Available online at www.sciencedirect.com
SCIENCE DIRECT®
Food Chemistry 91 (2005) 659–663
Food Chemistry
www.elsevier.com/locate/foodchem

Effects of O_2 and CO_2 concentrations on physiology and quality of litchi fruit in storage

Shi-Ping Tian *, Bo-Qiang Li, Yong Xu

Key Laboratory of Photosynthesis and Environmental Molecular Physiology, Institute of Botany, The Chinese Academy of Sciences, 20 Nanxincun, Xiangshan, Haidian District, Beijing 100093, China

Received 12 April 2004; received in revised form 25 June 2004; accepted 25 June 2004

Abstract

Litchi (*Litchi chinensis* Sonn. cv Heiye) fruit were stored in air, modified atmosphere packaging (MAP) and controlled atmospheres (CA) at 3 °C to determine the effects of different O_2 and CO_2 atmospheres on physiology, quality and decay during the storage periods. The results indicated that CA conditions were more effective in reducing total phenol content, delaying anthocyanidin decomposition, preventing pericarp browning, and decreasing fruit decay in comparison with MAP treatment. Polyphenol oxidase (PPO), peroxidase (POD), anthocyanin and total phenols were involved in cellular browning. High O_2 treatment significantly limited ethanol production of litchi flesh in the early period of storage. The fruit stored in CA conditions for 42 days maintained good quality without any off-flavour.

Keywords: Litchi fruit (*Litchi chinensis* Sonn. cv Heiye); Physiological properties; Quality attributes; O_2 and CO_2 concentration; Storage

1. Introduction

Litchi (*Litchi chinensis* Sonn.), native to southern China, is adapted to the warm subtropics, cropping best in regions with brief cool dry frost-free winters and long hot summers with high rainfall and humidity (Menzel & Simpson, 1987). The fruit easily loses its commercial value after harvest due to pericarp browning, quality deterioration and decay (Ray, 1998). Browning of litchi pericarp is still considered to be a major problem affecting its market value. During the past two decades, a considerable amount of research work has been carried out on litchi fruit, including analyzing the role of pigments, hormones and some other closely related factors responsible for postharvest browning of litchi fruit (Underhill & Critchley, 1994; Zhang & Quantick, 1997), selecting suitable storage temperature and postharvest chemical treatments to prevent browning, control decay and extend storage life (Paull & Chen, 1987; Ray, 1998; Zauberman et al., 1991).

Browning of litchi pericarp was thought to be due to degradation of anthocyanidin by polyphenol oxidase (PPO) and peroxidase (POD) (Chen & Wang, 1989; Nip, 1988) and was primarily the result of PPO activity degrading the anthocyanins and producing brown-coloured by-products (Huang, Hart, Lee, & Wicker, 1990). Since PPO can not oxidize monophenols on *o*-diphenols (Mayer & Harel, 1978), the POD was also suggested to play an important role in the browning of litchi pericarp (Gong & Tian, 2002). In general, sulfur dioxide treatments have been widely used to control saprophytic surface fungi and prevent peel browning of litchi fruit (Huang et al., 1990; Underhill, Critchley, & Simons, 1992).

Modified atmosphere packaging (MAP) has been considered to be beneficial to maintain high humidity,

* Corresponding author. Tel.: +86 10 6259 1431; fax: +86 10 8259 4675.
E-mail address: tsp@ibcas.ac.cn (S.-P. Tian).

0308-8146/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2004.06.038

essential for prevention of water loss and browning of litchi pericarp (Kader, 1994; Pesis et al., 2002). Controlled atmospheres (CA), with low-O_2 and high-CO_2, have been successfully used to reduce decay, maintain quality and extend storage life in many fruits (Beaudry, 1999). In recent years, high O_2 treatment was considered to be effective in inhibiting enzymic discoloration, preventing anaerobic fermentation reactions, and limiting aerobic and anaerobic microbial growth (Day, 1996). In the previous study, we found that CA conditions could effectively extend storage life of longan fruit; particularly, CA with 70% O_2 concentration significantly inhibited peel browning (Tian, Xu, Jiang, & Gong, 2002). However, there are no published data on the effects of CA with high-O_2 or low-O_2 and high-CO_2 atmospheres on physiology, metabolism and quality of litchi fruit.

The objective of this study was to investigate the effects of different storage conditions, such as MAP, CA with high-O_2 or low-O_2 and high-CO_2 atmospheres, on physiological properties, quality attributes and storability of litchi fruit, and to evaluate the relationship between pericarp browning and activities of PPO and POD, as well as contents of total phenol and anthocyanidin in pericarp of litchi fruit.

2. Materials and methods

2.1. Fruit and storage conditions

Litchi fruit were harvested from a commercial orchard in Maoming, Guangdong province, China. The fruit were precooled immediately at 5 °C after harvest, sorted without wounded fruit, put in the foam-boxes with ice and sealed, then transported to Beijing by air (about 3–5 h). The storage conditions included the following: MAP, 0.03 mm thick polyethylene film bag (25×35 mm for 1 kg fruit, 15–19% O_2 + 2–4% CO_2); CA-I, 5% O_2 + 5% CO_2; CA-II, fruit were stored in the high oxygen concentration atmosphere (70% O_2 + 0% CO_2) for the first week, then kept in 5% O_2 + 5% CO_2, and at room temperature in air as the control.

CA cabinets ($105 \times 55 \times 100$ cm^3), with CO_2 and ethylene absorbers, were linked with an atmosphere analyzer (FC-701, Italy). Initial O_2 and CO_2 levels in the cabinets were established by a flow-through system, mixing N_2 (100%) and O_2 (99.5%), or N_2 and CO_2 via pressure regulators, then automatically controlled and regulated by the analyzer. The concentrations of O_2 and CO_2 in the MAP bags were measured by an atmosphere analyzer (CYES-II, Shanghai, China) each 14 days during the experimental periods. The fruit were stored in CA conditions and MAP at 3 °C with approximately 95% relative humidity (RH), and in air as the control. There were 150 kg fruit in each CA cabinet, 50 kg of fruit in MAP and 30 kg of fruit in air, respectively, with three replications. According to the treatments, about 5 kg of fruits were used for each analysis after the specified intervals of 14 days.

2.2. Measurements of browning and decay

Peel browning severity was determined as: 1 = no browning; 2 = less than 1/4 browning; 3 = less than 1/4–1/2 browning; 4 = more than 1/2 browning. The browning index was calculated by the following formula:

$$\text{Browing Index} = \frac{\Sigma(\text{Browning level}) \times \text{number of fruit at the browning level}}{\text{Total number of fruit in the treatment}} \times 100\%.$$

In each treatment, 30 fruits with three replications were assessed for every 14 days to determine browning severity and decay rate.

2.3. Determination of ethanol and soluble solids contents

Ethanol content in litchi flesh was determined by headspace gas chromatography, as described by Tian, Folchi, Pratella, and Bertolini (1996). A 0.5 ml sample of the head space gas was removed by syringe and injected into the gas chromatograph equipped with a flame ionization detector (FID) and glass column (2 mm × 4 m). The experimental conditions were: 85 °C oven temperature, 130 °C injector temperature, 250 °C detector temperature. The components were identified individually by comparing retention times against standards, concentrations being determined by a regression equation calculated on four samples of standard concentrations.

Soluble solids content (SSC) in flesh was determined using an Abbe refractometer (10481 S/N, USA). There were three replicates for each analysis per treatment.

2.4. Determination of PPO and POD activities

Fruit skins (5 g) from 10 fruit were ground with 40 ml of 0.2 M sodium phosphate buffer (pH 6.4) and homogenised using a Kinematica tissue grinder (Crl-6010, Kriens-LU, Switzerland) at 4 °C. After filtration of the homogenate through one layer of cheesecloth, the filtrate was centrifuged at 12,000g for 30 min. PPO and POD activities were measured at 25 °C and determined

by monitoring the rate of dopachrome formation at 398 and 460 nm, respectively, using an UV-160 spectrophotometer (Shimadzu, Japan) according to the methodology described by Galeazzi, Sgarbieri, and Costantinides (1981), and Tian et al. (2002). Protein content was determined according to Bradford (1976) using the Bio-Rad protein assay kit with bovine serum albumin (Sigma) as standard. One unit of enzymatic activities was defined as an increase in one absorbance unit per minute at 25 °C. There were three replicates for each analysis per treatment.

2.5. Assays of contents of total phenol and anthocyanins

Contents of anthocyanins were measured with a spectrophotometer (UV-160 Shimadzu, Japan) by the method of Proctor (1974). A 2 g portion of litchi peel was cut from 10 fruits, homogenized in 50 ml of 0.1% HCl in methanol at low speed for 2 min, and extracted for 20 h at room temperature. Absorption of the extracts was measured at 530, 620 and 650 nm. The anthocyanin absorbance of the extracts was determined by means of the formula: $OD = (A_{530}-A_{620})-0.1\ (A_{650}-A_{620})$ (Lee & Wicker, 1991). One unit of anthocyanin content was expressed as a change of 0.1 OD (unit $\times 10^3$/g fw).

Contents of total phenol in the pericarp of litchi fruit were determined according to the method described by Pirie and Mullins (1976). Litchi peels (4 g) were extracted with 1% HCl–methanol (40 ml), then homogenized and filtered. Absorption of the extracts was measured at 492 nm for total phenol, and calculated from a standard curve made with gallic acid.

2.6. Statistical analysis

All storage treatments were done with three replications, and the experiment was carried out over two years. Results were processed by analysis of variance as a one-factor general linear model procedure (ANOVA). The treatment means were separated using the least significant difference method. Differences at $P = 0.05$ were considered to be significant.

3. Results

3.1. Changes of browning and decay

The results indicated that the fruit kept in MAP showed a more rapid browning than those in CA conditions ($P = 0.05$). There was no significant difference in preventing peel colour from browning between CA-I and CA-II (Fig. 1(a) and (b)).

Fruit decay rates gradually increased with storage time (Fig. 1(c)). The fruit stored in MAP had a more rapid increase in disease incidence compared to CA

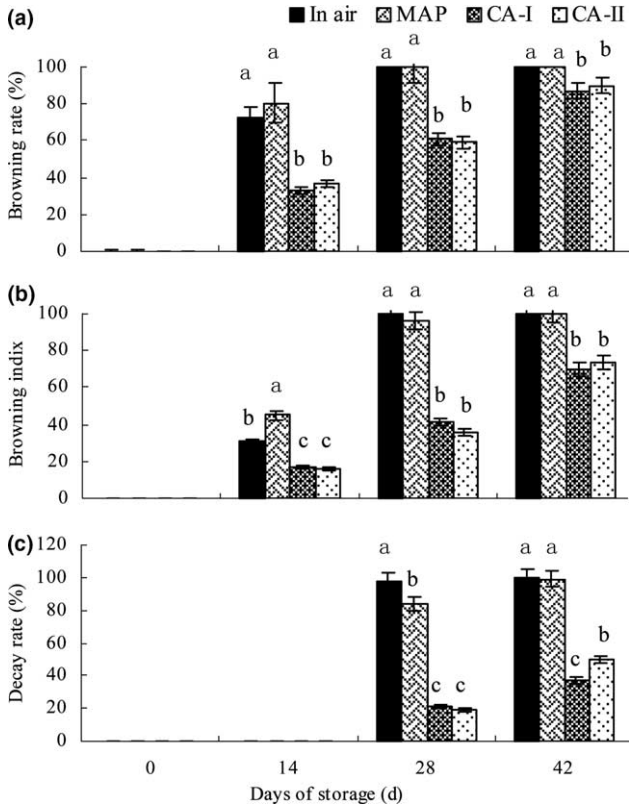

Fig. 1. Effects of different storage conditions on browning rate (a); index (b); decay (c) of litchi fruit. Data are of the means of three replicates for each treatment. In the same time the different letter represented significant difference according to LSD test ($P = 0.05$).

treatments ($P = 0.05$). The pathogens causing litchi fruit decay in storage were mainly *Peronophythora litchi* and *Geotrichum candidum*.

3.2. Changes of ethanol contents and SSC

CA-I, with 5% O_2 + 5% CO_2, significantly stimulated ethanol content of litchi flesh as compared to MAP and CA-II ($P = 0.05$). In addition, litchi fruits stored in CA-II conditions, where the fruits were kept in 70% O_2 concentration for one week at first, showed a significantly lower ethanol production than CA-I directly stored in CA, with 5% O_2 + 5% CO_2 in 14 days of storage (Fig. 2(a)).

CA conditions were more beneficial for maintaining SSC than were MAP, although SSC decreased gradually with storage time (Fig. 2(b)). But there was no significant difference in SSC of the fruit stored in CA-I and CA-II at the end of storage ($P = 0.05$).

3.3. Changes in activities of PPO and POD

PPO activity of litchi pericarp proved to be high before browning of the peel occurred, then decreased rapidly (Fig. 3(a)). POD activity in litchi peel increased at first, then gradually decreased with the same tendency

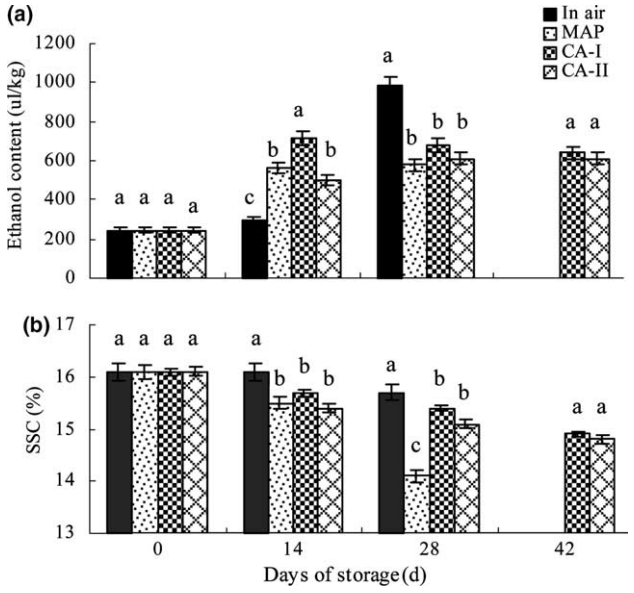

Fig. 2. Changes in ethanol (a) and soluble solids contents (b) of litchi fruit stored in air, MAP, CA-I and CA-II at 3 °C. Data are of the means of three replicates for each treatment. In the same time the different letter represented significant difference according to LSD test ($P = 0.05$).

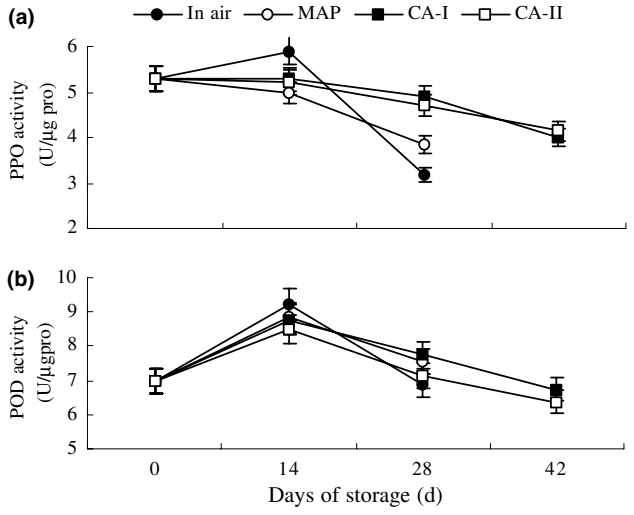

Fig. 3. Changes in activities of PPO (a); POD (b) in the pericarp of litchi fruit stored in air, MAP, CA-I and CA-II at 3 °C. Data are the means of three replicates for each treatment. Bars represented standard deviations of the means.

under all conditions at 3 °C (Fig. 3(b)). There were no significant differences among the different treatments.

3.4. Changes in contents of total phenol and anthocyanidin

Total phenol content of the fruits stored under CA conditions decreased to 42.0–43.8 μg/g after 14 days (Fig. 4(a)). There was no difference in phenol contents of the fruit kept under CA conditions during storage periods. Meanwhile, anthocyanidin contents in litchi

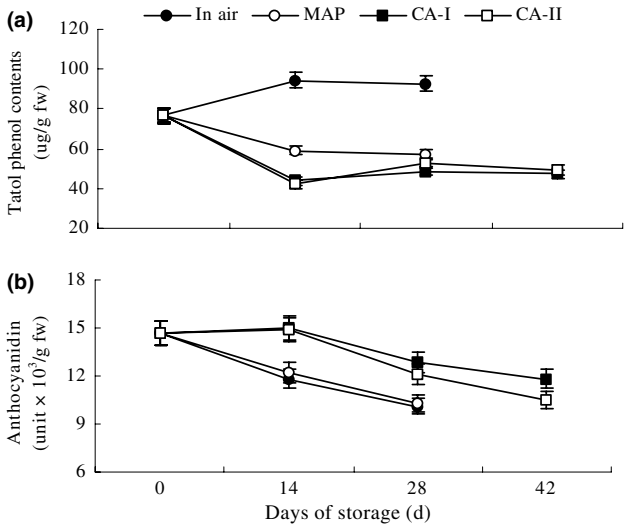

Fig. 4. Changes in contents of total phenol (a); anthocyanidin (b) in the pericarp of litchi fruit stored in air, MAP, CA-I and CA-II at 3 °C. Data are the means of three replicates for each treatment. Bars represented standard deviations of the means.

peel decreased gradually with storage time, and also showed the same tendency in all treatments. But anthocyanidin contents in peel of the fruits stored under CA conditions decreased more slowly than in those with MAP treatment (Fig. 4(b)).

4. Discussion

Pericarp browning of litchi could affect its storability and market value. Colour deterioration in litchi pericarp was due to browning reactions, and active PPO and POD were reported as being present in the fruit (Kader, 1994). Robards, Prenzler, Tucker, Swatsitang, and Glover (1999) considered that the main oxidative phenomenon of this type was enzymatic browning which involves an initial enzymatic oxidation of phenolic compounds, located predominantly in the vacuole, by PPO located in cytoplasm, to form slightly coloured quinines. The result of the experiment indicated that PPO might not be a unique factor involved in pericarp browning of litchi fruit because there was no obvious increase in PPO activity of fruit under different storage conditions (Fig. 3(a)). Other factors such as decay, desiccation and low temperature injury, also stimulated browning (Ray, 1998; Underhill et al., 1992). The fact, that POD activity in litchi pericarp was low at harvest and significantly increased when the peel browned (Fig. 3(b)) indicated POD played a role in enzymatic browning of litchi fruit, and further supported our previous result that POD can rapidly oxidize 4-methylcatechol in the presence of H_2O_2, and form brown polymeric pigments (Gong & Tian, 2002).

Regarding the browning mechanism, there are two different biochemical processes that contribute to browning, one involves the action of enzymes; the other

involves changes of the red pigment molecules. Lee and Wicker (1991) found that PPO could not oxidize anthocyanins, whereas the oxidative product of 4-methylcatechol, caused by PPO, resulted in an accelerated degradation of anthocyanins. Meanwhile, anthocyanase contributed to pericarp browning of litchi fruit by making the major phenolic constituents (anthocyanina) accessible to PPO or POD (Zhang, Pang, & Ji, 2001). Stress caused by water loss leads to changes in the red pigment molecules, and is easier to manipulate than the other biochemical processes. Desiccation also prompts the breakdown of vacuole and leakage of anthocyanin and destroys the compartmentalization of browning-related enzymes and their substrates (Underhill & Critchley, 1994). These observations suggested that heat-induced browning might result from non-selective or non-enzymic degradation of a range of compounds, including anthocyanins. The existence of other anthocyanin-related compounds present in the pericarp may also be involved in colour losses (Lee & Wicker, 1991). Degradation of anthocyanin pigments by condensation with quinones, formed from endogenous phenolics due to the action of PPO, has been reported as a possible mechanism of colour loss of strawberry fruit (Wesche-Ebeling & Montgomery, 1990). Litchi fruit, during senescence, may lose the compartmentalization of enzymes and this finally leads to enzymatic browning.

Pericarp browning of litchi fruit can be affected by biotic and abiotic factors, which occur a series of physiological and biochemical changes in fruit, such as senescence, enzymatic browning and degradation of anthocyanin pigments. The results of this experiment indicate that CA conditions were more effective in preventing pericarp browning and decreasing fruit decay than other treatments. Beneficial effects included delaying senescence, limiting PPO and POD activities, reducing total phenol content and maintaining high anthocyanidin levels.

Acknowledgements

The research was supported by the National Science Fund for Distinguished Young Scholars of China (Grant No. 30225030) and by the Ministry of Science and Technology (Grant No. 2001BA501A09).

References

Beaudry, R. M. (1999). Effect of O_2 and CO_2 partial pressure on selected phenomena affecting fruit and vegetable quality. *Postharvest Biology and Technology, 15*, 293–303.

Bradford, M. M. (1976). A rapid and sensitive method for the quantisation of microgram quantities of protein utilising the principle of protein-dye binding. *Analytical Biochemistry, 72*, 248–254.

Chen, Y. Z., & Wang, Y. R. (1989). Study on peroxidases in litchi pericarp. *Acta Batanica Austro Sinica, 5*, 47–52.

Day, B. P. E. (1996). High oxygen modified atmosphere packaging for fresh prepared produce. *Postharvest News and Information, 7*(3), 31–34.

Galeazzi, M. A. M., Sgarbieri, V., & Costantinides, S. M. (1981). Isolation, purification and physiocochemical characterization of polyphenol oxidase from dwarf variety of banana (*Musa cavendishii*). *Journal of Food Science, 46*, 150–155.

Gong, Q. Q., & Tian, S. P. (2002). Partial characterization of soluble peroxidase in pericarp of litchi fruit. *Progress in Biochemistry and Biophysics, 29*, 613–620.

Huang, S., Hart, H., Lee, H., & Wicker, L. (1990). Enzymatic and color changes during post-harvest storage of lychee fruit. *Journal of Food Science, 55*, 1762–1763.

Kader, A. A. (1994). Modified and controlled atmosphere storage of tropical fruit. In: Postharvest Handling of Tropical Fruit. *ACIAR Proceedings* (vol. 50, pp. 239–249).

Lee, H., & Wicker, L. (1991). Anthocyanin pigments in the skin of lychee fruit. *Journal of Food Science, 56*, 466–468.

Mayer, A. M., & Harel, E. (1978). Polyphenol oxidases in plants. *Phytochemistry, 18*, 193–215.

Menzel, C. M., & Simpson, D. R. (1987). Lychee nutrition: a review. *Scientific Horticulture, 31*, 195–224.

Nip, W. K. (1988). Handling and perservation of lychee (*Litchi chinensis* Sonn.) with emphasis on color relation. *Tropical Science, 28*, 5–11.

Paull, R. E., & Chen, N. J. (1987). Effect of storage temperature and wrapping on quality characteristics of litchi fruit. *Scientific Horticulture, 33*, 223–236.

Pesis, E., Dvir, O., Feygenberg, O., Arie, R. B., Ackerman, M., & Lichter, A. (2002). Production of acetaldehyde and ethanol during maturation and modified atmosphere storage of litchi fruit. *Postharvest Biology and Technology, 26*, 157–162.

Pirie, A., & Mullins, M. G. (1976). Change in anthocyanin and phenolic content of grapvine leaf and fruit tissue treated with sucrose, nitrate and abscisic acid. *Plant Physiology, 58*, 468–472.

Proctor, J. T. A. (1974). Color stimulation in attached apples with supplementary light. *Canadian Journal of Plant Science, 54*, 499–503.

Ray, P. K. (1998). Post-harvest handling of litchi fruits in relation to colour retention – A critical appraisal. *Journal of Food Science and Technology, 35*, 103–116.

Robards, K., Prenzler, P. D., Tucker, G., Swatsitang, P., & Glover, W. (1999). Phenolic compounds and their role in oxidative processes in fruits. *Food Chemistry, 66*, 401–436.

Tian, S. P., Folchi, A., Pratella, G. C., & Bertolini, P. (1996). The correlation of some physiological properties during ultra low oxygen storage in nectarine. *Acta Horticulture, 374*, 131–140.

Tian, S. P., Xu, Y., Jiang, A. L., & Gong, Q. Q. (2002). Physiological and quality responses of longan fruits to high-O_2 or high-CO_2 atmospheres in storage. *Postharvest Biology and Technology, 24*, 335–340.

Underhill, S. J. R., & Critchley, C. (1994). Anthocyanin decolorisation and its role in lychee pericarp browning. *Australian Journal of Experimental Agriculture, 34*, 115–122.

Underhill, S. J. R., Critchley, C., & Simons, D. H. (1992). Postharvest pericarp browning of lychee (*Litchi chniensis* Sonn.). *Acta Horticulture, 321*, 718–725.

Wesche-Ebeling, P., & Montgomery, M. W. (1990). Strawberry polyphenoloxidadase: its role in anthocyanin degradation. *Journal of Food Science, 55*, 731–734.

Zhang, D. G., & Quantick, P. C. (1997). Effects of chitosan coating on enzymatic browning and decay during postharvest storage of litchi fruit. *Postharvest Biology and Technology, 12*, 195–202.

Zhang, Z. Q., Pang, X. Q., & Ji, Z. L. (2001). Role of anthocyanin degradation in litchi pericarp browning. *Food Chemistry, 75*, 217–221.