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## Changes in anthocyanin concentration in Lychee (*Litchi chinensis* Sonn.) pericarp during maturation

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### Abstract

Lychee has an edible aril surrounded by an anthocyanin-rich pericarp. Chemical and physical changes during lychee ripening were studied focusing on anthocyanins (Acy). Length increased up to the 17th week after anthesis (waa), and width and weight increased continuously. Pericarp % decreased until the 17th waa while aril % increased until the 19th waa. The fruit became darker during ripening and changed from light green to yellow-green (12–15th waa), and from yellow-red to dark red (16–20th waa). pH decreased from 3.18 to 2.68 (12–15th waa), finally increasing to 4.49. Highest titratable acidity occurred in the 14th waa (4.44% malic acid) decreasing to a final 0.16%. Soluble solids were highest at ripeness (18 °Bx). Increases in monomeric (mAcy) and total Acy levels were observed up to the 17th waa. Green fruits contained only malvinidin-3-acetylglucoside and polymeric pigment while the ripe fruit contained cyanidin-3-rutinoside (>75%), cyanidin-3-glucoside (<17%) and malvinidin-3-acetylglucoside (<9%). © 1999 Elsevier Science Ltd. All rights reserved.

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### 1. Introduction

The lychee (*Litchi chinensis* Sonn.) is an exotic fruit from southeast Asian origin that has been grown in the last 30 years in the semitropical coastal lands of Sinaloa, a northwestern state of Mexico. The non-climateric fruit has a white, translucent aril, with a sweet and citrus flavor, that can be eaten fresh or processed as jelly or wine, or used in ice creams and sherberts (Salunke & Desai, 1984). It is covered by a leather-like pericarp with a bright red color due to the presence of anthocyanins (Lee & Wicker, 1991).

The high post-harvest losses reported of the ripe lychee fruit are due to the rapid color degradation of the pericarp. Some post-harvest treatments have been proposed to decrease color losses including a benomyl dip – plastic bag storage (Scott, Brown, Chaplin, Willcox & Bain, 1982; Huang & Scott, 1985; Campbell, 1959) combined with the use of anti-microbials to extend sto-

rage time (Gaur & Bajpai, 1978). Blanching or dehydration resulted in accelerated color degradation (Akamine, 1960). Color deterioration in the pericarp could be due to browning reactions, and active polyphenol oxidase and peroxidase have been reported to being present in the fruit (Huang, Hart, Lee & Wicker, 1990). Lee & Wicker (1991) mention the existence of other anthocyanin related compounds present in the pericarp that may also be involved in color losses. Degradation of anthocyanin pigments by condensation with quinones formed from endogenous phenolics due to the action of polyphenol oxidase has been reported as a possible mechanism of color loss (Wesche-Ebeling & Montgomery, 1990).

Lychee pericarp is a byproduct of lychee processing and the presence of anthocyanins make it a potential source of these pigments for the food industry, but no reports exist on the pre-harvest chemical changes in the lychee fruit, including the anthocyanin pigments. The objective of our study was to study the physical and chemical changes in the lychee fruit during ripening including the individual anthocyanin pigments.

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## 2. Materials and methods

### 2.1. Materials

Lychee (*Litchi chinensis* Sonn. var. Brewster) fruits were collected at a commercial farm in Culiacán, Sinaloa, México during the 1996 season. Fruits were randomly sampled using a Z-pattern (González-San José, Barron & Diez, 1990). The first samples were collected at 12 weeks after anthesis (waa, immature) and thereafter every week until 20 waa (start of senescence). The samples were washed, packed in polyethylene bags, transported in ice to the lab and kept frozen ( $-30^{\circ}\text{C}$ ) until used.

### 2.2. Physical measurements

**Color measurements:** The Hunter  $L^*$ ,  $a^*$  and  $b^*$  values were measured 10 times on each of 25 fresh fruits using a Minolta colorimeter (Chromameter CR-210, Japan) and the average Hunter values were calculated.

**Weight:** An OHAUS Precision Plus TP2KS (Ohaus Corp., Florham Park, NJ) was used to determine the average weight (g) of the 25 sample fruits used for color measurement.

**Physical dimensions:** A vernier was used to measure the length and average width of the 25 sample fruits and calculated in cm.

**Proportions of the fruit components:** The pericarp, aril and seeds of the 25 sample fruits were manually separated and the average % contribution in weight (g) of each was determined

### 2.3. Chemical characterization

**Chemical changes in the aril during development:** The 25 arils obtained for each development stage were placed inside an Ultramar juice extractor (Turmix de México) to obtain a juice. pH was obtained by direct immersion of the electrode according to the method described by AOAC (1990). Titratable acidity was determined according to AOAC method 942.15 titrating a 20 ml juice sample + 100 ml distilled water with 0.1N NaOH until the turning point of phenolphthalein and reported as % malic acid. Soluble solids were determined from a filtered (Whatman # 1) juice sample placed on an ABBE refractometer (Bellingam + Stanley Limited, England) and reported as  $^{\circ}\text{Bx}$ .

**Anthocyanin extraction:** A 20 g sample was homogenized in a 50 ml extraction solvent in a Waring blender at low speed for 2 min, extracted for 2 or 12 h at room temperature, and the extracts filtered through the medium followed by fine sintered glass funnels. The extraction solvents tried were: (A) Ethanol 95%:HCl 1.5 N (85:15) for 2 h, (B) Ethanol 95%:HCl 1.5 N (85:15) for 12 h, and (C) HCl:Methanol (1:99) for 2 h.

**Total anthocyanins, monomeric anthocyanins and degradation index:** Total anthocyanins (tAcy) were determined according to the single pH method reported by Fuleki and Francis (1968a) using 522 nm and the absorption coefficient reported for cranberry anthocyanin. This method includes all red-colored pigments and monomeric Acy.

Monomeric Acy (mAcy) were determined according to the method reported by Fuleki and Francis (1968b). 3 ml of the MeOH–HCl extract were diluted to 100 ml using the same solvent. Two 1 ml aliquots were taken and 6 ml of a pH 1 buffer (0.2 N KCl:0.02 N HCl (25:67)) were added to the first and 6 ml of a pH 4.5 buffer (1 N sodium acetate:1 N HCl:water (100:60:90)) to the second. The difference between the 510 nm absorbance at pH 1 and pH 4.5 was used to determine the mAcy concentration. mAcy change to the uncolored carbinol pseudobase at pH 4.5 and the red color from other pigments is then subtracted from tAcy obtaining the concentration of mAcy.

The degradation index (DI) was determined according to Fuleki and Francis (1968b) using the equation  $[\text{DI} = \text{tAcy}/\text{mAcy}]$ .

### 2.4. HPLC identification and quantification of the individual Acy

**Extraction of Acy:** Extraction of the Acy was done according to the methodology of Chandra, Muraleedharan and Iezzoni (1992) with slight modifications. Fruit from each stage was thawed and 20 g of the manually-removed pericarp homogenized in 50 ml HCl:MeOH (1:99). The mixture was homogenized in a Waring blender at low speed for 2 min, left standing for 2 h and centrifuged (Sorvall RC5C, Sorvall Instruments) at  $10,000 \times g$  for 10 min at  $20^{\circ}\text{C}$ . The supernatant was separated and the precipitate washed twice with 20 ml MeOH–HCl (1:99) and recentrifuged. The supernatants were combined, diluted to 100 ml with MeOH–HCl 1% and stored at  $-30^{\circ}\text{C}$  until used.

**Purification of Acy:** 10 ml of the extracted Acy were evaporated to dryness in a Büchi rotovapor model RE-121 (Brinkman Instrumentes, NY) and redissolved in 10 ml distilled water. The purification procedure was done according to the methodology of Dussy, Sugar and Wrolstad (1995) with slight modifications. 1 g of polyvinylpyrrolidone (PVPP) was preconditioned with 15 ml distilled water overnight and poured in a Gooch crucible lined with Whatman No. 1 filter paper. The Acy sample was absorbed onto the PVPP and the PVPP was washed with distilled water until the eluent became uncolored. The absorbed Acy was eluted with three washes of 10 ml MeOH–HCl each, the washes were combined and centrifuged as above. The supernatant was evaporated to dryness in the rotovapor (as above) and the Acy redissolved in 2 ml aqueous 4% phosphoric

acid and filtered through a 0.45 µm membrane (Millipore Corp., Bedford, MA).

**Chromatographic conditions:** A Varian Star 9050 HPLC system attached to a UV-Vis detector (Varian Star 9050) and a Hewlett-Packard 4400 integrator were used for the analyses. Acy were separated according to the methodology described by Lee & Wicker (1991). A Nova-Pak (Waters Corp., Millford, MA) reversed phase column (C18, 4 µm, 4.5 × 250 mm<sup>2</sup>) was used. Eluent A (aqueous 4% phosphoric acid), Eluent B (Acetonitrile); gradient 6% B for 5 min, 6–20% B for 45 min, 20% B for 10 min, 20–100% B for 1 min and 100% B for 10 min; sample size 10 µl, flow rate 1 ml/min and detection at 520 nm.

### 2.5. Statistical analysis

25 fruits were collected at each maturity stage. Physical analysis was done on each fresh fruit and samples collected for chemical analysis were taken from the 25 fruits and reported as the average of duplicate analyses. Analysis of variance and Duncan's Multiple Range Test were used for least significant difference at the 0.05% level (Statgraphics SGPlus).

## 3. Results and discussion

The lychee fruit used for this study was collected weekly from a commercial farm starting at the 12th waa. The fruit showed green color from the 12th through the 15th waa, changing to dark-red on the 20th waa, when the fruit was considered to be at the beginning of senescence.

### 3.1. Physical evaluation

**Changes in fruit dimensions and proportions of pericarp, aril and seed:** The lychee fruit showed a progressive increase in length up to the 17th waa but no significant increases occurred afterwards, whereas the

average fruit width and weight increased progressively during ripening (Table 1). Values between 3 and 4 cm length and 19 g weight have been reported for ripe lychee fruits by Galan-Sauco (1990).

Table 1 also shows the changes in the proportions of pericarp, aril and seed during ripening. The % pericarp decreased gradually ( $p \leq 0.05$ ) until the 17th waa while the % aril increased gradually ( $p \leq 0.05$ ) until the 19th waa, both remaining constant thereafter. The % seed increased significantly ( $p \leq 0.05$ ) to a maximum between the 13th and 15th waa, decreasing gradually ( $p \leq 0.05$ ) thereafter. Ripe lychee fruit v. Mauricio is reported by Holcroft and Mitcham (1996) to consist of 70–75% aril, 14–16% pericarp and 11–14% seed, while values > than 10% aril were reported by Galan-Sauco (1990) for v. Brewster.

**Changes in Hunter  $L^*$ ,  $a^*$ , and  $b^*$  values:** The changes in pericarp color during ripening, expressed as the Hunter  $L^*$ ,  $a^*$  and  $b^*$  values, are shown in Table 2. Hunter  $L^*$  values show a gradual ( $p \leq 0.05$ ) darkening of the fruit, while the Hunter  $a^*$  and  $b^*$  values indicate the gradual ( $p \leq 0.05$ ) change in color from light green-yellow (12th to the 15th waa), to yellow-red (15th to 17th waa) and to dark red (20th waa). Holcroft and Mitcham (1996) report the disappearance of chlorophyll coinciding with the active synthesis of flavonoids, particularly water-soluble anthocyanins during maturation of lychee.

**Changes in pH, titratable acidity and soluble solids:** A decrease ( $p \leq 0.05$ ) in pH was observed from 3.18 in the 12th to 2.68 in the 15th waa, while an increase ( $p \leq 0.05$ ) occurred up to pH 4.49 in the 20th waa (Table 2). Titratable acidity as % malic acid increased to a maximum of 4.44% in the 14th waa decreasing significantly to a low 0.16% value at the 20th waa. A significant increase in soluble solids (°Bx) was observed from the 14th (9.9 °Bx) to the 17th waa (18 °Bx), with no changes thereafter. These results indicate a behavior also seen in other non-climateric fruits as part of the normal metabolic changes occurring during ripening (Wills, Lee, Graham, McGlason & Hall, 1982). The

Table 1  
Changes in physical characteristics of the lychee fruit at the different ripening stages<sup>1</sup>

Week	Length (cm)	Avg. width (cm)	Weight (g)	(% portion of the fruit)		
				Pericarp	Aril	Seed
12	2.19 <sup>a</sup>	1.11 <sup>a</sup>	1.35 <sup>a</sup>	83.2 <sup>a</sup>	1.8 <sup>a</sup>	15.0 <sup>a</sup>
13	2.59 <sup>b</sup>	1.43 <sup>b</sup>	3.01 <sup>a</sup>	72.6 <sup>b</sup>	3.6 <sup>a</sup>	23.8 <sup>b</sup>
14	3.20 <sup>c</sup>	1.91 <sup>c</sup>	5.76 <sup>b</sup>	50.6 <sup>c</sup>	7.7 <sup>b</sup>	41.9 <sup>c</sup>
15	3.43 <sup>d</sup>	1.91 <sup>d</sup>	9.42 <sup>c</sup>	39.6 <sup>d</sup>	21.7 <sup>c</sup>	38.7 <sup>c</sup>
16	3.52 <sup>d</sup>	2.66 <sup>c</sup>	14.09 <sup>d</sup>	28.5 <sup>c</sup>	48.8 <sup>d</sup>	22.7 <sup>d</sup>
17	3.69 <sup>e</sup>	2.84 <sup>f</sup>	17.06 <sup>e</sup>	25.0 <sup>f</sup>	54.4 <sup>e</sup>	20.6 <sup>de</sup>
18	3.69 <sup>e</sup>	3.01 <sup>g</sup>	18.94 <sup>f</sup>	24.1 <sup>f</sup>	57.6 <sup>f</sup>	18.3 <sup>ef</sup>
19	3.70 <sup>e</sup>	3.10 <sup>g</sup>	20.18 <sup>f</sup>	22.9 <sup>f</sup>	60.9 <sup>g</sup>	16.2 <sup>fa</sup>
20	3.81 <sup>e</sup>	3.22 <sup>h</sup>	22.41 <sup>g</sup>	24.5 <sup>f</sup>	61.0 <sup>g</sup>	14.5 <sup>g</sup>

<sup>1</sup> Average of two measurements; means within columns with the same letters are not significantly different ( $p < 0.05$ ).

Table 2  
Changes in Hunter color parameters and chemical composition of the lychee fruit at the different ripening stages<sup>1</sup>

Week	Hunter color parameters				Titratable acidity <sup>2</sup>	Soluble solids <sup>3</sup>
	L*	a*	b*	pH		
12	35.8 <sup>a</sup>	−6.5 <sup>a</sup>	26.0 <sup>a</sup>	3.18 <sup>a</sup>	2.53 <sup>a</sup>	ND <sup>4</sup>
13	32.0 <sup>b</sup>	−5.8 <sup>a</sup>	22.3 <sup>b</sup>	2.99 <sup>b</sup>	3.22 <sup>b</sup>	ND
14	30.8 <sup>c,d</sup>	−5.9 <sup>a</sup>	20.8 <sup>b</sup>	2.79 <sup>c,d</sup>	4.44 <sup>c</sup>	9.9 <sup>a</sup>
15	32.5 <sup>b,c</sup>	−4.2 <sup>b</sup>	22.5 <sup>b</sup>	2.68 <sup>d</sup>	3.46 <sup>b</sup>	12.8 <sup>b</sup>
16	30.4 <sup>d</sup>	16.2 <sup>c</sup>	16.4 <sup>c</sup>	2.84 <sup>c</sup>	1.61 <sup>d</sup>	16.9 <sup>c</sup>
17	26.1 <sup>e</sup>	25.6 <sup>d</sup>	11.0 <sup>d</sup>	3.15 <sup>a</sup>	0.87 <sup>e</sup>	18.0 <sup>d</sup>
18	21.4 <sup>f</sup>	25.1 <sup>d,f</sup>	7.1 <sup>e</sup>	3.58 <sup>c</sup>	0.45 <sup>e,f</sup>	18.3 <sup>d</sup>
19	21.0 <sup>f</sup>	27.6 <sup>e</sup>	6.1 <sup>e,f</sup>	4.14 <sup>f</sup>	0.23 <sup>f</sup>	18.6 <sup>d</sup>
20	19.3 <sup>g</sup>	23.9 <sup>f</sup>	4.6 <sup>f</sup>	4.49 <sup>g</sup>	0.16 <sup>g</sup>	18.1 <sup>d</sup>

<sup>1</sup> Average of two measurements; means within columns with the same letters are not significantly different ( $p < 0.05$ ).

<sup>2</sup> Expressed as % malic acid.

<sup>3</sup> Expressed in °Bx.

<sup>4</sup> ND: not determined.

results also indicate that sugars accumulate only until the 17th waa, but for the fruit to be considered fully ripe the acidity would have to be lower and the red color due to anthocyanins fully developed.

### 3.2. Changes in anthocyanins

No reports were found on the changes in anthocyanin (Acy) pigment type and concentration during the development of the lychee fruit. Lee & Wicker (1991) reported the presence of cyanidin-3-glucoside (cyd-3-glc), cyanidin-3-rutinoside (cyd-3-rut) and malvinidin-3-acetylglucoside (mvd-3-Acglc) in ripe lychee pericarp, as well as the presence of small amounts of polymerized Acy pigment.

*Total and monomeric anthocyanins – Degradation index (DI):* Three different solvent systems were compared in order to obtain the maximum Acy extraction from lychee pericarp, but no significant differences were found between the use of acidified ethanol for 2 h (47.4 mg/100 g) or 12 h (44.7 mg/100 g), or acidified methanol for 2 h (43.1 mg/100 g); the last solvent system was used in this work.

Two methods were employed to measure anthocyanin concentrations; the single pH method (total Acy, tAcy) takes into account the absorption of the acidified sample at 520 nm and the pH differential method reports the concentration of monomeric Acy (mAcy) since it subtracts the color due to the presence of non-monomeric anthocyanin pigments that are not bleached at pH 4.5 (Wrolstad, 1993).

Only traces of mAcy were observed in the green to green-yellow stages of the fruit (12th to 15th waa, Table 3), although the single pH method reported the presence of red color – reported as total Acy – between 6 and 9 mg/100 g. Very high DI values were therefore obtained at these stages and they could be due to the presence of 520 nm absorbing condensed or polymeric pigments (Somers, 1971; Lee & Wicker, 1991). Once the green color disappeared from the pericarp between the 15th and 16th waa, both mAcy and tAcy levels increased significantly ( $p \leq 0.05$ ) and the DI values showed that the contribution of non-mAcy remained constant from the 17th waa onwards. The final mAcy concentration of 46 mg/100 g pericarp places it as a potentially very good source of Acy since it compares

Table 3  
Changes in total Acy, monomeric Acy, DI and in individual Acy in the lychee pericarp at the different ripening stages<sup>1</sup>

Week	Acy (mg/100 g)			Individual Acy (mg/100 g)		
	Total	Monomeric	DI	Cyd-3-Glc	Cyd-3-rut	Mvd-3-ac-glc
12	6.5	0.1	49.0	0.00	0.00	1.0 ± 0.00 <sup>a</sup>
13	9.0	0.5	18.5	0.00	0.00	5.0 ± 0.02 <sup>b</sup>
14	8.0	0.6	14.5	0.00	0.00	6.0 ± 0.04 <sup>b</sup>
15	6.0	0.3	20.5	0.00	0.00	3.0 ± 0.02 <sup>a</sup>
16	15.5	10.0	1.6	1.1 ± 0.08 <sup>a</sup>	4.3 ± 0.01 <sup>a</sup>	4.6 ± 0.12 <sup>b</sup>
17	27.0	23.0	1.2	2.8 ± 0.21 <sup>a,b</sup>	14.5 ± 0.05 <sup>b</sup>	5.7 ± 0.26 <sup>b</sup>
18	34.0	32.0	1.1	4.6 ± 0.14 <sup>b,c</sup>	24.5 ± 0.24 <sup>c</sup>	2.9 ± 0.13 <sup>a</sup>
19	44.0	42.0	1.1	6.9 ± 0.15 <sup>c,d</sup>	32.7 ± 0.24 <sup>c</sup>	2.4 ± 0.41 <sup>a</sup>
20	52.0	46.0	1.1	7.8 ± 0.34 <sup>d</sup>	35.3 ± 0.15 <sup>c</sup>	2.9 ± 0.20 <sup>a</sup>

<sup>1</sup> Average of two measurements; means within columns with the same letters are not significantly different ( $p < 0.05$ ).

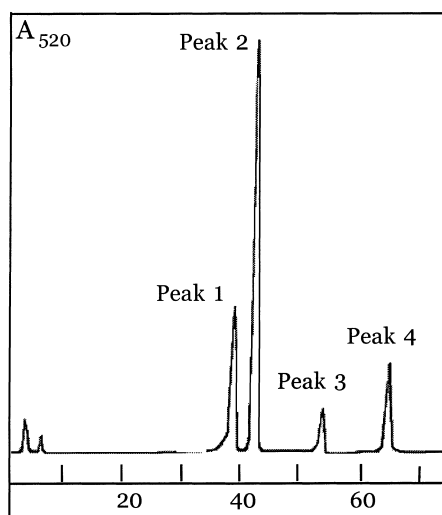


Fig. 1. HPLC chromatogram of lychee pericarp anthocyanins. Peak identification: Peak 1=Cyanidin-3-glucoside, Peak 2=Cyanidin-3-rutinoside, Peak 3=Cyanidin, and Peak 4=Malvinidin-3-acetylglucoside. Chromatographic conditions: Nova-Pak C18 column; 4% phosphoric acid (A) and acetonitrile (B)=6% B/10 min, linear gradient 6–20% B/45 min, 20% B/10 min, linear gradient 20–100% B/1 min, 100% B/10 min; 1 ml/min.

with sources such as strawberry (15–42 mg/100 g) (Rwabahizi & Wrolstad, 1988) and red raspberry (25–50 mg/100 g) (Torre & Barrit, 1977).

*Changes of the individual Acy during pericarp development:* Reversed-phase HPLC was used to separate and quantify the individual Acy during the ripening period. The chromatogram obtained (Fig. 1) was very similar to that reported by Lee & Wicker (1991) except that peak 5 reported by them was not observed. The individual Acy were therefore identified as cyd-3-glc (Peak 1, Rt = 39.2 min), cyd-3-rut (Peak 2, Rt=41.8 min), cyd (Peak 3, Rt=52.2 min) and mvd-3-Acglc (Peak 4, Rt=63.7 min). The differences in Rt values from those reported by Lee & Wicker (1991) may be due to the smaller particle size used in the column in our work. We agree with these authors in that the presence of Peak 3 represented by free cyd most probably is an artifact formed due to degradation of the cyd-glycosides.

The changes in concentration of the individual Acy are shown in Table 3 and Fig. 1. Mvd-3-Acglc could be detected at very low levels at all stages ranging between 1 and 5.7 mg/100 g. This Acy was the only one detected in the green fruits (up to the 15th waa) when the synthesis of the two cyd-glycosides started. The % contribution of Mvd-3-Acglc decreased significantly from 100% at 15 waa to around 6% from the 19th waa on. Cyd-3-rut became the main Acy increasing in concentration from 0 to 35.3 mg/100 g from the 15th to the 20th waa, and in the last three weeks represented over 75% of the Acy present. Cyd-3-glc showed the same kind of increase from 0 to 7.8 mg/100 g from the 15th to the 20th waa, but represented only 11–17% of the Acy

during the same period. This information on individual Acy appearance may be of help in elucidating the Acy biosynthetic pathways in lychee pericarp.

A different behavior was observed in another non-climateric fruit (grapes) by Fernández-López, Hidalgo, Almela and López-Roca (1992) González-San José, Barron & Diez (1990) who report a decrease in total Acy and in cyd-3-rut during the intermediate stages of maturation, with an increase in the final stages. A constant increase in Acy concentration during ripening was reported for pomegranate (Gil, García-Viguera, Artés & Tomás-Barberan, 1995).

#### 4. Conclusions

Lychee fruit growth represented by width, weight, and % aril continued until the 19th waa. Fruit length and % pericarp increased only until the 17th waa. Pericarp color changed from light green to yellow-green (16th waa), to yellow-red (15th to 17th waa), and to dark red (20th waa). At ripeness the fruits showed a very low titratable acidity (0.16%, malic acid) and a pH of 4.49. Soluble solids increased to a maximum of 18 °Bx at the 17th waa not changing thereafter. Traces of mAcy and very high DI values possibly due to the presence of polymeric red pigments were observed in the green to green-yellow stage. Lychee pericarp mAcy concentration (46 mg/100 g) at ripeness makes them a potentially good source of Acy. Individual Acy identified were cyd-3-glc, cyd-3-rut and mvd-3-Acglc. Only mvd-3-Acglc was detected in the green fruits. Cyd-3-rut became the main Acy (> 75%), while cyd-3-glc represented less than 17%, and mvd-3-Acglc less than 9%.

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