Available online at www.sciencedirect.com
SCIENCE DIRECT®
Food Chemistry 98 (2006) 539–544
Food Chemistry
www.elsevier.com/locate/foodchem

Identification of the major flavonoids from pericarp tissues of lychee fruit in relation to their antioxidant activities

Mouming Zhao [a], Bao Yang [a], Jinshui Wang [a], Baozhen Li [a], Yueming Jiang [b,*]

[a] College of Light Industry and Food Science, South China University of Technology, Guangzhou 510640, People's Republic of China
[b] South China Botanic Garden, The Chinese Academy of Sciences, Guangzhou Le Yi Ju 510650, People's Republic of China

Received 14 February 2005; received in revised form 15 June 2005; accepted 15 June 2005

Abstract

Large amount of polyphenolic compounds with strong antioxidant activity was present in the pericarp of harvested lychee fruits. Flavonoids were extracted with 85% ethanol:15% HCl from lychee fruit pericarp tissues. Most of the lychee flavonoids were partitioned into the ethyl acetate fraction. Three major components of the ethyl acetate fraction were obtained by reverse phase high-performance liquid chromatography and determined to be flavanol by their ultraviolet/visible spectra. Furthermore, these three components were identified as proanthocyanidin B4, proanthocyanidin B2 and epicatechin by nuclear magnetic resonance and mass spectrometry. The ethyl acetate fraction, proanthocyanidin B4, proanthocyanidin B2 and epicatechin exhibited a good antioxidant capability. The hydroxyl radical and superoxide anion scavenging activities of proanthocyanidin B2 was greater than those of proanthocyanidin B4 and epicatechin, while the epicatechin had the highest DPPH scavenging activity.

Keywords: Antioxidant activity; Epicatechin; Flavonoids; Litchi pericarp; Proanthocyanidin B4; Proanthocyanidin B2

1. Introduction

Lychee (*Litchi chinensis* Sonn.) is a tropical and subtropical fruit that has high commercial value due in part to its white and translucent aril and attractive red color (Holcroft & Mitcham, 1996). However, the fruit will rapidly lose its bright color and turn brown once harvested (Jaiswal, Sah, & Prasad, 1986; Jiang, 2000; Nip, 1988). Lychee fruit pericarp contains a large amount of pigments which are responsible for the red color (Lee & Wicker, 1991). Prasad and Jha (1978), using thin-layer chromatography (TLC), first reported that the red color of the lychee fruit was probably due to a mixture of cyanidin and pelargonidin. The red pigments were later identified as cyanidin-3-rutiside by TLC and high-performance liquid chromatography (HPLC) (Lee & Wicker, 1991). Zhang, Quantick, and Grigor (2000) reported that cyanidin-3-glucoside and malvidin-3-glucoside may be present in these anthocyanins. Sarni-Manchado, Roux, Guerneve, Lozano, and Cheynier (2000) identified the anthocyanins as cyanidin-3-rutinoside, cyanidin glucoside, quercetin-3-rutinoside, and quercetin glucoside, using low-pressure chromatography, HPLC, UV–visible light spectral analysis, mass spectrometry (MS), and nuclear magnetic resonance (NMR). Recently, Zhang, Pang, Yang, Ji, and Jiang (2004) reported that the major anthocyanin of lychee fruit pericarp was cyanidin-3-rutinoside. Differences in the identification of the anthocyanins may be attributed to differences in the extraction and purification procedures (Jiang, Duan, Joyce, Zhang, & Li, 2004). In our previous study, most of the red pigments in lychee fruit pericarp were present in the ethyl acetate fraction and the pigments were identified as flavanols. Identification

* Corresponding author. Tel.: +86 20 37252525; fax: +86 20 37252831.
E-mail address: ymjiang@scib.ac.cn (Y. Jiang).

0308-8146/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2005.06.028

of structures of flavanols was needed to explain the loss in the skin color of harvested lychee fruit, in relation to their antioxidant activity.

Recent research has shown that pigments in postharvest fruits exhibit a strong antioxidant activity (Einbond, Reynertson, Luo, Basile, & Kennelly, 2004; Bao, Cai, Sun, Wang, & Corke, 2005). Antioxidative properties of pigments resulted from their high reactivity as hydrogen or electron donors and from their ability to chelate transition metal ions (termination of the Fenton reaction) (Rice-Evans, Miller, & Paganga, 1997; Wada & Ou, 2002). It is well established that enzymatic browning of postharvest fruits is related to antioxidant activity (Martinez & Whitaker, 1995). Unfortunately, little information on the antioxidant activity of skin pigments from harvested lychee fruit is available.

The objective of this study was to extract and purify the major flavonoids of lychee fruit pericarp, using 85% ethanol:15% HCl, hexane, ethyl acetate and butanol and reverse phase HPLC, and then determine the molecular weight and chemical structure by MS and NMR analyses. The antioxidant activities of the major flavonoids were also evaluated.

2. Materials and methods

2.1. Materials

Fresh fruits of lychee (*Litchi chinensis* Sonn.) cv. Huaizhi at the commercially mature stage were picked from a commercial orchard in Guangzhou, China. Fruits were selected for uniformity of shape and color.

2.2. Chemicals

1,1-Diphenyl-2-picryldydrazyl (DPPH), nitro blue tetrazolium (NBT), phenazine methosulphate (PMS), dihydronicotineamidadenine dinucleotide (NADH), thiobarbituric acid (TBA) and deoxyribose were purchased from Sigma Chemical Co. (St Louis, MO, USA). All other chemicals used were of analytical grade.

2.3. Extraction of flavonoids

Fresh lychee pericarp tissues (5 g) were extracted for 2 h at 4 °C in 400 ml extraction medium consisting of 85% ethanol:15% HCl by the methods of Lee and Wicker (1991) and Argolo, Asnt'Ana, Pletsch, and Coelho (2004), with a minor modification. After filtering the extract through Whatman no. 1 paper, the residue was re-extracted and filtered a second time. Filtrates were combined and dried using a rotary evaporator at 40 °C. The dried extract was re-dissolved in 100 ml water and then partitioned with 300 ml of hexane, ethyl acetate and butanol squentially. The different fractions of hexane, ethyl acetate, butanol and water were separately collected. The flavonoid content in each extract was determined by aluminum nitrate method (Moreno, Isla, Sampietro, & Vattuone, 2000). In this study, most of flavonoids were detected in the ethyl acetate fraction (Table 1), and, thus, the ethyl acetate fraction of the flavonoids was used for further purification.

Table 1
Comparison of flavonoid content in hexane, ethyl acetate, butanol and water fractions

Solvent fraction	Flavonoid content (mg/g DW)	Percent flavonoids
Hexane	0.04 ± 0.00	0.2 ± 0.01
Ethyl acetate	21.3 ± 0.1	83.1 ± 0.5
Butanol	3.5 ± 0.05	13.6 ± 0.2
Water	0.8 ± 0.02	3.1 ± 0.06

Data were presented as means ± standard deviations of three replication determinations.

2.4. Purification of major flavonoids

The ethyl acetate fraction was dried using a rotary evaporator at 40 °C. The dried extract was re-dissolved in a small volume of ethanol. A 100 μl of samples was injected to an AKTA^{TM} purifier HPLC system (Amersham biotechnology Co., Swiss). The HPLC analysis was carried out on a reverse phase polystyrene/divinyl column (100 × 6.4 mm). Solvent A (water/formic acid = 98:2, v/v) and solvent B (acetonitrile/water/formic acid = 80:19:1, v/v/v) were used as mobile phases. The elution was allowed to run for 4 min with 3% B and 97% A, and then solvent B increased from 3% to 50% B while solvent B decreased from 97% to 50% for 14 min, linearly. The flavonoids were detected at 280 nm, and three major fractions (P1, P2 and P3 in Fig. 1) was collected and then freeze–dried separately.

2.5. UV–visible spectrophotpmetric analysis

Each of three major fractions (1 mg) was dissolved in 10 ml of ethanol. The sample solution was scanned at from 200 to 580 nm, using a UV-2102 PC UV–visible spectrophotometer (Unico, China) while the spectra were recorded.

2.6. Molecular weight estimation

MS system (LCQ^{DECA}, Finigan company, USA), equipped with a Hewlett–Packard 9000 computer system, was used to determine the molecular weight. Sample (1 mg) was dissolved in 10 ml of ethanol. A 100 μl of sample solution was injected into the MS system. Mass spectroscopy was recorded with a heat capillary voltage of 4.5 kV, a heat capillary temperature of 270 °C, sheath gas flow rate of 70 units and auxiliary gas flow rate of 10 units. The scan range of *m/z* was 200–1200.

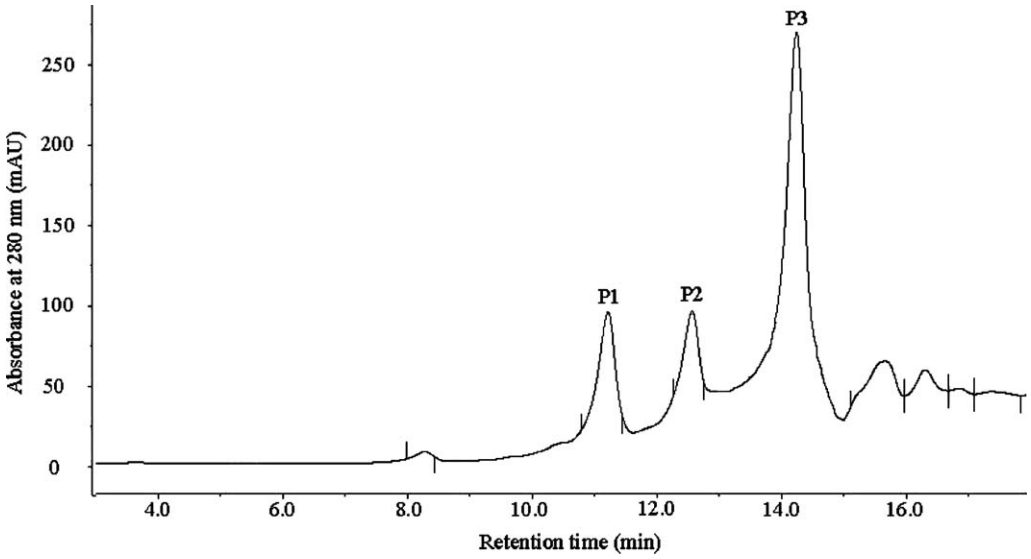

Fig. 1. Elution profiles of the ethyl acetate fraction of litchi flavonoids separated by reverse phase HPLC.

2.7. NMR spectroscopy

^{13}C NMR spectra were recorded with a Bruker AC 300 Instrument at 30 °C using 3 mm tubes. Samples (10 mg) were dissolved in 0.5 ml of MeOH-d4. ^{13}C chemical shifts were expressed in parts per million (ppm) relative to tetramethyl silane (TMS) as an internal standard.

2.8. Determination of antioxidant capability

2.8.1. DPPH scavenging activity

The free radical scavenging activity was measured using the method of Shimada, Fujikawa, Yahara, and Nakamura (1992) with some modification. Samples were dissolved in 10 ml ethanol at 0 (control), 20 or 40 μg/ml. A 2 ml of 0.2 mM DPPH in ethanol was added to 1 ml of the sample solution. The absorbance at 517 nm was measured after 20 min of incubation at 25 °C. The inhibition of DPPH radicals by the samples was calculated according to the following equation: DPPH scavenging activity (%) = [1 − absorbance of sample/absorbance of control] × 100.

2.8.2. Evaluation of hydroxyl radical scavenging activity

The hydroxyl radical scavenging activity was measured by the method of Ghiselli, Nardini, Baldi, and Scaccini (1998). Samples were dissolved in 10 ml ethanol at 0 (control), 200 or 400 μg/ml. Sample solution (0.1 ml) was mixed with 0.8 ml of reaction buffer [0.2 M phosphate buffer (pH 7.4), 1.75 μmol deoxyribose, 0.1 μmol iron ammonium sulphate and 0.1 μmol EDTA]. 0.1 ml of 1.0 mM ascorbic acid and 0.1 ml of 0.01 M H_2O_2 was then added to the reaction solution. The reaction solution was incubated for 10 min at 37 °C before 0.5 ml of 1% thiobarbituric acid and 1 ml of 2.8% trichloroacetic acid were added to the mixture. The mixture was boiled for 10 min and cooled on ice. The absorbance of the mixture was measured at 532 nm. Percent inhibition of deoxyribose degradation was calculated as (1 − absorbance of sample/absorbance of control) × 100.

2.8.3. Determination of superoxide anion scavenging activity

The superoxide anion scavenging activity was measured by the method of Robak and Gryglewski (1988) with a minor modification. Samples were dissolved in 10 ml ethanol at 0 (control), 20 or 40 μg/ml. A 1 ml aliquot of each sample solution was mixed with 1 ml of 0.1 M phosphate buffer (pH 7.4) containing 150 μM NBT, 60 μM PMS and 468 μM NADH. After 5 min of incubation at 25 °C the absorbance was measured at 560 nm. The superoxide anion scavenging activity was calculated as follows: scavenging activity (%) = (1 − absorbance of sample/absorbance of control) × 100.

2.9. Data handling

All the data were expressed as means ± standard deviations of three replication determinations.

3. Results and discussion

3.1. Comparison of flavonoid content in four fractions

Hayder et al. (2004) reported that application of hexane, chloroform and ethyl acetate can partition flavonoids from *Myrtus communis*. Based on different

polarities, lychee pericarp flavonoids could be partitioned into four fractions of hexane, ethyl acetate, butanol and water. In this study, the ethyl acetate fraction of flavonoids accounted for 83.1% of the total quantity (Table 1). This study corroborated that of Zhang et al. (2004) who reported that part of lychee anthocyanins had solubility in water. However, the composition of the butanol fraction of flavonoids is needed to be identified further.

3.2. Separation of ethyl acetate fraction of flavonoids

A reverse phase HPLC gave a successful resolution of major flavonoids (Robards & Antolovich, 1997; Rohr, Meier, & Sticher, 1999; Merken & Beecher, 2000; Maatta, Kamal-Eldin, & Torronen, 2003). In this analysis, the HPLC profiles exhibited three major peaks (P1, P2 and P3) of the ethyl acetate fraction (Figs. 1 and 2), with their levels being 3.3, 3.0 and 8.7 mg/g pericarp tissue on the basis of dry weight (DW), respectively. Thus, these three fractions were collected separately for further identification.

3.3. UV–visible spectrophotpmetric analysis

Flavanol and flavonol have maximum absorbances at about 280 and 350 nm, respectively, while anthocyanin has a maximum absorbance at about 280 and 520 nm (Pascale, Erwan, Christine, Yves, & Veronique, 2000). The UV–visible spectrophotometric analysis showed that P1, P2 and P3 had the maximum absorbance around 280 nm, which suggested that they could be flavanols.

3.4. Identification of flavonoids

P1, P2 and P3 were identified as proanthocyanidin B4, proanthocyanidin B2 and epicatechin, respectively, by UV, MS and NMR analyses, based on following characteristics.

P1: UV_{max} at 279 nm; $[M - H]^-$ peak at *m/z* 577.1; ^{13}C NMR: 29.6, 38.9, 67.7, 73.7, 79.9, 83.8, 96.3, 97.5, 97.7, 99.5, 101.2, 108.2, 114.2–115.9, 119.2, 120.3, 131.2–132.6, 145.6–146.5 and 155.4–158.7. P1 gave $[M - H]^-$ at *m/z* 577.1 on negative-ion ES–MS, which was consistent with a proanthocyanidin dimmer (Baba, Osakabe, Natsume, & Terao, 2002). Its dimeric constitution was apparent from the presence of the four clearly defined carbon signals in the heterocyclic region, with the C-2 carbon signals at δ 83.8 and 79.9 ppm being a 2,3-*trans* flavan unit with an appended substitute at C-4 and a 2,3-*cis* terminating unit (Foo, Newman, Waghorn, McNabb, & Ulyatt, 1996). The C-3 carbon signals at δ 67.7 and 73.7 ppm were a flavan terminal unit and an extended unit.

P2: UV_{max} at 279.5 nm; $[M - H]^-$ peak at *m/z* 577.3; ^{13}C NMR: 29.3, 36.9, 66.4, 72.5, 76.3, 79.2, 95.8, 96.3, 97.2, 100.5, 102.1, 107.4, 114.9–115.8, 119.2, 119.3, 131.4, 131.6, 145.0–146.1 and 155.1–157.9. P2 exhibited a $[M - H]^-$ at *m/z* 577.3 by negative-ion ES–MS, also indicating the presence of a proanthocyanidin dimer. Its ^{13}C NMR spectrum showed a similar chemical constitution to P1. C-2 (δ 76.3 and 79.2 ppm) indicated two flavan units which possessed the 2,3-*cis* configuration. The observation of two upfield methine carbon signals (δ 114.9–115.8 ppm), together with those of the hydroxylated carbon signals (δ 145.0–146.1 ppm), was evidence for the presence of a catechol B-ring.

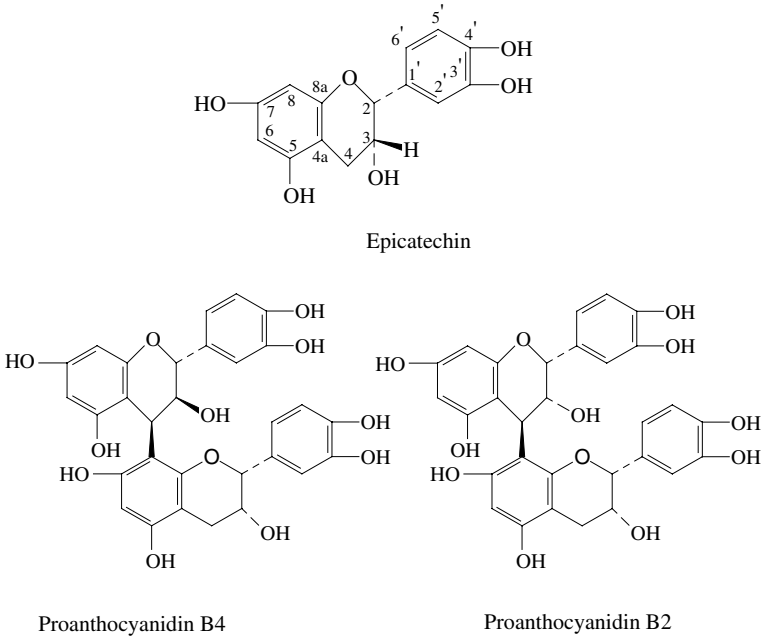

Fig. 2. Molecular formula of epicatechin, proanthocyanidin B2 and proanthocyanidin B4.

Table 2
Comparison of radical scavenging activity (%) of the ethyl acetate fraction, proanthocyanidin B2, proanthocyanidin B4 and epicatechin

Sample	Amount (μg)	DPPH scavenging activity	Hydroxyl radical scavenging activity	Superoxide anion scavenging activity
Ethyl acetate fraction	20	50.9 ± 1.0	76.3 ± 0.6	81.4 ± 1.0
	40	74.6 ± 1.3	95.7 ± 0.8	91.0 ± 1.1
Proanthocyanidin B4	20	46.1 ± 1.0	42.6 ± 0.7	62.2 ± 0.8
	40	54.5 ± 1.0	56.3 ± 1.2	71.3 ± 1.2
Proanthocyanidin B2	20	58.6 ± 0.5	72.9 ± 1.1	79.4 ± 1.6
	40	73.8 ± 0.9	96.8 ± 1.3	93.2 ± 1.4
Epicatechin	20	60.4 ± 0.6	38.7 ± 0.3	54.1 ± 1.0
	40	76.0 ± 0.8	52.3 ± 0.9	68.4 ± 0.9

Data were presented as means ± standard deviations of three replication determinations.

P3: UV_{max} at 278 nm; $[M - H]^-$ peak at *m/z* 289.1; ^{13}C NMR: 28.9 (C-4), 66.9 (C-3), 79.4 (C-2), 95.5 (C-8), 96.2 (C-6), 99.7 (C-4a), 115.3 (C-2′), 115.6 (C-5′), 119.3 (C-6′), 132.1 (C-1′), 145.3 (C-3′), 145.5 (C-4′), 157.0 (C-5), 157.5 (C-7) and 157.6 (C-8a). Identification of P3 as epicatechin was apparent from the ^{13}C NMR spectrum. A methylene carbon in the upfield region (δ 28.9 ppm) and two oxygenated methine carbons in the heterocyclic region (δ 66.9 and 79.4 ppm) were characteristic of the pyran C-ring of flavanols while the upfield position of the C-2 carbon (δ 79.4 ppm) was characteristic of the epicatechin chemical shift (Lu & Foo, 1997). The chemical structure of P3 was further corroborated by ES–MS, which gave a $[M - H]^-$ at *m/z* 289.1 using a negative-ion probe.

3.5. Antioxidant activities of P1, P2, P3 and ethyl acetate fractions

Flavonoids have exhibited a strong antioxidant capability (Robards & Antolovich, 1997; Ghiselli et al., 1998; Bao et al., 2005). In this study, proanthocyanidin B2 had higher scavenging activities of hydrogen radicals and superoxide anion than proanthocyanidin B4 and epicatechin, while the epicatechin had the highest DPPH scavenging activity (Table 2). The ethyl acetate fraction of the flavonoids, comprising mainly of proanthocyanidin B2, proanthocyanidin B4 and epicatechin, had good scavenging activity. The scavenging activity enhanced with increasing concentration used. In addition, synergistic effect of the antioxidant activity, particularly in scavenging activities of hydrogen radicals and superoxide anions could exist among proanthocyanidin B2, proanthocyanidin B4 and epicatechin.

4. Conclusion

The ethyl acetate fraction contained most of flavonoids present in lychee fruit pericarp. The major flavonoids were separated by reverse phase HPLC and identified as proanthocyanidin B4, proanthocyanidin B2 and epicatechin by UV, NMR and MS analyses. In addition, proanthocyanidin B2, proanthocyanidin B4 and epicatechin exhibited a strong antioxidant activity.

Acknowledgements

The financial support provided by National Natural Science Foundation of China (No. 30425040), the International Foundation for Science (Grant No. E2265/3F) and Guangdong Provincial Agricultural Research Foundation was greatly appreciated.

References

Argolo, A. C. C., Asnt'Ana, A. E. G., Pletsch, M., & Coelho, L. C. B. (2004). Antioxidant activity of leaf extracts from *Bauhinia monandra*. *Bioresource Technology, 95*, 229–233.

Baba, S., Osakabe, N., Natsume, M., & Terao, J. (2002). Absorption and urinary excretion of procyanidin B2 [epicatechin-(4β-8)-epicatechin] in rats. *Free Radical Biology and Medicine, 33*, 142–148.

Bao, J. S., Cai, Y. Z., Sun, M., Wang, G. Y., & Corke, H. (2005). Anthocyanins flavonols and free radical scavenging activity of Chinese bayberry (*Myrica rubra*) extracts and their color properties and stability. *Journal of Agricultural and Food Chemistry, 53*, 2327–2332.

Einbond, L. S., Reynertson, K. A., Luo, X. D., Basile, M. J., & Kennelly, E. J. (2004). Anthocyanin antioxidants from edible fruits. *Food Chemistry, 84*, 23–28.

Foo, L. Y., Newman, R., Waghorn, G., McNabb, W. C., & Ulyatt, M. J. (1996). Proanthocyanidins from *lotus corniculatus*. *Phytochemistry, 41*, 617–624.

Ghiselli, A., Nardini, M., Baldi, A., & Scaccini, C. (1998). Antioxidant activity of different phenolic fractions separated from an Italian red wine. *Journal of Agriculture and Food Chemistry, 46*, 361–367.

Hayder, N., Abdelwahed, A., Kilani, S., Ammar, R. B., Mahnoud, A., Ghedira, K., et al. (2004). Anti-genotoxic and free-radical scavenging activities of extracts from (Tunisian) Myrtus communis. *Mutation Research, 564*, 89–95.

Holcroft, D. M., & Mitcham, E. J. (1996). Postharvest physiology and handling of litchi (*Litchi chinensis* Sonn.). *Postharvest Biology and Technology, 9*, 265–281.

Jaiswal, B. P., Sah, N. L., & Prasad, U. S. (1986). Studies on phenolics of rind and aril during ripening and senescence of litchi fruits. *Plant Physiology and Biochemistry, 13*, 40–45.

Jiang, Y. (2000). Role of anthocyanins, polyphenol oxidase and phenols in lychee pericarp browning. *Journal of the Science of Food and Agriculture, 80*, 305–310.

Jiang, Y. M., Duan, X. W., Joyce, D., Zhang, Z. Q., & Li, J. R. (2004). Advances in understanding enzymatic browning of harvested litchi fruit. *Food Chemistry, 88*, 443–446.

Lee, H. S., & Wicker, L. (1991). Anthocyanin pigments in the skin of lychee fruit. *Journal of Food Science, 56*, 466–468.

Lu, Y., & Foo, L. Y. (1997). Identification and quantification of major polyphenols in apple pomace. *Food Chemistry, 59*, 187–194.

Maatta, K. R., Kamal-Eldin, A., & Torronen, A. R. (2003). High-performance liquid chromatography (HPLC) analysis of phenolic compounds in berries with diode array and electrospray ionization mass spectrometric (MS) detection. *Journal of Agricultural and Food Chemistry, 51*, 6736–6744.

Martinez, M. V., & Whitaker, J. R. (1995). The biochemistry and control of enzymatic browning. *Trends in Food Science and Technology, 6*, 195–200.

Merken, H. M., & Beecher, G. R. (2000). Measurement of food flavonoids by high-performance liquid chromatography: a review. *Journal of Agricultural and Food Chemistry, 48*, 577–599.

Moreno, M. I. N., Isla, M. I., Sampietro, A. R., & Vattuone, M. A. (2000). Comparison of the free radical-scavenging activity of propolis from several regions of Argentina. *Journal of Ethnopharmacology, 71*, 109–114.

Nip, W. K. (1988). Handling and preservation of lychee (*Litchi chinensis* Sonn.) with emphasis on colour retention. *Tropical Science, 28*, 5–11.

Pascale, S. M., Erwan, L. R., Christine, L. G., Yves, L., & Veronique, C. (2000). Phenolic composition of litchi fruit pericarp. *Journal of Agriculture and Food Chemistry, 48*, 5995–6002.

Prasad, U. S., & Jha, O. P. (1978). Changes in pigmentation patterns during litchi ripening: flavonoid. *The Plant Biochemical Journal, 5*, 44–49.

Rice-Evans, C., Miller, N. J., & Paganga, G. (1997). Antioxidant properties of phenolic compounds. *Trends in Plant Science, 2*, 152–159.

Robak, J., & Gryglewski, R. J. (1988). Flavonoids are scavengers of superoxide anions. *Biochemical Pharmacology, 37*, 837–841.

Robards, K., & Antolovich, M. (1997). Analytical chemistry of fruit bioflavonoids: a review. *Analyst, 122*, 11–34.

Rohr, G. E., Meier, B., & Sticher, O. (1999). Quantitative reverse-phase high-performance liquid chromatography of procyanidins in crataegus leaves and flowers. *Journal of Chromatography A, 835*, 59–65.

Sarni-Manchado, P., Roux, E. L., Guerneve, C. L., Lozano, Y., & Cheynier, V. (2000). Phenolic composition of litchi fruit pericarp. *Journal of Agricultural and Food Chemistry, 48*, 5995–6002.

Shimada, K., Fujikawa, K., Yahara, K., & Nakamura, T. (1992). Antioxidative properties of xanthan on the autoxidation of soybean oil in cyclodextrin emulsion. *Journal of Agriculture and Food Chemistry, 40*, 945–948.

Wada, L., & Ou, B. X. (2002). Antioxidant activity and phenolic content of oregon caneberries. *Journal of Agricultural and Food Chemistry, 50*, 3495–3500.

Zhang, D. L., Quantick, P. C., & Grigor, J. M. (2000). Changes in phenolic compounds in Litchi (*Litchi chinensis* Soon.) fruit during postharvest storage. *Postharvest Biology and Technology, 19*, 165–172.

Zhang, Z. Q., Pang, X. Q., Yang, C., Ji, Z. L., & Jiang, Y. M. (2004). Purification and structural analysis of anthocyanins from litchi pericarp. *Food Chemistry, 84*, 601–604.