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Changes in Flavonoids and Nonphenolic Pigments during On-Tree Maturation and Postharvest Pericarp Browning of Litchi (*Litchi chinensis* Sonn.) As Shown by HPLC-MS^n

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Supporting Information

ABSTRACT: Polyphenols, chlorophylls, and carotenoids were characterized by HPLC-DAD-MS^n in the pericarp of unripe to overripe 'Hong Huey' and 'Chacapat' litchi fruit at harvest and during subsequent storage (5 °C, 90% RH, 21 days). (−)-Epicatechin and A-type procyanidins always predominated quantitatively. Besides these *ortho*-diphenolic compounds, minor novel litchi flavonoids included monohydroxylated structures. Chlorophyll degradation by 73−92% and 7−38-fold anthocyanin accumulation affected pericarp color throughout the last 15−20 days of on-tree maturation. Postharvest, anthocyanins and (−)-epicatechin largely degraded within the first 3 days, accompanied by severe pericarp browning. Without packaging of the fruit, desiccation initially accelerated polyphenol oxidase-induced oxidation of (−)-epicatechin, but then hindered its further progress. Constant levels of the monohydroxylated (epi)afzelechin indicated no involvement of peroxidase. Acting as antioxidants, anthocyanins retarded (−)-epicatechin degradation. Hence, pinkish-red fruit with a molar ratio of cyanidin 3-*O*-rutinoside to (−)-epicatechin of >3:100 retained flavonoids best. However, brown polymers masked remaining red pigments.

KEYWORDS: anthocyanin, chlorophyll, enzymatic browning, (−)-epicatechin, fresh produce, HPLC-MS/MS, *Litchi chinensis* Sonn., proanthocyanidin

INTRODUCTION

Pericarp color is a key attribute in harvest maturity specification of litchi fruit.[1] At the same time, it is crucial for the buying incentive of the fresh fruit. At the end of on-tree maturation, pinkish-red anthocyanins[2,3] have accumulated in the vacuoles of the outer mesocarp, whereas green chlorophylls have degraded.[4] Usually, the attractive pericarp color of this fruit disappears within 3−5 days of postharvest cold storage.[1] Litchi polyphenol oxidase (PPO) can rapidly oxidize the (−)-epicatechin that abounds in the pericarp,[5−7] but neither litchi pericarp PPO nor peroxidase (POD) can directly oxidize anthocyanins.[8,9] Loss of red pericarp pigments was hence attributed to pH-induced decolorization,[10] degradation by reactive oxygen species,[11] hydrolysis by an anthocyanase,[12] or coupled oxidation in the presence of polyphenols and PPO or POD.[6,8,9,13]

Export of litchi fruit requires shelf-life extension, particularly conservation of the attractive appearance.[1] Sulfur dioxide fumigation is a common practice, but increasing legal and consumer restrictions demand alternative methods for color preservation. Development of suitable postharvest processes requires detailed knowledge of the maturity-dependent pericarp pigment composition and resulting pericarp browning products in vivo. However, analyses have mainly been focused on individual compounds such as anthocyanins[3,14] or (−)-epicatechin,[15,16] either throughout on-tree maturation[3,15] or during storage.[6,14,16] Detailed characterization of pericarp pigments by high-performance liquid chromatography (HPLC) and mass spectrometry (MS) was largely performed irrespective of maturity, harvest time, and storage.[5,17,18] Evidence of pericarp discoloration mechanisms shown in vitro[8,9,11−13] has hardly been provided in vivo.[6,10]

The impact of harvest maturity on the eating quality and shelf life of litchi fruit in terms of fruit size, color, and aril properties, as well as pericarp moisture and activities of browning enzymes (PPO, POD), was previously described for two cultivars, differing in pericarp morphology.[1] The present follow-up study aimed at exploring their rapid pericarp browning during cold storage with respect to changes in pigments and potential browning substrates. Therefore, polyphenols and nonphenolic pigments were to be characterized by HPLC-DAD-MS^n. Their contents in the pericarp were to be quantified at harvest after different maturation periods on the tree and throughout subsequent cold storage of the fruit. Proposed mechanisms of pericarp browning were finally to be scrutinized by linking pericarp composition at harvest to postharvest pigment degradation.

MATERIALS AND METHODS

Plant Material. Litchi (*Litchi chinensis* Sonn.) fruit ('Hong Huey' and 'Chacapat') was harvested every fifth day during the main harvest season of 2007 in Mae Sa Mai, northern Thailand. Picking, precooling, transportation, debranching, and sorting of the fruit lots of five ('Hong

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Huey', H1−H5) and four ('Chacapat', C1−C4) harvest dates into four maturity categories [green (G), breaker (B), pink (P), and red (R)] were detailed previously.[1] The sorted lots were stored for 21 days in open mesh baskets in a CAT 610/620 climate simulator (Contherm Scientific, Lower Hutt, New Zealand) at 5 °C and 90% relative humidity (RH). After 0, 3, 8, 13, and 21 days of storage, 20−25 fruits were immersed into liquid nitrogen. The shock-frozen pericarp was removed from the deep-frozen aril without contamination by juice. The isolated pericarp was stored at −80 °C until lyophilization and sent deep-frozen (−40 °C) and vacuum-packed to Hohenheim University by airfreight. There, it was kept at −80 °C until fine-grinding in a water-cooled mill type A10 (IKA, Staufen, Germany) just before pigment and polyphenol extraction. CIELab color measurements and analyses of moisture and activities of PPO and POD have already been described in detail for the pericarp of the same fruit samples by Reichel et al.[1]

Sample Preparation. The pulverized pericarp (2.5 g) was extracted with methanol (40 mL) for 30 min under continuous stirring to dissolve low-molecular-weight phenolics, carotenoids, and chlorophylls. After centrifugation at 4000 rpm (3309*g*) for 10 min in a Varifuge 3.0 (Heraeus, Osterode, Germany), the pellet was suspended in methanol (20 mL) before further centrifugation (10 min, 4000 rpm). The solid residue was extracted with 40 mL of acetone/water (7:3, v/v) for 60 min, centrifuged (10 min, 4000 rpm), and re-extracted for 30 min under the same conditions to obtain anthocyanins and proanthocyanidins. The suspension was filtered through a Buchner funnel with filter paper no. 4 (Whatman, Maidstone, U.K.), followed by washing of the residue with acetone/water (∼20 mL). All extractions were carried out under nitrogen atmosphere and acidic conditions to prevent oxidation of phenolic compounds. For this reason, 0.01 and 0.1% (v/v) concentrations of trifluoroacetic acid were added to methanol and aqueous acetone, respectively, and subsequently removed during evaporation.

The methanolic supernatants of the first two centrifugation steps were combined in an amber glass separation funnel with purified water (10 mL) and extracted three times, using 1 × 40 mL and 2 × 20 mL of petroleum ether (boiling range of 40−60 °C). The pooled organic phases were dried with sodium sulfate (2 g) and evaporated in vacuo at ambient temperature without exposure to light. The dried residue was dissolved in 2-propanol (5 mL), membrane-filtered (0.45 μm), and transferred to an amber glass vial for HPLC analysis of carotenoids and chlorophylls. The aqueous phase was combined with supernatant and filtrate from the extractions with aqueous acetone and evaporated to dryness in vacuo at ambient temperature. The residue was dissolved in deionized water (20 mL) containing 0.01% (v/v) HCl. An aliquot was membrane-filtered (0.45 μm) for direct analysis of flavonols, proanthocyanidins, and anthocyanins by reversed-phase HPLC. All samples were analyzed in duplicate. All solvents and reagents were of analytical or gradient grade and obtained from VWR (Darmstadt, Germany). Deionized water was used throughout.

HPLC Analyses. A series 1100 HPLC system (Hewlett-Packard, Waldbronn, Germany), as described by Schütz et al.,[19] was used for polyphenol analyses. The signals of the diode array detector G1315A (acquisition range of 200−600 nm) were processed with ChemStation LC 3D software (Agilent Technologies, Waldbronn, Germany). Separation of the analytes was achieved at 25 °C and a flow rate of 0.4 mL/min on a 150 mm ×3.0 mm i.d., 4 μm, Synergi 4u Hydro-RP 80A column, with a 4 mm × 2.0 mm i.d. SecurityGuard C18 Aq guard column (Phenomenex, Torrance, CA). Two different gradient methods were adapted for the separation of proanthocyanidins and flavonols (method 1) and anthocyanins (method 2). In method 1, the mobile phase consisted of 2% (v/v) acetic acid in water (eluent A_1) and acetonitrile/0.5% acetic acid in water (50:50, v/v, eluent B_1). The gradient of method 1 was from 10 to 17% B_1 (5 min), from 17 to 48% B_1 (45 min), from 48 to 100% B_1 (10 min), 100% B_1 isocratic (2 min), and from 100 to 10% B_1 (3 min), with the total run time being 70 min. Proanthocyanidins and flavonols were monitored at 280 and 370 nm, respectively. Gradient elution in method 2, based on water/formic acid/acetonitrile (87:10:3, v/v/v, eluent A_2) and water/formic acid/acetonitrile (40:10:50, v/v/v, eluent B_2), was from 10 to 25% B_2 (10 min), from 25 to 31% B_2 (5 min), from 31 to 40% B_2 (5 min), from 40 to 50% B_2 (10 min), from 50 to 100% B_2 (10 min), and from 100 to 0% B_2 (5 min) within a total run time of 50 min. Anthocyanins were monitored at 520 nm. The injection volume was 4 μL for both methods.

HPLC analyses of chlorophylls and carotenoids were performed on the Waters 2690 separation module (Waters, Milford, MA) described by Kurz et al.[20] A 150 mm ×3.0 mm i.d., 3 μm, C30 reversed-phase column (YMC, Wilmington, MA) was used at 25 °C. Two eluents based on methanol/methyl *tert*-butyl ether/water (81:15:4, v/v/v, eluent A_3; 4:92:4, v/v/v, eluent B_3) were used for a gradient from 5 to 20% B_3 (10 min), from 20 to 40% B_3 (5 min), from 40 to 55% B_3 (7 min), from 55 to 100% B_3 (0.5 min), 100% B_3 isocratic (2.5 min), and from 100 to 5% B_3 (2 min). The total run time was 30 min at a flow rate of 0.42 mL/min. The injection volume was 10 μL. Carotenoids as well as chlorophyll *b* and its derivatives were detected at 450 nm and chlorophyll *a* and derivatives at 660 nm.

Quantitation was based on 7−10 point calibration curves of respective standard substances. Procyanidin A2, cyanidin 3-*O*-rutinoside, and cyanidin 3-*O*-glucoside were purchased from Extrasynthèse (Lyon, France) and (−)-epicatechin, all-*trans*-β-carotene, and chlorophylls *a* and *b* from Sigma-Aldrich (St. Louis, MO). Quercetin 3-*O*-rutinoside was from Carl Roth (Karlsruhe, Germany). When reference substances were unavailable, calibration curves of structurally related compounds were used with a molar mass correction factor. All anthocyanins, except cyanidin 3-*O*-glucoside (0.05−500 mg/L), were calibrated as cyanidin 3-*O*-rutinoside (0.05−500 mg/L), flavonols as quercetin 3-*O*-rutinoside (0.1−200 mg/L), monoflavanols as (−)-epicatechin (10−4000 mg/L), oligoflavanols as procyanidin A2 (0.1−1000 mg/L), and carotenes as all-*trans*-β-carotene (0.1−28 mg/L). Chlorophyll *a* and *b* derivatives were quantitated without molar mass correction as chlorophyll *a* (0.05−30 mg/L) and *b* (0.04−20 mg/L), respectively.

HPLC-MS Analyses. For HPLC-MS analyses, the series 1100 HPLC system was coupled online to an Esquire 3000+ ion trap mass spectrometer (Bruker, Bremen, Germany), which was fitted with an electrospray ionization (ESI) source for polyphenol analyses or with an atmospheric pressure chemical ionization (APcI) source for chlorophyll and carotenoid analyses. Identical columns, eluents, flow rates, and gradient programs were used as described above for the respective HPLC analyses. Esquire Control software was applied for data acquisition and processing. Negative ion mass spectra of the eluates were recorded in the mass-to-charge (m/z) range of 50−2400 for proanthocyanidins and flavonols, whereas positive polarity was chosen for anthocyanins (m/z range of 50−2400) as well as chlorophylls and carotenoids (m/z range of 50−1000). Scan speed was 13000 (m/z)/s throughout. In the ESI method, nitrogen served as the drying gas at a flow rate of 9 L/min and as nebulizing gas at a pressure of 310.3 kPa. The nebulizer temperature was set to 365 °C. Helium was used as collision gas for collision-induced dissociation (CID) at a pressure of 10^{-3} Pa. When using the APcI source, parameters were set as described by Kurz et al.[20]

Statistical Analysis. Statistical analyses of the data were computed using SAS 9.1 software (SAS Institute, Cary, NC). Significant effects and changes ($P \leq 0.05$) were determined on the basis of Tukey's multiple-comparison test.

RESULTS AND DISCUSSION

Characteristic Compounds of Litchi Pericarp As Identified by HPLC-MS^n. Three classes of phenolic compounds were found in the pericarp of the litchi cultivars 'Hong Huey' and 'Chacapat',

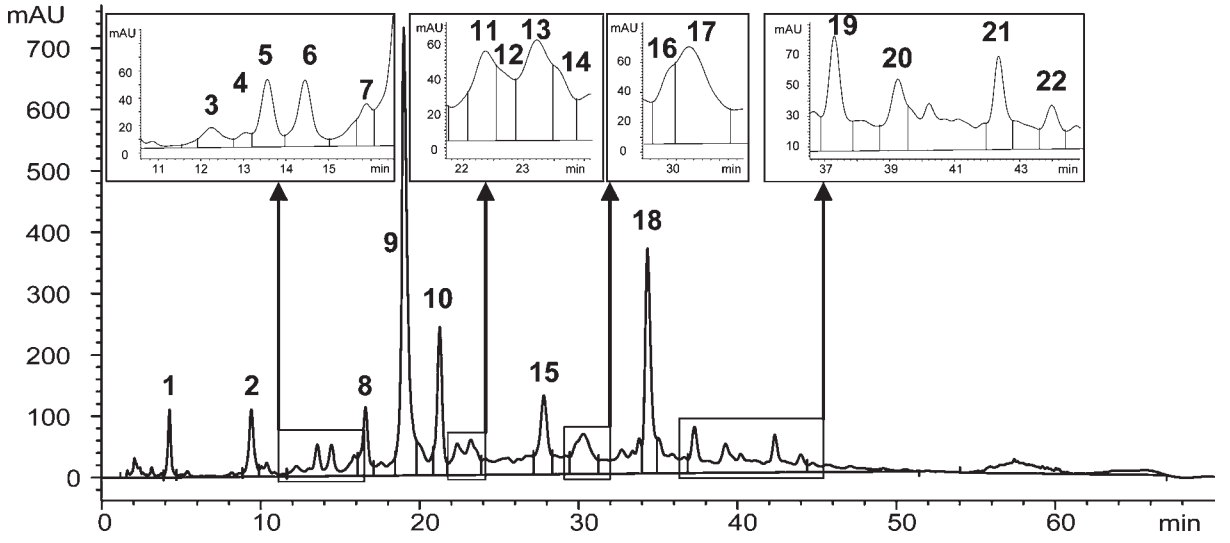

Figure 1. Reversed-phase HPLC separation of proanthocyanidins (280 nm) extracted from pericarp of freshly harvested pink 'Chacapat' litchi fruit. For peak assignment, cf. Table 1.

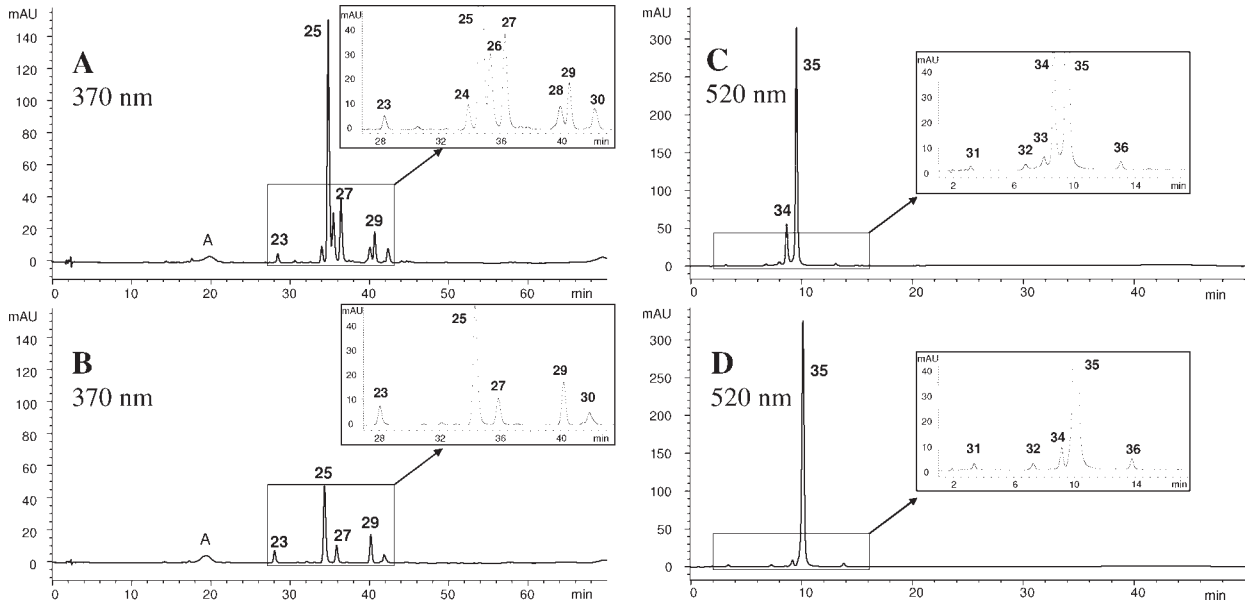

Figure 2. Reversed-phase HPLC separation of (A, B) flavonols (370 nm) and (C, D) anthocyanins (520 nm) extracted from pericarp of freshly harvested pink 'Hong Huey' (A, C) and 'Chacapat' (B, D) litchi fruit. For peak assignment, cf. Tables 1 and 2. A, anthocyanins.

which widely differ in outer peel characteristics:[1] (1) colorless flavan-3-ols and proanthocyanidins (Figure 1) comprising the precursors of polymeric brown pigments, (2) yellowish flavonols (Figure 2A,B), and (3) anthocyanins (Figure 2C,D), which constitute the attractive red pericarp color of litchi. In conformity with Sarni-Manchado et al.,[5] no further polyphenol classes were identified in litchi pericarp. Flavan-3-ols were by far prevailing. Thus, characterization of the phenolic extract by HPLC-DAD-MS^n without previous fractionation was facilitated. However, nonphenolic pigments including chlorophylls and carotenoids had to be removed from the crude extract by solvent extraction to avoid interferences. This allowed ancillary analyses of the green and yellowish pigments, which are crucial for the appearance of green-red-shelled fruit harvested early.

Structure of Flavan-3-ols and Proanthocyanidins in Litchi Pericarp. Reversed-phase HPLC allowed satisfactory separation of 22 monomeric to pentameric flavanols (**1**−**22** in Figure 1). Their mass spectral data are compiled in the Supporting Information (Table S1). Condensed tannins with degrees of polymerization of >5 were not detected, probably due to insufficient solubility. Coupled with multistage electrospray ionization mass spectrometry (ESI-MS^n), this method was favored over quantitation after chemical degradation or direct flow injection ESI-MS, because rapid qualitative and quantitative analysis of small samples was feasible without tedious purification or derivatization.

At harvest, (−)-epicatechin, **9** (Figure 3), is the major phenolic compound in litchi pericarp followed by procyanidin A2, **18** (Figure 3).[5,6] Both were unambiguously identified by comparison with reference substances and ESI-MS analyses (**9** and **18** in Figure 1). As **4** (Figure 1) involved a molecular ion $[M - H]^-$ at m/z 289 similar to that of (−)-epicatechin

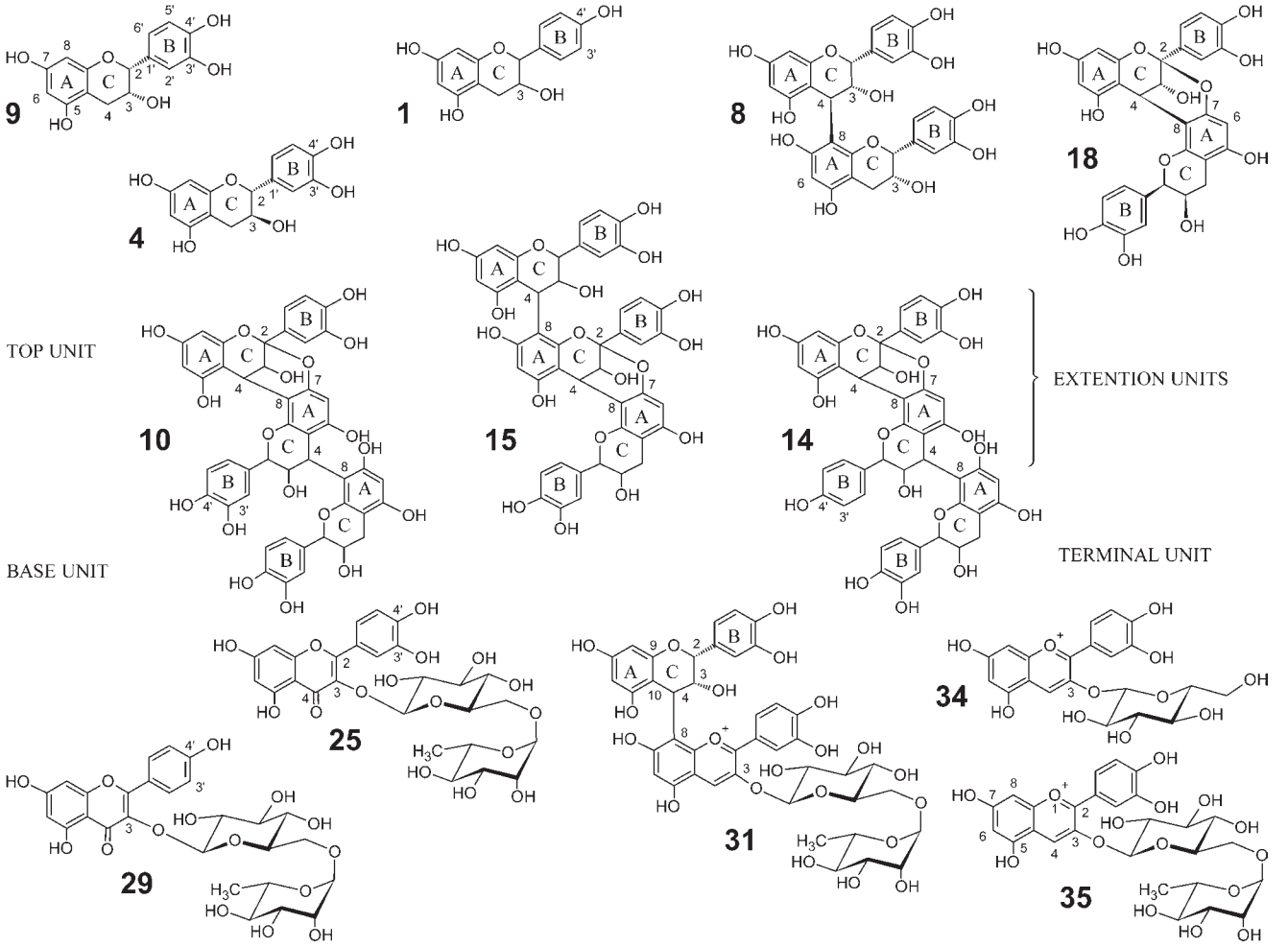

Figure 3. Chemical structures of characteristic flavonoids (cf. Figures 1 and 2 and Tables 1 and 2) in litchi pericarp: **9**, (−)-epicatechin (M = 290 g/mol); **4**, (+)-catechin (M = 290 g/mol); **1**, (epi)afzelechin (M = 274 g/mol); **8**, procyanidin B2 (M = 578 g/mol); **18**, procyanidin A2 (M = 576 g/mol); **10**, (epi)catechin-A-(epi)catechin-B-(epi)catechin (M = 864 g/mol); **15**, (epi)catechin-B-(epi)catechin-A-(epi)catechin (M = 864 g/mol); **14**, (epi)catechin-A-(epi)afzelechin-B-(epi)catechin (M = 848 g/mol); **25**, quercetin 3-*O*-rutinoside (M = 610 g/mol); **29**, kaempferol 3-*O*-rutinoside (M = 594 g/mol); **31**, (epi)catechin-cyanidin 3-*O*-rutinoside (M = 884 g/mol); **34**, cyanidin 3-*O*-glucoside (M = 449 g/mol); **35**, cyanidin 3-*O*-rutinoside (M = 595 g/mol).

(2*R*,3*R*), but eluted earlier, it was assigned to the (2*R*,3*S*)-epimer (+)-catechin, **4** (Figure 3). For all other flavanols, the bracketed prefix (epi) was used, because MS does not differentiate chirality at C3 (e.g., **9** and **4** in Figure 3). To our knowledge, (epi)afzelechin, **1** (Figure 3), the monohydroxylated flavan-3-ol monomer with $[M - H]^-$ at m/z 273, was detected in litchi pericarp for the first time (**1** in Figure 1). In contrast, the presence of (epi)gallocatechin in litchi pericarp[21] was not substantiated, because the extracted ion chromatogram of m/z 305 (trihydroxylated flavan-3-ol) showed no distinct peak.

Ions obtained in the MS^1 experiment (ESI-(−)-MS^1) indicated the number (n) and type of subunits and linkages in the oligomers. Di- and trimeric procyanidins showed typical single-charged molecular ions $[M - H]^-$ at m/z $[(290 + (n - 1) \times 288) - 1]$, that is, m/z 577 and 865, respectively. All tetra- and pentamers exhibited double-charged base peaks $[M - 2H]^{2-}$, which were distinguished from the former, because their ion series displayed differences of 0.5 amu (m/z 1/2) instead of 1 amu in series of single-charged ions due to the natural ^{13}C isotopes included in each oligomer. Besides A-type linkages comprising an additional C2−*O*−C7 ether bond (e.g., **18** in Figure 3), subunits were linked through B-type single bonds at C4−C8 (e.g., **8** in Figure 3) rather than C4−C6.[22] A-type procyanidins yielded characteristic ions $[M - H]^-$ with a mass being lower by 2 amu per double linkage than that of ions from solely B-type-linked flavanols. Aside from procyanidin A2 (**18** in Figure 1), another A-type-linked (**21**) and a B-type-linked procyanidin dimer (**8**), presumably procyanidin B2, **8** (Figure 3),[6] were found. Among the 15 tri- to pentameric procyanidins detected, 9 possessed one and 4 even two A-type linkages, confirming the reported conclusion from direct flow injection ESI-MS^1 that A-type procyanidins are predominant in litchi pericarp.[17] Even a heterogeneous proanthocyanidin trimer (**14** in Figure 1) composed of one (epi)afzelechin and two (epi)catechin subunits, **14** (Figure 3), was linked by a double and a single linkage ($[M - H]^-$ at m/z 847 = $[(274 + 2 \times 288) - 2 - 1]$). Such compounds are known from cinnamon,[22] but have not been reported for litchi pericarp so far.

The monomer sequence in all oligomers was assessed on the basis of characteristic product ions, which appeared in the (−)-MS^2 and (−)-MS^3 experiments (cf. Supporting Information) due to quinone methide (QM) cleavage, retro-Diels−Alder

reaction (RDA), and heterocyclic ring fission (HRF), respectively. Figure 4 exemplarily illustrates the fragmentation pattern of a procyanidin tetramer comprising one A-type linkage (**2** in Figure 1). B-type interflavan bonds were cleaved under release of the remaining base as flavanol ion (**I a**, m/z 863 in Figure 4) by QM formation (**I**) through ring A of the upper C4-linked subunit (**I b**, m/z 287; **I c**, m/z 573). Cleavage of A-type linkages involved double QM formation of the upper subunit through ring B to open the ether bond and through ring A as described above (**I d**, m/z 285).[23] A loss of 152 amu was characteristic of the elimination of ring B by RDA fission (**II**) of an (epi)catechin subunit (**II a**, m/z 711 in Figure 4), whereas RDA of (epi)afzelechin resulted in a loss of 136 amu ($[M - H - 136]^-$ at m/z 711 for compound **14**).[22] After RDA fission, the ions lost a water molecule that was most likely based on the free hydroxyl at C3[22] (**II b**, m/z 693). Likewise, the product ion of m/z 490 resulted from RDA (**II c**). RDA preferably occurred at the top unit (**II c**), unless it was inhibited by an A-type linkage (**II a**).[22] HRF also cleaved ring C, but eliminated ring A (126 amu) of a flavan-3-ol subunit (**III** in Figure 4), unlike RDA. When HRF took place in an A-type-linked extension unit, it was not only the ion of the base unit (**III a**, m/z 451) that was produced, but also the conjugate ion of the upper unit (**III b**, m/z 411).[22] The positions of the double bonds between subunits resulted from asymmetric cleavages by HRF and QM formation. Oligomers with the same linkage pattern (dimers **18** and **21**; trimers **15** and **19** as well as **7** and **11**; tetramers **17** and **22** as well as **2** and **13**; and pentamers **5** and **3** in Tables 1 and 2) may differ in conformation of epimers or in comprising C4−C6 instead of C4−C8 bonds. Consistent with Liu et al.,[6] the main trimer in litchi pericarp (**10** in Figure 1) was epicatechin-(4β-8,2β-O)-epicatechin-(4β-8)-epicatechin, **10** (Figure 3). A-type linkages were ubiquitous in litchi proanthocyanidins. They often linked the terminal unit (e.g., **15** in Figure 3), but also occurred among extension units (e.g., **10** in Figure 3; Figure 4). The pericarp flavanol patterns of both Thai litchi cultivars were similar, but notably differed from those deduced from HPLC-DAD analyses of Chinese 'Huaizhi' and 'Baila' pericarp, which included epicatechin gallate and solely B-type-linked procyanidins.[7,21] Gallocatechin, epicatechin gallate, and procyanidin B2, besides (−)-epicatechin, were likewise detected in litchi seeds.[24] As shown by ESI-MS and NMR spectroscopy, the dimers and trimers isolated from 'Heiye' litchi seeds though comprised A-type-linked (−)-epicatechin and epiafzelechin,[25] consistent with the pericarp proanthocyanidins described above.

Cultivar-Specific Flavonol Pattern of Litchi Pericarp. Major differences between the cultivars studied became evident in the flavonol pattern (Figure 2A,B). The Supporting Information (Table S2) lists the retention times, absorbance maxima, and mass spectral data for all flavonols **23**−**30**. Generally, the extracts of 'Hong Huey' pericarp showed a more complex pattern and larger peaks at 370 nm (Figure 2A) than those of 'Chacapat' peels. Reliable reports on flavonols of litchi pericarp have largely been limited to 'Kwai Mi' fruit.[5] In accordance, the maximum peak at 370 nm of the extracts of both Thai cultivars (**25** in Figure 2A,B) resulted from quercetin 3-O-rutinoside, **25** (Figure 3), as concluded from the same retention time as well as UV−vis and mass spectra being similar to those of the reference substance. Further comparison of the chromatograms with those published for 'Kwai Mi'[5] and the characteristic ions in the (−)-MS and (−)-MS^2 experiment gave evidence of compound **27** being quercetin 3-O-glucoside. However, the linkage position and the exact structure of the sugar moiety could not be assigned solely by mass spectrometry. In the 'Hong Huey' pericarp, another quercetin hexoside, which displayed a fragmentation pattern similar to that of compound **27**, and another quercetin disaccharide with a fragmentation pattern similar to that of quercetin 3-O-rutinoside were identified (**24** and **26** in Figure 2A). Fragments $[M - 146]^-$ at m/z 609 and $[M - 146 - 308]^-$ at m/z 301 of compound **23** indicated a quercetin with two sugar moieties, supposedly rutinoside and rhamnoside (**23** in Figure 2A,B). Substantiating the tentative findings of Sarni-Manchado et al.[5] and former studies based on thin layer chromatography,[26] the minor compounds eluting later than quercetins (**28**−**30** in Figure 2A,B) were assigned to kaempferol derivatives (MS^2, m/z 285). Mass analyses indicated two kaempferol hexosides, **28** and **30**, and one desoxy hexosylhexoside, **29**. Considering the characteristic glycosylation pattern of litchi polyphenols and the general occurrence of glycosides in fruit,[5] the two late-eluting flavonols that were present in both cultivars were tentatively identified as kaempferol 3-O-rutinoside, **29** (Figure 3), and -glucoside, **30**, respectively.

Cultivar-Specific Anthocyanin Pattern of Litchi Pericarp. HPLC analysis of the phenolic extract of 'Hong Huey' pericarp at 520 nm led to six clearly separated peaks (Figure 2C). Five of them were also detected in 'Chacapat' pericarp (Figure 2D). The Supporting Information (Table S3) itemizes the retention times, absorbance maxima, and ESI-(+)-MS^n data for all anthocyanins **31**−**36**. The major red pigments of both cultivars (**34** and **35** in Figure 2C,D) were identified as cyanidin 3-O-rutinoside, **35** (Figure 3), and cyanidin 3-O-glucoside, **34** (Figure 3), on the basis of the comparison of retention times and UV−vis absorbance spectra with those of reference substances. Peak assignment was confirmed by ESI-(+)-MS^n. These anthocyanins had already been detected in 'Brewster',[2,3] 'Kwai Mi',[5] and 'Huaizhi'[18] litchi fruit. By contrast, malvidin 3-acetylglucoside, which had been considered characteristic of unripe 'Brewster' fruit,[2,3] was not detectable in either green or in red fruit of the Thai litchi cultivars explored. Among four other pigments with visible-light absorbance maxima around 520 nm, two possessed typical anthocyanin fragmentation patterns (**33** and **36** in Figure 2C,D). Compound **33**, found only in 'Hong Huey' pericarp (Figure 2C), was tentatively identified as a cyanidin glycoside because of the characteristic aglycone fragment at m/z 287 in the MS^2 experiment. Further fragments $[M - 146]^+$ at m/z 595 and $[M - 308]^+$ at m/z 433 indicated the presence of two sugar moieties, supposedly rutinoside and rhamnoside, as found for quercetin, **23**. Collision-induced dissociation of the molecular ion of compound **36** produced a main fragment at m/z 301 and a further ion with 15 amu less at m/z 286 in the MS^2 and MS^3 experiments, suggesting peonidin as the aglycone fragment. Because the MS^2 fragmentation pattern resembled that of cyanidin 3-O-rutinoside, **35**, in terms of characteristic sugar losses ($[M - 146]^+$, $[M - 308]^+$), compound **36** was tentatively identified as peonidin 3-O-rutinoside. Mass spectrometric analysis of the pigment eluting first (**31** in Figure 2C,D) revealed a base peak at m/z 884. The loss of a sugar moiety $[M - 308]^+$ and a flavanol unit $[M - 308 - 289]^+$ resulted in the product ion at m/z 287 in the MS^2 and MS^3 experiments. Fragment ions at m/z 423 and 329 arose from RDA fission and concurrent cleavage of the C2−C3 and C4−C10 bonds in the heterocyclic ring of a top (epi)catechin unit, respectively.[27] Thus, compound **31** (Figure 3) may be an adduct of the major litchi flavanol, (−)-epicatechin, linked through its C4 to the major

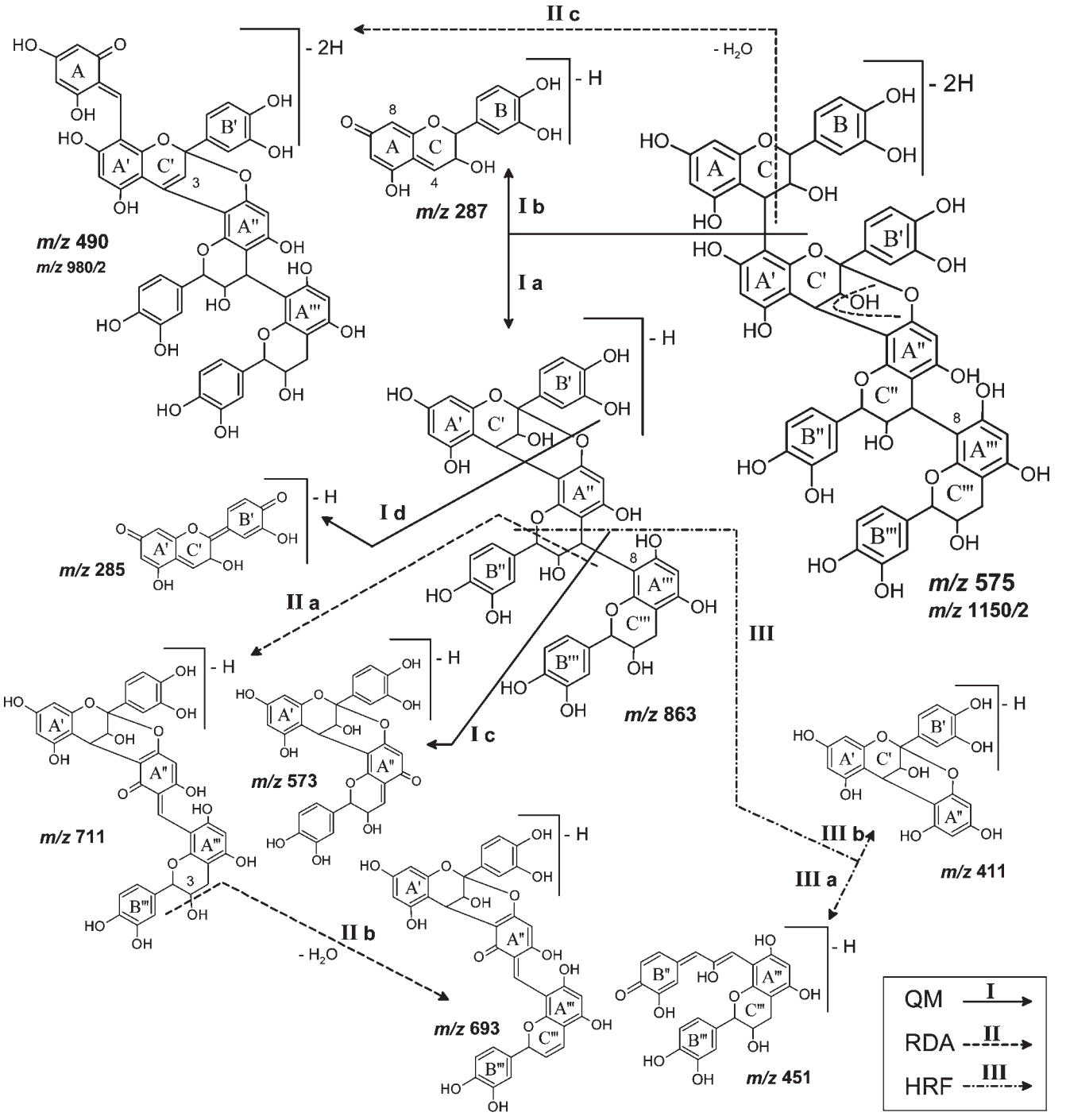

Figure 4. Fragmentation pathway proposed for a tetrameric procyanidin (cf. **2** in Tables 1 and 2) in litchi pericarp. Characteristic ions were derived from quinone methide cleavage (QM, **I**), retro-Diels−Alder reaction (RDA, **II**), and heterocyclic ring fission (HRF, **III**).

litchi anthocyanin, cyanidin 3-*O*-rutinoside. Similar tannin adducts have been described for malvidin and peonidin glycosides in red wine.[27]

Detection of Nonphenolic Pigments in Litchi Pericarp. Besides phenolic pigments, six chlorophyll derivatives and two carotenoids were found in unripe fruit of both litchi cultivars studied. Chlorophylls *a* and *b* (peaks 38 and 37 in Figure 5A,B), pheophytins *a*, *a*′, and *b* (peaks 42, 41, and 40), and a mono-*O*-allomer of pheophytin *a* (peak 39) were identified on the basis of their retention times, their UV−vis absorbance spectra, and the results of the APcI mass spectrometric analyses.[28,29] The latter mainly featured product ions resulting from the loss of the phytyl chain ($[M + H - 278]^+$) and additional loss of acetic acid ($[M + H - 278 - 60]^+$) or propanoic acid together with the β-keto ester group ($[M + H - 278 - 134]^+$).[30] Identification of chlorophylls *a* and *b* was assured by comparison with reference compounds. The main carotenoid peak (43 in Figure 5B) displayed the characteristic UV−vis absorbance spectrum of β-carotene[20] with maxima at 430, 452, and 478 nm and a molecular ion peak at *m*/*z* 537. As the standard substance eluted at the same retention time, it was assigned to all-*trans*-β-carotene. A smaller

Table 1. Maturity-Dependent Contents (mg/hg Dry Weight) of Polyphenols (1−36 in Figures 1 and 2A,C) in Pericarp of 'Hong Huey' Litchi Fruit

		'Hong Huey'[a]																	
		H1G		H1B		H2B		H2P		H3B		H3P		H4P		H4R		H5R	
total polyphenols		7502	d	8796	ab	8657	abc	8789	ab	9448	a	8978	a	7658	d	7748	cd	8014	bcd
flavanols		7243	c	8418	ab	8309	ab	8438	ab	9104	a	8560	a	7278	c	7302	c	7597	bc
monomers		1743	bc	2011	a	1878	abc	1761	bc	1960	ab	2019	a	1727	c	1826	abc	1773	bc
9	epicat	1571	c	1839	a	1700	abc	1621	bc	1810	ab	1870	a	1597	c	1714	abc	1630	bc
1	(epi)afz	157	ab	161	ab	168	a	130	cd	143	bc	142	bc	118	d	98	e	132	cd
4	cat	15	a	11	bc	10	c	10	c	7	d	7	d	12	b	14	a	11	bc
dimers		1262	ab	1339	a	1320	a	1251	ab	1391	a	1315	a	1134	b	1136	b	1273	ab
18	$[(epi)cat]_2$: A	1037	ab	1071	a	1045	ab	918	bcd	1062	a	1011	abc	889	cd	875	d	1006	abcd
8	$[(epi)cat]_2$: B	168	c	206	b	199	b	266	a	258	a	257	a	183	bc	202	b	196	b
21	$[(epi)cat]_2$: A	58	d	63	cd	75	a	67	bc	71	ab	46	e	62	cd	59	d	71	ab
trimers		2023	ab	2152	a	2095	a	2076	a	2110	a	2006	ab	1819	b	1818	b	1837	b
10	$[(epi)cat]_3$: A-B	869	a	868	a	820	ab	775	ab	795	ab	802	ab	738	b	733	b	722	b
15	$[(epi)cat]_3$: B-A	609	bc	585	cd	656	ab	696	a	701	a	659	ab	516	d	522	d	557	cd
19	$[(epi)cat]_3$: B-A	271	c	325	b	328	b	346	b	378	a	325	b	277	c	284	c	283	c
7	$[(epi)cat]_3$: B-B	105	cde	168	a	90	f	94	ef	97	def	93	f	116	bc	118	b	107	bcd
14	(epi)cat-(epi)afz-(epi)cat: A-B	122	a	106	b	98	bc	96	c	66	e	58	e	83	d	85	d	83	d
11	$[(epi)cat]_3$: B-B	48	f	101	ab	102	a	70	e	71	e	68	e	89	bc	76	de	86	cd
tetramers		1603	e	1923	cd	2005	c	2278	b	2599	a	2265	b	1615	e	1569	e	1699	de
17	$[(epi)cat]_4$: A-B-A	538	cd	552	cd	627	b	596	bc	764	a	638	b	493	de	456	e	482	de
2	$[(epi)cat]_4$: B-A-B	410	bc	469	ab	450	abc	507	a	469	ab	501	a	392	c	384	c	406	bc
13	$[(epi)cat]_4$: B-A-B	296	b	333	b	336	b	463	a	506	a	513	a	290	b	270	b	287	b
20	$[(epi)cat]_4$: A-A-B	173	d	248	c	245	c	351	b	442	a	335	b	148	d	166	d	218	c
12	$[(epi)cat]_4$: A-B-B	76	d	191	bc	238	a	225	a	239	a	175	c	184	bc	193	bc	209	ab
22	$[(epi)cat]_4$: A-B-A	109	cd	130	bc	109	cd	137	b	179	a	103	d	108	d	100	d	97	d
pentamers		611	c	993	ab	1011	ab	1072	a	1044	ab	955	b	982	ab	953	b	1015	ab
16	$[(epi)cat]_5$: B-A-B-A	234	c	352	a	343	ab	373	a	308	b	233	c	348	ab	309	b	356	a
6	$[(epi)cat]_5$: B-A-B-B	154	e	283	cd	301	bc	316	abc	347	a	330	ab	275	d	270	d	286	cd
5	$[(epi)cat]_5$: B-B-A-B	147	c	262	ab	241	ab	267	ab	272	ab	282	a	232	b	247	ab	249	ab
3	$[(epi)cat]_5$: B-B-A-B	76	d	96	c	127	a	116	ab	117	ab	110	b	126	a	127	a	124	ab
flavonols		230.7	c	281.2	ab	288.0	a	263.7	abc	275.3	ab	290.0	a	244.7	bc	234.1	c	231.9	c
25	qrc 3-*O*-rut	136.3	b	168.3	a	169.3	a	154.5	ab	158.2	ab	169.6	a	146.1	ab	137.0	b	136.5	b
27	qrc 3-*O*-glc	29.4	b	36.9	a	37.2	a	33.5	ab	38.3	a	36.9	a	30.2	b	29.3	b	27.8	b
26	qrc-hex	19.4	d	23.8	bc	25.8	abc	25.4	abc	27.8	ab	29.2	a	22.4	cd	23.4	cd	22.5	cd
29	kpf 3-*O*-rut	16.1	bcd	18.1	abc	18.8	a	18.4	a	18.4	ab	19.4	a	16.0	cd	15.1	d	14.6	d
24	qrc-gly	8.5	d	9.8	ab	10.4	a	9.5	abcd	8.8	bcd	10.2	a	9.0	bcd	8.8	cd	9.6	abc
28	kpf-hex	8.2	cd	9.9	ab	10.0	a	7.0	d	8.8	abc	8.9	abc	8.5	bc	8.2	cd	8.4	cd
30	kpf 3-*O*-glc	7.2	d	7.9	bcd	9.6	a	7.8	bcd	8.5	bc	8.6	b	7.5	d	7.7	cd	7.8	bcd
23	qrc-gly	5.5	c	6.5	b	6.8	ab	7.5	a	6.5	b	7.2	ab	5.1	c	4.7	c	4.7	c
anthocyanins		28.5	g	96.6	d	59.9	f	87.3	de	69.1	ef	128.6	c	135.6	c	211.8	a	184.8	b
35	cyd 3-*O*-rut	23.3	f	81.6	c	51.7	e	73.8	cd	57.6	de	110.2	b	119.3	b	183.9	a	163.9	a
34	cyd 3-*O*-glc	4.4	g	10.9	d	6.5	fg	9.7	de	8.5	ef	13.5	b	11.1	cd	19.7	a	13.1	bc
33	cyd-gly	0.9	d	2.9	a	1.1	cd	2.4	ab	1.9	bc	2.9	a	2.3	ab	2.7	a	2.3	ab
36	peo 3-*O*-rut	tr		0.5	de	0.3	e	0.6	d	0.5	de	1.1	c	1.2	c	2.3	a	1.6	b
32	unknown compound	tr		0.8	b	0.3	c	0.7	b	0.5	bc	0.8	b	0.9	b	1.4	a	1.7	a
31	(epi)cat-cyd-rut	tr		0.1	c	0.1	c	0.1	c	0.1	c	0.2	c	0.7	b	1.8	a	2.1	a

[a] Fruit from different harvests (H1−5) after sorting according to peel color categories (G, green; B, breaker; P, pink; R, red). Abbreviations: (epi)cat, (epi)catechin; (epi)afz, (epi)afzelechin; qrc, quercetin; rut, rutinoside; glc, glucoside; hex, hexoside; kpf, kaempferol; gly, glycoside; cyd, cyanidin; peo, peonidin; tr, traces. Different letters (a−g) refer to significant differences ($P \leq 0.05$) due to harvest time and peel color category.

peak eluting later (**44** in Figure 5B) had the same mass, but showed a slight hypsochromic shift of the UV−vis spectrum and an additional absorbance maximum at 340 nm, typical for *cis*-isomers.[20] It was tentatively identified as 9-*cis*-β-carotene.

Table 2. Maturity-Dependent Contents (mg/hg Dry Weight) of Polyphenols (1−36 According to Figures 1 and 2B,D) in Pericarp of 'Chacapat' Litchi Fruit

		'Chacapat'[a]															
		C1G		C1B		C2B		C2P		C3B		C3P		C3R		C4R	
total polyphenols		9138	ab	8689	b	8004	c	8052	c	9141	ab	9363	a	9231	ab	9299	a
flavanols		9040	a	8509	b	7864	c	7791	c	8988	ab	9119	a	8883	ab	8953	ab
monomers		2051	a	1879	c	1789	d	1869	c	2037	a	2043	a	1974	ab	1919	bc
9	epicat	1901	a	1714	c	1676	c	1728	c	1936	a	1876	ab	1870	ab	1811	b
1	(epi)afz	133	b	154	a	101	c	133	b	95	cd	146	a	85	d	87	d
4	cat	17	b	11	c	11	c	8	d	6	d	21	a	19	ab	20	a
dimers		1576	a	1492	ab	1379	cd	1289	d	1471	bc	1452	bc	1405	bc	1438	bc
18	$[(epi)cat]_2$: A	1162	a	1120	a	1026	b	925	c	1036	b	972	bc	969	bc	974	bc
8	$[(epi)cat]_2$: B	261	d	214	e	217	e	253	d	295	c	331	ab	307	bc	352	a
21	$[(epi)cat]_2$: A	153	ab	158	a	136	cd	111	e	140	bcd	149	abc	129	d	111	e
trimers		2076	bc	2057	bc	1940	cd	1820	d	2213	ab	2293	a	2267	a	2273	a
10	$[(epi)cat]_3$: A-B	862	a	812	ab	794	b	683	c	864	a	842	ab	861	a	855	ab
15	$[(epi)cat]_3$: B-A	662	bc	696	ab	628	cd	594	d	702	ab	697	ab	679	ab	720	a
19	$[(epi)cat]_3$: B-A	306	ab	323	a	281	c	250	d	295	bc	328	a	310	ab	300	bc
11	$[(epi)cat]_3$: B-B	84	d	68	d	89	cd	112	c	154	b	199	a	191	a	182	a
7	$[(epi)cat]_3$: B-B	99	b	86	c	72	d	87	c	102	b	118	a	112	a	113	a
14	(epi)cat-(epi)afz-(epi)cat: A-B	63	c	71	c	76	bc	94	ab	96	a	109	a	114	a	102	a
tetramers		2309	a	2015	bc	1726	d	1864	cd	2104	b	2152	ab	2083	b	2119	b
17	$[(epi)cat]_4$: A-B-A	667	a	651	ab	539	c	578	bc	671	a	686	a	676	a	643	ab
2	$[(epi)cat]_4$: B-A-B	550	a	406	b	365	b	405	b	560	a	544	a	555	a	525	a
13	$[(epi)cat]_4$: B-A-B	518	a	318	c	287	c	405	b	377	b	384	b	376	b	389	b
20	$[(epi)cat]_4$: A-A-B	296	ab	259	bc	223	c	224	c	233	c	268	bc	245	c	323	a
12	$[(epi)cat]_4$: A-B-B	169	c	263	a	204	b	159	c	157	cd	135	e	127	e	140	de
22	$[(epi)cat]_4$: A-B-A	109	c	117	b	107	cd	93	e	106	cd	134	a	103	cd	99	de
pentamers		1028	bc	1066	b	1031	b	949	c	1163	a	1180	a	1155	a	1205	a
6	$[(epi)cat]_5$: B-A-B-B	298	b	306	b	276	b	268	b	368	a	370	a	368	a	367	a
5	$[(epi)cat]_5$: B-B-A-B	353	a	331	ab	309	bc	291	c	317	b	328	b	319	b	316	b
16	$[(epi)cat]_5$: B-A-B-A	263	e	319	bc	340	b	284	de	330	b	338	b	300	cd	368	a
3	$[(epi)cat]_5$: B-B-A-B	114	c	110	c	106	c	106	c	147	b	146	b	168	a	154	ab
flavonols		92.4	e	122.9	bc	111.3	cd	125.2	bc	94.0	e	101.1	de	135.0	ab	145.3	a
25	qrc 3-*O*-rut	50.6	f	69.1	cd	61.6	de	71.3	bc	53.9	ef	59.1	ef	79.6	ab	87.0	a
29	kpf 3-*O*-rut	18.1	cd	24.5	a	20.8	bc	23.7	a	15.4	e	17.4	de	22.6	ab	23.4	ab
27	qrc 3-*O*-glc	8.2	e	12.2	c	12.9	bc	12.2	c	8.8	de	9.9	d	14.2	ab	15.0	a
23	qrc-gly	11.1	ab	11.5	ab	11.2	ab	11.3	ab	11.6	ab	9.7	b	12.4	a	11.5	ab
30	kpf 3-*O*-glc	4.3	e	5.6	cd	4.7	de	6.6	b	4.3	e	5.0	de	6.2	bc	8.4	a
anthocyanins		5.6	e	57.0	c	29.0	d	135.6	b	58.9	c	142.7	b	213.2	a	200.9	a
35	cyd 3-*O*-rut	5.6	e	54.8	c	28.5	d	129.9	b	57.4	c	137.0	b	204.4	a	193.1	a
34	cyd 3-*O*-glc	tr		1.1	b	0.4	c	2.2	a	0.6	c	1.9	a	2.1	a	2.2	a
36	peo 3-*O*-rut	tr		0.4	d	0.05	e	1.4	c	0.6	d	1.6	c	2.7	a	2.0	b
32	unknown compound	tr		0.5	d	tr		1.1	c	0.4	d	1.0	c	1.8	a	1.3	b
31	(epi)cat-cyd-rut	nd		0.1	c	tr		1.1	b	tr		1.2	b	2.2	a	2.4	a

[a] Fruit from different harvests (C1−4) after sorting according to peel color categories (G, green; B, breaker; P, pink; R, red). Abbreviations: (epi)cat, (epi)catechin; (epi)afz, (epi)afzelechin; qrc, quercetin; rut, rutinoside; kpf, kaempferol; glc, glucoside; gly, glycoside; cyd, cyanidin; peo, peonidin; nd, not detectable; tr, traces. Different letters (a−f) refer to significant differences ($P \leq 0.05$) due to harvest time and peel color category.

Characteristic Compounds in Litchi Pericarp at Harvest. Chlorophylls *a* and *b*, pheophytins *a* and *b*, and *β*-carotene compose the yellowish-green color of unripe litchi fruit. Polyphenols constitute the attractive pinkish-red color of ripe litchi, but also cause their susceptibility to pericarp browning. All polyphenols detected were flavonoids, most of them procyanidins, cyanidin glycosides, or quercetin glycosides. The predominance of highly oxidizable *ortho*-diphenolic structures[5] in litchi pericarp is obvious. Besides, flavonoids with a monohydroxylated ring B, such as (epi)afzelechin and kaempferol, were detected in both Thai cultivars. A-type linkages are seldom in fruit tannins,[31] but common in litchi procyanidins. Especially their ubiquitous

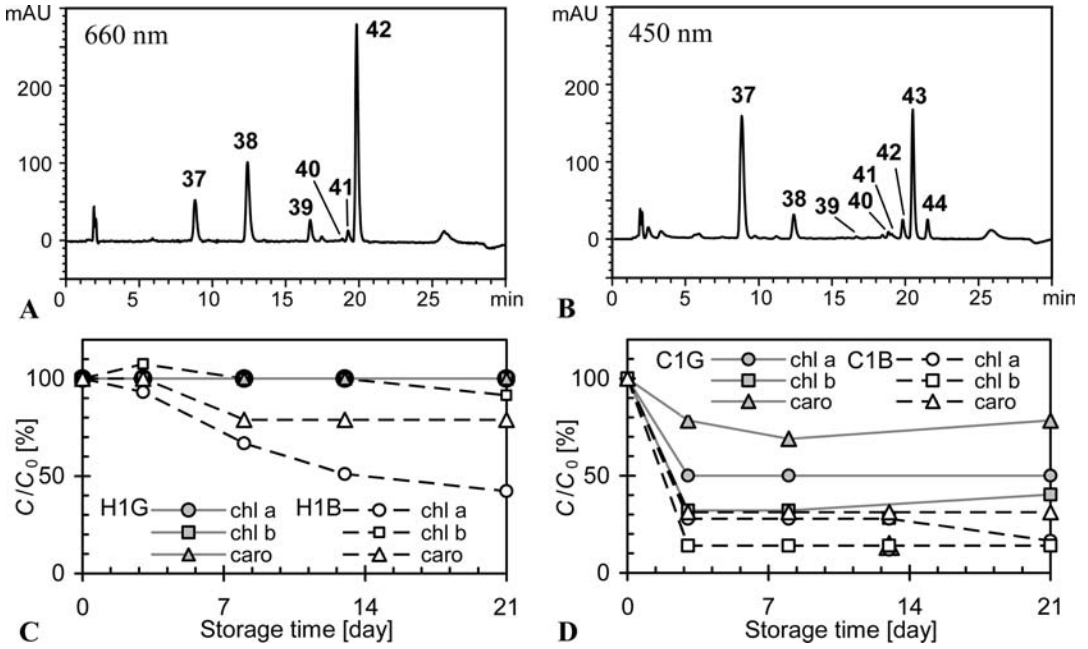

Figure 5. Nonphenolic pigments in litchi pericarp: (A, B) reversed-phase HPLC separation of chlorophylls (660, 450 nm) and carotenoids (450 nm) extracted from freshly harvested green 'Hong Huey' fruit (for peak assignment, cf. Table 3); (C, D) relative contents (C/C_0, %) of summed chlorophyll *a* (chl a), chlorophyll *b* (chl b), and β-carotene (caro) derivates in green (1G) or green-red shelled (1B) 'Hong Huey' (C) and 'Chacapat' (D) fruit during cold storage (5 °C, 90% RH). Relative contents were calculated on the basis of the amounts (mg/hg) measured directly after harvest, after insignificantly different amounts had been averaged throughout storage.

occurrence in terminal and extension units is rarely found in food.[31] Whereas anthocyanidins or flavonol aglycones were not detected in the pericarp, even a glycosylated (epi)-catechin−cyanidin adduct was identified. Throughout, sugar moieties consisted of hexosides and desoxyhexosides. Rutinosides and glucosides prevailed. Cultivar-specific differences in pericarp pigment patterns (Figure 2) exclusively referred to glycosylation.

Quantitative Changes in Pericarp Flavonoids and Nonphenolic Pigments during Maturation. Evidently, maturity did not influence the total amount of soluble polyphenols (flavanols, flavonols, and anthocyanins). In 'Hong Huey' pericarp, maximum amounts of 87−94 g/kg dry weight (DW) were recorded in breaker and pink fruit from harvests 1−3 (H1B, H2B/P, H3B/P; Table 1). By contrast, maximum accumulation (91−94 g/kg DW) was observed in 'Chacapat' pericarp for late-harvested fruit (C3B/P/R, C4R; Table 2). However, equal quantities were found in the pericarp of green fruit (C1G).

Maturity-Dependent Contents of Monomeric and Oligomeric Flavan-3-ols. A flavan-3-olic skeleton characterized 94−97% of the total polyphenols in 'Hong Huey' (H4R−H1G) and 96−99% in 'Chacapat' (C3/4R−C1G) pericarp. Irrespective of the cultivar, 23% of the flavan-3-ols on average were monomers. The prevailing (−)-epicatechin, **9**, attained large amounts of 16−19 g/kg DW (Tables 1 and 2) in pericarp of freshly harvested litchi, equaling 4−6 g/kg fresh weight (FW). This was in the range described for premature 'Nuomici' (4−15 g/kg FW)[15] and 'Feizixiao' pericarp (4 g/kg FW).[6,15] Smaller amounts had been reported for 'Kwai Mi' (2 g/kg FW)[5] and 'Huaizhi' (3 g/kg FW).[21] A decrease in (−)-epicatechin, as reported throughout fruit development,[15] was not confirmed during on-tree maturation. Only the amount of (epi)afzelechin, **1** (Tables 1 and 2), was lower in late-harvested red than in partly colored premature fruit. The portion of the latter thus dropped from 9.0% (H1G) and 8.2% (C1B) to 5.4% (H4R) and 4.3% (C3R) of the flavan-3-ol monomers. As the contents of (+)-catechin, **4** (Tables 1 and 2), were 100 times lower than those of its epimer, biosynthesis of (−)-epicatechin was clearly preferred. Likewise, accumulation of procyanidin A2, **18**, was favored over procyanidin B2, **8** (Tables 1 and 2). In both cultivars, the pericarp contents of the A-type-linked dimer were 3−6 times higher than the B-type procyanidin levels. This ratio declined during on-tree maturation, particularly in 'Chacapat' pericarp. However, in ripe to over-ripe fruit (C3P/R, C4R), it was still higher than reported for 'Feizixiao'.[6] The contents of procyanidin A2 (∼3 g/kg FW) and the A-B-linked trimer (∼2 g/kg FW) generally exceeded literature data.[5,6] Apart from the predominant flavanols, 16 proanthocyanidins were quantified in litchi pericarp for the first time to our knowledge. In both cultivars, the same tri- and tetramers accumulated preferably. The linkage pattern A-B was favored over B-A in trimer biosynthesis, whereas A-B-A prevailed over B-A-B, A-A-B, or A-B-B in tetramers (Tables 1 and 2). According to the analytical method used, the total amounts of trimers and tetramers, respectively, represented the highest percentages by weight on average ('Chacapat', 24%; 'Hong Huey', 25%). When normal-phase HPLC and thiolytic degradation were used, penta- to heptamers were reported to be the predominant polymeric compounds in litchi pericarp.[17] However, structural features of higher oligomers and polymers can be extrapolated from low oligomers.[31] Overall, 88−94% (mol/mol) of all di- to pentameric molecules contained at least one A-type linkage. In unripe fruit (H1G/B, H2/3B, C1G/B, C2B), >50% of the interflavanoid linkages had the additional ether bond. Like in dimers, the total proportion of double bonds decreased with ongoing maturation. Hence, over-ripe fruit were characterized by maximal B-type linkages (H4R, 51%; C4R, 52%).

Maturity-Dependent Flavonol Contents. High flavonol contents were already present in the pericarp of unripe 'Hong Huey' fruit (H1/2B; Table 1). By contrast, their amount in 'Chacapat' pericarp increased by 57% throughout maturation (C1G−C4R; Table 2), mainly due to accumulation of quercetin glycosides. Irrespective of cultivar and maturity, quercetin 3-*O*-rutinoside accounted for 55−60% of the flavonols. Its average content in red 'Chacapat' (0.25 g/kg FW; C3/4R) was comparable with that found in red 'Kwai Mi' pericarp,[5] whereas a markedly higher level was detected in red 'Hong Huey' (0.40 g/kg FW; H4/5R). Nevertheless, the molar ratio of quercetin 3-*O*-rutinoside to quercetin 3-*O*-glucoside was 4.3 in 'Chacapat', but only 3.6 in 'Hong Huey' as reported for 'Kwai Mi' (3.4).[5] 'Chacapat' is a variety with flat protuberances, whereas 'Kwai Mi' ('Kwai May') and 'Hong Huey' ('Tai So') are cultivars with protruding skin segments.[32] Another cultivar-specific difference was seen in the molar ratio of quercetin to kaempferol amounting to 6:1 for 'Hong Huey' and 3:1 for 'Chacapat'. The former cultivar accumulated similar total amounts of the monohydroxylated flavonol, particularly as rutinoside, but almost 2−3 times as much quercetin glycosides.

Maturity-Dependent Anthocyanin Accumulation. Maturity exerts essential influence on the anthocyanin content in litchi pericarp. In ripe 'Chacapat' fruit (C3/4R), the red pigment concentration was 36−38 times higher than in unripe fruit (C1G; Table 2). In 'Hong Huey' pericarp, the amount increased only 7-fold (H1G-H4R) due to elevated initial contents (Table 1). Comparable increments within 3 weeks prior to full maturity to equal final total anthocyanin contents ≥0.5 g/kg FW had been reported for 'Brewster' [3] and 'Kwai May'.[4] As formerly described for Indian litchi,[26] maximum anthocyanin contents were detected in fully ripe to over-ripe, but not yet senescent, red fruit (C3/4R, H4R). Cyanidin 3-*O*-rutinoside was predominant and contributed most to color development (Tables 1 and 2). It comprised >95% of the red pericarp pigments in 'Chacapat' (C1G−C4R) and 82−89% in 'Hong Huey' (H1G−H5R). As demonstrated for flavonols, the ratio of the 3-*O*-rutinoside to the 3-*O*-glucoside was cultivar-specific. For cyanidin in 'Hong Huey' pericarp, the molar ratio rose from 4:1 (H1G) to 9:1 (H5R). Equal glycosylation patterns were reported for 'Brewster' (3−8:1)[2,3] and 'Kwai Mi' (6:1).[5] 'Chacapat' again displayed higher ratios of 36:1 (C1B) to 73:1 (C3R). Likewise, 94% cyanidin 3-*O*-rutinoside and very low amounts of the glucoside had been detected in 'Huaizhi' ('Wai Chee') pericarp,[18] another cultivar with flat protuberances.[32] The glycosylated (epi)-catechin−cyanidin adduct, **31**, occurred in pericarp of both cultivars studied when the red color increasingly prevailed at the end of on-tree maturation (Tables 1 and 2).

Quantitative Changes in Nonphenolic Pigments during Maturation. To our knowledge, individual nonphenolic pigments were quantitated in litchi pericarp for the first time (Table 3). Amounts of chlorophyll *a* or *b* derivatives were calculated as chlorophyll *a* or *b*, because loss of the Mg^{2+} ion during extraction under slight acid conditions could not be excluded. Irrespective of maturity, the total chlorophyll *a*/*b* ratios were 2.0 ± 0.4 and 1.4 ± 0.2 in pericarp of 'Hong Huey' and 'Chacapat', respectively. Changing ratios, as reported for ripening Indian litchi,[26] were not observed. Generally, photometrically determined amounts of chlorophylls[4,26] were not directly comparable with the contents quantitated by HPLC. As expected,[4] total chlorophylls *a* and *b* degraded, but were still detectable at the end of on-tree maturation. In the case of 'Hong Huey' pericarp, red fruit (H4/5R) contained 27% of the contents found in green fruit (H1G). In contrast, chlorophylls in 'Chacapat' pericarp were metabolized by 92% (C1G−C3/4R). However, no dephytylized degradation products and, hence, no evidence for chlorophyllase activity[30] were found. Carotenoids decreased to an equal extent as chlorophylls, by 70 and 89% in 'Hong Huey' and 'Chacapat', respectively, and thus seemed to be part of the pigment−protein complex of the photosystems in the chloroplasts.

Impact of Pigment Contents on Pericarp Color at Harvest. Chlorophylls and anthocyanins mainly affect color appearance of ripening litchi fruit. Hence, the molar ratio of total anthocyanins (A_t) to total chlorophylls (Ch_t) should best describe objective pigment changes from green to red. The litchi maturity index [$LMI = 10 \times (a^* (a^{*2} + L^{*2})^{-1/2})$] based on the CIELab color space proved best to express visual impression of pericarp color at harvest.[1] According to the Weber−Fechner law,[33] the logarithmic relationship between stimulus (A_t/Ch_t) and perception (LMI; data cf. ref 1) was calculated for 'Chacapat' with R^2 = 0.99 [$LMI_{\text{'Chacapat'}} = 1.19 \times \ln(A_t/Ch_t) - 0.04$] and for 'Hong Huey' with R^2 = 0.94 [$LMI_{\text{'Hong Huey'}} = 2.23 \times \ln(A_t/Ch_t) - 3.01$]. When the total amounts of anthocyanins and chlorophylls were equal [ln(1)], 'Hong Huey' fruit ($-3.01 < LMI_{H1G}$) appeared greener than 'Chacapat' litchi ($-0.04 > LMI_{C1G}$), presumably because of its different pericarp morphology.[34] Anthocyanins accumulate first near the apex of protuberances.[4] Hence, the visible red area is smaller for fruit with spiny pericarp ('Hong Huey') than for those with warty peel ('Chacapat'). However, the color of 'Chacapat' pericarp changed more uniformly, and fewer fruits with green and red parts were found.[1]

Effects of Cold Storage on Characteristic Compound Classes in Litchi Pericarp. Postharvest, the pericarp rapidly lost moisture, accompanied by visually perceivable browning within 3−5 days of fruit storage.[1] The three polyphenol classes were differently involved in these changes (Table 4). Flavonol amounts hardly changed, except for some 'Hong Huey' variants (H1/2B, H3P). Anthocyanin contents drastically decreased. For all lots except H5R, maximum loss occurred within the first 3 days of storage (Table 4), being consonant with visible discoloration.[1] Later, anthocyanin degradation proceeded only in the pericarp of unripe 'Hong Huey' (H1G/B) and pink 'Chacapat' (C3P) fruit. These variants had shown slower browning than other samples.[1] At the end of storage, anthocyanin retention was highest in late-harvested pink 'Hong Huey' (H4P) and red fruit of both varieties (C4/3R, H3R; Table 4). Total flavanol degradation was also most pronounced in premature fruit (C1G, H/C1B, H/C2B/P, H/C3B, H3P), except H1G. The degradation rates of flavanol mono- and oligomers differed widely. The strong decline in monomeric flavanols resembled that of anthocyanins, but progressed after 3 days. The contents of di- to pentamers only decreased in unripe, particularly breaker, fruit of 'Hong Huey' (H1/2/3B). Likewise, 'Chacapat' fruit harvested early (C1/2) lost most oligomers. Changes in nonphenolic pigments in fruit harvested first are shown in Figure 5C, D. By analogy to the phenolic pigments, 'Chacapat' fruit catabolized chlorophyll in the first days of storage, whereas green 'Hong Huey' litchis better retained the green pigments. The latter fruit had a thick, not yet dilated, pericarp being less prone to desiccation and browning.[1]

Storage-Dependent Changes in Major Pericarp Polyphenols. As shown in Tables 5 and 6, the contents of (epi)afzelechin rather increased than decreased throughout storage. The

Table 3. Maturity-Dependent Contents (mg/hg Dry Weight) of Nonphenolic Pigments (37−44 According to Figure 5A,B) in Pericarp of 'Hong Huey' and 'Chacapat' Litchi Fruit

		'Hong Huey'[a]																	
		H1G		H1B		H2B		H2P		H3B		H3P		H4P		H4R		H5R	
nonphenolic pigments		27.60	a	20.81	ab	20.16	ab	10.62	cd	17.45	bc	13.45	bcd	8.20	d	6.95	d	7.90	d
chlorophyll *a* derivatives[b]		15.86	a	13.43	ab	10.25	bcd	6.00	de	10.88	bc	7.70	cde	4.72	e	4.04	e	4.45	e
42	pheophytin *a*[b]	11.20	a	6.49	bc	7.66	ab	4.17	bcd	6.62	bc	6.20	bcd	3.84	cd	2.80	d	2.67	d
38	chlorophyll *a*	3.45	abc	6.33	a	1.71	bc	1.38	bc	3.85	ab	0.73	bc	0.31	c	0.91	bc	1.45	bc
41	pheophytin *a'*[b]	0.53	a	0.47	ab	0.44	ab	0.29	ab	0.31	ab	0.40	ab	0.18	ab	0.11	b	0.25	ab
39	mono-*O*-pheophytin *a*[b]	0.67	a	0.15	ab	0.44	ab	0.16	ab	0.10	b	0.36	ab	0.38	ab	0.21	ab	0.09	b
chlorophyll *b* derivatives[c]		8.71	a	4.93	abc	7.53	ab	3.39	c	4.72	bc	4.19	bc	2.36	c	2.07	c	2.47	c
37	chlorophyll *b*	8.06	a	4.71	abc	7.00	ab	3.23	c	4.47	abc	3.78	bc	2.13	c	1.96	c	2.32	c
40	pheophytin *b*[c]	0.65	a	0.22	bc	0.53	ab	0.16	bc	0.25	bc	0.41	abc	0.23	bc	0.10	c	0.15	bc
carotenoids		3.04	a	2.45	ab	2.39	ab	1.23	cd	1.86	bc	1.57	bcd	1.13	cd	0.85	d	0.98	cd
43	all-*trans*-β-carotene	2.63	a	2.14	ab	2.12	ab	1.11	cd	1.66	bc	1.41	bcd	1.02	cd	0.76	d	0.88	cd
44	9-*cis*-β-carotene	0.41	a	0.30	ab	0.27	bc	0.12	d	0.19	bcd	0.15	d	0.11	d	0.09	d	0.10	d

		'Chacapat'[a]													
		C1G		C1B		C2B		C2P		C3B		C3P		C3R		C4R	
nonphenolic pigments		23.51	a	14.71	b	8.59	c	4.06	de	6.65	cd	5.56	d	2.03	e	1.87	e
chlorophyll *a* derivatives[b]		12.31	a	6.88	b	4.63	c	2.10	e	3.46	d	2.85	d	0.97	f	0.89	f
42	pheophytin *a*[b]	11.26	a	6.38	b	4.21	c	2.03	e	3.10	d	2.79	d	0.94	f	0.85	f
38	chlorophyll *a*	0.19	a	0.04	c	0.07	b	tr		0.03	c	tr		tr		tr	
41	pheophytin *a'*[b]	0.33	a	0.29	a	0.17	b	0.04	cd	0.08	c	0.03	cd	tr		0.01	d
39	mono-*O*-pheophytin *a*[b]	0.53	a	0.17	ab	0.18	ab	0.02	b	0.25	ab	0.02	b	0.02	b	0.02	b
chlorophyll *b* derivatives[c]		8.72	a	5.96	b	2.66	c	1.41	c	2.27	c	1.92	c	0.78	c	0.73	c
37	chlorophyll *b*	7.82	a	2.99	b	1.62	bc	1.32	bc	2.18	bc	1.86	bc	0.76	c	0.73	c
40	pheophytin *b*[c]	0.90	ab	2.97	a	1.04	ab	0.09	b	0.09	b	0.05	b	0.03	b	tr	
carotenoids		2.48	a	1.87	b	1.29	c	0.55	e	0.92	d	0.79	d	0.28	f	0.26	f
43	all-*trans*-β-carotene	2.29	a	1.70	b	1.16	c	0.52	e	0.87	d	0.75	d	0.27	f	0.26	f
44	9-*cis*-β-carotene	0.20	a	0.17	b	0.13	c	0.03	e	0.06	d	0.04	de	0.01	f	tr	

[a] Fruit from different harvests ('Hong Huey', H1−5; 'Chacapat', C1−4) after sorting according to peel color categories (G, green; B, breaker; P, pink; R, red). [b] Quantitated as chlorophyll *a*. [c] Quantitated as chlorophyll *b*. Abbreviations: tr, traces. Different letters (a−f) refer to significant differences ($P \leq 0.05$) due to harvest time and peel color category.

amounts of (+)-catechin (data not shown) varied at a constant low level. Hence, the drastic decline in flavanol monomers was solely due to (−)-epicatechin degradation. At the end of storage, different maturity stages could be grouped according to percentage losses of (−)-epicatechin (Tables 5 and 6): The contents in pericarp of fruit harvested early (C/H1G/B, C/H2B/P) were reduced by 70%, in bicolored fruit harvested later (C3B, H3B/P) by 60%, in pink to red 'Chacapat' (C3P/R, C4R) and in over-ripe 'Hong Huey' (H5R) by 50%, but in pink to red 'Hong Huey' only by 40% (H3R, H4P/R). On average, three-fifths ('Hong Huey') and four-fifths ('Chacapat') of the total decline occurred within the first 3 days of storage. Sun et al.[16] had shown that (−)-epicatechin declined by half in pericarp of mature 'Huaizhi' litchi within 1 week at room temperature. In contrast, no degradation was observed in pericarp of mature 'Feizixiao' litchi stored in PPE bags at 3 °C until day 22 of storage.[6] However, only 21% of the initial (−)-epicatechin amount was found in the pericarp after 36 days. In this bagged fruit, the flavanol dimers, particularly procyanidin B2, disappeared even more rapidly and to a larger extent than the monomer.[6] In fruit stored without packaging, procyanidin B2 was depleted slightly more rapidly than procyanidin A2 (Tables 5 and 6), but both dimers were much more stable than (−)-epicatechin. At the end of storage, procyanidin A2 was thus the major polyphenol in the groups of early-harvested and bicolored fruit. In pericarp of fruit picked early, even the final amounts of the A-B-linked trimer exceeded those of (−)-epicatechin (Tables 5 and 6). The contents of the glycosylated (epi)catechin−cyanidin adduct, **31**, fell throughout storage (data not shown). A postharvest increase in anthocyanins, as described for litchis stored in PE bags,[14] was not observed. Cyanidin 3-*O*-rutinoside was though less prone to oxidation than cyanidin 3-*O*-glucoside (Tables 5 and 6), as reported for 'Brewster' litchi.[14] Its amounts in pericarp of red fruit (H3/4/5R, C3/4R) after 21 days were equal to or even higher than initial contents in pink fruit (H2/3/4P, C2/3P; Tables 5 and 6). Quercetin 3-*O*-rutinoside hardly decreased throughout.

Table 4. Relative Polyphenol Contents (C/C_0, %) after 3 and 21 Days of Storage (5 °C, 90% RH) in Pericarp of 'Hong Huey' and 'Chacapat' Litchi Fruit

	'Hong Huey'[a]										'Chacapat'[a]							
day	H1G	H1B	H2B	H2P	H3B	H3P	H3R[b]	H4P	H4R	H5R	C1G	C1B	C2B	C2P	C3B	C3P	C3R	C4R
flavanols[c]																		
3	95	72	80	78	69	74	90	96	98	100	66	76	70	79	nsa	80	86	78
21	95	71	67	78	69	74	90	96	98	86	64	75	75	75	69	79	82	78
monomers[c]																		
3	88	64	55	63	51	55	71	83	70	97	43	54	51	53	nsa	63	68	60
21	45	30	37	45	51	48	56	65	68	59	35	44	37	40	42	48	55	53
dimers[c]																		
3	88	73	84	100	75	80	93	100	100	94	69	75	71	82	nsa	84	90	77
21	81	73	80	100	75	80	93	100	100	81	68	73	78	79	71	84	86	77
trimers[c]																		
3	96	78	86	92	78	92	96	102	100	101	76	85	79	98	nsa	89	96	86
21	96	78	74	95	78	96	96	102	100	97	76	81	86	96	79	89	93	86
tetramers[c]																		
3	100	82	94	89	67	78	95	109	101	105	75	98	86	88	nsa	87	96	82
21	100	82	82	94	67	78	95	109	100	99	67	97	100	88	80	87	94	82
pentamers[c]																		
3	100	83	87	100	78	91	97	99	100	98	76	82	74	94	nsa	88	100	81
21	100	83	68	100	78	91	97	99	100	100	73	73	77	89	75	88	87	83
anthocyanins[c]																		
3	59	59	47	66	39	53	75	87	68	100	53	36	79	46	nsa	69	75	78
21	26	21	43	100[d]	59	53	75	87	68	64	53	36	70	46	37	47	75	91
flavonols[c]																		
3	100	81	78	100	100	76	95	99	105	100	91	100	68	97	nsa	100	100	94
21	100	82	82	100	100	76	95	99	97	100	91	100	96	100	100	100	100	98

[a] Fruit from different harvests ('Hong Huey', H1−5; 'Chacapat', C1−4) after sorting according to peel color categories (G, green; B, breaker, P, pink; R, red); nsa, no sample available. [b] Because sample of day 0 deteriorated due to rupture of the bag during airfreight transport, percentages are based on averaged initial amounts of H3P, H4P/R, H5R. [c] Relative contents were calculated on the basis of the amounts (mg/hg) measured directly after harvest, after insignificantly different amounts had been averaged throughout storage. [d] This value is presumably an outlier, because 66% were constantly determined on days 3, 8, and 13.

Postharvest Browning Mechanisms As Deduced from Changes in Pericarp Composition. Many theories on pericarp browning exist. Various processes presumably coincide and interfere in vivo. The postharvest flavonoid changes noted in this study indicated some basic principles as discussed below.

Impact of Pericarp Moisture on Pericarp Browning Reactions. Desiccation had a vast effect on pericarp composition: flavanols and anthocyanins degraded much earlier in litchi stored without packaging (Tables 5 and 6) than reported for bagged ones.[6,14] However, their residual levels were higher, because degradation stopped after a few days, when the moisture dropped below ∼30%.[1] Conversely, when the pericarp was rehydrated, major phenolic compounds, especially dimeric procyanidins, declined in a similar way as described for bagged fruit.[6] This effect became obvious in a sample (H3R, day 0; Table 5) contained in a bag that ruptured during airfreight transport.

Role of Anthocyanin Decoloration in Pericarp Browning. According to the anthocyanin contents, litchi harvested when red would appear at least pink at the end of storage. Nevertheless, they were dark brown.[1] Underhill and Critchley[10] explained this browning as a result of an increase in pH by 0.4−0.6 unit in the vacuole, inducing conversion of anthocyanin cations to colorless chromenols. These approximated pH values[10] were though still within the usual range of fruit vacuoles (pH 4−6).[34] Moreover, with increasing concentration due to water loss, flavylium cations are better stabilized by intermolecular copigmentation with other anthocyanins or flavonols.[34] Above all, green litchi (H1G) appeared light brown after 3−5 days despite chlorophyll retention. The pigment-containing outer layers of the mesocarp thus seemed to be entirely masked by the epicarp, where browning had chiefly been localized,[10] or the pigments were obliterated by brown products produced in the same cells.

Indication for Enzymes Involved in Pericarp Browning. High initial activities of PPO and especially POD, followed by a decrease after 3−8 days of storage, had been detected in the same pericarp samples.[1] Concomitantly, only (−)-epicatechin, but not the (epi)afzelechin, declined (Tables 5 and 6). This gave strong evidence for oxidation by litchi pericarp PPO, which has high substrate specificity for *ortho*-diphenolic structures and cannot oxidize monohydroxy phenols.[8] Oxidation by POD[9] would occur less selectively. Furthermore, compositional changes in 'Hong Huey' pericarp, which possessed higher POD activity,[1] did not markedly differ from those in 'Chacapat' fruit. Hence, the role of POD in litchi pericarp browning remains unclear. The existence of an aglycone-forming anthocyanase[12] was not corroborated. β-Glucosidases universally occur in plants and hydrolyze β-glucosidic bonds between two glycones or glucose and aglycones.[35] All samples contained neither

Table 5. Contents of Major Phenolic Compounds (mg/hg) after 0, 3, and 21 Days of Storage (5 °C, 90% RH) in Pericarp of 'Hong Huey' Litchi Fruit, Including Relative Contents (C/C_0, %) Based on the Initial Amounts (Day 0) in Parentheses

	'Hong Huey'[a]									
day	H1G	H1B	H2B	H2P	H3B	H3P	H3R	H4P	H4R	H5R
(−)-epicatechin, **9**										
0	1571 a	1839 a	1700 a	1621 a	1810 a	1870 a	[345 c (20[b])][c]	1597 a	1714 a	1630 a
3	1091 b (69)	1128 b (61)	883 b (52)	892 b (55)	977 b (54)	1060 b (57)	1342 a (79[b])	968 b (61)	1158 bc (68)	1553 a (95)
21	434 c (28)	458 d (25)	479 d (28)	544 c (34)	774 bc (43)	784 c (42)	1030 b (60[b])	983 b (62)	1026 c (60)	872 d (53)
(epi)afzelechin, **1**										
0	157 ab	161 a	168 b	130 c	143 b	142 ab	[170 a (139[b])][c]	118 a	98 c	132 b
3	143 ab (91)	145 a (90)	162 b (96)	164 abc (126)	115 b (81)	106 c (75)	152 a (124[b])	112 a (95)	103 bc (104)	120 c (91)
21	148 ab (94)	159 a (99)	197 a (117)	191 ab (148)	218 a (152)	145 a (102)	154 a (126[b])	113 a (96)	118 b (121)	143 a (108)
procyanidin A2, **18**										
0	1037 a	1071 a	1045 a	918 a	1062 a	1011 a	[376 b (40[b])][c]	889 a	875 a	1006 a
3	860 ab (83)	854 b (80)	894 b (86)	833 a (91)	900 ab (85)	848 b (84)	944 a (100[b])	821 a (92)	894 a (102)	896 b (89)
21	653 b (63)	790 b (74)	813 b (78)	807 a (88)	844 b (79)	837 b (83)	939 a (99[b])	835 a (94)	835 a (96)	796 c (79)
procyanidin B2, **8**										
0	168 a	206 a	199 a	266 a	258 a	257 a	[61 b (29[b])][c]	183 a	202 a	196 d
3	149 ab (89)	134 bc (65)	180 b (90)	186 b (70)	174 b (68)	193 b (75)	226 a (108[b])	137 c (75)	179 bc (89)	259 a (132)
21	103 ab (62)	105 d (51)	136 c (68)	186 b (70)	160 b (62)	181 b (70)	222 a (106[b])	147 bc (80)	159 c (79)	166 e (84)
(epi)catechin trimer: A-B, **10**										
0	869 a	868 a	820 a	775 a	795 a	802 a	[305 b (41[b])][c]	738 a	733 a	722 a
3	648 a (75)	673 b (78)	701 b (85)	680 ab (88)	703 a (88)	662 b (83)	728 a (97[b])	645 a (87)	790 a (108)	737 a (102)
21	659 a (76)	692 b (80)	642 b (78)	747 ab (96)	660 a (83)	688 ab (86)	722 a (96[b])	715 a (97)	731 a (100)	685 b (95)
cyanidin 3-*O*-rutinoside, **35**										
0	23.3 a	81.6 a	51.7 a	73.8 a	57.6 a	110.2 a	[28.9 b (20[b])][c]	119.3 a	183.9 a	163.9 a
3	14.3 b (61)	51.5 b (63)	25.0 b (48)	53.3 b (72)	23.6 c (41)	59.3 b (54)	135.3 a (94[b])	92.5 b (78)	145.3 ab (79)	188.4 a (115)
21	7.1 c (31)	18.4 d (23)	22.7 bc (44)	74.1 a (100)	42.4 ab (74)	66.3 b (60)	135.0 a (94[b])	51.8 d (43)	105.7 b (57)	115.1 b (70)
cyanidin 3-*O*-glucoside, **34**										
0	4.4 a	10.9 a	6.5 a	9.7 a	8.5 a	13.5 a	[1.8 d (13[b])][c]	11.1 a	19.7 a	13.1 a
3	2.0 b (45)	7.7 b (71)	2.5 b (39)	4.3 c (44)	2.1 bc (24)	5.7 b (43)	11.1 ab (78[b])	7.6 b (69)	10.9 bc (56)	12.4 a (94)
21	0.7 c (17)	1.6 e (15)	2.2 bc (34)	7.5 b (77)	3.7 b (43)	5.9 b (44)	11.6 a (81[b])	4.7 c (43)	8.8 c (45)	7.5 b (57)
quercetin 3-*O*-rutinoside, **25**										
0	136.3 a	168.3 a	169.3 a	154.5 a	158.2 a	169.6 a	[120.7 a (82[b])][c]	146.1 a	137.0 bc	136.5 a
3	124.1 a (91)	122.1 c (73)	127.7 bc (75)	134.7 a (87)	132.4 a (84)	125.0 b (74)	145.6 a (99[b])	136.7 a (94)	147.8 abc (108)	133.3 a (98)
21	134.8 a (99)	133.9 bc (80)	139.6 b (82)	144.3 a (93)	142.6 a (90)	136.1 b (80)	147.7 a (100[b])	141.4 a (97)	132.0 c (96)	133.2 a (98)

[a] Fruit from different harvests (H1−5) after sorting according to peel color categories (G, green; B, breaker; P, pink; R, red). [b] Percentage based on averaged initial amounts of H3P, H4P/R, H5R. [c] Sample was deteriorated, because the bag ruptured during airfreight transport. Different letters (a−e) refer to significant differences ($P \leq 0.05$) due to storage (data of days 8 and 13 not shown).

anthocyanidins nor their degradation products[36] or the more stable flavonol aglycones that would be produced at least in small amounts by side activities of an anthocyanase. Thus, it is unlikely that the β-glucosidase found in vitro[12] catalyzes deglycosylation of cyanidin 3-*O*-glucoside in vivo. The relevance of this enzyme is questionable in any case, because the ability to deglycosylate cyanidin 3-*O*-rutinoside was never shown.

Substrates and Products of Postharvest Browning in Litchi Pericarp. At harvest, 40% (mol/mol) of all detected polyphenol molecules were (−)-epicatechin. Its role as main substrate of litchi PPO[6,13,15,16] was confirmed. During pericarp browning, (−)-epicatechin losses were 2−8 times higher than that of all other polyphenols together. Hypothetical involvement of quercetins in pericarp browning due to their *ortho*-diphenolic structure[5] was thus not substantiated. As procyanidin di- and trimers are poor substrates of litchi PPO, coupled oxidation with the enzymatically generated *o*-quinone of (−)-epicatechin was suggested.[6] Screenings based on specific fragment ions in (−)-MS^n experiments[37] for proposed flavanolic oxidation products, for example, dehydrodiepicatechin A,[13] remained without discovery. Even though some amounts of individual tri- to pentameric procyanidins were sporadically enhanced after 3, 8, 13, or 21 days (data not shown), no clear trend became evident. Thus, in vivo condensation of *o*-quinones derived from (−)-epicatechin with other mono- or oligomeric flavanols were regarded as insignificant. *o*-Quinones of (−)-epicatechin and co-oxidized oligomers rather seemed to react with matrix components, for example, proteins, to insoluble brown complexes. Coupled oxidation of anthocyanins by (−)-epicatechin *o*-quinone[8,9] was considered to be more essential than their degradation by peroxide and hydroxyl radicals,[11] because abundant flavanols also possess high scavenging activity.[16] Acceleration of enzymatic

Table 6. Contents of Major Phenolic Compounds (mg/hg) after 0, 3, and 21 Days of Storage (5 °C, 90% RH) in Pericarp of 'Chacapat' Litchi Fruit, Including Relative Contents (C/C_0, %) Based on the Initial Amounts (Day 0) in Parentheses

	'Chacapat'[a]																							
day	C1G			C1B			C2B			C2P			C3B			C3P			C3R			C4R		
(−)-epicatechin, **9**																								
0	1901	a		1714	a		1676	a		1728	a		1936	a		1876	a		1870	a		1811	a	
3	739	b	(39)	822	b	(48)	781	b	(47)	851	b	(49)	nsa			1112	b	(59)	1209	b	(65)	1037	b	(57)
21	514	d	(27)	614	d	(36)	537	d	(32)	532	d	(31)	745	d	(38)	874	d	(47)	1002	d	(54)	914	d	(50)
(epi)afzelechin, **1**																								
0	133	b		154	b		101	bc		133	b		95	a		146	b		85	bc		87	a	
3	163	a	(123)	185	a	(120)	110	bc	(109)	132	b	(100)	nsa			158	a	(108)	114	a	(135)	91	a	(105)
21	163	a	(123)	188	a	(122)	113	b	(112)	156	a	(118)	94	a	(99)	100	d	(69)	74	c	(88)	88	a	(101)
procyanidin A2, **18**																								
0	1162	a		1120	a		1026	a		925	a		1036	a		972	a		969	a		974	a	
3	836	bc	(72)	829	bc	(74)	690	d	(67)	783	b	(85)	nsa			876	ab	(90)	885	b	(91)	776	b	(80)
21	734	c	(63)	825	bc	(74)	808	b	(79)	734	b	(79)	766	b	(74)	869	ab	(89)	813	c	(84)	784	b	(80)
procyanidin B2, **8**																								
0	261	a		214	a		217	a		253	a		295	a		331	a		307	a		352	a	
3	158	b	(61)	205	a	(96)	184	b	(85)	201	b	(79)	nsa			251	b	(76)	275	b	(90)	249	bc	(71)
21	151	bc	(58)	144	c	(67)	169	bc	(78)	177	b	(70)	180	b	(61)	236	c	(71)	267	b	(87)	264	b	(75)
(epi)catechin trimer: A-B, **10**																								
0	862	a		812	a		794	a		683	a		864	a		842	a		861	a		855	a	
3	618	b	(72)	641	cd	(79)	566	c	(71)	632	a	(92)	nsa			783	ab	(93)	800	ab	(93)	731	bc	(86)
21	580	b	(67)	716	bc	(88)	664	b	(84)	662	a	(97)	671	b	(78)	756	ab	(90)	752	b	(87)	750	b	(88)
cyanidin 3-*O*-rutinoside, **35**																								
0	5.6	a		54.8	a		28.5	a		129.9	a		57.4	a		137.0	a		204.4	a		193.1	a	
3	3.4	b	(60)	20.7	b	(38)	19.3	abc	(68)	63.6	b	(49)	nsa			106.8	b	(78)	165.2	b	(81)	159.1	c	(82)
21	2.8	b	(50)	22.7	b	(41)	14.1	bc	(49)	52.4	b	(40)	21.6	b	(38)	61.8	d	(45)	145.9	b	(71)	176.9	b	(92)
cyanidin 3-*O*-glucoside, **34**																								
0	tr			1.1	a		0.4	a		2.2	a		0.6	a		1.9	a		2.1	a		2.2	a	
3	tr			0.2	b	(17)	0.2	b	(42)	0.9	b	(39)	nsa			1.1	b	(61)	1.4	d	(64)	1.5	b	(69)
21	tr			0.2	b	(20)	0.05	c	(10)	0.6	b	(26)	0.05	b	(8)	0.5	c	(29)	1.8	b	(85)	1.7	b	(78)
quercetin 3-*O*-rutinoside, **25**																								
0	50.6	a		69.1	ab		61.6	a		71.3	a		53.9	a		59.1	a		79.6	a		87.0	a	
3	39.9	b	(79)	66.9	ab	(97)	39.6	d	(64)	62.8	ab	(88)	nsa			57.8	a	(98)	83.4	a	(105)	76.3	bc	(88)
21	40.7	b	(80)	76.6	a	(111)	53.5	bc	(87)	68.3	ab	(96)	50.8	a	(94)	65.0	a	(110)	81.1	a	(102)	81.2	ab	(93)

[a] Fruit from different harvests (C1−4) after sorting according to peel color categories (G, green; B, breaker; P, pink; R, red). Abbreviations: nsa, no sample available; tr, traces. Different letters (a−d) refer to significant differences ($P \leq 0.05$) due to storage (data of days 8 and 13 not shown).

browning by anthocyanin degradation products[12] was not supported. In fact, anthocyanins rather acted as natural antioxidants as shown by model systems in vitro.[13,36] Despite rather constant PPO activity during monitored on-tree maturation[1] and (−)-epicatechin excess (Tables 1 and 2), increasing anthocyanin contents at harvest reduced the degradation of the flavanol monomer (Tables 5 and 6). In return, higher amounts of anthocyanins were co-oxidized, until a maximum was reached. The absolute amounts of anthocyanins degraded in red pericarp ('Chacapat', 0.2−0.6 g/kg; 'Hong Huey', 0.6−0.9 g/kg) were of the same magnitude as in pink pericarp ('Chacapat', 0.8 g/kg; 'Hong Huey', 0.5−0.8 g/kg), thus being almost constant in pink (C2/3P, H3/4P) and red variants (C3/4R, H4/5R), respectively. Liu et al.[13] observed such plateaus in vitro for molar ratios of cyanidin 3-*O*-rutinoside to (−)-epicatechin of >2:5. In vivo, molar ratios of >3:100 displayed by ripe to over-ripe, pink to red fruit (H3P/R, H4P/R, H5R and C2P, C3P/R, C4R) were already adequate. Consequently, harvest of pink to red-shelled litchis should be preferred to harvest of greenish-red breaker fruit in terms of both aril[1] and pericarp quality and storability.

In conclusion, the abundance of *ortho*-diphenolic structures, especially enormous contents of (−)-epicatechin, various mainly A-type-linked procyanidins, cyanidins, and flavonols, constitutes the typical polyphenol pattern of litchi pericarp, besides the absence of phenolic acids. Maturity-dependent pericarp color at harvest was directly linked to accumulation of anthocyanins and degradation of chlorophylls on the tree. In contrast, postharvest pericarp browning could not be deduced from pericarp pigment composition, because brown fruit still contained significant amounts of anthocyanins or chlorophylls, whereas brown polymers masking these pigments were not extractable. Cold storage affected only a few components, chiefly (−)-epicatechin. This indicated highly specific oxidation of the latter catalyzed by PPO. Antioxidant activity of anthocyanins restricted (−)-epicatechin

degradation, thus making red fruit more suitable for long supply chains. Postharvest processes should henceforth focus on inhibition of PPO-induced (−)-epicatechin oxidation and minimization of matrix-bound brown polymers because of their masking effect.

■ ASSOCIATED CONTENT

Supporting Information. Three supplemental tables including retention times, mass spectral data, and fragmentation patterns of all flavan-3-ols **1**−**22** (Table S1), as well as retention times, absorbance maxima, and ESI-MS^n data of all flavonols **23**−**30** (Table S2) and anthocyanins **31**−**36** (Table S3). This material is available free of charge via the Internet at http://pubs.acs.org.

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■ REFERENCES

(1) Reichel, M.; Carle, R.; Sruamsiri, P.; Neidhart, S. Influence of harvest maturity on quality and shelf-life of litchi fruit (*Litchi chinensis* Sonn.). *Postharvest Biol. Technol.* **2010**, *57*, 162–175.

(2) Lee, H. S.; Wicker, L. Anthocyanin pigments in the skin of lychee fruit. *J. Food Sci.* **1991**, *56*, 466−468, 483.

(3) Rivera-López, J.; Ordorica-Falomir, C.; Wesche-Ebeling, P. Changes in anthocyanin concentration in lychee (*Litchi chinensis* Sonn.) pericarp during maturation. *Food Chem.* **1999**, *65*, 195–200.

(4) Underhill, S. J. R.; Critchley, C. The physiology and anatomy of lychee (*Litchi chinensis* Sonn.) pericarp during fruit development. *J. Hortic. Sci.* **1992**, *67*, 437–444.

(5) Sarni-Manchado, P.; Le Roux, E.; Le Guernevé, C.; Lozano, Y.; Cheynier, V. Phenolic composition of litchi fruit pericarp. *J. Agric. Food Chem.* **2000**, *48*, 5995–6002.

(6) Liu, L.; Cao, S.; Xu, Y.; Zhang, M.; Xiao, G.; Deng, Q.; Xie, B. Oxidation of (−)-epicatechin is a precursor of litchi pericarp enzymatic browning. *Food Chem.* **2010**, *118*, 508–511.

(7) Prasad, K. N.; Yang, B.; Zhao, M.; Ruenroengklin, N.; Jiang, Y. Application of ultrasonication or high-pressure extraction of flavonoids from litchi fruit pericarp. *J. Food Process Eng.* **2009**, *32*, 828–843.

(8) Jiang, Y. Role of anthocyanins, polyphenol oxidase and phenols in lychee pericarp browning. *J. Sci. Food Agric.* **2000**, *80*, 305–310.

(9) Zhang, Z.; Pang, X.; Xuewu, D.; Ji, Z.; Jiang, Y. Role of peroxidase in anthocyanin degradation in litchi fruit pericarp. *Food Chem.* **2005**, *90*, 47–52.

(10) Underhill, S.; Critchley, C. Anthocyanin decolorisation and its role in lychee pericarp browning. *Aust. J. Exp. Agric.* **1994**, *34*, 115–122.

(11) Ruenroengklin, N.; Yang, B.; Lin, H.; Chen, F.; Jiang, Y. Degradation of anthocyanin from litchi fruit pericarp by H_2O_2 and hydroxyl radical. *Food Chem.* **2009**, *116*, 995–998.

(12) Zhang, Z.; Pang, X.; Ji, Z.; Jiang, Y. Role of anthocyanin degradation in litchi pericarp browning. *Food Chem.* **2001**, *75*, 217–221.

(13) Liu, L.; Cao, S.; Xie, B.; Sun, Z.; Wu, J. Degradation of cyanidin 3-rutinoside in the presence of (−)-epicatechin and litchi pericarp polyphenol oxidase. *J. Agric. Food Chem.* **2007**, *55*, 9074–9078.

(14) Lee, H. S.; Wicker, L. Quantitative changes in anthocyanin pigments of lychee fruit during refrigerated storage. *Food Chem.* **1991**, *40*, 263–270.

(15) Sun, J.; Xiang, X.; Yu, C.; Shi, J.; Peng, H.; Yang, B.; Yang, S.; Yang, E.; Jiang, Y. Variations in contents of browning substrates and activities of some related enzymes during litchi fruit development. *Sci. Hortic.* **2009**, *120*, 555–559.

(16) Sun, J.; Jiang, Y.; Shi, J.; Wei, X.; Xue, S. J.; Shi, J.; Yi, C. Antioxidant activities and contents of polyphenol oxidase substrates from pericarp tissues of litchi fruit. *Food Chem.* **2010**, *119*, 753–757.

(17) Le Roux, E.; Doco, T.; Sarni-Manchado, P.; Lozano, Y.; Cheynier, V. A-type proanthocyanidins from pericarp of *Litchi chinensis*. *Phytochemistry* **1998**, *48*, 1251–1258.

(18) Zhang, Z.; Xuequn, P.; Yang, C.; Ji, Z.; Jiang, Y. Purification and structural analysis of anthocyanins from litchi pericarp. *Food Chem.* **2004**, *84*, 601–604.

(19) Schütz, K.; Kammerer, D.; Carle, R.; Schieber, A. Identification and quantification of caffeoylquinic acids and flavonoids from artichoke (*Cynara scolymnus* L.) heads, juice and pomace by HPLC-DAD-ESI/MS^n. *J. Agric. Food Chem.* **2004**, *52*, 4090–4096.

(20) Kurz, C.; Carle, R.; Schieber, A. HPLC-DAD-MS^n characterisation of carotenoids from apricots and pumpkins for the evaluation of fruit product authenticity. *Food Chem.* **2008**, *110*, 522–530.

(21) Zhang, D.; Quantick, P. C.; Grigor, J. M. Changes in phenolic compounds in litchi (*Litchi chinensis* Sonn.) fruit during postharvest storage. *Postharvest Biol. Technol.* **2000**, *19*, 165–172.

(22) Gu, L.; Kelm, M. A.; Hammerstone, J. F.; Zhang, Z.; Beecher, G.; Holden, J.; Haytowitz, D.; Prior, R. L. Liquid chromatographic/electrospray ionization mass spectrometric studies of proanthocyanidins in foods. *J. Mass Spectrom.* **2003**, *38*, 1272–1280.

(23) Karchesy, J. J.; Foo, L. Y.; Barofsky, E.; Arbogast, B.; Barofsky, D. F. Negative-ion fast-atom-bombardment mass spectrometry of procyanidin oligomers. *J. Wood Chem. Technol.* **1989**, *9*, 313–331.

(24) Prasad, K. N.; Yang, B.; Yang, S.; Chen, Y.; Zhao, M.; Ashraf, M.; Jiang, Y. Identification of phenolic compounds and appraisal of antioxidant and antityrosinase activities from litchi (*Litchi chinensis* Sonn.) seeds. *Food Chem.* **2009**, *116*, 1–7.

(25) Xu, X.; Xie, H.; Wang, Y.; Wei, X. A-type proanthocyanidins from lychee seeds and their antioxidant and antiviral activities. *J. Agric. Food Chem.* **2010**, *58*, 11667–11672.

(26) Jaiswal, B. P.; Sah, N. L.; Prasad, U. S. Regulation of colour break during litchi (*Litchi chinensis* Sonn.) ripening. *Indian J. Exp. Biol.* **1987**, *25*, 66–72.

(27) Pati, S.; Losito, I.; Gambacorta, G.; La Notte, E.; Palmisano, F.; Zambonin, P. G. Simultaneous separation and identification of oligomeric procyanidins and anthocyanin-derived pigments in raw red wine by HPLC-UV-ESI-MS^n. *J. Mass Spectrom.* **2006**, *41*, 861–871.

(28) Harris, P. G.; Carter, J. F.; Head, R. N.; Harris, R. P.; Eglinton, G.; Maxwell, J. R. Identification of chlorophyll transformation products in zooplankton faecal pellets and marine sediment extracts by liquid chromatography/mass spectrometry atmospheric pressure chemical ionization. *Rapid Commun. Mass Spectrom.* **1995**, *9*, 1177–1183.

(29) Gauthier-Jaques, A.; Bortlik, K.; Hau, J.; Fay, L. B. Improved method to track chlorophyll degradation. *J. Agric. Food Chem.* **2001**, *49*, 1117–1122.

(30) van Breemen, R. B.; Canjura, F. L.; Schwartz, S. J. Identification of chlorophyll derivates by mass spectrometry. *J. Agric. Food Chem.* **1991**, *39*, 1452–1456.

(31) Gu, L.; Kelm, M. A.; Hammerstone, J. F.; Zhang, Z.; Beecher, G.; Holden, J.; Haytowitz, D.; Prior, R. L. Screening of foods containing proanthocyanidins and their structural characterization

using LC-MS/MS and thiolytic degradation. *J. Agric. Food Chem.* **2003**, *51*, 7513–7521.

(32) Menzel, C. M.; Simpson, D. R. Performance and improvement of lychee cultivars: a review. *Fruit Varieties J.* **1990**, *44*, 197–215.

(33) Fechner, G. T. *Elemente der Psychophysik*; Breitkopf und Härtel: Leipzig, Germany, 1860; Vol. 1−2.

(34) Stintzing, F. C.; Carle, R. Functional properties of anthocyanins and betalains in plants, food, and in human nutrition. *Trends Food Sci. Technol.* **2004**, *15*, 19–38.

(35) Esen, A. β-Glucosidase. In *Handbook of Food Enzymology*; Whitaker, J. R., Voragen, A. G. J., Wong, D. W. S., Eds.; Dekker: New York, 2003; pp 791−803.

(36) Sadilová, E.; Carle, R.; Stintzing, F. C. Thermal degradation of anthocyanins and its impact on color and in vitro antioxidant capacity. *Mol. Nutr. Food Res.* **2007**, *51*, 1461–1471.

(37) He, F.; Pan, Q. H.; Shi, Y.; Zhang, X. T.; Duan, C. Q. Identification of autoxidation oligomers of flavan-3-ols in model solutions by HPLC-MS/MS. *J. Mass Spectrom.* **2009**, *44*, 633–640.