Reversibility of lychee pericarp red color in relation to pericarp pH, activity of polyphenol oxidase, and particle size of brown pigment

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Reversibility of lychee pericarp red color in relation to pericarp pH, activity of polyphenol oxidase, and particle size of brown pigment.

Abstract — Introduction. The peel of lychee fruit turns from its attractive bright red color to a dull brown color when the fruit starts to age. This study reports how the color change of lychee pericarp from red to brown is associated with several factors and may be reversed depending on its pH, anthocyanin content and brown pigments. **Materials and methods**. After transport by air transit from China to Canada, arriving 4 d after harvest, the anthocyanin, brown pigment concentrations and the pH values in the lychee (*Litchi chinensis* Sonn.) pericarp were determined over a period of 5 d at 25 °C and 65% RH. The macromolecular sizes of the brown pigment extract were measured in solutions between a pH of 4 and 6. **Results and discussion**. The acidity in the lychee pericarp decreased from a pH of 4.3 to 5.3, at the end of the 5-day period. The anthocyanins in the lychee pericarp decreased and the brown pigment increased. Polyphenol oxidase in the pericarp was active when its pH was between 4.1 and 4.6, and became less active when its pH was above 4.6. The diameter of the brown pigment molecules in the solutions increased when the pH of the solutions was increased and maintained for 5 d. The anthocyanins returned to their original redness and concentrations if they were placed in solutions with a pH value ranging between 4 and 6 for 10 min or they were placed in solutions with a pH value of 4 for 5 d. However, the anthocyanins did not return to their original redness and concentration if they were placed in solutions with a pH value ranging between 5 and 6 for 5 d. **Conclusion**. This study suggests that the bright red color of lychee peel could be maintained if its pericarp pH could be maintained at a pH of 4. If the pericarp pH is above 4, the reversibility of its bright red color from brown pigments is dependent on storage time.

Canada / *Litchi chinensis* / fruits / coloration / pigments / anthocyanins / browning

Réversibilité de la couleur rouge du péricarpe de litchi en fonction du pH, de l'activité polyphénol oxydase et de la taille des particules de pigments bruns.

Résumé — Introduction. La peau du litchi tourne d'une belle couleur rouge vif à une couleur brune mate quand le fruit commence à vieillir. Notre étude montre que, dans le péricarpe de litchi, le changement de couleur du rouge au brun est associé à plusieurs facteurs et qu'il peut être réversible selon le pH et la teneur en anthocyanes et en pigments bruns de l'écorce du fruit. **Matériel et méthode**. Quatre jours après la récolte du fruit transporté par avion de Chine au Canada, les concentrations en anthocyanes et en pigments bruns et les valeurs de pH du péricarpe de litchis (*Litchi chinensis* Sonn.) entreposés à 25 °C et 65% d'humidité relative ont été suivies pendant 5 j. La taille macromoléculaire des pigments bruns ont été mesurées dans des solutions de pH 4 à 6. **Résultats et discussion**. En 5 j de stockage, le pH dans le péricarpe de litchi a diminué de 4,3 à 5,3. Dans le même temps, les anthocyanes ont diminué et la teneur en pigments bruns a augmenté. La polyphénol oxydase du péricarpe a été active pour un pH compris entre 4,1 et 4,6 et elle est devenue moins active lorsque le pH de l'épicarpe a dépassé la valeur de 4,6. Le diamètre des molécules de pigments bruns des extraits analysés a augmenté quand le pH des solutions augmentait et était maintenu pendant 5 j. Les anthocyanes ont récupéré leur couleur rouge et leur concentration initiale lorsqu'elles ont été placées dans des solutions avec un pH s'échelonnant de 4 à 6 pendant 10 min, ou avec un pH de 4 pendant 5 j. Ce même phénomène n'a pas été observé en solutions maintenues à pH 5 ou 6 pendant 5 j. **Conclusion**. Cette étude suggère que la couleur rouge vif de la peau de litchi pourrait être conservée si le pH de son péricarpe pouvait être maintenu à 4. Au-dessus de cette valeur, la réversibilité de la couleur rouge à partir des pigments bruns dépend du temps d'entreposage.

Canada / *Litchi chinensis* / fruits / coloration / pigment / anthocyane / brunissement

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Received 7 January 2003
Accepted 27 June 2003

Fruits, 2004, vol. 59, p. 17–23

DOI: 10.1051/fruits:2004002

***RESUMEN ESPAÑOL**, p. 23*

1. Introduction

Lychee (*Litchi chinensis* Sonn.) fruit is an exotic fruit, which originates from Southern China. The freshly picked fruit has a bright red pericarp (peel) and sweet white pulp. Lychee fruit normally have 3 d of shelf life at 25 °C. The pH of lychee pericarp increases during storage [1–3]. When the fruit starts to age, the peel of lychee fruit turns from its attractive bright red color to a dull brown color [2].

The discoloration process of the red anthocyanins in lychee is pH-dependent. The acidification of lychee pericarp modifies the anthocyanins of lychee, thereby inhibiting the degradation of the pigments [2]. Anthocyanin pigments from lychee change from highly colored cations in a strong acid media to a colorless carbinol base as the pH of the media increases [4, 5]. The stability of the red anthocyanins in lychee is a direct function of pH [6, 7]. Anthocyanins are also very unstable and lose their red appearance over time [8–11]. Cyanidin-3-glucoside, which has a maximum absorbance at 515 nm, is the major monomeric red anthocyanin in lychee [12–14].

The color of the anthocyanin pigments is changeable between the red color at a pH of 3 and the dull brownish color at a pH of 4.5 [15]. In addition, anthocyanins in the lychee pericarp might be recovered from their brown pigment product upon low pH acidification with 1 M HCl [2].

The browning of lychee pericarp is associated with enhanced polyphenol oxidase (PPO) activity [9, 11, 16, 17] and the oxidation and polymerization of the degraded red anthocyanins in lychee pericarp [18]. The acidification of lychee pericarp has been known to greatly reduce the PPO activity [10].

The objectives of our study were: (1) to examine the change in pH, anthocyanin, and brown pigments in lychee pericarp at 25 °C for 5 d; (2) to examine the change in size of the brown pigments at various pH levels, and (3) to study the reversibility of anthocyanin redness at various pH levels over a 5-d period.

2. Materials and methods

2.1. Lychee fruit

'Yook Ho Pow' lychee fruits (also called 'San Yue Hong' in Mandarin) were picked in a commercial orchard in China, and they were shipped immediately in sealed polyfoam boxes filled with ice bags by air cargo transport, arriving 4 d after harvest in the laboratories at the University of Toronto and the University of Guelph. The fruits were then sorted visually for uniform pericarp color and size.

2.2. Pericarp color

Fifteen lychee fruits, five in each of three replicates, were used to examine their pericarp color at 25 °C and 65% relative humidity (RH) over a period of 5 d. The pericarp redness was graded visually [9] as grade 1: < 10% red area; grade 2: 25% red area; grade 3: 50% red area; grade 4: 75% red area; and grade 5: > 90% red area.

2.3. Pericarp pH

Fifteen lychee fruits, five in each of three replicates, were used to prepare samples for the measurement of pericarp pH. From five fruits, 6 g of pericarp tissue were peeled and finely sliced, and washed in distilled water to remove excessive juice. The sliced pericarp tissues were homogenized in 50 mL of distilled water for 1 min with a homogenizer (Waring–7011, CT, USA) [2, 3]. While stirring the homogenate, the pH was measured using a pH meter (Model Accumet-610, Fisher Scientific, PA, USA) with a combination pH probe (Orion 91–05, MA, USA).

2.4. Anthocyanin

Fifteen lychee fruits, five in each of three replicates, were used to prepare anthocyanin samples. Pericarp (5 g) from five lychee fruits were finely sliced, extracted and well stirred with 50 mL of 1% weight HCl for 2 h at a pH of 1 [15]. The extract was centrifuged at 4500 rpm for 20 min and filtered

through Whatman #1 paper. The absorbance reading of the anthocyanins was then measured spectrophotometrically at 515 nm [2].

2.5. Brown pigments

Pericarp browning was measured spectrophotometrically according to Jiang *et al.* [10]: 5 g of pericarp tissue from five peeled fruits in each of three replicates were finely sliced, ground and extracted with 17 mL of 60% methanol (v/v) in a 0.1 M sodium phosphate buffer (pH 6.8) and 0.5 g of polyvinylpyrrolidone (PVP). The extract was centrifuged at 4500 rpm for 20 min and filtered through Whatman # 1 filter paper, and the supernatant collected and diluted to a 1:4 ratio with a phosphate buffer and measured spectrophotometrically at 410 nm (Model 8452, Hewlett Packard, CA, USA) [3].

2.6. Polyphenol oxidase

Fifteen lychee fruits, five in each of three replicates, were used to prepare PPO activity samples. Peeled and finely sliced lychee pericarp (4 g) from five fruits was frozen with liquid nitrogen and stored overnight at –20 °C. The frozen pericarp was powdered the next day and homogenized with 30 mL of a 0.1 M sodium phosphate buffer at a pH of 6.8. The supernatant was collected after 20 min of centrifugation at 4500 rpm. The solution was filtered through Whatman #1 filter paper and the PPO activity was assayed using 4-methycatechol. One unit of PPO activity was defined as a change in 0.001 absorbance units per min at 410 nm at 25 °C under a pH of 6.8 [11].

2.7. Brown pigment size analysis

One milliliter of anthocyanin extract solution was mixed with 9 mL of buffer solutions to achieve a pH value of between 4 and 6. Each mixture was transferred into a cuvet and the size of the solute particles in the solution was analyzed optically by using a light scattering particle analyzer (Model 90 plus, Brookhaven Instrument, NY, USA). The anthocyanin extracts in the solutions were allowed to degrade and form brown pigments for 5 d before any measurements were conducted. The particle size distribution of lychee pigment under different acidic conditions (i.e., pH of 4, 5 and 6 adjusted using phosphate buffer/ potassium hydroxide) was analyzed. The detection limit for the device was between 2 nm and 10 nm. The data were analyzed using Particle Sizing software, ver. 2.31 (Brookhaven Instrument, NY, USA).

2.8. Reversibility of anthocyanin degradation from its brown pigment

Fifteen lychee fruits, five in each of three replicates, were used to prepare samples for determining anthocyanin reversibility. Three milliliters of anthocyanin extract from five fruits were mixed with buffer solutions (phosphate buffer/ potassium hydroxide) to achieve a combined volume of 30 mL with an endpoint pH of 4, 5 or 6. Each solution was mixed thoroughly for either 10 min or 5 d, followed by a pH adjustment to 1 using HCl. Spectrophotometric measurements at 410 nm (for brown) and 515 nm (for red) were recorded. The visual color of each extract solution at different pH values was also determined and recorded before and after their pH adjustment.

2.9. Statistical analysis

SPSS software (SPSS® Inc., IL, USA), release 7.5.1, was used for linear and nonlinear regression analyses in all experimental runs.

3. Results and discussion

3.1. Pericarp redness

After air transit from China to Canada, arriving 4 d after harvest, the pericarp redness of lychees then stored at 25 °C and 65% RH decreased rapidly during the first day of shelf life (*figure 1*). Jiang and Fu [3] and Underhill and Critchley [2] also reported similar results from storage at 25 °C and 60% RH. However, our study showed that over

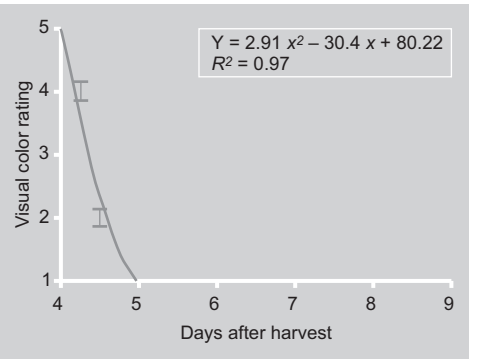

Figure 1.
Changes in red color in the pericarp of lychee stored at 25 °C and 65% RH for 5 d in the laboratory, after transport by air transit from China to Canada, arriving 4 d after harvest. Each data point and its corresponding vertical bar represent the mean and standard error of three replicates. Regression equation is based on data points between day 0 and day 1 (Visual color scale: 1: < 10% red area; 2: 25% red area; 3: 50% red area; 4: 75% red area; 5: > 90% red area).

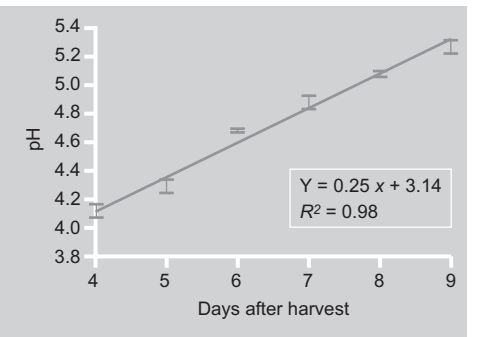

Figure 2.
Changes in the pH values of the pericarp of lychee stored at 25 °C and 65% RH for 5 d in the laboratory, after transport by air transit from China to Canada, arriving 4 d after harvest. Each data point and its corresponding vertical bar represent the mean and standard error of three replicates.

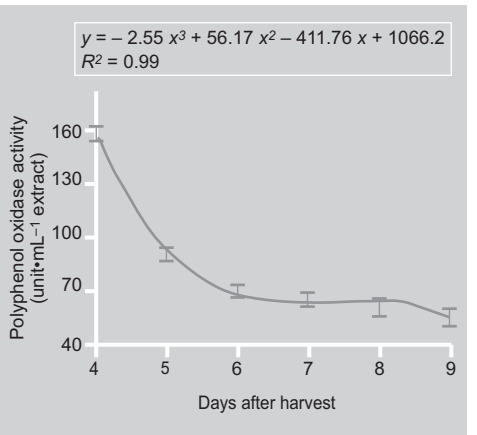

Figure 3.
Changes in polyphenol oxidase activity in the pericarp of lychee stored at 25 °C and 65% RH for 5 d in the laboratory, after transport by air transit from China to Canada, arriving 4 d after harvest. Each data point and its corresponding vertical bar represent the mean and standard error of three replicates.

50% of the original pericarp redness disappeared within 0.5 d as compared with 3 d [3] and 1.5 d [2]. We used 'Yook Ho Pow' lychee as compared with 'Wai Chee' lychee [3] and 'Bengal' lychee [2]. Lychee fruit samples were transported to our laboratories in approximately 4 d as compared to within 1 h with Jiang and Fu [3] and Underhill and Critchley [2]. We realized the 4-d delay before the initiation of our experiments could limit the interpretation and application of our results. However, a 4-d delay of a postharvest treatment is very likely in a commercial operation if lychee can be ice-cooled and stored in cold storage (at approximately 5 °C) immediately after its picking.

3.2. Effect of pH on PPO activity

After air transit from China to Canada, arriving 4 d after harvest, the pH value of the pericarp of lychees then stored at 25 °C and 65% RH changed from 4 to 5.3 during the 5-d period (*figure 2*). The PPO activity declined from 160 units·mL^{-1} of extract to 80 units·mL^{-1} during the first day and then remained essentially at the same level after the second day (*figure 3*). These results show that the PPO in the pericarp was active when its pH was between 4.1 and 4.3 during the first day, and became less active after that, when its pH was between 4.3 and 5.3. This may suggest that the senescence, the desiccation, and the pH of the pericarp might interactively affect the PPO activity in the pericarp. Jiang [19] reported that the optimum pH to obtain the maximum PPO activity was 6.8 with 4-methylcatechol, and the relative activity of PPO increased from 0% to approximately 20% when the pH of the pericarp changed from 4.0 to 5.0. Our results show that, in natural conditions, PPO activity could be affected by many factors and not by the pH of the pericarp alone.

3.3. Anthocyanin and brown pigment

After air transit from China to Canada, arriving 4 d after harvest, anthocyanins in the pericarp of lychees then stored at 25 °C and 65% RH decreased significantly during the first 3 d (*figure 4*). However, the lychee pericarp lost its red color in 1 d (*figure 1*). In other words, the lychee pericarp lost its visual red color much faster than the loss of its anthocyanin content. Underhill and Critchley [2] also reported that visual color and Hunter *a* values were not consistent with the total anthocyanin concentration.

Brown pigment increased during the 5 d (*figure 5*). The absorbance of brown pigment at 410 nm almost doubled from day 1 to day 2. The presence of brown pigment might mask the red color that could have been revealed from the presence of anthocyanins, therefore showing the brown pericarp color after the first day.

Jiang [19] found that lychee PPO showed activity when the pH of the pericarp was above 4.0. In addition, we would expect that lychee could rapidly lose water while stored at 25 °C and 65% RH for 5 d. Browning of the pericarp could be enhanced due to the increase in water loss.

3.4. Brown pigment size

The mean diameter of the brown pigments analyzed under different acidic conditions, after 1 mL anthocyanin extract solution was mixed with 9 mL of buffer solutions to achieve a pH value of between 4 and 6, was less than 2 nm in a pH 4.5 solution; however, it increased to approximately 1 µm in a pH 6 solution (*figure 6*). While the pH of the lychee pericarp increased from 4.1 to 5.5 during the 5 d (*figure 2*), the size of brown pigment also increased from less than 2 nm to approximately 400 nm. This change in brown pigment size might have contributed to the change from red peel color to brown peel color.

3.5. Reversibility from brown color to red

The absorbance at 515 nm of samples placed in solutions at a pH of 4, 5, and 6 decreased after 10 min. When the pH of these solutions was adjusted to a pH of 1, the absorbance at 515 nm increased to a level similar to that obtained from samples maintained at a pH of 1 all the time (i.e., the control sample) (*figure 7*). In the meantime, the absorbance at 410 nm was essentially unaffected by the pH change, remaining at about the same level as the control sample. However, when the exposure time was lengthened from 10 min to 5 d, only extract solutions exposed to a pH of 4 had similar absorbance levels at 515 nm as the control sample after the pH adjustment (*figure 8*). The extract solutions exposed to a pH of 5 or 6 for 5 d showed a loss in anthocyanin absorbance and an increase in brown pigment absorbance after the adjustment of pH to 1.

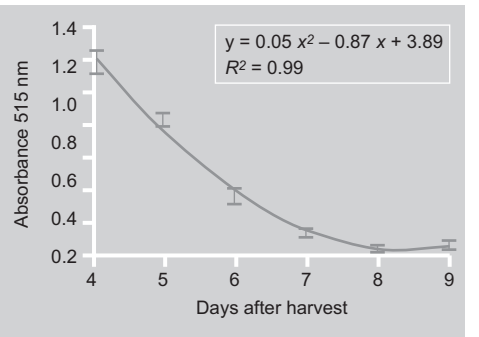

Figure 4.
Changes in anthocyanin in the pericarp of lychee fruits stored at 25 °C and 65% RH for 5 d in the laboratory, after transport by air transit from China to Canada, arriving 4 d after harvest. Each data point and its corresponding vertical bar represent the mean and standard error of three replicates.

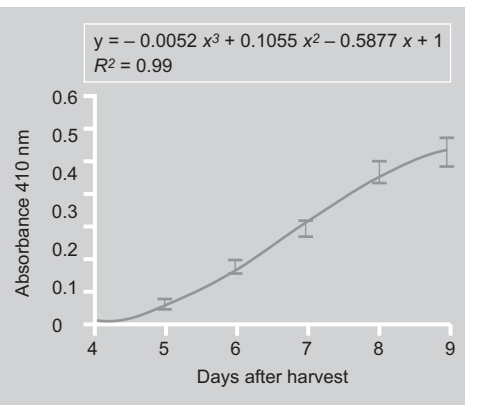

Figure 5.
Changes in brown pigment content of lychee fruits stored at 25 °C and 65% RH for 5 d in the laboratory, after transport by air transit from China to Canada, arriving 4 d after harvest. Each data point and its corresponding vertical bar represent the mean and standard error of three replicates.

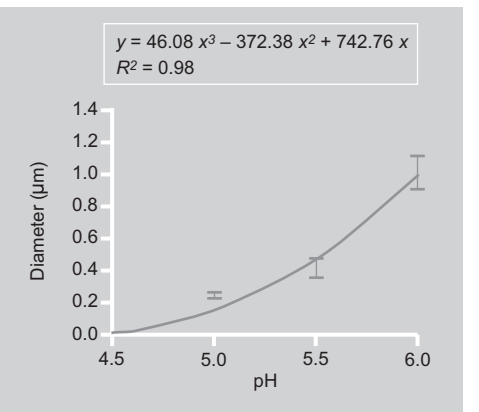

Figure 6.
Mean diameter of the brown pigment of lychee fruits after being in solutions of various pH values for 5 d. Each data point and its corresponding vertical bar represent the mean and standard error of three replicates.

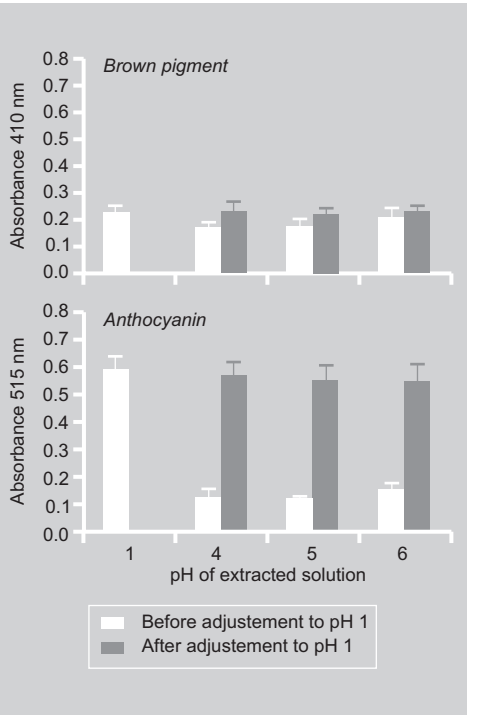

Figure 7.
Anthocyanin (515 nm) and brown pigment content (410 nm) of lychee fruits before and after placement of samples in various pH solutions for 10 min, followed by pH adjustment to 1. Each data point and its corresponding vertical bar represent the mean and standard error of three replicates.

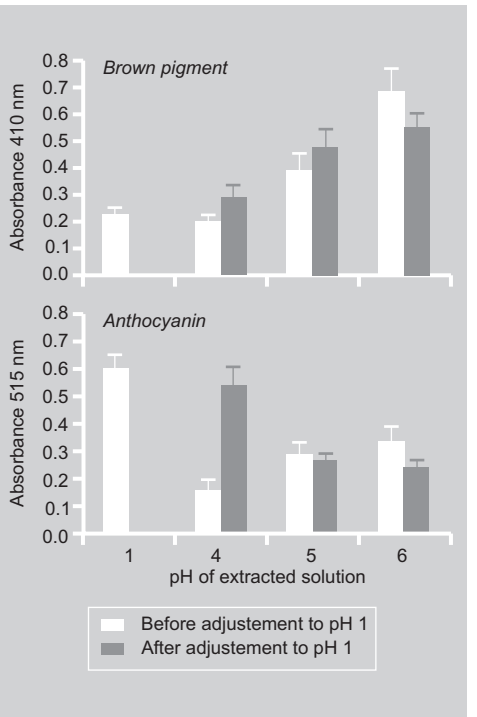

Figure 8.
Anthocyanin (515 nm) and brown pigment content (410 nm) of lychee fruits before and after placement of samples in various pH solutions for 5 d, followed by pH adjustment to 1. Each data point and its corresponding vertical bar represent the mean and standard error of three replicates.

4. Conclusion

Our study suggests that the bright red color of lychee could be obtained from acidification (to pH 1) of the fruit, if the pH of the pericarp was maintained at a pH of 4 for 5 d or less. The effectiveness of acidification could be decreased significantly if the pH of the lychee pericarp reached 5 or 6 for 5 d. This could be due to the larger size of the brown pigment developed in such pH conditions, limiting the reversibility of brown peel color to red peel color. Our results also suggest that the PPO-induced brown peel color, while the pericarp pH was less than 4, could be reversed by acidification.

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Reversibilidad del color rojo del pericarpio de litchi en función del pH, de la actividad polifenol oxidasa y del tamaño de las partículas de pigmentos pardos.

Resumen — Introducción. La piel del litchi evoluciona de una bonita capa de color rojo vivo a un color pardo mate cuando el fruto comienza a envejecer. Nuestro estudio muestra que, en el pericarpio del litchi, el cambio de color del rojo al pardo está asociado a varios factores y que puede ser reversible según el pH y el contenido de antocianos y de pigmentos pardos de la corteza del fruto. **Material y métodos**. Cuatro días después de la recogida del fruto, transportado en avión desde China a Canadá, se efectuó el seguimiento, durante 5 d, de las concentraciones de antocianos y pigmentos pardos así como los valores de pH del pericarpio de los litchis (*Litchi chinensis* Sonn.) almacenados a 25 °C y con un 65% de humedad relativa. Se midieron los tamaños macromoleculares de los pigmentos pardos en soluciones de pH 4 a 6. **Resultados y discusión**. En 5 d de almacenamiento, el pH en el pericarpio de litchi disminuyó de 4,3 a 5,3. Al mismo tiempo, los antocianos disminuyeron y el contenido de pigmentos pardos aumentó. La polifenol oxidasa del pericarpio fue activa con un pH comprendido entre 4,1 y 4,6 y se volvió menos activa cuando el pH del epicarpio superó el valor de 4,6. El diámetro de las moléculas de pigmentos pardos de los extractos analizados aumentó cuando el pH de las soluciones aumentaba y se mantenía durante 5 d. Los antocianos recuperaron su color rojo y su concentración inicial cuando se introdujeron en soluciones con un pH comprendido entre 4 a 6 durante 10 min, o con un pH de 4 durante 5 d. Este fenómeno no se observó en soluciones mantenidas con un pH 5 a 6 durante 5 d. **Conclusión**. Este estudio apunta que el color rojo intenso de la piel de litchi podría conservarse si el pH de su pericarpio se mantuviera a 4. Por encima de este valor, la reversibilidad del color rojo a partir de los pigmentos pardos depende del tiempo de almacenamiento.

Canadá / *Litchi chinensis* / frutas / coloración / pigmentos / antocianinas / oscurecimiento