Variability for Free Sugars and Organic Acids in *Capsicum chinense*

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Variability in the concentrations of the chemical constituents that contribute to fruit flavor in *Capsicum chinense* is poorly documented in the scientific literature. We surveyed fruit of 216 landraces and cultivated varieties of *Capsicum chinense* acquired from North, Central, and South America, and analyzed these for concentrations of the simple sugars sucrose, glucose, and fructose, and citric, malic, succinic, fumaric, and ascorbic acids. Concentrations (mg/100 g Fresh Weight (FW) of whole fruit) of sucrose, glucose, and fructose in fruit of this species ranged from 0.0 to 150, 68 to 701, and 101 to 823, respectively. The total of these sugars (sucrose + glucose + fructose) ranged from 198 to 1543 mg/100 g FW. Concentrations of organic acids ranged from 0.0 (not detected) to 818, 430, 340, and 232 mg/100 g FW for citric, malic, fumaric, and succinic acids, respectively. However, the relative ranking in the concentrations of the individual acids was genotype-dependent. Total ascorbic-acid values ranged from 30 to 1466 mg/100 g FW. These data serve to document the range in the concentrations of individual sugars and acids present in mature *C. chinense* fruit and suggest that this variability may lend itself to studies involving the synthesis and/or metabolism of compounds associated with fruit flavor.

Introduction. – Many consumers are familiar with *Capsicum annuum* L. (common bell, jalapeno, banana, poblano, cherry, etc., type peppers) and *C. frutescens* L. (*Tabasco* sauce), but are less familiar with *C. chinense* Jacq. *Capsicum chinense* is, however, an important food and spice crop in Mexico, South America, and the Caribbean, and rivals *C. annuum* in importance as a crop in those areas [1]. *Capsicum chinense* has been referred to as the most important cultivated pepper in South America, east of the Andes [2], where fruits of the species are generally referred to as habanero, Scotch Bonnet, or goat pepper. In the eastern Caribbean, the various landraces of this species are referred to as Congo peppers (Trinidad) or booney peppers (Trinidad). In the western Caribbean, they are referred to as Scotch bonnets (Jamaica), rocotillos (Puerto Rico), and cachucha (Cuba). Fruit of this species is generally regarded as quite pungent [2], but this is not always the case.

*Capsicum chinense* is closely related to *C. frutescens* and, to a lesser extent, *C. annuum* [1][3]. The species may have originated in the western Amazon River basin [4] and domesticated forms appear in early agricultural sites in coastal Peru [5]. The species is now cultivated from Mexico and the Caribbean south to and including Peru, Bolivia and Brazil [6–8]. Rosland et al. [9] noted that the species was widely grown in the Yucatan and popular in Jamaica, with great diversity present in Brazil.

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Fruits of members of the genus *Capsicum* play a nutritional role in many cultures by serving as a source of Vitamins A, C, and other nutrients [10–16]. In addition, the pungency associated with many forms makes the fresh or dried fruit a desirable spice and a valuable international commodity [2]. Morphological variation among mature fruit of *C. chinense* [2][8][17][18] has been examined. However, the literature contains remarkably little information on the range of variability present for compounds that contribute to flavor and/or vitamin content in this crop plant.

Concentrations of free sugars and organic acids in pepper fruit affect fruit flavor [19]. However, published reports describing free sugar or organic acid concentrations in the fruit of *C. chinense* are not available with the exception of Howard et al. [20] who examined the concentrations of ascorbic acid in two cultivars of *C. chinense* and found them to be ca. 120 mg/100 g Fresh Weight (FW). This study was undertaken to examine the range in the concentrations of free sugars and organic acids present in New World cultivars and landraces of *C. chinense*.

**Results and Discussion.** — The changes in free sugars and organic acids that occur during the ripening process have been characterized [16][19–22]. As determined by Luning et al. [19] and Navarro et al. [16], free sugars and organic acids are important components contributing to the flavor characteristics and nutritional value of pepper fruit. In addition, carbohydrates promote ascorbic-acid stability, and thus enhance vitamin content [23]. Studies examining these characteristics in pepper have typically utilized only a limited range of plant material (generally fewer than three cultivars) and have focused on commercially cultivated bell-type *C. annuum* [20][24] or a single regional/local favorite [25].

**Sugars.** Concentrations of sucrose in mature fruits of the *C. chinense* examined averaged 18.1 and ranged from 0 to 150 mg/100 g FW (*Table 1*). Whereas the distributions of the concentration values of glucose, fructose, and total sugars were normal, the distribution of the concentration values of sucrose was highly skewed (*Table 1* and *Fig. 1,a–d*) with a median of 9.6 mg/100 g FW. Luning et al. [19] reported a decrease in sucrose levels of from 310 (immature green) to 650 (turning) to 190 mg/100 g FW (mature red) in maturing fruit of *C. annuum* cv. MAZURKA. Lopez-Hernandez et al. [25] noted the absence of sucrose in immature fruits of cv. PADRON. Others [16] noted that sucrose decreased through the maturation process to non-detectable levels in fully mature fruits of *C. annuum* cv. ORLANDO. Hence, it is generally agreed that sucrose levels decrease in pepper fruit as the ripening process proceeds. Since only fully mature fruits were utilized in the present study, low concentrations of this sugar were expected. However, concentrations of sucrose in mature fruit were not uniformly low (*Tables 1* and 2; *Fig. 1,a*). Sucrose as a percentage of total sugars ranged from 0 (Grif 9261) to 15% (Grif 9277), and sucrose concentrations were always lower than either glucose or fructose.

Concentrations of fructose, glucose, and total sugar, but not sucrose, were found to be correlated with the attribute sweetness in the red mature stage of pepper fruit [19] and to increase during the ripening process [19][21][25]. Lopez-Hernandez et al. [25] reported D-glucose to be the most abundant (850 mg/100 g FW) carbohydrate in immature fruit of cv. PADRON peppers, while fructose concentrations were lower (750 mg/100 g FW). Navarro et al. [16] noted that levels of glucose and fructose were
Fig. 1. Distributions of the concentrations of sucrose (a), glucose (b), fructose (c), and total sugars (d) among 216 accessions of Capsicum chinense.

Table 1. General Statistics of Fruit Quality Characteristics among 216 Accessions of Capsicum chinense

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Mean*</th>
<th>Min.*</th>
<th>Max.*</th>
<th>Median*</th>
<th>Skewness</th>
<th>Kurtosis</th>
<th>K-S Dist.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sucrose (S)</td>
<td>18.1</td>
<td>0.00</td>
<td>150.0</td>
<td>9.6</td>
<td>3.970</td>
<td>13.17</td>
<td>0.236b)</td>
</tr>
<tr>
<td>Glucose (G)</td>
<td>321.9</td>
<td>67.7</td>
<td>701.0</td>
<td>318.0</td>
<td>0.475</td>
<td>0.39</td>
<td>0.03</td>
</tr>
<tr>
<td>Fructose (F)</td>
<td>451.9</td>
<td>101.3</td>
<td>822.5</td>
<td>464.4</td>
<td>-0.261</td>
<td>0.385</td>
<td>0.054</td>
</tr>
<tr>
<td>S+G+F</td>
<td>792.0</td>
<td>197.8</td>
<td>1542.7</td>
<td>805.7</td>
<td>0.163</td>
<td>0.707</td>
<td>0.044</td>
</tr>
<tr>
<td>Citric acid</td>
<td>244.2</td>
<td>0.00</td>
<td>818.3</td>
<td>213.8</td>
<td>0.617</td>
<td>-0.296</td>
<td>0.093b)</td>
</tr>
<tr>
<td>Malic acid</td>
<td>69.5</td>
<td>0.00</td>
<td>429.9</td>
<td>43.4</td>
<td>1.628</td>
<td>3.094</td>
<td>0.201b)</td>
</tr>
<tr>
<td>Fumaric acid</td>
<td>49.9</td>
<td>0.00</td>
<td>339.9</td>
<td>37.6</td>
<td>1.688</td>
<td>5.220</td>
<td>0.154b)</td>
</tr>
<tr>
<td>Succinic acid</td>
<td>24.5</td>
<td>0.00</td>
<td>231.9</td>
<td>3.9</td>
<td>2.524</td>
<td>7.219</td>
<td>0.276b)</td>
</tr>
<tr>
<td>Total ascorbic acid</td>
<td>391.6</td>
<td>29.6</td>
<td>1465.6</td>
<td>333.2</td>
<td>1.832</td>
<td>4.867</td>
<td>0.133b)</td>
</tr>
</tbody>
</table>

* Expressed as mg/100 g FW. b) Failed K-S test for normality.

generally similar in fruit of C. annuum cv. ORLANDO. Total sugar concentration also increased with maturation of that cultivar. The values for glucose and fructose reported by Lopez-Hernandez et al. [25] were near the extreme upper range of the concent-
Table 2. Lowest and Highest Values\(^a\) for Various Sensory Attributes Observed among Fruit of 216 Accessions\(^b\) of Capsicum chinense

<table>
<thead>
<tr>
<th>Compound</th>
<th>Lowest</th>
<th>Highest</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sucrose (S)</td>
<td>439449 (≤ 0.0), 439475 (≤ 0.0),</td>
<td>594139 (114.5), 315021 (132.5),</td>
</tr>
<tr>
<td></td>
<td>355819 (1.75), 438642 (2.0)</td>
<td>281338 (137.5), 281441 (150.0)</td>
</tr>
<tr>
<td>Glucose (G)</td>
<td>Griff 9277 (67.8), 439452 (71.5),</td>
<td>315021 (664.0), 585252 (665),</td>
</tr>
<tr>
<td></td>
<td>260478 (87.5), 257115 (104.7)</td>
<td>281338 (674.5), 281441 (701)</td>
</tr>
<tr>
<td>Fructose (F)</td>
<td>Griff 9277 (101.3), 257115 (140.7),</td>
<td>281441 (691.8), 438610 (701),</td>
</tr>
<tr>
<td></td>
<td>439452 (143.5), 260513 (146.8)</td>
<td>439445 (711.3), 439479 (822.5)</td>
</tr>
<tr>
<td>Total sugars</td>
<td>Griff 9277 (197.8), 439452 (247.0),</td>
<td>585252 (1317), 315021 (1439),</td>
</tr>
<tr>
<td>(S+G+F)</td>
<td>260513 (255.0), 257115 (256.0)</td>
<td>439479 (1458), 281441 (1543)</td>
</tr>
<tr>
<td>Total ascorbic</td>
<td>238048 (29.6), Griff 1518 (58.6),</td>
<td>281317 (1465), 439417 (1438),</td>
</tr>
<tr>
<td></td>
<td>238049 (62.9), 260523 (77.3)</td>
<td>438619 (1328), 594139 (1187)</td>
</tr>
<tr>
<td>Citric acid</td>
<td>543184 (0.0), 439453 (0.0),</td>
<td>315008 (818), 281423 (725),</td>
</tr>
<tr>
<td></td>
<td>315021 (0.0), 281417 (0.0)</td>
<td>281441 (723), 439425 (676)</td>
</tr>
<tr>
<td>Malic acid</td>
<td>640894 (0.0), 640893 (0.0),</td>
<td>441625 (429), 257091 (394),</td>
</tr>
<tr>
<td></td>
<td>593611 (0.0), 441640 (0.0)</td>
<td>257085 (387), 260558 (338)</td>
</tr>
<tr>
<td>Succinic acid</td>
<td>640894 (0.0), 640893 (0.0),</td>
<td>441625 (429), 257091 (394),</td>
</tr>
<tr>
<td></td>
<td>441629 (0.0), 441603 (0.0)</td>
<td>281423 (231), 439397 (221)</td>
</tr>
<tr>
<td>Fumaric acid</td>
<td>159246 (0.0), 257085 (0.0)</td>
<td>280464 (339), 439465 (205),</td>
</tr>
<tr>
<td></td>
<td>281303 (0.0), 441612 (0.0)</td>
<td>281438 (177), 315024 (168)</td>
</tr>
</tbody>
</table>

\(^a\) All values expressed as mg/100 g FW. \(^b\) Plant Introduction (PI) or Griffin (Grif) inventory numbers as indicated.

Table 2. Lowest and Highest Values\(^a\) for Various Sensory Attributes Observed among Fruit of 216 Accessions\(^b\) of Capsicum chinense

The mean concentration of fructose across all samples (451 mg/100 g FW) was higher than for glucose (321 mg/100 g FW), although the glucose/fructose ratio in individual samples varied from 0.38 (PI 290972) to 1.23 (PI 281338). Also, the percentage of total sugars represented by the combined values of glucose and fructose varied from 87 (PI 594139) to 100% (i.e., PI 439475).

Vitamin C. Vitamin C is essential for human health [26]. In recognition of the importance of this vitamin, numerous investigators have examined changes in ascorbic-acid content during pepper-fruit ripening [14] [16], as a result of storage/processing [10] [12] [13], and to a lesser extent in response to genotypic effects [13] [14] [20] [27–29]. Concentrations of ascorbic acid either remained constant or increased as pepper fruit matured [13] [19–21] [28].

The concentrations of ascorbic acid in the materials examined ranged from ca. 30 to 1465 mg/100 g FW with a mean of 391, and were negatively skewed (Fig. 2, and Tables 1 and 2). This upper value was higher than that reported previously [30], when a total ascorbic acid concentration of 155 mg/100 g FW in fresh red pepper (C. annuum) was observed. Ukhun and Dibie [12] reported an ascorbic-acid concentration of 86.8 mg/100 g FW in whole red pepper of cvs. of Capsicum annuum grown in Nigeria. Howard et al. [13] noted concentrations of total ascorbic acid among twelve cvs. of C. annuum...
(various commercial types) ranging from 50 to 168 mg/100 g FW. Simonne et al. [24] reported ascorbic-acid values of 73 to 124 mg/100 g FW in the edible portion of fruit of 17 commercial varieties of bell-type *C. annuum*. Values of 400–500 mg/100 g dry weight (DW) in fruit of a local cv. of *C. annuum* were reported by Tchiegang et al. [31]. Howard et al. [20] reported concentrations of ascorbic acid in seven cultivars of *Capsicum* spp. (four *C. annuum*, one *C. frutescens*, and two *C. chinense*) that ranged from 75 to 203 mg/100 g FW. Concentrations of ascorbic acid among the two varieties of *C. chinense* examined by Howard et al. [20] were ca. 120 mg/100 g FW. It should be noted that fruit concentrations of this and other compounds contributing to flavor and/ or nutritional attributes are often subject to strong environmental effects [27][32][33].

![Figure 2](image)

**Fig. 2. Distribution of total ascorbic acid values among 216 accessions of Capsicum chinense**

**Organic Acids.** The contribution of organic acids to *Capsicum* fruit flavor was recognized and characterized by Luning et al. [19]. They attributed fruit sourness to the sum of the organic acids present. These investigators [19] also noted that, as fruit turned from green to red, concentrations of citric acid increased, whereas levels of malic, fumaric, oxalic, and pyroglutamic acids decreased, although cultivar differences were observed. However, the literature contains little information on the range in the concentrations of these compounds present in mature fruit. We observed that the relative concentrations of organic acids across all samples were citric > malic > fumaric > succinic acid, with means of 244.2, 69.5, 49.9, and 24.5 mg/100 g FW, respectively (*Tables 1 and 2, and Fig. 3*). However, other rankings were observed and included: citric > malic > succinic > fumaric acid (Grif 9269), malic > citric > fumaric > succinic acid (Grif 9291), citric > fumaric > malic > succinic acid (PI 238046), succinic > citric > malic > fumaric acid (PI 257171), and other combinations.

The observed concentrations of malic, fumaric, and succinic acids were highly skewed and not normally distributed as determined by the Kolmogorov–Smirnov (K–S) goodness-of-fit test (*Table 1 and Fig. 3*). Mean values for the four acids were generally higher than those reported in the two cvs. of *C. annuum* examined by Luning et al. [19]. Lopez-Hernandez et al. [25] reported levels of citric, fumaric, and malic acid as 28.0, 1.1,
Fig. 3. Distributions of the concentrations of citric (a), malic (b), succinic (c), and fumaric acid (d) among 216 accessions of Capsicum chinense.

and 208 mg/100 g FW, respectively, in immature fruit of C. annuum cv. PADRON. However, large (80-fold) variations in the quantity of the various constituents contributing to the quality of pepper fruit are not uncommon [25].

Conclusions. – The data presented indicate that great diversity exists within the Capsicum chinense genepool for the concentrations of free sugars and organic acids that are believed to contribute to the flavor and health-promoting benefits of mature fruit. We suggest that the moderate levels of sugars and the relatively high levels of organic acids found in most fruits of the C. chinense examined contribute to the typically non-sweet taste of fruit of this species. However, variability for the concentrations of free sugars and organic acids is sufficiently large that genetic manipulation may enable the development of C. chinense cultivars with high levels of vitamin C and novel flavor(s). The C. chinense genetic materials characterized here may represent useful tools in future studies to examine sugar and acid synthesis or metabolism.

We acknowledge the expert technical assistance of Mrs. Sarah Moon, Mr. Chris Tatum, and Ms. Holly Sisson in the preparation of fruit extracts and analysis.
Plant Culture. Seeds were obtained from the USDA/ARS Capsicum spp. germplasm collection in Griffin, GA [34]. Fifteen to 20 plants of each genotype were transplanted to and grown in the field in Woodland, CA, in May 2006. Plants received periodic fertilization and irrigation, as required. Mature fruits were harvested from all plants of each genotype, and the fruits were shipped overnight to Griffin, GA where they were counted, measured, and weighed. Only sound, fully mature fruits were utilized in subsequent analyses. Mature fruits were defined as those that had achieved full and uniform color development typical for mature fruit of each respective genotype. Calyxes were first removed from a minimum of 100 fruits (or 200 g). These were chopped into small (~2.5 mm³) pieces and subsequently ground to a slurry using a commercial food grinder. A complete list of all plant materials utilized in this study is available from R. L. J.

Extraction of Carbohydrates and Organic Acids. Carbohydrates and organic acids were extracted from aliquots of freshly ground fruit slurry as described in [35]. After centrifugation, the supernatant was brought to a final volume of 50 ml with 80% EtOH. For HPLC analysis, aliquots of this preparation were filtered sequentially through a pre-prepared Sep-Pak Plus C18 cartridge filter (Waters Corp., Milford, MA) and a 0.45-µ syringe filter. Filtrates were stored in amber glass vials at −20° for up to one week prior to analysis.

HPLC Analysis of Carbohydrates and Organic Acids. HPLC Analysis of sugars was conducted as described by Baldwin et al. [36] and Jarret et al. [35]. Individual sugars were analyzed by injecting 20 µl of extract filtrate on a Sugar Pak column at 90° (Waters, Millipore Corp., Milford, MA) with a mobile phase of 0.0001 ethylenediaminetetraacetic acid disodium-calcium salt (CaEDTA), a flow rate of 0.5 ml/min, and a refractive-index detector (Agilent 1100 series, Agilent Technologies, Palo Alto, CA).

HPLC Analysis of acids was conducted by injecting 20 µl of extract filtrate on a ThermoFinnigan SpectrSystem UV6000UL (Thermo Electron Corp., San Jose, CA) with a Prevail Organic Acid column at 35° (Alltech, Deerfield, IL), a mobile phase of 0.1N H₂SO₄, a flow rate of 0.2 ml/min, and a photo-diode array (PDA, Thermo Electron Corp., San Jose, CA) spectrophotometric detector at 210 nm (280 nm for fumaric acid).

Data Analysis. Statistical data and graphs were generated using SigmaStat 3.1. The experiment was partially replicated (50%). The mean of the coefficients of variation across all treatments did not exceed 5%.

REFERENCES

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