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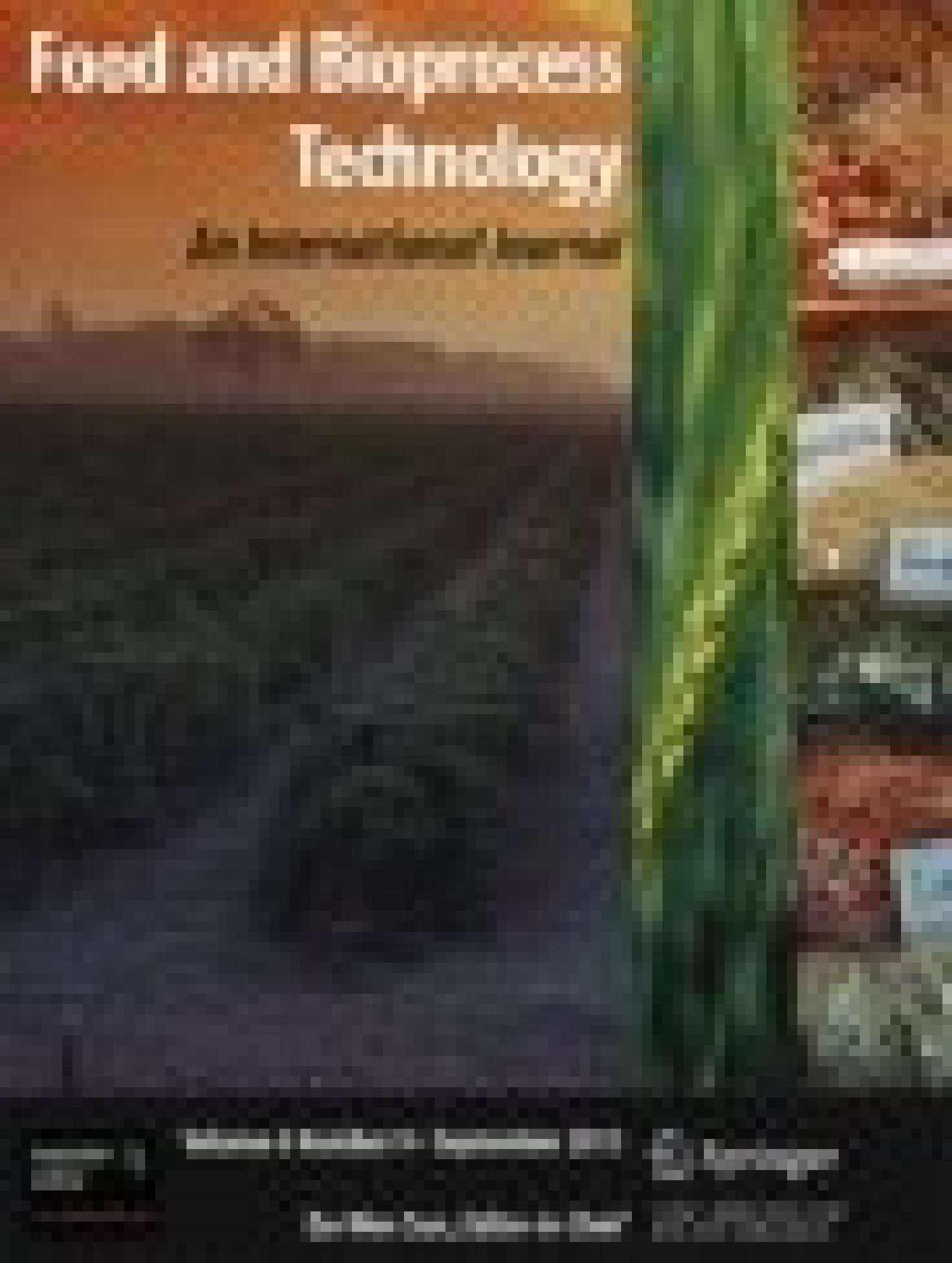

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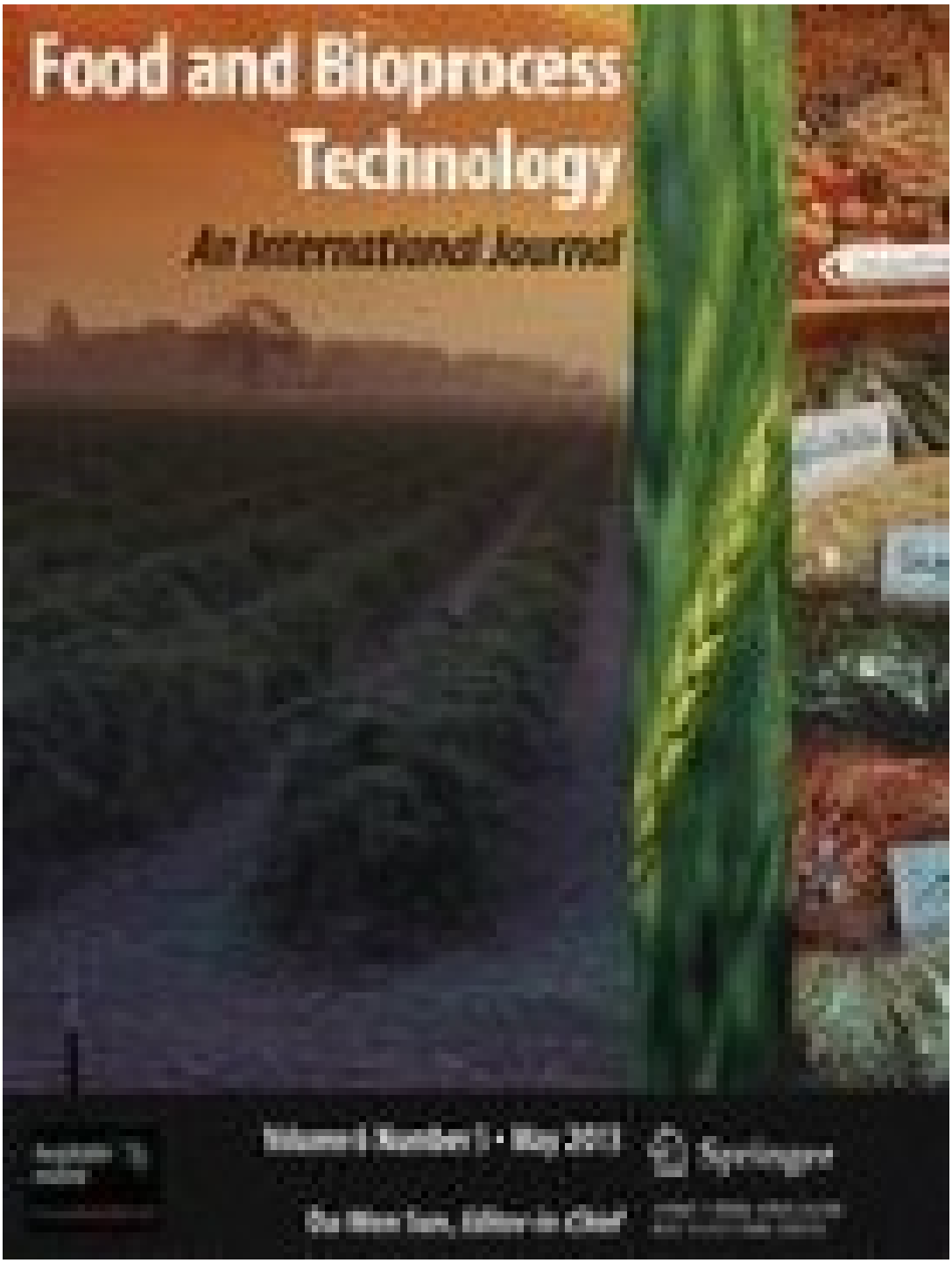

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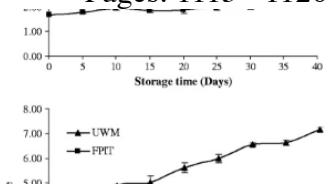

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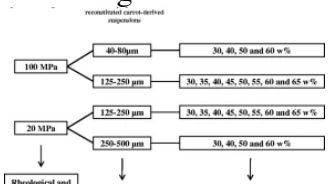

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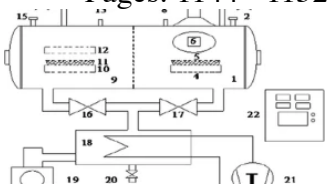

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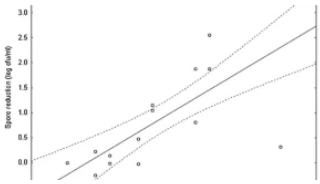

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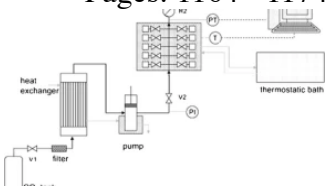

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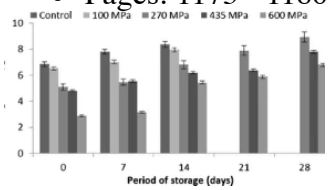

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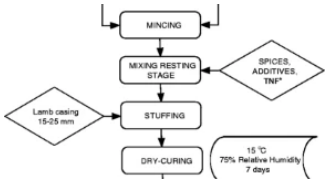

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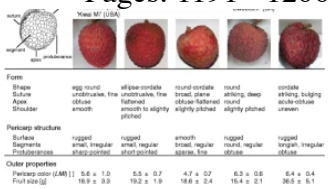

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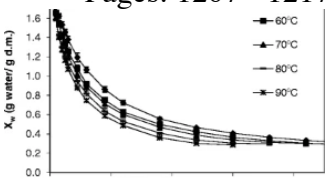

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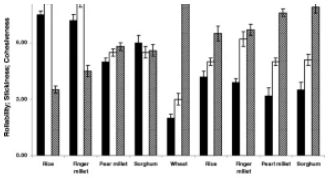

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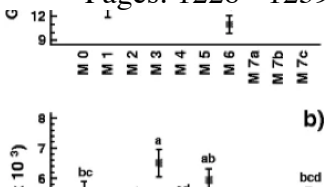

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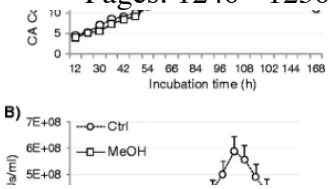

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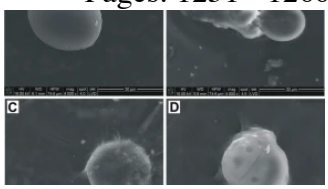

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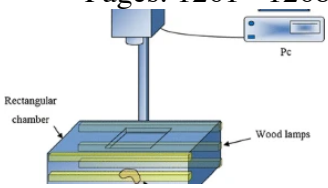

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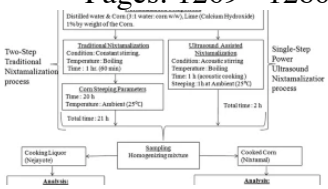

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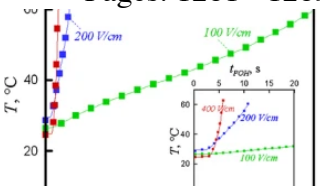

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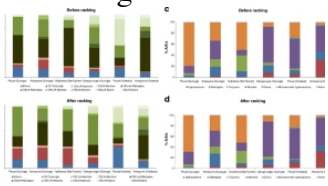

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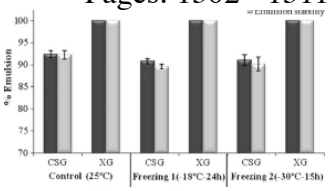

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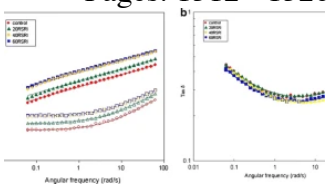

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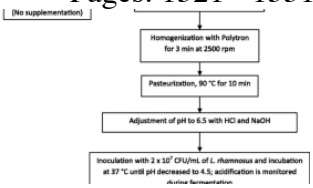

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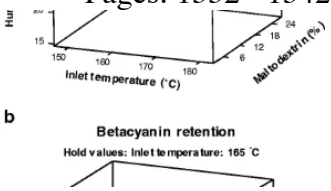

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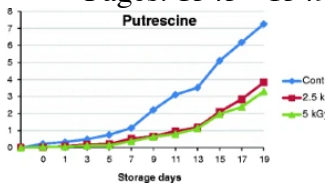

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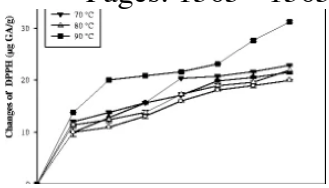

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ORIGINAL PAPER

Vital Characteristics of Litchi (*Litchi chinensis* Sonn.) Pericarp that Define Postharvest Concepts for Thai Cultivars

Mareike Reichel · Rini Triani · Julia Wellhöfer · Pittaya Sruamsiri · Reinhold Carle · Sybille Neidhart

Received: 21 September 2011 / Accepted: 12 December 2011

Abstract To assess the fruit-specific determinants of pericarp browning, litchi pericarp was characterized in terms of appearance, the polyphenol pattern as specified by HPLC-DAD-MS^n without and after thiolysis, and the activities of polyphenol oxidase (PPO) and peroxidase (POD) by exploring "Kwang Jao," "O-Hia," "Kim Cheng," and "Chacapat" fruit on the respective harvest day, "Hong Huey" fruit also throughout 52 days of cold storage (5 °C, 95% relative humidity). At harvest, PPO activity was maximum for "Kim Cheng" pericarp (126 μkat/hg), whereas POD activity was striking for that of "O-Hia" (512 μkat/hg, including membrane-bound isoforms). Flavan-3-ol and proanthocyanidin patterns were consistent for all cultivars. However, cultivars with sharp-pointed and round–obtuse protuberances differed in pericarp anthocyanin and flavonol glycosylation patterns. The molar ratio of cyanidin 3-*O*-rutinoside to its glucoside was ≤6:1 for "Hong Huey" and "Kwang Jao," but ≥43:1 for "Kim Cheng" and "Chacapat" pericarp. Long-term storage gave evidence of two key processes involved in pericarp browning: (1) PPO-mediated oxidation of abundant (−)-epicatechin (1.4–2.0 g/hg), resulting in dark brown pigments, and (2) microcrack-induced formation of light brown surface scurf, supposably with involvement of POD. Accordingly, an improved scheme for litchi pericarp browning was proposed. As regards recommendable postharvest concepts for each cultivar, "Chacapat" suited most for long-distance transports due to its overall low susceptibility to pericarp browning. Properties of "O-Hia" litchi, being prone to surface scurf formation, suggested preferred distribution via domestic markets. High contents of flavonols (e.g., quercetin glycosides, 166 mg/hg) and A-type-linked procyanidins (e.g., procyanidin A2, 1,092 mg/hg) qualified pericarp of "Hong Huey" litchi as raw material for polyphenol extracts exerting antioxidant properties.

Keywords Enzymatic browning · Flavonoids · Fresh fruit · Peroxidase · Polyphenol oxidase · By-product valorization

M. Reichel · R. Triani · J. Wellhöfer · R. Carle · S. Neidhart (✉)
Institute of Food Science and Biotechnology,
Chair of Plant Foodstuff Technology, Hohenheim University,
Garbenstrasse 25,
70599 Stuttgart, Germany
e-mail: sybille.neidhart@uni-hohenheim.de

P. Sruamsiri
Department of Crop Science and Natural Resources,
Faculty of Agriculture, Chiang Mai University,
Chiang Mai 50200, Thailand

Present Address:
R. Triani
School of Life Science and Technology,
Bandung Institute of Technology,
Jalan Tamansari Nomor 64,
Bandung 40116, Indonesia

Introduction

Litchi (*Litchi chinensis* Sonn.) fruit production has considerable commercial relevance in Thailand, particularly in the northern provinces where 91% of the fruit was harvested in 2008 (CAI and OAE 2009). The nation's litchi crop of that year was estimated at 53,175 t with a farm gate value of ~US $16 million (CAI and OAE 2009; Bank of Thailand 2011). Fruit was mainly sold in domestic markets with marginal added value. Only 5,880 t of fresh litchi were exported in 2008, but totaled ~US$5 million (MOC 2011). Besides exports within Southeast Asia and to China (50%), minor quantities were sold to markets in Europe (36%), Oceania (6%), Western Asia (4%), and Northern America (3%) (MOC 2011). Exports to distant markets are notably

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impaired by fruit quality beyond legal and customers' requirements of the importing countries due to fast pericarp browning (Reichel et al. 2010) or noxious residues from common sulfur dioxide and fungicide treatments. Besides predominant fresh fruit marketing, litchi is processed into juices, purees (Neidhart et al. 2007), canned (Hoppe et al. 2006), frozen, and dried fruit (Nagle et al. 2011). Pericarp percentages ≥15% of litchi fresh weight result in notable processing waste volumes that are hardly absorbed by any utilization concept (Nagle et al. 2011).

Rapid postharvest pericarp browning (Reichel et al. 2010) is mainly caused by polyphenol oxidase (PPO)-catalyzed oxidation of (−)-epicatechin that abounds in the pericarp (Liu et al. 2010; Reichel et al. 2011). Pinkish-red anthocyanins, providing the attractive pericarp color, are weak substrates of litchi PPO, but function as antioxidants (Liu et al. 2007b; Reichel et al. 2011). In contrast, flavonols and oligomeric proanthocyanidins (Sarni-Manchado et al. 2000) seem to have minor importance for the appearance of the litchi fruit in view of their poor quantitative changes during fruit storage (Reichel et al. 2011). However, they contribute to the quality of litchi pericarp that might be destined for polyphenol extracts. Such litchi-derived preparations, particularly those being rich in flavanol oligomers, are expected to have health-promoting potential as regards lifestyle-related and obesity-derived diseases through their effect on lipid metabolism (Miura et al. 2010) and might be produced from by-products of litchi processing. Hence, postharvest concepts for litchi fruit intended for both fresh fruit markets and processing must consider initial pericarp quality in terms of polyphenols and browning enzymes. However, comprehensive characterization of litchi pericarp polyphenol patterns has so far been limited to a few cultivars (Sarni-Manchado et al. 2000; Reichel et al. 2011) and has hardly been linked with enzymatic studies (Liu et al. 2010). PPO activity has largely been determined, using the usual standard substrates, which are rather unspecific for litchi pericarp PPO (Underhill and Critchley 1995; Jiang and Fu 1999; Ducamp-Collin et al. 2007; Reichel et al. 2010), and the role of peroxidase (POD) in browning processes has not yet been fully elucidated (Reichel et al. 2011).

However, recent exploration of the impacts exerted by on-tree maturation and cold storage on fruit quality (Reichel et al. 2010) and pericarp polyphenol pattern (Reichel et al. 2011) of two litchi cultivars indicated some cultivar-specific differences in the initial resistance to pericarp desiccation and browning of the fruit during cold storage without packaging and in the flavonol and anthocyanin patterns. Therefore, the present study aimed at the classification of five major northern Thai cultivars according to their pericarp properties at harvest in order to assess their individual susceptibility to pericarp browning and the respective fruit-specific determinants. Activities of pericarp PPO and POD were thus appraised in detail. Concurrently, polyphenol patterns and quantities were specified by HPLC-DAD-MS^n, the proanthocyanidins also after thiolytic pericarp degradation. In addition, the stability of all flavonoids throughout long-term fruit storage was exemplarily substantiated for "Hong Huey" and related to respective enzyme activities in the pericarp. By this multistage approach, theories concerning pericarp browning were to be verified, aiming at suitable approaches for retardation of pericarp browning. Depending on their individual pericarp characteristics, appropriate marketing strategies were finally to be deduced for the cultivars studied.

Materials and Methods

Chemicals

Unless otherwise stated or specified elsewhere (Reichel et al. 2010, 2011), reagents and solvents were of analytical or gradient grade and supplied by VWR International (Darmstadt, Germany), benzyl mercaptan, polyvinylpolypyrrolidone (PVPP), and Triton X-100 [4-(1,1,3,3-tetramethylbutyl)phenyl-polyethylene glycol] by Sigma-Aldrich (St. Louis, MO, USA). (−)-Epicatechin benzyl thioether was kindly provided by Matthias Fromm (Hohenheim University). Deionized water was used throughout.

Plant Material

Mature, red-colored fruit of *Litchi chinensis* Sonn. cultivars "O-Hia," "Kwang Jao," "Kim Cheng," and "Chacapat" (1 kg per variety) was obtained from a local fresh fruit market in Chiang Mai, Northern Thailand. Fruit had been harvested in Fang, Northern Thailand ("O-Hia" and "Kwang Jao" on 15 May 2008; "Kim Cheng" and "Chacapat" on 2 June 2008), and transported to the market on the same day in traditional bamboo baskets. "Hong Huey" litchi fruit (~12 kg) was harvested at commercial maturity (3 and 8 May 2008; 95 and 100 days after anthesis) from five trees of the research orchard with weather station in Mae Sa Mai, Northern Thailand, as described by Reichel et al. (2010). Harvested fruit lots were precooled during transportation (≤1 h) as detailed previously (Reichel et al. 2010). After debranching in the laboratory (20 °C), litchis of all cultivars were sorted for uniformity and soundness. A litchi maturity index [LMI=10 a^* $(a^{*2}+L^{*2})^{-0.5}$] on the basis of the pericarp color, which was measured with a CR 300 colorimeter (Minolta, Osaka, Japan; CIELab, D65 illuminant, 2° standard observer angle), and fruit size were determined for 25 fruits of each cultivar as reported by Reichel et al. (2010). To obtain pericarp samples, further 20–25 shock-frozen fruits were peeled after immersion into liquid nitrogen and

subsequently lyophilized, stored, and pulverized as described by Reichel et al. (2011). Additionally, half of the "Chacapat" pericarp pieces were kept at −20 °C before lyophilization, so that the white, spongy tissue could manually be removed from the outer red part of the pericarp for separate polyphenol analyses of both pericarp parts.

To monitor pericarp browning during cold fruit storage, "Hong Huey" fruit was stored in open mash baskets (21×16×12 cm; ~2 kg/basket) in a climate simulator (CAT 610/620, Contherm Scientific, Lower Hutt, New Zealand) at 5 °C and 90% relative humidity (RH). Samples were taken after 0, 1, 2, 3, 8, 13, and 26 days of storage. After 52 days, the remaining fruit was sorted according to visually rated pericarp color into light brown (fruit sample 52/o) and dark brown fruit (sample 52/c), which mainly originated from the outer (52/o) and the central zones (52/c) of the storage baskets. Respective pericarp samples were prepared from the stored fruit as described above. Pericarp properties during cold fruit storage were monitored according to Reichel et al. (2010) by analysis of moisture (in grams per hectogram), thickness (in millimeters), brittleness (in newtons per millimeter) (3365 Series IX, Instron, Canton, MA, USA), the postharvest litchi color index [PLCI=$(a^{*2}+L^{*2})^{0.5}$], and the microscopic structure (MX-5040RZ, AD-5040RVS, Horox, Tokyo, Japan) of the pericarp.

Enzyme Activities

Enzyme Extraction

Pulverized pericarp (2.0 g) was extracted under continuous stirring for 2 h at 4 °C with McIlvaine buffer [citric acid (0.1 mmol/L)/sodium dihydrogen phosphate (0.2 mmol/L), 3:7 (*v*/*v*), pH 6.5; 25 g] containing 0.7 g PVPP (Reichel et al. 2010). For the assays, the extracts were diluted with McIlvaine buffer (1:2, 1:5, or 1:10), if necessary.

Pericarp of "Hong Huey," "O-Hia," and "Kim Cheng" was additionally extracted in the presence of 1% Triton X-100, which was added to solubilize plasma membrane proteins and to enhance extractability of those oxidases, which are ionically bound to the cell wall, without extensive cellular destruction (Sun et al. 2008; Ruenroengklin et al. 2009). These extracts were prepared as described above and subsequently purified on a nonpolar polystyrene adsorbent (Bio-Beads SM-2, 20–50 mesh, biotechnology grade, Bio-Rad Laboratories, Hercules, CA, USA) because Triton X-100 otherwise interferes with the photometric assays. The adsorbent (5 g) was suspended in McIlvaine buffer, degassed, and filled into a polypropylene column (1.5×12 cm, Econo-Pac, Bio-Rad Laboratories). After settling of the adsorbent, it was covered with a 30-μm polyethylene upper bed support (Bio-Rad Laboratories) and washed with two bed volumes (2×8 mL) of McIlvaine buffer. An aliquot of the enzyme extract (8 mL) was placed on top of the bed and eluted at a rate of ~0.35 mL/min. The adsorbent was finally rinsed with McIlvaine buffer (5 mL). After discarding of the first 3 mL of the eluate, 10 mL of purified enzyme extract were collected. The column was regenerated by washing with three bed volumes of methanol, deionized water, and McIlvaine buffer, respectively. For comparison, an enzyme extract obtained without Triton X-100 was always treated by analogy.

Spectrophotometric Assays

Protein contents of the enzyme extracts were quantitated, using the method of Bradford (1976) with bovine serum albumin as the standard. The PPO assay with 4-methylcatechol as the substrate ($PPO_{methcat}$) and the POD assay were performed according to Reichel et al. (2010). The latter focused on the maximum linear increase in absorbance at 418 nm during incubation of a reaction mixture, which contained tropolone (10.4 mmol/L), H_2O_2 (2.86 mmol/L), and the five or ten times diluted enzyme extract (0.2/1.5 mL) in McIlvaine buffer (pH 6.5), for 10 min at 25 °C. The reaction mixture of the PPO assay (2 mL) consisted of 4-methylcatechol (2.5 mmol/L), sodium dodecyl sulfate (SDS; 0.475 mmol/L), L-proline (50 mmol/L), and enzyme extract (0.05 L/L) in McIlvaine buffer (pH 6.5) and was analyzed at 525 nm and 25 °C. PPO activity towards the natural substrate in litchi (PPO_{epicat}) was likewise determined by incubation of a reaction mixture (2 mL), which comprised (−)-epicatechin (1.3 mmol/L), SDS (0.6 mmol/L), and the enzyme extract (0.05 L/L) without L-proline, for 10 min at 25 °C and spectrophotometric analysis at 390 nm. Activities [in microkatals per hectogram of pericarp dry weight (DW)] were computed from the maximum linear increase in absorbance (POD, $\varepsilon_{418\ nm}$=2,075 Lmol^{-1} cm^{-1}; $PPO_{methcat}$, $\varepsilon_{525\ nm}$=1,550 L mol^{-1} cm^{-1}; PPO_{epicat}, $\varepsilon_{390\ nm}$=7,022 L mol^{-1} cm^{-1}) after subtraction of the absorbance slopes recorded for two blanks without enzyme extract and substrate, respectively (spectrophotometer Cary 100, Varian, Mulgrave, Australia).

Polyphenol Analyses

Polyphenol Extraction of Litchi Pericarp

Extraction of lyophilized pericarp powder (2.5 g) according to Reichel et al. (2011) was for 30 min with 40 mL of methanol containing 0.01% (*v*/*v*) trifluoroacetic acid, followed by washing with 20 mL and further extraction (60 and 30 min) with acetone/water (7:3, *v*/*v*) including 0.1% (*v*/*v*) trifluoroacetic acid (2×40 mL), always under nitrogen protection and stirring. For polyphenol analysis without consideration of carotenoids and chlorophylls, all four extracts were combined irrespective of solvent type,

evaporated to dryness in vacuo at ambient temperature, and redissolved in 20 mL of water containing 0.01% (*v*/*v*) HCl. An aliquot was membrane-filtered (0.45 μm) for HPLC analysis of anthocyanins, flavonols, and proanthocyanidins. All samples were extracted in duplicate.

Thiolytic Degradation of Litchi Pericarp

A method based on those described by Le Roux et al. (1998), Ferreira et al. (2002), and Gu et al. (2002) was used for thiolytic degradation of litchi pericarp proanthocyanidins. Benzyl mercaptan (5% *v*/*v*) and concentrated HCl (3.3% *v*/*v*) dissolved in 800 and 400 μL of methanol, respectively, were added to 20 mg of dry pericarp powder. The suspension was heated to 90 °C under nitrogen protection in a thermoblock (TM-S-300-36/MHB-36-16, Liebisch Labortechnik, Bielefeld, Germany). After a dwell period of 2 min at this temperature, incubation at 22 °C followed for 10 h to ensure complete degradation. The mixture was membrane-filtered (0.45 μm) and directly analyzed by HPLC. Thiolytic degradation of samples was performed in duplicate.

HPLC-DAD and HPLC-DAD-MS^n Analyses of Polyphenols and Thiolytic Degradation Products

HPLC-DAD and HPLC-DAD-MS^n analyses of anthocyanins, flavonols, and proanthocyanidins were carried out as described by Reichel et al. (2011) on a series 1100 HPLC system (Hewlett Packard, Waldbronn, Germany) that was coupled online to an Esquire 3000+ ion trap mass spectrometer (Bruker, Bremen, Germany) with an electrospray ionization (ESI) source. Separation of the analytes was achieved at a flow rate of 0.4 mL/min on a 150×3.0 mm i.d., 4 μm, Synergi 4u Hydro-RP 80A column (Phenomenex, Torrance, CA, USA), using two different gradient methods (Reichel et al. 2011) for proanthocyanidins and flavonols [eluents A_1, 2% (*v*/*v*) acetic acid in water; B_1, acetonitrile/0.5% acetic acid in water (50:50, *v*/*v*)] and anthocyanins [water/formic acid/acetonitrile eluents A_2 (87:10:3, *v*/*v*/*v*), B_2 (40:10:50, *v*/*v*/*v*)], respectively. Proanthocyanidins were monitored at 280 nm, flavonols at 370 nm, and anthocyanins at 520 nm. With parameters set as detailed previously (Reichel et al. 2011), negative ion mass spectra were recorded for proanthocyanidins and flavonols, whereas the positive mode was chosen for anthocyanins.

In addition, separation of the thiolytic degradation products was achieved at 25 °C and a flow rate of 0.4 mL/min on the same device, but with 2% (*v*/*v*) acetic acid in water (eluent A_3) and methanol/methyl *tert*-butyl ether/acetic acid (94.5:5:0.5, *v*/*v*/*v*; eluent B_3). The gradient was from 10% to 21% B_3 (6 min), from 21% to 45% B_3 (24 min), from 45% to 60% B_3 (10 min), from 60% to 72% B_3 (10 min), from 72% to 100% B_3 (20 min), 100% B_3 isocratic (2 min), and from 100% to 10% B_3 (1 min) with a total run time of 75 min. Flavanols, proanthocyanidins, and their benzyl thioethers were monitored at 280 nm. (−)-ESI-MS^n parameters were set as described for proanthocyanidins (Reichel et al. 2011). To optimize the detection of the flavanol benzyl thioethers, additional mass spectra were recorded at 50% compound stability and 25% ion trap drive according to Gu et al. (2002).

Polyphenols were quantified by means of seven- to ten-point calibration curves of respective standard substances. When reference substances were unavailable, calibration of structurally related compounds was considered 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 and their benzyl thioethers as (−)-epicatechin (10–4,000 mg/L), oligoflavanols and diflavanols and their benzyl thioethers as procyanidin A2 (0.1–1,000 mg/L).

Statistical Analysis

Significant effects and changes (P=0.05) were identified by means of Tukey's multiple comparison tests, using SAS 9.1 software (SAS Institute, Cary, NC, USA).

Results and Discussion

Cultivars Under Study

The five litchi cultivars "Hong Huey," "Kwang Jao," "O-Hia," "Kim Cheng," and "Chacapat," being of commercial relevance in Northern Thailand, were characterized in terms of their visual and chemical pericarp characteristics. They were easily distinguishable from each other by morphological features (Fig. 1), as described by the Thai Plant Germplasm Database (Plant Varieties Protection Division 2003). The pericarp structure was of particular interest because the shape of skin segments and protuberances is a reliable and stable genetic characteristic that hardly varies due to climate, soil type, and orchard management (Menzel and Simpson 1990). This feature even indicates revealing relationships among cultivars of different growing regions (Fig. 1). "Hong Huey" and "Kwang Jao" had pointed protuberances and "Kim Cheng" and "Chacapat" had obtuse ones, whereas skin segments of "O-Hia" were flat. Consistent with pericarp structure, fruit of the three types significantly differed in color on the harvest day, as numerically described by the LMI (Reichel et al. 2010) (Fig. 1). Whereas "O-Hia" pericarp was less red (minimum LMI of 4.7), "Kim Cheng" and "Chacapat" fruit had a darker red pericarp (maximum LMI of 6.4) than "Hong Huey" and "Kwang Jao" litchi (LMI=5.6). However, fruit

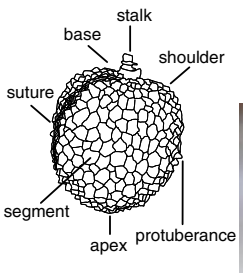
Cultivar	'Hong Huey'	'Kwang Jao'	'O-Hia'	'Kim Cheng'	'Chacapat'
		'Tai So' (AU), 'Da Zhao' (CN), 'Mauritius' (SA), 'Kwai Mi' (USA)	'Haak Yip' (AU), 'Heiye' (CN)	'Wei Chee' (AU), 'Huaizhi' (CN), 'Sweetcliff' (SA)	'Emperor'
					
Form					
Shape	egg round	ellipse-cordate	round-cordate	round	cordate
Suture	unobtrusive, fine	unobtrusive, fine	broad, plane	striking, deep	striking, bulging
Apex	obtuse	flattened	obtuse-flattened	round	acute-obtuse
Shoulder	smooth	smooth to slightly pitched	slightly pitched	slightly pitched	uneven
Pericarp structure					
Surface	rugged	rugged	smooth	rugged	rugged
Segments	small, irregular	small, regular	broad, regular	round, regular	longish, irregular
Protuberances	sharp-pointed	short-pointed	sparse, fine	obtuse	obtuse
Outer properties					
Pericarp color (*LMI*) [ ]	5.6 ± 1.0	5.5 ± 0.7	4.7 ± 0.7	6.3 ± 0.6	6.4 ± 0.4
Fruit size [g]	18.9 ± 3.3	19.2 ± 1.9	18.6 ± 2.4	15.4 ± 2.1	36.5 ± 5.1
Enzyme activities in the pericarp [μkat/hg of dry weight]					
POD	49.5 ± 2.9	125.3 ± 0.7	155.5 ± 5.7	44.8 ± 0.04	47.1 ± 0.7
$PPO_{methcat}$	2.25 ± 0.03	1.45 ± 0.03	1.55 ± 0.06	1.64 ± 0.06	1.18 ± 0.06
PPO_{epicat}	27.7 ± 1.1	31.6 ± 0.2	51.8 ± 0.6	125.7 ± 0.8	50.0 ± 0.1

Fig. 1 Characterization of northern Thai litchi cultivars [synonymic denominations and their origins according to Menzel and Simpson (1990)]: illustration (not to scale), description, pericarp color as average litchi maturity index (LMI; $n=3\times25$), fruit size as average fruit mass ($n=25$), and activities of peroxidase (POD) and polyphenol oxidase, the latter determined either with (−)-epicatechin (PPO_{epicat}) or 4-methylcatechol ($PPO_{methcat}$) as the substrate ($n=2$). All values are given as the mean±standard deviation

size differed most between small "Kim Cheng" and big "Chacapat" fruit (Fig. 1). Nevertheless, all fruit batches were of commercial grade "A," irrespective of the cultivar, when obtained from local markets as presorted fruit. Color and size of "Chacapat" litchi conformed to data obtained 1 year before for red fruit from the same region on the harvest day (Reichel et al. 2010). Ripe "Hong Huey" fruit harvested in 2007 at a similar pericarp color had been slightly larger (21.3 g; Reichel et al. 2010) than the fruit of season 2008 (Fig. 1) from the same orchard. During the last 10 days before harvest, the rainfall measured there in 2007 was >4 times as much as in the following year.

Activities of Browning Enzymes on the Harvest Day

Remarkable POD activity was noticed for the pericarp of "O-Hia" fruit, followed by "Kwang Jao," whereas the oxidoreductase was less active in the other three cultivars (Fig. 1). The widely applied PPO enzyme assay based on 4-methylcatechol as the substrate revealed only low PPO activities in litchi pericarp ($PPO_{methcat}$ in Fig. 1). The preponderance of POD confirmed previous results for "Hong Huey" and "Chacapat" pericarp (Reichel et al. 2010) and was in accordance with studies of litchi from Florida (Huang et al. 1990), "Kwai May" from Reunion, and "Wai Chee" from Spain (Ducamp-Collin et al. 2007). However, as expected from literature (Liu et al. 2007a), higher activities than in the presence of 4-methylcatechol were measured throughout, when the natural substrate (−)-epicatechin (Reichel et al. 2011) was used (Fig. 1). Furthermore, the activities towards the different substrates ($PPO_{methcat}$, PPO_{epicat}) did not correlate, confirming the high specificity of litchi pericarp PPO for (−)-epicatechin and the unreliability of the 4-methylcatechol-based assay. In the presence of (−)-epicatechin, PPO of "Kim Cheng" pericarp was more than twice as active as that of "O-Hia" and "Chacapat" peel, and its activity was even three to four times higher than those of the cultivars with spiky skin segments ("Hong Huey," "Kwang Jao"). Only in the pericarp of "Kim Cheng" and "Chacapat," which were the two cultivars having obtuse protuberances as well as high genetic similarity (Cutler et al. 2006), the activity of PPO (PPO_{epicat}) surpassed that of POD.

To appraise the percentages of membrane-bound enzymes and those noncovalently linked with the cell wall, pericarp samples were also extracted with the addition of Triton X-100, using those of "Hong Huey," "O-Hia," and "Kim Cheng" as examples. In this way, 4- to 11-fold amounts of protein were solubilized (variants +T/P in Table 1). The purification method, which had been found to satisfactorily remove the octylphenol ethylene oxide condensate from the extract (data not shown), significantly reduced PPO activity in all extracts (variants −T/P vs. −T in Table 1). Detergent-assisted extraction (variants +T/P) did not increase PPO activity compared to extraction with pure buffer (−T/P), thus suggesting that litchi pericarp PPO mainly exists as a soluble fraction in the cytoplasm. In contrast, POD activity per hectogram of pericarp rose to 2.5- to 3.1-fold levels, when Triton X-100 was added to the extraction buffer (+T/P vs. −T/P in Table 1). Especially in "Chacapat" pericarp, bound POD forms were prevailing (+T/P vs. −T/P in Table 1), leading to total POD activities comparable to those in "Hong Huey." The pericarp sample of the latter fruit, which had been harvested later than the "Hong Huey" fruit batch of Fig. 1 analyzed before, showed similar POD activity (−T in Table 1) as the other cultivar with spiky pericarp ("Kwang Jao"; Fig. 1) did and twice as much soluble POD as "Chacapat" (−T in Table 1). Maximum total POD activity (512 μkat/hg) was again observed for the flat "O-Hia" pericarp, with the ratio of soluble to bound POD being 1:2. For "Huaizhi" and "Nuomici," Huang et al. (2006) had detected soluble and ionically wall-bound POD at a ratio of ~5:3.

Litchi Pericarp Polyphenols

The total polyphenol contents in litchi pericarp [sum of flavanols (Table 2), flavonols, and anthocyanins (Table 3)] ranged from 82 g/kg of DW for "Chacapat" to 93 g/kg for "Kim Cheng." The polyphenol pattern of "Hong Huey" and "Chacapat" pericarp had comprehensively been characterized by HPLC-ESI-MS^n for fruit harvested in year 2007 (Reichel et al. 2011). With fruit of season 2008, analysis of the pericarp flavonoids of these two cultivars revealed the same compounds (Tables 2 and 3). Particularly in "Chacapat" pericarp, contents were overall below the levels previously reported for the red fruit of crop year 2007. However, the main harvest season of year 2008 began approximately 2 weeks earlier than in the prior year. Moreover, "Chacapat" fruit was obtained from different orchards in both years. Various environmental factors, such as light stress and shading, day and night temperature, solar radiation, as well as nitrogen, water, and inorganic phosphate deficiency exert significant influence on proanthocyanidin biosynthesis (He et al. 2008). Since the flavan-3-ol derivatives (Table 2) always accounted for 96–97% of the total litchi pericarp flavonoids, differences in total polyphenol contents of litchi pericarp among the cultivars were mainly attributed to environmental factors. As reported previously (Liu et al. 2010; Reichel et al. 2011), the predominant polyphenol compound in litchi pericarp was (−)-epicatechin, being maximum for "Kwang Jao" with 20 g/kg DW. The general prevalence of *ortho*-diphenolic structures (Sarni-Manchado et al. 2000; Reichel et al. 2011) was confirmed because (epi)

Table 1 Activities of polyphenoloxidase (PPO_{epicat}) and peroxidase (POD) per extracted protein and per dry weight of "Hong Huey," "O-Hia," and "Chacapat" litchi pericarp after extraction without (−T) or with (+T) Triton X-100 and subsequent purification (P) on polystyrene-divinyl-benzene adsorbent columns

		Protein		PPO_{epicat}				POD			
		In the extract		Per pericarp		Per protein		Per pericarp		Per protein	
		[mg/mL]	[%][a]	[μkat/hg]	[%][a]	[μkat/hg]	[%][a]	[μkat/hg]	[%][a]	[μkat/hg]	[%][a]
"Hong Huey"	−T	0.04[b]	(100)	43.77±0.56	(100)	105.85±1.36	(100)	189.57±2.32	(100)	458.51±5.62	(100)
	−T/P	0.05[b]	(110)	3.34±0.02	(8)	5.83±0.04	(6)	144.85±4.26	(76)	253.19±7.45	(55)
	+T/P	0.55[b]	(1,281)	1.38±0.06	(3)	0.21±0.01	(0)	366.64±8.56	(193)	54.84±1.28	(12)
"O-Hia"	−T	0.04[b]	(100)	48.94±1.75	(100)	113.43±4.05	(100)	258.46±2.12	(100)	599.01±4.92	(100)
	−T/P	0.05[b]	(102)	4.98±0.17	(10)	9.05±0.31	(8)	162.84 ±1.03	(63)	295.95±1.88	(49)
	+T/P	0.37[b]	(841)	0.82±0.01	(2)	0.18±0.00	(0)	511.56±1.62	(198)	111.86±0.36	(19)
"Chacapat"	−T	0.04[b]	(100)	36.39±0.65	(100)	94.18±1.69	(100)	89.01±0.81	(100)	230.33±2.10	(100)
	−T/P	0.06[b]	(151)	31.74±0.30	(87)	42.86±0.40	(46)	122.40±1.58	(138)	165.25±2.13	(72)
	+T/P	0.21[b]	(540)	15.63±0.09	(43)	5.82±0.03	(6)	340.34±1.03	(382)	126.78±0.39	(55)

Results are expressed as the mean±standard deviation (n=2)

[a] Based on the unpurified extract without Triton X-100 (−T)

[b] Standard deviation ≤3%

Table 2 Cultivar-specific contents of flavan-3-ols and proanthocyanidins in the pericarp of "Hong Huey," "Kwang Jao," "O-Hia," "Kim Cheng," and "Chacapat" litchi fruit [in milligrams per hectogram of dry weight]

	RT [min]	"Hong Huey"	"Kwang Jao"	"O-Hia"	"Kim Cheng"	"Chacapat"
Flavanols		8,442±115ab	8,687±239a	8,876±229a	8,914±66a	7,879±104b
Monomers		1,785±13b	2,153±79a	2,049±26a	1,849±3b	1,587±54c
9 epicatechin	19.0	1,712±14c	2,027±75a	1,927±24ab	1,788±2bc	1,438±52d
1 (epi)afzelechin	4.3	57±1c	104±4b	98±2b	45±0.3d	138±1a
4 catechin	13.1	16±1b	22±1a	24±0.3a	16±0.4b	11±0.3c
Dimers		1,390±42ab	1,369±44b	1,394±48ab	1,548±43a	1,176±13c
18 $[(epi)cat]_2$: A	34.4	1,092±46a	1,076±33a	1,040±36a	1,115±35a	863±6b
8 $[(epi)cat]_2$: B	16.7	238±7c	217±7cd	306±10a	268±6b	202±6d
21 $[(epi)cat]_2$: A	42.4	60±3d	76±4c	48±2e	165±2a	112±0.5b
Trimers		2,271±97ab	2,034±51b	2,293±74a	2,155±31ab	2,085±16ab
10 $[(epi)cat]_3$: A-B	21.3	981±67a	764±35b	839±34ab	844±24ab	926±5a
15 $[(epi)cat]_3$: B-A	27.9	589±15b	657±10a	697±25a	646±6a	514±6c
19 $[(epi)cat]_3$: B-A	37.3	324±8a	340±9a	339±15a	306±1a	263±4b
11 $[(epi)cat]_3$: B-B	22.5	162±4b	68±1d	181±0.4a	128±6c	166±6ab
7 $[(epi)cat]_3$: B-B	15.9	148±15a	95±0.3b	108±2b	102±5b	145±8a
14 (epi)cat-(epi)afz-(epi)cat: A-B	23.5	67±4c	110±3b	129±2a	129±1a	71±1c
Tetramers		1,953±32c	2,216±42b	1,889±31c	2,385±9a	1,926±19c
17 $[(epi)cat]_4$: A-B-A	30.4	574±6c	686±5b	484±8e	745±9a	534±14d
2 $[(epi)cat]_4$: B-A-B	9.5	509±22a	492±14a	492±8a	539±7a	490±3a
13 $[(epi)cat]_4$: B-A-B	23.3	159±2c	335±9b	347±6b	429±8a	173±5c
20 $[(epi)cat]_4$: A-A-B	39.3	203±2c	306±6b	300±4b	338±14a	224±3c
12 $[(epi)cat]_4$: A-B-B	22.7	377±15a	232±5b	165±0.4c	210±0.4b	383±0.2a
22 $[(epi)cat]_4$: A-B-A	44.0	132±4b	164±4a	102±5c	124±1b	121±3b
Pentamers		1,044±21bc	915±23d	1,250±50a	978±1cd	1,105±29b
16 $[(epi)cat]_5$: B-A-B-A	29.9	360±3a	250±9c	386±19a	290±0.1b	379±4a
6 $[(epi)cat]_5$: B-A-B-B	14.5	295±1b	311±3b	376±10a	364±8a	313±8b
5 $[(epi)cat]_5$: B-B-A-B	13.6	269±23b	229±7bc	333±13a	192±4c	275±10b
3 $[(epi)cat]_5$: B-B-A-B	12.4	120±5b	125±4b	155±8a	132±3b	138±7ab

Subunits are indicated as (epi)catechin [(epi)cat] and (epi)afzelechin [(epi)afz]. The sequence of A- or B-type linkages is listed from the top to the base unit of the oligomers. Results are expressed as the mean±standard deviation (n=2). Different letters (a–e) refer to significant differences (P= 0.05) between the cultivars

afzelechin only accounted for 3–9% of the total flavanol monomers in all cultivars (Table 2). The triphenolic (epi)gallocatechin that had been reported to occur in pericarp of Chinese "Huaizhi" (Zhang et al. 2000) was again (Reichel et al. 2011) undetectable both in "Hong Huey" and "Chacapat" as well as the other Thai litchi cultivars studied. Concerning the B-ring hydroxylation pattern of litchi flavonoids, the pericarp thus seemed to have strong flavonoid 3′-hydroxylase activity, but not the respective 3′,5′-enzyme (He et al. 2008). Small amounts of (+)-catechin in the pericarp of all cultivars (Table 2) might have resulted from epimerization of (−)-epicatechin during extraction and concentration and thus hardly indicated any leucoanthocyanidin reductase activity (He et al. 2008).

For deeper specification of the litchi pericarp polyphenols beyond the previous structure determination (Reichel et al. 2011), thiolytic degradation of "Hong Huey" pericarp was performed to calculate the average degree of polymerization (DP) and the proportions of the individual subunits of all proanthocyanidins. Thiolysis releases the terminal units and the benzyl thioethers of B-type-linked extension units, whereas A-type interflavan linkages are resistant to thiolysis (Gu et al. 2002). Approximately 23% of the terminal subunits of the litchi procyanidins were B-type-linked (+)-catechins (Table 4). Presumably, their amount is slightly overestimated due to the rude reaction conditions that promoted inevitable epimerization of (−)-epicatechin (Gu et al. 2002). Besides (+)-catechin, **4′**, and (−)-epicatechin, **9′**, three A-type-linked terminal *ortho*-dihydroxy flavanols ($[M–H]^-$ at m/z 575), **18′**, **37**, and **38**, were detected (Fig. 2). Among them, **18′** coeluted with procyanidin A2. As reported for bilberry extracts (Gu et al. 2002), benzyl thioethers linked to the former C2 position of terminal

Table 3 Cultivar-specific contents of flavonols and anthocyanins in the pericarp of "Hong Huey," "Kwang Jao," "O-Hia," "Kim Cheng," and "Chacapat" litchi fruit [in milligrams per hectogram of dry weight]

	RT [min]	"Hong Huey"	"Kwang Jao"	"O-Hia"	"Kim Cheng"	"Chacapat"
Flavonols		190.2±6.9a	136.0±4.6c	91.1±3.7d	165.3±5.8b	120.4±2.0c
25 quercetin 3-*O*-rutinoside	33.4	114.1±4.3a	81.2±2.7b	65.0±2.7c	104.3±3.8a	74.1±1.9bc
27 quercetin 3-*O*-glucoside	34.9	24.9±1.2a	14.6±0.5b	7.6±0.4c	7.7±0.3c	9.0±0.2c
26 quercetin hexoside	33.9	14.8±0.3a	12.1±0.4b			
29 kaempferol 3-*O*-rutinoside	39.1	10.1±0.2c	7.0±0.3d	8.2±0.4cd	38.3±1.3a	19.1±0.3b
24 quercetin glycoside	32.7	7.3±0.3a	5.6±0.2b			
28 kaempferol hexoside	38.3	7.1±0.2a	4.1±0.2b	0.01±0.003c		
30 kaempferol 3-*O*-glucoside[a]	40.8	5.6±0.2b	5.2±0.1bc	3.3±0.04d	4.7±0.2c	6.2±0.1a
23 quercetin glycoside	27.4	4.5±0.2c	5.0±0.1c	6.2±0.3b	2.5±0.03d	10.9±0.3a
Anthocyanins		111.90±0.16b	195.26±8.87a	188.24±2.60a	200.23±3.30a	188.70±7.32a
35 cyanidin 3-*O*-rutinoside	9.9	95.41±0.19c	168.75±8.00b	172.90±2.23ab	191.30±3.32a	180.42±7.10ab
34 cyanidin 3-*O*-glucoside	8.8	13.23±0.005b	20.40±0.97a	6.35±0.19c	2.63±0.02d	3.20±0.10d
33 cyanidin glycoside	8.2	1.19±0.01c	2.00±0.06a	1.66±0.06b	1.09±0.05c	0.03±0.03d
36 peonidin 3-*O*-rutinoside	13.6	0.54±0.01e	1.71±0.04c	1.12±0.05d	3.24±0.07a	2.06±0.05b
32 unknown compound	7.0	0.61±0.02d	0.72±0.01c	0.93±0.02b	0.57±0.01d	1.19±0.04a
31 (epi)catechin cyanidin rutinoside	3.2	0.93±0.03d	1.69±0.01b	5.27±0.06a	1.41±0.02c	1.80±0.06b

[a] Quantified as kaempferol 3-*O*-glucoside, coeluting with isorhamnetin 3-*O*-rutinoside. Results are expressed as the mean±standard deviation (n=2). Different letters (a–e) refer to significant differences (P=0.05) between the cultivars

subunits were formed under heterocyclic ring fission (**41**, **42**, and **45** in Fig. 2); this was indicated by characteristic product ions at m/z 413 and 289 (**41**, **42**) as well as m/z 699 and 575 (**45**) for B- and A-type-linked units, respectively. As expected from other thiolytic analyses of litchi polyphenols (Le Roux et al. 1998), (+)-catechin extension units were not found (Table 4; Fig. 2). Peak **43** at 37.9 min (Fig. 2) with typical molecular ions at m/z 411 and 287 in the MS^n experiment (Gu et al. 2002) was tentatively identified as 4-benzylthio-epicatechin (Fig. 2) by comparison with the reference substance. In

Table 4 Characterization of procyanidins after thiolytic degradation of pericarp isolated from "Hong Huey" litchi fruit after 0, 3, 26, and 52 days of storage (5 °C, 90% RH)

Day		0	3	26	52/o[a]	52/c[a]
Total subunits [mol/hg]	$S=T_1+E_1+2(T_2+E_2)$	39.5±0.4a	35.9±0.3ab	30.0±0.4cd	27.7±1.2d	33.9±2.6bc
Terminal units [mol/hg]	$T=T_1+T_2$	13.7±0.1a	12.3±0.0001b	8.6±0.02c	7.7±0.3c	8.8±0.7c
(+)-catechin (B-type-linked) [%]	$c(\mathbf{4'})/T$	23±0.1a	23±0.1a	18±0.3b	18±0.2b	17±0.5b
(−)-epicatechin [%]	$[c(\mathbf{9'})+c(\mathbf{18'})]/T$	43±0.4b	42±0.5b	47±0.1a	48±0.1a	48±0.5a
B-type-linked [%]	$c(\mathbf{9'})/T$	19±0.1a	17±0.5a	17±0.1ab	17±0.1ab	15±1.1b
A-type-linked [%]	$c(\mathbf{18'})/T$	24±0.3c	24±0.02c	30±0.2b	31±0.03b	33±0.7a
Extension units [mol/hg]	$E=E_1+2E_2+T_2$	25.8±0.3a	23.6±0.3abc	21.3±0.4bc	20.0±0.9c	25.1±2.0ab
(−)-epicatechin, B-type-linked [%]	$(E_1+E_2)/E$	53±0.2b	53±0.2b	53±0.4b	53±0.2b	55±0.4a
(−)-epicatechin, A-type-linked [%]	$(E_2+T_2)/E$	47±0.2a	47±0.2a	47±0.4a	47±0.2a	45±0.4b
$DP_{absolute}$	S/T	2.9±0.001c	2.9±0.02c	3.5±0.05b	3.6±0.03b	3.9±0.01a
$DP_{procyanidins}$	$(S-M)/(T-M)$	4.3±0.004c	5.2±0.03a	4.6±0.1b	4.7±0.01b	4.8±0.1b
$DP_{oligomers}$	$(S-M-2D)/(T-M-D)$	5.4±0.1c	7.6±0.01a	5.9±0.2bc	6.0±0.03bc	6.1±0.3b

Molar contents (c, in moles per hectogram) of monomeric terminal units [$T_1=c(\mathbf{4'})+c(\mathbf{9'})+c(\mathbf{41})+c(\mathbf{42})$; cf. Fig. 2], dimeric terminal units [$T_2=c(\mathbf{18'})+c(\mathbf{37})+c(\mathbf{38})+c(\mathbf{45})$; cf. Fig. 2], monomeric extension units [$E_1=c(\mathbf{39})+c(\mathbf{43})$; cf. Fig. 2], dimeric extension units [$E_2=c(\mathbf{44})$; cf. Fig. 2], monomers quantified directly after extraction without thiolytic degradation [$M=c(\mathbf{9})+c(\mathbf{4})$; cf. Table 2], dimers quantified directly after extraction without thiolytic degradation [$D=c(\mathbf{8})+c(\mathbf{18})+c(\mathbf{21})$; cf. Table 2]. Results are expressed as the mean±standard deviation (n=2). Different letters (a–d) refer to significant differences (P=0.05) due to fruit storage. *DP* degree of polymerization

[a] After 52 days, two samples were taken from the outer (52/o) and the central (52/c) zones of the storage basket, respectively

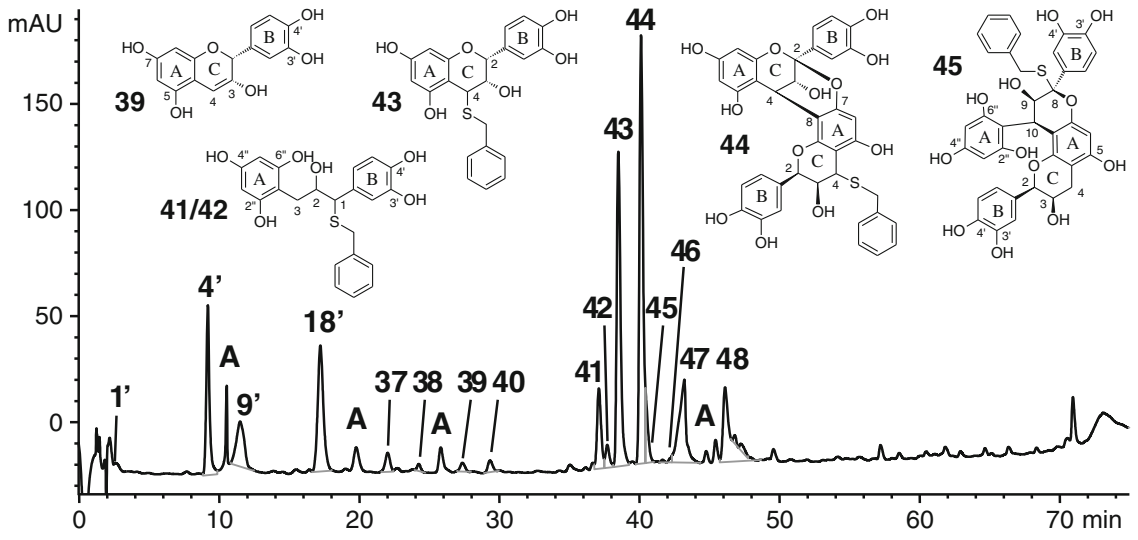


Fig. 2 Reversed-phase HPLC separation (280 nm) of thiolytic degradation products of proanthocyanidins from pericarp of freshly harvested "Hong Huey" litchi fruit. Peak and compound assignment as derived from Gu et al. (2002): **1′**, (epi)afzelechin; **4′**, (+)-catechin; **9′**, (−)-epicatechin; **18′**, procyanidin A2; **37**, **38**, A-type procyanidin dimers; **39**, 3,5,7,3′,4′-pentahydroxy-3,4-flavene; **40**, unknown (epi)catechin dehydration product; **41**, **42**, stereoisomers of 1-benzylthio-1-(3′,4′-dihydroxyphenyl)-3-(2″,4″,6″-trihydroxyphenyl)-propan-2-ol; **43**, 4-benzylthio-epicatechin; **44**, 4-benzylthio-procyanidin A2; **45**, 8-benzylthio-3,5,9-trihydroxy-2,8-bis(3′,4′-dihydroxyphenyl)-10-(2″,4″,6″-trihydroxyphenyl)-3,4,9,10-tetrahydro-2H,8H-pyrano<2,3-h>chromene; **46**, 4-benzylthio-(epi)afzelechin; **47**, **48**, thiolysis reagent peaks. Peaks marked with **A** showed additional absorption maxima at 520 nm

accordance with Le Roux et al. (1998), peak **44** at 39.4 min (Fig. 2), displaying a molecular ion at *m/z* 697 and the fragments *m/z* 573 and 411 in the MS^2 experiment, was tentatively made out as the 4-benzyl thioether of procyanidin A2 (Fig. 2). Furthermore, an unknown dehydration product, **40** (*m/z* 271→229, 161, 203), and a 3,4-flavene, **39** (*m/z* 287→125, 161, 269), of the epicatechin extension units (Gu et al. 2002) were recorded (Fig. 2). Minor traces of (epi)afzelechin, **1′** ($[M–H]^-$ at *m/z* 273) and its 4-benzyl thioether, **46** ($[M–H]^-$ at *m/z* 395; [M–benzyl thiol]$^-$ at *m/z* 271) were detected, but were not quantifiable. From the ratio of total to terminal subunits, the DP of the flavan-3-ol monomers to polymers ($DP_{absolute}$ in Table 4) was estimated to be 2.9 in the pericarp of freshly harvested "Hong Huey" litchi. Due to the huge amounts of flavan-3-ol monomers in the fresh pericarp (Table 2), the DP was corrected ($DP_{procyanidins}$) by subtracting the contents of (+)-catechin and (−)-epicatechin detected after simple extraction (Table 2) from the sum of terminal units obtained after thiolytic degradation. According to a $DP_{procyanidins}$ of 4.3, the average procyanidin thus consisted of one terminal and 3.3 extension units, with 47% of the latter being A-type-linked (Table 4). However, the estimated size of the "Hong Huey" procyanidins ($DP_{procyanidins}$) was still below the DP of 6.4±1.1 reported for procyanidins in "Kwai Mi" litchi (Le Roux et al. 1998). The latter was concluded from thiolytic degradation of a purified procyanidin fraction without low molecular flavonoids and thus came much closer to a DP calculated for "Hong Huey" proanthocyanidins after correction of $DP_{absolute}$ for the amounts of both the free flavanol monomers and dimers ($DP_{oligomers}$=5.4; Table 4).

Without thiolytic degradation, the number and positions of A-type linkages and the kind of subunits in the individual proanthocyanidin dimers to pentamers were determined by HPLC-DAD-MS^n. Concurrently, this method (Reichel et al. 2011) allowed separate quantitation of all compounds (Table 2). Regardless of the cultivar studied, the pericarp had three to five times higher procyanidin A2, **18**, than procyanidin B2 contents, **8**. Confirming the thiolytic degradation products (Table 4), 14 out of 16 trimers to pentamers exhibited at least one A-type linkage. Irrespective of the cultivar, the trimer **10**, presumably epicatechin-(4*β*-8,2*β*-*O*)-epicatechin-(4*β*-8)-epicatechin (Reichel et al. 2011), clearly topped over **15** and **19** in litchi pericarp. By analogy, the A-B-A-linked tetramer **17** exceeded the B-A-B-linked one, **2**. Procyanidin biosynthesis, particularly of A-type linkages, is still unclear (He et al. 2008), but the constant procyanidin pattern, irrespective of cultivar and crop year (Reichel et al. 2011), suggested consistent, genetically controlled formation.

The total anthocyanin contents were ~2 g/kg DW for all cultivars, except "Hong Huey" (Table 3). However, in the previous year, such high amounts had equally been found in pink- to red-colored fruit of the latter variety (Reichel et al. 2011). Strikingly high contents of flavonols were confirmed for this cultivar (Table 3). In contrast, only half of this total flavonol amount was found in pericarp of "O-Hia" litchi (Table 3; Fig. 3a). The previous study of anthocyanins and

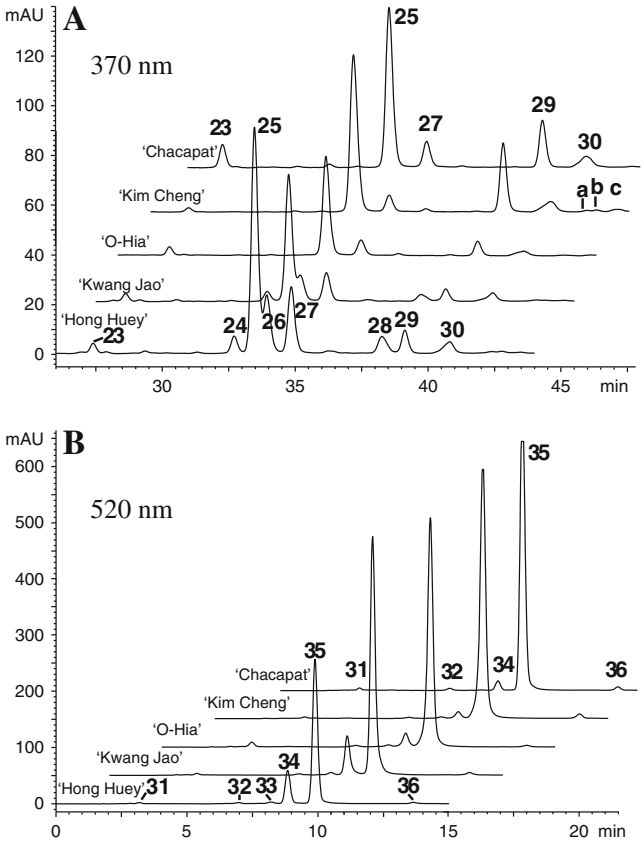


Fig. 3 Reversed-phase HPLC separation of **A** flavonols (370 nm) and **B** anthocyanins (520 nm) from pericarp of freshly harvested "Hong Huey," "Kwang Jao," "O-Hia," "Kim Cheng," and "Chacapat" litchi fruit. For peak assignment, cf. Table 3. Peaks **a–c** refer to unknown compounds

flavonols of "Hong Huey" and "Chacapat" pericarp and comparison with literature data had suggested that cultivars with warty and spiky peel generally differ in their pigment patterns (Reichel et al. 2011). This hypothesis was verified for the Thai cultivars studied, particularly in terms of pigment glycosylation (Fig. 3). The molar ratio of cyanidin 3-*O*-rutinoside to its glucoside was 5:1 and 6:1 for "Hong Huey" and "Kwang Jao" pericarp, whereas "Kim Cheng" and "Chacapat" reached high ratios of 55:1 and 43:1, respectively, because of lower contents of cyanidin 3-*O*-glucoside (Table 3). Likewise, the molar ratio of the main flavonol quercetin 3-*O*-rutinoside to the respective glucoside was 3–4:1 for pericarp with pointed protuberances, but 6–10:1 for pericarp with obtuse ones. Other quercetin glycosides, **24** and **26**, as well as a kaempferol hexoside, **28**, were only detected in "Hong Huey" and "Kwang Jao" pericarp (Fig. 3a). The chromatogram of the "Kim Cheng" pericarp extract revealed three additional minor peaks, a, b, and c, at 370 nm, which eluted later than kaempferols (Fig. 3a) did and accounted for 4% of the total flavonols, but could not be identified (not listed in Table 3). In contrast to our previous study (Reichel et al. 2011), peak **30** (Fig. 3a) tentatively identified as kaempferol 3-*O*-glucoside revealed an additional molecular ion peak at *m*/*z* 623 in the MS^n experiments for all cultivars, with fragmentation to *m*/*z* 315 and 300. Hence, the minor compound coeluting with the kaempferol glucoside was tentatively assigned to isorhamnetin 3-*O*-rutinoside, corresponding to the anthocyanin peonidin 3-*O*-rutinoside, **36** (Fig. 3b). Overall, 21% and 26% of the total flavonols in "Chacapat" and "Kim Cheng" pericarp were kaempferol glycosides, whereas in that of "Hong Huey" and "Kwang Jao," only 12% had the monohydroxylated B-ring (Table 3). The assumption that wild litchi may have evolved into two general classes of litchi, cultivars with either obtuse or sharp-pointed protuberances (Menzel and Simpson 1990), was corroborated by the flavonoid pattern. However, the cultivar with quite smooth skin segments, "O-Hia," seemed to have an intermediate position because quercetin 3-*O*-rutinoside dominated among the flavonols and compounds **24** and **26** were absent (Fig. 3a), as observed for cultivars with warty pericarp. But the ratio of quercetin glycosides to kaempferol ones was 6:1 like in fruit with spiky peels (Table 3). Furthermore, the ratio of cyanidin rutinoside to cyanidin glucoside was 20:1. A high amount of the glycosylated anthocyanidin-(epi)catechin adduct, **31**, was particularly characteristic for "O-Hia" pericarp (Table 3). The lower values in pericarp redness *a** (LMI in Fig. 1) were neither explainable by the total amounts of anthocyanins nor by the anthocyanin pattern. Instead, red pigments in "O-Hia" pericarp seemed to be masked by light brown scurf on the broad peel segments, even when the fruit was freshly harvested (cf. photo in Fig. 1). Such scurf was also visible on microscopic pictures of "Hong Huey" and "Kwang Jao" pericarp on the respective harvest days, more sparsely for "Kim Cheng" and "Chacapat," but always only as small radial lines on the protuberances where it supposably marked microcracks (LM micrographs not shown). The darker red color (LMI in Fig. 1) of "Chacapat" and "Kim Cheng" in comparison to "Hong Huey" and "Kwang Jao" pericarp might be caused by different pigment patterns or by morphological differences in surface structure, influencing the color measurements.

Besides the outer red part, litchi pericarp includes a white spongy inner tissue, which is particularly pronounced in "Chacapat" fruit. Therefore, the polyphenol composition of both parts was separately analyzed for this cultivar. The inner hardly colored fraction accounted for ~10% (DW/DW) of the entire pericarp, but only contained 5.5% of the total procyanidins, 4.5% of the flavan-3-ol monomers, 3.1% of the flavonols, and 1.5% of the total anthocyanins.

Pericarp Characteristics and Cultivar-Specific Browning Susceptibility

In litchi pericarp (Tables 2 and 3), flavonols ranged from ~200 mg/kg of fresh pericarp weight (FW) ("O-Hia") to ≥500 mg/kg FW ("Hong Huey"). These levels were

comparable to the maximum contents reported per fresh weight for the edible parts of vegetables and were ~10 times those in whole apples or other fresh fruit (Hollman and Arts 2000). The total anthocyanin contents (~300–550 mg/kg FW) were of the same magnitude as those described for some red berries and grapes (Clifford 2000). The striking amounts of flavan-3-ol monomers (~4,200–6,000 mg/kg FW) in pericarp of the Thai litchi cultivars were ≥10 times higher than the contents reported for 60 fruit, vegetable, and food samples (Arts et al. 2000). In particular, the litchi pericarp was an abundant source of proanthocyanidins (~17–18 g/kg FW), comparable with cocoa and sorghum (Prior and Gu 2005). However, proanthocyanidins in litchi pericarp best resembled those described for cranberry fruit, which have an average DP of 4.7–8.5 with (−)-epicatechin as the prevailing subunit and mostly one to multiple A-type linkages (Pappas and Schaich 2009). Together with anthocyanins (140–1,710 mg/kg), flavan-3-ols (~70 mg/kg), and especially flavonols (200–400 mg/kg), the proanthocyanidins (~4 g/kg) are made responsible for the potential health benefits of cranberries, such as anti-adhesion towards bacterial and viral pathogens, anticancer, anti-inflammatory and antiulcer effects (Pappas and Schaich 2009). Not surprisingly, polyphenol extracts of litchi fruit or pericarp and antioxidant or nutraceutical products thereof have recently attracted medical research interest, after studies had supported their safety for human consumption (Miura et al. 2010). For these extracts and derived products, improvement of blood flow and markers of oxidative stress, favorable effects in glucose and lipid metabolism, as well as immunomodulatory and anticancer activities have been shown by in vitro studies, animal experiments, or clinical trials (Zhao et al. 2007; Miura et al. 2010). However, their industrial production requires consistent, high-quality raw materials. The results of this work and the previous study (Reichel et al. 2011) support the suitability of Thai litchi for such purposes because the high contents and particularly the composition of flavan-3-ol monomers and oligomers were rather constant in the pericarp, irrespective of cultivar, maturity, and season. Exploitation of high-quality by-products from fruit processing as a source of natural antioxidants as technical food additives or dietary supplements and ingredients for food fortification might be a promising approach (Schieber et al. 2001) for the hardly used litchi processing waste, such as the "Hong Huey" peels arising from the Thai juice and canning industry (Nagle et al. 2011). "Hong Huey" pericarp would particularly be suitable for polyphenol preparations because of its combination of abundant A-type procyanidins and high flavonol contents (Table 3), comparable with cranberries (Pappas and Schaich 2009).

However, fresh fruit marketing of litchi is by far prevailing for this precious fruit species, though limited by the high susceptibility of the pericarp to browning. The latter is enhanced by high PPO_{epicat} activities and high contents of the main substrate (−)-epicatechin, whereas the antioxidant potential of the anthocyanins has some protective effect (Reichel et al. 2011). The relative importance of these three biochemical factors that are directly involved in pericarp browning is shown by Fig. 4 for all five cultivars. Since the enzyme activities tended to depend not only on the cultivar but also on harvest time and fruit lot (Fig. 1; Table 1), Fig. 4 only allowed rough classification of the pericarp browning potential of the samples. Accordingly, proneness to browning was maximum for "Kim Cheng" pericarp due to highly active PPO and abundant (−)-epicatechin. The contributions of enzyme and substrate were lowest for "Hong Huey" and "Chacapat." However, the sample of the former variety contained less anthocyanins than the other cultivars in year 2008. A molar ratio of cyanidin 3-*O*-rutinoside to (−)-epicatechin >3:100 was found to retain flavonoids best (Reichel et al. 2011). This molar ratio was only 2.7:100 for the pericarp of the "Hong Huey" fruit in crop year 2008. But, this sample additionally contained cyanidin 3-*O*-glucoside, which has an antioxidant potency exceeding that of the rhamnoglucoside (Wang et al. 1997), and maximum quercetin glycoside levels. For the 3-*O*-glucosides of cyanidin and quercetin, equally high antioxidant activities were recorded (Zheng and Wang 2003). However, among their 3-*O*-rutinosides, glycosylation-induced lowering of the notable antioxidant activities of both aglycones was less for the cyanidin glycoside than for the quercetin glycoside (Rice-Evans et al. 1996; Heo et al. 2007). Because of the decline of anthocyanin contents and constancy of flavonol glycoside levels during fruit storage, the counteractive role in litchi pericarp browning as fruit-inherent antioxidant was mainly ascribed to the anthocyanins (Reichel et al. 2011; Fig. 4) rather than to the flavonols. Consistently, the contents of cyanidin 3-*O*-rutinoside usually exceeded those of quercetin 3-*O*-rutinoside (Table 3), in addition to its higher antioxidant activity.

The Impact of Long-Term Storage on Pericarp Characteristics and Browning

Pericarp browning throughout long-term cold storage of the fruit without packaging was exemplarily analyzed for the commercially most important Thai cultivar "Hong Huey" (harvest 2008) in order to scrutinize the roles of the three factors directly involved (cf. Fig. 4) and further peel properties. As expected (Jiang and Fu 1999; Reichel et al. 2010), subjectively rated (BI) and objectively measured (PLCI) pericarp color changed within the first three storage days (Table 5). However, striking discoloration, especially loss in pericarp redness a^*, was primarily observed between days 3 and 8, concurrent with loss of pericarp moisture and thickness (Table 5). First, the pericarp softened, but then

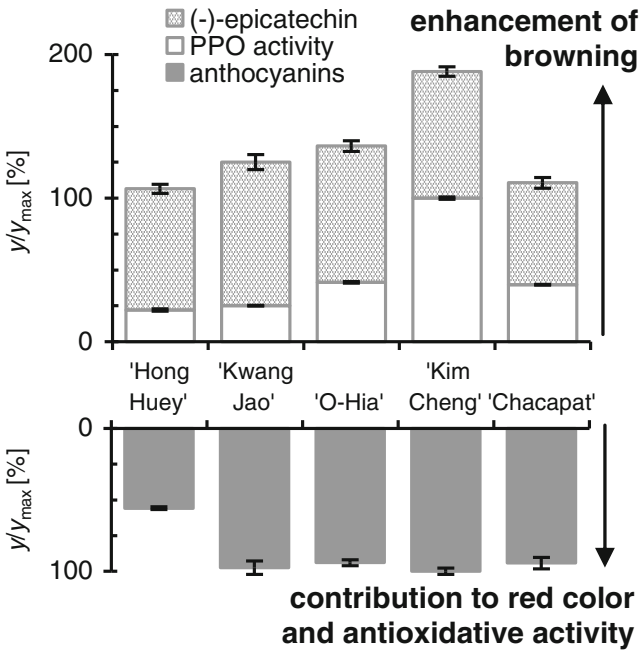


Fig. 4 Susceptibility to pericarp browning of "Hong Huey," "Kwang Jao," "O-Hia," "Kim Cheng," and "Chacapat" litchi fruit in terms of (−)-epicatechin contents, (−)-epicatechin-specific PPO activities (PPO_{epicat}), and total anthocyanin contents. All values are displayed as the ratio relative to the respective maximum measured. *Error bars* represent the standard deviations calculated based on error propagation

embrittled with ongoing desiccation (Britt; Table 5). As from day 8, the physical pericarp properties did not change significantly (Table 5). But although mean values for the whole fruit lots remained constant, differences in peel color and surface properties became evident between fruit located in the outer zones of the mesh baskets and those in the central ones throughout long-term storage. Hence, after 52 days, two separate samples corresponding to these two zones were analyzed instead of a randomly mixed one. Fruit in the center of the storage baskets was dark brownish-red (Fig. 5d–f), whereas fruit in the outer zone had a light brown, scurfy surface (Fig. 5a–c). These differences could presumably be ascribed to different rates of moisture loss (Sivakumar et al. 2010) because fruit next to the netted container wall lost pericarp water more rapidly than the litchi in the center of the basket. Enzyme activities of PPO and POD remained high until day 3 of fruit storage, but also declined afterwards with ongoing pericarp desiccation (Table 6). However, even after 52 days, ≥15% of residual PPO_{epicat} and ≥43% of residual POD activity were measured. Similar rates of activity loss had commonly been recorded for litchi stored without packaging (Jiang and Fu 1999; Reichel et al. 2010), whereas PPO and POD activities of bagged fruit had been reported to be stable or even increase during storage (Huang et al. 1990; Liu et al. 2010). Contents of pericarp polyphenols, particularly (−)-epicatechin, mostly decreased within the first 3 days of open cold fruit storage in the previous study (Reichel et al. 2011). In the present case, slightly elevated amounts in polyphenols were determined in "Hong Huey" pericarp after 3 days (Table 6). However, the differences were rather due to sample variations than to postharvest biosynthesis because the contents of all polyphenol classes studied were consistently lower in the pericarp of freshly harvested fruit than in that of the stored ones (day 3; Table 6). The typical rapid decline in polyphenols occurred after day 3, concurrent with the moisture loss. The contents of the main PPO substrate (−)-epicatechin dropped by 55% until day 26 and by further 6% and 8% until day 52 in light brown, scurfy and dark brown fruit (samples 52/o and 52/c in Table 6), respectively. As described previously (Reichel et al. 2011), high amounts

Table 5 Pericarp features of "Hong Huey" litchi fruit after 0, 1, 2, 3, 8, and 13 days of cold storage (5 °C, 90% RH)

Day	0	1	2	3	8	13
Thick[a]	1.2±0.1a	1.0±0.1b	1.0±0.2b	0.8±0.2b	0.4±0.1c	0.3±0.1c
MC[b]	73.2±0.8a	72.4±2.1a	68.5±2.6a	68.1±4.8a	31.7±1.3b	28.5±2.7b
Britt[c]	5.8±0.7b	3.3±0.5d	4.8±1.1bc	4.0±0.7cd	7.8±1.5a	8.8±1.7a
PLCI[d]	50.9±2.2a	49.0±2.3a	44.4±2.0b	39.6±2.2c	32.2±1.1d	35.1±1.3d
a^*[e]	30.2±3.5a	29.2±2.6a	26.6±3.4ab	24.0±3.0b	18.6±1.5c	18.2±1.4c
BI[f]	1.1±0.3e	1.8±0.4d	2.6±0.5c	4.2±0.4b	5.0±0a	5.0±0a

Results are expressed as the mean±standard deviation. Different letters (a-e) refer to significant differences (P=0.05) due to fruit storage

[a] Thickness [in millimeters], n=10

[b] Moisture content [in grams per hectogram], n=5

[c] Brittleness as the quotient of maximum load and maximum extension [in newtons per millimeter], n=10

[d] Postharvest litchi color index (Reichel et al. 2010), n=3×10

[e] CIE color coordinate a^*, n=3×10

[f] Subjective browning index (Jiang and Fu 1999; Reichel et al. 2010), n=10

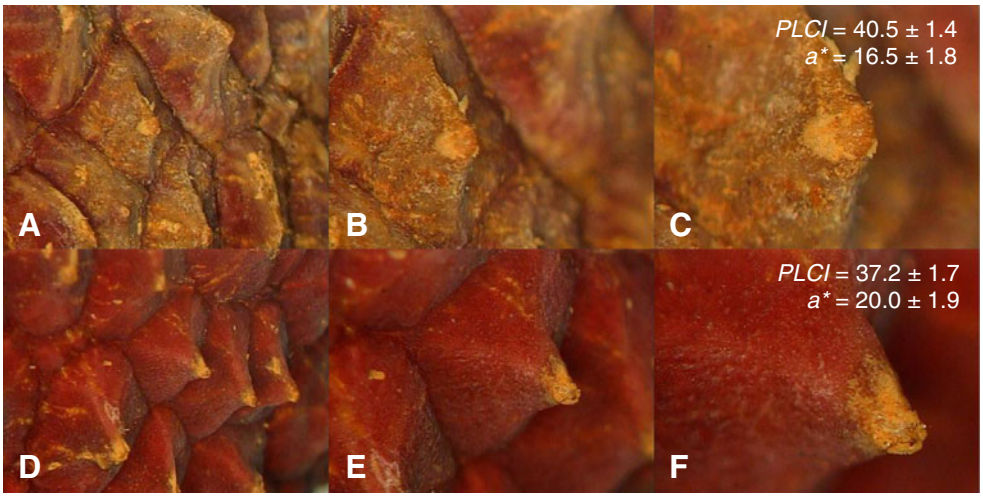


Fig. 5 Microscopic structure of "Hong Huey" pericarp after 52 days of fruit storage (5 °C, 90% RH): magnification **A**, **D** ×50, **B**, **E** ×100, and **C**, **F** ×200. Two separate fruit samples were taken from the outer (**A**–**C**) and the central (**D**–**F**) zones of the storage baskets. Pericarp color is indicated as the postharvest litchi color index (PLCI) and the CIE color coordinate *a**. Values are given as the mean±standard deviation (n=3×15)

of anthocyanins were still detectable in brown fruit after ≥3 weeks of storage. The extent by which cyanidin 3-*O*-glucoside degraded was similar to that of cyanidin 3-*O*-rutinoside. After 7.5 weeks, 87% of the initial anthocyanin content was still found in light brown, scurfy fruit (sample 52/o), whereas only 54% of the red pigments were then present in the dark brown ones (sample 52/c; Table 6), despite the color impression of the fruit in terms of objectively measured pericarp redness *a** (Fig. 5). In contrast, contents of flavanol oligomers (DP 2–5) declined by 20% in scurfy pericarp, but only by 4.9% in dark brown litchi without scurf (Table 6). In pericarp of the latter, the decrease was mainly due to a decline in B-type-linked procyanidins such as procyanidin B2. Similar changes had been reported for fruit stored in PPE bags for 36 days (Liu et al. 2010). In the light brown, scurfy fruit (sample 52/o), degradation of A-type-linked flavanols such as procyanidin A2 was also pronounced (Table 6). Thiolytic degradation equally revealed stronger procyanidin loss in scurfy, light brown litchi (Table 4). However, no evidence for the exact nature of light brown or dark brown products was found by HPLC-DAD-MS^n analysis. Procyanidin polymerization or reversible linkage of flavanols to matrix structures were unlikely because the monomer-corrected DP was stable and

Table 6 Activities of polyphenol oxidase (PPO_{epicat}) and peroxidase (POD) [in microkatals per hectogram of dry weight] and contents of major phenolic compounds [in milligrams per hectogram of dry weight] after 0, 3, 26, and 52 days of fruit storage (5 °C, 90% RH) in the pericarp of "Hong Huey" litchi fruit

Day	0	3	26	52/o[a]	52/c[a]
PPO_{epicat}	27.7±1.1a	30.0±4.9a	2.9±0.2b	6.3±0.3b	4.1±0.9b
POD	49.5±2.9a	52.3±1.3a	15.3±5.3b	21.2±0.2b	23.9±0.1b
Total polyphenols	8,745±122b	9,353±21a	7,274±83c	6,325±81d	7,236±36c
Flavanols	8,442±115b	9,018±20a	6,972±76c	6,023±84d	6,996±38c
Monomers	1,785±13b	1,986±7a	824±9c	711±8d	667±0.3e
Epicatechin	1,712±14b	1,895±7a	772±8c	668±7d	628±1e
Dimers	1,390±42a	1,425±8a	1,165±3b	1,029±22c	1,176±1b
$[(epi)cat]_2$: A	1,092±46a	1,125±8a	937±1b	828±21c	964±3b
$[(epi)cat]_2$: B	238±7a	240±1a	174±4b	148±1c	153±1c
Trimers	2,271±97ab	2,398±1a	2,113±22b	1,810±15c	2,128±19b
$[(epi)cat]_3$: A-B	981±67a	966±6a	884±11a	748±13b	889±1a
Tetramers	1,953±32ab	2,066±15a	1,825±44c	1,563±30d	1,926±3bc
Pentamers	1,044±21b	1,143±9a	1,045±2b	912±9c	1,098±16a
Anthocyanins	111.9±0.2ab	120.1±0.3a	105.9±5.8ab	97.9±5.4b	60.6±1.4c
Cyanidin 3-*O*-rutinoside	95.4±0.2ab	104. 9±0.2a	89.2±5.1b	84.5±4.7b	49.4±1.1c
Cyanidin 3-*O*-glucoside	13.2±0.005a	11.4±0.01a	12.3±0.8a	8.7±0.6b	9.1±0.3b
Flavonols	190.2±6.9cd	215.2±0.6a	196.7±1.1bc	204.3±2.1ab	179.9±0.2d
Quercetin 3-*O*-rutinoside	114.1±4.3c	129.9±0.9a	117.1±0.3bc	122.5±1.4ab	103.8±0.03d
Quercetin 3-*O*-glucoside	24.9±1.2c	29.3±0.3a	26.4±0.1bc	27.6±0.7ab	25.8±0.2bc
Kaempferol 3-*O*-rutinoside	10.1±0.2c	10.7±0.1b	10.1±0.03c	11.2±0.1a	8.9±0.02d

Results are expressed as the mean±standard deviation (n=2). Different letters (a–e) refer to significant differences (P=0.05) due to fruit storage

[a]After 52 days, two separate samples were taken from the outer (52/o) and the central (52/c) zones of the storage baskets, respectively

procyanidin contents determined after thiolytic pericarp degradation decreased (Table 4). A similar decline in procyanidin contents had been reported for pears after sun-drying due to oxidation-induced, irreversible complex formation of the oligomers with matrix compounds (Ferreira et al. 2002).

Postharvest Browning Mechanisms Deduced from Changes in Pericarp Features

A scheme for postharvest browning of litchi pericarp is proposed in Fig. 6, based on the present and previous results (Reichel et al. 2011). In contrast to former theories (Jiang et al. 2004; Sivakumar et al. 2010), the role of (−)-epicatechin as the specific substrate of litchi pericarp PPO was corroborated. Instead of anthocyanase-mediated degradation of anthocyanins, their role as antioxidants and masking of the red pigments by polymeric brown products is indicated. Whereas the importance of PPO in litchi pericarp browning has widely been approved, direct involvement of POD remained unclear (Reichel et al. 2011). Pericarp POD presumably contributed to cell-specific lignification in vascular bundles of the mesocarp and the subepidermal sclerenchyma (Underhill and Critchley 1995). Besides, POD activity localized in the epicarp had been reported (Huang et al. 1990; Underhill and Critchley 1995). Amino acid composition of structural litchi pericarp proteins suggested accumulation of extensin throughout fruit development (Huang et al. 2006). Plant POD is known to catalyze the H_2O_2-dependent cross-linking of the latter in response to wounding (Almagro et al. 2009). Huge percentages of POD activity were bound to the cell wall (Table 1), supporting the involvement in cell wall repair. Microcracking of the pericarp due to desiccation (Underhill and Simons 1993) may stimulate such POD activity in the litchi epicarp. The fact that "O-Hia" pericarp, which is prone to tension-induced microcracks because of its flat, broad skin segments being maximally stretched throughout fruit growth, showed maximum POD activity corroborated this hypothesis. Surface scurf was already present on the day of harvest for this cultivar. Similar scurf formation was only observed along radial microcracks on protuberances of the other cultivars on the harvest days, but particularly for "Hong Huey" fruit when it was exposed to vast desiccation during long-term storage (Fig. 5a–c). Decline in procyanidin contents was barely specific in such fruit and losses rather concerned other compounds than (−)-epicatechin and anthocyanins (sample 52/o in Table 6). Hence, scurf formation remained unclear, but was probably based on various wound-induced repair processes such as POD- and PPO-induced polyphenol oxidation, POD-mediated cross-linking of matrix compounds, and deposition of polysaccharides and proteins (Almagro et al. 2009) (Fig. 6). Nonenzymatic reactions of proanthocyanidins with proteins to insoluble complexes (Taylor et al. 2007) or binding of the former to cell wall polysaccharides (Renard et al. 2001; Gu et al. 2002) were most likely. The rate of water loss seemed to control which of the two key browning processes (Fig. 6) prevailed (Sivakumar et al. 2010). Whereas rapid water loss favored surface scurf formation, retarded desiccation resulted in prolonged PPO-catalyzed oxidation of (−)-epicatechin and co-oxidation of anthocyanins in deeper cell layers (Table 6) (Reichel et al. 2011). From the present scheme of postharvest pericarp browning, the following basic approaches for

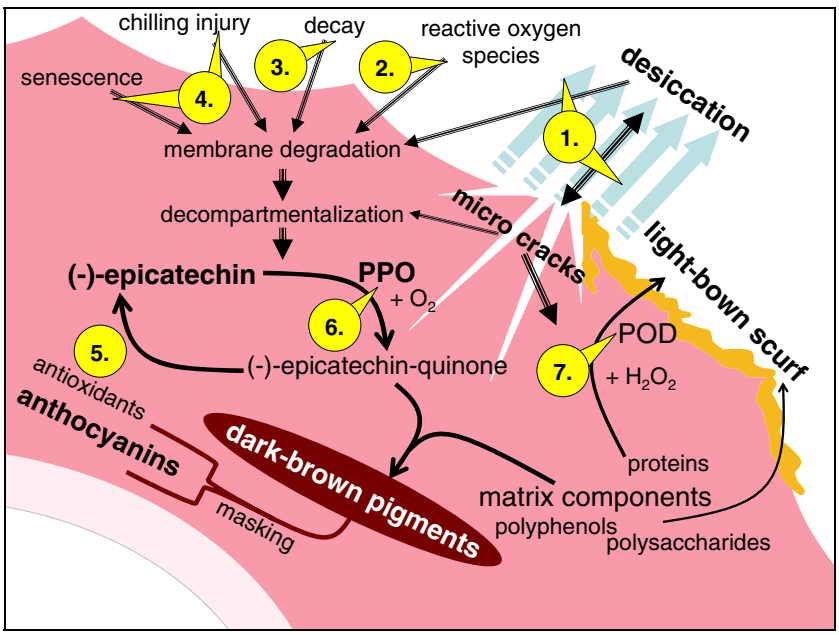


Fig. 6 Proposed scheme for postharvest pericarp browning in litchi fruit based on PPO-induced polyphenol oxidation and scurf formation as the two main processes. Possible postharvest measures **1**–**7** deduced thereof are explained in the text

postharvest concepts for retarding browning of litchi fruit became evident (1–7 in Fig. 6): (1) protection from desiccation to avoid microcracks and rapid surface scurf formation, (2) application of radical scavengers to protect membranes from lipid peroxidation, (3) prevention of microbial decay, (4) retarding of senescence and avoiding of chilling injury by suitable cold storage conditions, (5) supplementation of antioxidants to promote reduction of (−)-epicatechin quinones prior to polymerization, (6) addition of PPO inhibitors to minimize enzymatic browning, and (7) minimizing wounding stress and/or application of POD inhibitors. Respective measures, advantageously concerted, may result in postharvest concepts that efficiently retard pericarp browning throughout the postharvest chain.

Conclusions

The pericarp of five representative Thai litchi cultivars differing in protuberance morphology was characterized in terms of appearance, specific PPO and POD activities, as well as detailed polyphenol patterns. Their susceptibility to browning was rated by the three factors: (−)-epicatechin, PPO_{epicat} activity, and anthocyanin contents. Additional observations throughout the long-term storage experiment helped to identify the role of other factors involved in pericarp browning such as those related to POD activity and pericarp structure. Consequently, "O-Hia" fruit, the pericarp of which showed highest POD activity and was particularly prone to rapid surface scurf formation, was recommended for short transportation and local markets. Proneness to pericarp browning in interior layers was maximal for "Kim Cheng" samples due to striking PPO_{epicat} activity, suggesting marketing under consideration of a shelf life of ~3–10 days. The pericarp characteristics of "Hong Huey" and "Kwang Jao" made both cultivars appropriate for export to more distant markets, provided the application of suitable postharvest measures. "Hong Huey" pericarp as a by-product of litchi processing could further be considered as a rich source of polyphenols based on its availability and high contents of both A-type procyanidins and flavonols. However, among the cultivars studied, the robust, thick pericarp structure, low POD activity, and low general browning susceptibility qualified "Chacapat" best as regards marketability in terms of necessary shelf life for prolonged transportation.

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