Relationship between Procyanidin and Flavor Contents of Cocoa Liquors from Different Origins

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The flavor of eight cocoa liquors of different origins (Africa, America, and Asia) and different varieties (Fine grades: criollo, trinitario, and nacional. Bulk-basic grade: forastero.) was analyzed by headspace solid-phase microextraction mass spectrometry (HS-SPME-MS). Their procyanidin contents were quantified by HPLC–UV (280 nm). Fine varieties with short fermentation processes proved to contain more procyanidins, while criollo from New Guinea and forastero beans showed the highest aroma levels. The levels of cocoa aroma compounds formed during roasting are shown to vary directly with bean fermentation time and inversely with residual procyanidin content in cocoa liquor. Measurement of antioxidant activity in cocoa liquor proved to be a useful tool for assessing residual polyphenols.

**KEYWORDS:** Cocoa origins; chocolate; procyanidins; flavor; AAPH antioxidant activity

**INTRODUCTION**

The flavor and polyphenol contents of cocoa liquors depend on cocoa bean variety (genotype), postharvest processes (fermentation and drying), and roasting conditions (1–4). Mainly three varieties of cocoa beans are produced worldwide: forastero (bulk grade, 70% of the world production), criollo (fine grade), and their hybrid, trinitario (fine grade) (5). Cacao from Ecuador (Arriba) is viewed as a third fine variety: nacional (6). Bulk cocoas usually exhibit strong, harsh flavors while fine cocoas are perceived as more aromatic or smoother (4). Cocoa bean fermentation and drying contribute to developing cocoa–chocolate flavors by increasing levels of amino acids and sugars, reagents of the Maillard reactions that occur during roasting. Through these postharvest processes, polyphenols are usually lost by diffusion, browning, and oxidative polymerization.

Dried fermented cocoa beans from Southeast Asia and the South Pacific are characterized by a higher acidity (higher levels of lactic and acetic acids) than those of South African origin. Of the four acids analyzed (lactic, acetic, citric, and oxalic), only oxalic acid seems to have a positive impact on chocolate taste (7).

Each cocoa liquor exhibits its own organoleptic properties. For instance, Cameroon liquors are famous for their bitterness while Ecuador liquors are known for their raisin–fruity flavor. Bailey et al. (1) detected only quantitative variations in aroma between origins of dried fermented cocoa beans (these authors investigated the Accra, Arriba, Bahia, Sanchez, and Trinidad origins). According to Ziegleder (8), fine dried fermented cocoa beans (Ecuador, Trinidad, and Venezuela) contain up to 8 times more linalool than bulk-basic ones (Ghana, Ivory Coast, Brazil). Linalool might thus be responsible for the flowery–tea note found in fine varieties. In addition, Muggler-Chavan et al. (9) found higher butanol, isopentanol, hexanol, and octanol levels in criollo–trinitario–nacional dried fermented cocoa beans (Puerto Cabello, Arriba, Trinidad, and Caracas) than in four forastero origins (Carupano, Bahia, Para, and Accra). On the other hand, high concentrations of phenol, guaiacol, 2-phenylbutenal, and γ-butyrolactone characterize the Bahia beans known for their typical smoked ham odor. Also noteworthy are the higher levels of 2-methylpropanal and 3-methylbutanal in Caracas and Trinidad dried fermented beans (10). As far as other Maillard products are concerned, Reineccius et al. (11) report that roasting leads to higher levels of pyrazines in well-fermented beans (Ghana, Bahia) than in less-fermented (Arriba) or unfermented (Sanchez, Tabasco) materials.

Owing to their lower astringency and bitterness, mainly imparted by polyphenols according to refs 3 and 12, criollo beans are often less fermented than the forastero varieties. The N index, defined as the soluble nitrogen percentage [(N_{solute}/N_{total}) \times 100] is used to assess the intensity of the fermentation process. On a scale of shorter to longer fermentation times, the order is usually as follows: Machala (Ecuador) < Sanchez (Dominican Republic) < Para (Brazil) < Costa Rica < Carupano (Venezuela) < Indonesia < Grenada < Jamaica < Fernando Po (Republic of Ecuador) < Puerto Cabello (Venezuela) < Trinidad < Arriba (Ecuador) < Ivory Coast < Accra (Ghana) < New Guinea < Cameroon < Lagos (Nigeria) < San Thome < Bahia (Brazil) (13).

As recently shown by Counet et al. (14), procyanidins, the main class of polyphenols in cocoa products (Figure 1), impart not only astringency and bitterness to chocolate but also its exceptional antioxidant activity. The aim of the present work was therefore to investigate how the procyanidin level of cocoa liquors of different origins might be predicted (14). Eight different samples were analyzed by HS-SPME-GC-MS for their
aroma content and by HPLC–UV/MS so as to quantify procyanidin in aqueous acetone extracts of defatted cocoa liquors. Measurement of antioxidant activity was also performed by using the AAPH [2,2′-azobis(2-amidinopropane)dihydrochloride]-induced linoleic oxidation assay.

EXPERIMENTAL PROCEDURES

Materials. Eight cocoa liquors from different origins and varieties were supplied by Belcolade (Erembodegem, Belgium): Ivory Coast and Ghana (forastero), New Guinea, Java, Venezuela and Madagascar (criollo), Ecuador (nacional), Trinidad (trinitario).

Chemicals. Acetone (99.9%), (−)-epicatechin, (+)-catechin, and most flavor compounds were from Sigma-Aldrich (Bornem, Belgium). Only 1-methylpropyl acetate, 2-pentanol acetate, 3-methyl-1-butanol acetate, and 2-methyl-1-butanol acetate were synthesized from the corresponding alcohol and acids in the presence of sulfuric acid. Methanol (99.9%) and dichloromethane (99.9%) were purchased from Romil (Cambridge, U.K.). Acetic acid (99.8%) was from Acros (Geel, Belgium), and diethyl ether (99.5%) from Fluka (Buchs, Switzerland). Aqueous solutions were made with Milli-Q (Millipore, Bedford, MA) double-distilled water (resistance = 18 mΩ).

Procyanidin Analysis of Cocoa Liquors (14). Lipid Removal. Cocoa liquor (70 g) was reduced to a powder with a mixer and introduced into the Soxhlet extractor (Waterkeyn, Belgium) by using a filtration cartridge (Schleicher & Schüll, Germany). Lipids were removed for 24 h with diethyl ether (375 mL). Defatted cocoa liquor (50 g) was finally obtained.

Procyanidin Extraction. Defatted cocoa liquor (10 g) was extracted three times with 50 mL of solvent (3 × 1 h, 25 °C). The organic solvent used for procyanidin extraction was acetone mixed with water and acetic acid (70:28:2, v/v). After each extraction, the suspension was centrifuged for 10 min at 3000g, and the supernatant was collected. After filtration to remove residual particles, the combined supernatants were concentrated by rotary evaporation under partial vacuum (40 °C) to obtain about 50 mL of extract.

Procyanidin Extracts Purification. A 10 g C18 Sep-Pack cartridge (Waters, Millipore) was preconditioned with methanol and then with deionized water. About 50 mL of a procyanidin mixture was loaded on the cartridge, and sugars were removed with 200 mL of deionized water. Procyanidins were then eluted with 30 mL of acetic acid (70:28:2, v/v). The eluates were concentrated by rotary evaporation under partial vacuum (40 °C) and freeze-dried.

High Performance Liquid Chromatography Analysis of Procyanidin Extracts (HPLC–UV). A SpectraSystem (Finnigan Mat, San Jose, CA) equipped with a SCM degasser, an AS3000 autosampler, a P4000 quaternary pump, and a diode array detector UV6000LP at 280 nm was used for quantification (identification previously checked by mass spectrometry according to ref 14). The system was controlled with Xcalibur software version 1.2 (Finnigan Mat). Procyanidins were separated on a Phenomenex 5 μm normal-phase Luna silica column, 250 × 4.6 mm i.d. (Bester, Holland) at 25 °C. Separations were carried out at a flow rate of 1 mL/min with a linear gradient from A (dichloromethane) to B (methanol) and a constant 4% level of C (acetic acid and water, 1:1, v/v). Gradient elution was 14–28% B, 0–30 min; 28–50% B, 30–60 min; 50–86% B, 60–65 min; 65–70 min isocratic. Procyanidin extract (10 mg) was diluted in 1 mL of methanol before injections in duplicate with the 20 μL Rheodyne loop (Berkeley, CA).

Antioxidant Assay: AAPH Method. The reduction power of cocoa liquor extracts was measured by a method developed in our laboratory by Liégeois et al. (15). The oxidation of linoleic acid was induced by 2,2′-azobis(2-amidinopropane)dihydrochloride (AAPH) in an aqueous...
dispersion in the absence or presence of antioxidant. The rate of oxidation at 37°C was monitored by recording the increase in absorption at 234 nm caused by conjugated diene hydroperoxides. A Shimadzu (Antwerp, Belgium) UV-visible 240 spectrophotometer equipped with an automatic sample positioner allowed analysis of six samples per minute. In all cases, the measurements were run in duplicate against the buffer and compared with the case of a separate AAPH-free control to check for any spontaneous oxidation.

Flavor Analysis of Cocoa Liquors. Headspace Solid-Phase Microextraction (HS-SPME). Twenty-three millimeters of PDMS/Carboxen fiber (Supelco, Bellefonte, PA) was introduced into the headspace of vials (Chromacol Ltd, Lokeren, Belgium) containing 2 g of cocoa liquor and equipped with a magnetic CrimpCap (20 mm in diameter) and a silicon—Teflon septum (Interscience, Louvain-la-Neuve, Belgium). The volatile compounds of 2 g of cocoa liquor were adsorbed onto the fiber for 20 min at 25°C. They were then automatically desorbed for 5 min at 250°C in the gas chromatography injector (penetration of the fiber: 54 mm).

Gas Chromatography—Mass Spectrometry Analysis (GC-MS) (18). The gas chromatograph was a Trace GC (Finnigan Mat, San Jose, CA) equipped with a split—splitless injector and an MS detector linked to a computer with Xcalibur software version 1.2 (Finnigan Mat). Compounds were separated using a 50 m × 0.32 mm i.d., wall coated open tubular (WCOT) nonpolar CP-Si5-CB capillary column (film

Figure 3. Total (a and b) and detailed (c) concentrations of procyanidins (P1 to P8 = monomeric to octameric procyanidins) in extracts of cocoa liquors of different origins (extraction and HPLC—UV injection each in duplicate, CV = 2.8−1.7%): (a) mg/100 g of dry extract; (b and c) mg/kg of cocoa liquor (recovery factor set at 100%). (C) = criollo, (T) = trinitario, (F) = forastero, and (N) = nacional.
thickness: 1.2 µm) (Varian, Sint-Katelijne-Waver, Belgium). The oven temperature was programmed from 36 to 85 °C at 2 °C/min, to 145 °C/min, and then was held constant at 250 °C for 30 min. The injection temperature, the split flow, and the splitless time were respectively 250 °C, 20 mL/min, and 1 min.

The retention index (RI) was calculated by using n-alkanes as references. MS analyses were carried out with a Trace MS quadrupole mass spectrometer (Finnigan Mat, San Jose, CA). Electron impact mass spectra were recorded at 70 eV (2.45 scan per second) with a 40-400 amu range.

**Flavor Quantification.** Before analysis, increasing concentrations of commercial standards (aromas) were added to the Venezuelan cocoa liquor. The standard addition curves obtained in this way were then used to quantify compounds in other cocoa samples.

**RESULTS AND DISCUSSION**

**Procyanidins in Cocoa Liquor Extracts.** As depicted in Figure 2 for the Madagascar origin, procyanidin monomers (P1), dimers (P2), and so forth up to octamers (P8) were found in our eight cocoa liquor extracts (HPLC-UV data compared with MS results previously reported for chocolate extracts (14)). Yet significant differences (as much as 8-fold) in total procyanidin concentration were observed between the origins, with the order being Madagascar > Java > Trinidad > Ecuador > Venezuela > Ivory Coast > Ghana > New Guinea (Figure 3a). Most of the fine-cocoa liquor extracts (less fermented) emerged at the top of the range [Madagascar (criollo), Java (criollo), Trinidad (trinitario), and Ecuador (nacional)] while bulk cocoa liquors from Africa [Ivory Coast and Ghana (forastero), highly fermented] proved very poor in such compounds. Although it belongs to the criollo variety, the New Guinea extract ranked lowest on our scale. This is probably also due to the long fermentation applied.

Taking into account the yield of each cocoa liquor, milligrams of procyanidins per 100 g of dry extract were converted into

<table>
<thead>
<tr>
<th>T (min)</th>
<th>RI</th>
<th>compound</th>
<th>T (min)</th>
<th>RI</th>
<th>compound</th>
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<tr>
<td>6.01</td>
<td>665</td>
<td>3-methylbutanal&lt;sup&gt;a,b,d&lt;/sup&gt;</td>
<td>18.31</td>
<td>928</td>
<td>5-methyl-2-furancarboxaldehyde&lt;sup&gt;a,c&lt;/sup&gt;</td>
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<tr>
<td>6.12</td>
<td>668</td>
<td>2-methylbutanal&lt;sup&gt;f&lt;/sup&gt;</td>
<td>18.63</td>
<td>933</td>
<td>benzaldehyde&lt;sup&gt;a,c,f&lt;/sup&gt;</td>
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<tr>
<td>6.71</td>
<td>686</td>
<td>pentanal&lt;sup&gt;f&lt;/sup&gt;</td>
<td>20.16</td>
<td>949</td>
<td>dimethyl trisulfide&lt;sup&gt;a,d&lt;/sup&gt;</td>
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<tr>
<td>6.82</td>
<td>689</td>
<td>2-pentanol&lt;sup&gt;d&lt;/sup&gt;</td>
<td>21.82</td>
<td>973</td>
<td>2-ethyl-6-methylpyrazine&lt;sup&gt;a,b&lt;/sup&gt;</td>
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<tr>
<td>8.26</td>
<td>732</td>
<td>dimethyl disulfide&lt;sup&gt;a&lt;/sup&gt;</td>
<td>22.23</td>
<td>978</td>
<td>trimethylpyrazine&lt;sup&gt;a,b&lt;/sup&gt;</td>
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<td>8.46</td>
<td>738</td>
<td>1-methylpropyl acetate</td>
<td>22.62</td>
<td>982</td>
<td>myrcene&lt;sup&gt;c&lt;/sup&gt;</td>
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<td>8.95</td>
<td>752</td>
<td>toluene&lt;sup&gt;a&lt;/sup&gt;</td>
<td>24.54</td>
<td>1005</td>
<td>benzy alcohol&lt;sup&gt;a,c&lt;/sup&gt;</td>
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<tr>
<td>9.71</td>
<td>775</td>
<td>dihydro-2-methyl-3(2H)-furanone&lt;sup&gt;a&lt;/sup&gt; and hexanal&lt;sup&gt;d,f&lt;/sup&gt;</td>
<td>26.14</td>
<td>1021</td>
<td>2-acetylpyrrole&lt;sup&gt;a,c&lt;/sup&gt;</td>
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<td>3-methylbutanolic acid&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>limonene&lt;sup&gt;c&lt;/sup&gt;</td>
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<td>10.91</td>
<td>806</td>
<td>2,5-dimethylpyrazine&lt;sup&gt;a&lt;/sup&gt;</td>
<td>27.82</td>
<td>1037</td>
<td>3(or 2)-ethyl-2(or 3)-5-dimethylpyrazine&lt;sup&gt;p,f&lt;/sup&gt;</td>
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<td>2-pentanol acetate&lt;sup&gt;c&lt;/sup&gt;</td>
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<td>1070</td>
<td>2-nonanone&lt;sup&gt;c&lt;/sup&gt;</td>
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<td>oxyde de linol&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>856</td>
<td>2,6-dimethyl-1-butanol acetate</td>
<td>32.78</td>
<td>1084</td>
<td>β-phenylethanol&lt;sup&gt;a,c&lt;/sup&gt;</td>
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<td>865</td>
<td>2-heptane&lt;sup&gt;a&lt;/sup&gt;</td>
<td>33.80</td>
<td>1094</td>
<td>2-isopropyl-5-methylhex-2-enal</td>
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<tr>
<td>14.95</td>
<td>880</td>
<td>2-heptanone&lt;sup&gt;a&lt;/sup&gt;</td>
<td>38.82</td>
<td>1138</td>
<td>2,3,5-trimethyl-6-ethylpyrazine</td>
</tr>
<tr>
<td>15.32</td>
<td>887</td>
<td>2,5-dimethylpyrazine&lt;sup&gt;a&lt;/sup&gt;</td>
<td>43.62</td>
<td>1178</td>
<td>or 3,5-diethyl-2-methylpyrazine&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>15.64</td>
<td>893</td>
<td>ethylpyrazine&lt;sup&gt;a&lt;/sup&gt;</td>
<td>49.43</td>
<td>1226</td>
<td>ethyloctanolate&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>15.88</td>
<td>897</td>
<td>2,3-dimethylpyrazine&lt;sup&gt;a&lt;/sup&gt;</td>
<td>50.84</td>
<td>1237</td>
<td>2-phenylethanol acetate&lt;sup&gt;a,c&lt;/sup&gt;</td>
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<tr>
<td>16.25</td>
<td>903</td>
<td>4-hydroxy-2-butane</td>
<td>74.07</td>
<td>1485</td>
<td>α-ethylidene benzene acetaldehyde (2-phenylbut-2-enal)</td>
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</tbody>
</table>

<sup>a</sup> Compound previously identified in a dark chocolate by (18). <sup>b</sup> Compound previously reported as key odorant in bitter chocolate by (19). <sup>c</sup> Compound previously identified in a dark semi-sweet chocolate before conching by (23). <sup>d</sup> Compound previously reported as key odorant in milk chocolate by (20). <sup>e</sup> Compound previously identified in milk chocolate by (24). <sup>f</sup> Compound previously identified in chocolate (type not defined) by (25).

Figure 4. Volatile compounds potential (scored as the sum of all peak areas) of each cocoa liquor. (C) = criollo, (T) = trinitario, (F) = forastero, and (N) = nacional.
milligrams per kilogram of cocoa liquor (extraction yield set at 100%; Figure 3b). The total amount of procyanidins calculated in this way ranged between 2200 and 8300 ppm (0.22–0.83%). This means a difference as high as 6100 ppm between origins. The richer origin (Madagascar) was found to contain at least 3524 ppm P1, 1522 ppm P2, 1236 ppm P3, 821 ppm P4, 513 ppm P5, 326 ppm P6, 169 ppm P7, and 118 ppm P8 (Figure 3c). Because of the current unavailability of procyanidin oligomer standards, no standard addition was applied except for \((-\text{e})\)-epicatechin (100% recovery factor). Hence, the lower levels measured for P4 to P8 might be partially due to less efficient recovery after extraction.

**Flavor Analysis of Cocoa Liquors.** SPME-GC-MS was used to identify 43 compounds in each cocoa liquor studied. The main compounds investigated were alcohols, esters, aldehydes, ketones, hydrocarbons, nitrogen and oxygen heterocycles, nitriles, and sulfides (Table I). Among them, five had never been described before in chocolate: 1-methylpropyl acetate, 3-methyl-1-butanol acetate, 2-methyl-1-butanol acetate, 4-hydroxy-2-butanone, and 2-isopropyl-5-methylhex-2-enal.

According to their volatile compounds potential (scored as the sum of all peak areas), cocoa liquors should be classified as follows: New Guinea ≫ Ivory Coast ≈ Ghana ≫ Venezuela, Java, Trinidad, Ecuador, Madagascar (Figure 4). Fine cocoa liquors emerged as much less rich than the bulk-basic ones.
and (N)
Venezuela. The former might impart the typical flowery
(512 ppm), respectively, in the cocoa liquors from Java and
ylpyrazine was obtained for four fine varieties. Only
nacional
depicted in
the procyanidin content ([PC]) of a cocoa liquor, we tried to
Contents of Different Cocoa Liquors.
To predict more easily
note usually perceived with the Venezuelan cocoa liquor.

Figure 5 is depicted in

Figure 7. Correlation between the procyanidin level and (a) the
2,5-dimethylpyrazine content and (b) the total level of five compounds
synthesized during roasting. (C) = criollo, (T) = trinitario, (F) = forastero,
and (N) = nacional.
despite their higher quality according to consumer preferences
(16). The chemical nature of the most potent flavors and how they are balanced by others (1, 17) are probably of prime
importance in allowing perception of the fine fruity profile
characterizing criollo varieties (16).
An enlargement of the 9–13 min zone of the chromatograms is depicted in Figure 5. The well-fermented cocoa liquors from
New Guinea, Ghana, and Ivory Coast gave rise to more
numerous and intense peaks than the fine varieties. Especially
dihydro-2-methyl-3-(2H)-furanone (no. 1) and methylpyrazine
(no. 2) proved specific to these three polyphenol-low liquors.
Five flavors synthesized by Maillard reactions during the
roasting step (18–20) were quantified in each cocoa liquor:

methylpyrazine (hazelnut-green), 2,5-dimethylpyrazine, trimethyl-
ylpyrazine (cocoa-roasted-green), 3-ethyl-2,5-dimethylpyrazine
(roasted-smoky-praline), and dimethyl disulfide (derived from methional and recognized as exhibiting a cocoa-like odor in
synergy with 3-methylbutanal (21)) (see Figure 6). Bulk
varieties from Africa and the New Guinea sample contained
higher amounts of all these compounds, most probably due to
higher levels of precursors synthesized through fermentation.

Also to be emphasized in the SPME data are the huge
amounts of tetramethylpyrazine (590 ppm) and α-phenylethanol
(512 ppm), respectively, in the cocoa liquors from Java and
Venezuela. The former might impart the typical flowery—lemon
note usually perceived with the Venezuelan cocoa liquor.

Correlation between the Procyanidin Level and Flavor
Contents of Different Cocoa Liquors. To predict more easily
the procyanidin content ([PC]) of a cocoa liquor, we tried to
establish a mathematical correlation with its flavor content. As
depicted in Figure 7a, a negative correlation with 2,5-dimeth-
ylpyrazine was obtained for four fine varieties. Only nacional
cocoa liquor from Ecuador appears very different, but literature
indicates that it is an independent variety from a genotype point
of view (22). In the three long-fermentation origins, the best
indicators of P1 to P8 procyanidin content proved to be the
levels of five major compounds synthesized during roasting
(Figure 7b). A large number of samples should of course be
analyzed to confirm these preliminary results.

Correlation between the Procyanidin Level and Antioxidant
Activities of Different Cocoa Liquors. As Figure 8 shows,
quick measurement of the inhibition time observed in the
AAPH-induced (2,2′-azobis(2-aminopropane)dihydrochlo-
rine) oxidation assay after addition of cocoa liquor extract is
another interesting way to predict the procyanidin level. The
higher the antioxidant activity, the higher the level of polyphen-
ols, suggesting that procyanidins are the best radical scavengers
in cocoa liquors, despite the presence of high amounts of
melanoids (14).

CONCLUSIONS
The fermentation time of cocoa beans seems to be the key
factor controlling the synthesis of aromas such as methylpyra-
azine, 2,5-dimethylpyrazine, trimethylpyrazine, 3-ethyl-2,5-di-
 methylpyrazine, and dimethyl disulfide. Expectedly, therefore,
a negative correlation between procyanidins and some of these
compounds emerged from our data. The antioxidant activity
measured as the inhibition time found in the AAPH-induced
linoleic oxidation assay appears as an easy way to predict the
procyanidin level in chocolate factories.

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