Anthocyanin decolorisation and its role in lychee pericarp browning

S. Underhill[AB] *and C. Critchley*[B]

[A] Queensland Department of Primary Industries, Horticulture Postharvest Group, 19 Hercules Street, Hamilton, Qld 4007, Australia.

[B] Department of Botany, The University of Queensland, Qld 4072, Australia.

Summary. Mature red lychee fruit were stored at 3 different temperature and relative humidity regimes. Total anthocyanin concentration, pigment distribution, pH of the pericarp homogenate, Hunter *a* values (redness index), and visual colour were measured as a function of pericarp weight loss. Pericarp colour rapidly deteriorated during both ambient and high temperature storage, resulting in a uniform browning of the pericarp surface. The degree of tissue browning was proportional to the rate of pericarp desiccation. Although anthocyanin degradation occurred concurrently with tissue browning, visual colour and Hunter *a* values were not consistent with total anthocyanin concentration. Instead, a more significant correlation was seen between Hunter *a* values and the pH of the pericarp homogenate.

Pericarp colour could be altered by external pH. Acidification of whole fruit increased pericarp redness, whereas alkaline treatment caused discoloration. Both colour responses occurred independently of anthocyanin synthesis and degradation and were completely reversible.

These results question the current theory that browning is due to anthocyanin degradation. No evidence of browning was observed in the anthocyanin-containing mesocarp, and acidification of already brown tissue significantly increased pericarp redness independently of anthocyanin synthesis. We believe that anthocyanin pigments were progressively decolorised during ambient storage, possibly due to changes in pericarp pH. Once colourless, independent tissue browning became visual and was enhanced.

Introduction

Lychee fruit rapidly deteriorate once harvested, resulting in a uniform browning of the pericarp surface. Browning has been attributed to rapid fruit desiccation (Scott *et al.* 1982) and has been shown to be proportional to temperature, humidity and storage time (Huang and Scott 1985; Nip 1988; Huang *et al.* 1990). Resultant tissue browning was thought to be caused by a rapid degradation of the red pigments by polyphenol oxidase (PPO), producing brown-coloured byproducts (Akamine 1960). Prasad and Jha (1978) and Lee and Wicker (1991) identified the red pigments in lychee pericarp as anthocyanins: cyanidin-3-rutinoside, cyanidin-3-glucoside, malvidin-3-acetylglucoside, pelargonidin 3-glycoside, and pelargonidin 3,5-diglucoside. Anthocyanins are common plant pigments (Pecket and Small 1980), vacoule-bound and relatively unstable even within intact tissue. During storage, anthocyanin concentration was shown to decrease (Jaiswal *et al.* 1987) while PPO activity increased (Lin *et al.* 1988; Huang *et al.* 1990). Lychee tissue browning is therefore commonly attributed to enzymic oxidation of anthocyanins.

Although PPO can degrade anthocyanin (Debicki-Pospišil *et al.* 1983; Sistrunk and Gascoigne 1983; Morris *et al.* 1986), it has a low affinity for the pigment (Markakis 1982). Where anthocyanin degradation has been studied, it has involved sequential structural changes to the molecule, which resulted in decolorisation. This was initially reversible under low pH conditions (Markakis 1982); however, the colourless anthocyanin forms were more degradable.

Studies by Fuchs *et al.* (1993) showed that lychee pericarp colour after sulfur dioxide treatment was significantly improved by acid treatment. Underhill and Critchley (1990) also provided circumstantial evidence for a positive correlation between increased cellular pH and a reduction in pericarp colour. The present study was conducted to examine the relationship between anthocyanin degradation and pericarp browning during fruit storage under ambient conditions.

Materials and methods

Mature lychee fruit (*Litchi chinensis* Sonn. cv. Wai Chee) were obtained from 2 commercial orchards in south-eastern Queensland (lat 27°S.). Fruit were transported to the laboratory within 1 h and sorted for uniform pericarp colour.

Temperature and humidity treatments

Time-courses of pericarp browning were studied under 3 different temperature and relative humidity (RH) regimes. Fruit were stored in open containers for 1 week at 5°C, 95% relative humidity (RH) (low level of fruit desiccation); 25°C, 60% RH (moderate 'ambient' desiccation); or 48°C, 70% RH (severe fruit desiccation). Visual pericarp colour, Hunter *a* values (redness index), pH of the pericarp homogenate, total anthocyanin concentration (TAcy), and anthocyanin distribution were determined as a function of storage time after 0, 6, 21, 30, 48, and 98 h, and 1 week.

pH treatments

The relationship between pericarp colour and pH was examined by immersing whole fruit into 1 mol HCl/L or 2 mol NaOH/L for 2 min. Pericarp pH, Hunter *a* values, and TAcy were determined immediately before and after initial treatments. Treated fruit were then re-immersed after 10 min into either NaOH or HCl for 2 min to assess treatment reversibility. Acidification of brown fruit was also undertaken to investigate possible colour recovery.

Measurements

Pericarp tissue (4 g) from a minimum of 5 fruit was washed in distilled water to remove juice. The tissue was finely sliced and homogenised in 40 mL distilled water for 1 min with an ultra-turrax. The pH was determined while stirring the homogenate.

Pericarp visual colour was assessed by rating 20 fruit on a 1–5 scale: 1, pericarp surface completely brown; 2, one-quarter red; 3, half red; 4, three-quarters red; 5, pericarp surface completely red, no browning. Fruit were rated individually by a 5-member panel. Following the first rating, samples were randomised and visual colour was reassessed. Pericarp redness was also measured using a spectrocolorimeter (Hunterlab, Labscan 6000) that was fitted with a 13-mm orifice, 10° observer, and D65 illuminant and was standardised daily. Spectral reflectance in the green–red range was determined and results were expressed as Hunter *a* values. A reduction in pericarp redness equates with a decrease in the associated Hunter *a* value, with *a* values <12 representing the completely brown pericarp. Readings were taken from 2 random sites on the pericarp surface and from 20 fruit.

Anthocyanin concentration was measured according to Fuleki and Francis (1968). Pericarp tissue (1 g) was finely sliced and extracted with 50 mL HCl–methanol (1% v/v) for 2 h. The extract was filtered and diluted, and its absorbance measured at 530 nm selective for cyanidin-3-glucoside. Results were expressed as absorbance at 530 nm/g FW (Paull *et al.* 1984). Anthocyanin distribution was determined at each removal using an Olympus BH-2 light microscope. Tissue was hand-sectioned and mounted unstained in 30% glycerol. Pericarp moisture loss was determined progressively during storage using 20 fruit. Results were expressed as percentage weight loss, based on a comparison of mean fresh weight.

Statistical analyses

Statistical evaluations of TAcy, pH of the pericarp homogenate, Hunter *a* values, and sensory colour data were conducted by analysis of variance and least significant difference using the STATISTIX 3.1 program. The relationships between TAcy, Hunter *a* values, and pH were determined using second-order regression analysis.

Results and discussion

Lychee pericarp was highly susceptible to desiccation (Table 1). Under ambient conditions (25°C, 60% RH), 50% of the initial pericarp fresh weight was lost in the first 48 h. Increased storage temperature resulted in a concurrent rise in the rate of desiccation. Both the visual colour and Hunter *a* values (pericarp redness) decreased proportionally with the degree of desiccation. Total anthocyanin concentration also decreased during storage; however, the rate of degradation was not linearly correlated with pericarp desiccation.

Table 1. Changes in per cent pericarp weight, anthocyanin concentration, homogenate pH, and colour of lychee pericarp following whole fruit storage at three temperature–relative humidity (RH) regimes

Within columns means followed by same letter are not significantly different at $P = 0.05$

Storage time (h)	Per cent weight loss ($n = 20$)	Anthocyanin concentration[A] ($n = 3$)	pH ($n = 3$)	Hunter *a* value (redness) ($n = 20$)	Visual colour ($n = 5$)
		Storage at 5°C, 95% RH			
0	0	0.49a	4.15a	21.74a	4.8ab
6	0	0.53a	4.29ab	25.91b	4.3ab
21	0	0.32b	4.42b	25.97b	4.3ab
30	0	0.28b	4.42b	25.08b	4.9a
48	0	0.37b	4.41b	25.94b	4.2b
		Storage at 25°C, 60% RH			
0	0	0.49a	4.15a	21.74a	4.8a
6	3.6	0.36ab	4.18a	17.76b	3.9b
21	22.5	0.29 bc	4.46b	15.96bc	3.2bc
30	32.0	0.22 bc	4.46b	13.89c	3.0c
48	50.5	0.21c	4.52c	16.71bc	2.5c
		Storage at 48°C, 70% RH			
0	0	0.49a	4.15a	21.74a	4.8a
6	1.7	0.41a	—[B]	17.34b	3.9b
21	20.2	0.21b	4.55b	10.61c	2.7c
30	30.0	0.18b	4.55b	13.02c	2.7c
48	64.7	0.14b	4.74c	11.27c	1.6d

[A] Absorbance at 530 nm/g FW. [B] No data.

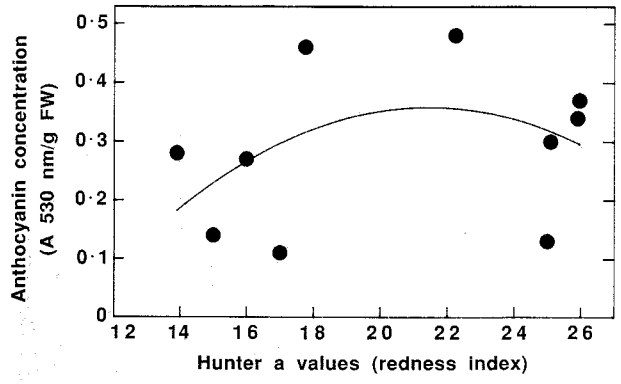

Fig. 1. Correlation between Hunter *a* values (redness index) and total anthocyanin concentration (absorbance at 530 nm/g FW). Data were obtained during 1 week storage at 25°C, 60% RH. The equation for the curve is:

$$Y = 11.3X^2 + 41.7X - 38.5 \quad (n = 15, R^2 = 0.44, P<0.05)$$

Although anthocyanins are responsible for the red pigmentation (Lee and Wicker 1991), the relationship between TAcy and pericarp redness was not consistent (Table 1). When fruit were held at 5°C, 95% RH, TAcy decreased in the period between 6 and 48 h, while the Hunter *a* values did not change. When TAcy values were compared between the 3 storage conditions, associated Hunter *a* values at equal TAcy were consistently higher in the cool-stored fruit. This suggests some independence between TAcy and pericarp redness. When the 2 parameters were compared at 25°C, 60% RH, the correlation between TAcy and Hunter *a* values was relatively low (Fig. 1). Similarly, the relationship between TAcy and the degree of visual colour was also inconsistent (Table 1). Colour (Hunter *a* value) was better correlated with pericarp pH in an inversely proportional relationship (Fig. 2).

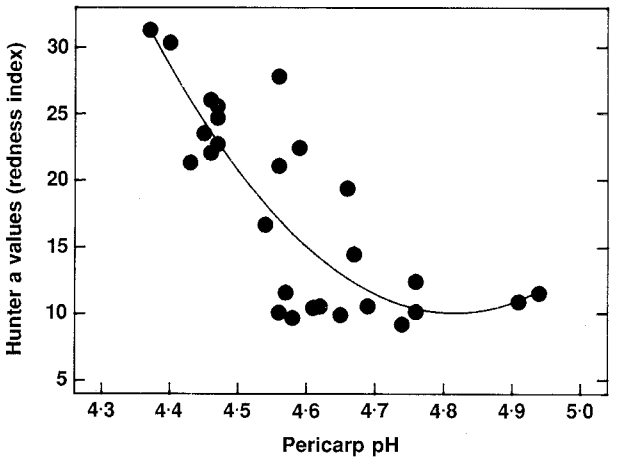

Fig. 2. Correlation between Hunter *a* values (redness index) and pH of the pericarp homogenate. Data were obtained during 1 week storage at 25°C, 60% RH. The equation for the curve is:

$$Y = -2218X^2 - 916X + 95 \quad (n = 15, R^2 = 0.75, P<0.05)$$

Anthocyanin structure and, more importantly, colour are directly dependent on pH (Nakayama and Powers 1972). Under alkaline conditions, anthocyanins are rapidly converted to less stable anhydro bases, resulting in the formation of colourless chromenols (Jurd 1972). It was for this reason that pH of the pericarp homogenate was determined as a crude index of cellular pH. Although the pH of the pericarp homogenate became slightly less acidic under non-desiccating conditions, it increased significantly with pericarp desiccation (Table 1). Where pH changes have been demonstrated in other tissue [e.g. senescent floral epidermal cells (Stewart *et al.* 1975)], distinct changes in tissue coloration were observed. Anthocyanin stability is greatest at pH<3 (Brouillard 1982), and an increase of 0.5 pH units would dramatically affect pigment structure and, hence, colour. Although a more precise measure of cellular pH is required, it can be assumed that increased pericarp pH reflects changes in vacuole pH, since the mature parenchyma tissue of the mesocarp is highly vacuolated (Fig. 3*a*). Under conditions of vacuole alkalisation, the anthocyanins would decolorise rapidly and become more vulnerable to enzymic degradation. The correlation between tissue pH and colour may highlight the importance of anthocyanin structure in overall pericarp coloration.

Anthocyanin distribution was assessed qualitatively during fruit storage at 25°C, 60% RH for 96 h. Pigmentation was initially seen in the upper mesocarp and the epicarp (Fig. 4). There were no anthocyanins in the aril. During storage, pigment distribution was significantly reduced, such that the mesocarp tissue became progressively colourless (Fig. 4). Independent tissue browning has been previously shown to be highly localised and restricted to the epicarp (Underhill and Critchley 1993). With the exception of the epicarp, the incidence of cellular browning was not consistent with previous anthocyanin distribution. The absence of tissue browning in the mesocarp (Fig. 3) was contradictory to expectations of byproducts of anthocyanin degradation causing browning. It is therefore unlikely that anthocyanin degradation alone is responsible for the production of brown pigmentation.

Clearly, pericarp colour can be manipulated by changing cellular pH. When pericarp pH was lowered by immersing the fruit in 1 mol HCl/L, TAcy was significantly reduced although pericarp redness (Hunter *a* values) was not affected (Fig. 5). Increasing pericarp pH using NaOH, however, resulted in instantaneous loss of red colour independent of any significant reduction in TAcy. Both treatments were

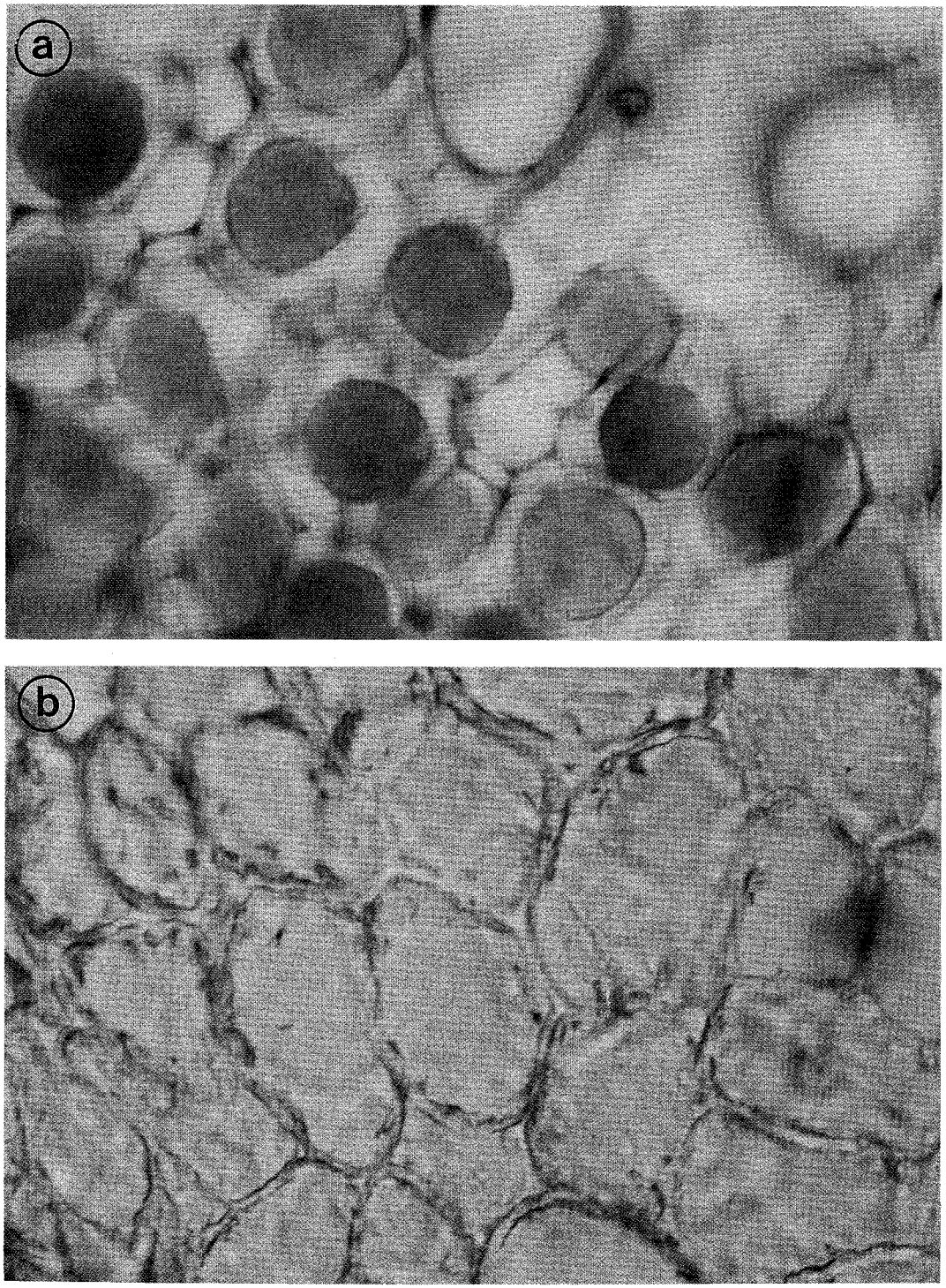

Fig. 3. Lychee parenchyma tissue from the upper mesocarp region illustrating pigment decolorisation: (*a*) tissue before storage, (*b*) fruit stored at 25°C, 60% RH for 96 h. Magnification 800 x.

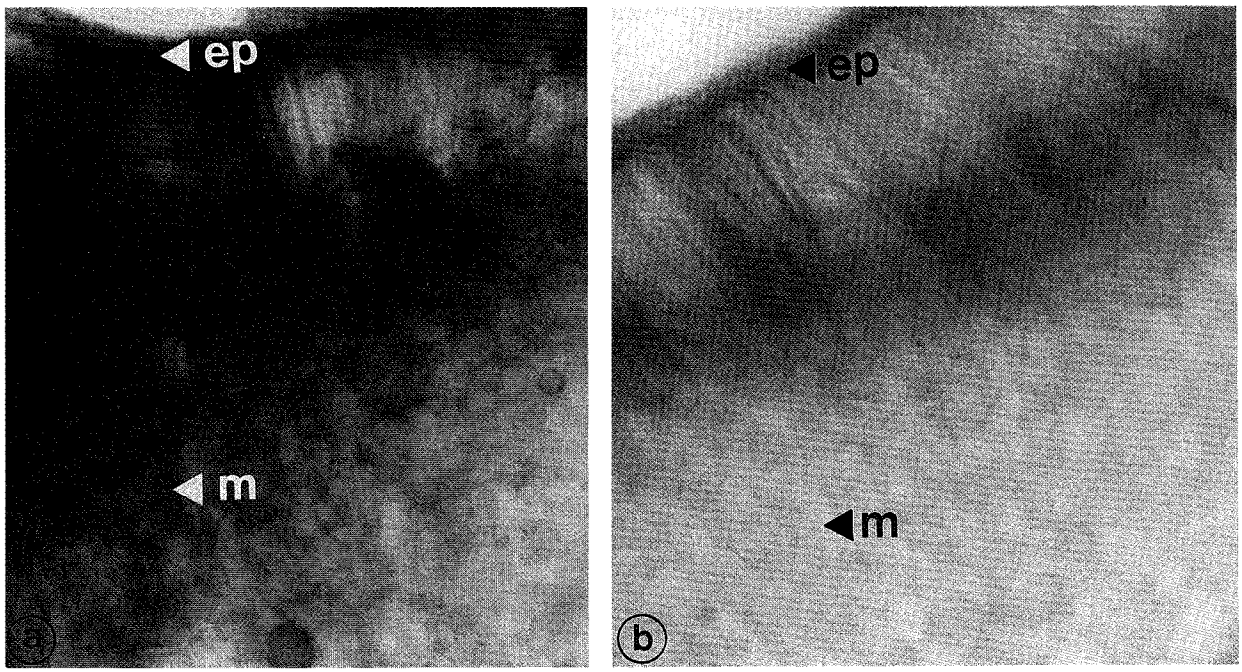

Fig. 4. Light microscope sections of lychee pericarp tissue illustrating anthocyanin distribution: (*a*) fresh pericarp tissue, (*b*) fruit stored at 25°C, 60% RH for 96 h. Mesocarp (m); epicarp (ep). Tissue was hand-sectioned (unstained) and mounted in 30% glycerol. Magnification 200 x.

reversible upon further pH treatment. Increasing the pH of previously acid-treated fruit resulted in an immediate loss of red colour without change in TAcy. Acidification of NaOH-treated fruit resulted in total recovery of pericarp colour. In both treatments, acidification of the pericarp resulted in a significant reduction in TAcy. However, pericarp colour changed independently of TAcy. The pH environment of the anthocyanins therefore has an important influence on both the intensity and colour of the anthocyanins.

Following the work of Denny *et al.* (1986) in peaches, we acidified already-brown lychee pericarp tissue (Fig. 6). Application of HCl immediately increased pericarp redness to a Hunter *a* value similar to that before browning. Colour improvement occurred independently of any increase in TAcy, which excluded the possibility of anthocyanin synthesis. Colour recovery under these conditions, however, was not stable, and colour deteriorated further with storage. If fruit were allowed to desiccate further before acidification, colour recovery was less intense. No colour recovery was observed following acidification of either heat- or chilling-induced browning (S. Underhill unpublished data).

The reversibility of what has been considered a degradation process necessitates a re-evaluation of the current theory. We believe that colour recovery involves some form of pH-induced structural change to the anthocyanins, whereby decolorisation is reversed. As enzymic degradation also facilitates colour loss, colour recovery must be somewhat proportional to TAcy and is therefore unlikely to occur where complete degradation of the pigment has occurred.

The fact that HCl treatment can increase mean pericarp redness above that measured in control fruit (Fig. 5) requires further investigation. Brouillard *et al.* (1990) suggested that tissue pigmentation was the result of an equilibrium between coloured anthocyanins and colourless chromenols. This assumes that a portion of the total anthocyanin content of the tissue is colourless. Acidification may cause a shift towards the coloured form so that colour intensity is increased. The conversion of colourless chromenols to coloured anthocyanins due to a reduction in pH is well documented (Lukton *et al.* 1956; Markakis 1982; Osawa 1982). This conversion would not increase anthocyanin concentration, as anthocyanins are normally extracted in the presence of HCl and this reaction would occur during extraction. Although the presence of a coloured–colourless anthocyanin equilibrium in the mature lychee fruit needs to be confirmed, it is consistent with an increased colour intensity following acidification, independent of anthocyanin synthesis.

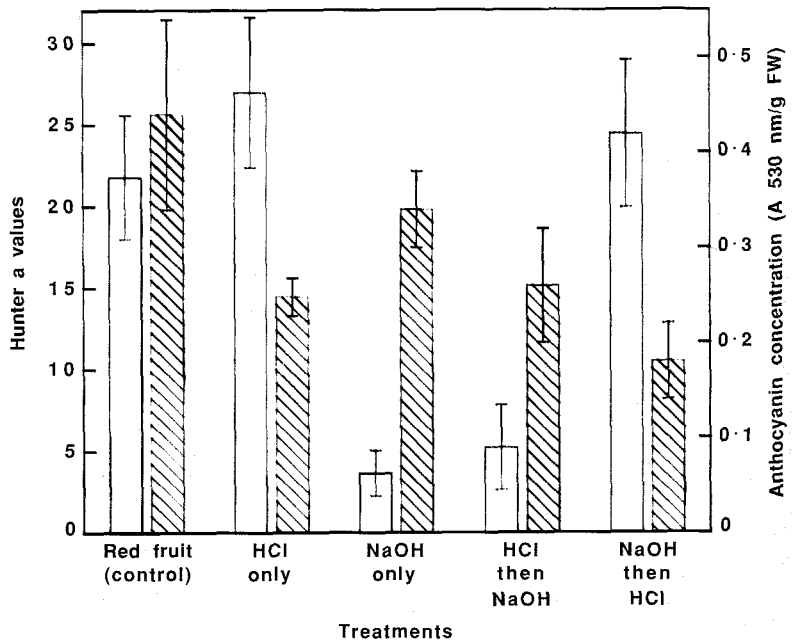

Fig. 5. Hunter *a* values (redness index) (open bars) and anthocyanin concentration (absorbance at 530 nm/g FW) (hatched bars) of red fruit immersed in 1 mol HCl or NaOH/L, or sequentially in the 2 solutions, for 2 min. Vertical bars are standard errors (n = 10).

Anthocyanin decolorisation due to changes in pH have been reported both *in vitro* (Markakis 1982) and *in vivo* (Proctor and Creasy 1969). Similar decolorisation systems are also important factors in the visual colour of flowers (Proctor and Creasy 1969) and fruit (Pifferi and Cultrera 1974; Denny *et al.* 1986). Although pH appears fundamental to *in vivo* anthocyanin colour, Brouillard *et al.* (1990) highlighted the importance of anthocyanin co-pigmentation. Stewart *et al.* (1975), although also acknowledging the significance of the anthocyanin–pH relationship, further emphasised the existence of an anthocyanin co-pigmentation system.

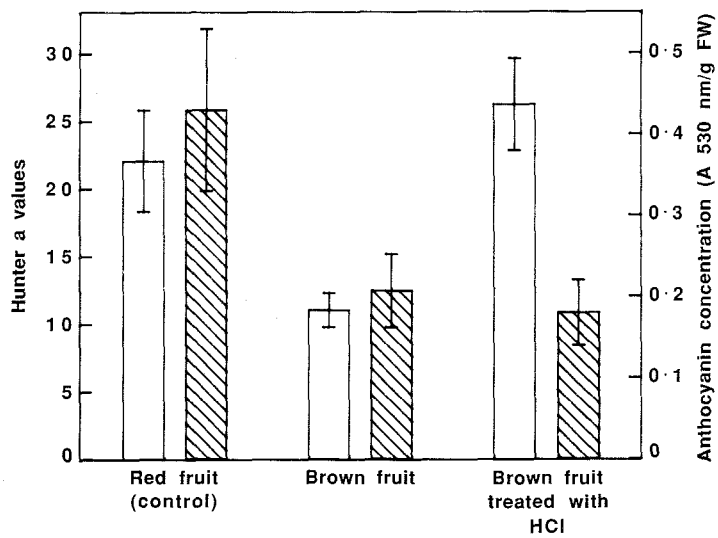

Fig. 6. Hunter *a* values (redness index) (open bars) and total anthocyanin concentration (absorbance at 530 nm/g FW) (hatched bars) of control red fruit compared with untreated brown fruit (stored at 25°C, 60% RH for 1 week), and brown fruit treated with 1 mol HCl/L for 2 min. Vertical bars are standard errors (n = 10).

Co-pigments are colourless compounds that combine with the anthocyanin molecule to result in a bathochromic shift of the absorption maximum (λ_{max}), thereby enhancing colour. Without some form of complexing, anthocyanins within the common vacuolar pH range 4–6 would appear colourless (Asen *et al.* 1972). The red colour of the anthocyanins at an homogenate pH of 4.15 (Table 1) may therefore provide circumstantial evidence for the existence of an anthocyanin co-pigmentation system in the lychee pericarp. Changes in cellular pH may affect the capacity of the anthocyanins to retain co-pigmentation, resulting in colour loss. Further work is required to measure changes in vacuolar pH more accurately, and to determine whether increased pH, both during storage and as a consequence of pH treatment, affects the capacity of the anthocyanins to form co-pigments.

We have provided evidence that pH-induced anthocyanin decolorisation may contribute to ambient desiccation browning of the lychee pericarp. We suggest that this decolorisation occurs as a consequence of reversible anthocyanin structural changes and changes in co-pigmentation, as well as irreversible enzymic degradation. Changes in pH were shown to have a significant effect on tissue colour. Although the mechanism of anthocyanin degradation is unclear, it is unlikely that tissue browning was due to any byproducts of this reaction. We believe that as a consequence of anthocyanin decolorisation, the visualisation of independent tissue browning is enhanced. Rapid pericarp browning is therefore probably a result of both anthocyanin decolorisation and concurrent tissue browning.

Acknowledgments

The authors wish to thank Dr D. H. Simons, The University of Queensland, Gatton College, Mr B. C. Peacock, formerly Horticulture Postharvest Group, Queensland Department of Primary Industries, and Dr G. Zauberman, Volcani Centre Israel, for technical advice. The financial support from the Australian Centre for International Agricultural Research and the Rural Industries Research and Development Corporation is gratefully acknowledged.

References

Akamine, E.K. (1960). Preventing the darkening of fresh lychees prepared for transport. Hawaii Agricultural Experimental Station, University of Hawaii, Technical Programme Report No. 127.

Asen, S., Stewart, R. N., and Norris, K. H. (1972). Co-pigmentation of anthocyanins in plant tissues and its effect on colour. *Phytochemistry* **11**, 1139–44.

Brouillard, R., Wigand, M-G., and Cheminat, A. (1990). Loss of colour, a prerequisite to plant pigmentation by flavonoids. *Phytochemistry* **29**, 3457–60.

Debicki-Pospišil, J., Lovrić, T., Trinajstić, N. and Sabljić, A. (1983). Anthocyanin degradation in the presence of furfural and 5-hydroxymethylfurfural. *Journal of Food Science* **48**, 411–16.

Denny, E. G., Coston, D. C., and Ballard, R. E. (1986). Peach skin discolouration. *Journal of the American Society of Horticultural Science* **111**, 549–53.

Fuchs, Y., Zauberman, G., Ronen, R., Rot, I., Weksler, A., and Akerman, M. (1993). The physiological basis of litchi fruit pericarp colour retention. *Acta Horticulture* **343**, 29–33.

Fuleki, T., and Francis, F. J. (1968). Quantitative methods for anthocyanin. 1. Extraction and determination of total anthocyanin in cranberries. *Journal of Food Science* **33**, 72–7.

Huang, P. Y., and Scott, K. J. (1985). Control of rotting and browning of litchi fruit after harvest at ambient temperatures in China. *Tropical Agriculture* (Trinidad) **62**, 2–4.

Huang, S., Hart, H., Lee, H., and Wicker, L. (1990). Enzymatic and colour changes during post-harvest storage of lychee fruit. *Journal of Food Science* **55**, 1762–3.

Jaiswal, B. P., Sah, N. L., and Prasad, U. S. (1987). Regulation of colour break during *Litchi chinensis* Sonn. ripening. *Indian Journal of Experimental Biology* **25**, 66–72.

Jurd, L. (1972). Some advances in the chemistry of anthocyanin-type plant pigments. *In* 'The Chemistry of Plant Pigments.' (Ed. C. O. Chichester.) pp. 123–42. (Academic Press: New York.)

Lee, H. S., and Wicker, L. (1991). Anthocyanin pigments in the skin of lychee fruit. *Journal of Food Science* **56**, 466–8, 483.

Lin, Z., Li, S., Chang, D., Lin, G., Li, Y., Liu, S., and Chen, M. (1988). The changes of pigments, phenolic content and activities of polyphenol oxidase and phenylalanine ammonia-lyase in pericarp of postharvest litchi fruit. *Acta Botanica Sinica* **30**, 40–5.

Lukton, A., Chichester, C. O., and Mackinney, G. (1956). The breakdown of strawberry anthocyanin pigment. *Food Technology* **10**, 427–32.

Markakis, P. (1982). Stability of anthocyanin in food. *In* 'Anthocyanin as Food Color.' (Ed. P. Markakis.) pp. 163–80. (Academic Press: New York.)

Morris, J. R., Sistrunk, W. A., Junek, J., and Sims, C. A. (1986). Effects of fruit maturity, juice storage, and juice extraction temperature on quality of 'concord' grape juice. *Journal of the American Society for Horticultural Science* **111**, 742–6.

Nakayama, T. O. M., and Powers, J. J. (1972). Absorption spectra of anthocyanin *in vivo*. *In* 'The Chemistry of Plant Pigments.' (Ed. C. O. Chichester.) pp. 193–9. (Academic Press: New York.)

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

Osawa, Y. (1982). Co-pigmentation of anthocyanins. *In* 'Anthocyanins as Food Colors.' (Ed. P. Markakis.) pp. 41–65. (Academic Press: New York.)

Paull, R. E., Chen, N. J., Deputy, J., Huang, H., Cheng, G., and Goa, F. (1984). Litchi growth and compositional changes during fruit development. *Journal of the American Society of Horticultural Science* **109**, 817–21.

Pecket, R. C., and Small, C. J. (1980). Occurrence, location and development of anthocyanoplasts. *Phytochemistry* **19**, 2571–6.

Pifferi, P. G., and Cultrera, R. (1974). Enzymatic degradation of anthocyanins: the role of sweet cherry polyphenol oxidase. *Journal of Food Science* **39**, 786–91.

Prasad, U. S., and Jha, O. P. (1978). Changes in pigmentation patterns during litchi ripening: flavonoid production. *Plant Biochemical Journal* **5**, 44–9.

Proctor, J. T. A., and Creasy, L. L. (1969). An anthocyanin-decolourising system in florets of *Cichorium intybus*. *Phytochemistry* **8**, 1401–3.

Scott, K. J., Brown, B. I., Chaplin, G. R., Wilcox, M. E., and Bain, J. M. (1982). The control of rotting and browning of litchi fruit by hot benomyl and plastic film. *Scientia Horticulturae* **16**, 253–62.

Sistrunk, W. A., and Gascoigne, H. L. (1983). Stability of colour in 'concord' grape juice and expression of colour. *Journal of Food Science* **48**, 430–3, 440.

Stewart, R. N., Norris, K. H., and Asen., S. (1975). Microspectrophotometric measurement of pH and pH effect on colour of petal epidermal cells. *Phytochemistry* **14**, 937–42.

Underhill, S. J. R., and Critchley, C. (1990). Anthocyanin pH regulation and its role in postharvest browning of lychee pericarp. *In* 'Proceedings of the Australian Society for Plant Physiology.' 30th Annual General Meeting, University of New South Wales. Abstract No. 113.

Underhill, S. J. R., and Critchley, C. (1993). Lychee pericarp browning caused by heat injury. *HortScience* **28**, 721–2.

Received 20 November 1992, accepted 25 June 1993