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ARTICLE

Impact of Fermentation, Drying, Roasting, and Dutch Processing on Epicatechin and Catechin Content of Cacao Beans and Cocoa Ingredients

Mark J. Payne,* W. Jeffrey Hurst, Kenneth B. Miller, Craig Rank, and David A. Stuart

Hershey Center for Health & Nutrition, Hershey Technical Center, 1025 Reese Avenue, Hershey, Pennsylvania 17033-0805

Low molecular weight flavan-3-ols are thought to be responsible, in part, for the cardiovascular benefits associated with cocoa powder and dark chocolate. The levels of epicatechin and catechin were determined in raw and conventionally fermented cacao beans and during conventional processing, which included drying, roasting, and Dutch (alkali) processing. Unripe cacao beans had 29% higher levels of epicatechin and the same level of catechin compared to fully ripe beans. Drying had minimal effect on the epicatechin and catechin levels. Substantial decreases (>80%) in catechin and epicatechin levels were observed in fermented versus unfermented beans. When both Ivory Coast and Papua New Guinea beans were subjected to roasting under controlled conditions, there was a distinct loss of epicatechin when bean temperatures exceeded 70 °C. When cacao beans were roasted to 120 °C, the catechin level in beans increased by 696% in unfermented beans, by 650% in Ivory Coast beans, and by 640% in Papua New Guinea fermented beans compared to the same unroasted beans. These results suggest that roasting in excess of 70 °C generates significant amounts of (−)-catechin, probably due to epimerization of (−)-epicatechin. Compared to natural cocoa powders, Dutch processing caused a loss in both epicatechin (up to 98%) and catechin (up to 80%). The epicatechin/catechin ratio is proposed as a useful and sensitive indicator for the processing history of cacao beans.

KEYWORDS: Dutch processing; cocoa; cacao; epicatechin; catechin; epimerization; procyanidins; roasting; *Theobroma cacao*

INTRODUCTION

Cacao beans, cocoa powder, and dark chocolates contain substantial amounts of the monomeric flavan-3-ols (+)-catechin and (−)-epicatechin (**Figure 1**) as well as oligomeric and polymeric procyanidins. These natural components are associated with the cardiovascular health benefits of cocoa powder and dark chocolate. The health benefits have been summarized in recent reviews (*1*, *2*). Evidence for these health benefits comes from epidemiological studies with elderly men (*3*), postmenopausal women (*4*), and, recently, survivors of a first heart attack (*5*) in which the risk of cardiovascular disease, heart attack, or death were lower among chocolate consumers compared to nonconsumers. Cocoa and chocolate consumption has been shown, in meta-analysis, to consistently lower blood pressure and increase vasodilation (*6*). Acute and long-term consumption of either chocolate or cocoa has been shown to decrease blood pressure (*7*−*9*). Studies demonstrating improved blood flow are numerous and include measurement by peripheral artery tonography (*10*) and brachial artery flow mediated dilation (*11*, *12*) as well as by increased blood flow to the left anterior descending coronary artery (*13*). Consumption of cocoa-containing food or drink also can positively affect platelet aggregation, slow the rate of oxidation of serum cholesterol, and increase the level of serum HDL (*2*).

Of the flavan-3-ol compounds found in cocoa, the monomeric and, to a lesser degree, the dimeric flavan-3-ols are thought to account for at least part of the effects. Evidence for this comes from bioavailability studies in which epicatechin and catechin concentrations peak in the blood at 2 h postconsumption (*14*, *15*). The peak serum concentration of epicatechin also coincides with the maximum vasodilation observed (*15*). Additionally, the B-2 flavanol dimer has been shown to appear in blood with a similar time course (*16*). Finally, it has been postulated that the cardiovascular effects of cocoa and dark chocolate are due, in part, to epicatechin (*15*).

Given the importance of the low molecular weight flavanols for these health benefits, one can question the impact of food processing on these compounds. It has long been known that fresh cacao beans contain between 12 and 18% total flavanols (*17*−*19*). Common processing steps such as fermentation (*17*, *18*), roasting (*20*), alkaline treatment (Dutch processing) (*21*, *22*), and baking in the presence of baking soda (*23*) all have been shown to reduce both the level of total procyanidins and the level of low molecular weight flavanols. Several papers indicate that the level of epicatechin and catechin can vary independently from one another (*24*, *25*). A number of studies have been reported on other

*Corresponding author. E-mail: mpayne@hersheys.com. Phone: (717) 534-5212.

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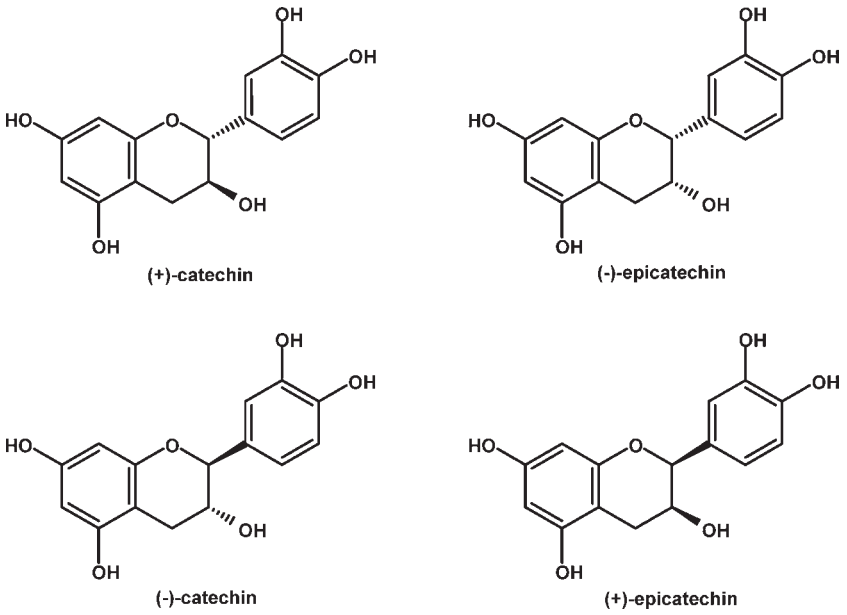

Figure 1. Structures of (+)-catechin, (−)-catechin, (−)-epicatechin, and (+)-epicatechin.

foods, particularly tea (*26*, *27*), demonstrating a similar reduction in monomeric flavanols when exposed to high temperatures or increased pH. It has been postulated that the ratio of epicatechin to catechin (epi/cat) possibly could be associated with the degree of processing of cocoa (*25*). However, to date, we are not aware of a systematic study of conventional food processing on the monomeric flavanols in cocoa from bean to Dutch-processed cocoa powder.

Here the levels of epicatechin and catechin have been measured throughout the processing of cocoa beans, beginning with fresh cocoa beans through fermentation, drying, and roasting to Dutch processing of cocoa powders. The roasted beans, which subsequently are ground into chocolate liquor, natural cocoa powder, and Dutch-processed cocoa powder, are the main ingredients used for all chocolates, cocoa beverages, ice creams, and confections including cakes, biscuits, and cookies.

MATERIALS AND METHODS

Chemicals and Materials. (+)-Catechin hydrate and (−)-epicatechin were purchased from Sigma-Aldrich. HPLC-grade methanol and hexanes, 99% trifluoroacetic acid, and ACS-grade acetic acid were purchased from Thermo Fisher. Stock solutions of (+)-catechin hydrate and (−)-epicatechin were prepared by weighing accurately approximately 0.01 g of (+)-catechin hydrate or (−)-epicatechin into a 50 mL volumetric flask and dissolving into 1:9 methanol/water. Solutions were subdivided into 2 mL glass vials and stored at ≤0 °C. From these stock solutions, working solutions were prepared covering the range from 0.01 to 10 ppm. To prepare working standards, a vial containing the stock solution was thawed and diluted using 1:1 methanol/H_2O + 0.1% acetic acid. The working standards were injected to establish catechin and epicatechin calibration curves.

Cocoa Bean Samples. Fresh cacao pods of the Forestaro type were obtained from the USDA Field Station, Mayaguez, Puerto Rico. Cocoa beans from either unripe or ripe pods were hand dissected using a scalpel and freed from the surrounding, foamy pulp (in the case of unripe beans) or watery pulp (in the case of ripe beans). The seed coat was cut away leaving the cotyledons, hypocotyl axis, and root radicle. Beans from unripe and ripe pods were freeze-dried to moisture levels at or below 5%. Fresh beans were also laboratory-dried for 6−8 h to < 5% moisture in a 70 °C water-jacketed Ross Mixer with agitation. Washed and dried beans, here referred to as cacao lavado, were commercially available from AMSA in Mexico. Fermented beans from Ivory Coast and Papua New Guinea (PNG) were from commercially available fair to average quality beans typical of the origin. Commercial beans are nominally ≤8% moisture. The degree of fermentation of beans was evaluated by extraction and colorimetric determination using the method of Gourieva and Tserevitinov (*28*).

For the roasting study to various temperatures, about 11 kg of nibs were placed in a Barth pilot scale roaster with 105 °C circulating air. Water (300 mL) was added to the nibs, and the mixture was held for 30 min, at which time the nibs had achieved a temperature of 45 °C. The air-circulating temperature then was adjusted to 250 °C, and nibs were allowed to roast to increasing temperatures; triplicate samples were taken at 60, 70, 80, 90, 100, 110, and 120 °C. Dutch-processed cocoas reported here were commercially available cocoa obtained from J. B. Cocoa, and the results of their analysis are available from Miller et al. (*22*).

Sample Preparation. Bean samples were ground in a coffee mill to reduce particle size to ≤1 mm. Samples (~10 g) were defatted using 3 × 35 mL portions of hexanes (*29*). After the solvent had been allowed to evaporate completely, the sample was stored in a closed container. A portion (~0.25 g) of the defatted sample was weighed into a screw-capped test tube, and the sample mass was recorded. Sample extraction solvent (49.5:49.5:1 v/v/v methanol/H_2O/acetic acid, 5.0 mL) was added to the tube. The sample was vortexed and placed in a sonicating bath at 50 °C for 15 min. The sample was vortexed again and centrifuged at 2500 rpm for 4 min. After the supernatant had been decanted, the residue was extracted with a second 5 mL portion of sample extraction solvent. After extraction and centrifugation, the combined supernatants were filtered through a 0.45 μm PTFE syringe filter and then diluted with sample extraction solvent prior to analysis. Dilutions were carried out so that the concentration of analytes fell within the linear range of the method.

Instrumentation and Conditions. The analytical method used to determine catechin and epicatechin data reported in this paper was developed on the basis of previous work reported by Ho et al. (*30*), Arts and Hollman (*31*), Nelson and Sharpless (*32*), the International Standards Organization (*33*), and Cheong et al. (*34*). Chromatography was carried out on a Waters Alliance 2695 separations module with column heater set to 30 °C, using a Phenomenex Luna 5 μm phenylhexyl (250 mm × 4.6 mm) column. A Phenomenex guard column of the same phase also was used. A binary mobile phase consisting of water + 0.05% trifluoroacetic acid (solvent A) and methanol + 0.05% trifluoroacetic acid (solvent B) was employed. The flow rate was established at 1.0 mL/min, and the following linear gradient program was used: t = 0 min, 84% A + 16% B; from t = 4 min to t = 14 min, B was increased to 50% A + 50% B and held until t = 18 min; from t = 18 min to t = 22 min, B was increased to 100% B until t = 26 min and then returned to the initial gradient (84% A + 16% B). The injection volume was 10 μL. Peaks were detected on a Waters 2475 fluorescence detector set to the following wavelengths: excitation at 280 nm, emission at 315 nm. Under these conditions catechin elutes at approximately 12.0 min and epicatechin at 14.5 min. Catechin and epicatechin results are reported on a whole product basis.

Table 1. Epicatechin and Catechin Contents of Unfermented Cocoa Beans That Were Dried in the Sun on the Farm (Sun-Dried) or Dried in the Laboratory (Ross Mixer)[a]

bean treatment	no. of samples	epicatechin, mg/g	% epicatechin vs sun-dried	catechin, mg/g	% catechin vs sun-dried	total monomers, mg/g	epicatechin/ catechin
freeze-dried unripe	3	16.0 ± 0.32	129	0.46 ± 0.01	100	16.5 ± 0.33	34.9 ± 0.49
freeze-dried ripe	3	12.8 ± 0.06	103	0.39 ± 0.01	84	13.2 ± 0.06	33.0 ± 0.35
sun-dried	13	12.4 ± 4.05	**100**	0.46 ± 0.17	**100**	12.8 ± 4.10	29.3 ± 10.3
laboratory-dried	3	12.0 ± 0.00	97	0.35 ± 0.01	76	12.4 ± 0.01	34.3 ± 0.98

[a] Sun-dried unfermented beans represent reference beans (in bold) to which others are compared. Results are expressed as mean value ± standard deviation.

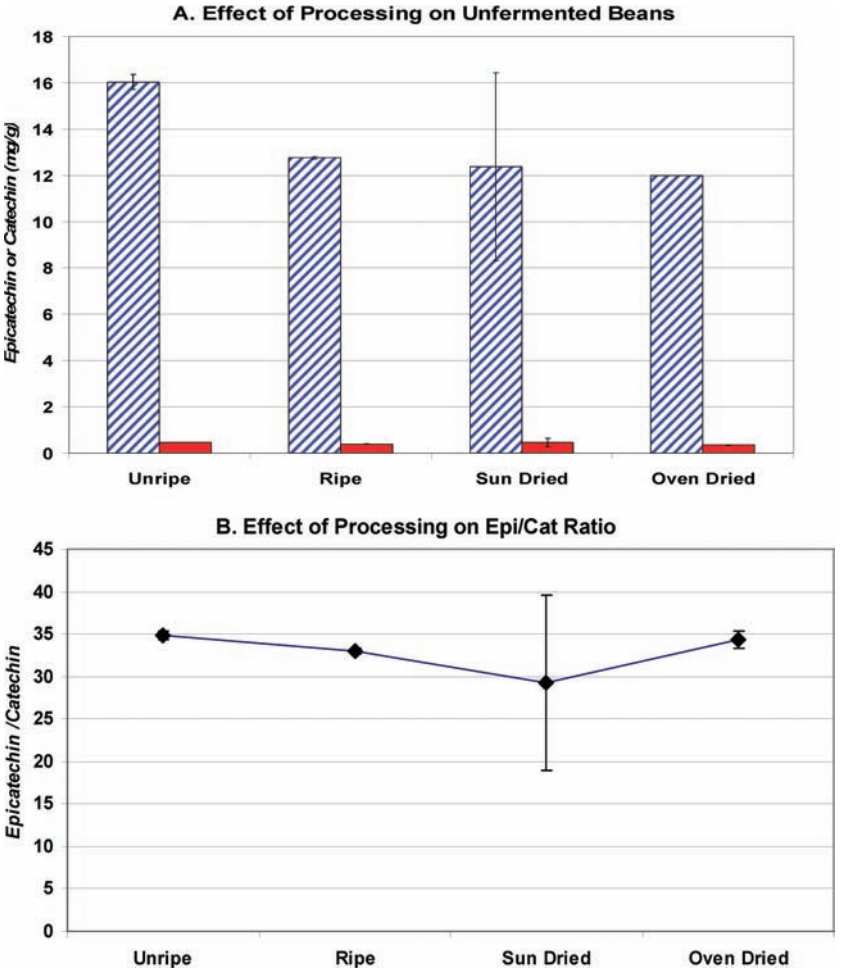

Figure 2. Processing of unfermented cocoa beans through drying: (**A**) blue, slashed bars, epicatechin; solid red bars, catechin; (**B**) epicatechin to catechin ratio. Error bars are ± standard deviation of the mean.

RESULTS AND DISCUSSION

Unripe and Ripe Beans. Fresh beans of Forestaro-type cacao removed from both unripe green pods and ripe pods were subjected to analysis, and the results are shown in **Table 1** and **Figure 2**. Epicatechin and catechin concentrations are based on the weight of the beans after drying (freeze-drying for unripe and ripe beans, sun-drying or laboratory-drying). Beans from unripe pods had epicatechin levels of 16.0 mg/g, which is 26 and 29% higher than that of freeze-dried ripe beans (12.8 mg/g) and beans dried on the farm (sun-dried beans, 12.4 mg/g), respectively. By comparison, the level of catechin was low in both the freeze-dried unripe and the ripe beans. As a consequence, the epicatechin to catechin (epi/cat) ratio in unripe beans was significantly higher (34.9) compared to that in freeze-dried ripe beans (33.0). The results also are shown graphically in **Figure 2A** for epicatechin, catechin, and total monomers (catechin + epicatechin) and in **Figure 2B** for the epi/cat ratio.

Farm-Dried Unfermented Beans. Much of the world's supply of cacao undergoes farm fermentation, but significant quantities of cacao are intentionally not fermented and are immediately dried. These beans are routinely available commercially from the states of Chiapas and Tabasco in southern Mexico, the Dominican Republic, Ecuador, the Amazonia region of Brazil, and Sulawesi in Indonesia. Typically these beans are either immediately dried with the pulp adhering to the bean or are water-washed to remove the pulp using an ancient, traditional process of washing, referred to locally in southern Mexico as cacao lavado (from Spanish, lavar meaning "to wash"). Either unfermented or fermented beans are then dried to moistures ranging from 5 to 8%, typically in the sun or in wood-fired ovens. Beans dried this way can be shipped or stored and represent the cocoa beans of worldwide commerce.

The average epicatechin concentration of representative samples of unfermented beans dried in the sun on the farm was

Table 2. Effect of Roasting Temperature on Unfermented Dried Cocoa Beans from Three Replicate Samples[a]

temp, °C	epicatechin, mg/g	% epicatechin vs sun-dried	catechin, mg/g	% catechin vs sun-dried	total monomers, mg/g	epicatechin/catechin
	8.87 ± 0.23	**100**	0.28 ± 0.01	**100**	9.14 ± 0.23	31.8 ± 0.81
45	9.50 ± 0.23	107	0.30 ± 0.03	107	9.80 ± 0.67	31.3 ± 0.62
60	8.69 ± 0.30	98	0.39 ± 0.02	139	9.07 ± 0.38	22.5 ± 0.65
70	7.76 ± 0.25	87	0.52 ± 0.02	186	8.28 ± 0.27	14.9 ± 0.14
80	6.68 ± 0.35	75	0.98 ± 0.03	350	7.66 ± 0.38	6.81 ± 0.18
90	5.36 ± 0.12	60	1.46 ± 0.06	527	6.81 ± 0.17	3.67 ± 0.07
100	3.98 ± 0.29	45	1.89 ± 0.06	675	5.87 ± 0.38	2.11 ± 0.09
110	2.76 ± 0.06	31	2.26 ± 0.06	808	5.02 ± 0.12	2.11 ± 0.09
120	1.58 ± 0.06	18	1.95 ± 0.06	696	3.23 ± 0.38	1.22 ± 0.01

[a] Results are expressed as mean value ± standard deviation.

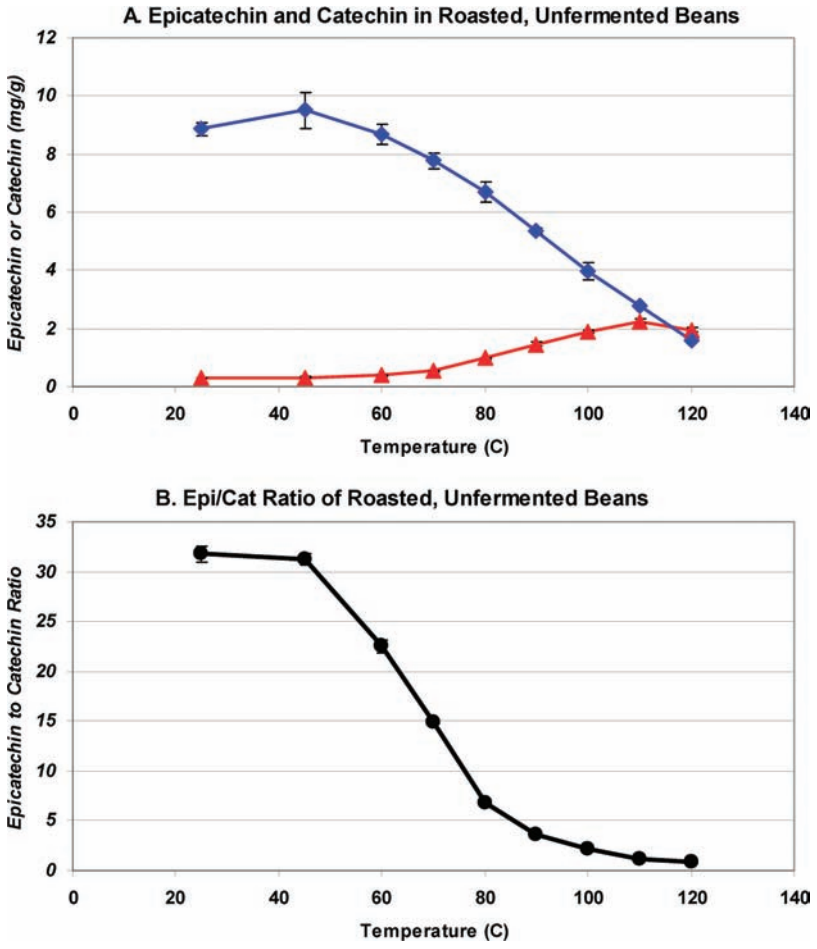

Figure 3. Impact of roast temperature on monomer content of unfermented beans: (**A**) epicatechin (blue diamonds) and catechin (red triangles); (**B**) epicatechin/catechin ratio of unfermented cocoa beans. Error bars are ± standard deviation of the mean.

12.4 mg/g (**Table 1**), indicating that these beans have about the same epicatechin content as the freshly harvested, freeze-dried cocoa beans. This also indicates that the farm drying process had a negligible effect on the level of epicatechin. Finally, note that the catechin levels were slightly, but not significantly, higher at 0.46 mg/g and the epi/cat ratio of these beans is 29.3. Throughout the remainder of this paper we set the epicatechin and catechin content of unfermented beans dried in the sun on the farm (sun-dried, unfermented) as the 100% reference (**reference beans**) to compare treatments.

Processing of Unfermented Beans. Unfermented beans were submitted to laboratory-drying in a Ross Mixer at 70 °C for 6−8 h under vacuum to decrease moisture to about 2.5−4.1%. The results of this drying process are shown in **Table 1**. The laboratory-dried beans had an epicatechin content of 12.0 mg/g and a catechin content of 0.35 mg/g, yielding an epi/cat ratio of 34.3, which compared favorably to that of sun-dried beans. Thus, there was a minimal impact of laboratory-drying compared to sun-drying on the farm.

Controlled Roasting of Unfermented Beans. In **Table 2** are shown the results of sequential roasting of a single lot of farm-dried unfermented cocoa beans to various temperatures. The initial epicatechin content of this lot of beans was 8.87 mg/g. A drop in the level of epicatechin and total monomers began to occur at roast temperatures above 70 °C, and losses increased as internal bean temperatures reached 120 °C (see **Figure 3A**). At the highest temperature, only 18% of the original epicatechin remained (1.58 mg/g). Conversely, the level of catechin started at 0.28 mg/g in unroasted beans and increased at roast temperatures above 60 °C, rising to an average of 1.95 mg/g at 120 °C.

Table 3. Epicatechin and Catechin Contents of Fermented Cocoa Beans Compared to Unfermented Reference Beans[a]

bean type	epicatechin, mg/g	% epicatechin vs sun-dried	catechin, mg/g	% catechin vs sun-dried	total monomers, mg/g	epicatchin/catechin
sun-dried unfermented	12.4 ± 4.05	**100**	0.46 ± 0.17	**100**	12.8 ± 4.10	29.3 ± 10.3
Ivory Coast	1.69 ± 0.10	14	0.08 ± 0.00	20	1.78 ± 0.10	20.1 ± 0.63
Papua New Guinea	0.78 ± 0.04	6	0.05 ± 0.00	11	0.83 ± 0.05	17.1 ± 0.22

[a] A minimum of three replicate samples were analyzed. Results are expressed as mean value ± standard deviation.

Table 4. Effect of Roasting Temperature on Epicatechin and Catechin in Fermented Cocoa Beans[a]

temp, °C	epicatechin, mg/g	% epicatechin vs sun-dried	catechin, mg/g	% catechin vs sun-dried	total monomers, mg/g	epicatechin/catechin
			(**A**) Roasting Study Using Ivory Coast Beans			
25	1.69 ± 0.10	**100**	0.08 ± 0.00	**100**	1.78 ± 0.10	20.1 ± 0.63
45	1.78 ± 0.10	105	0.09 ± 0.00	112	1.88 ± 0.10	19.5 ± 0.81
60	1.80 ± 0.06	107	0.12 ± 0.01	150	1.92 ± 0.06	14.9 ± 0.65
70	1.72 ± 0.06	102	0.15 ± 0.00	188	1.87 ± 0.06	11.1 ± 0.38
80	1.40 ± 0.06	83	0.24 ± 0.02	300	1.64 ± 0.08	5.94 ± 0.21
90	1.22 ± 0.10	72	0.35 ± 0.02	438	1.57 ± 0.12	3.35 ± 0.20
100	0.96 ± 0.07	57	0.42 ± 0.04	525	1.38 ± 0.11	2.29 ± 0.09
110	0.77 ± 0.07	46	0.54 ± 0.05	675	1.30 ± 0.11	1.43 ± 0.05
120	0.50 ± 0.03	30	0.52 ± 0.02	650	1.02 ± 0.06	0.96 ± 0.02
			(**B**) Roasting Study Using Papua New Guinea Beans			
25	0.78 ± 0.04	**100**	0.05 ± 0.00	**100**	0.83 ± 0.05	17.1 ± 0.22
45	0.94 ± 0.03	121	0.05 ± 0.00	100	0.99 ± 0.03	18.1 ± 0.33
60	1.08 ± 0.10	138	0.07 ± 0.01	140	1.15 ± 0.11	14.9 ± 1.28
70	0.93 ± 0.03	119	0.07 ± 0.00	140	1.00 ± 0.03	13.5 ± 0.76
80	0.82 ± 0.02	105	0.08 ± 0.00	160	0.90 ± 0.03	10.5 ± 0.21
90	0.76 ± 0.06	97	0.11 ± 0.01	220	0.89 ± 0.07	7.18 ± 0.24
100	0.72 ± 0.06	92	0.17 ± 0.02	340	0.89 ± 0.07	4.24 ± 0.08
110	0.51 ± 0.04	65	0.24 ± 0.02	480	0.75 ± 0.06	2.14 ± 0.06
120	0.46 ± 0.02	59	0.32 ± 0.02	640	0.78 ± 0.03	1.42 ± 0.03

[a] Three replicate samples used for each determination. Results are expressed as mean value ± standard deviation.

This represented a 696% increase for roasted beans compared to unroasted beans. The epi/cat ratio of unroasted beans was 31.8 (**Table 2**; **Figure 3B**), but as beans were exposed to heating as high as 120 °C, the epi/cat ratio dropped to 1.22 at 120 °C (**Figure 3**).

Analysis of Fermented Beans. On the cocoa farm, freshly harvested cocoa beans and their adhering pulp typically are scooped out as a mass from the cacao pod and placed into heaps, baskets, or boxes at a location on or near the farm. Beans then are allowed to ferment. Fermentation is typically done for a period of 2 to as long as 10 days. Cocoa beans from two sources were chosen for analysis because they represent a range in the degree of fermentation typical of local custom; Ivory Coast beans are typically fermented for 4−5 days, which is considered medium fermentation, whereas Papua New Guinea (PNG) beans are fermented for up to 10 days, which is considered to be a long or heavy fermentation. In **Table 3** are shown the levels of epicatechin and catechin for samples of commercially available, dried beans compared to the average sun-dried unfermented beans. The average level of epicatechin in farm-dried Ivory Coast beans was 1.69 mg/g and that for catechin was 0.08 mg/g. These values were 14 and 20%, respectively, of reference beans. The PNG beans had only 0.78 mg/g epicatechin and 0.05 mg/g catechin, which represent 6 and 11% of the sun-dried unfermented reference cocoa beans. The loss of epicatechin and/or catechin as a result of fermentation has been reported earlier (*17*−*19*). The epi/cat ratio of the fermented beans ranged from 20.1 for Ivory Coast to 17.1 for PNG beans, which represented a significant decrease in this ratio compared to the unfermented beans, all of which were above 29 (**Tables 1** and **3**).

Controlled Roasting of Fermented Beans. A roasting experiment similar to the one above for unfermented beans was performed with the medium fermented Ivory Coast and heavily fermented PNG beans, and the results are shown in **Table 4** and **Figure 4**. A single lot of Ivory Coast beans was subjected to increasingly higher temperatures, and samples of the beans were taken for analysis. The epicatechin content of beans heated above 70 °C decreases (**Table 4A**; **Figure 4A**). After roasting to 120 °C, the level of epicatechin dropped to 0.50 mg/g, or 30% of the level of unroasted Ivory Coast beans and 4% of the level of the sun-dried unfermented reference beans. The catechin content of beans after roasting rose to 0.52 mg/g, or 650% of the content of unroasted Ivory Coast beans and 113% of the content of sun-dried unfermented reference beans. The epi/cat ratio of the Ivory Coast beans roasted to 120 °C was 0.96 mg/g. For the heavily fermented PNG beans the epicatechin content of beans roasted to 120 °C was 0.46 mg/g, or 59% of the content of unroasted PNG beans and only 3.7% of the content of the sun-dried unfermented reference beans. The catechin content of PNG beans roasted to 120 °C was 0.32 mg/g, or 640% of the content of unroasted PNG beans and 70% of the content of sun-dried unfermented reference beans shown in **Figure 2**. The epi/cat ratio for PNG beans after roasting was 1.42. Thus, whereas the level of epicatechin measured in fermented and roasted beans dropped below 1 mg/g, there was an *increase* in catechin as a result of roasting compared to the reference sun-dried unfermented cocoa beans. As a result, the epi/cat ratio of fermented and roasted beans dropped to about 1.

Analysis of Dutch (Alkali) Processed Cocoa Powders. A series of commercially available cocoa powders was purchased and characterized as previously described by Miller et al. (*22*). The data from four to six independent determinations of these powders were grouped and classified as natural powders, lightly Dutch-processed,

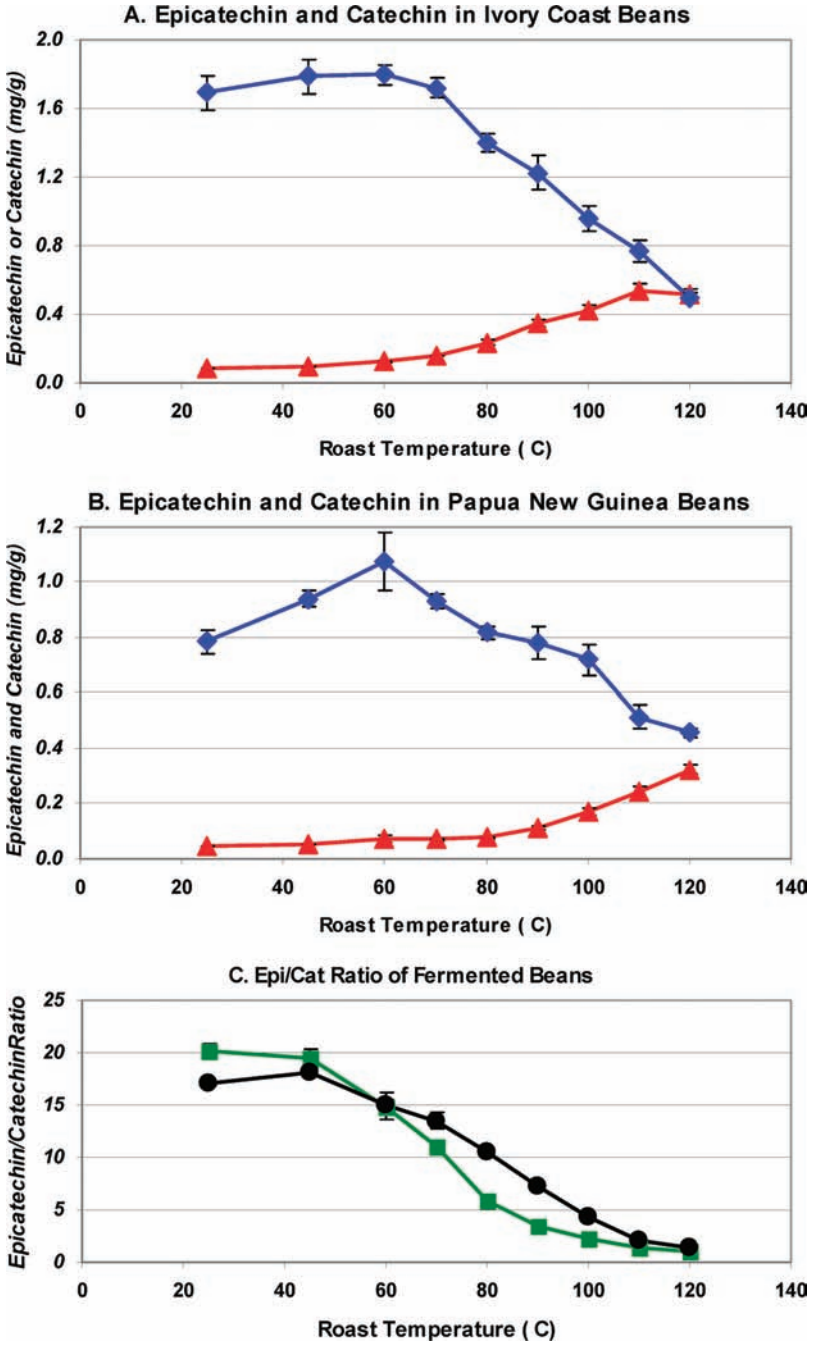

Figure 4. Effect of roasting on monomeric flavanol content of fermented cocoa beans: (**A**) Ivory Coast beans and (**B**) Papua New Guinea beans (blue diamonds, epicatechin; red triangles, catechin); (**C**) epicatechin/catechin ratio (green squares, Ivory Coast; black circles, Papua New Guinea). Error bars are ± standard deviation of the mean.

Table 5. Effect of Dutch Processing on the Epicatechin and Catechin Content of Cocoa Powders[a]

Dutch Processing	extractable pH	epicatechin, mg/g	catechin, mg/g	total monomers, mg/g	epicatechin/catechin
none (natural)	5.59	2.23 ± 0.46	0.88 ± 0.14	3.11 ± 0.54	2.57 ± 0.50
light	6.96	0.69 ± 0.55	0.70 ± 0.48	1.38 ± 1.03	0.93 ± 0.09
medium	7.36	0.26 ± 0.29	0.36 ± 0.34	0.62 ± 0.63	0.71 ± 0.17
heavy	7.89	0.04 ± 0.04	0.09 ± 0.08	0.13 ± 0.11	0.40 ± 0.34

[a] Results are expressed as mean value ± standard deviation.

medium-Dutch-processed, or heavily alkali treated depending upon the final extractable pH of the powder (see Miller et al. (*22*) for details). The results of these analyses are shown in **Table 5** and **Figure 5**. The natural cocoa powders had an average pH of 5.59, 2.23 mg/g of epicatechin, 0.88 mg/g of catechin, 3.11 mg/g of total monomers, and an epi/cat ratio of 2.57. With progressive Dutch processing there was a decrease in epicatechin, catechin, and total monomers. With Dutch processing there also was a decrease in the epi/cat ratio to 0.40 for the powders receiving the heaviest Dutch processing.

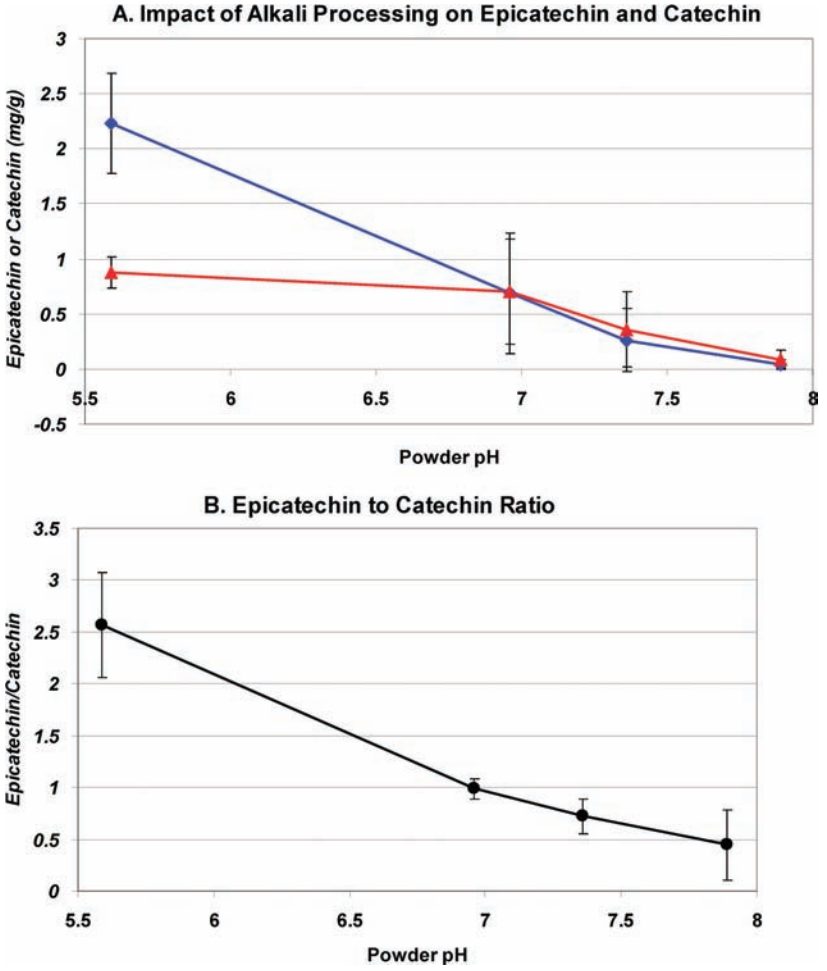

Figure 5. Effect of alkali processing on epicatechin, catechin, and total flavanol monomers in cocoa powder: (**A**) epicatechin (blue diamonds), catechin (red triangles); (**B**) epicatechin/catechin ratio. Natural powder is pH 5.59, lightly alkalized is pH 6.96, medium alkalized is pH 7.36, and heavily alkalized is pH 7.89. Error bars are ± standard deviation of the mean.

Summarization of Processing Effects. The data in **Figure 6** summarize selected data in this paper and place data on an equal bean equivalent basis by correcting cocoa powders back to a whole bean, assuming that average cocoa beans are 53% cocoa butter and that typical cocoa powders range between 10 and 12% cocoa butter. The epicatechin content dropped substantially as beans were fermented (compare lavado, sun-dried, to fermented, sun-dried), when beans were then roasted to 120 °C (compare fermented, sun-dried, to fermented, roasted), and when beans were Dutch processed (compare natural powder to medium Dutch-processed powder). The epi/cat ratio showed that the highest ratios were found in unripe and ripe, unfermented dried beans (lavado) with a range of 32−35. Fermentation lowered the epi/cat ratio to about 20. Epi/cat ratios were further reduced to 0.90−1.40 by roasting. The lowest epi/cat ratios were found in Dutch-processed cocoa powders and range from about 0.4 to slightly less than 1.

The data reported here are, to our knowledge, the first comprehensive report on the changes in the monomeric flavan-3-ols, namely, epicatechin and catechin, in cocoa beans ranging from unripe to ripe, dried, fermented, roasted, and Dutch-processed powders. Furthermore, this is the first detailed study of the fate of epicatechin and catechin during the roasting process, a process that develops chocolate flavor and is an essential step in ensuring microbiological safety of chocolate- and cocoa-containing products. We find that the loss of epicatechin began before the cocoa pod is fully ripe (**Table 1**). Once ripe, the epicatechin content of beans was roughly the same regardless of whether beans were freeze-dried, sun-dried, or laboratory-dried. Roasting caused the epicatechin content of unfermented beans to drop 82% when roasted to a terminal temperature of 120 °C and in medium fermented beans to 18% (roasting to 120 °C) of reference beans. Catechin content also decreased as beans ripened. Although the catechin values are low throughout processing, their levels rose by 640−696% in beans as a result of roasting (**Tables 2** and **4**).

When beans are fermented, there is a typical loss in epicatechin that is dependent on the length of fermentation. As a result of variation in the degree of fermentation, the average values for epicatechin, catechin, and the epi/cat ratio in fermented cacao beans show a high degree of variation, as indicated by the large standard deviation of the mean. Because the data shown are the average of 13 lots of beans from origins that have different traditional methods of fermentation, this variation likely was the result of differences in fermentation (**Figure 6**). Roasting of fermented beans caused an additional loss in the epicatechin content of beans such that the Ivory Coast and the Papua New Guinea beans both have about 0.5 mg/g epicatechin after roasting. The catechin content also was reduced as a result of fermentation, but not to the same degree as that of epicatechin, as is reflected in the lower percentage loss and the lower epi/cat ratios. After roasting, the catechin content increased so that roasted beans have more catechin than sun-dried unfermented beans.

The analytical methods employed throughout this study used a common and standard method of Nelson and Sharpless

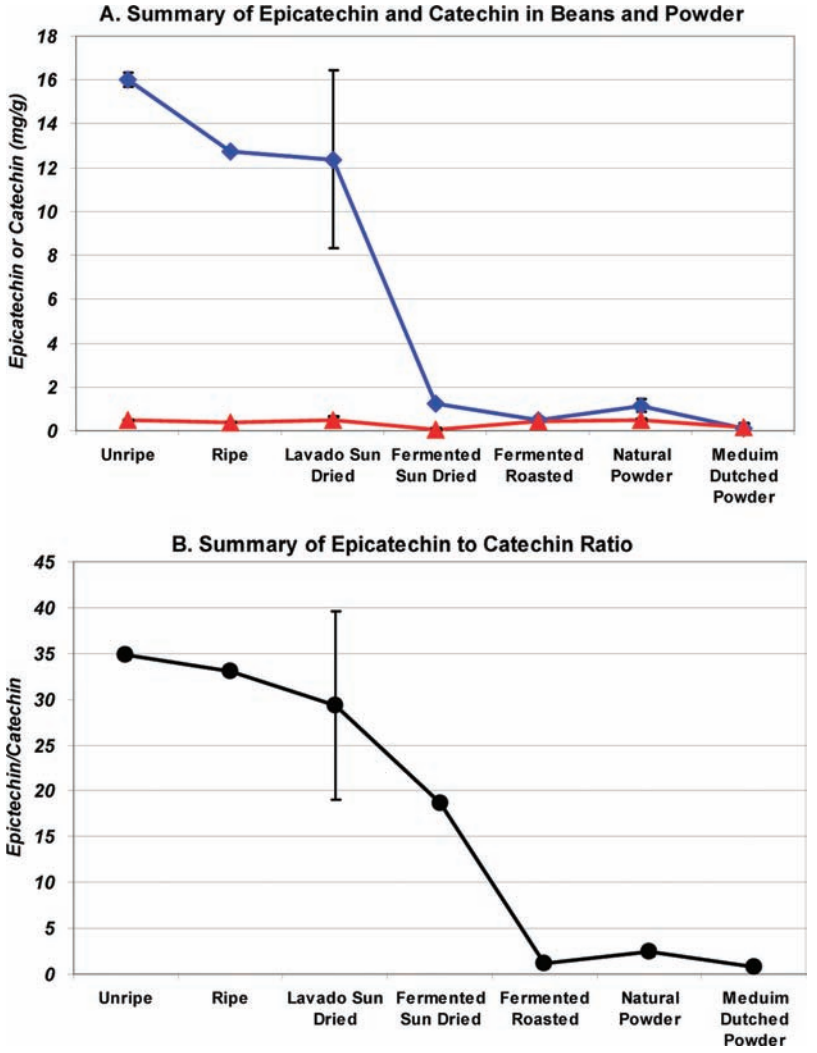

Figure 6. Summary of processing of beans and cocoa powder on total monomers, epicatechin, catechin, and epicatechin/catechin ratio: (**A**) epicatechin (blue diamonds), catechin (red triangles); (**B**) epicatechin/catechin ratio. Error bars are ± standard deviation of the mean.

(*32*), employing HPLC coupled to a fluorescence detector. Fluorescence is an established detection technique that offers excellent sensitivity and selectivity for catechin and epicatechin. Although this method does not resolve the stereochemical configuration of epicatechin or catechin, more recent findings that resolve the (+)- and (−)-forms of epicatechin and catechin show that (a) unfermented cocoa contains primarily (−)-epicatechin and (+)-catechin (*35*) and (b) roasting and/or alkaline treatment can cause an epimerization of (−)-epicatechin to (−)-catechin (*36*, *37*). When the data reported here, and especially the epi/cat ratios, are interpreted in light of these findings, we feel that the ripening process of the cocoa beans causes loss in epicatechin due to biological oxidation and or polymerization of monomers to procyanidins. During fermentation of beans, temperatures in excess of 60 °C for as long as 2 days can be achieved (*38*). The disproportionate losses in epicatechin compared to catechin may be partly accounted for by epimerization of (−)-epicatechin to (−)-catechin due to the heat of fermentation as suggested by the change in the epi/cat ratio from between 29 and 32 in fresh beans to about 20 for fermented beans. To our knowledge this is the first time that the heat of fermentation has been implicated in the epimerization of (−)-epicatechin to (−)-catechin, but real evidence based on stereoseparation of the enantiomers is needed.

Increases in the level of catechin that resulted from roasting unfermented and fermented beans were almost certainly the result of the epimerization of (−)-epicatechin to (−)-catechin at roast temperatures in excess of 70 °C. Thus, the apparent increase in catechin as a result of roasting probably is best described as the loss of (+)-catechin due to roasting and the appearance of (−)-catechin due to the epimerization of (−)-epicatechin to (−)-catechin during the roasting process. Resolution of this awaits analysis of these samples for the (−)- and (+)-enantiomers. The amount and form of catechin probably has dietary significance for consumers of cocoa and chocolate because the bioavailability of epicatechin and catechin in mammalian systems is (−)-epicatechin > (+)-catechin > (−)-catechin (*39*).

Substantial research has been carried out on processing effects of flavan-3-ols in tea, including exposure to high temperatures and increased pH. Results reported have been similar to those observed here, namely, a significant decrease in the concentration of (−)-epicatechin and an increase in (−)-catechin, attributed primarily to epimerization. Although it is reasonable to assume that the same mechanism applies to the changes observed in cacao flavanols, it is important to note that tea leaves and extracts are compositionally different from cacao. Additionally, the processing steps normally applied to cacao are not applied to tea leaves.

But even without resolving the stereochemistry of epicatechin and catechin, a useful diagnostic for several bean-processing steps emerges from these data—namely, the epi/cat ratio. Fresh and dried, unfermented beans have an epi/cat ratio of ≥29. The

examples of fermented beans reported here have epi/cat ratios in the range of 17−20. When beans are roasted to 120 °C, the epi/cat ratio drops to between 1.22 and 4.0 for unfermented beans and ranges between 0.96 and 1.42 for fermented beans. Dutch processing of cocoa beans or powder may also show a similar epimerization reaction, and we observe that lightly Dutch processed powders have epi/cat ratios pf slightly less than 1, whereas heavily Dutch processed powders have an epi/cat ratio of < 0.5. Indeed, several papers indicate that commercially available cocoa powders and chocolates have significant variation in catechin content specifically, which we know can be affected by heating and Dutch processing (*40*). This pattern of change in the epi/cat ratio suggests that the epi/cat metric might be a useful and sensitive indicator of the processing history of beans.

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