Phenolic Composition of Litchi Fruit Pericarp

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Litchi (*Litchi chinensis*, Sapindaceae) is a nonclimacteric subtropical fruit that, once harvested, loses its red pericarp color because of browning reactions probably involving polyphenols. Low-pressure chromatography, high-pressure liquid chromatography, UV–visible spectral analysis, mass spectrometry, and nuclear magnetic resonance studies have allowed the determination and quantification of the polyphenolic composition of litchi pericarp. Litchi skins contain significant amounts of polyphenolic compounds. The principal characteristic of the litchi skin polyphenolic compounds is their ortho-diphenolic structure, which gives them high oxidability. Four major pigments were formally identified as cyanidin 3-rutinoside, cyanidin glucoside, quercetin 3-rutinoside (rutin), and quercetin glucoside. The tannin content was characterized after the depolymerization thiolysis reaction. Tannins (polymeric proanthocyanidins) are mainly constituted with epicatechin units linked by A- and B-type bonds. The different phenolic compounds of litchi cv. Kwai Mi were quantified by HPLC. Condensed tannins were the most abundant (4 mg g^{-1} of fresh skin), followed by epicatechin and procyanidin A2 (1.7 and 0.7 mg g^{-1} of fresh pericarp, respectively). The amount of anthocyanins was found to be comparable to that of flavonols, with a value of ~0.4 mg g^{-1} of fresh pericarp.

Keywords: *Litchi; polyphenols; anthocyanins; flavonols; proanthocyanidins; condensed tannins; characterization; quantification*

INTRODUCTION

Litchi (*Litchi chinensis*, Sapindaceae) is a tropical fruit originating from China having a red bright attractive pericarp surrounding a white gelatinous aril. During the past decade, the litchi industry has shown a considerable expansion (Loeillet, 1994). However, the production of high-quality fruits is affected by the excessive discoloration of the red pericarp, which rapidly turns brown after harvest, resulting in a significant postharvest loss (Snowdon, 1990). The majority of the litchi crop is exported far from its production areas to European markets, which are strongly influenced by aesthetic "eye-appeal" (Holcroft and Mitcham, 1996). Therefore, the postharvest browning of the pericarp, which is known to occur during ambient desiccation and senescence (Underhill, 1992), is a major inconvenient (Kaiser, 1994) for litchi commercial export. Consequently, studies have been developed to find solutions to limit it. Therein, anthocyanins have been largely studied primarily as they are responsible for red color. However, skin color and its preservation do not only depend on them but may involve other phenolic compounds that can act as copigments and enhance anthocyanin color (Brouillard and Dangles, 1993). Moreover, browning is related to oxidation reactions that involve numerous polyphenolic compounds directly or by means of their coupled oxidation (Mathew and Parpia, 1971; Nicolas et al., 1993; Sarni et al., 1995). It is usually associated with enzymatic activity, especially polyphenol oxidase (PPO) and peroxidase (POD) (Huang et al., 1990; Underhill and Critchley, 1995; Yue-Ming et al., 1997), the substrates of which are polyphenolic compounds. Analysis of phenols in litchi pericarp indicates numerous compounds, but information existing on the phenolic composition is poor because of the lack of formal identification of the cited compounds. Studies have primilarly been carried out by thin-layer chromatography (TLC) (Prasad and Jha, 1978; Jaiswal et al., 1987) and then by high-pressure liquid chromatography (HPLC) (Lee and Wicker, 1991). Until now, in litchi skin, the anthocyanins have been the most studied compounds. The literature indicates the presence of cyanidin 3,5-diglucoside, cyanidin 3-glucoside, cyanidin 3-rutinoside (Jaiswal et al., 1987; Lee and Wicker, 1991), cyanidin 3-galactoside (Lee and Wicker, 1991; Underhill and Critchley, 1994), pelargonidin 3,7-diglucoside (Prasad and Jha, 1978; Jaiswal et al., 1987), and malvidin 3-acetylglucoside (Lee and Wicker, 1991). Flavonols have been mentioned, but data are based only on their R_f on TLC and absorbance maxima (Jaiswal et al., 1987; Macheix et al., 1990a; Mazza and Miniati, 1993). These authors reported quercetin 3-rhamnoside, quercetin 5-glucoside, and kaempferol. The presence of condensed tannins was demonstrated by Le Roux et al. (1998), who showed that polymeric proanthocyanidins in litchi pericarp are epicatechin units linked by both A- and B-type interflavanoid bonds.

It appears that studies of polyphenolic composition of litchi skin are necessary in order to examine these conflicting data. Moreover, the anthocyanins that are reported to be largely involved in litchi pericarp browning are poor substrates of oxidation enzymes (Wesche-

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Ebeling and Montgomery, 1990; Sarni et al., 1995). They may be involved in coupled oxidation with other polyphenols, which are better substrates of enzymes. Thus, as a first step toward understanding the browning mechanisms, we report here the determination and characterization of the polyphenolic composition of the pericarp of *Lichi chinensis* cv. Kwai Mi, with the particularity of examining its proanthocyanidin content.

MATERIALS AND METHODS

Materials. Deionized water was purified with a Milli-Q water system (Millipore, Bedford, MA). All of the solvents and reagents were of analytical grade quality (Merck, Darmstadt, Germany; and Prolabo, Fontenay S/Bois, France).

(+)-Catechin and (−)-epicatechin were provided by Sigma (St. Louis, MO). Cyanidin, flavonols, and flavonol glycosides were purchased from Sigma or Extrasynthèse (Genay, France). Cyanidin 3-*O*-glucoside was purified from blackberry by PVP chromatography using a method adapted from Glories (1976). Flavanols and their thiol derivatives were obtained and characterized as already described (Prieur et al., 1994; Souquet et al., 1996; Le Roux et al., 1998).

β-Glucosidase and α-rhamnosidase were gifts from Dr. Le Traon and Dr. Günata, respectively.

Plant Material. Fruits of litchi (*L. chinensis* cv. Kwai Mi) were picked at commercial maturity (red color, diameter = 30−35 mm) and were not submitted to any postharvest treatment before air transportation from Réunion to France. The fresh fruits were selected for uniformity of shape and color, and unhealthy fruits were eliminated. They were then peeled at 4 °C, and the recovered pericarps were frozen in liquid nitrogen before drying in a vacuum to yield stabilized material. They were then stored at −80 °C to avoid deterioration until extraction and analysis.

Extraction of Phenolic Compounds. Phenolic compounds were prepared using a method adapted from Lee and Wicker (1991). Three grams of lyophilized pericarp ground in liquid nitrogen were extracted three times with 50 mL of methanol/1.5 N HCl (85:15, v/v) for 20 min at 4 °C. The three extracts were pooled and filtered on GF/C filters (Whatman, Maidstone, Kent, U.K.) before concentration under vacuum at 30 °C. During the concentration procedure, water was added to avoid hydrolysis by residual acid. The dried extract was dissolved in 40 mL of ethanol/water/TFA (55:45:0.005, v/v/v) for further purification.

Purification of Polyphenolic Compounds. The total polyphenolic extract (40 mL in ethanol/water/TFA) was fractionated by low-pressure chromatography on a Toyopearl HW40 (F) column (160 × 25 mm) (Tosohaas, Japan). The flow rate was set at 2 mL min^{-1}. The monomeric polyphenols were recovered in the fraction eluted by 800 mL of ethanol/water/TFA (55:45:0.005, v/v/v) designated FI. The crude tannin (proanthocyanidins) fraction (FII) was then eluted by 350 mL of acetone/water (60:40, v/v) and then taken to dryness under vacuum.

Fraction FI was used for further purification and characterization of major monomeric polyphenolic compounds. After being taken to dryness and dissolved in methanol/water (30:70, v/v), it was submitted to a semipreparative scale HPLC procedure on a reverse phase Microsorb Fast PCLC C18 column (3 *μ*m packing, 50 × 21.4 mm i.d.) (Rainin, Walnut Creek, CA). The analyses were performed with a system including Gilson pumps (models 305 and 306, Gilson, Villiers Le Bel, France), a UV−visible detector (Jasco 875), and a Hewlett-Packard intregator (model 3396A). Elution conditions were adapted for each class of compounds from the following gradient: 10 mL min^{-1} flow rate at 30 °C; solvent A, water/formic acid (98:2, v/v); solvent B, methanol/solvent A (80:20, v/v); isocratic for 2 min with 5% B, from 5 to 40%B in 13 min, from 40 to 60% B in 5 min, and from 60 to 90% B in 5 min. The compounds were detected at their maximum absorbance wavelength. The elution volume of major peaks was collected, evaporated under vacuum, and freeze-dried to obtained the corresponding compound in a pure dried state.

Purification of Oligomeric and Polymeric Proanthocyanindins. The 50 mg of crude tannin fraction (FII) dissolved in 10 mL of ethanol/water/TFA was submitted to a second step of low-pressure chromatography on Toyopearl HW40 (F) under the same conditions as above. The first elution with ethanol/water/TFA (140 mL) allowed recovery of the oligomers (FIIa), whereas the polymers were further eluted with acetone/water (FIIb).

Thiolysis. Tannins (100 *μ*L of FII or FIIb extract redissolved in 2 mL of methanol) were added to an equal volume of thiolytic reagent (toluene-α-thiol 5% in 0.2 N HCl in methanol), stirred, heated for 2 min at 90 °C, and analyzed by HPLC under conditions described earlier (Le Roux et al., 1998). Calibration curves were established using standards purified by semiprepreparative HPLC and identified by liquid chromatography−electrospray ionization mass spectrometry (LC-ESI-MS). To remove excess acid and toluene-α-thiol prior to MS analysis, the samples were treated as described by Le Roux et al. (1998). The average number of units in the procyanidin fraction (mDP) was determined by calculating the ratio between total (terminal plus extension) units and terminal units.

Analytical HPLC-DAD Analyses. Reverse phase HPLC analyses were performed on a Kontron Instrument system (Bio-Tek-Kontron, Milano, Italy) including a 460 autosampler, a 325 pump system, a 440 diode array detector (DAD), and an MSTI 450 data system. The column was a Nucleosil column (3 *μ*m, 125 × 4 mm i.d.) (Merck) protected with a guard column of the same material and maintained at 30 °C. Phenolic compounds were eluted under the following conditions: flow rate = 1 mL min^{-1}; solvent A, 2% formic acid in water; solvent B, acetonitrile/water/formic acid (80:15:5, v/v/v), starting with 3% B isocratically for 7 min, then from 3 to 35% B in 40 min, from 35% to 50% B in 5 min, from 50% to 80% B in 5 min followed by washing and reconditioning of the column. UV−visible spectra were recorded from 250 to 600 nm and peak areas measured at the maximum absorbance wavelength of each compound. The different fractions were analyzed in triplicate. Calibrations were performed for each compound by injection of known dilutions. Quantifications were based on peak areas at 280 nm for flavan-3-ols (response factor of epicatechin and procyanidin A2), at 350 nm for flavonols (response factor of quercetin 3-*O*-rutinoside), and at 520 nm for anthocyanins (response factors of cyanidin 3-*O*-rutiniside and cyanidin 3-*O*-glucoside).

UV−Visible Spectophotometric Analyses. All spectra were measured on a Uvikon 930 (Bio-Teck-Kontron) and were recorded from 250 to 600 nm and from 250 to 500 nm for anthocyanins and flavonols, respectively. All solutions of flavonoids were prepared in methanol at a concentration of 1.8 mM except cyanidin 3-*O*-glucoside solution, which was used at 62 mM. The spectrum with sodium methoxide (NaOMe, 2.5 g of metallic sodium in 100 mL of methanol) was obtained by adding three drops of NaOMe to a cuvette containing a 1.8 mM solution of flavonoid. After 5 min, the spectrum was rerun to check flavonoid decomposition. The spectrum with aluminum chloride ($AlCl_3$) was obtained by adding six drops of a 5% methanolic solution of $AlCl_3$ (5 g of fresh anhydrous $AlCl_3$ in 100 mL of methanol) to a cuvette containing the flavonoid solution. The $AlCl_3$/HCl spectrum was recorded immediately after the addition of three drops of HCl 37% to the cuvette containing the $AlCl_3$−flavonoid solution.

LC-ESI-MS Analyses. The apparatus was a Sciex API I Plus (Sciex, Thornhill, ON, Canada) simple quadrupole mass spectrometer with a nominal mass range up to *m*/*z* 2400, equipped with an electrospray ionization source (ESI). The conditions used were adapted to the compounds with a negative ion mode for flavanols (−4000 V applied to the electrospray needle and orifice voltage set at −60 or −120 V) and a positive ion mode for anthocyanins and flavonols (+5000 V applied to the electrospray needle and orifice voltage set at

+60 or +120 V). The instrument was scanned from 250 to 1200 amu, in steps of 0.25 amu and with a dwell time of 0.75 ms per scan.

Chromatographic separations were done on a Superspher 100RP18 (5 μm, 125 × 2 mm) (Merck) using an Applied Biosystem 140B pump (Applied Biosystem, Foster City, CA) and a 785 UV detector set at 280 nm under the following elution conditions: flow rate = 250 μL min^{-1}; temperature = 30 °C; solvent A, 2% formic acid in water; solvent B, acetonitrile/water/formic acid (80:15:5, v/v/v), starting with 15% B isocratic for 2 min, then from 15 to 40% B in 7 min, from 40% to 60% B in 1 min, and from 60% to 90% B in 2 min followed by washing and reconditioning of the column. The eluent was split at 50 μL min^{-1} going through the mass detector and at 200 μL min^{-1} into the UV detector. Continuous flow injection was performed with a medical syringe infusion pump (Harvard Apparatus, model 22, South Natick, MA) with a constant flow rate of 5 μL min^{-1} and scanned from *m*/*z* 200 to 2400 with steps of 0.2 amu and a dwell time of 1 ms.

NMR Spectroscopy. Spectra were recorded on a Varian Unity Inova 500 MHz spectrometer (Varian, Palo Alto, CA) operating at 500 MHz for ^{1}H and at 125.75 MHz for ^{13}C. One-dimensional (1D) ^{1}H and ^{13}C spectra as well as two-dimensional (2D) correlation spectra (homonuclear ^{1}H DQCOSY, heteronuclear $^{1}H-^{13}C$ gradient sensitivity-enhanced HSQC, and HMBC) were performed with a 3 mm indirect detection probe. Chemical shifts (δ) are reported in parts per million relative to the solvent peak: DMSO-d_6 at 25 °C (^{1}H, δ 2.50; ^{13}C, δ 39.5) or MeOH-d_4 at 30 °C (^{1}H, δ 3.30; ^{13}C, δ 49.0). The solvent used to dissolve the samples and the probe temperature for experiments were as followes: flavonol (DMSO-d_6, 25 °C); anthocyanin [DMSO-d_6/TFA (9:1, v/v), 25 °C]. Spectra were processed using Felix (Biosym Technologies, San Diego, CA) on a Silicon Graphics workstation.

Enzymatic Treatment of Glycosylated Polyphenols. Enzymatic incubations were carried out in 0.05 M potassium acetate buffer, pH 3.6, at 30 °C. Hydrolysis of monomeric polyphenol glycosides (final concentration = 1 mM) were performed with β-glucosidase (0.2 nkat mL^{-1}) for 60 min, whereas α-rhamnosidase (Günata et al., 1988) was at a final concentration of 0.12 nkat mL^{-1} for 120 min. The reactions were stopped by the addition of 50 μL of 1 M HCl. The samples were then analyzed by HPLC as described earlier.

Sugar Analyses. After hydrolysis of the compound by heating at 120 °C for 75 min in 2 M TFA (Albersheim et al., 1967), neutral sugars were determined by gas chromatography (GC) of the alditol acetate derivatives (Harris et al., 1984) at 210 °C on a fused-silica DB-225 capillary column (30 m × 0.32 mm i.d., 25 μm film; J&W Scientific, Folsom, CA) with hydrogen as the carrier gas.

Quantification of Polyphenols. Extraction was performed in triplicate. Polyphenolic extracts were prepared from 2 g of lyophilized pericarps as described above. Twenty-five milliliters was withdrawn, evaporated under vacuum, redissolved in 5 mL of methanol, and injected onto the HPLC system to quantify monomeric and oligomeric polyphenols. For quantification purposes, the detector was set at 280 nm for flavanols, at 520 nm for anthocyanins, and at 350 nm for flavonols. Calibration curves were performed for each compound by injections of known dilutions.

After evaporation to dryness, the rest of the extract medium was dissolved in 30 mL of ethanol/water/TFA (55:45:0.005, v/v/v). Two milliliters was submitted to low-pressure chromatography on a column of Toyopearl HW40 (F) (10 × 1 cm). The elution with ethanol/water.TFA (FI) was prolonged (50 mL) to recovere monomeric and oligomeric flavanols, whereas the polymeric tannins (FII) were obtained after elution with 20 mL of acetone/water. After the addition of *p*-hydroxybenzoyl methyl ester (used as an internal standard), the FII fraction was evaporated to dryness under vacuum and recovered with 2 mL of methanol for thiolysis reaction. The tannin concentration was then calculated from the HPLC analysis of the thiolysis reaction. The amount of condensed tannins was calculated by summing the amount of each monomer unit obtained from thiolysis degradation. Calibration curves (based on peak areas at 280 nm) were established using flavanols and benzyl thioether standards as described earlier (Prieur et al., 1994).

RESULTS AND DISCUSSION

The complex phenolic composition of litchi pericarp is demonstrated by the HPLC chromatogram recorded at 280 nm (Figure 1A). Some of the numerous peaks detected at 280 nm exhibit additional absorption maxima as shown by chromatographic profiles registrated at 520 nm (Figure 1B) and 350 nm (Figure 1C) and by UV–visible spectra. Therefore, three classes of compounds were clearly present: flavanols, as shown by the 280 nm trace; anthocyanins, 520 nm trace; and flavonols, 350 nm trace. We have to note the absence of hydroxycinnamic acids in the pericarp of litchi fruit because no compound was detected at 310 nm except the anthocyanins and flavonols. Figure 2 shows the chemical structures of the compounds thereafter identified.

Low-pressure chromatography of litchi pericarp extract on Toyopearl HW40 (F) allowed us to separate these different major classes of phenolic compounds.

Characterization of Polyphenols from Litchi Pericarp. *Monomeric Polyphenols.* The first fraction eluted by ethanol/water/TFA(FI) contained the monomeric flavanols, the anthocyanins, and the flavonols, which were further purified for characterization.

Within the compounds eluted by ethanol/water/TFA, the presence of monomeric flavanols (Figure 1A) was demonstrated, and the LC-ESI-MS analysis showed that the major peak (2) corresponds to epicatechin (Figure 2), whereas the minor peak (1) is catechin.

The two compounds presenting the maximum absorbance around 520 nm (Figure 1B) were purified and submitted to UV–visible spectrophotometry, LC-ESI-MS, and NMR analyses to ascertain their identifications. LC-ESI-MS (positive ion mode) showed that the first anthocyanin has an *m*/*z* value of 449 corresponding to $[M]^+$. With higher orifice voltage (120 V) a characteristic fragmentation $[M - 162]^+$ at 287 corresponds to the cleavage of a hexosyl moiety from the glycosylated cyanidin. For the other anthocyanin, the ion peak is detected at *m*/*z* 595, 146 amu more than the *m*/*z* value of the first one, which corresponds to an additional rhamnosyl moiety. The UV–visible studies showed that these compounds present the same spectrum as cyanidin 3-glucoside, and their $AlCl_3$ complexations (Mabry et al., 1970) are consistent with no substitution on the B ring (data not shown). GC analysis after hydrolysis and peracetylation of the sugar residues demonstrated that glucose is the only glycosyl residue encountered for anthocyanin (1′) and glucose and rhamnose were found in equimolecular ratio for anthocyanin (2′). For the latter, the sugar linkage positions were finally established by using 1D ^{1}H and ^{13}C and 2D COSY, HSQC, and HMBC NMR experiments. The chemical shifts and coupling constants of the aglycon protons determined from the 1D ^{1}H spectrum confirmed its cyanidin structure. After all rhamnose and glucose proton signals had been attributed by using COSY correlations, the chemical shifts of protonated and nonprotonated carbons were attributed by HSQC and HMBC spectra. ^{1}H and ^{13}C chemical shifts are summarized in Table 1. The presence of a long-distance correlation between the glucose anomeric proton (5.35 ppm) and the cyanidin C3 (144 ppm), itself correlated with the H4 proton, enabled us to establish that the glucose C1″ was attached to the

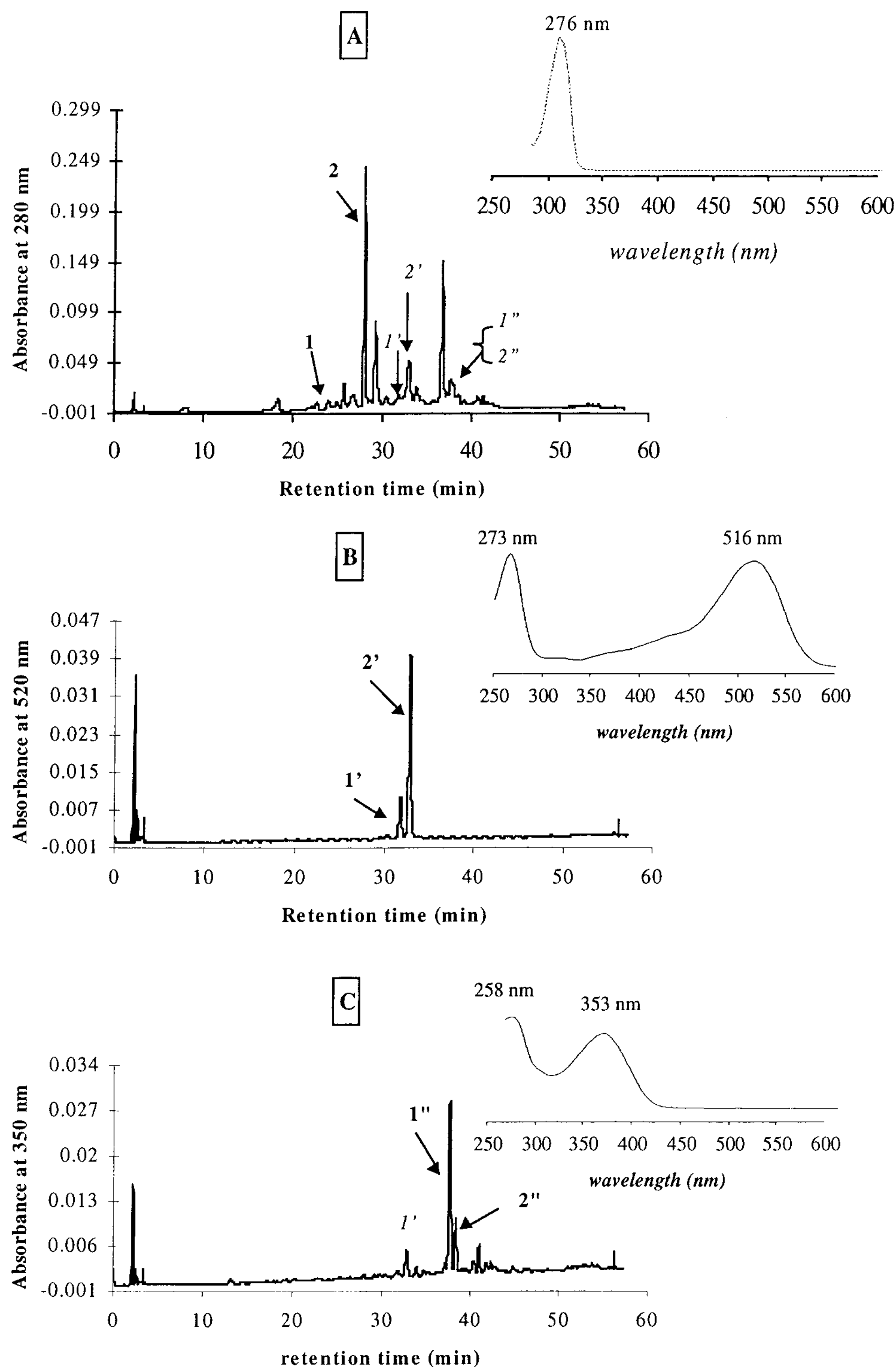

Figure 1. HPLC traces at 280 nm (A), 520 nm (B), and 350 nm (C) of polyphenol extract of litchi pericarp. Peaks showing an absorbance at 520 nm are noted with a prime (′), whereas those showing an absorbance at 350 nm are noted with a double prime (″). Both are reported in italics on the 280 nm trace. (Insets) UV−visible spectrum of the major compound detected at the corresponding wavelength.

aglycon in the 3-position. Moreover, the existence of a correlation between the glucose H6″ and the rhamnose C1‴ showed that both sugars were linked through a 1‴→6″ linkage. The anthocyanin (2′) was thus unambiguously identified as cyanidin 3-rutinoside (Figure 2). The low amount of anthocyanin (1′) purified did not allow us to perform NMR analysis. However, the data from sugar GC analysis, chromatography, UV−visible spectrophotometry, and mass spectrometry demonstrated that anthocyanin (1′) is a cyanidin glucoside, probably cyanidin 3-*O*-glucoside, as its behavior was similar to that of cyanidin 3-*O*-glucoside control and the glycosylation of anthocyanidins almost always occurs in the 3-position (Macheix et al., 1990b). The presence of these anthocyanins in litchi pericarp has been reported by Lee and Wicker (1991), but the anthocyanins were identified on the basis of the comparison of their retention time and UV−visible spectrum with external standards. In contrast with these authors, we did not detect cyanidin 3,5-diglucoside, pelargonidin 3-glycoside or 3,5-diglucoside, or malvidin 3-acetylglucoside. The lack of observation of malvidin 3-acetylglucoside may

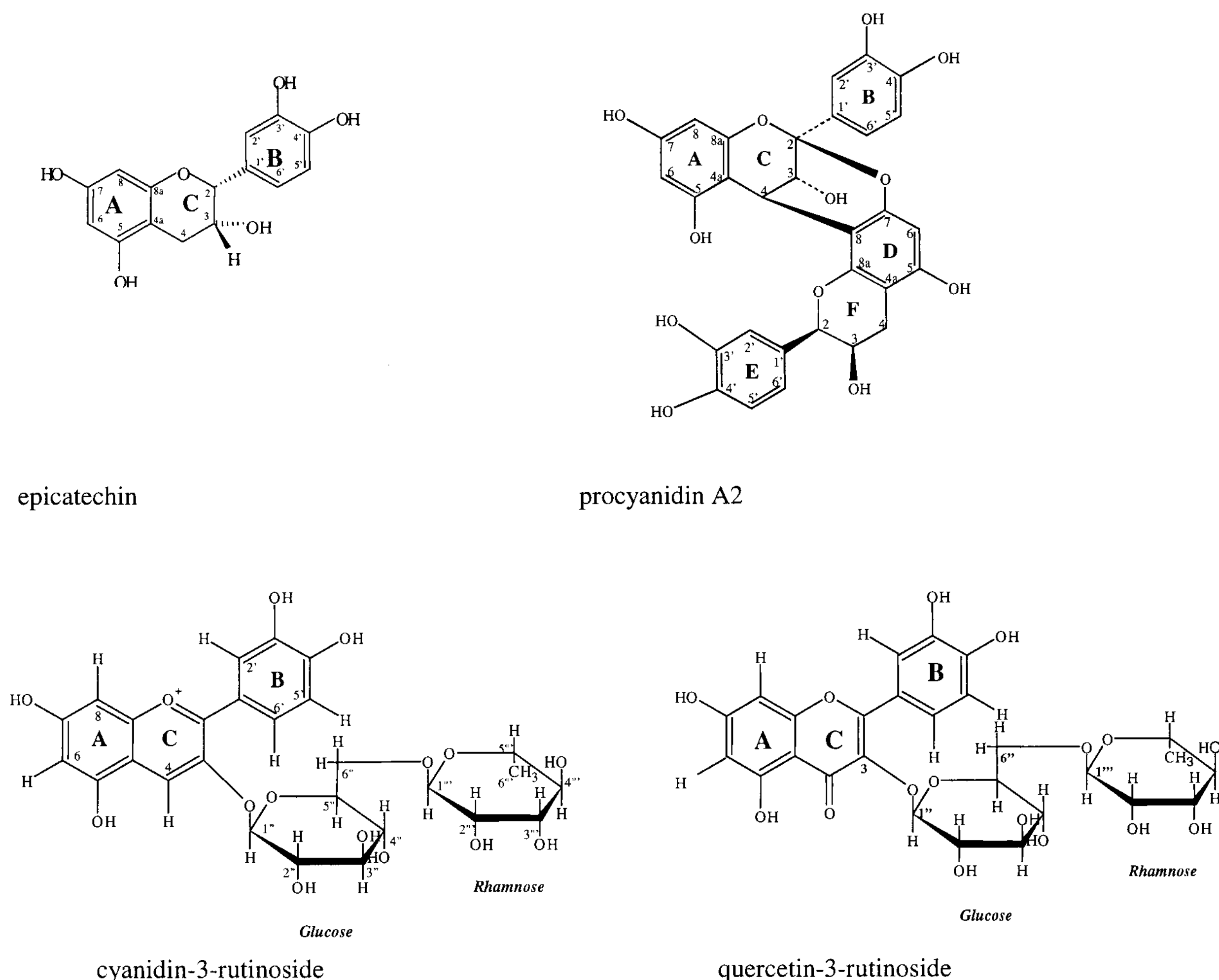

Figure 2. Chemical structures of the principal polyphenols of litchi pericarp.

be related either to its very low amount encountered, as it was found to decrease during the maturation of the fruit (Rivera-Lopez et al., 1999), or to its absence in our cultivar.

Another group of compounds present in one of the fractions eluted by the ethanol/water/TFA solvent with maximum absorbance at 350 nm corresponded to flavonols. In the Kwai Mi pericarp extract, two major compounds were detected by HPLC analysis (peaks 1″ and 2″, Figure 1C). The LC-ESI-MS spectrum (positive ion mode) showed that the most polar compound (1″) was detected at *m*/*z* 611 and compound 2″ at *m*/*z* 465, corresponding to $[M + H]^+$ ions. The increase of the orifice voltage at +120 V allowed us to obtain the same fragment ion peak at *m*/*z* 303. This fragment corresponds to the molecular ion of quercetin ($[M + H]^+$ at *m*/*z* 303). The difference of mass found between the nonfragmented and the fragmentation peaks corresponds to a diglycosyl moiety for the first flavonol (308) and to a hexosyl moiety for the second one (162). Sugar analysis of these compounds showed the presence of glucose and rhamnose on the flavonol (1″) with a molecular ratio (glucose/rhamnose) of 1.3 [this ratio is due to contamination by flavonol (2″)] and of glucose in the case of the flavonol (2″). With regard to these data, the flavonol (2″) was identified as quercetin *O*-glucoside. For flavonol (1″), the linkage position of the two glycosyl moieties has been first examined by use of enzymatic activities. The action of glucosidase activity on the flavonol (1″) was not possible without the prior action

Table 1. ^{1}H and ^{13}C Chemical Shifts and Coupling Constant (*J*) of Cyanidin 3-Rutinoside in DMSO-d_6/TFA (9:1, v/v) at 25 °C

	δ ^{1}H	*J* (Hz)	δ ^{13}C
cyanidin			
2			162.1
3			144.1
4	8.80 (s)		134.4
4a			111.8
5			157.9
6	6.70 (s)		102.6
7			168.4
8	6.87 (s)		94.2
8a			156.3
1′			154.6
2′	7.98 (d)	$J_{2',6'} = 2.0$	117.3
3′			146.6
4′			119.8
5′	7.02 (d)	$J_{5',6'} = 9.0$	116.8
6′	8.21 (dd)	$J_{6',5'} = 9.0$, $J_{6',2'} = 2.0$	123.0
glucose			
1″	5.35 (d)	$J = 7.6$	101.7
2″	3.50 (m)		73.1
3″	3.39 (m)		76.4
4″	3.22 (m)		69.8
5″	3.66 (m)		76.0
6″	3.88/3.46 (d)	$J = 10.8$	66.5
rhamnose			
1‴	4.51 (s)		100.9
2‴	3.59 (m)		70.4
3‴	3.42 (m)		70.7
4‴	3.13 (m)		72.1
5‴	3.37 (m)		68.5
6‴	1.04 (s)		17.8

Table 2. ^{1}H and ^{13}C Chemical Shifts and Coupling Constant (J) of Quercetin 3-Rutinoside in DMSO-d_6 at 25 °C

	δ ^{1}H	J (Hz)	δ ^{13}C
quercitin			
6	6.16 (s)		98.96
8	6.35 (s)		93.37
2′	7.51 (d)	$J_{2',6'} = 2.0$	115.28
5′	6.83 (d)	$J_{5',6'} = 8.3$	115.28
6′	7.54 (dd)	$J_{6',5'} = 8.3$, $J_{6',2'} = 2.0$	121.34
glucose			
1″	5.00 (d)	$J = 6.9$	101.29
2″	3−3.60 (m)		
3″	3−3.60 (m)		
4″	3−3.60 (m)		
5″	3−3.60 (m)		70.07
6″	3.69/3.26 (d)	$J = 10.5$	66.81
rhamnose			
1‴	4.37 (s)		100.36
2‴	3−3.60 (m)		
3‴	3−3.60 (m)		
4‴	3−3.60 (m)		
5‴	3−3.60 (m)		
6‴	0.98 (s)		17.40

of rhamnosidase, which gave rise to quercetin glucoside as shown by the HPLC analysis of the intermediate reaction mixture (data not shown). The end product obtained after the consecutive action of both enzymes was quercetin. These data mean that flavonol (1″) corresponds to a rhamnosyl glucoside of quercetin. Final identification of this flavonol was obtained by NMR spectrometry using the 1D ^{1}H spectrum and heteronuclear 2D HSQC and HMBC correlation spectra (Table 2). The 1D ^{1}H spectrum allowed us to assign all flavonol aglycon protons, the rhamnose methyl protons, and the glucose and rhamnose anomeric protons. The glycosidic linkage positions were determined by long-distance correlations. First, a correlation between the H-6″ of glucose the rhamnose C-1‴ confirmed that the disaccharide was a rutinose. The linkage of the disaccharide in the 3-position was then clearly demonstrated by the presence of a correlation between glucose H-1″ and the flavonol C3. The major litchi flavonol was thus unambiguously identified as quercetin 3-*O*-rutinoside (Figure 2). Due to the low amount of flavonol (2″), no NMR analysis could be performed. However, it is probably a quercetin 3-*O*-glucoside (coelution and similar UV−visible and mass analyses with control quercetin 3-*O*-glucoside). The literature on litchi polyphenolic composition cited other flavonols (Mazza and Miniati, 1993), but until now, some doubts remained on their definitive identification due to the paucity of the method used 13 years ago (Jaiswal et al., 1987). The use of UV−visible spectra and of chromatographic retention is not sufficient to clearly ensure the identity of a peak. No kaempferol glycoside was detected in litchi cv. Kwai Mi skin, whereas it was mentioned by Mazza and Miniati (1993). However, the chromatographic profile showed the presence of other minor compounds showing absorbance at 350 nm and eluting later than quercetin derivatives. Further work is required to identify these molecules. In fruits, the most common flavonol glycosides are the 3-glycosides of quercetin and kaempferol. Our results characterizing quercetin 3-*O*-rutinoside (rutin) and quercetin glucoside, probably 3-glucoside (isoquercitrin), are consistent with this general feature and with the fact that the frequency of occurrence of glycosides in fruits generally decreases in the order 3-glucosides > 3-rutinosides > 3-galactosides > 3-rhamnosides > 3-glucuronides (Dawes and Keene, 1999).

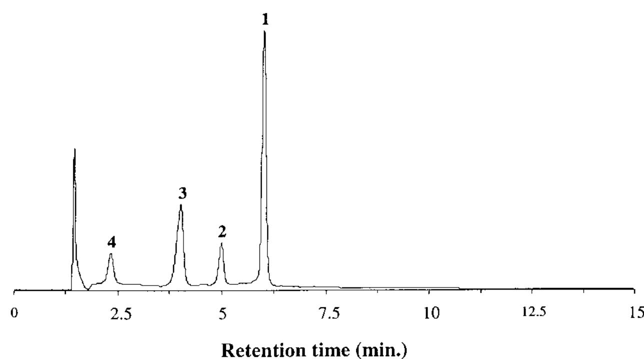

Figure 3. HPLC chromatogram at 280 nm of the FIIa fraction obtained after Fractogel chromatography.

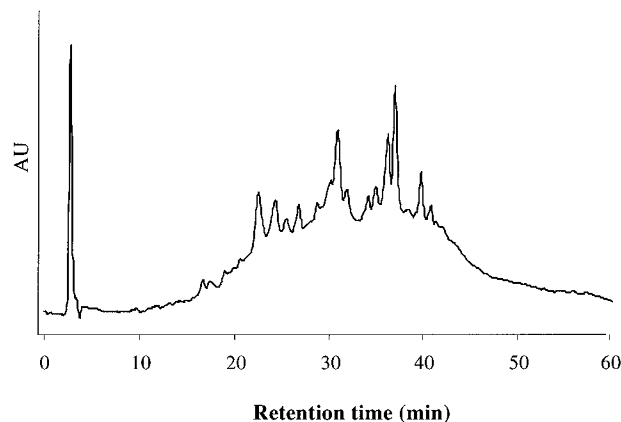

Figure 4. HPLC chromatogram at 280 nm of the FIIb fraction obtained after Fractogel chromatography.

Oligomeric and Polymeric Polyphenols. Further fractionation of the polymeric tannin extract (FIIa, Figure 3) allowed us to detect four major compounds showing characteristic flavanol spectra (λ_{max} = 276 nm). Compound **1** was purified and was identified as procyanidin A2 (Figure 2) as the ^{1}H and ^{13}C NMR spectral data corresponded with those previously described (Vivas et al., 1996). The other three compounds were detected at m/z 863 (**2** and **3**) and m/z 1151 in the negative ion mode and thus presumably correspond to (epi)catechin trimers and tetramers containing one A-type linkage. Identification of these molecules is underway. In previous literature on litchi skin composition, the term “condensed tannins” has been mentioned, but the first definitive demonstration was performed by Le Roux et al. (1998). The HPLC profile of FIIb [fraction eluted from Toyopearl HW40 (F) by acetone/H_2O] showed numerous compounds eluted as a hump between 15 and 50 min (Figure 4). The UV−visible spectra recorded along this hump showed a single absorbance band at 276.8 nm corresponding to the spectrum of flavanols. As described earlier (Le Roux et al., 1998), thiolytic degradation followed by ESI-MS and NMR analysis indicated the presence of catechin, epicatechin, and proanthocyanidin A-type constitutive units. The mDP of this fraction has been calculated at a value of 6.4, procyanidin A2 being taken into account as 2 units.

Quantitative Determination of Polyphenols from Litchi Pericarp. The quantitative contribution (Table 3) of each class of polyphenols was determined by HPLC analyses. As reported by Underhill and Critchley (1993), cyanidin 3-*O*-rutinoside was found to be the anthocyanin present at the highest concentration in fresh skin of ripe litchi (97% of total anthocyanin). The cyanidin 3-*O*-

Table 3. Concentration of the Phenolic Compounds Characterized in the Pericarp of Litchi (Mean ± SD)

compound	mg g^{-1} of fresh pericarp
epicatechin	1.720 ± 0.05
procyanidin A2	0.683 ± 0.01
trimer	0.40 ± 0.029
condensed tannins	4.02 ± 0.15
cyanidin 3-rutinoside	0.429 ± 0.068
cyanidin glucoside	0.054 ± 0.005
quercetin 3-rutinoside	0.223 ± 0.071
quercetin glucoside	0.05 ± 0.018

rutinoside concentration measured was 0.4 mg g^{-1} of fresh pericarp, which is consistent with the concentrations determined for cv. Bengal (Underhill and Critchley, 1993) and those previously reported by Rivera-Lopez et al. (1999) (0.35 mg g^{-1} of fresh pericarp) but much lower than that reported by Lee and Wicker (1991) (1.41 mg g^{-1} of fresh pericarp*)*. The additional peak of cyanidin 3-*O*-glucoside was found to represent 0.06 mg g^{-1} as observed by Rivera-Lopez et al. (1999), whereas Lee and Wicker (1991) have determined a concentration of ~0.12 mg g^{-1}. The differences may arise either from the use of different cultivars, as these authors did not mention the cultivars they studied, or from the methods used to quantify (total absorbance at 505 or 530 nm on a total polyphenol extract, HPLC area at 520 nm). Until now, the flavonol concentration had never been determined. Quercetin 3-rutinoside and quercetin glucoside contribute a relatively important part of the polyphenolic pool of litchi pericarp with a concentration of 0.3 mg g^{-1} of fresh pericarp. These compounds, which are ortho-diphenolic structures, may be involved in postharvest browning of pericarp.

Epicatechin is the most important monomeric flavanol (1.7 mg g^{-1} of fresh pericarp). Catechin and epicatechin are widely present in numerous fruits, and several studies have dealt with their oxidation in the presence of PPO or via coupled oxidation. The generated *o*-quinones are highly reactive species involved in different reaction pathways leading to many color products (Guyot et al., 1996; Amiot et al., 1997). The amount of procyanidins was determined from the data of thiolysis reaction of procyanidin extracts. The presence of high levels of oligomeric compounds (trimers plus procyanidin A2 =1.1 mg g^{-1} of fresh pericarp) has to be noted. Our investigations demonstrate that the flavanol polymers represent the major part of the polyphenols of litchi pericarp with a level of 4 mg g^{-1} of fresh skin. Thus, polymeric flavanols are found to account for 90% of the total phenolic compounds of litchi cv. Kwai Mi pericarp.

Until now, it has been stressed that the most representative compounds of litchi pericarp are anthocyanins because of their red color and their supposed involvement in postharvest browning reaction. Actually, although anthocyanins were not direct substrates for PPO (Cheynier et al., 1994), anthocyanins with ortho-diphenolic B rings were oxidized via the enzymatically generated *o*-quinones of other polyphenols (Sarni et al., 1995). We have demonstrated the abundance of flavanols, some of which may be good substrates for PPO and lead to *o*-quinones involved, on the one hand, in the formation of flavanol-derived color products and, on the other hand, in the degradation of anthocyanins via coupled oxidations and condensation reactions.

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