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Chemical and Morphological Characterization of Costa Rican Papaya (*Carica papaya* L.) Hybrids and Lines with Particular Focus on Their Genuine Carotenoid Profiles

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ABSTRACT: Papaya (*Carica papaya* L.) F1 hybrids and inbred lines grown in Costa Rica were screened for morphological and nutritionally relevant fruit traits. The qualitative composition of carotenoids showed great similarity, being mostly composed of free and esterified β-cryptoxanthins accompanied by β-carotene, lycopene, and biosynthetic precursors. High levels of (*all-E*)-lycopene and its isomers were distinctive for red-fleshed hybrids, whereas yellow-fleshed fruits were virtually devoid of lycopenes. Because carotenoid levels among the investigated hybrids and lines differed significantly, this study supports the hypothesis of an exploitable genetic variability, and a potential heterotic effect regarding carotenoid expression may be instrumental in papaya-breeding programs. Due to significantly higher levels of provitamin A carotenoids and coinciding high levels of total lycopene, particularly red-fleshed hybrids might represent prospective sources of these compounds. Furthermore, the nutritional value of some genotypes was boosted by substantial amounts of ascorbic acid (up to 73 mg/100 g of fresh weight), which correlated to total soluble solids (R^2 = 0.86).

KEYWORDS: *papaya, carotenoids, lycopene, hybrid, lines, heterosis, ascorbic acid*

INTRODUCTION

During the past two decades, high growth rates for papaya (*Carica papaya* L.) production were reported worldwide. For instance, Central America increased its production by almost 3 times from 0.39 to 1.08 million tons (mt), whereas growth in India rose by even more than 8 times from 0.45 to 3.91 mt. On average, total papaya world production approximately tripled from 3.26 mt in 1990 to 10.02 mt in 2009, illustrating the expanding importance of this crop.[1] Besides its economic value, papaya fruit provides valuable amounts of micronutrients, in particular several pro-vitamin A carotenoids. Consequently, in tropical developing and transition countries, papaya fruit represents a fundamental pro-vitamin A source, because vitamin A-rich animal foods such as eggs and milk products are unavailable for the majority of the population.[2] On the basis of the prevalence of night blindness and biochemical vitamin A deficiency (VAD) associated with serum retinol concentrations <0.70 μmol/L, VAD was reported to be one of the major nutritional issues worldwide. Generally, infants, young children, and pregnant women are strongly affected due to the crucial role of vitamin A in development and reproduction.[3] In papaya, the vitamin A precursors β-carotene and β-cryptoxanthin occur in high amounts, and bioavailability of carotenoids from this tropical fruit was assumed to be high as shown by a high accumulation rate of vitamin A in rat liver after 2 weeks of a papaya-rich diet.[4] This assumption was recently supported by Schweiggert et al.,[5] who found significant differences in the deposition of pro-vitamin A carotenoids in papaya, tomato, and carrot chromoplasts.

As a further health benefit, red-fleshed papaya genotypes contain high contents of lycopene, which was found to reduce the incidence of degenerative diseases and cancer.[5,6] The prevention mechanism is related to the efficient quenching of singlet oxygen by carotenoids containing more than seven conjugated double bonds.[7] Lycopene exerted extremely strong antioxidative properties being of utmost interest for human health, because this carotene was found to accumulate in various human organs and compartments such as the prostate.[8,9] Therefore, red papaya genotypes containing both lycopene and pro-vitamin A carotenoids may represent a valuable nutritional component of the human diet.[10]

To date, the evolutionary origin of papaya is supposed to be located between southern Mexico and northwestern South America and,[11] therefore, particularly Central American papaya genotypes may reveal an exceptional diversity. However, information on detailed and systematic characterizations of Central American papaya fruits is still scarce, because most research has been focused on Hawaiian genotypes. Therefore, the main objective of this study was reporting technologically and nutritionally important morphological and chemical fruit traits of Costa Rican papaya genotypes. Yellow- and red-fleshed fruits of female and hermaphrodite plants were included in this study with emphasis on their carotenoid profile. Consideration of closely related F1 hybrids and inbred lines should provide insights into the genetic variability of papaya genotypes with regard to their carotenoid profiles. Besides carotenoids, ascorbic acid, glucose, fructose, sucrose, and a range of further physicochemical and morphological parameters, including fruit size, cavity size, and pulp firmness, should also be assessed

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Table 1. Morphological Traits and Further Characteristics of Fruits from Different *Carica papaya* L. Genotypes at Full Ripeness

	Criolla	Industrial 10P	Industrial 10G	Pococí	MHR 21-4-6	Silvestre	Sunset
pulp color class	red	red	red	red	yellow	yellow	red
gender[a]	hermaphrodite	hermaphrodite	hermaphrodite	hermaphrodite	hermaphrodite	female	female
hybrid/line	line	hybrid	hybrid	hybrid	line	line	line
relative RI/%	98 ± 2 D	99 ± 1 D	99 ± 1 CD	101 ± 1 BC	102 ± 1 AB	99 ± 2 CD	102 ± 0 A
fruit weight/g	2053 ± 601 A	1862 ± 287 A	1729 ± 243 A	1501 ± 240 A	692 ± 221 C	1031 ± 76 B	604 ± 98 C
pulp/% (w/w)	76.0 ± 6.9 A	73.2 ± 6.6 A	73.2 ± 5.6 A	69.1 ± 7.0 A	77.0 ± 9.3 A	71.4 ± 5.1 A	58.4 ± 3.8 B
seed/% (w/w)	2.4 ± 0.5 E	5.3 ± 0.7 D	8.1 ± 1.2 BC	8.3 ± 1.8 BC	7.2 ± 1.7 CD	10.2 ± 3.8 BC	18.2 ± 2.4 A
peel/% (w/w)	21.7 ± 6.9 A	21.6 ± 6.4 A	18.7 ± 5.1 A	22.5 ± 6.0 A	15.8 ± 7.9 A	18.4 ± 3.9 A	23.4 ± 3.5 A
fruit volume/cm^3	2318 ± 670 A	2152 ± 349 A	1933 ± 396 AB	1667 ± 266 B	756 ± 233 C	1138 ± 61 C	679 ± 116 C
cavity volume/cm^3	261 ± 83 A	277 ± 80 A	299 ± 64 A	276 ± 74 A	93 ± 53 C	156 ± 38 BC	184 ± 39 B
cavity index/% (v/v)	9.9 ± 2.7 D	13.0 ± 2.0 CD	15.6 ± 1.4 BC	16.5 ± 3.0 B	11.4 ± 3.7 D	13.7 ± 2.8 BCD	27.0 ± 2.4 A
L_{max}/cm	32.5 ± 6.0 A	24.7 ± 1.2 B	24.1 ± 1.3 B	23.6 ± 2.0 B	17.0 ± 2.2 C	15.5 ± 0.3 CD	12.6 ± 0.5 D
D_{max}/cm	11.8 ± 1.1 AB	12.6 ± 0.7 A	12.3 ± 0.8 A	12.4 ± 0.9 A	8.8 ± 1.5 A	12.5 ± 0.5 A	10.8 ± 0.6 B
D_{min}/cm	4.1 ± 0.5 B	4.3 ± 0.5 B	3.8 ± 0.4 BC	3.8 ± 0.4 BC	3.5 ± 0.3 C	4.8 ± 0.7 A	3.4 ± 0.2 C
L_{Dmax}/cm	15.1 ± 3.5 A	11.6 ± 1.8 BC	13.8 ± 1.9 AB	12.7 ± 1.9 BC	10.9 ± 1.0 C	7.4 ± 0.7 D	6.4 ± 0.9 D
0.5 D_{max}/L_{Dmax}	0.41 ± 0.09 C	0.55 ± 0.09 B	0.45 ± 0.04 BC	0.50 ± 0.09 BC	0.40 ± 0.06 C	0.85 ± 0.08 A	0.87 ± 0.15 A

[a]Fruits were collected from hermaphrodite or female plants. Different letters indicate significant difference of means.

to conclusively evaluate the economic and nutritional potential of the respective genotypes.

MATERIALS AND METHODS

Plant Material. Red- and yellow-fleshed papaya (*C. papaya* L.) fruits from female and hermaphrodite plants of various F1 hybrids and inbred lines, subsequently denoted "hybrids" and "lines", respectively, were obtained from the Agricultural Experiment Station "Los Diamantes" (Guápiles, Costa Rica) during September−November 2009. Red-fleshed fruits of the commercial line 'Sunset' were obtained from female plants. The Costa Rican commercial line 'Criolla', the commercial hybrid 'Pococí', mainly marketed as "Papaya Perfecta", and the newly bred hybrids 'Industrial 10P' and 'Industrial 10G' represented red-fleshed fruits from hermaphrodite plants. The latter two hybrids both descend from the lines 'Criolla' (red), 'Maradol' (red), and 'Sunset' (red). Yellow-fleshed fruits of a Costa Rican wild type line named 'Silvestre' were obtained from female plants. Originating from cross-breeding of the 'Silvestre' (yellow), 'Sunset' (red), and 'Maradol' (red) genotypes, fruits of the experimental yellow-fleshed line 'MHR 21-4-6' were obtained from hermaphrodite plants. Table 1 summarizes the gender, their genetic origin (F1 hybrid or inbred line), and the fruit pulp color of the plants investigated.

Mimicking commercial Costa Rican practice, fruits of all genotypes were harvested at color break stage approximately 150 days after anthesis, subsequently dipped into an aqueous solution of 0.1% (v/v) ethephone (Ethrel 48 SL, Bayer Crop Science, Monheim, Germany), air-dried, and stored for 5−7 days at approximately 25 °C until peel color was >90% yellow. Complementing visual estimation of full ripeness, the ripening index (RI) as proposed by Schweiggert et al.[10] was calculated for each fruit from corresponding total soluble solids and pulp firmness (Table 1). Of each genotype, a minimum of five fruits was harvested for evaluation, except for 'Silvestre', for which only three fully ripe fruits were available.

Reagents. *β*-Apo-8′-carotenal, *β*-carotene, *β*-cryptoxanthin, and (*all-E*)-lycopene standards were obtained from Sigma Aldrich Chemie GmbH (Taufkirchen, Germany). Besides methyl *tert*-butyl ether (MTBE, Merck Chemicals, Darmstadt, Germany), 2,6-di-*tert*-butyl-*p*-cresol and 3-*tert*-butyl-4-hydroxyanisole (BHT and BHA, respectively; Fluka Chemie GmbH, Buchs, Switzerland), all further reagents or solvents were purchased from VWR International GmbH Darmstadt, Germany), at least of analytical or HPLC grade. Deionized water was used throughout.

Morphological Traits. Total fruit, peel, seed, and pulp weights were gravimetrically determined. Maximum length (L_{max}), minimum fruit diameter (D_{min}), maximum fruit diameter (D_{max}), and distance from the stem end to maximum fruit diameter (L_{Dmax}) were assessed as described previously and schematically illustrated in Figure 1.[10] Furthermore, fruit volume (V_{fruit}) was assessed gravimetrically by displacement of water (~20 °C). By analogy, determination of cavity volume (V_{cav}) was carried out after seed removal, and the cavity index (%) was calculated as follows: cavity index = $V_{cav}/V_{fruit} \times 100\%$.

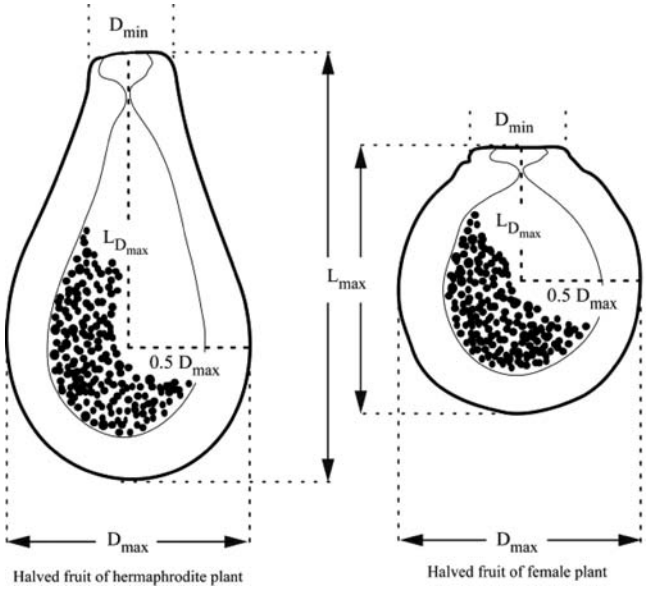

Figure 1. Morphological characteristics of fruits from hermaphrodite and female plants according to Schweiggert et al.[10].

Pulp and Peel Firmness and Color Analyses. Pulp and peel firmness and color determination as applied in this study were described in detail previously.[10] Analyses of fruit peel and pulp firmness were carried out on the intact fruit using a TA-XT plus Texture Analyzer (Stable Microsystems, Surrey, U.K.). CIE L^*, C^*, and $h°$ color values were assessed using a Colorflex instrument (Hunterlab, Reston, VA).

Chemical Analyses. First, peels and seeds of the fruits were manually separated. Subsequently, the fresh papaya mesocarp was cut into small cubes (~1 cm^3). Aliquots of approximately 100 g were immediately frozen with liquid nitrogen, lyophilized, and ground with liquid nitrogen. The obtained powders were filled into aluminum pouches, sealed under vacuum, and stored at −80 °C until carotenoid analysis. Parts of the remaining fresh mesocarp cubes were

immediately used for the determination of sucrose, D-glucose, and D-fructose as described below. Prior to ascorbic acid determination, fresh mesocarp cubes were rapidly cooled and stored at −20 °C until analyses. Residual mesocarp cubes were homogenized and immediately used for the determination of total soluble solids (TSS), pH, and titratable aciditiy (TA). TSS and pH were determined at 20 °C using an Abbé refractometer type 1T (Atago Co. Ltd., Tokyo, Japan) and a pH-meter lab 827 (Metrohm, Herisau, Switzerland), respectively. TA, expressed as citric acid (CA) in grams per 100 g of fresh weight (FW), was determined by titration with 0.25 N NaOH to a pH of 8.1, applying an automatic titration system of the type Titrino 718 STAT (Metrohm). If not described differently, chemical parameters of each single fruit investigated were determined at least in duplicate for each fruit, except for cv. 'Sunset', where 2−3 fruits of a total of 15 fruits had to be pooled to obtain sufficient sample material for all analyses.

Sucrose, D-Glucose, and D-Fructose. For the determination of sucrose, D-glucose, and D-fructose, rapid inactivation of invertase activity was necessary as reported by Chan and Kwok.[12] For this purpose, aliquots of the freshly cut mesocarp cubes were halved to obtain a size of ∼0.5 cm^3 and immediately heated by microwave in plastic tubes for 25 s to 90−95 °C. The cubes were subsequently cooled to room temperature and stored at −20 °C until further analysis. The evaporation loss caused by heating was recorded for correction of the sugar contents. Subsequent analyses of sucrose, D-glucose, and D-fructose contents were carried out in duplicate for each single fruit using an enzyme kit (no. 10 716 260 035) from R-Biopharm (Darmstadt, Germany). Following the instructions of the kit for solid samples (e.g., potato), the extraction of sugars was carried out as follows: An aliquot of 10.00 ± 0.01 g of the heated sample was homogenized with 30 mL of double-distilled water and stirred for 15 min at room temperature (∼20−25 °C). After adjustment of the pH to 7.5−8.5 with 1 mol/L NaOH, 5 mL of aqueous potassium hexacyanidoferrate(II) (85 mmol/L) and 5 mL of aqueous zinc sulfate (250 mmol/L) were added for sample clarification. The solution was made up to 100 mL and filtered for removal of precipitate. The filtrate was diluted (1:20) and used for enzymatic determination of sucrose, D-glucose, and D-fructose following the supplier's instructions.

Ascorbic Acid. Analyses of ascorbic acid contents were carried out using an enzyme kit (no. 10 409 677 05, R-Biopharm). Aliquots of two fruits (10.0 g each) were pooled and used for sample preparation according to a modified method of the kit's instructions for ascorbic acid extraction from potatoes. Briefly, an aliquot of 10.00 ± 0.01 g of the pooled sample was homogenized with 10 mL of metaphosphoric acid (15% w/v) and 0.02 mL of *n*-octanol using a Tissue Tearer (Biospec Products, Bartlesville, OK). Subsequently, the solution was adjusted to pH 3.5−4.0 by 2 mol/L aqueous KOH, made up to 100 mL, and filtered (pore size = 12−25 μm). The filtrate was used for enzymatic determination of ascorbic acid according the supplier's instructions.

Carotenoid Analyses. Sample preparation as well as HPLC-DAD analyses for qualitative and quantitative determination of carotenoids was carried out as described in detail previously.[10] Briefly, a mixture of methanol, ethyl acetate, and light petroleum (bp 40−60 °C) containing 0.1 g/L of both BHT and BHA was repeatedly used as extraction solvent until the sample residue appeared colorless using β-apo-8′-carotenal as internal standard (ISTD). After washing with H_2O and drying with Na_2SO_4, the combined organic phases were evaporated to dryness, made up to 5 mL with MTBE/methanol (9:1, v/v), membrane filtered (0.45 μm) into amber glass vials, and subsequently stored at −20 °C until HPLC analysis. For separation of carotenoids, a series 1100 HPLC (Agilent, Waldbronn, Germany) equipped with a G1379A degasser, a G1312A binary gradient pump, a G1313A autosampler, a G1316A column oven, and a G1315B diode array detector was used. Operating at 25 °C, the column employed was a 150 × 3.0 mm i.d., 3 μm particle size, analytical scale YMC C30 reversed phase column (YMC Europe, Dinslaken, Germany), which was protected by a 10 × 3.0 mm i.d., 3 μm particle size, YMC C30 guard column (YMC Europe). The mobile phase consisted of methanol/MTBE/water (91:5:4, v/v/v; eluent A) and methanol/MTBE/water (6:90:4, v/v/v; eluent B). The gradient applied was as follows: isocratic at 100% A for 10 min, from 100 to 35% A in 55 min, from 35 to 15% A in 10 min, isocratic at 0% A for 5 min, from 0 to 100% A in 5 min, and isocratic at 100% A for 5 min. Total run time was 90 min at a flow rate of 0.42 mL/min. Injection volume was 10 μL. Free and esterified carotenoids were monitored at 450 nm, and additional UV−vis spectra were recorded in the range of 200−600 nm.

On the basis of the method described by Schweiggert et al.,[13] the HPLC system was coupled online to a Bruker 3000+ ion trap mass spectrometer (Bruker Daltronic, Bremen, Germany) operating in positive mode and an APcI source. Mass spectra were recorded in the range of m/z 100−1100 at a scan rate of 13000 Th/s (peak width = 0.6 Th, fwhm). Nitrogen was used as both drying and nebulizing gas at a flow rate of 3.5 L/min and a pressure of 50 psi, respectively. Nebulizer temperature was set at 350 °C, and a potential of −2779 V was applied on the capillary. Corona and vaporizer temperature were set to 2000 nA and 400 °C, respectively. Collision gas for CID was helium at a pressure of 4.9×10^{-6} mbar. CID mass spectra were obtained with an isolation width of 2.0 Th for precursor ions and a fragmentation amplitude of 1.0 V.

Identification of carotenoids was accomplished by comparison of UV−vis absorption spectra, retention times, and mass spectra with those of authentic standards. When standards were unavailable, pigments were tentatively identified by comparing their UV−vis absorption spectra and mass spectral behavior with data published previously.[13−16] Quantification was carried out after obtaining linear calibration curves of β-carotene, β-cryptoxanthin, and (*all-E*)-lycopene. Contents of β-cryptoxanthin esters were calculated on the basis of the β-cryptoxanthin calibration, and (*Z*)-isomers of lycopene were quantified by (*all-E*)-lycopene calibration. Concentrations of stock solutions were measured spectrophotometrically by applying their specific absorption coefficients ($A_{1\,cm}^{1\%}$) as reported previously.[14] For calculation of retinol equivalents, 1 μg of β-carotene corresponded to 0.167 μg of retinol equivalents (RE) and 1 μg of other pro-vitamin A to 0.084 μg RE. Masses of the acyl moieties of carotenoid esters were not included in the calculation of retinol equivalents.[5]

Statistics. Determination of significant differences between means was carried out using SAS 9.1 (SAS Institute, Cary, NC). First, a Shapiro−Wilk's test for normality was conducted ($P < 0.05$), and homogeneity of variances was assessed by Levene's test ($P < 0.01$). With regard to normally distributed data sets with homogeneous variances, Duncan's multiple-range test was conducted, whereas a pairwise Welch's test ($P < 0.05$) was applied for samples with unequal variances ($P < 0.05$).

RESULTS AND DISCUSSION

Morphological Fruit Traits. The different papaya genotypes investigated are displayed in Figure 2. Fruits from hermaphrodite plants showed elongated forms, whereas female plants developed round fruits. This phenomenon was earlier related to the shape of their either slender or globose ovary, respectively.[17] With regard to the geometric differences between elongated and round-shaped fruits as shown in Figure 1, the ratio of fruit radius 0.5 D_{max} and L_{Dmax} delivered significantly distinguishable values from 0.31 to 0.65 and from 0.67 to 1.15 for single fruits of hermaphrodite and female plants, respectively. The corresponding means of all fruits of a genotype illustrate the difference described (cf. Table 1).

The cavity index of fruits of hermaphrodite origin ranged from 9.9 to 16.5%, whereas the index of papayas from female plants reached from 13.7 to 27.0% (Table 1). By analogy, fruits from female plants revealed a higher proportion of seeds (10.2−18.2%, cf. Table 1). Because papaya seeds contain high levels of benzylglucosinolates, being enzymatically converted into pungent, earthy, and cress-like flavor components such as benzyl isothiocyanate and phenylacetonitrile, respectively, damaged seeds may affect the quality of processed fruit.[18] Moreover, extracts of papaya seeds were shown to affect the

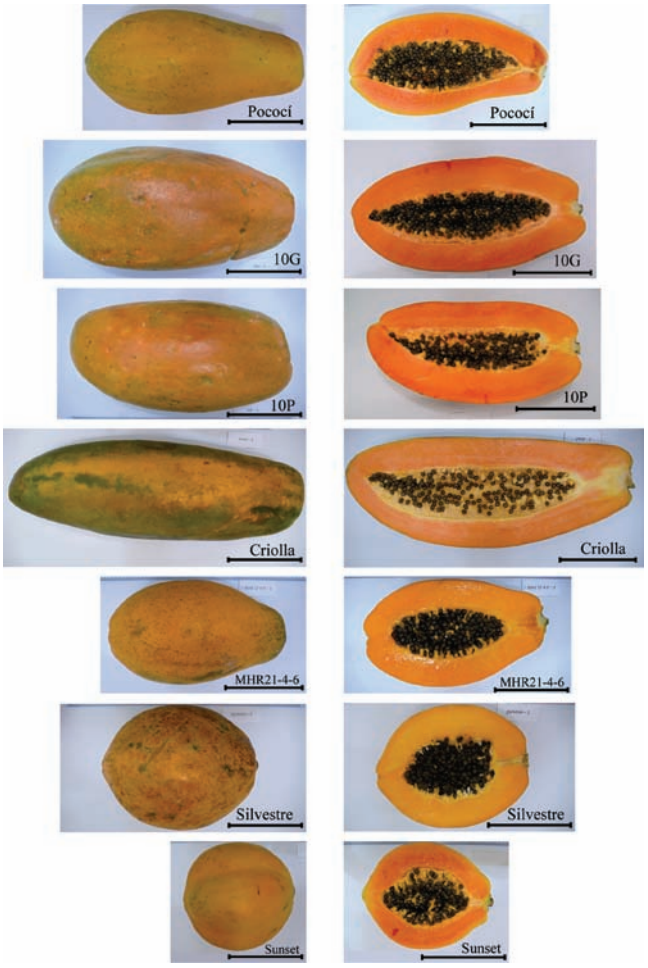

Figure 2. Papaya genotypes investigated. Bars mark 10 cm.

fertility of several mammals including humans, ranging from reduced sperm motility to extensive azoospermia.[19,20] Therefore, papaya seeds should be removed carefully during pulp finishing and genotypes with low proportions of seeds are desirable. Consequently, the genotypes 'Criolla' and 'Industrial 10P' (from 2.4 to 5.3% seeds) should be of great interest for food processors. In general, papaya fruits of hermaphrodite plants are more popular in the fresh fruit trade, as the lower amount of seeds and the smaller cavity result in higher yields of edible mesocarp.[21] As exemplified for the genotypes 'Sunset' and 'Criolla' derived from female and hermaphrodite plants, respectively, a minimum of ∼58% and a maximum of ∼76% of edible pulp could be obtained from the respective fruits (cf. Table 1).

Most morphological traits of the investigated hybrids did not show significant differences to the investigated lines. For instance, 'Criolla' fruits derived from an inbred line reached similar or even bigger fruit sizes than the closely related F1 hybrids 'Industrial 10P' and 'Industrial 10G' (Table 1). In contrast, Marin et al.[22] found heterotic effects for several fruit traits, for example, increase of total fruit production of a plant.[22]

Physicochemical Traits. Besides morphological traits, consumer acceptance of papaya fruit depends on various physicochemical properties. For instance, TSS of >11.5° Brix are a minimum grade requirement for traded Hawaiian papayas.[17] Whereas in our study the highest average TSS values (∼13.5° Brix) were reached for the Hawaiian cultivar 'Sunset', TSS of individual fruits of all genotypes varied between 7.9 and 13.6 °Brix (cf. Table 2). A similar range from 9.0 to 13.0 °Brix was reported for Bangladeshi papayas.[23] Titratable acidity of the genotypes included in our study was low (0.09−0.19 g CA/100 g of FW), but comparable to the report of Bron and Jacomino, who found 0.09−0.13 g CA equivalents in 100 g of FW.[24] Because pH values of the pulp varied only slightly between 4.9 and 5.4 for the fruits under investigation, a relationship between pH and TA could not be established (correlation R^2_{linear} = 0.07; $R^2_{exponential}$ = 0.04).

In agreement with the visual appearance (Figure 2), pulp color measurements revealed only minor variability within red- or yellow-fleshed genotypes (Figure 3A). For red-fleshed papaya, a correlation between CIE color values of the pulp and corresponding carotenoid contents was previously reported.[10] Compared to the other red-fleshed types showing CIE C^* values in the range of 59.7−61.7, the 'Criolla' genotype was characterized by a weak red tint (C^* = 57.6). As described below, the 'Criolla' genotype contained the minimum amount of total carotenoids.

In contrast to pulp color, peel color generally showed significantly higher variance, due to large, separated green and yellow areas on the peel. Due to its enormous fruit size, the peel color of the genotype 'Criolla' was particularly heterogeneous (Figures 2 and 3B). Due to the described variance within the same genotype, significant differences of peel color between the red- and yellow-fleshed genotype groups were not found in our study. Like color values, pulp and peel firmness revealed a high variability among fruits within the same genotype (Figure 3C).

Whereas mean values of all genotypes investigated are listed in Table 2, contents of D-glucose, D-fructose, and sucrose of individual fruit samples ranged from 2.2 to 4.2 g/100 g of FW, from 1.8 to 3.5 g/100 g of FW, and from 0.3 to 2.2 g/100 g of FW, respectively. Interestingly, average total sugar contents comprising the three saccharides added up to only 55−65% of TSS. Schweiggert et al.[10] described an extensive tissue softening during fruit ripening. According to Lazan et al.,[25] this softening

Table 2. Chemical Parameters of the Investigated Genotypes[a]

	TSS (°Brix)	pH	TA (g/100 g FW)	TSS/TA	ascorbic acid (mg/100 g FW)	D-glucose (g/100 g FW)	D-fructose (g/100 g FW)	sucrose (g/100 g FW)
Criolla	8.8 ± 0.6 D	5.4 ± 0.2 AB	0.09 ± 0.02 C	103 ± 16 AB	24.9 ± 0.4 D	2.9 ± 0.3 BCD	2.2 ± 0.3 BC	0.5 ± 0.2 C
Industrial 10P	10.7 ± 0.6 C	4.9 ± 0.2 D	0.11 ± 0.02 BC	104 ± 18 AB	48.6 ± 0.5 BC	3.3 ± 0.3 B	2.6 ± 0.3 B	0.8 ± 0.4 BC
Industrial 10G	10.6 ± 0.9 C	5.1 ± 0.1 C	0.10 ± 0.02 C	110 ± 31 AB	45.6 ± 1.1 C	2.9 ± 0.3 BCD	2.6 ± 0.4 B	1.3 ± 0.7 AB
Pococí	11.2 ± 0.4 C	5.3 ± 0.1 BC	0.10 ± 0.02 C	117 ± 19 A	46.1 ± 2.0 BC	3.1 ± 0.5 BC	2.6 ± 0.4 B	1.2 ± 0.2 AB
Silvestre	10.4 ± 0.7 C	5.2 ± 0.1 BC	0.13 ± 0.02 B	77 ± 4 BC	50.0 ± 5.9 B	2.6 ± 0.4 D	1.9 ± 0.2 C	1.7 ± 0.8 A
MHR 21-4-6	12.6 ± 0.7 B	5.4 ± 0.0 AB	0.19 ± 0.02 A	67 ± 7 C	69.6 ± 0.6 A	2.6 ± 0.3 CD	2.6 ± 0.3 B	1.8 ± 0.5 A
Sunset	13.5 ± 0.2 A	5.2 ± 0.0 C	0.11 ± 0.01 BC	127 ± 12 A	72.9 ± 1.5 A	3.8 ± 0.4 A	3.5 ± 0.4 A	1.5 ± 0.5 A

[a]Different letters indicate significant difference of means.

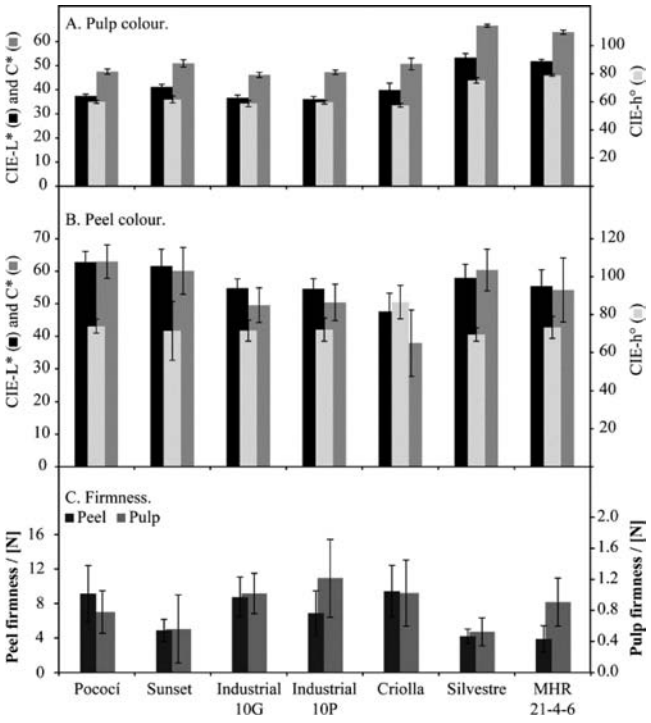

Figure 3. CIE color and firmness values of peel and pulp of the investigated genotypes.

process is related to cell wall degradation by native β-galactosidase, pectinesterase, and polygalacturonase activities. Because papaya contains very low amounts of starch independent of fruit maturity,[25] carbohydrates other than D-glucose, D-fructose, and sucrose are very likely to contribute significantly to the TSS measured by refractometry.

As illustrated in Figure 4, TSS values correlated with ascorbic acid contents ($R^2_{polynomial}$ = 0.856), which ranged from ~24.9

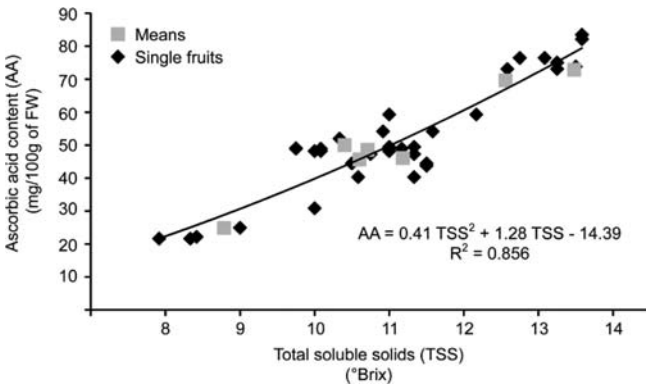

Figure 4. Correlation of ascorbic acid contents and total soluble solids. The denoted equation corresponds to ascorbic acid contents of single fruits versus TSS.

mg ('Criolla') to ~72.9 mg ('Sunset') per 100 g of FW (Table 2). Linear regression yielded similar correlation coefficients (R^2_{linear} = 0.851, not shown). Because both ascorbic acid and TSS were previously found to rise during papaya fruit ripening,[10,26] the observed correlation is in agreement with previous expectations. Plant biosynthesis of ascorbic acid follows the Smirnoff–Wheeler pathway via low molecular weight precursors such as D-glucose and L-galactose.[27] Interestingly, a direct relationship was shown for papaya by Barata-Soares et al.,[26] who infiltrated papaya slices with several isotope-labeled carbohydrate precursors, proving their bioconversion to ascorbic acid. Increasing precursor concentrations during ripening might trigger the biosynthesis of ascorbic acid. Furthermore, because ascorbic acid levels of individual papaya fruits range widely from 21.6 to 83.5 mg/100 g of FW, vitamin C content may be relevant for their nutritional classification (cf. Table 2). Although L-dehydroascorbic acid, also contributing to vitamin C values, was not determined in our study, fresh and particularly "high-Brix" papaya fruits should be considered a good nutritional source for vitamin C due to their high ascorbic acid contents. For comparison, ascorbic acid contents of apples, mangoes, and oranges varied from 1.4 to 3.5 mg/100 g of FW, from 9.1 to 18.6 mg/100 g of FW, and from 42.1 to 62.4 mg/100 g of FW, respectively.[28] In agreement with the upper range of our observations, Franke et al.[28] reported 62.7–80.7 mg/100 g of FW for Hawaiian papaya genotypes.

Qualitative Carotenoid Composition Analyzed by HPLC-DAD-MS^n. The characterization of the genuine carotenoid profiles of various red- and yellow-fleshed Costa Rican papaya genotypes from hermaphrodite and female plants was a major objective of this study. Figure 5 exemplarily highlights two chromatograms of nonsaponified extracts of the genotypes 'Industrial 10P' (red-fleshed) and 'MHR 21-4-6' (yellow-fleshed). Irrespective of gender and genetic ancestors of the mother plant, the pigment composition of all genotypes investigated revealed only minor qualitative diversity. The most outstanding difference was indicated by either the presence or the almost complete absence of lycopene, which is clearly illustrated by the (*all-E*)-lycopene peak (compound **19**) in the HPLC chromatograms (Figure 5). Concomitantly to high levels of (*all-E*)-lycopene in red-fleshed genotypes, several lycopene (*Z*)-isomers (compounds **13**, **15**–**18**, and **20**) were tentatively assigned according to their UV–vis and mass spectral fragments listed in Table 3. In good agreement with the fragmentation of the authentic (*all-E*)-lycopene standard, the MS^2 experiments of quasi-molecular (*Z*)-lycopene ions at *m/z* 537 revealed characteristic losses of 56, 70, 82, 110, 124, 138, and 150 Da. However, differentiation of (*Z*)-isomers by their MS^2 fragmentation pattern was not feasible. For instance, the product ion $[M + H - 82]^+$ at *m/z* 455 occurred consistently at highest abundance for all lycopenes. Similarly, the peak intensity of most other fragments varied only slightly.

Although a mutual exclusion has been ascertained for lycopene in red- and yellow-fleshed papaya as early as 1964 by Yamamoto,[29] Devitt et al.[30] and Schweiggert et al.[5] recently provided new insights into the biosynthesis and cellular deposition of lycopene, respectively. In red papaya, one of two identified lycopene β-cyclase genes encodes a dysfunctional enzyme (LCY-2) due to a frame shift mutation of the corresponding gene.[30] As a result, lycopene is accumulated in the presence of the dysfunctional LCY-2 enzymes, leading to the formation of lycopene crystals in papaya chromoplasts.[5] Nevertheless, the remaining functional enzyme (LCY-1) expresses full activity, converting lycopene to β-carotene and, consequently, all characteristic yellow papaya carotenoids were also found in red-fleshed genotypes.[5,30] In yellow papaya, both LCY-1 and LCY-2 represent functional enzymes, converting almost 100% of the lycopene to β-carotene, which is subsequently converted to further typical free and esterified papaya xanthophylls. Because yellow papaya color is known to

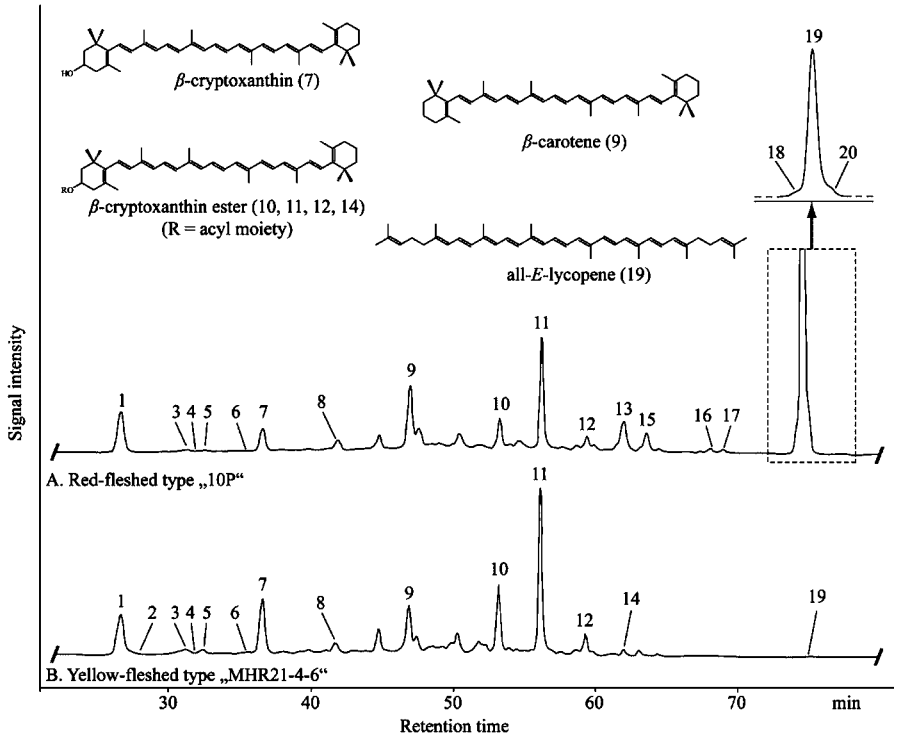

Figure 5. HPLC separation of carotenoids and carotenoid esters from a red (A) and a yellow (B) papaya pulp monitored at 450 nm. For peak assignment see Table 3.

be inherited dominantly,[31] cross-breeding may enable the repair of the defect gene encoding LCY-2. Accordingly, the 'MHR 21-4-6' hybrid descended from various red genotypes and an ultimate cross-breeding with the yellow 'Silvestre' genotype, resulting in yellow flesh color.

In both the yellow- and red-fleshed genotypes, β-carotene (compound **9**) and β-cryptoxanthin (compound **7**) were unequivocally identified and quantified in all genotypes (Tables 3 and 4). Similarly, capric (**10**), lauric (**11**), myristic (**12**), and palmitic acid (**14**) esters of β-cryptoxanthin were characterized according to their UV−vis absorption identical to free β-cryptoxanthin and their characteristic fragments in MS^n experiments (Table 3). In contrast to violaxanthin diesters characteristic of mango fruit, in which unusual xanthophyll dibutyrates (C4:0) were previously detected,[32] fatty acids ranging from C10:0 to C16:0 were typical acyl moieties of β-cryptoxanthin esters in the papaya fruit. Protonated ester molecules $[M + H]^+$ were observed at comparably low intensities by applying the positive mode, whereas neutral fatty acid fragments were released from the quasi-molecular ion $[M + H]^+$ during in-source fragmentation, which resulted in abundant deacylated product ions at m/z 535 $[M + H -$ fatty acid$]^+$. MS^2 experiments confirmed the origin of the ions at m/z 535 from the corresponding quasi-molecular ion $[M + H]^+$ at m/z 707 (caprate), 735 (laurate), 763 (myristate), and 791 (palmitate) after CID. Moreover, the in-chain elimination of toluene from both the acylated $[M + H - 92]^+$ and deacylated ions $[M + H -$ fatty acid $- 92]^+$ confirmed the carotenoid nature of the compounds (cf. Table 3).

Furthermore, various cryptoxanthin epoxides (compounds **2**, **3**, and **5**) were tentatively identified by comparison of their UV−vis absorption and mass spectral characteristics to those reported previously.[33,34] Whereas the cryptoxanthin-5,6-epoxide was predominantly found in the yellow-fleshed types, cryptoxanthin-5,8-epoxides (*syn* cryptoflavines), the furanoid oxide derivatives of the 5,6-epoxide, were detected in trace amounts in all genotypes investigated. However, cryptoxanthin-5,6-epoxide was reported to readily undergo a furanoid rearrangement to form the cryptoxanthin-5,8-epoxide during the analytical workup procedure.[35,36] The configuration at the C5 atom remains unchanged in the course of the structural reorganization, whereas at C8 both the 8*R* and 8*S* configurations were found for various carotenoid 5,8-epoxides by Deli and Ösz.[35] Both showing an identical, typical UV−vis and mass spectral behavior of β-cryptoxanthin-5,8-epoxides,[35,36] compounds **3** and **5** might represent C8 epimers of such epoxides.

Precursors such as phytoene (compound **4**), phytofluene (compound **6**), and ζ-carotene (compound **8**) were tentatively identified in all genotypes by their UV−vis absorption spectra and coinciding detection of protonated molecular ions (Table 3). CID fragmentation of the quasi-molecular ion $[M + H]^+$ of phytofluene (m/z 543) revealed a characteristic loss of 69 Da, presumably formed by bis-allylic cleavage of the C3,C4 single bond as reported by Enzell and Back.[15] A further product ion $[M + H - 82]^+$ at m/z 461 was observed at high abundance (100% base peak intensity) supposedly corresponding to the loss of a C_6H_{10} group by cleavage of the C4,C5 or the C4′,C5′ phytofluene single bond followed by a hydrogen atom transfer. Similarly, a fragment ion $[M + H - 82]^+$ appeared during CID fragmentation of phytoene and ζ-carotene, resulting in dominating signals at m/z 463 and 459, respectively, possibly indicating an analogous elimination of a C_6H_{10} group.

Quantitative Variability of Nutritionally Relevant Carotenoids. On the basis of a broad range of epidemiological studies, Willet[37] estimated that 32% of all cancer deaths might be avoided by changing dietary habits, recommending an abundant consumption of fruits and vegetables. Particularly,

Table 3. UV−Vis Spectra and MS Data of Carotenoids from Yellow and Red Papaya

no.	retention time (min)	compound identity	HPLC-DAD UV−vis spectrum λ_{max} (nm)	$[M + H]^+$ m/z	HPLC/APCI(+)MS^n experiment m/z[a]	genotypes that contained the pigment[c]
1	26.5	β-apo-8′-carotenal (ISTD)	464	417	MS^2 [417]: 399 (83), 389 (11), 325 (94)	none
2	28.3	β-cryptoxanthin-5,6-epoxide[b]	424/448/474	569	MS^2 [569]: 551 (18), 535 (5), 512 (8), 477 (8), 221 (2)	SIL, MHR
3	32.4	cryptoflavin isomer I[b]	404/426/452	569	MS^2 [569]: 551 (76), 533 (10), 477 (15), 221 (20)	all
4	32.4	phytoene[b]	274/286/298	545	MS^2 [545]: 503 (11), 489 (28), 475 (17), 463 (68)	all
5	33.3	cryptoflavin isomer II[b]	404/426/452	569	MS^2 [569]: 551 (90), 533 (5), 513 (28), 477 (7), 221 (28)	all
6	35.3	phytofluene[b]	332/348/368	543	MS^2 [543]: 501 (18), 487 (31), 474 (18), 461 (100)	all
					MS^3 [543→461]: 433 (5), 405 (19), 323 (10)	
7	36.4	β-cryptoxanthin	428/452/478	553	MS^2 [553]: 535 (100), 497 (15), 479 (11), 460 (80), 461 (34), 429 (42), 415 (15)	all
					MS^3 [553→535]: 535 (5), 479 (47), 413 (10), 397 (11)	
8	41.4	ζ-carotene[b]	380/400/424	541	MS^2 [541]: 485 (54), 471 (31), 459 (100), 417 (84), 403 (66), 391 (58)	all
9	46.7	β-carotene	428/450/476	537	MS^2 [537]: 481 (25), 445 (39), 444 (100), 399 (50), 387 (23), 347 (36)	all
10	53.1	β-cryptoxanthin caprate	428/452/478	707	MS^2 [707]: 615 (27), 535 (100), 443 (11), 442 (16)	all
11	56.0	β-cryptoxanthin laurate	428/452/478	735	MS^2 [735]: 643 (3), 535 (100), 443 (6), 413 (4)	all
12	59.2	β-cryptoxanthin myristate	428/452/478	763	MS^2 [763]: 671 (29), 535 (100), 443 (4), 442 (5), 413 (7)	all
13	61.9	(Z)-lycopene isomer 1[b]	442/468/496	537	MS^2 [537]: 481 (51), 467 (49), 455 (100), 427 (15), 413 (43), 399 (64), 387 (11)	red
14	63.2	β-cryptoxanthin palmitate	428/450/476	791	MS^2 [791]: 699 (39), 535 (100), 443 (4), 413 (46)	MHR, SIL, POC
15	63.4	(Z)-lycopene isomer 2[b]	442/468/496	537	MS^2 [537]: 481 (42), 467 (35), 455 (100), 427 (61), 413 (88), 399 (24), 387 (42)	red
16	67.9	(Z)-lycopene isomer 3[b]	442/468/496	537	MS^2 [537]: 481 (5), 467 (42), 455 (86), 427 (22), 413 (4), 399 (86), 387 (11)	red
17	68.8	(Z)-lycopene isomer 4[b]	440/468/496	537	MS^2 [537]: 481 (11), 467 (32), 455 (79), 427 (48), 413 (30), 399 (42), 387 (38)	red
18	74.1	(Z)-lycopene isomer 5[b]	446/472/502	537	MS^2 [537]: 481 (49), 467 (20), 455 (100), 427 (24), 413 (60), 399 (21), 387 (19)	red
19	74.5	(*all-E*)-lycopene	446/472/502	537	MS^2 [537]: 481 (41), 467 (16), 455 (100), 427 (32), 413 (85), 399 (30), 387 (15)	red
					MS^3 [537→455]: 413 (10), 388 (41)	
20	75.0	(Z)-lycopene isomer 6[b]	446/472/502	537	MS^2 [537]: 481 (17), 467 (32), 455 (100), 427 (23), 413 (61), 399 (40), 387 (17)	red

[a]MS^n peak intensity was taken from data of the red-fleshed cv. 'Industrial 10P'; for compounds **2**, **3**, **5**, and **14**, data of the yellow-fleshed cv. 'Silvestre' were used. [b]Tentatively identified. Abbreviations: ; [c]SIL, Silvestre; MHR, MHR 21-4-6; POC, Pococí.

Table 4. Contents of Major Carotenoids from Yellow and Red Papaya Genotypes

		carotenoid content in various papaya genotypes in μg/100 g of FW[d]						
no.	identity[a]	Pococí (hybrid)	Criolla (line)	Sunset (line)	Industrial 10P (hybrid)	Industrial 10G (hybrid)	Silvestre (line)	MHR 21-4-6 (line)
7	β-cryptoxanthin	233 ± 15 C	191 ± 75 C	160 ± 21 C	246 ± 67 C	296 ± 52 C	393 ± 31 B	494 ± 51 A
9	β-carotene	514 ± 114 A	200 ± 57 B	283 ± 77 B	534 ± 138 A	554 ± 114 A	270 ± 79 B	508 ± 80 A
10	β-cryptoxanthin caprate	329 ± 63 B	116 ± 33 C	364 ± 69 AB	269 ± 39 B	272 ± 49 B	229 ± 83 B	540 ± 109 A
11	β-cryptoxanthin laurate	899 ± 188 BC	243 ± 64 D	1080 ± 184 AB	870 ± 116 BC	839 ± 148 BC	747 ± 132 C	1218 ± 226 A
12	β-cryptoxanthin myristate	218 ± 55 BC	77 ± 21 D	203 ± 27 BC	178 ± 25 C	197 ± 35 BC	271 ± 35 A	242 ± 22 AB
19	(*all-E*)-lycopene	3264 ± 362 A	1981 ± 325 B	1861 ± 321 B	3861 ± 691 A	3858 ± 614 A	12 ± 5 C	9 ± 5 C
	total (Z)-lycopenes	436 ± 82 A	194 ± 41 B	453 ± 97 A	412 ± 23 A	448 ± 83 A	nd	nd
	total lycopene	3700 ± 384 A	2175 ± 322 B	2314 ± 325 B	4273 ± 697 A	4307 ± 682 A	12 ± 5 C	9 ± 5 C
	unidentified carotenoids[b]	662 ± 130 C	328 ± 100 D	447 ± 158 CD	1206 ± 234 AB	972 ± 247 B	1159 ± 265 AB	1354 ± 136 A
	total carotenoids[c]	6496 ± 753 A	3309 ± 331 C	4852 ± 777 B	7690 ± 1199 A	7436 ± 1240 A	3079 ± 438 C	4581 ± 448 B
	retinol equivalents	197 ± 34 B	77 ± 14 C	165 ± 31 B	193 ± 38 B	200 ± 34 B	157 ± 22 B	253 ± 32 A

[a]See Table 3 for identification parameters. [b]Quantified by β-cryptoxanthin calibration. [c]Including traces and unknown pigments after summarized quantification by β-cryptoxanthin calibration. [d]nd, not detected. Different letters indicate significant difference of means.

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regular intake of food containing lycopene and vitamin A precursor carotenoids was shown to be inversely related to the incidence of several cancer types such as prostate and breast cancer.[8,9,37,38] The red-fleshed papaya genotypes investigated in the present study contained lycopene in the range of 2.2 (cv. 'Criolla') to 4.3 mg/100 g (cv. 'Industrial 10G') of FW (Table 4). According to the classification of Britton and Khachik,[39] all red-fleshed papaya genotypes of our study represent "very good" nutritional sources of lycopene (>2 mg/100 g of FW). In several previous studies on lycopene in papaya, levels of 1.9–4.3 mg per 100 g of FW[10,40,41] were reported. For comparison, in commercial tomato varieties lycopene levels ranged between 1.9 and 6.5 mg/100 g of FW.[42]

Besides their important antioxidant properties, various carotenoids such as β-carotene and β-cryptoxanthin contribute to a further health-promoting characteristic of papaya fruit through their vitamin A potential. β-Carotene contents ranged from 200 to 554 μg/100 g of FW (Table 4). Among free and esterified β-cryptoxanthins, its laurate ester occurred in highest amounts, reaching 1218 μg/100 g of FW (cv. 'MHR 21-4-6'). Also finding highest levels of the β-cryptoxanthin laurate ester in papaya (892 μg/100 g of FW), Breithaupt et al.[43] screened various fruits and vegetables for several β-cryptoxanthin esters previously.

As a result of variable β-carotene and β-cryptoxanthin contents (cf. Table 4), retinol equivalents spanned from 77 μg (cv. 'Criolla') to 253 μg/100 g of FW (cv. 'MHR 21-4-6'). Because FAO/WHO[44] suggested 500 and 600 μg RE/day as daily recommended safe intakes for women and men (19–65 years), respectively, the consumption of ca. 200–240 g of the yellow-fleshed papaya genotype 'MHR 21-4-6' would suffice to meet these requirements. However, this calculation does not consider variable bioavailability of the corresponding pro-vitamin A carotenoids. For instance, parsley and spinach were reported to have approximately 8–17 times higher β-carotene levels than mango and papaya, respectively. Nevertheless, after β-carotene from mango and papaya had been supplied to rats, a more efficient vitamin A bioconversion and deposition in the liver was observed when compared to an equal dose of β-carotene provided by spinach and parsley.[4] In addition, Schweiggert et al.[5] hypothesized a different bioavailability of lycopene and pro-vitamin A carotenoids from tomato and carrots when compared to papaya (cv. 'Pococí'), due to dissimilarities of the carotenoid deposition in their chromoplasts. Thus, nutritional evaluation simply based on carotenoid levels of a food is questionable, and many further factors, for example, the use of dietary fat for meal preparation and differing food matrices, will strongly influence the bioavailability of pro-vitamin A carotenoids.[45]

Notably, the genotype 'Pococí' along with the other F1 hybrids 'Industrial 10G' and 'Industrial 10P' stood out by their high contents of lycopene and pro-vitamin A carotenoids. Their (*all-E*)-lycopene contents ranged from 3.7 to 4.3 mg/100 g of FW, whereas the lines 'Criolla' and 'Sunset' contained this bioactive compound to a lesser extent (2.2–2.3 mg/100 g of FW). By analogy, more than 500 μg of β-carotene per 100 g of FW was found in the above-mentioned F1 hybrids and <300 μg/100 g of FW was detected in the studied inbred lines (cf. Table 4). Although our study did not aim at investigating heterosis and, moreover, environmental factors might play a certain role, a pronounced genetic effect on the expression of nutritionally relevant carotenoids in papaya was visible and would merit deeper investigation. A heterotic effect on carotenoid accumulation was already shown for, for example, African marigold (*Tagetes erecta* L.) and tomato (*Lycopersicon esculentum* Mill.).[46,47] Exploiting possible hybrid vigor on carotenoid contents in papaya may be utilized for breeding of new varieties with significantly enhanced nutritional properties, for example, increased lycopene and pro-vitamin A levels.

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