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Compositional Characterization of Native Peruvian Chili Peppers (*Capsicum* spp.)

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Supporting Information

ABSTRACT: The national *Capsicum* germplasm bank of Peru at INIA holds a unique collection of more than 700 *Capsicum* accessions, including many landraces. These conserved accessions have never been thoroughly characterized or evaluated. Another smaller collection exists at UNALM, and CIDRA provided taxonomically characterized fruits from the Amazon region of Ucayali. Of these collections, 147 accessions have been selected to represent the biodiversity of Peruvian *Capsicum annuum*, *Capsicum baccatum*, *Capsicum chinense*, and *Capsicum frutescens* by morphological traits as well as by agronomic characteristics and regional origin. All fruits from the selected accessions have been oven-dried and ground in Peru and analyzed in Germany. Results are reported for each accession by total capsaicinoids and capsaicinoid pattern, total polyphenol content, antioxidant capacity, specific flavonoids (quercetin, kaempferol, luteolin, apigenin), fat content, vitamin C, surface color, and extractable color. A wide variability in phytochemical composition and concentration levels was found.

KEYWORDS: *chili pepper, capsicum, capsaicin, vitamin C, quercetin, color*

INTRODUCTION

Belonging to the botanical family of Solanaceae together with other plants such as tomato, eggplant, potato, or tobacco, plants of the genus *Capsicum* are some of the oldest cultivated plants. For over 6000 years their fruits have been used for many purposes and not only as spice or food in the human diet.[1] On the basis of taxonomic classification the genus today includes at least 37 species.[2,3] These include the five domesticated species *Capsicum annuum*, *Capsicum frutescens*, *Capsicum chinense*, *Capsicum baccatum*, and *Capsicum pubescens*.

Due to their characteristic pungency, aromas, and flavors, chili pepper fruits (syn. chile, chilli, red pepper, hot pepper, spicy pepper) are an important ingredient in millions of people's daily diets (perhaps even billions considering India). In addition, they are good sources of the antioxidant vitamins C and E and provitamin A, as well as excellent sources of other antioxidants, which counter the oxidation of lipids via scavenging free radicals and thus are discussed as protection against cancer, anemia, diabetes, and cardiovascular diseases.[2] The concentration and pattern of these health-promoting phytonutrients are influenced by genotype and environmental factors as well as by processing parameters in the production of chili powders, such as sample treatment, drying conditions, and milling.[4,5] Health-promoting attributes are not the only important usage of chilies. The xanthophylls capsanthin and capsorubin are the dominating carotenoids that allow the production of natural colorants such as oleoresins. These products are used in the food and cosmetics industries.[6,7]

According to molecular analyses of domesticated and wild species of *Capsicum*, it is concluded that the genus *Capsicum* most likely originated in arid regions of the Andes Mountains, in what became Peru and Bolivia, and then migrated to tropical lowland regions of the Americas.[2,8] The centers of domestication are still under discussion: *C. baccatum* and *C. pubescens* are postulated to have been domesticated in Bolivia, the putative center of crop origin of *C. annuum* is in current Mexico, and *C. chinense* and *C. frutescens* are thought to have originated from the Amazon.[9] Peru is a center of diversification and probably the country with the highest diversity of cultivated chili peppers in the world because of the long pre-Columbian cultural history and the fact that this is one of the few countries where varieties of all five cultivated species are grown and used in local diets. Today Peru is also one of the leading export countries for paprika (*C. annuum*) using conventional varieties that have been introduced to Peru more recently. The pricing for paprika in international trade relies mainly on its extractable color with the quality categorized by the color value as determined by American Spice Trade Association (ASTA) method 20.1.[10]

Being the world's most important center of cultivated *Capsicum* diversity, Peru holds a wealth of local *Capsicum* varieties, each with specific phytochemical characteristics. More

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than 700 *Capsicum* accessions of the five cultivated species, genetic material collected on farms and from home gardens, are kept in the Peruvian national *Capsicum* germplasm bank being managed by the Instituto Nacional de Innovación Agricola (INIA). Of these ∼700 *Capsicum* accessions, 90 have been selected to represent the biodiversity of native Peruvian chili peppers domiciled in the three climatic zones: coast, Andes, Amazon. To expand the set, 37 accessions from the Universidad Nacional Agraria La Molina (UNALM) collection as well as 20 accessions collected in smallholder farms in the Amazon region from the Centro de Investigación y Desarrollo Rural Amazónico (CIDRA) were included. These 147 accessions belong to the four domesticated species *C. annuum*, *C. baccatum*, *C. chinense*, and *C. frutescens* and are commonly named *ajíes* in Peru, as they are called in most other South American countries as well. Accessions of the species *C. pubescens* can easily be differentiated from other cultivated species due to their black seeds and are commonly named *rocoto*. A set of rocoto samples is currently under investigation, and the results will be reported in a separate study.

The study characterizes the phytochemical biodiversity of native Peruvian chili peppers (ajíes). The following attributes have been investigated: pungency by total capsaicinoids, capsaicinoid pattern (capsaicin, dihydrocapsaicin, and nordihydrocapsaicin), total polyphenol content using the Folin−Ciocalteu assay, antioxidant capacity (TEAC assay), total and specific flavonoids (quercetin, kaempferol, luteolin, and apigenin), fat content as indicator for vitamin E, vitamin C (sum of ascorbic and dehydroascorbic acid), surface color (CIE L^*, a^*, b^*), and extractable color (ASTA method 20.1). To comply with national restrictions concerning the export of indigenous biological material, all samples were dried and crushed in Peru and shipped via air courier to Wuppertal, Germany, for analysis.

The results of this study contribute to characterize Peruvian *Capsicum* varieties for potentially commercial traits. The biochemical descriptions can be used to identify in a participatory approach with small-scale farmers and local entrepreneurs promising material for the development of high-value products and to start market specialization. The results can also be a starting point to target accessions for further breeding activities. The study results thus add value to *Capsicum* diversity to generate income for small-scale farmers. At the same time, this can provide an incentive to conserve local *Capsicum* varieties *through* use. It would also confirm the important role of gene banks in conservation *for* use. Ex situ conservation of Peruvian *Capsicum* varieties is necessary because not all accessions have direct commercial value.

MATERIALS AND METHODS

Chemicals. Acetone, methanol, and acetonitrile of HPLC grade, disodium hydrogen phosphate, ammonium acetate, and disodium carbonate were purchased from VWR International (Darmstadt, Germany); Folin−Ciocalteu's phenol reagent, formic acid, *tert*-butylhydroquinone, 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), gallic acid (3,4,5-trihydroxybenzoic acid), 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox), 3′,4′,5,7-tetrahydroxyflavone (luteolin), 3,4′,5,7-tetrahydroxyflavone (kaempferol), 4′,5,7-trihydroxyflavone (apigenin), nonanoic acid vanillylamide, and natural capsaicin (65% 8-methyl-*N*-vanillyl-*trans*-6-nonenamide, 30% 8-methyl-*N*-vanillylnonanamide, 5% *N*-vanillyl-7-methyloctanamide) were obtained from Sigma-Aldrich (Steinheim, Germany); and ascorbic acid, 3,3′,4′,5,6-pentahydroxyflavone (quercetin monohydrate), acetic acid, 1,4-dimercapto-2,3-butanediol(DL-dithiothreitol), and ethanol p.a. were purchased from Carl Roth (Karlsruhe, Germany). Water was obtained from a Milli-Q Gradient A10 - System (Millipore, Schwalbach, Germany). If not noted, all chemicals were purchased in the highest commercially available grade.

Plant Material and Postharvest Treatment. One hundred and forty-seven different accessions provided by the three Peruvian organizations (INIA, UNALM, and CIDRA) were characterized. INIA collected fruits from single plants grown in the experimental station of Donoso in the Peruvian coastal zone, Huaral, Lima (11° 31′ 25″ S, 77° 14′ 01″ E). CIDRA also collected fruits from single plants that were grown by local farmers in the community of Campo Verde of the Ucayali region in the Peruvian Amazon (08° 31′ 50″ S, 74° 04′ 43″ E). Samples from UNALM came from several plants of the same accession to collect a sufficient amount of material. Plants were grown in two experimental stations: (1) El Huerto, La Molina, Lima (12° 04′ 60″ S, 76° 56′ 32″ E) and (2) Casma, Ancash (09° 28′ 54″ S, 78° 17′ 34″ E) . Fully ripe fruits were harvested in the years 2010, 2011, and 2012 (detailed information about the harvest year of the accession is given in the Supporting Information). Peduncles were removed, and fruits were oven-dried at 60 °C to constant weight for approximately 72 h, crushed, and sent to Wuppertal by air courier in sealed bags. After sieving, material with particle size >850 μm was remilled to obtain 99% <850 μm according to ASTA method 1.0.[10] Milling was performed under cooling using a knife mill (IKA Universal Mill M20 for batches >10 g and IKA Analytical Mill A10 for batches <10 g, IKA-Werke Staufen, Germany). Samples were stored in black polyethylene plastic bags at −25 °C until analysis. Table 1 shows the number of accessions per species received from the different organizations. Plants were taxonomically classified to belong to the four domesticated species *C. annuum*, *C. baccatum*, *C. chinense*, and *C. frutescens*.[11] Classification of the plants was done by David Williams for INIA, by Victor Mendoza for UNALM, and by Maarten van Zonneveld for CIDRA. Detailed information including accession code, growing region, taxonomical classification, harvest year, and the full set of analytical results are presented in the Supporting Information.

Table 1. Number of Accessions per Species and Organization

organization	total	*C. annuum*	*C. baccatum*	*C. chinense*	*C. frutescens*
INIA	90	19	26	43	2
UNALM	37	2	8	27	0
CIDRA	20	0	2	15	3
total	147	21	36	85	5

Extraction and Analysis of Capsaicinoids. The analysis of the capsaicinoid content was done by high-performance liquid chromatography (HPLC) with fluorescence detection. Two separate samples of each accession were analyzed. For the extraction, 500 mg of sample was placed in a glass centrifuge tube. One milliliter of a disodium hydrogen phosphate buffer (0.5 M, pH 11) and 15 mL of acetonitrile and methanol (50:50, v/v) were added. After 16 h at 4 °C in the dark, the sample was placed in an oven at 80 °C for 4 h and vortexed every 30 min. The crude extract was diluted with methanol/water (1:1, v/v) from 1:1 to 1:40 to fit into the calibration curve and filtered through a 0.2 μm polyvinylidene difluoride (PVDF) syringe filter (Carl Roth) before HPLC analysis. Separation of the capsaicinoids was performed by injecting 10 μL into a Merck-Hitachi HPLC system (interface L-7000, quaternary pump L-7100, autosampler L-7250, fluorescence detector L-7485, and a CIL column oven) with a Kinetex RP-18 column (2.6 μm, 100 mm × 3 mm) equipped with a 0.5 μm inline filter (Phenomenex, Aschaffenburg, Germany) at 50 °C. The fluorescence detector was set to 280 nm for excitation and to 320 nm for detection.[12] Separation of capsaicinoids was achieved by isocratic elution with acetonitrile and 0.5% acetic acid (38:62, v/v) at a flow rate of 0.7 mL/min and a total run time of 11 min. Nonanoic acid vanillylamide was used as standard for an external calibration curve for quantification, because of the identical fluorescence characteristic like

other capsaicinoids and the availability in high purity. Peak identification was done by injecting a solution of natural capsaicin. The capsaicinoid content was calculated as the sum of nordihydrocapsaicin, capsaicin, and dihydrocapsaicin. Minor capsaicinoids were not considered in this study.

Determination of Total Polyphenol Content. The method was based on the Folin−Ciocalteu procedure.[13] The crude extract from the capsaicinoid determination was used for analysis. One hundred microliters was placed in a 15 mL centrifuge tube and was diluted with 900 μL of water. Five milliliters of the Folin−Ciocalteu phenol reagent (1:10, v/v, diluted with water) was added. After an incubation time between 3 and 8 min, 4 mL of disodium carbonate solution (7.5 g/100 mL) was added. After 1 h at 30 °C, 250 μL of the solution, each, was transferred to two wells of a 96-well microtiter plate for a duplicate reading of the absorbance at 750 nm with a model 680 microtiter plate reader (Bio-Rad, Munich, Germany). For external calibration gallic acid was used. The results were expressed as gallic acid equivalents (GAE).

Trolox Equivalent Antioxidant Capacity (TEAC). The procedure described by Re et al.[14] was applied. The crude extract from the capsaicinoid determination was used. One hundred microliters of the extract was diluted with 900 μL of ethanol. Twenty microliters of each solution was transferred to two wells of a 96-well microtiter plate for duplicate measurement, and 200 μL of the diluted ABTS-radical solution was added. After an incubation time of 6 min at 20 °C, the absorbance was read at 750 nm with the microtiter plate reader. For external calibration Trolox was used. The ABTS radical stock solution was prepared by dissolving 192 mg of ABTS in 50 mL of water. The radical is produced by adding 33 mg of potassium peroxydisulfate to the solution. The mixture was placed in the dark at room temperature for 16 h to generate the radical. One milliliter of ABTS stock solution was diluted with approximately 50 mL of water, and the absorbance was adjusted to 0.70 ± 0.02 at 750 nm before use.

Analysis of Ascorbic Acid by HPLC. Five hundred milligrams of sample material was placed in a 15 mL centrifuge tube, and 10 mL of a mixture of acetonitrile and an ammonium acetate buffer (100 mM, pH 6.8) (70:30, v/v, containing 1 g/100 mL *tert*-butylhydroquinone and 1 g/100 mL dithiothreitol) was added. The suspension was shaken for 2 h at room temperature. Subsequently, the solution was centrifuged at 2000*g* for 10 min and filtered through a 0.2 μm PVDF syringe filter before HPLC analysis using hydrophilic interaction liquid chromatography (HILIC) as described by Nováková et al.[15] for ascorbic acid analysis. Five microliters was injected on the same Merck-Hitachi system but equipped with a Merck-Hitachi L-7455 photodiode array detector. The separation for half the sample pool was performed on a sulfobetaine ZIC-HILIC column (3.5 μm, 150 mm × 4.6 mm) (SeQuant, Umeå, Sweden) at 35 °C. Isocratic elution was done using a mixture of acetonitrile and an ammonium acetate buffer (100 mM, pH 6.8) (70/30, v:v) at a flow rate of 0.5 mL/min with a run time of 17 min. With the availability of core−shell HILIC columns, the separation of the remaining samples was performed on a sulfobetaine Nucleoshell HILIC column (2.7 μm, 100 mm × 3 mm) (Macherey-Nagel, Dueren, Germany) at 35 °C with acetonitrile and the same buffer (80:20, v/v) at a flow rate of 0.4 mL/min with a total run time of 9.5 min. Quantification was performed in both cases at the absorption maximum of 260 nm. Ascorbic acid was used as external standard for calibration. Dehydroascorbic acid is reduced by dithiothreitol to ascorbic acid; therefore, this method detects the sum of both.

Flavonoid Analysis. A slightly modified method described by Miean and Mohamed[16] was used to analyze quercetin, kaempferol, luteolin, and apigenin aglycons. For extraction and hydrolysis of the flavonoid glycosides, 750 mg of the sample was weighed into a centrifuge tube, and 10 mL of a mixture of methanol, water, and 12.5 M hydrochloric acid (70:20:10, v/v/v, containing 0.4 g/100 mL *tert*-butylhydroquinone) was added. The suspension was kept at 80 °C for 3 h and vortexed every 30 min. Five hundred microliters of the crude extract was diluted with a disodium hydrogen phosphate buffer (50 mM Na_2HPO_4, pH 12)/methanol solution (1:1, v/v) to a final volume of 2000 μL. After filtration through a 0.2 μm PVDF filter, 10 μL was injected in the same Merck-Hitachi HPLC system being used for ascorbic acid determination but equipped with a Kinetex PFP (pentafluorophenyl) column (2.6 μm, 100 mm × 3 mm) with a 0.5 μm inline filter (Phenomenex) at 50 °C. Methanol (solvent A) and water both with 0.1% formic acid were used as mobile phase by applying the following gradient program at a flow rate of 0.5 mL/min: 0−5 min, ramp from 40 to 45% A; 5−8 min, 45% A; 8−22 min, ramp from 45 to 95%; 22−22.1 min, ramp from 95 to 40% A; and 22.1−31 min, 40% A (column re-equilibration). Quantification was performed at 360 nm for all four flavonoids. For external calibration quercetin, kaempferol, luteolin, and apigenin were used. The sum of the four individual flavonoids is expressed as total flavonoids.

Measurement of Surface Color (CIE *L, *a**, *b**, Hue Angle).** Measurement was performed on a Jasco UV/Vis-NIR-Spektrometer V-670 (Gross-Umstadt, Germany) in the reflection mode equipped with the PSH-001/02 powder holder. Three hundred milligrams of the sample was placed in the powder holder with subsequent spectra recording. CIE *L**, *a**, *b**, hue angle, and chroma C^* [17] were calculated from the obtained UV−vis spectra by Jasco Spectramanager V.2.07.00.

Determination of Extractable Color According to ASTA Method 20.1.[10] On the basis of the surface color data, the amount of sample material was chosen to achieve the required absorption between 0.3 and 0.7. Typically 70−700 mg of sample material was used and placed in a 100 mL volumetric flask. Ninety milliliters of acetone was added, and the flask was shaken. After 16 h at room temperature in the dark, the flask was filled to the mark with acetone and shaken again. After particles had settled, the absorbance of the clear supernatant was measured with a Hach DR/2000 spectrophotometer (Duesseldorf, Germany) at 460 nm and ASTA 20.1 values were calculated.

Determination of Fat Content. The method described by Schulte[18] was used. A 1.2 g sample of the chili powder was placed in a glass centrifuge tube. After addition of 10 mL of 4 M hydrochloric acid and 5 mL of toluene, the tube was placed in an oven at 120 °C for 2 h and vortexed every 20 min. Samples were cooled to room temperature and centrifuged at 2800*g* for 10 min. One milliliter of the toluene phase was evaporated under a nitrogen stream at 115 °C until a constant weight for the residue was obtained.

Determination of Moisture Content. Two grams of the sample was exactly weighed into a weighing bottle and dried in a vacuum oven for 1 h at 60 °C at 100 mbar. The sample was allowed to cool in a desiccator for 1 h and weighed again. Moisture content was calculated as the difference of the sample mass before and after drying.

Statistical Analysis. All determinations were carried out as duplicates (two extracts), except for ascorbic acid. Table 2 shows the mean coefficients of variation determined from 147 duplicate analyses.

Analyses were carried out on dried material. Accordingly, the results refer to 100 g of the dry sample material as obtained after milling. The moisture content of this material was also determined and is recorded in the Supporting Information.

Box plot analysis was done using the software tool "R 2.15.1" (R Foundation for Statistical Computing, Vienna, Austria), freely available at http://www.r-project.org. The box plot shows the range minimum−maximum, 25th percentile, median, and 75th percentile. Outliers were identified as 1.5 times the interquartile range. Outliers can be regarded as samples with outstanding attributes. Statistical box plot analysis was not carried out for *C. frutescens* accessions because of their small number (n = 5).

RESULTS AND DISCUSSION

Capsaicinoid Content and Pattern. The pungency of chili peppers and spice paprika is an important quality parameter. The amount of the capsaicinoids capsaicin, dihydrocapsaicin, and nordihydrocapsaicin, which are responsible for the pungent taste, shows a wide variability among all Peruvian species and varieties from nonpungent to very hot (Figure 1). The highest capsaicinoid concentration was found in a *C. frutescens* accession (no. 146) with 1560.1 mg/100 g of total capsaicinoids and a pattern of 68.5% capsaicin, 29.5%

Table 2. Analytical Precision Data

parameter	CV[a] (%)
capsaicinoids	4.0
nordihydrocapsaicin	7.1
capsaicin	4.0
dihydrocapsaicin	4.4
total polyphenols	1.9
TEAC	2.7
total flavonoids	2.3
quercetin	2.3
luteolin	2.6
kaempferol	2.2
apigenin	9.0
ASTA-20.1	2.1
a^*	1.6
b^*	2.2
C^*	1.7
h	0.8
L^*	0.7
fat content	2.5
moisture content	5.2

[a]CV, coefficient of variation; average CV from all 147 duplicate analyses.

dihydrocapsaicin, and 1.7% nordihydrocapsaicin. This is equivalent to ca. 250,000 Scoville heat units (SHU). In *C. chinense* the maximum amount was 1411.6 mg/100 g (no. 131) and that in *C. annuum*, 809.0 mg/100 g (no. 102). *C. baccatum* was the least pungent of the three species with the highest value at 711.7 mg/100 g (no. 78). In two *C. annuum* and two *C. chinense* accessions, no capsaicinoids at all could be detected. Figure 1 also shows the wide concentration range for each individual capsaicinoid. It is remarkable that *C. chinense* samples in general had very low nordihydrocapsaicin content but also the two varieties with the highest content.

Figure 2 presents the pattern (percentage distribution) of the capsaicinoids. Capsaicin and dihydrocapsaicin were the dominating capsaicinoids in all accessions. *C. chinense* accessions contain smaller amounts of nordihydrocapsaicin compared to the accessions of the other two species. Multivariate data analysis did not show any correlation between species and patterns of individual capsaicinoids (data not shown). This has been described before by Zewdie and Bosland.[19] One of the five *C. frutescens* (no. 144) from our set is remarkable for a capsaicinoid composition of 37.6% capsaicin, 43.2% dihydrocapsaicin, and 19.2% nordihydrocapsaicin, which is very untypical with regard to nordihydrocapsaicin when compared with the literature data.[19]

Total Polyphenols and Antioxidant Capacity. In the modern human diet phytonutrients with the ability to scavenge free radicals and with further health-promoting attributes become more and more important. The antioxidant activity and total polyphenols are attributed to different compounds such as flavonoids, phenolic acids, capsaicinoids, and vitamins C and E, together with other antioxidants found in chili. Assays such as the determination of the total polyphenol content using the Folin−Ciocalteu assay or the TEAC assay are key parameters for the assessment of the health-benefit potential. The advantage is that these assays assess the mixtures of the extracted phytonutrients in total and do not focus on a single antioxidant or group.[20] This allows chili accessions to be rated by the degree of their antioxidant properties. However, these tests have the disadvantage of providing only very limited comparability with data of other studies. Slightly changed conditions for extraction or minor modifications in the assay procedures have a strong influence on the results of the unspecific sum parameters.[21]

Our results for total polyphenols and TEAC show a wide variation across the different accessions and species (Figure 3). The highest levels were found in accession 2 (*C. chinense*) with 3.69 g gallic acid equivalents (GAE)/100 g of total polyphenols and a TEAC value of 9.2 mmol Trolox/100 g. The majority of samples are in the ranges between 1.5 and 2.0 g GAE/100 g and between 3.0 and 5.0 mmol Trolox/100 g for TEAC value. Hervert-Herández et al.[22] reported comparable data for extractable polyphenols of four dried hot pepper varieties (*C. annuum*) with 0.97−1.4 g GAE/100 g and 1.9−3.6 mmol Trolox/100 g. Additionally, they observed a correlation between total polyphenols and the corresponding TEAC values with $R^2 = 0.98$. We also obtained a positive correlation (Figure 4) but with a significantly lower coefficient of correlation (R^2 =

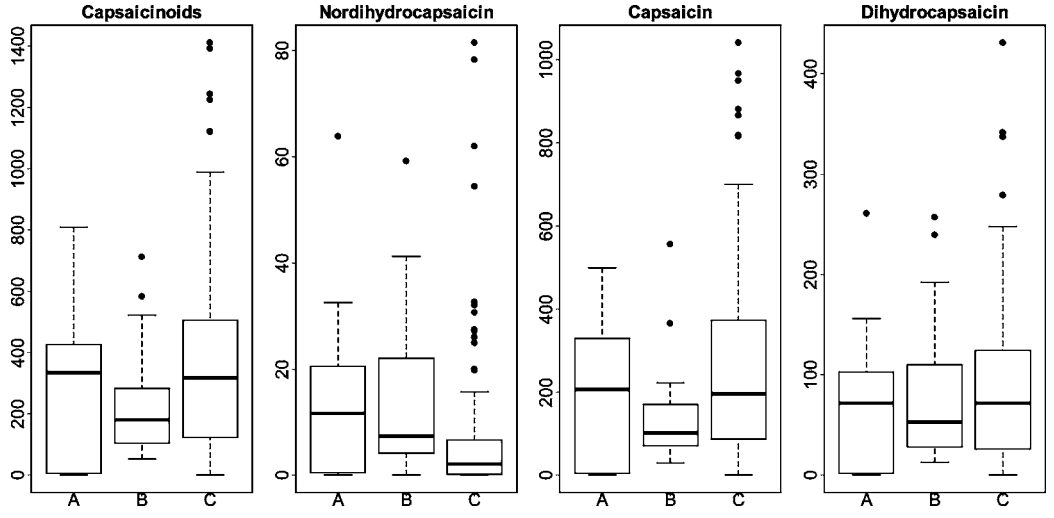

Figure 1. Box plot of capsaicinoid concentrations. Twenty-fifth percentile, median (thick line), 75th percentile, and range minimum−maximum; outliers (•) were identified as 1.5 times the interquartile range. All results are expressed in mg/100 g. A, *C. annuum*; B, *C. baccatum*; C, *C. chinense*.

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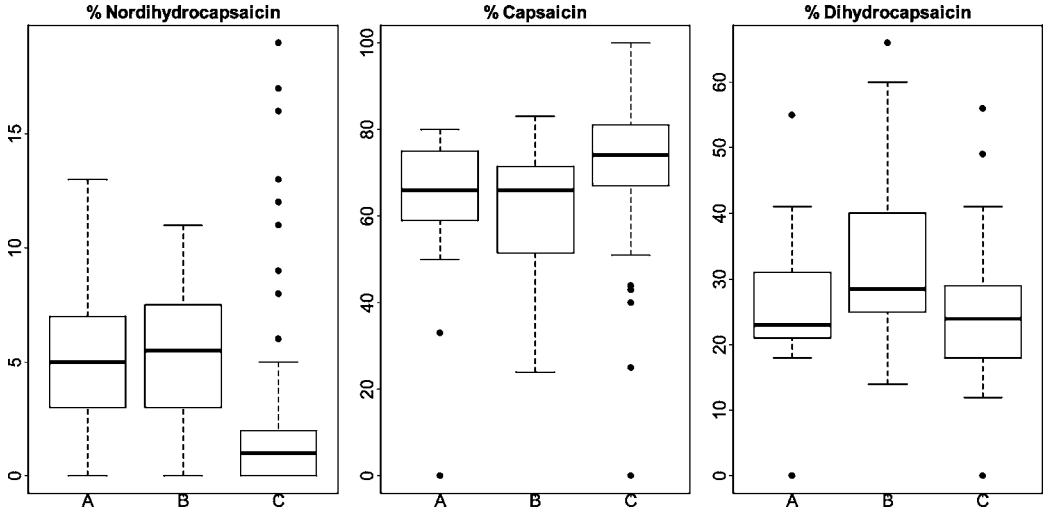

Figure 2. Box plot analysis of percentage of capsaicinoid distribution. A, *C. annuum*; B, *C. baccatum*; C, *C. chinense*.

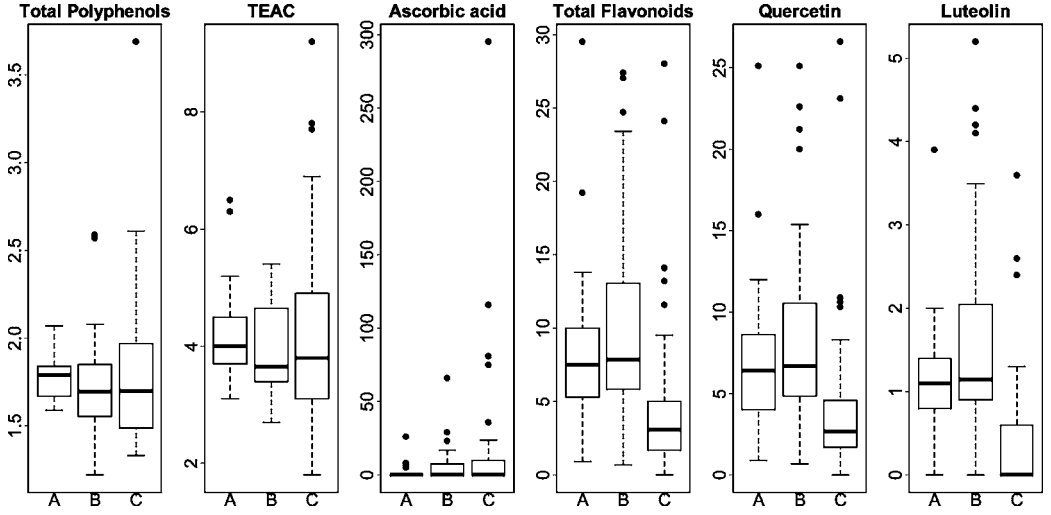

Figure 3. Box plot of antioxidant relevant parameters. Units: total polyphenols, g GAE/100 g, TEAC mmol Trolox/100 g; ascorbic acid, total flavonoids, quercetin, and luteolin, mg/100 g. A, *C. annuum*; B, *C. baccatum*; C, *C. chinense*.

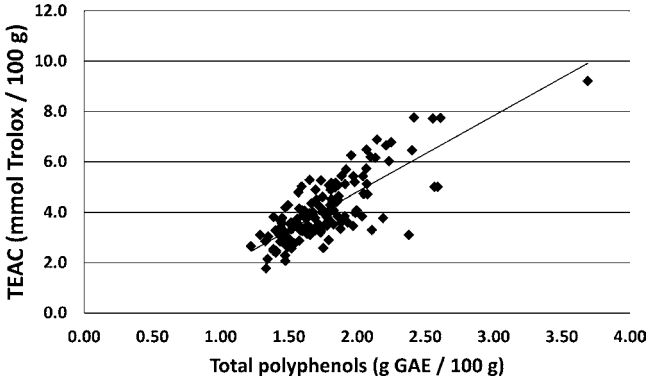

Figure 4. Correlation between TEAC and total polyphenols, R^2 = 0.61.

0.61). This could be due to the much higher number of samples and greater diversity of species and varieties in our study.

Those accessions of this study that showed outstanding high levels of the two sum parameters could be a good source of antioxidants in the human diet.

Ascorbic Acid. Fresh chili peppers are an extremely rich source of ascorbic acid (vitamin C). Fresh fruits typically contain up to 250 mg/100 g fresh weight. The content is influenced by the degree of ripeness.[2] During the drying process most of the ascorbic acid is degraded to residual levels of only 10%.[23] Therefore, the ascorbic acid content was screened for by only a single determination for each accession. In 83 of the 147 varieties we did not find any ascorbic acid, whereas some accessions surprisingly showed outstanding high ascorbic acid concentrations. The maximum of ascorbic acid was found in no. 25 (*C. chinense*) with 295 mg/100 g. A high-resolution mass spectrometric analysis confirmed the identity of the HPLC peak ascribed to ascorbic acid with an m/z of 175.0246 for the $[M - H]^-$ ion. To finally confirm this outstanding result it will be necessary to analyze fresh fruits of this and other exceptionally vitamin-rich accessions. Besides the health-promoting effects of ascorbic acid as vitamin and antioxidant, such high concentrations help to protect and preserve other valuable compounds, for example, carotenoids, and, thereby, color intensity during the drying process and storage of chili powder.[5]

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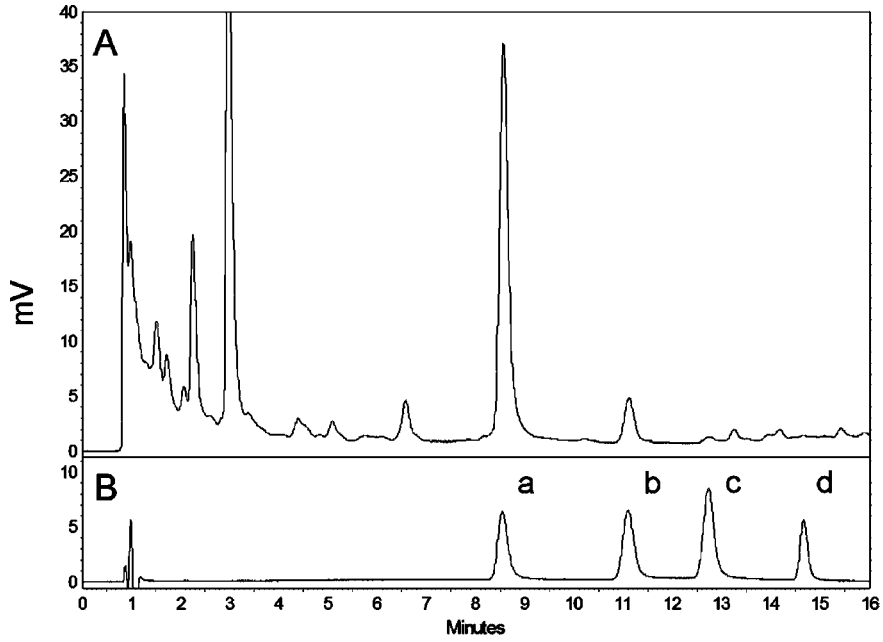

Figure 5. Typical HPLC profile obtained for flavonoid analysis: (A) sample 41 containing 20.0 mg/100 g quercetin, 3.0 mg/100 g luteolin, 0.4 mg/100 g kaempferol, and traces of apigenin; (B) standard solution. Peaks: a, quercetin; b, luteolin; c, kaempferol; d, apigenin.

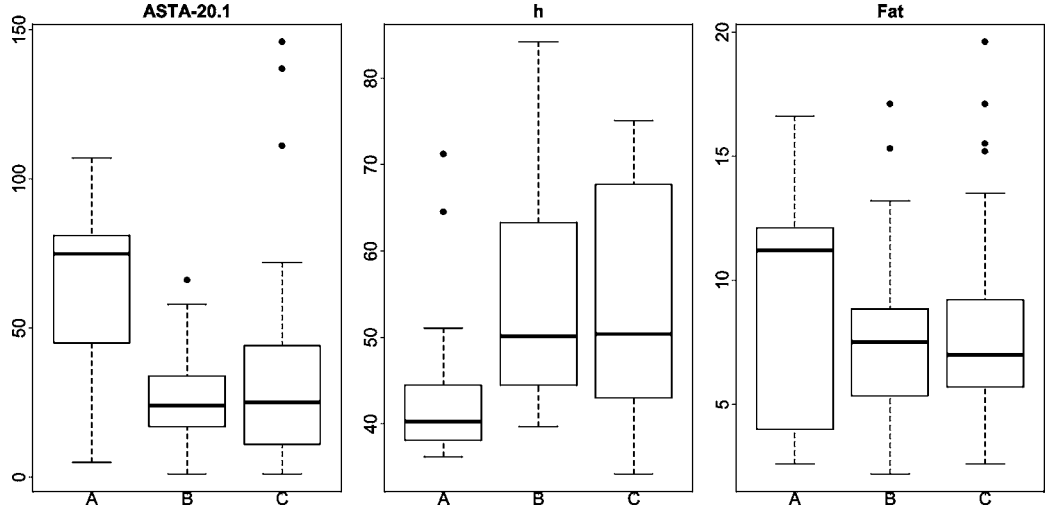

Figure 6. Box plot of ASTA 20.1 value, hue angle (h), and fat content in g/100 g. A, *C. annuum*; B, *C. baccatum*; C, *C. chinense*.

Specific Flavonoids. Chili peppers are also a good source of flavonoids. This class of phytonutrients has different health-promoting effects. Besides their antioxidant properties and free radical scavenging activity, they have anti-inflammatory and anticarcinogenic effects, which make them interesting for the human diet and highly valuable compounds in chili.[24] We focused the analysis of flavonoids on the major flavones quercetin, luteolin, kaempferol, and apigenin contained in the fruits as free flavones and as glycosides. We report on the total content of specific flavones due to a hydrolysis step in the analytical procedure. A typical HPLC profile is shown in Figure 5. Quercetin is the dominating flavonoid and was found in 141 accessions with concentrations up to 26.6 mg/100 g. In six accessions neither quercetin nor any other of the three flavonoids could be found. Luteolin was the second dominating flavonoid but with much lower concentrations between 0.4 and 5.2 mg/100 g. Kaempferol and apigenin were found in just a limited number of chilis with concentrations from 0.1 to 0.6 mg/100 g for kaempferol and from 0.2 to 0.7 mg/100 g for apigenin. The highest amount of an individual flavonoid was found in a *C. chinense* accession (no. 18) with 26.6 mg quercetin/100 g, whereas the maximum total flavonoid concentration was measured in a *C. annuum* accession with 29.5 mg/100 g (no. 76). Remarkably, 64% of all *C. chinense* accessions did not contain detectable amounts of luteolin, kaempferol, or apigenin. The five *C. frutescens* accessions showed in all cases rather low levels of flavonoids, with quercetin as the dominating flavonoid.

Miean and Mohamed[16] analyzed three *C. annuum* (green chili, red chili, and bell pepper) and one *C. frutescens* (bird chili) market samples. Quercetin was found in three samples (40−80 mg/100 g), luteolin in green chili (3.3 mg/100 g), and a remarkably high content of luteolin in bird chili (103 mg/100 g). Apigenin was present in bell pepper (27.2 mg/100 g) as was kaempferol (3.3 mg/100 g). As with capsaicinoids a wide range is to be expected for flavonoids in *Capsicum* fruits.

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Color and Fat Content. Besides the capsaicinoids, the content of carotenoids as classified by the ASTA 20.1 value is another important quality parameter. Carotenoids are potent antioxidants, and some have provitamin A activity. The xanthophylls capsanthin and capsorubin in fruits of the genus *Capsicum* are responsible for the intense red color of a wide range of varieties. Concentrated extracts are used as important colorants for the food and cosmetics industry. Color measurement by the CIE $L^*a^*b^*$ system is best suited to describe the surface color objectively and reproducibly. The hue angle (h) can be calculated from the L^*, a^*, and b^* values and describes the relationship between red, orange, and yellow pigments. Due to the fact that the yellowish seeds have been milled together with the dried pericarp, the color of the powders will be shifted into the yellow range.

In general, a hue angle of 90° describes a pure yellow color and one of 0°, a pure red color, with orange in between. Most of the samples were in the orange range with only a few appearing red. The most intense red sample was a *C. chinense* accessions (no. 49) with a hue angle of 36.4 and also the maximum ASTA 20.1 value of 146 for extractable color. This is remarkable for chili powders but far from paprika, reaching ASTA values beyond 200. As can be seen in Figure 6, the median of extractable color by ASTA units is significantly higher in *C. annuum* than in *C. baccatum* and *C. chinense*. Perhaps more promising materials for extractable color can be found in native samples from Mexico and Central America, which is considered a secondary center of cultivated *Capsicum* diversity and the principal center of *C. annuum* domestication.

Nieto-Sandoval et al.[25] noted a correlation between surface color and the corresponding natural logarithm of the ASTA 20.1 value with a good correlation of $R^2 = 0.97$ for typical red paprika from *C. annuum*. On the basis of surface color measurement, we used this correlation in our study for getting an estimate of the ASTA value. This allowed us to find a suitable sample weight for the ASTA 20.1 determination for achieving the required absorption of 0.3−0.7.

The fat content in the samples depends on the amount of seeds in comparison to the content of pericarp in the powder. Fat content ranged between 2.2 and 19.6 g/100 g (no. 131; *C. chinense*) with the median for *C. annuum* at ca. 12 g/100 g and median values at ca. 7 g/100 g for *C. baccatum* and *C. chinense*. Because vitamin E is a fat-soluble complex of different tocopherols, a high fat content is correlated with a high content in vitamin E.[2]

We found *Capsicum* accessions with pungency from nonpungent to extremely pungent and with outstanding content in valuable health-related phytonutrients. This still under-utilized diversity of native *Capsicum* varieties should be a starting point for high-value product differentiation and income generation for poor small-scale farmers and local entrepreneurs in Peru by strengthening local value chains. Today, most consumers will buy fruits and vegetables on the basis of appearance and not nutritional quality. This may be changing, however, as consumers begin to look to fruits and vegetables as insurance against illness.[2] Promising accessions are replanted to further evaluate their health-promoting potential.

■ ASSOCIATED CONTENT

Supporting Information

Sample description and quantification data of 19 parameters for all 147 accessions. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

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■ ABBREVIATIONS USED

TEAC, Trolox equivalent antioxidant capacity; ABTS, 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt; GAE, gallic acid equivalents; A, *C. annuum*; B, *C. baccatum*; C, *C. chinense*

■ REFERENCES

(1) Perry, L.; Dickau, R.; Zarrillo, S.; Holst, I.; Pearsall, D. M.; Piperno, D. R.; Berman, M. J.; Cooke, R. G.; Rademaker, K.; Ranere, A. J.; Raymond, J. S.; Sandweiss, D. H.; Scaramelli, F.; Tarble, K.; Zeidler, J. A. Starch fossils and the domestication and dispersal of chili peppers (*Capsicum* spp. L.) in the Americas. *Science* **2007**, *315*, 986−988.

(2) Bosland, P. W.; Votava, E. J. *Peppers. Vegetable and Spice Capsicums*, 2nd ed.; CABI: Cambridge, UK, 2012.

(3) USDA, ARS National Genetic Resources Program. *Germplasm Resources Information Network − (GRIN) [on-line database]. National Germplasm Resources Laboratory*; http://www.ars-grin.gov/cgi-bin/npgs/html/econ.pl (accessed Nov 21, 2012).

(4) Gnayfeed, M. H.; Daood, H. G.; Biacs, P. A.; Alcaraz, C. F. Content of bioactive compounds in pungent spice red pepper (paprika) as affected by ripening and genotype. *J. Sci. Food Agric.* **2001**, *81*, 1580−1585.

(5) Biacs, P. A.; Czinkotai, B.; Hoschke, A. Factors affecting stability of colored substances in paprika powders. *J. Agric. Food Chem.* **1992**, *40*, 363−367.

(6) Minguez-Mosquera, M. I.; Jaren-Galan, M.; Garrido-Fernandez, J. Color quality in paprika. *J. Agric. Food Chem.* **1992**, *40*, 2384−2388.

(7) Minguez-Mosquera, M. I. Color quality in paprika oleoresins. *J. Agric. Food Chem.* **1998**, *46*, 5124−5127.

(8) Walsh, B. M.; Hoot, S. B. Phylogenetic relationships of *Capsicum*. *Int. J. Plant Sci.* **2001**, *162*, 1409−1418.

(9) Moscone, E. A.; Scaldaferro, M. A.; Grabiele, M.; Cecchini, N. M.; Sánchez García, Y.; Jarret, R.; Daviña, J. R.; Ducasse, D. A.; Barboza, G. E.; Ehrendorfer, F. The evolution of chili peppers (*Capsicum* Solanaceae): a cytogenic perspective. *Acta Hortic.* **2007**, *745*, 137−169.

(10) American Spice Trade Association. *Official Analytical Methods of the American Spice Trade Association*; ASTA: Englewood Cliffs, NJ, 1997.

(11) Ibiza, V. P.; Blanca, J.; Cañizares, J.; Nuez, F. Taxonomy and genetic diversity of domesticated *Capsicum* species in the Andean region. *Genet. Resour. Crop Evol.* **2012**, *59*, 1077−1088.

(12) Kirschbaum-Titze, P.; Hiepler, C.; Mueller-Seitz, E.; Petz, M. Pungency in paprika (*Capsicum annuum*). 1. Decrease of capsaicinoid content following cellular disruption. *J. Agric. Food Chem.* **2002**, *50*, 1260−1263.
(13) Singelton, V. M.; Rossi, J. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *Am. J. Enol. Vitic.* **1965**, *16*, 144−158.
(14) Re, R.; Pellegrini, N.; Proteggente, A.; Pannala, A.; Yang, M.; Rice-Evans, C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biol. Med.* **1999**, *26*, 1231−1237.
(15) Nováková, L.; Solichová, D.; Pavlovičová, S.; Solich, P. Hydrophilic interaction liquid chromatography method for the determination of ascorbic acid. *J. Sep. Sci.* **2008**, *31*, 1634−1644.
(16) Miean, K. H.; Mohamed, S. Flavonoid (myricetin, quercetin, kaempferol, luteolin, and apigenin) content of edible tropical plants. *J. Agric. Food Chem.* **2001**, *49*, 3106−3112.
(17) McGuire, R. G. Reporting of objective color measurements. *HortScience* **1992**, *12*, 1254−1255.
(18) Schulte, E. Mikromethode zur schnellen gravimetrischen Bestimmung des Fettgehaltes von Lebensmitteln nach Säureaufschluss. *Dtsch. Lebensm.-Rundsch.* **2001**, *97*, 85−89.
(19) Zewdie, Y.; Bosland, P. W. Capsaicinoid profiles are not good chemotaxonomic indicators for *Capsicum* species. *Biochem. Syst. Ecol.* **2001**, *29*, 161−169.
(20) Wang, S.; Meckling, K. A.; Marcone, M. F.; Kakuda, Y.; Tsao, R. Synergistic, additive, and antagonistic effects of food mixtures on total antioxidant capacities. *J. Agric. Food Chem.* **2011**, *59*, 960−968.
(21) Dawidowicz, A. L.; Olszowy, M. Antioxidant properties of BHT estimated by ABTS assay in systems differing in pH or metal ion or water concentration. *Eur. Food Res. Technol.* **2011**, *232*, 837−842.
(22) Hervert-Hernández, D.; Sáyago-Ayerdi, S. G.; Goñi, I. Bioactive compounds of four hot pepper varieties (*Capsicum annuum* L.), antioxidant capacity, and intestinal bioaccessibility. *J. Agric. Food Chem.* **2010**, *58*, 3399−3406.
(23) Ramesh, M. N.; Wolf, W.; Tevini, D.; Jung, G. Influence of processing parameters on the drying of spice paprika. *J. Food Eng.* **2001**, *49*, 63−72.
(24) Yao, L. H.; Jang, Y. M.; Shi, J.; Tomás-Barberán, F. A.; Datta, N.; Singanusong, R.; Chen, S. S. Flavonoids in food and their health benefits. *Plant Foods Hum. Nutr.* **2004**, *59*, 113−122.
(25) Nieto-Sandoval, J. M.; Fernandez-Lopez, J. A.; Almela, L.; Muñoz, J. A. Dependence between apparent color and extractable color in paprika. *Color Res. Appl.* **1999**, *24*, 93−97.