Changes in Key Aroma Compounds of Criollo Cocoa Beans During Roasting

FELIX FRAUENDORFER AND PETER SCHIEBERLE*
Deutsche Forschungsanstalt für Lebensmittelchemie, Lichtenbergstrasse 4, D-85748 Garching, Germany

Application of a comparative aroma extraction dilution analysis on unroasted and roasted Criollo cocoa beans revealed 42 aroma compounds in the flavor dilution (FD) factor range of 1–4096 for the unroasted and 4–8192 for the roasted cocoa beans. While the same compounds were present in the unroasted and roasted cocoa beans, respectively, these clearly differed in their intensity. For example, 2- and 3-methylbutanoic acid (rancid) and acetic acid (sour) showed the highest FD factors in the unroasted beans, while 3-methylbutanal (malty), 4-hydroxy-2,5-dimethyl-3(2H)-furanone (caramel-like), and 2- and 3-methylbutanoic acid (sweaty) were detected with the highest FD factors in the roasted seeds. Quantitation of 30 odorants by means of stable isotope dilution assays followed by a calculation of odor activity values (ratio of the concentration/odor threshold) revealed concentrations above the odor threshold for 22 compounds in the unroasted and 27 compounds in the roasted cocoa beans, respectively. In particular, a strong increase in the concentrations of the Strecker aldehydes 3-methylbutanal and phenylacetaldehyde as well as 4-hydroxy-2,5-dimethyl-3(2H)-furanone was measured, suggesting that these odorants should contribute most to the changes in the overall aroma after roasting. Various compounds contributing to the aroma of roasted cocoa beans, such as 3-methylbutanoic acid, ethyl 2-methylbutanoate, and 2-phenylethanol, were already present in unroasted, fermented cocoa beans and were not increased during roasting.

KEYWORDS: Cocoa beans; roasting process; aroma extraction dilution analysis; stable isotope dilution assay

INTRODUCTION

The attractive aroma of roasted cocoa is the result of a sophisticated technological process applied to the seeds of the cocoa tree (cocoa beans, Theobroma cacao L.). Among the manufacturing steps, both, fermentation and roasting are considered to be the most important with respect to flavor formation, because, e.g., roasting of nonfermented cocoa does not deliver the characteristic cocoa aroma (1). During fermentation, aroma precursors, such as free amino acids, short-chain peptides, and reducing sugars, are formed (2–7), from which the typical cocoa aroma is suggested to be generated during the subsequent roasting process. Besides, also a significant increase in volatile compounds, such as alcohols, organic acids, and aldehydes, was found after fermentation (8). In particular, 2-phenylethanol is formed in significant amounts, a compound which has later been reported as an important aroma compound in cocoa mass (9). Moreover, also an increase in the concentration of 3-methylbutanal has been observed (10, 11), a compound quite often proposed as an important aroma compound in roasted cocoa (3, 12, 13).

Roasting of the fermented seeds fulfills mainly two purposes: the removal of undesired compounds with low boiling points, such as acetic acid, and the formation of the typical roasty, sweet odorants of cocoa (14). The thermal treatment initiates reactions between reducing sugars and free amino acids or short-chain peptides, consequently leading to a significant reduction in the concentration of free amino acids and reducing sugars (17–19). Ziegleder and Biehl (18) observed that the formation of 3-methylbutanal during roasting depends upon the quality of the preceding fermentation procedure. While the concentration of 3-methylbutanal was only 4–10 mg/kg in poorly fermented beans, well-fermented seeds delivered 60 mg/kg after roasting. Further compounds formed during roasting are alcohols, ethers, furans, thiazoles, pyrones, acids, esters, imines, amines, oxazoles, and pyroles (19, 20).

However, although numerous investigations on the aroma of cocoa have been published thus far (21), only a very few authors have used a comprehensive combination of analytical and sensory methods to differentiate key odorants from the numerous non-odor active volatiles. Schnerrmann and Schieberle (9) were the first to apply the aroma extract dilution analysis (AEDA) on a commercial cocoa mass. Their results revealed, in particular, 3-methylbutanal, ethyl 2-methylbutanoate, hexanal, 2-isopropyl-3-methoxypyrazine, 2-ethyl-3,5-dimethylpyrazine,

* To whom correspondence should be addressed. Telephone: +49-89-289-13265. Fax: +49-89-289-14183. E-mail: peter.schieberle@ch.tum.de.
Table 1. Selected Ions and Response Factors Used in the Stable Isotope Dilution Assays

<table>
<thead>
<tr>
<th>odorant&lt;sup&gt;a&lt;/sup&gt;</th>
<th>ion (m/z)</th>
<th>internal standard</th>
<th>number</th>
<th>ion (m/z)</th>
<th>RF&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-methylbutanal&lt;sup&gt;b&lt;/sup&gt;</td>
<td>69</td>
<td>[2H-2]-methylbutanal</td>
<td>d-2a</td>
<td>70 + 71</td>
<td>0.78</td>
</tr>
<tr>
<td>3-methylbutanal&lt;sup&gt;b&lt;/sup&gt;</td>
<td>69</td>
<td>[2H-3]-methylbutanal</td>
<td>d-2b</td>
<td>71</td>
<td>1.0</td>
</tr>
<tr>
<td>ethyl-l-methylpropanoate</td>
<td>117</td>
<td>[2H]-ethyl-2-methylpropanoate</td>
<td>d-3</td>
<td>120</td>
<td>0.92</td>
</tr>
<tr>
<td>ethyl-2-methylbutanoate</td>
<td>137</td>
<td>[2H]-ethyl-2-methylbutanoate</td>
<td>d-4</td>
<td>140</td>
<td>0.95</td>
</tr>
<tr>
<td>1-octen-3-one</td>
<td>127</td>
<td>[2H]-1-octen-3-one</td>
<td>d-8</td>
<td>129</td>
<td>0.90</td>
</tr>
<tr>
<td>2-heptanol</td>
<td>99</td>
<td>[2H]-2-heptanol</td>
<td>d-9</td>
<td>103</td>
<td>0.79</td>
</tr>
<tr>
<td>2-acetyl-1-pyrroline&lt;sup&gt;c&lt;/sup&gt;</td>
<td>112</td>
<td>[2H&lt;sub&gt;2&lt;/sub&gt;]-2-acetyl-1-pyrroline</td>
<td>d-10</td>
<td>114 - 117</td>
<td>0.83</td>
</tr>
<tr>
<td>dimethyl trisulfide</td>
<td>127</td>
<td>[2H]-dimethyl trisulfide</td>
<td>d-11</td>
<td>133</td>
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</tr>
<tr>
<td>2,3,5-trimethylpyrazine</td>
<td>123</td>
<td>[2H]-2,3,5-trimethylpyrazine</td>
<td>d-13</td>
<td>126</td>
<td>0.93</td>
</tr>
<tr>
<td>2-ethyl-3,6-dimethylpyrazine</td>
<td>137</td>
<td>[2H&lt;sub&gt;2&lt;/sub&gt;]-2-ethyl-3,6-dimethylpyrazine</td>
<td>d-15</td>
<td>140</td>
<td>0.93</td>
</tr>
<tr>
<td>2-ethyl-3,5-dimethylpyrazine</td>
<td>137</td>
<td>[2H&lt;sub&gt;2&lt;/sub&gt;]-2-ethyl-3,5-dimethylpyrazine</td>
<td>d-15</td>
<td>140</td>
<td>0.95</td>
</tr>
<tr>
<td>2,3-diallyl-5-methylpyrazine&lt;sup&gt;d&lt;/sup&gt;</td>
<td>151</td>
<td>[2H&lt;sub&gt;3&lt;/sub&gt;]-2,3-diallyl-5-methylpyrazine</td>
<td>d-17</td>
<td>154</td>
<td>0.95</td>
</tr>
<tr>
<td>acetic acid&lt;sup&gt;d&lt;/sup&gt;</td>
<td>61</td>
<td>[13C&lt;sub&gt;2&lt;/sub&gt;]-acetic acid</td>
<td>d-18</td>
<td>64</td>
<td>0.87</td>
</tr>
<tr>
<td>3-isobuty-2-methoxyprazine</td>
<td>167</td>
<td>[2H&lt;sub&gt;3&lt;/sub&gt;]-3-isobuty-2-methoxyprazine</td>
<td>d-19</td>
<td>170</td>
<td>0.90</td>
</tr>
<tr>
<td>methylpropanoic acid&lt;sup&gt;d&lt;/sup&gt;</td>
<td>89</td>
<td>[2H]-methylpropanoic acid</td>
<td>d-22</td>
<td>91</td>
<td>1.0</td>
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<tr>
<td>butanoic acid&lt;sup&gt;d&lt;/sup&gt;</td>
<td>103</td>
<td>[2H]-butanoic acid</td>
<td>d-23</td>
<td>105</td>
<td>0.85</td>
</tr>
<tr>
<td>2-methyl-3-(methylthiol)furan&lt;sup&gt;d&lt;/sup&gt;</td>
<td>161</td>
<td>[2H&lt;sub&gt;4&lt;/sub&gt;]-2-methyl-3-(methylthiol)furan</td>
<td>d-25</td>
<td>164</td>
<td>0.90</td>
</tr>
<tr>
<td>(E)-4,2-nonadial</td>
<td>139</td>
<td>[2H&lt;sub&gt;2&lt;/sub&gt;]-2-methyl-2,4-nonadial</td>
<td>d-26</td>
<td>141</td>
<td>0.87</td>
</tr>
<tr>
<td>2/3-methylbutanoic acid&lt;sup&gt;d&lt;/sup&gt;</td>
<td>103</td>
<td>[2H]&lt;sub&gt;3&lt;/sub&gt;-methylbutanoic acid</td>
<td>d-27</td>
<td>105</td>
<td>0.67</td>
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<tr>
<td>2-methoxyphenol&lt;sup&gt;d&lt;/sup&gt;</td>
<td>125</td>
<td>[2H]-2-methoxyphenol</td>
<td>d-29</td>
<td>128</td>
<td>0.98</td>
</tr>
<tr>
<td>2-phenylthanol&lt;sup&gt;d&lt;/sup&gt;</td>
<td>105</td>
<td>[2H]-2-phenylthanol</td>
<td>d-30</td>
<td>107</td>
<td>0.94</td>
</tr>
<tr>
<td>δ-octenolactone</td>
<td>141</td>
<td>[2H&lt;sub&gt;2&lt;/sub&gt;]-δ-octenolactone</td>
<td>d-32</td>
<td>145</td>
<td>0.98</td>
</tr>
<tr>
<td>4-methylphenol&lt;sup&gt;d&lt;/sup&gt;</td>
<td>109</td>
<td>[2H]&lt;sub&gt;2&lt;/sub&gt;-4-methylphenol</td>
<td>d-35</td>
<td>115 + 116</td>
<td>1.0</td>
</tr>
<tr>
<td>δ-decanolactone&lt;sup&gt;d&lt;/sup&gt;</td>
<td>169</td>
<td>[2H&lt;sub&gt;3&lt;/sub&gt;]-δ-decanolactone</td>
<td>d-38</td>
<td>171</td>
<td>0.91</td>
</tr>
<tr>
<td>linalool&lt;sup&gt;d&lt;/sup&gt;</td>
<td>137</td>
<td>[13C&lt;sub&gt;2&lt;/sub&gt;]-linalool</td>
<td>c-21</td>
<td>139</td>
<td>1.0</td>
</tr>
<tr>
<td>phenylacetalddehyde</td>
<td>121</td>
<td>[13C&lt;sub&gt;2&lt;/sub&gt;]-phenylacetalddehyde</td>
<td>c-24</td>
<td>123</td>
<td>1.0</td>
</tr>
<tr>
<td>2-phenylethyl acetate&lt;sup&gt;d&lt;/sup&gt;</td>
<td>166</td>
<td>[2H&lt;sub&gt;2&lt;/sub&gt;]-2-phenylethyl acetate</td>
<td>c-28</td>
<td>168</td>
<td>1.0</td>
</tr>
<tr>
<td>2-phenylphenol&lt;sup&gt;d&lt;/sup&gt;</td>
<td>105</td>
<td>[2H&lt;sub&gt;2&lt;/sub&gt;]-2-phenylphenol</td>
<td>c-30</td>
<td>107</td>
<td>1.0</td>
</tr>
<tr>
<td>4-hydroxy-2,5-dimethyl-3(2H)-furanone&lt;sup&gt;d&lt;/sup&gt;</td>
<td>129</td>
<td>[2H&lt;sub&gt;2&lt;/sub&gt;]-4-hydroxy-2,5-dimethyl-3(2H)-furanone</td>
<td>c-34</td>
<td>131</td>
<td>1.0</td>
</tr>
<tr>
<td>3-hydroxy-4,5-dimethyl-2(5H)-furanone&lt;sup&gt;d&lt;/sup&gt;</td>
<td>129</td>
<td>[2H&lt;sub&gt;3&lt;/sub&gt;]-3-hydroxy-4,5-dimethyl-2(5H)-furanone</td>
<td>c-39</td>
<td>131</td>
<td>1.0</td>
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<tr>
<td>phenylacetic acid&lt;sup&gt;d&lt;/sup&gt;</td>
<td>137</td>
<td>[2H&lt;sub&gt;3&lt;/sub&gt;]-phenylacetic acid</td>
<td>c-42</td>
<td>139</td>
<td>1.0</td>
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</table>

<sup>a</sup> Compounds were quantified after separation on an OV-1701 GC stationary phase by mass spectrometry in the MS–Cl mode.<sup>b</sup> The response factor (RF) was determined as reported previously (37).<sup>c</sup> The quantitation was performed by means of two-dimensional GC–MS.<sup>d</sup> The quantitation was performed on the FFAP column.

as well as 2- and 3-methylbutanoic acids as important contributors to the aroma. The important role of organic acids for the cocoa aroma was later confirmed by Kring et al. (22).

Recently, on the basis of the odor activity value concept, the key aroma compounds of a commercial cocoa powder were identified (23). Among the compounds identified and quantified, 24 odorants were present in concentrations above their odor thresholds, among which acetic acid, 2- and 3-methylbutanal, 3-methylbutanoic acid, and phenylacetaldehyde showed the highest odor activity values (ratio of concentration/odor threshold). Finally, reconstitution experiments, in which the 24 odorants were combined in the same concentration as they occurred in the cocoa powder, revealed that the aroma of the cocoa powder could be successfully mimicked.

Despite the fact that roasting is among the most important steps in generating the key odorants of cocoa, comprehensive studies aimed at clarifying changes in the key aroma compounds between fermented, unroasted and fermented, roasted beans performed on the same batch are scarcely available. Thus, the aim of this work was to compare the key odorants present in raw fermented cocoa beans to those formed after roasting. The results should give insights into which odorants are delivered by the fermented, raw bean itself and which are formed from odorless during roasting.

**MATERIALS AND METHODS**

**Cocoa Beans.** Fermented and dried Criollo cocoa beans (*Theobroma cacao L.*) from Grenada were supplied by a chocolate producing company. Roasting of the beans was performed in a laboratory scale with a coffee-roaster (Probart BRZ 4, Emmerich, Germany). Cocoa beans were freshly roasted for each experiment, and roasting conditions were optimized by variation of roasting time and temperature. The overall aroma generated was checked by sensorial evaluation of the roasted samples in comparison to industrially roasted samples. A temperature of 95 °C applied for 14 min was finally judged to deliver the characteristic and desired aroma of roasted cocoa beans.

**Reference Odorants.** The following reference compounds were obtained from the sources given in parentheses: p-cresol, (ROS)-γ-decalactone, (ROS)-δ-decalactone, 2,3-diethyl-5-methylpyrazine, dimethyl trisulfide, 2-ethyl-5-methylpyrazine, benzyl alcohol, 2,3,5-trimethylpyrazine, (Sigma-Aldrich Chemie, Taufkirchen, Germany); butanoic acid, acetic acid, and ethyl 2-methylpropanoate (Merck, Darmstadt, Germany); 2-methoxyphenol (Serva, Heidelberg, Germany); and 3-methylbutanal (Lancaster, Mühlenheim, Germany). The following odorants were synthesized as previously reported (23): 2-acetyl-1-pyrroline, 2-methyl-3-(methylthiol)furan, and δ-decanolactone.

**Isotopically Labeled Internal Standards.** The isotopically labeled internal standards, either labeled with deuterium or carbon-13, were synthesized as described in the references given in parentheses. Numbering refers to the numbers of the respective odorants described in Table 1 but assigned as “d” for deuterated isotopologues and “c” for carbon-13-labeled ones: d-2a (24); d-2b (25); d-3 and d-4 (26); d-5 and d-10 (27); d-11 (28); d-13 and c-28 (Czerny, unpublished); d-15, d-17, and d-29 (29); d-19 (30); c-21 (31); d-22 (32); d-23 (33); d-25 and d-38 (24); d-27 (34); d-30 and c-24 (35); d-32 (36); c-34 (37); c-39 (38). [2H<sub>3</sub>]-Acetic acid (d-18), [13C<sub>2</sub>]-methylpropanoic acid (d-29), and [13C<sub>2</sub>]-phenylacetic acid (c-42) were purchased from Sigma-Aldrich Chemie, Taufkirchen, Germany.

**Syntheses.** [2H<sub>2</sub>]-2-Heptanol (d-9). In a laboratory autoclave equipped with a glass vial (100 mL), tris-(triphenylphosphine)-rhodium(1)-chloride (Wilkinson catalyst, 0.8 g) was suspended in toluene (15 mL) under a nitrogen atmosphere. Then, nitrogen was replaced by deuterium,
and the mixture was stirred until the dark-red-colored suspension turned into orange. 4-Heptene-2-ol (2.5 g) was added; the mixture was stirred for 8 h; and finally, pentane (50 mL) was added. The catalyst was removed by high vacuum distillation, and the distillate was concentrated to 15 mL by distilling off the solvent using a Vigreux column (60 × 1 cm). To remove the toluene, the distillate was applied onto a water-cooled column (30 × 1.5 cm) filled with silica gel (30 g). The column was first flushed with pentane (250 mL), and then the target compound was eluted with diethyl ether (150 mL). The eluate was dried over anhydrous sodium sulfate and concentrated under reduced pressure.

The incorporation of the deuterium atoms was confirmed by mass spectrometry (MS–CI): m/z in %, 103 (100), 102 (77), 60 (50), 101 (36), 100 (20), 65 (14). The main fragment m/z 103 is formed by elimination of water (M + 18 + 1 = 103) from the molecular ion of [2H2]-2-heptenol m/z 120.

[2H2]-(E,E)-2,4-Nonadienal (d-26). [2H2]-(E,E)-2,4-nonadienal was synthesized via a Grignard reaction using 1-methoxybut-1-en-3-ine and [2H2]-pentanal following a procedure described for [2H2]-(E,E)-2,4-decadienal (26). 1-Methoxybut-1-en-3-ine was synthesized according to Shostakvskii and Khomenko (39). [2H]2-Pentanal was synthesized as described above for [2H2]-2-heptenol but using 2-pentenal instead of 4-heptin-2-ol in the deuteration process. The incorporation of the two deuterium atoms was checked by MS–CI: m/z in %, 141 (100), 142 (18), 140 (13), 123 (8). The molecular ion m/z 141 (M + 1) shows a shift of two mass units as compared to the unlabeled dienal (m/z 139), thus confirming the success of the labeling procedure.

Isolation of the Volatile Fraction. For solvent extraction, cocoa beans were frozen with liquid nitrogen and then ground into a fine powder in a laboratory mill (Model A 10, Jahnke and Kunkel, Staufen, Germany). The material (50 g) was extracted with diethyl ether (2 L), and the neutral/basic volatiles (NBV) and concentration of the two fractions were calculated using a series of µl aliquots of 0.5 µl. To avoid wrong identifications because of coeluting compounds (41), MS–CI was performed using the moving column stream switching system (MCSS) (Fisons Instruments, Mainz, Germany), as recently described (42). The concentrations were calculated from the relative abundances of the ions selected for the analyte and the internal standards, and the data were corrected by means of response factors, determined from mixtures containing known amounts of the respective labeled and unlabeled compound (37).

Sensory Evaluation. Sensory analyses were performed at 21 ± 1 °C in a sensory panel room equipped with single booths. A total of 10 assessors with experience in sensory evaluation were recruited from the German Research Center of Food Chemistry in Garching, Germany. Aroma profile analyses were performed by orthonasally scoring 12 odor qualities on a 7 point scale from 0 to 3 (from 0, 0.5, 1,..., to 3) selected in a previous session for the evaluation of unroasted and roasted Criollo cocoa beans. The values given by the panelists were averaged. Oronasal odor thresholds were determined in sunflower oil as described recently (23).

RESULTS AND DISCUSSION

Aroma Profile Analysis. An orthonasal evaluation of the main odor qualities of the fermented, unroasted Criollo cocoa beans revealed the odor quality “vinegar-like” with the highest intensity, followed by smoky, roasty, caramel-like, honey-like, coconut-like, flowery, seasoning-like, and earthy, which all were perceived at nearly the same intensity (Figure 1). Fruity, rancid-sweaty, and smoky aroma notes were scored somewhat lower. After the seeds were roasted, the aroma profile was substantially changed. The strong vinegar-like note predominating in unroasted cocoa was scored significantly lower, while the smoky note was predominating, followed by caramel-like, earthy, honey-like, and roasty aroma qualities (Figure 1).

Identification of Odor-Active Compounds in Raw, Fermented Cocoa Beans. The volatile fraction from fermented, raw cocoa beans (50 g) was isolated by solvent extraction followed by high vacuum distillation. This distillate fully represented the typical aroma of the raw cocoa beans when sniffed on a strip of filter paper. Application of HRGC–MS on the distillate revealed 34 aroma-active regions in the neutral/basic volatile fraction (NBF). Eight further odorants were detected in the fraction containing the acidic compounds (AF). By sniffing of serial dilutions, compound 27, exhibiting a rancid aroma, was detected with the highest flavor dilution (FD) factor of 4096, followed by 18 (sour) with a FD factor of 2048. Further important odorants showing somewhat lower FD factors were 31 (flowery), 39 (seasoning-like), and 29 (smoky) (Figure 2).

For the identification experiments, an aroma distillate was prepared from 500 g of ground cocoa beans and the neutral/basic fraction was fractionated on silica using solvent mixtures of different polarity (9). After GC–O and mass spectrometric analysis, structures of odor-active compounds were suggested.
on the basis of data available in an in-house database. Correct identifications of compounds were finally performed by comparison of the retention indices on two columns of different polarities, their odor qualities, and odor activities, as well as their mass spectra (MS−EI and MS−CI), to reference compounds (41). Thus, the most odor-active compounds among the neutral/basic odorants were identified as 2-phenylethanol (31, flowery) and 2-methoxyphenol (29, smoky), followed by ethyl 2-methylbutanoate (4, fruity), 2-heptanol (9, citrus-like), 2-methyl-3-(methyldithio)furan (25, cooked meat-like), and 2-phenylethyl acetate (28, flowery), which were all detected with a FD factor of 256 (Figure 2). In the acid fraction, compound 27, showing a rancid odor quality, was identified as a mixture of 2- and 3-methylbutanoic acid, compound 18, with a sour odor as acetic acid, and compound 39, eliciting a seasoning-like note as 3-hydroxy-4,5-dimethyl-2(5H)-furanone (sotolon). In addition, 4-hydroxy-2,5-dimethyl-3(2H)-furanone (34, caramel-like) and phenylacetic acid (42, sweet) were characterized as further important odorants.

Identification of Odor-Active Compounds in Roasted Cocoa Beans. The aroma extract dilution analysis applied on a distillate from the same batch of cocoa beans, which were roasted for 14 min, revealed 42 odor-active areas. On the basis of the identification experiments in correlation with the FD factors, 2- and 3-methylbutanal (2), both exhibiting a malty aroma note, followed by phenylacetaldehyde (24, honey-like) and 2-phenylethanol (31, flowery) were identified with the highest FD factors between 1024 and 8192, respectively (Figure 3). A somewhat lower FD factor of 256 was found for 2-heptanol (9, citrus-like), 2-acetyl-1-pyrroline (10, popcorn-like), 2,3,5-trimethylpyrazine (13, earthy), 2-ethyl-3,6-dimethylpyrazine (15, earthy), 2,3-diethyl-5-methylpyrazine (17, earthy), 2-methyl-3-(methyldithio)furan (25, cooked meat-like), and 2-phenylethyl acetate (28, flowery), and 2-methoxyphenol (29, smoky). In the acidic fraction, 2- and 3-methylbutanoic acid (27, rancid) and 4-hydroxy-2,5-dimethyl-3(2H)-furanone (34, caramel-like) appeared with the highest FD factor of 4096, followed by 3-hydroxy-4,5-dimethyl-2(5H)-furanone (39, sea-
The results obtained for the unroasted cocoa beans showed that acetic acid was by far the most abundant odorant (1.1 g/kg), followed by methylpropanoic acid and 3-methylbutanoic acid with amounts of 9.7 and 9.3 mg/kg, respectively (Table 3). Quite high concentrations were also found for 2-phenylethanol (3.5 mg/kg), 3-methylbutanal (1.6 mg/kg), and 2-heptanol (1.16 mg/kg). On the other hand, some components were only present in trace amounts, such as 1-octen-3-one (0.21 µg/kg) and 2-methyl-3-(methyldithio)-furan (0.13 µg/kg). As expected from the AEDA results, quantitation of the odorants after roasting revealed significant differences in the concentrations of several aroma compounds. For example, phenylacetaldehyde increased by a factor of more than 90, while 3-methylbutanal increased by a factor of more than 16 in the roasted sample. In addition, the amount of 4-hydroxy-2,5-dimethyl-3(2H)-furanone was increased more than 70 times during roasting. Interestingly, pyrazines, commonly suggested as compounds only formed during thermal food processing, were already present in significant amounts and increased only moderately during roasting. Only acetic acid was significantly lowered, while even the very volatile esters ethyl 2-methylbu-
tanoate and ethyl methylpropanoate remained nearly constant. The same was true for 2- and 3-methylbutanoic acid, 2-phenylethanol, 2-phenylethylacetate, or 2-heptanol, respectively (Table 3).

### Calculation of Odor Activity Values (OAVs)

To elucidate whether the quantified compounds were present in concentrations above their odor thresholds and may, therefore, contribute to the overall aroma of the cocoa beans, odor activity values (ratios of concentration/orthonasal threshold of the aroma compounds were calculated. Because cocoa beans contain about 54% fat (43), sunflower oil was chosen as an appropriate matrix for threshold determination. The threshold values for most compounds have previously been determined by our group (23, 44), except the value for 2-heptanol, which was newly determined in this study (Table 4).

In unroasted cocoa beans, 22 aroma compounds were present in concentrations above their odor thresholds (Table 4). By far the highest OAV of 8870 was calculated for acetic acid, followed by 3-methylbutanoic acid (424), ethyl 2-methylbutanoate (138), and 3-methylbutan-1-ol (123). Methylpropanoic acid, 3-hydroxy-4,5-dimethyl-2(5H)-furanone, ethyl 2-methylpropanoate, 2-methylbutanoic acid, 2-phenylethanol, and 2-phynylacetic acid showed OAVs > 10 and are, thus, also likely to contribute to the aroma of the unroasted cocoa beans.

In roasted cocoa beans, 27 compounds were present in concentrations exceeding their odor thresholds (Table 4). Because of the changes in concentrations of several compounds caused by the roasting process, however, the ranking of compounds on the basis of their OAVs was clearly different. Although the highest value was still calculated for acetic acid (ratios of concentration/orthonasal odor threshold) of the aroma compounds were present in concentrations above their odor thresholds (Table 4). By far the highest OAV of 8870 was calculated for acetic acid, followed by 3-methylbutanoic acid (424), ethyl 2-methylbutanoate (138), and 3-methylbutan-1-ol (123). Methylpropanoic acid, 3-hydroxy-4,5-dimethyl-2(5H)-furanone, ethyl 2-methylpropanoate, 2-methylbutanoic acid, 2-phenylethanol, and 2-phynylacetic acid showed OAVs > 10 and are, thus, also likely to contribute to the aroma of the unroasted cocoa beans.

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had only minor influence on the concentrations of the rancid-smelling 3-methylbutanoic acid (Table 3). Ziegleder and Biehl (18) had proposed that 3-methylbutanoic acid is formed at the end of the fermentation from the amino acid leucine by aerobic putrefactive bacteria. The esters ethyl 2-methylbutanoate, ethyl 2-methylpropanoate, and 2-phenylethyl acetate were present in similar concentrations in the unroasted as well as roasted cocoa beans (Table 3). The esters might be present already in the unfermented beans but may also be generated during fermentation. Interestingly, no losses occurred during roasting.

The largest impact on the aroma caused by the roasting process was observed for the Strecker aldehydes 3-methylbutanal (malty) and phenylacetaldehyde (honey-like), as well as for 4-hydroxy-2,5-dimethyl-3(2H)-furanone (caramel-like), the concentrations of which were significantly higher in the roasted beans. These results were well-correlated with the data of the aroma profile analyses, indicating that the malty and caramel-like qualities of these compounds were also rated clearly higher in the unroasted cocoa beans (Table 3 and Figure 1). A contribution of 3-methylbutanal to the aroma of cocoa and cocoa products has previously been proposed by numerous authors (3, 11, 47, 48), but its key role for the aroma of cocoa mass or cocoa powder has only previously been confirmed (9, 23). Furthermore, although the formation pathway leading to 3-methylbutanal and phenylacetaldehyde, respectively, has been suggested by a Strecker degradation of the parent amino acids leucine and phenylalanine in many studies, no clear quantitative correlation is yet available between the amounts of the precursors degraded and the amounts of the odorants formed in model experiments using the “natural” concentrations of these precursors. Furthermore, as very recently found by us (Weigl and Schieberle, unpublished) besides the free amino acids, also the Amadori products and certain dipeptides act as potent precursors of the Strecker aldehydes.

4-Hydroxy-2,5-dimethyl-3(2H)-furanone, an odorant present in many thermally processed foods, such as coffee, meat, or bread crust, was first described as cocoa constituent by Ziegleder in many thermally processed foods, such as coffee, meat, or bread crust, was first described as cocoa constituent by Ziegleder (18). Semi-quantitative determinations had revealed concentrations of <0.1 mg/kg for unroasted and 0.5–2 mg/kg for roasted cocoa beans, which were in the same concentration range as reported in Table 3. Besides a thermal degradation of glucose or fructose, 4-hydroxy-2,5-dimethyl-3(2H)-furanone can more effectively be formed in a thermal degradation of either rhamnose or fructose-1,6-bisphosphate (50). However, it is yet unclear whether these precursors do exist in cocoa beans.

In general, our results suggest that the differences in the aroma profiles of unroasted and roasted Criollo beans are caused by quantitative rather than a qualitative change in the composition of only a quite small set of key aroma compounds. Of the 30 aroma compounds quantified, only acetic acid was significantly reduced by the roasting process, while 15 compounds increased in their concentrations by more than a factor of 2. Most compounds, however, were scarcely influenced by the heat treatment. The results of our study might also explain why an experiment by Lopez and Quesnel (48) to create the characteristic aroma of roasted cocoa beans by thermally reacting reducing sugars and amino acids was not successful, because important compounds in the typical roasted cocoa aroma, such as acids, esters, and alcohols, are not formed from precursors during the roasting process but are delivered by the fermented unroasted cocoa bean. Further studies to clarify which of the odorants are already present in the unfermented cocoa beans are currently under investigation.

LITERATURE CITED

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Aroma Compounds in Roasted and Unroasted Cocoa Beans


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