JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

J. Agric. Food Chem. 2010, 58, 6209–6218 6209
DOI:10.1021/jf100023x

Altered Physiology and Biochemistry of Imported Litchi Fruit Held under Different Vapor Pressure Deficits

Nettra Somboonkaew and Leon A. Terry*

Plant Science Laboratory, Cranfield University, Bedfordshire, MK43 0AL, United Kingdom

The effects of vapor pressure deficit (VPD) on litchi fruit quality have not yet been fully defined. The aim of this study was to detail the changes in physiology, sugars, organic acids, and individual anthocyanin concentrations in imported litchi fruit held at various controlled relative humidity (RH) and VPD levels. SO_2-fumigated (but not acid-treated) litchi imported from Thailand (cv. Kom) and from Israel (cv. Mauritius) were air freighted to the United Kingdom and then stored for 9 days at either 5 or 13 °C to simulate shelf-life conditions. Fruits were stored under a series of controlled RH conditions for the duration of the trial using different concentrations of glycerol in deonized water. Respiration rates and weight losses of both fruit lots were greater in litchi stored at 13 °C and a VPD of 0.274 kPa. At 5 °C and a VPD of 0 or 0.042 kPa, sugars and organic acids in aril and pericarp tissue and individual anthocyanins in pericarp were better maintained. This is the first piece of work that has systematically evaluated the effect of a series of VPDs on litchi fruit biochemistry such that implications for designing systems to better maintain the physiological quality of imported litchi fruit are discussed.

KEYWORDS: Anthocyanins; lychee; organic acids; RH; sugar; VPD

INTRODUCTION

Although litchi (*Litchi chinensis* Sonn.) fruit has been supplied and consumed worldwide for decades, pericarp browning and dehydration still persist as major postharvest problems. Storage at low temperature can slow down fruit metabolism and also the rate of growth and spread of pathogens, thus prolonging shelf life. However, there is still a lack of detailed information concerning the effects of other storage parameters on storage disorders in litchi.

Vapor pressure deficit (VPD) in the storage environment is an important factor in influencing the postharvest life of fresh produce. The VPD is defined as the difference between the moisture content in fruit tissue and the water vapor in the environment, which mainly depends on temperature and relative humidity (RH). However, effects of VPD on the biochemistry of harvested litchi fruit have infrequently been investigated. This might be due partly to the practical difficulties in maintaining defined levels of RH in a storage environment. The air exchange rate, temperature distribution, fresh produce type, and packaging material used in the storage room can influence the range of RH encountered (*1*).

Jiang and Fu (*2*) reported the influence of RH on pericarp browning of litchi cv. Huaizhi fruit. They supplied dry (35%) and wet (> 95%) air streams into storage containers (2.12 m^3) held at 20 °C and mixed these air streams to achieve 60, 70, 80, or 90% RH levels. The RH was measured at a position midway between the top of the fruit in the container and the exhaust port by an electrohygrometer sensor calibrated using saturated salt solutions. Litchi stored at 90% RH showed the lowest water loss, tissue pH level, relative membrane leakage, polyphenol oxidase (PPO) activity, and browning scale with a higher total anthocyanin content after storage. RH at 90% (20 °C; humidification method not stated) was also shown to inhibit browning in litchi cv. Hong Huay fruit by limiting water loss, PPO and phenylalanine ammonia lyase (PAL) activities, and total anthocyanin and phenol degradation and maintaining higher a^* (red color), followed by the fruit stored at 80 and 70% RH, respectively (*3*).

Although storage at elevated RH has been shown to maintain the postharvest quality of litchi, the specific effects of a range of controlled VPD levels on physiological and biochemical changes during storage have not been completely described. Thus, the aim of this study was to detail the explicit spatial and temporal physiological and biochemical changes in imported litchi fruit as affected by different storage VPDs (temperature and RH), with particular emphasis on sugars and nonvolatile organic acids in aril and pericarp tissue and anthocyanins in pericarp tissue.

MATERIALS AND METHODS

Sample Preparation. Thailand litchi cv. Kom fruits, grown in Samutsongkram province, were exported by the River Kwai International Food Industry Ltd. (Bangkok, Thailand) to the United Kingdom (Minor Weir and Wills Ltd., Birmingham, United Kingdom). Fruits were harvested (fruit core temperature = 29 °C) on April 14, 2007, at the color-break stage ($h° = 50-55$), hydrocooled (fruit core, 6 °C), and transported to the pack-house by refrigerated lorry (ca. 12 °C). As per standard practice, fruits were treated with SO_2 (1 g SO_2 for 1 kg fruit; temperature and time were not measured) within 7 h of harvest before being stored at 2 °C and transported to the airport by refrigerated truck within 24 h of harvest. Israeli cv. Mauritius litchi fruit were grown in Western Galilee and imported to the United Kingdom by Agrexco Agricultural Export Ltd. (Middlesex, United Kingdom). Fruits were harvested at the color-break stage (ca. $h° = 55-60$) on July 17, 2007, and treated with SO_2 (1 g SO_2 for 1 kg fruit at 20 °C for 30 min) within 5 h of harvest. Afterward, fruits were

*To whom correspondence should be addressed. Tel: +44-7500-766-490. E-mail: l.a.terry@cranfield.ac.uk.

Published on Web 05/04/2010

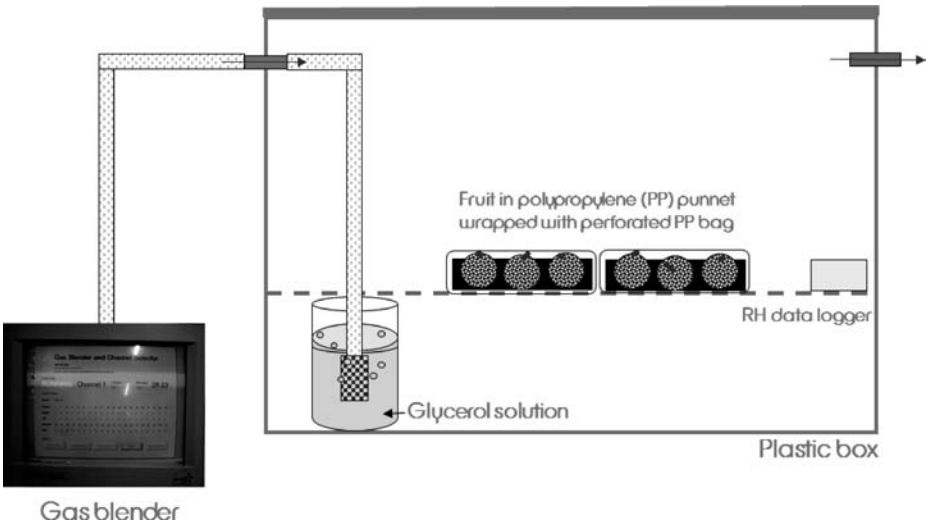

Figure 1. Set up for the modification of RH in the closed plastic containers using different concentrations of glycerol solution with 3 L min^{-1} flow rate of regular air.

precooled at 1 °C for 8 h and air-freighted to the United Kingdom. Upon arrival in the United Kingdom, litchi cv. Kom fruits were transported by refrigerated truck to Minor Weir and Wills Ltd. and cv. Mauritius to Agrexco (Middlesex, United Kingdom) and stored at 1 °C. Importantly, both litchi cultivars were not pretreated with acid as this practice is becoming increasingly less desirable for U.K. consumers. Fruit cvs. Kom and Mauritius were transported to Cranfield University within 2 h and thus arrived at the laboratory within 4 and 6 days of harvest, respectively, to simulate the real world supply chain. Fruits were sorted for uniformity of size and freedom from defects.

Both experiments were arranged as randomized complete blocks. Thai (n = 300) and Israeli fruits (n = 300) were separated into 10 groups. Each group was divided equally into six polypropylene (PP) plastic punnets: 115 mm × 140 mm (Nicholas Ltd., Derbyshire, United Kingdom). Each punnet was then individually placed in a microperforated PP bag (Nicholas Ltd.): 150 mm × 200 mm with 25 μm thickness as per standard commercial practice in the United Kingdom and heat-sealed using a hand-operated heat sealer (Hulme Martin Ltd., Surrey, United Kingdom). Fruit punnets were stored at 80, 85, 90, 95, and 100% RH in 25 L polyethylene boxes (HK-Plastics BV, Oldenzaal, The Netherlands) for 9 days at either 5 or 13 °C (as common shelf temperature in U.K. supermarkets and average supply chains, respectively).

The RH in containers was controlled according to Pateraki et al. (*4*) with modification. Regular air was bubbled through 500 mL of glycerol solution [116, 82, 53, and 24% (v/v) glycerol in distilled water to achieve 80, 85, 90, and 95% RH, respectively] or pure distilled water (for ca. 100% RH) for 3 min at 30 min intervals for 9 days and exhausted continuously at a 3 L min^{-1} flow rate using a gas mixing blender (Signal Series 850, Signal Instrument Ltd., Surrey, United Kingdom) (**Figure 1**). The experiments only started once the RH had equilibrated for 4 days (96 h). After the boxes were opened and the fruit was put inside, desired RH levels returned within minutes. The RH in each container was monitored using Tiny Tag Ultra 2 data loggers (Gemini Data Logger, W. Sussex, United Kingdom). The VPDs were then calculated using eq 1 (*5*), where the vapor pressure at saturated condition (VP(s); eq 2) was based on the measured temperature (T in °C) and the vapor pressure in the storage (VP; eq 3) was based on the controlled RH (%).

$$\mathrm{VPD} = \mathrm{VP(s)} - \mathrm{VP\ (kPa)} \quad (1)$$

$$\mathrm{VP(s)} = 0.6108e[(17.27 \times T)/(T + 265.5)] \quad (2)$$

$$\mathrm{VP} = \mathrm{VP(s)} \times \mathrm{RH}/100 \quad (3)$$

Fruits (n = 300 each cultivar) were individually weighed, the color was measured, and the total soluble solids (TSS) were analyzed on days 0, 1, 3, 6, and 9 of storage. The colors of fruit pericarps [lightness (L*), intensity (C*), and red color (h°)] were measured using a Konica Minolta colorimeter (Chroma meter model CR-400 and data processor model DP-400, Konica Minolta Sensing, Japan). Each value was the average of three randomized measurements on the fruit surface. Fruits were then peeled, and pericarp, aril tissues, and stones were separated. Pericarp and aril tissues were weighed. The aril tissue was then gently squeezed to produce a few drops of juice to determine TSS using a digital refractometer (PR 301α, Atago Ltd., Japan). To limit the potential contamination of pericarp tissue with aril fruit juice, pericarp tissue was rinsed with deionized water before snap-freezing. Pericarp and aril tissues were immediately snap-frozen in liquid nitrogen and stored at −40 °C before being freeze-dried (Christ LOC-1, Germany) for 5 and 9 days, respectively. Lyophilized samples were subsequently weighed, ground [aril tissue using a hand-operated pestle and mortar and pericarp tissue using a motorized mortar grinder (RMO, Retsch, Germany)] into a fine powder, and stored at −40 °C until required.

Measurement of Respiration Rate. Three punnets (which acted as blocks) of fruit (n = 15) from each RH container were weighed, and the respiration rate (mL CO_2 kg^{-1} h^{-1}) was measured. Fruits (n = 5) from each punnet were placed into a 450 mL airtight plastic box with a lid fitted with a septum and sealed for 2 h at fruit initial storage temperature (5 or 13 °C). After this incubation period, gas samples were removed with repeated full withdrawal-injection displacements of a 30 mL plastic syringe. The gas sample was immediately analyzed for CO_2 according to Terry et al. (*6*). The gas chromatograph was calibrated with 10.06 kPa CO_2 (10 kPa CO_2, 2 kPa O_2, and 88 kPa N_2; Certified Standard from British Oxygen Co., Surrey, United Kingdom).

Extraction and Analysis of Sugars. Freeze-dried aril and pericarp powder (150 mg; aril n = 300; pericarp n = 300 each cultivar) was extracted with 3 mL of 62.5:37.5 HPLC grade methanol:water (v/v) and mixed well according to Terry et al. (*7*). Vials of the slurry were placed in a shaking water bath at 55 °C for 15 min. They were removed briefly and shaken for 20 s every 5 min to prevent layering and then left to cool. The cooled samples were filtered through a 0.2 μm Millex-GV syringe driven filter unit (Millipore Corp., MA), and the clear extract analyzed. The extracts were stored at −40 °C until required. Sugars were measured according to Terry et al. (*7*) using a high-performance liquid chromatography (HPLC) system comprised of a P580 pump and GINA 50 autosampler (Dionex, CA). Extracts were diluted 1:10 (v/v) with HPLC grade water immediately before analysis. The diluted extract (20 μL) was injected into a Rezex RCM monosaccharide Ca^{+} size exclusion column that was 300 mm × 7.8 mm diameter, 8 μm particle size (Phenomenex, CA), with a Carbo-Ca^{2+} security guard cartridge that was 4 mm × 3 mm diameter (Phenomenex). The mobile phase was degassed with HPLC grade water at a flow rate of 0.6 mL min^{-1}. The column temperature was maintained at 75 °C using a Dionex STH column thermostat. Eluted carbohydrates were monitored by an evaporative light scattering detector (ELSD 2420, Waters, MA) connected to the Dionex system using a UCI-50 universal chromatography interface. The presence and abundance of mannose (in pericarp only), fructose, glucose, and sucrose were automatically

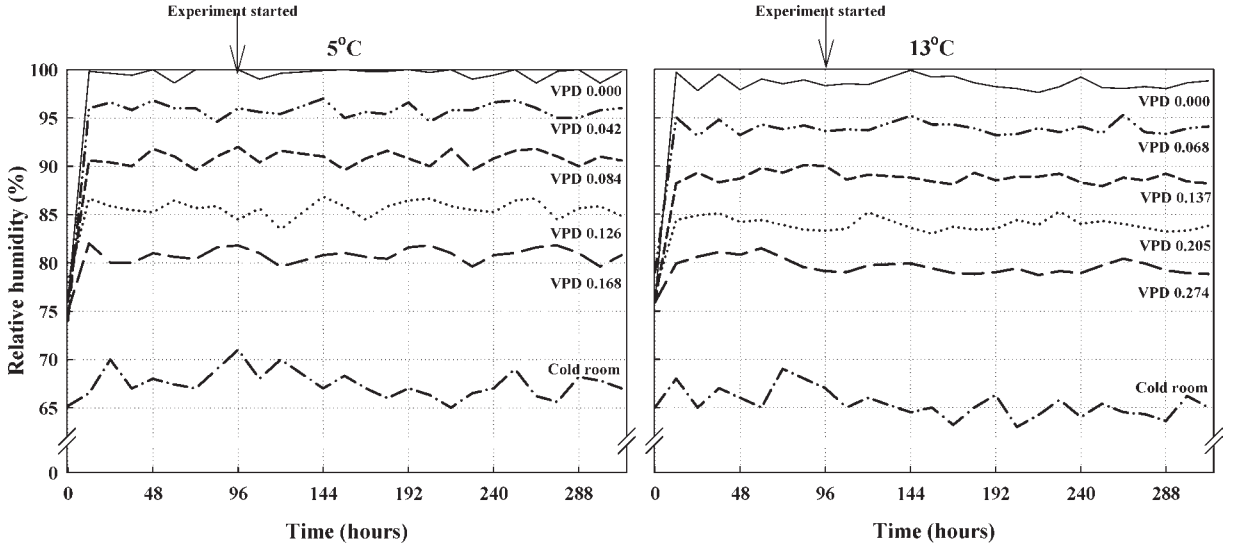

Figure 2. RH levels in the cold room and inside the storage containers set at 80, 85, 90, 95, and 100% RH at 5 or 13 °C.

calculated against external standards using Chromeleon version 4.6 software (Dionex, CA). Additionally, mannose was confirmed by an enzyme test kit (K-MANGL 01/05; Megazyme International Ireland Ltd., Co. Wicklow, Republic of Ireland) before it was analyzed by HPLC.

Extraction and Analysis of Nonvolatile Organic Acids. Nonvolatile organic acids were extracted according to Terry et al. (*7*) with modifications. Freeze-dried litchi aril (50 mg; $n = 300$ each cutivar) and pericarp (50 mg; $n = 300$ each cultivar) were mixed well with 3 mL of HPLC grade water. The samples were incubated at room temperature for 5 min and filtered through a 0.2 μm filter and stored at −40 °C until required. The aril extracts were measured using the same Dionex HPLC as previously described. Samples (20 μL) were injected into an Alltech Prevail Organic Acid column of 250 mm × 4.6 mm diameter, 5 μm particle size (Alltech, IL), with a guard column of 7.5 mm × 4.6 mm diameter. The mobile phase was degassed and filtered with 0.2% (v/v) metaphosphoric acid in HPLC grade water at a flow rate of 1.0 mL min^{-1}. The column temperature was held at 35 °C using a Dionex STH column thermostat. Eluted organic acids from aril extracts were monitored at 210 nm using a UV−vis diode array detector (UVD 170S/340S; Dionex). The presence and abundance of oxalic, tartaric, ascorbic, malic, and citric acids in aril and pericarp were automatically calculated against external standards using Chromeleon version 4.6 software.

Extraction and Analysis of Anthocyanins. The pericarp tissue was extracted and quantified according to Terry et al. (*7*) and Giné Bordonaba and Terry (*8*) with modifications. Freeze-dried litchi pericarp powder (150 mg; $n = 300$ each cultivar) was mixed well with 3 mL of 70:29.5:0.5 HPLC grade methanol:water:HCl (v/v/v). The samples were held at 35 °C for 1.5 h. They were shaken for 10 s every 15 min to prevent layering. The samples were filtered as before and stored at −40 °C until required. The pericarp extract (20 μL) was quantified using an Agilent 1200 series HPLC (Agilent, Berkshire, United Kingdom) and injected into a Zorbax column of 250 mm × 4.6 mm diameter, 5 μm particle size with 4 XDB-C18 (5 μm) guard column of 12.5 mm × 4.6 mm diameter (Agilent). The mobile phase was degassed and filtered with (A) 1% (v/v) phosphoric acid and 10% (v/v) acetic acid in water and (B) acetonitrile with a flow rate of 1 mL min^{-1}. The program followed a linear gradient from 2 to 20% of B in 25 min and then from 20 to 40% of B in 15 min. Anthocyanins were detected at 520 nm using a photodiode array detector (G1315D, Agilent). The column temperature was set at 40 °C, and the temperature of the autosampler was held at 4 °C. The presence and abundance of cyanidin 3-glucoside, cyanidin 3-rutinoside, and malvidin 3-glucoside were automatically calculated against external standards (Extrasynthese, Lyon, France) using Chemstation Rev. B.02.01 software (Agilent).

Fruit Decay. Disease was recorded by scoring the percentage of incidence on each fruit surface. Disease was scored using a 1−5 visual scale (1 = no incidence, 2 = one spot to 5% of disease on each fruit surface, 3 = 10% on fruit surface, 4 = 15% on fruit surface, and 5 = 20% on fruit surface).

Statistical Analyses. All statistical analyzes were carried out using Genstat for Windows Version 10.1 (VSN International Ltd., Herts., United Kingdom). Analysis of variance was performed on the data, extracting information about the main effects and interactions of storage RH, temperature, and storage duration. Least significant difference values (LSD; $P < 0.05$) were calculated for comparison of appropriate treatment means. Unless otherwise stated, significant differences were $P < 0.001$. Principal component analysis (PCA) (using group average linkage) was carried out on the autoscale data set of each experiment using Unscrambler Camo Software AS version 9.8 (free trial; www.camo.com), to understand the effect of temperature and RH level on the chemometric profile of spatial and temporal variation within each cultivar.

RESULTS AND DISCUSSION

The desired RH conditions of 80, 85, 90, 95, and 100% RH in containers at 5 or 13 °C remained relatively stable during 9 days of storage (**Figure 2**). Previous RH studies on litchi have either not stated how RH was controlled (*3*) or have used a different apparatus capable of defining a series of set levels (*2*). At constant RH, a lower temperature resulted in a lower VPD level, while at the same temperature, a higher RH caused a lower VPD level (**Figure 2**). Disease, mainly caused by *Penicillium* spp., was detected approximately 5% of surface of fruit stored at 13 °C and 100% RH (VPD = 0.000 kPa) for both cultivars on day 9.

Fruit Weight Loss and Pericarp Moisture Content. Weight loss of fruits from all regimes increased during 9 days of storage. Predictably, fruit cvs. Kom and Mauritius stored at 80% RH and 13 °C (VPD of 0.274 kPa) had a significantly higher weight loss than fruit at 85, 90, 95, and 100% RH, respectively (**Figure 3**). Kaewchana et al. (*3*) reported that low RH (40−50%) storage inevitably resulted in greater moisture loss in litchi fruit. In the case of litchi fruit, low RH could enhance pericarp microcracking (*9*) and damage cellular membranes, resulting in an increase in membrane leakage and cellular moisture loss (*10*). Temperature significantly affected fruit weight loss, whereby weight loss of fruit stored at 13 °C was 1.7- (cv. Kom) and 1.9-fold (cv. Mauritius) higher than in the 5 °C treatment. Lower weight loss of cv. Kom fruit could be partially due to hydrocooling at the pack house. Higher temperatures cause more free energy of water molecules, which increases water movement and potential for exchange to atmosphere around the fruit (*11*), resulting in faster evaporation. Higher temperatures require more moisture to saturate the air. A greater difference in vapor pressure between the fruit and the storage atmosphere therefore exists and leads to rapid moisture

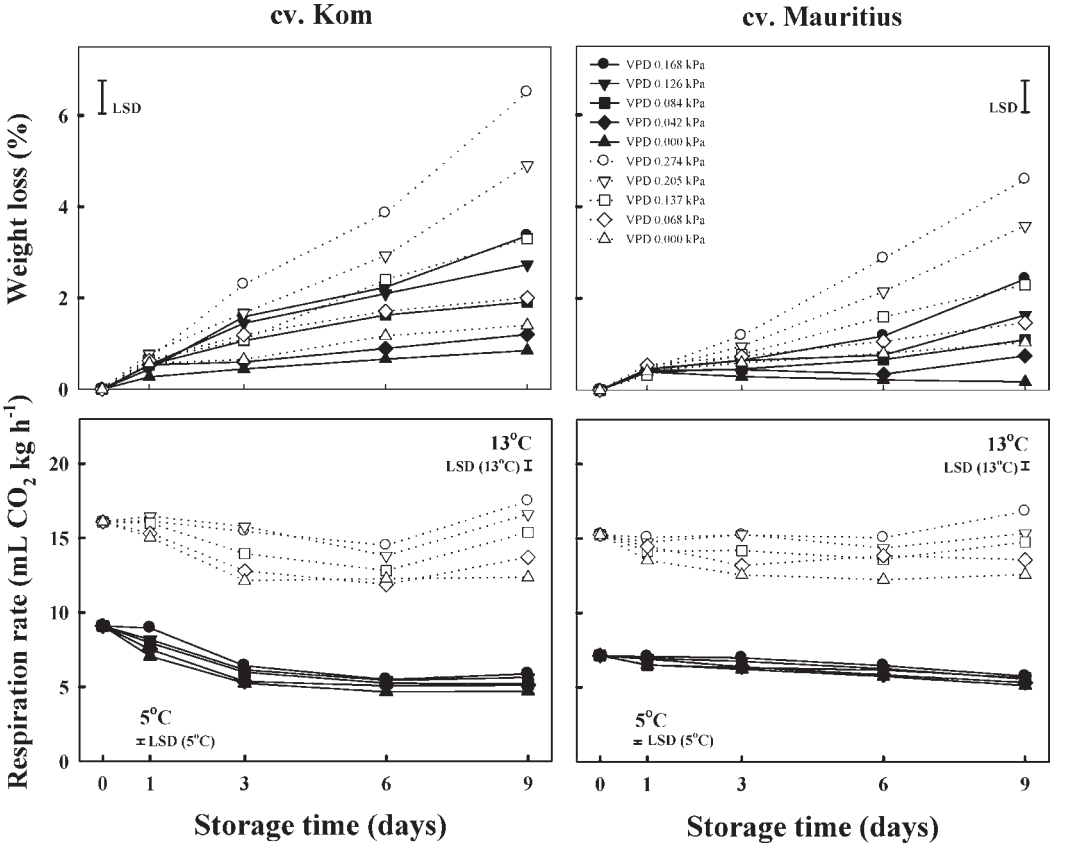

Figure 3. Respiration rate (mL CO_2 kg h^{-1}; each symbol $n = 3$) and weight loss (%; each symbol $n = 15$) in litchi cvs. Kom and Mauritius stored at 5 °C, 80 (●), 85 (▼), 90 (■), 95 (♦), and 100% (▲) RH, and at 13 °C, 80 (○), 85 (▽), 90 (□), 95 (◇), and 100% (△) RH, during 9 days of storage (LSD, $P < 0.05$).

loss from fruit to the environment (*12*). According to the present study, for instance, the air at 13 °C and 90% RH regimes (VPD = 0.137 kPa) would have been drier than the air at 5 °C and 90% RH (VPD = 0.084 kPa), resulting in faster fruit weight loss. The results indicated that a higher moisture loss was induced by a lower RH and higher temperature (VPD = 0.274 kPa). There were no significant differences in moisture contents of pericarp between RH treatments in both experiments.

Respiration Rate. Kom and Mauritius fruits stored at 100% RH at both 5 and 13 °C (VPD = 0.000 kPa) had the lowest respiration rate, while the highest rates were recorded at 80% RH (VPD = 0.168 and 0.274 kPa at 5 and 13 °C, respectively). Water stress (63 and 70% RH) was reported to accelerate respiration in litchi fruit cv. Heiye (*13*) and senescence in cv. Huaizhi (*10*). However, the impact of a wide range of controlled RH conditions on respiratory activity has not been reported. Unsurprisingly, storage temperature significantly affected the respiration rate, whereby fruit stored at 5 °C had a significantly lower respiration rate than fruit held at 13 °C (**Figure 3**). A decline in respiration rate was found in fruit from all treatments over storage time. A slight increase in respiration rate for both experiments kept at 13 °C was detected after day 6, which could be partially due to pathogenic rot (but less than 5% of fruit surface) but is more likely to have been influenced by browning (*14*, *15*) since fruit held at 80% RH and 13 °C showed no disease but the most severe browning. The results indicated that higher VPD (low RH and high temperature) encouraged greater fruit desiccation and moisture loss and accelerated respiratory rate during storage time, which was in agreement with previous work on litchi cv. Heiye (*13*). There was no correlation between respiration rate and weight loss in the current study.

Pericarp Color. The storage temperature, RH, storage duration, or their interactions did not affect the pericarp color of litchi cv. Mauritius but did influence cv. Kom. Kom fruits kept at 5 °C had significantly higher L^* values than those at 13 °C. The L^* and C^* values of pericarp of cv. Kom fruit stored at 5 or 13 °C with 100% RH (VPD = 0.000 kPa) were significantly higher than with 95, 90, 85, and 80% RH, respectively. However, fruits kept at 80 or 85% RH and 5 or 13 °C had higher $h°$ (more brown) than those held at 90, 95, and 100% RH. The results indicated that pericarp browning was most reduced at 5 °C and at 90−100% RH (VPD = 0.000−0.084 kPa) storage. Values of L^*, C^*, and $h°$ of cv. Kom fruit significantly decreased during 9 days of storage (**Figure 4**). The decrease in $h°$ recorded in the present study is in disagreement with previous work by Kaewchana et al. (*3*) who reported a very slight increase of $h°$ (220−225) in stored litchi cv. Hong Huay held at 20 °C and 50−90% RH between days 0 and 9; yet, it is unclear whether these fruit were acid dipped.

TSS and Sugar Concentrations. The storage RH, temperature, time, or their interactions did not affect TSS in cvs. Kom and Mauritius, which may be due to the fact that only slight changes in refractometric values occur in harvested litchi fruit (16.2−19.4%). Small changes in refractometric values were reported in litchi cvs. Da Zao, Hei Ye, Bengal, Huai Zhi (*16*), and Mauritius (*17*) during storage. The freeze-dried aril tissue of cvs. Kom and Mauritius contained mainly fructose (368.7 and 252.9 mg g^{-1} DW) followed by glucose (363.6 and 230.3 mg g^{-1} DW) and sucrose (193.8 and 221.5 mg g^{-1} DW). Sugar is an important energy source for respiration and other metabolism in fresh produces. Aril sucrose in both cultivars stored at 5 °C and 90% RH (VPD = 0.084 kPa) was considerably higher than for other RH regimes, while the lowest levels were found at 13 °C and 80% RH (VPD = 0.274 kPa). In contrast, all fruits held at 80% RH, irrespective of temperature, had the highest glucose and fructose levels. Fruits stored at 5 °C from both cultivars had

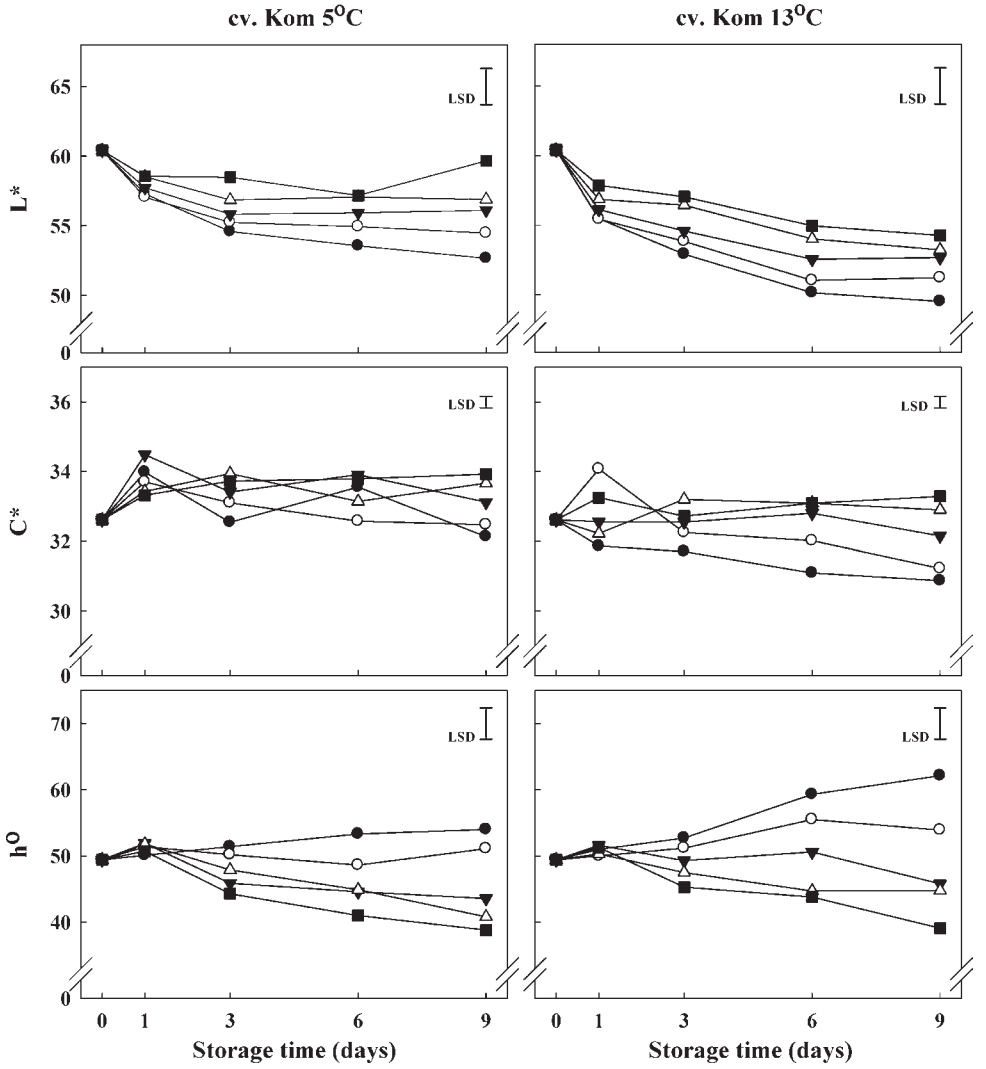

Figure 4. Lightness (L^*), chroma (C^*), and hue angle ($h°$) of litchi cv. Kom fruit stored at 80 (●), 85 (○), 90 (▼), 95 (△), and 100% (■) RH at 5 or 13 °C for 9 days (LSD, $P < 0.05$; each symbol $n = 15$).

significantly higher sucrose and lower glucose and fructose contents than those kept at 13 °C (**Figure 5A**,**C**). Fructose and glucose concentrations generally increased during 9 days of storage time, whereas sucrose decreased. These results may be explained by hydrolysis of sucrose to form fructose and glucose, together with a probable increase in sugar inverstase enzyme activity during storage (*18*). An enhancement of glucose and fructose and deterioration of sucrose during storage time was found in litchi cvs. Hei Ye, Chen Zi (*19*), and Rose (*20*) and may affect the perception of sweetness. However, there was no correlation between TSS and aril sugars in either cultivars, which is in agreement with Somboonkaew and Terry (*17*).

Major sugars in the pericarp of cvs. Kom and Mauritius were glucose (42.17 and 26.15 mg g^{-1} DW), mannose (39.00 and 28.66 mg g^{-1} DW), and fructose (30.90 and 28.87 mg g^{-1} DW) with trace amounts of sucrose. This is the first study to report the presence and abundance of these nonstructural carbohydrates in litchi pericarp. Both cultivars kept at 5 °C had higher sugar concentrations than those at 13 °C during 9 days (**Figure 5B**,**D**). Fruits from cv. Kom contained 1.56-fold higher glucose and 1.36-fold higher mannose than cv. Mauritius, whereas fructose levels did not differ. A high proportion (%) of mannose content in combination with other polysaccharides was reported in litchi pericarp cv. Huaizhi as a strong antioxidant source (*21*). Yet, the concentration of mannose in litchi pericarp has not been previously reported. Glucose and fructose concentrations were significantly different according to RH treatments. The RH of 80 and 85% resulted in lower glucose and fructose concentrations in both cultivars' pericarp than 90−100% RH. A higher moisture content in fruit pericarp at 90−100% RH could partially accelerate sucrose inversion, by the action of acid (low pH) or invertase enzyme, to form glucose and fructose (*22*), resulting in trace levels of sucrose in the present study. Although a decrease in glucose was recorded with increases of mannose and fructose concentrations in nonacid-treated and SO_2-free litchi fruit pericarp cv. Mauritius (*17*), the mannose content in the present study remained stable over time (**Figure 5B**,**D**). This could be because SO_2 residue in the pericarp was hydrolyzed to sulfurous acid (*23*), leading to inappropriate conditions for glucose−mannose−fructose transformation according to Lobry de Bruyn-Alberda van Ekenstein epimerization (*24*). Although fruit dehydration did not affect sucrolytic enzyme activities (*25*) or gluconeogenesis (*26*), an increase of fruit weight loss (cv. Mauritius) correlated (albeit weakly) to accumulation of glucose and fructose contents ($r = 0.61$ and 0.69, respectively).

Nonvolatile Organic Acids Concentration. Malic and tartaric acid (cv. Kom, 22.84 and 16.28 mg g^{-1} DW; and cv. Mauritius, 10.27 and 13.37 mg g^{-1} DW, respectively) were the most abundant organic acids found in aril tissue with small amounts of citric, ascorbic, and oxalic acids (**Figure 6**). Concentrations of

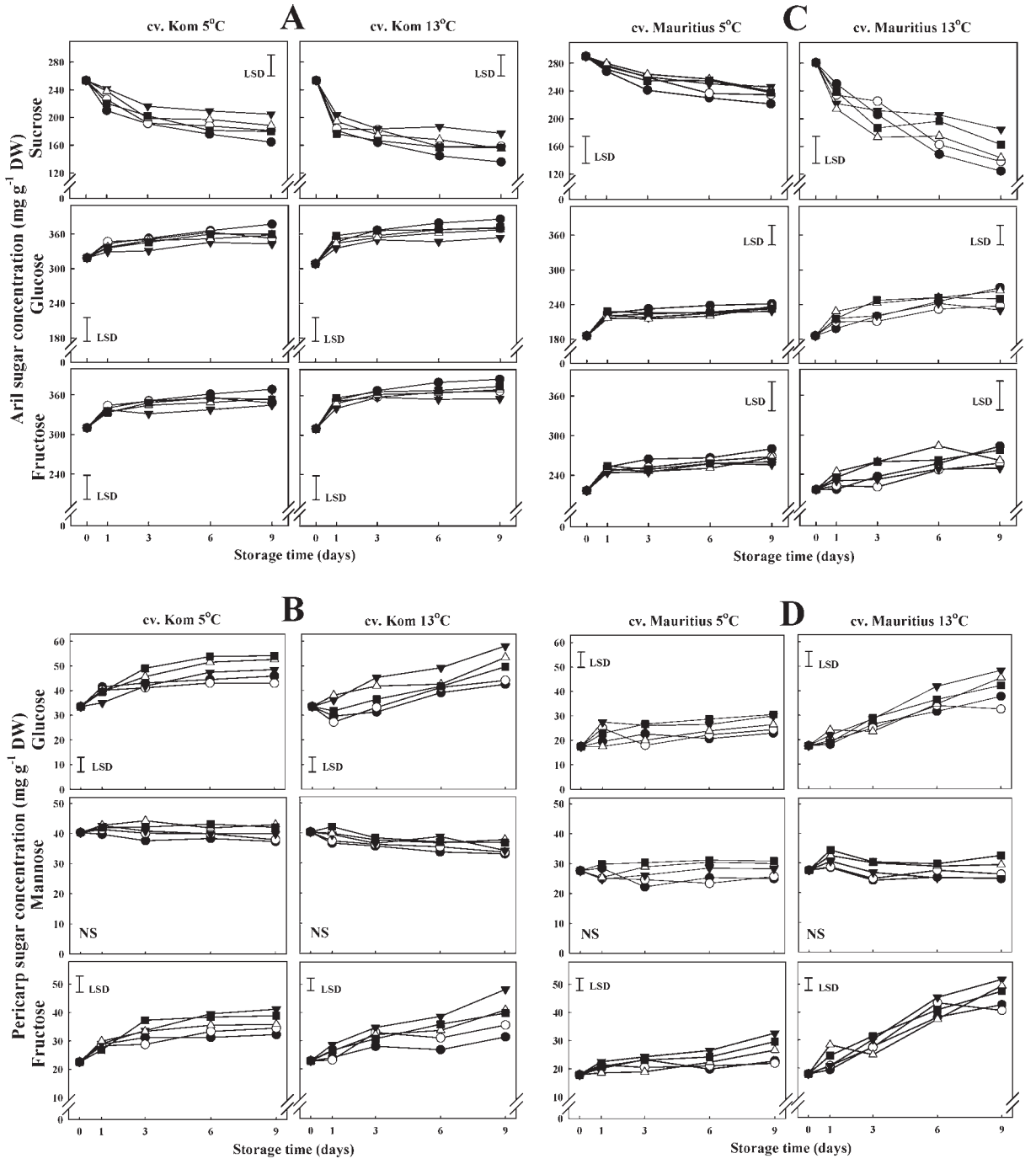

Figure 5. Concentrations of sucrose, glucose, mannose, and fructose in litchi cv. Kom aril (**A**) and pericarp (**B**) and cv. Mauritius aril (**C**) and pericarp (**D**) stored at 80 (●), 85 (○), 90 (▼), 95 (△), and 100% (■) RH at 5 or 13 °C during 9 days of storage (LSD, $P < 0.05$; each symbol $n = 15$).

all acids including total acids (malic + tartaric + citric + ascorbic + oxalic acid) in cv. Kom were 1.74 times higher than in cv. Mauritius. The storage RH significantly affected organic acids in the aril of both cultivars. Kom fruit at 100% RH and Mauritius fruit at 95% RH retained higher malic acid (26.56 and 14.64 mg g^{-1} DW, respectively) than those treated with other RH, whereas the highest tartaric acid concentrations were detected at 90% RH in both cultivars. Fruits stored at 80% RH contained the lowest concentrations of organic acids in both cultivars. Results indicated that higher RH storage (lower VPD) maintained higher aril organic acid contents. Both fruit cultivars stored at 5 °C retained higher malic, tartaric, oxalic, and total acids than those stored at 13 °C. Organic acids are a major source of energy for general metabolism, including the respiratory tricarboxylic acid cycle in harvested produce. Lower organic acid contents at higher storage temperatures could be due in part to the higher respiratory metabolism. The total organic acid concentrations in both cultivars significantly decreased during 9 days. A similar reduction in acids in aril tissue was also recorded in stored litchi aril cvs. Calcuttia (*27*) and Huaizhi (*15*). Besides, high temperature and low RH storage possibly resulted in an increase in SO_2 movement and absorption in litchi aril (*28*) and would lead to off-flavor (not measured) in aril tissue.

Alteration in organic acid levels is likely to influence pH and, therefore, possibly sugar transformation and anthocyanin rutino/glucosides in litchi pericarp. Tartaric, malic, and citric acids were the major organic acids found in pericarp of both litchi cultivars. Relatively small amounts of ascorbic and oxalic acids were

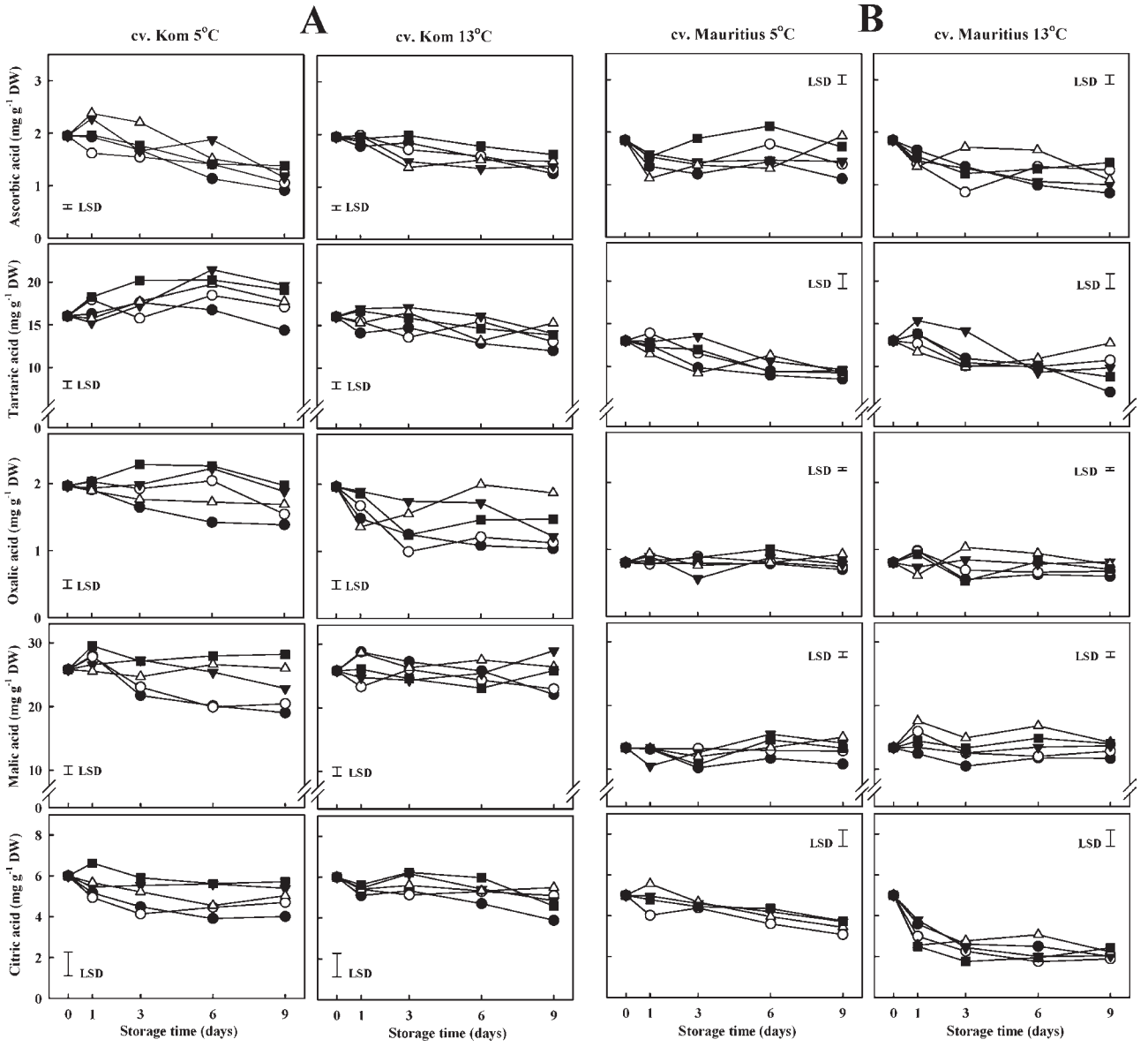

Figure 6. Organic acids in aril tissue of litchi cvs. Kom (**A**) and Mauritius (**B**) stored at 80 (●), 85 (○), 90 (▼), 95 (△), and 100% (■) RH at 5 or 13 °C during 9 days of storage (LSD, $P < 0.05$; each symbol $n = 15$).

observed, but higher concentrations were generally measured in cv. Mauritius. In general, pericarp acid contents in both cultivars declined over 9 days, in agreement with Joubert (*29*) and Caro and Joas (*30*), but acid levels remained significantly higher in cv. Mauritius at 5 °C or 90−100% RH (VPD = 0.000−0.084 kPa) than other regimes (**Figure 7**). Apart from pericarp endogenous organic acids, SO_2 can also inhibit enzymatic browning by decreasing oxidation and anthocyanin deterioration and increasing membrane integrity (*31*, *32*). SO_2 treatment may have slowed down the reduction of pericarp organic acids in the current study, which retained higher acid content than in pericarp of nonacid and SO_2-free fruit during storage (*17*). Greater pH changes would have been expected at lower RHs as the acid level decreased to a greater extent, especially tartaric and malic acids, which were dominant. In addition to the concentration of these two acids being high, they have low pK_a (pK_a malic = 3.40 and pK_a tartaric = 2.98) values, implying strong acid conditions. Reductions in malic and tartaric acids hence could possibly be linked to an increase in pH in the pericarp of fruit stored at low RH with resultant effects on color.

Anthocyanin Concentrations. The level of individual anthocyanins in litchi cv. Mauritius has been previously detailed (*17*); yet, no work to date has described anthocyanins in cv. Kom. Cyanidin 3-rutinoside, cyanidin 3-glucoside, and malvidin 3-glucoside were found in the pericarp of both cultivars. The concentrations of cyanidin 3-rutinoside (1678 μg g^{-1} DW) and malvidin 3-glucoside (19.84 μg g^{-1} DW) in cv. Kom were 1.74 and 1.55 times higher than in cv. Mauritius, respectively, while cyanidin 3-glucoside in litchi cv. Mauritius was 3.90-fold as compared to cv. Kom fruits (**Figure 8**). Total individual anthocyanin contents of cvs. Kom and Mauritius were lowest under the 80% RH regime. All anthocyanins in both cultivars generally remained stable or slightly declined over 9 days but were significantly higher in fruits stored at 5 °C (**Figure 8**). The decline in anthocyanins may have been accelerated by enzymatic activities of anthocyanin-β-glucosidase (*33*), PPO, and peroxidase activity (*34*), which can contribute to browning of litchi pericarp. However, SO_2 can prevent these enzymatic browning reactions by being hydrolyzed to colorless chromen-2 (or chromen-4) sulfonic acid (quinine−sulphite complex), which has a similar structure and property to the carbitol form of the anthocyanin (*23*, *35*). Although anthocyanins in pericarp can be oxidized to anthocyanidin and sugar moieties during storage time, the SO_2 residue interferes with

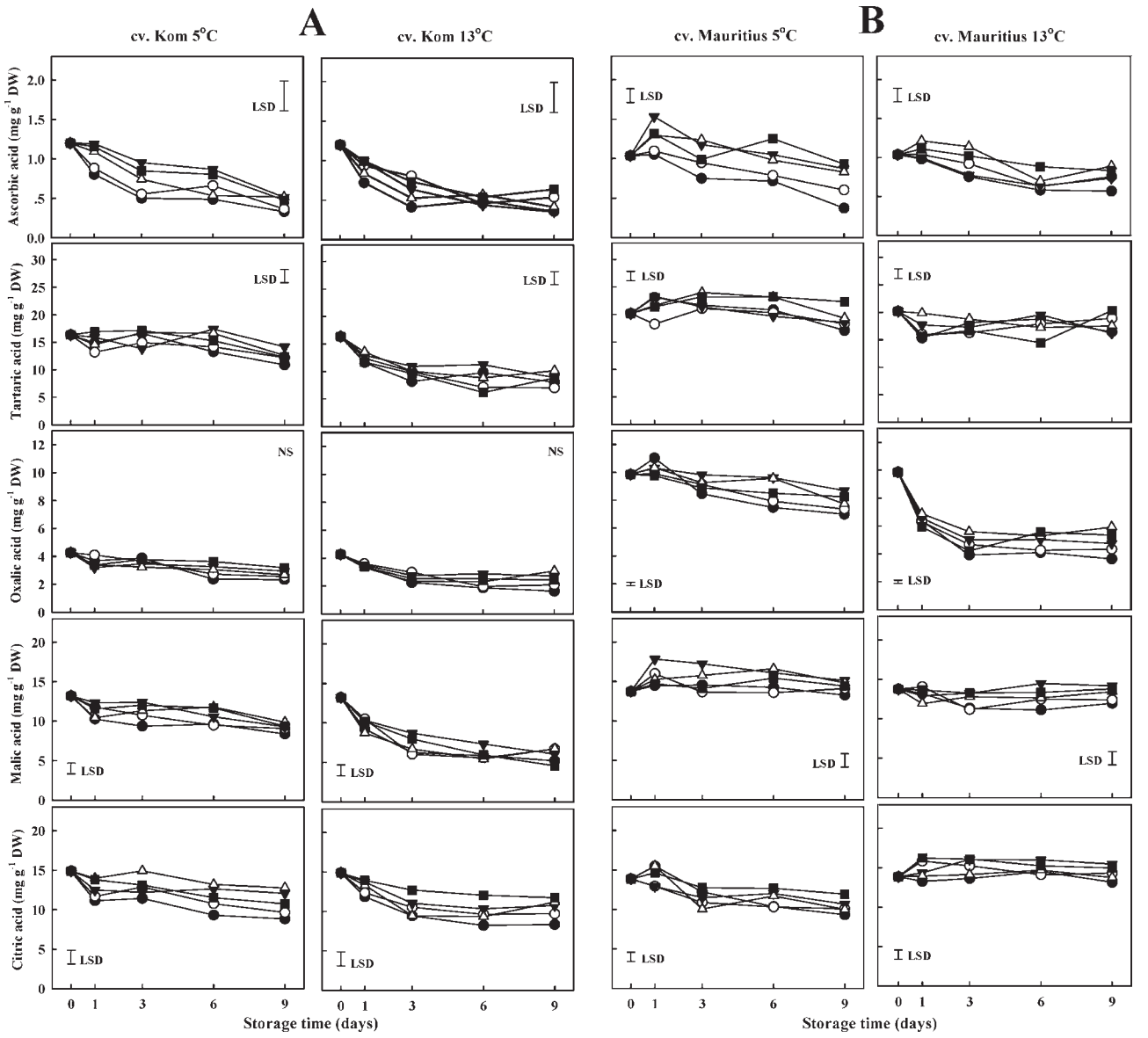

Figure 7. Organic acids in pericarp tissue of litchi cvs. Kom (**A**) and Mauritius (**B**) stored at 80 (●), 85 (○), 90 (▼), 95 (△), and 100% (■) RH at 5 or 13 °C during 9 days of storage (LSD, $P < 0.05$; each symbol $n = 15$).

the activities of PPO and anthocyanin, inhibiting quinine and melanin formation (*36*) and perhaps pericarp sugar reduction (**Figure 5B**,**D**). However, there were no reports fully describing the relation between SO_2 concentration in pericarp tissue and browning and/or anthocyanin degradation. The relation between anthocyanin degradation and pericarp discoloration in litchi has been recorded for cvs. Hong Huay (*3*) and Huaizhi (*36*); however, it is unclear whether these fruits were acid-treated. There was no correlation between pericarp discoloration and anthocyanin deterioration in the present study. The results could be due partly to the belief that visible pericarp discoloration is closely related to senescence-induced anthocyanin transformation rather than its degradation (*37*). Besides, the contradiction between fruit color and color pigments may be an artifact of the objective measurement system used since it may have been unable to account for the heterogeneity in coloration of nonacid-dipped fruit and the sampling system employed. Redness in stored litchi fruit (25 °C, 60% RH, and 48 h) was better correlated with pericarp pH than anthocyanin concentration (*38*). It is likely that changes in pH will have altered the stability, copigmentation, and spectra of the anthocyanins found in litchi fruit during storage. As a result and despite anthocyanins being responsible for red pigmentation, the relationship between anthocyanins and litchi pericarp color is not fully understood.

Chemometric Analysis. The PCA of litchi fruit clearly demonstrated the clustering of the samples on PC 1 and PC 2 (68 and 16% of the variance, respectively). Cv. Kom fruits were arranged away from cv. Mauritius fruits along PC 1 (**Figure 9**), indicating a different reaction of each cultivar. Fruits from cv. Mauritius kept at 5 °C and 95−100% RH (VPD = 0.000−0.084 kPa) were separated from those held at 13 °C and 80−90% RH (VPD = 0.137−0.274 kPa) treatments, respectively, along PC 1. Although cv. Kom fruit samples could not be differentiated along PC 1, the samples were grouped separately into 5 °C + 80−85% RH (VPD = 0.126−0.168 kPa), 5 °C + 90−100% RH (VPD = 0.000−0.084 kPa), 13 °C + 80−85% RH (VPD = 0.205−0.274 kPa), and 13 °C + 90−100% RH (VPD = 0.000−0.137 kPa) on PC 2. Respiration rate played the most important role in sample separation along PC 1, while aril glucose concentration was a key variable for PC 2 (data not shown). It is clear, therefore, that RH, temperature, and VPD affected not only senescence but also carbohydrate utilization through respiration.

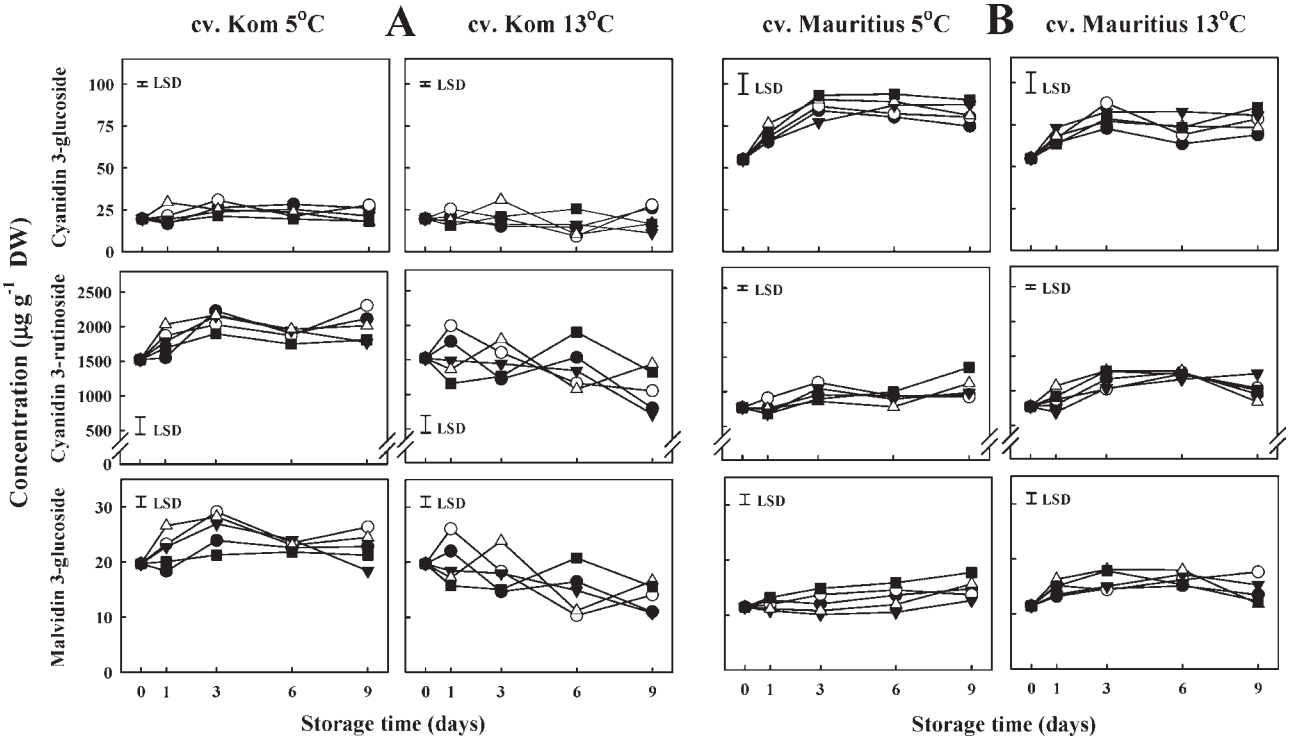

Figure 8. Anthocyanins in pericarp tissue of litchi cvs. Kom (**A**) and Mauritius (**B**) stored at 80 (●), 85 (○), 90 (▼), 95 (△), and 100% (■) RH at 5 or 13 °C during 9 days of storage (LSD, $P < 0.05$; each symbol $n = 15$).

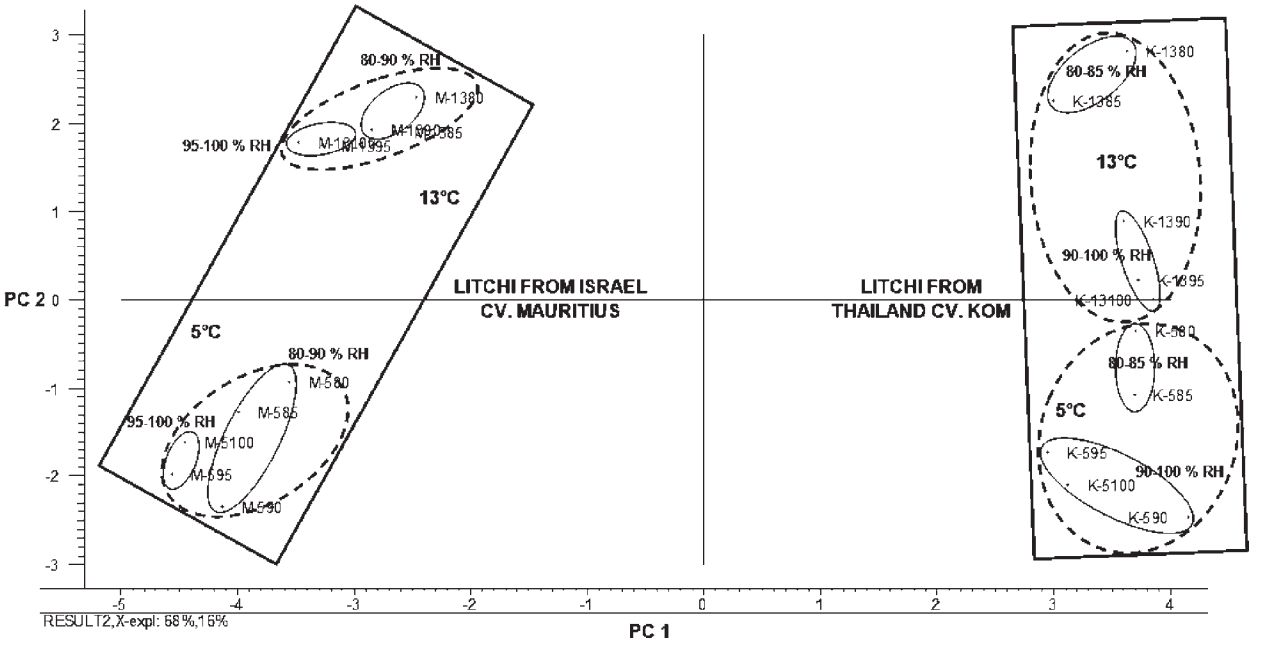

Figure 9. PCA of litchi fruit cvs. Kom and Mauritius. Clustering of 20 samples from stored fruit at 5 or 13 °C and 80, 85, 90, 95, and 100% RH demonstrated on the loading and score plot of PCA based on the similarities in spatial and temporal variation of weight loss, respiration rate, sugars, and acids in aril and pericarp and anthocyanins in pericarp. The outlines of the clusters have been added manually to aid interpretation.

Results suggested that low VPDs (high RH and low temperature) were important to maintain the quality of litchi cvs. Kom and Mauritius over 9 days of storage. Accompanying reduced weight loss and respiration rate and storage at 95−100% RH or 5 °C (VPD = 0.000−0.084 kPa) significantly controlled the reduction of aril and pericarp sugars and organic acids and retained anthocyanin concentrations. Recommendations are that storage conditions for litchi should not only be centered on maintaining the cool chain but should also consider controlling the VPD at less than 0.068 kPa to attain improved conservation of physiological and biochemical characteristics.

ACKNOWLEDGMENT

We thank Minor Weir and Willis Ltd. and Agrexco Ltd. (Dr. Anne-Do Bauchot) for supplying fruit. We thank Prof. Naresh Magan and Dr. Gemma A. Chope (Cranfield University; CU) for their advice and Allen Hilton and students at the Plant

Science Laboratory for their assistance. Balwinder Cook (CU) is especially thanked for help with HPLC analysis.

LITERATURE CITED

(1) Paull, R. E. Effect of temperature and relative humidity on fresh commodity quality. *Postharvest Biol. Technol.* **1999**, *15*, 263–277.

(2) Jiang, Y. M.; Fu, J. R. Postharvest browning of litchi fruit by water loss and its prevention by controlled atmosphere storage at high relative humidity. *LWT* **1999**, *32*, 278–283.

(3) Kaewchana, R.; Niyomlao, W.; Kanlayanarat, S. Relative humidity influences pericarp browning of litchi cv. Hong Huay. *Acta Hortic.* **2006**, *712*, 823–827.

(4) Pateraki, M.; Dekanea, A.; Mitchell, D.; Lydakis, D.; Magan, N. Efficacy of sulphur dioxide, controlled atmospheres and water availability on in vitro germination, growth and ochratoxin A production by strains of *Aspergillus carbonarius* from grapes and vine fruits. *Postharvest. Biol. Technol.* **2007**, *44*, 141–149.

(5) Thompson, A. K. *Controlled Atmosphere Storage of Fruits and Vegetables*; CABI: Oxon, United Kingdom, 1998.

(6) Terry, L. A.; Ilkenhans, T.; Poulston, S.; Rowsell, L.; Smith, A. W. J. Development of new palladium-promoted ethylene scavenger. *Postharvest Biol. Technol.* **2007**, *45*, 214–220.

(7) Terry, L. A.; Chope, G. A.; Giné Bordonaba, J. Effect of water deficit irrigation and inoculation with *Botrytis cinerea* on strawberry (*Fragaria* × *ananassa*) fruit quality. *J. Agric. Food Chem.* **2007**, *55*, 10812–10819.

(8) Giné Bordonaba, J.; Terry, L. A. Biochemical profiling and chemometric analysis of seventeen UK-grown black currant cultivars. *J. Agric. Food Chem.* **2008**, *56*, 7422–7430.

(9) Underhill, S. J. R.; Simons, D. H. Lychee (*Litchi chinensis* Sonn.) pericarp desiccation and the importance of postharvest microcracking. *Sci. Hortic.* **1993**, *54*, 287–294.

(10) Huang, X. M.; Wang, H. C.; Yuan, W. Q.; Lu, J. M.; Yin, J. H.; Luo, S.; Huang, H. B. A study of rapid senescence of detached litchi: Roles of water loss and calcium. *Postharvest Biol. Technol.* **2005**, *36*, 177–189.

(11) Kays, S. J.; Paull, R. E. *Postharvest Biology*; Exon Press: Georgia, 2004.

(12) Wills, R. B. H.; McGlasson, W. B.; Graham, D.; Joyce, D. C. Water loss and humidity. *Postharvest: An Introduction to the Physiology and Handling of Fruit, Vegetables and Ornamentals*; CABI: Oxon, United Kingdom, 2007; pp 67−82.

(13) Peng, Y. H.; Cheng, W. Effect of postharvest handling on fruit quality, mass loss and respiration rate of litchi. *Acta Hortic.* **2001**, *558*, 359–365.

(14) Chen, Y. Z.; Li, P.; Wang, Y. R.; Zeng, L. P. The effect of chilling temperature on respiration, electrolyte leakage and cold-storage life in litchi fruits. *Acta Hortic. Sin.* **1987**, *14*, 169–173.

(15) Zhang, D. L.; Quantick, P. C. Effect of low-temperature hardening on postharvest storage of litchi fruit. *Acta Hortic.* **2000**, *518*, 175–182.

(16) Batten, D. J. Maturity criteria for litchis (lychees). *Food Qual. Preference* **1989**, *1*, 149–155.

(17) Somboonkaew, N.; Terry, L. A. Physiological and biochemical profiles of imported litchi fruit under modified atmosphere packaging. *Postharvest Biol. Technol.* **2010**, *56*, 246–253.

(18) Chan, H. T.; Kwok, S. C. M.; Lee, C. W. Q. Sugar composition and invertase activity in lychee. *J. Food Sci.* **1975**, *40*, 772–774.

(19) Paull, R. E.; Chen, N. J. Effect of storage temperature and wrapping on quality characteristics of litchi fruit. *Sci. Hortic.* **1987**, *33*, 223–236.

(20) Shah, N. S.; Nath, N. Changes in qualities of minimally processed litchis: effect of antibrowning agents, osmo-vacuum drying and moderate vacuum packaging. *LWT* **2008**, *41*, 660–668.

(21) Yang, B.; Wang, J.; Zhao, M.; Liu, Y.; Wang, W.; Jiang, Y. Identification of polysaccharides from pericarp tissues of litchi (*Litchi chinensis* Sonn.) fruit in relation to their antioxidant activities. *Carbohydr. Res.* **2006**, *341*, 634–638.

(22) Shallenbuger, R. S. *Taste Chemistry*; Chapman and Hall: Glasgow, 1993.

(23) Bridle, P.; Timberlake, C. F. Anthocyanins as natural food colours: Selected aspects. *Food Chem.* **1997**, *58*, 103–109.

(24) Angyal, S. J. A short note on the epimerization of aldoses. *Carbohydr. Res.* **1997**, *300*, 279–281.

(25) Lafta, A. M.; Fugate, K. K. Dehydration accelerates respiration in postharvest sugarbeet roots. *Postharvest Biol. Technol.* **2009**, *54*, 32–37.

(26) Costantini, V.; Bellincontro, A.; De Santis, D.; Botondi, R.; Mencarelli, F. Metabolic changes of Malvasia grapes for wine production during postharvest drying. *J. Agric. Food. Chem.* **2006**, *54*, 3334–3340.

(27) Nagar, P. K. Physiological and biochemical studies during fruit ripening in litchi (*Litchi chinensis* Sonn.). *Postharvest Biol. Technol.* **1994**, *4*, 225–234.

(28) Lemmer, D.; Kruger, F. L. Factors influencing SO_2- Residues on commercially fumigated HLH Mauritius and McLean's Red litchi fruit. *S. Afr. Litchi Growers' Assoc. Yearbook* **2000**, *11*, 42–46.

(29) Joubert, A. J. Litchi. In *CRC Handbook of Fruit Set and Development*; Moselise, S. P., Ed.; CRC Press: Boca Raton, FL, 1986; pp 233−246.

(30) Caro, Y.; Joas, J. Postharvest control of litchi pericarp browning (cv. Kwai Mi) by combined treatments of chitosan and organic acids: II. Effect of the initial water content of pericarp. *Postharvest Biol. Technol.* **2005**, *38*, 137–144.

(31) Joas, J.; Caro, Y.; Ducamp, M. N.; Reynes, M. Postharvest control of pericarp browning of litchi fruit (*Litchi chinensis* Sonn cv. Kwaï Mi) by treatment with chitosan and organic acids: I. Effect of pH and pericarp dehydration. *Postharvest Biol. Technol.* **2005**, *38*, 128–136.

(32) Zheng, X.; Tian, S. Effect of oxalic acid on control of postharvest browning of litchi fruit. *Food Chem.* **2006**, *96*, 519–523.

(33) Jiang, Y. M.; Wang, Y.; Song, L.; Liu, H.; Lichter, A.; Kerdchoechuen, O.; Joyce, D. C.; Shi, J. Postharvest characteristics and handling of litchi fruit—An overview. *Aust. J. Exp. Agric.* **2006**, *46*, 1541–1556.

(34) Huang, S.; Hart, H.; Lee, H.; Wicker, L. Enzymatic and color changes during postharvest storage of lychee fruit. *J. Food Sci.* **1990**, *55*, 1762–1763.

(35) Jurd, L. Reactions involved in sulphite bleaching of anthocyanins. *J. Food Sci.* **1964**, *29*, 16–19.

(36) Zhang, Z.; Pang, X.; Ji, Z.; Jiang, Y. Role of anthocyanin degradation in litchi pericarp browning. *Food Chem.* **2001**, *75*, 217–221.

(37) Underhill, S. J. R.; Critchley, C.; Simons, D. H. Postharvest pericarp browning of lychee (*Litchi chinensis* Sonn.) fruit. *Acta Hortic.* **1992**, *321*, 718–725.

(38) Underhill, S. J. R.; Critchley, C. Anthocyanin decolorisation and its role in lychee pericarp browning. *Aust. J. Exp. Agric.* **1994**, *34*, 115–122.

Received for review January 4, 2010. Revised manuscript received March 25, 2010. Accepted April 20, 2010. We are grateful to the Agricultural Research Development Agency (ARDA), Royal Thai Government, for financial support.