of Cooper et al. (*21*). Not surprisingly, the flavanols are also associated with the calcd % cacao, a proxy for % cacao, of the products studied, although the correlation coefficients are not as high as those found for % NFCS and for total polyphenols. We attribute the lesser degree of correlation to the fact that milk chocolate, in particular, may have cocoa butter and milk fat added to typical commercial formations, which causes a systematic error in the calcd % cacao as determined by the methods described herein. Obviously, the best way to express the level of flavanols found in products is to systematically measure and report actual flavanol levels. Catechin is the flavanol that stands out from the other flavanols as being unique, being less correlated with % NFCS, total polyphenols, epicatechin, flavanol oligomers, or total procyanidins. Although this paper does not resolve the enantiomers of epicatechin or catechin, we speculate that the variation in catechin is due to epimerization reactions that may occur during cocoa-processing steps such as roasting or alkali processing.

## ABBREVIATIONS USED

% NFCS, percent nonfat cocoa solids; ORAC, oxygen radical absorbance capacity; VCEAC, vitamin C equivalence antioxidant capacity; PCA, principal component analysis; PC-1, principal component 1; GAE, gallic acid equivalents; TE, Trolox equivalents, Epi + Cat, sum of epicatechin and catechin; calcd % cacao, calculated percent cacao.

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