Food Chemistry **40** (1991) 263–270

Quantitative Changes in Anthocyanin Pigments of Lychee Fruit During Refrigerated Storage

H. S. Lee

Florida Department of Citrus, 700 Experiment Station Road, Lake Alfred, Florida 33850, USA

&

L. Wicker

Department of Food Science & Technology, University of Georgia, Athens, Georgia 30602, USA

(Received 8 June 1990; accepted 31 July 1990)

ABSTRACT

*Changes in cyanidin-3-glucoside, cyanidin-3-rutinoside, malvidin-3-acetyl-glucoside, total anthocyanins, percent polymeric color and browning indexes in stored lychee (*Litchi chinensis *Sonn.) fruits were determined. Total anthocyanin content declined from 1·77 to 0·73 mg/g fresh weight, and individual anthocyanins also decreased throughout the 48-day storage period. Decline in anthocyanins was accompanied by an increase in browning. Polymeric pigments also gradually increased from 20·9 to 53%.*

INTRODUCTION

The bright, red color of lychee fruit (*Litchi chinensis* Sonn.) is due to the anthocyanin pigments present in the skin of the fruit. Cyanidin-3-glucoside (cy-3-gl), cyanidin-3-rutinoside (cy-3-rt) and malvidin-3-acetylglucoside (mv-3-ac-gl) were identified as the major monomeric anthocyanin pigments in lychee fruit (Lee & Wicker, 1990). Besides monomeric pigments, polymeric pigments also contribute to the visual appearance of lychee. However, the lychee pigments, like all other anthocyanins, are relatively

Food Chemistry 0308-8146/91/$03·50

unstable and lose their bright attractive color during storage (Campbell, 1959). Desiccation, the accompanying loss of red color, and development of browning are the major problems (Campbell, 1959; Akamine, 1960). Browning of lychee fruit is due, most probably, to an oxidative polyphenoloxidase system (Joslyn & Ponting, 1951; Akamine, 1960; Shu *et al.*, 1990). Many treatments of the fruit to inhibit post-harvest browning have been attempted, and refrigerated storage in polyethylene bags was suggested for extension of shelf-life (Campbell, 1959; Akamine, 1960). Recently, Nip (1988) summarized the work done on the preservation of lychee fruit in conjunction with other pertinent factors affecting overall stability. Although this color defect is one of the main factors in shelf-life reduction during storage of lychee fruit, there has been no research aimed at evaluating these pigment changes. Since we observed significant decreases in Hunter *L*, *a*, *b* color parameters and saturation index values by non-destructive reflectance measurements from stored lychee fruit (Shu *et al.*, 1990), quantitative changes in anthocyanin were also expected. The purpose of this study was to determine the changes in anthocyanin pigments of lychee fruit during refrigerated storage (4°C) conditions.

MATERIALS AND METHODS

Source and treatment of fruit

Brewster variety lychee fruits were donated by J. R. Brooks and Sons, Homestead, Florida. The ripe fruit was harvested, packed in polyethylene bags, stored in 2·3 kg lots, transported to the Citrus Research and Education Center, Lake Alfred, Florida in June 1988, and stored at 4°C in a dark room. Three sets of ten fruits (*c.* 28 g) were taken randomly from the storage locker for analysis each time.

Preparation of lychee anthocyanin

An ethanolic solution of lychee skin extract was prepared by homogenizing the skin of lychee fruit with 50 ml of acidified ethanol (1·5N HCl: 95% ethanol; 15:85, v/v), refrigerating overnight, filtering through Whatman No. 41 paper, and diluting to 250 ml with acidified ethanol.

Preparation of standard anthocyanins

Natural source of standard anthocyanins (cy-3-gl and cy-3-rt) was prepared from individually quick-frozen blackberries (Big Valley Marketing Co.,

Fremont, CA) through repetitive (twice) column chromatography on a Sephadex (Pharmacia LKB, Piscataway, NJ) LH-20 column (1·0 cm × 60 cm) eluting with 10% formic acid at a flow rate of 24 ml/h (Lee & Wicker, 1990). The isolated anthocyanins were pooled and individually freeze-dried, dissolved with 10 ml of 0·1% formic acid/methanol and absorbance was measured at 528 nm or 541 nm by using a spectrophotometer. The concentration of each standard was calculated by using published molar absorptivity (ε), 29 600 for cy-3-gl (Blundstone & Crean, 1966), and 28 800 for cy-3-rt (Wrolstad, 1976).

HPLC analyses

Quantitative measurements of anthocyanins in lychee skin extract were done by the HPLC procedure of Lee & Wicker (1990). The conditions were as follows: a PLRP-S column (4·6 mm × 250 mm) and guard column from Polymer Lab. (Amherst, MA); gradient elution with 3·5% phosphoric acid/water (A) and acetonitrile (B); flow rate 0·8 ml/min; detection at 520 nm; and injection volume, 20 μl. The initial solvent system was 6% B for 5 min. After 5 min, the concentration was changed linearly to 20% B within 45 min. This condition was held for 10 min, followed by a change to 100% B within 1 min, held at 100% B for 10 min, then returned to the initial condition. The amounts of anthocyanins were calculated from the integration of the HPLC peak areas using standard anthocyanins of cy-3-gl and cy-3-rt and reported as mg/g fresh weight. Mv-3-ac-gl contents were estimated by comparision of peak areas to that of cy-3-gl.

Total anthocyanin, browning index and percent polymeric color

Total anthocyanin concentration was determined using a molar absorbtivity of cy-3-rt (Wrolstad, 1976) and reported as mg of cy-3-rt per g fresh weight. Percent polymeric color and browning index were determined from spectral measurements as described by Wrolstad *et al.* (1982). Lychee sample was diluted (2 g/25 ml) with 0·1M sodium acetate solution (pH 3·5) and filtered through 1·2 μm Acro disc filter (Gelman Science Inc., Ann Arbor, MI) before measurements. All spectral determinations were made on a Shimadzu UV-160 UV/Visible spectrophotometer.

RESULTS AND DISCUSSION

Lychee fruit has a bright, red color and contains a high level of anthocyanins. The level of total anthocyanin was 1·68 mg/g fresh weight on

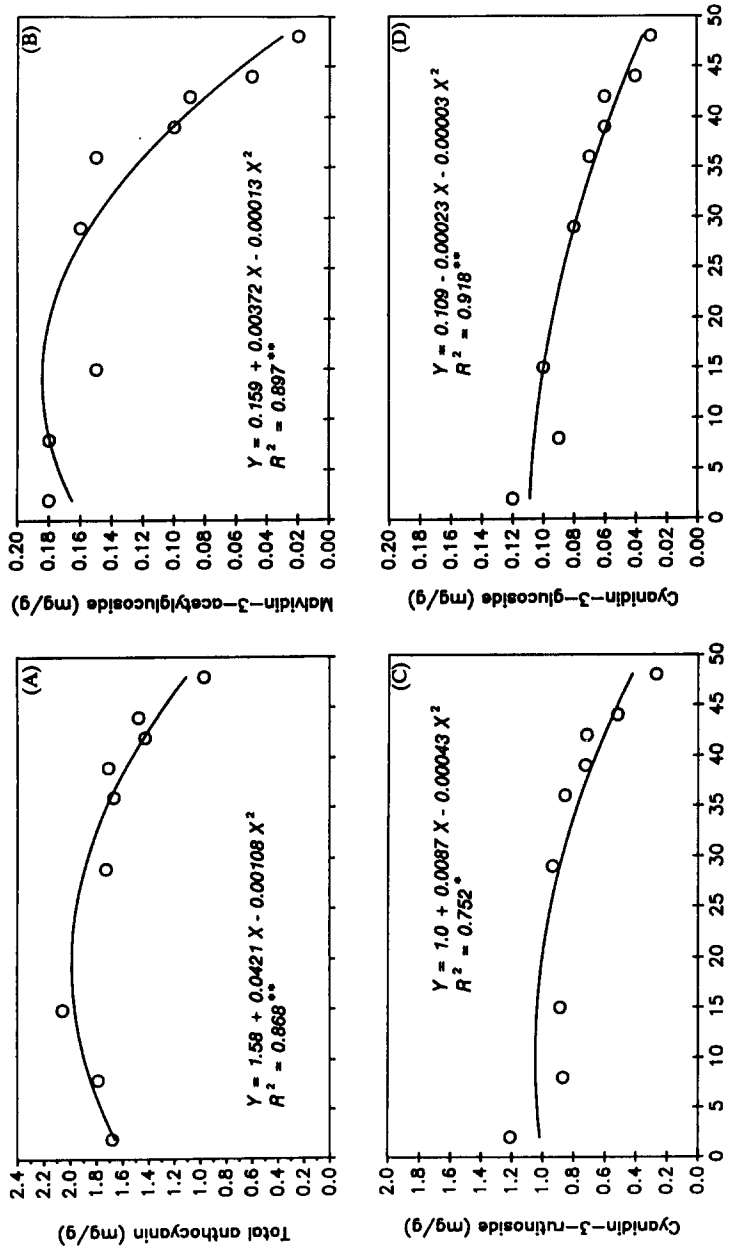

Fig. 1. Changes in total anthocyanin (A), malvidin-3-acetylglucoside (B), cyanidin-3-rutinoside (C) and cyanidin-3-glucoside (D) contents during storage. Regressions are significant at $P = 0{\cdot}01$ (**) or at $P = 0{\cdot}05$ (*). Each datum represents mean values for three replicate measurements.

the second day after harvest and gradually increased to 1·79 mg/g after 8 days of storage, representing a 6·5% increase (Fig. 1). After 15 days, the total anthocyanin concentration had increased to 2·06 mg/g (a 22·6% increase) and then decreased gradually thereafter. Similar observations in increase of total anthocyanin content were reported from stored red raspberries during cold storage (Sjulin & Robbins, 1987; Robbins *et al.*, 1989). The increase in total anthocyanin content was probably due to the enhanced anthocyanin biosynthesis during refrigerated storage. However, the decreases in monomeric anthocyanins and increase in percent polymeric color values seemed to be related to the formation of more intensely colored, condensed, polymer type pigments. Also, continual increases in percentage of polymeric peak in HPLC chromatogram appeared to support this speculation. A marked decline of total anthocyanin content was observed after 42 days of storage, and more than 42% of total anthocyanin pigments were lost after 48 days of storage.

The concentrations of all three major anthocyanins, cy-3-gl, cy-3-rt and mv-3-ac-gl, decreased during storage. Cy-3-rt was the major pigment, comprising about 68·4% of the total anthocyanin content. This pigment concentration was approximately 1·2 mg/g fresh weight on the second day after harvest and decreased to 0·9 mg/g after 15 days of storage (Fig. 1). After 48 days, the cy-3-rt content decreased to 0·27 mg/g, a loss of more than 77%.

The second principal anthocyanin pigment, mv-3-ac-gl, representing about 10·2% of the total anthocyanins in lychee fruit, was present at 0·18 mg/g and decreased to 0·16 mg/g (an 11·1% decrease) at 29 days of storage (Fig. 1). The initial concentration of cy-3-gl was 0·12 mg/g and decreased to 0·08 mg/g (a 33·3% decrease) after 29 days of storage. By 29 days, the loss of cy-3-gl during storage was much higher on a percentage basis (33·3%) compared with that lost from cy-3-rt (22·3% or mv-3-ac-gl (11·1%). Even though it is not possible to make a direct comparision as there is no nonacylated malvidin glucoside, mv-3-ac-gl seems to be more stable when compared to cyanidin glycosides between 2 and 36 days of storage. The stability of anthocyanins depends upon a large number of factors, but the methoxyl substitutions in the molecule appeared to be more stable than the hydroxyl substitution in the molecule, such as cyanidin glycoside (Hrazdina *et al.*, 1970). Also, the stability of acylated anthocyanins seems to increase on exposure to light compared to nonacylated anthocyanins (Van Buren *et al.*, 1968). But after 36 days of storage, the percent degradation appeared more pronounced, especially for mv-3-ac-gl.

Converse to gradual decreases in total and individual anthocyanins, the polymeric pigments increased from 20·9% to 35·7% after 29 days of storage and comprised over 50% of the lychee pigments at 48 days of storage (Fig. 2). Towards the end of storage, polymeric pigments were the

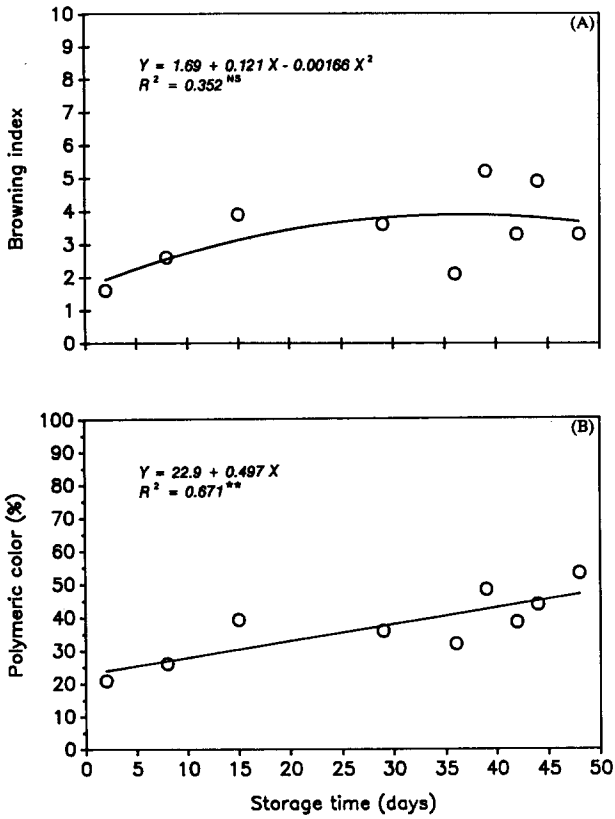

Fig. 2. Changes in browning index (A) and percent polymeric pigment (B) during storage. Regressions are significant at $P = 0{\cdot}01$ (**) or nonsignificant (NS). Each datum represent mean values for three replicate measurements.

predominant pigments in the lychee fruit, suggesting that during storage, the lychee anthocyanins initially responsible for lychee color are displaced, progressively and irreversibly, by more stable polymeric pigments.

Browning of lychee fruits also progressed during post-harvest storage. Most fruits containing anthocyanins are subject to rapid pigment degradation through enzymic and nonenzymic reactions, but post-harvest browning of lychee fruit seems to be mostly associated with enhanced activities of peroxidase and polyphenoloxidase (Shu *et al.*, 1990). Anthocyanins of the cyanidin type are poor phenolase substrates, but they are readily decolorized by this enzyme in the presence of a better substrate, such as catechol (Peng & Markakis, 1963). Browning index fluctuated considerably and as pigment degradation proceeded, the rate of browning appeared to slow down (Fig. 2).

Visual observation revealed mold spots on the skin of lychee fruits after 29 days of storage which progressed throughout the remainder of the

storage period. It has been shown that fungal growth is another problem associated with the browning reaction in lychee fruit (Nip, 1988). Quantitative analyses revealed that a large decrease in concentrations of the three anthocyanins was found after 39-day storage. This dramatic decrease may, in large part, be due to anthocyanin degradation by molds. Enzymes derived from molds are believed to play an important role in anthocyanin pigment degradation, polymerization and browning reactions (Huang, 1955; Pilando *et al.*, 1985). Considering the significant effects of molds on pigments, chemical control for fruit rot should be applied to maintain the bright color of lychee fruit even with storage at the recommended conditions.

ACKNOWLEDGEMENTS

Gratitude is extended to Mr Craig Campbell for donation of the fruits, Mr H. Shu for preparation of lychee skin extracts and Mr Gary Coates for technical assistance.

REFERENCES

Akamine, E. K. (1960). *Preventing the Darkening of Fresh Lychees Prepared for Export. Technical progress report 127.* Hawaii Agriculture Experiment Station. Univ. Hawaii.

Blundstone, H. A. W. & Crean, D. E. C. (1966). *The Pigments of Red Fruits. Report on Investigations Jan. 1964–July 1966.* The Fruit and Vegetable Preservation Research Association, Chipping Campden, Glos., UK.

Campbell, C. W. (1959). Storage behaviour of fresh Brewster and Bengal lychees. *Proc. Fla. State Hortic. Soc.*, **72**, 356–60.

Hrazdina, G., Borzell, A. J. W. & Robinson, W. B. (1970). Studies on the stability of the anthocyanin-3,5-diglucosides. *Am. J. Enol. Vitic.*, **21**, 201–4.

Huang, H. T. (1955). Decolorization of anthocyanins by fungal enzymes. *J. Agric. Food Chem.*, **3**, 141–6.

Joslyn, M. S. & Ponting, J. D. (1951). Enzyme catalyzed oxidative browning of fruit products. *Advances Food Res.*, **3**, 1–44.

Lee, H. S. & Wicker, L. (1990). Anthocyanin pigments in the skin of the lychee fruit. *J. Food Sci.* (in press).

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

Peng, C. Y. & Markakis, P. (1963). Effect of phenolase on anthocyanins. *Nature*, **199**, 597–8.

Pilando, L., Wrolstad, R. E. & Heatherbell, D. A. (1985). Influence of fruit composition, maturity and mold contamination on the color and appearance of strawberry wine. *J. Food Sci.*, **50**, 1121–5.

Robbins, J. A., Sjulin, T. M. & Patterson, M. (1989). Postharvest storage characteristics and respiration rates in five cultivars of red raspberry. *Hort Sci.*, **24**, 980–2.

Shu, H., Hart, H., Lee, H. & Wicker, L. (1990). Enzymatic and color changes during post-harvest storage of lychee fruit. *J. Food Sci.*, **55**, 1762–3.

Sjulin, T. M. & Robbins, J. A. (1987). Effects of maturity, harvest date and storage time on postharvest quality of red raspberry fruit. *J. Amer. Soc. Hort. Sci.*, **112**, 481–7.

Van Buren, J. P., Bertino, J. J. & Robinson, W. B. (1968). The stability of wine anthocyanins upon exposure to heat and light. *Am. J. Enol. Vitic.*, **19**, 147–54.

Wrolstad, R. E. (1976). Color and pigment analyses in fruit products. Agricultural Experiment Station 624, Oregon State University, Corvallis, Oregon.

Wrolstad, R. E., Culbertson, J. D., Cornwell, C. J. & Mattick, L. R. (1982). Detection of adulteration in blackberry juice concentrates and wines. *J. Assoc. Off. Anal. Chem.*, **65**, 1417–23.