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Effects of Pure Oxygen on the Rate of Skin Browning and Energy Status in Longan Fruit

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Summary

Postharvest pericarp browning is one of the main problems resulting in reduced shelf life of longan fruit. Experiments were conducted to examine the changes in concentrations of adenosine triphosphate (ATP), adenosine diphosphate (ADP) and adenosine monophosphate (AMP), energy charge levels and activities of polyphenol oxidase (PPO) and peroxidase (POD) in relation to pericarp browning of longan fruit. Fruit kept for 6 days in pure oxygen atmosphere at 28 °C showed lower browning indices and higher ATP concentrations but lower AMP concentrations and higher respiratory rates, compared to those kept in air. While energy charge decreased during storage, the decrease was delayed markedly by exposure to pure oxygen. There was a lower energy charge in the browned fruit, which was associated with rapid increase in malondialdehyde concentration. Enhanced respiration of longan fruit exposed to pure oxygen can result in the production of ATP. However, fruit exposed to pure oxygen exhibited higher activities of PPO and POD, which was not associated with reduced skin browning inhibition. These results supported the hypothesis that skin browning of postharvest longan fruit may be a consequence of membrane injury caused by the lack of maintenance energy.

Key words: browning, longan (*Dimocarpus longan* Lour.), energy, peroxidase, polyphenol oxidase, pure oxygen, respiration

Introduction

The longan (*Dimocarpus longan* Lour.) is an attractive subtropical fruit. The fruit is non-climacteric and is harvested when eating quality and visual appearance are optimal (*1*). However, longan fruit has a very short marketing shelf life under normal ambient conditions due to skin colour loss (browning) (*2,3*). There have been several studies on the effects of elevated O_2 on fruit parameters, such as respiration rate, ethylene production and colour formation (*4–6*). Day (*7*) proposed that any level of O_2 above ambient (21 kPa) would reduce decay and prevent anaerobic fermentation of commodities in storage, which should consequently prevent the development of off-flavours and odours. Tian *et al.* (*8*) reported that controlled atmosphere storage at 70 % O_2 reduced decay and prevented peel browning of longan fruit stored at 2 °C. However, in our preliminary investigations, exposure of longan fruit to 60 or 80 % O_2 accelerated the skin browning of the fruit but 100 % O_2 markedly extended shelf life at ambient temperature. The underlying biochemical and physiological effects of high O_2 atmospheres on longan pericarp browning need further investigation.

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Tissue browning has been attributed to oxidation of phenolic compounds resulting from the loss of membrane compartmentation. Lipids are essential components of plant cell membranes, and the involvement of adenylate nucleotides in fatty acid biosynthesis of membrane lipids is well established (*9,10*). While adenosine triphosphate participates in fatty acid synthesis, malondialdehyde (MDA) is a major product of membrane fatty acid oxidation and can act as a suitable index of membrane integrity (*11*). MDA can be formed by peroxidation of fatty acids with resulting free radicals during fruit senescence (*12*). It was reported that exposure of plant tissues to high O_2 atmospheres may stimulate, inhibit or may not affect MDA formation (*13–15*). Changes in membrane lipid components and associated alteration of biophysical and biochemical membrane properties may result in cellular decompartmentation (*16*). Saquet *et al.* (*17*) found that the delay in flesh browning of Conference pears stored under delayed controlled atmosphere conditions was associated with higher ATP concentrations that might maintain membrane integrity in the cells of fruit tissue. Thus, it was suggested that fruit browning may be a consequence of membrane injury caused by a lack of maintenance energy.

The involvement of PPO and POD in longan pericarp browning has been established (*2,3,18*). Previous studies indicated that sulphur fumigation and chitosan coating inhibited activities of PPO and POD and, thus, delayed skin browning of longan fruit (*19,20*). Therefore, delaying or inhibiting enzymatic oxidation could be an important means to extend storage life and maintain quality of longan fruit.

There are no published data on the effects of high O_2 concentrations on energy production related to fruit skin browning. Moreover, it is unclear if exposure of plant tissues to pure oxygen enhances or inhibits MDA formation caused by membrane lipid peroxidation. The objective of this study was to investigate effects of pure oxygen on skin browning inhibition, MDA formation and activities of PPO and POD, in association with tissue energy status, of harvested longan fruit.

Materials and Methods

Plant materials and treatments

Longan fruit (*Dimocarpus longan* Lour.) cv. Shixia at 90 % maturation were obtained from a commercial orchard in Guangzhou. Fruit were selected for uniformity, shape, colour and size, and any blemished or diseased fruits were discarded. Sixty individual fruits were put into a 4.5-L glass jar as one replicate. There were six replicates per treatment in a flow-through gas system. Pure oxygen gas was supplied by Guangzhou Gas Factory (China). The fruit samples were continuously kept in humidified air (control) or pure O_2 at 0 % CO_2 for up to 6 days at 28 °C and 80–90 % relative humidity (RH). The initial concentration of O_2 in the jars was verified using an O_2 and CO_2 detector (Model CYES-II, Shanghai Scientific Instruments). In this study the concentration of CO_2 was <0.002 % because a continuous gas flow system was used. Fruit from each replicate of each treatment removed after being held for 2, 4 and 6 days was immediately assessed. Prior to exposure to pure O_2 atmosphere, 60 fruits were sampled and then used for 0 day assessments. After 2, 4 and 6 days of storage, 20 fruits were removed from each jar and then used for measurements of respiration rate and concentrations of ATP, ADP, AMP and MDA. During the experimental period, all experiments for the fruit kept in air and pure oxygen were maintained at 28 °C and 80–90 % RH. The experiments were conducted in two sequential years. A similar result in the inhibition of the skin browning of longan fruit was obtained from the two experiments. The data from the experiment in 2003 are presented.

Skin browning evaluation

Fruit browning was assessed by measuring the extent of the total browned pericarp area on 60 individual fruits using the following visual appearance scale (*2*): 1 = no browning (excellent quality); 2 = slight browning; 3 = <1/4 browning; 4 = 1/4–1/2 browning; 5 = >1/2 browning (poor quality). The browning index was calculated as $\sum$(browning scale × proportion of corresponding fruit within each class). Fruit evaluated at a higher index than 2.0 (browning index) was considered to be unacceptable for visual marketing quality. In this study, skin browning of longan fruit does not mean entirely the loss of pulp eating quality, but, in practice, it reduces its commercial value (*2*).

Respiration rate measurement

For respiration rate measurement, 30 fruits (about 400 g) were sealed in a 2.3-L airtight glass jar for 2 h, with 3 replicates. A 1 mL of headspace gas sample was withdrawn with a gas-tight hypodermic syringe and analysed by gas chromatography (Shimadzu GC-9A) equipped with alumina packed with 80–100 mesh in a copper column (457.2 × 3.175 mm) and a thermal conductivity detector (*21*). CO_2 concentration was calibrated with a known standard CO_2 gas (20 mL/L), while respiratory rate was expressed as mg CO_2/h/kg (fresh weight, FW).

Determinations of ATP, ADP and AMP concentrations

ATP, ADP and AMP were extracted by the method of Ozogul *et al.* (*22*). Pericarp tissue (5 g) from 30 fruits was homogenized with 25 mL of 0.6 M perchloric acid for 1 min in an ice bath. The extraction mixture was then centrifuged for 10 min at 3000 × *g* at 4 °C. A volume of 10 mL of supernatant was taken and then quickly adjusted to pH=6.5 with 1 mol/L of KOH. The supernatant was allowed to stand for 30 min in an ice bath to precipitate most of the potassium perchlorate, which was then removed by centrifugation at 10 000 × *g* for 5 min. The supernatant was collected for measurements of ATP, ADP and AMP concentrations. Separation and determination of ATP, ADP and AMP were performed by high performance liquid chromatograph (HPLC, Beckman Gold 125) using a Nucleosil 100-5 C_{18} column (4.6 × 250 mm). The elution consisted of a linear gradient program from 0 to 25 % acetonitrile in a solution (pH=7.0) of 0.06 mol/L of K_2HPO_3 and 0.04 mol/L of KH_2PO_3 for 5.3 min and then returned to 0 % acetonitrile in 0.06

mol/L of K_2HPO_3 and 0.04 mol/L of KH_2PO_3 (pH=7.0) for 0.7 min. The flow rate was 1.2 mL/min. Sample of 20 μL was injected into the HPLC system for the analysis of ATP, ADP and AMP concentrations. The identification of individual ATP, ADP and AMP in the HPLC analysis was made after the addition of known standards based on comparisons of the retention time. The concentrations of ATP, ADP and AMP were calculated as peak areas × response factors. In this study, there was about 94 % recovery of the standards tested. The energy charge was calculated as

$$\frac{[\mathrm{ATP}]+0.5[\mathrm{ADP}]}{[\mathrm{ATP}]+[\mathrm{ADP}]+[\mathrm{AMP}]}\times 100$$

All the results in this study were reported on a fresh weight basis.

MDA determination

Pericarp tissue (5 g) from 30 fruits was homogenised with 25 mL of 50 g/L trichloroacetic acid (TCA) and centrifuged for 10 min at 4000 × *g*. The supernatant was collected to determine MDA concentration. MDA assay was performed by the method of Guidi *et al.* (*23*) with minor modifications. Thiobarbituric acid (TBA) reactivity was determined by adding 2.5 mL of 0.5 % TBA in 15 % TCA to 1.5 mL of the supernatant. The reaction solution was held for 20 min in a boiling water bath, then cooled quickly and finally centrifuged at 4000 × *g* for 10 min to clarify the solution. Absorbance was measured at 532 nm and corrected for non-specific turbidity by subtracting the absorbance at 600 nm. The MDA concentration was calculated with a molar absorption coefficient of 1.55 nmol/L/m on a fresh weight basis by the method of Guidi *et al.* (*23*).

Extraction and assays of PPO and POD activities

Fruit peel tissue (5 g) from 15 fruits was homogenized in 20 mL of 0.05 mol/L phosphate buffer (pH=6.8) and 0.5 g of polyvinylpyrrolidone (insoluble) at 4 °C. After filtration of the homogenate through a cotton cloth, the filtrate was centrifuged at 19 000 × *g* for 20 min at 4 °C. The supernatant was then collected as the crude enzyme extract. PPO activity was assayed by measuring the oxidation of 4-methylcatechol as the substrate according to the method of Jiang (*24*). One unit of enzyme activity was defined as the amount that caused a change in absorbance of 0.001 absorbance units per minute.

POD was extracted by homogenising the fruit pericarp (5 g) from 15 fruits with 10 mL of 0.05 mol/L phosphate buffer (pH=7.0) and 0.5 g of polyvinylpyrrolidone (insoluble). The homogenate was centrifuged for 20 min at 16 000 × *g* and 4 °C and then the supernatant was collected as the crude enzyme extract. POD activity, with guaiacol as a substrate, was assayed by the method of Zhang *et al.* (*25*) in a reaction mixture (3 mL), containing 25 μL of enzyme extract, 2.725 mL of 0.05 mol/L phosphate buffer (pH=7.0), 0.1 mL of 1 % H_2O_2 and 0.15 mL of 4 % guaiacol. The increase in absorbance at 470 nm due to the guaiacol oxidation was recorded for 2 min. Definition of one unit of the enzyme activity was the same as for PPO.

Protein content was determined according to the method of Bradford (*26*) with bovine serum as the standard.

Data handling

In all experiments, fruits were arranged in a completely randomized design, and each treatment comprised of 60 individual fruits for evaluations of skin browning and three replicates for measurements of respiration rate, concentrations of ATP, ADP and AMP, activities of PPO and POD, and MDA content. Data were subject to analysis of variance (ANOVA) using Genstat Version 5. Least significant differences were calculated to compare data mean values at the 5 % level.

Results

Skin browning

The browning index of longan fruit pericarp increased slowly within the first 2 days and then quickly over the next two days at 28 °C (Fig. 1). When storage time was extended to 4 days, the initial colour of fruit kept in air had largely disappeared, with a browning index of 2.7, and the fruit had unacceptable visual marketing quality. The pure oxygen treatment markedly extended visual marketing life of longan fruit in terms of browning index (Fig. 1). Fruit kept for 4 and 6 days in pure oxygen had browning indices of 1.6 and 2.8, respectively. The difference in the browning index between the fruit stored in air and pure oxygen for 4 and 6 days at 28 °C was significant (p=0.01).

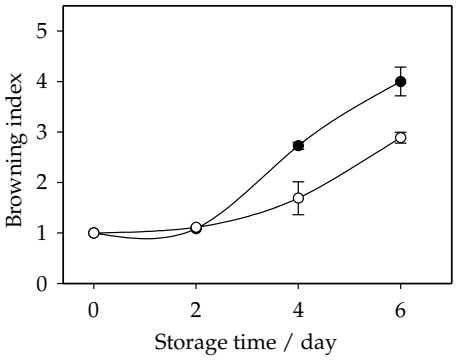

Fig. 1. Effects of pure oxygen treatment on browning index of harvested longan fruit during 6-day storage at 28 °C. Vertical bars indicate standard errors of the means where they exceed the symbol size. ●, air; ○, pure oxygen

Respiration rate

Exposure of longan fruit to pure oxygen markedly enhanced respiration rate over the first 2 days of storage, which then stayed relatively high (Fig. 2A). Fruits kept in air did not significantly change in their respiration rate over the first 2 days of storage, and the respiration rate maintained at lower level throughout the experiment. There were significant differences in the respiration rates between the fruits kept in air and those kept in pure oxygen during the experiment.

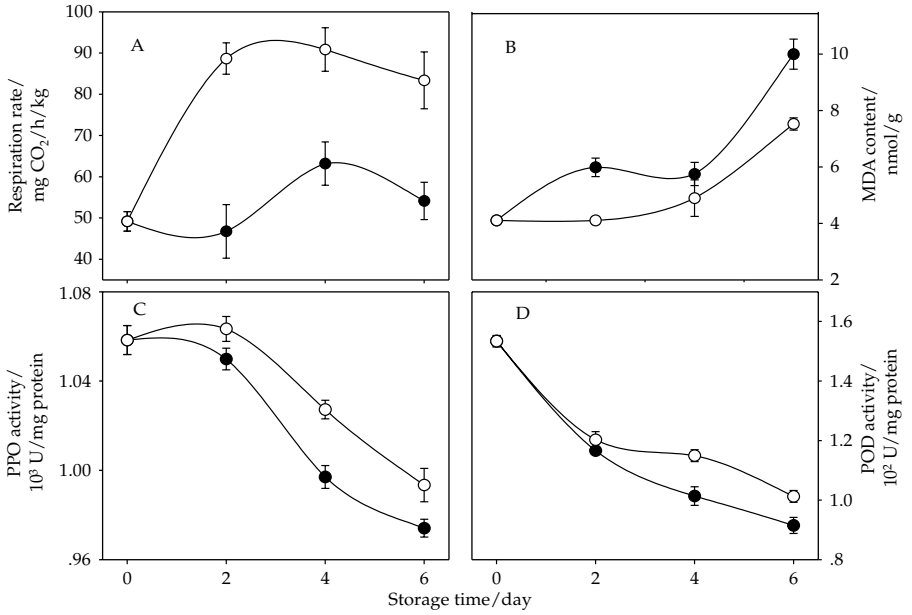

Fig. 2. Effects of pure oxygen treatment on respiratory rate (A), MDA concentration (B) and activities of PPO (C) and POD (D) of harvested longan fruit during 6-day storage at 28 °C. Vertical bars indicate standard errors of the means where they exceed the symbol size. ●, air; ○, pure oxygen

MDA concentration

MDA concentration in longan fruit pericarp tissues exhibited a tendency to increase as storage time progressed (Fig. 2B). The MDA concentration of control fruit increased within 2 days, then decreased a little, and finally increased rapidly after 4 days of storage. The pure oxygen treatment delayed the increase in the MDA concentration. Furthermore, fruit kept for 2 or 6 days in pure oxygen had a significantly lower MDA concentration, compared to those kept in air.

PPO and POD activities

PPO activity in control longan fruit decreased slowly over the 2 days of storage and then decreased rapidly (Fig. 2C). Fruit exposed to pure oxygen atmosphere exhibited a higher PPO activity compared to those kept in air. A similar effect of pure oxygen treatment on POD activity compared to that on PPO activity was observed (Fig. 2D). There were significant differences in the activities of PPO and POD between the fruit stored in air and in pure oxygen for 4 and 6 days.

ATP, ADP and AMP concentrations and energy charge

There were high ATP and ADP concentrations and energy charge level, but low AMP concentration, in longan fruit at harvest (Fig. 3).

ATP concentrations in pericarp tissues decreased rapidly within 2 days of storage and then were maintained at relatively low level. Similarly, ADP concentrations decreased markedly. AMP concentrations increased with storage time. Fruit kept in pure oxygen exhibited generally higher ATP concentrations and energy charge level during the experimental period. Significant differences at the 5 % level in the concentrations of ATP, ADP and AMP and the energy charge levels existed between the fruit kept in air and pure oxygen after 6 days of storage, which was associated well with browning index (Fig. 1). Moreover, the significant difference in the energy charge levels between control fruit and pure oxygen-treated fruit was more evident.

Discussion

Application of high oxygen atmospheres to inhibit browning has been reported when pure oxygen was applied to sliced apple fruit (*27*) and shredded lettuce (*28*). Similarly, we found that exposure of longan fruit to pure oxygen markedly inhibited skin browning (Fig. 1). Tian *et al.* (*8*) reported that 70 % O_2 plus high CO_2 was effective in preventing peel browning of longan fruit stored at 2 °C, compared to modified atmosphere packaging storage. They did not investigate 100 % O_2 effects. However, in our preliminary investigation, exposure of longan fruit to 60 or 80 % O_2 accelerated skin browning of the fruit at 28 °C (unpublished data). Different response of longan fruit to high O_2 concentrations may be due to different storage conditions.

Membrane deterioration leads to decompartmentation of enzymes and substrates, and thus is an inherent feature of senescence (*16*). Availability of energy of fruit tissue delayed the senescence process and maintained cellular compartmentation (*29*). Saquet *et al.* (*17*) and Veltman *et al.* (*30*) suggested that membrane damage could be induced by ATP deprivation. While involvement of ATP in biosynthesis of fatty acids in membrane lipids is well established (*9,10,31*), enhanced free radical production needs to be scavenged by enzymatic and non-enzymatic processes to maintain cell homeostasis (*32,33*). Furthermore, Saquet *et al.* (*17, 29*) suggested that high concentrations of ATP in pear fruit are likely to have resulted from elevated respiration. In this study, the enhanced respiration of longan fruit exposed to pure

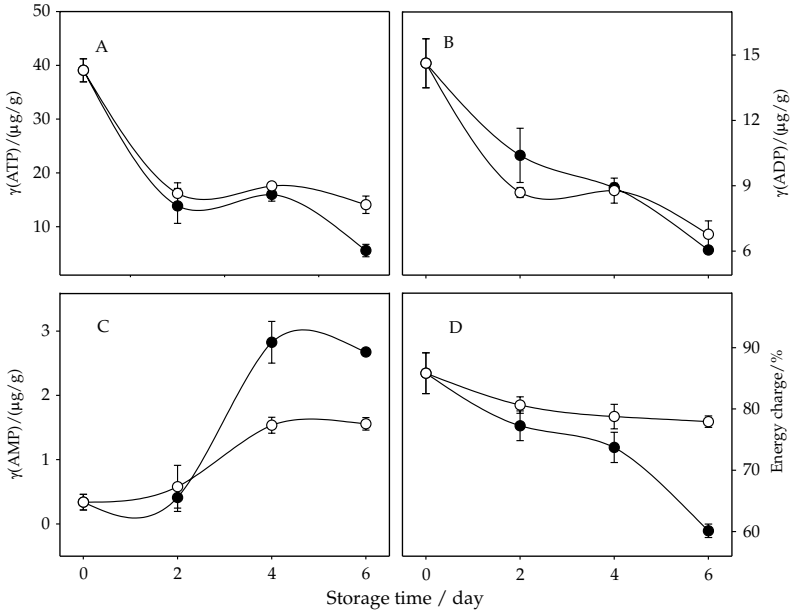

Fig. 3. Effects of pure oxygen treatment on the concentrations of ATP (A), ADP (B) and AMP (C), and energy charge (D) of harvested longan fruit during 6-day storage at 28 °C. Vertical bars indicate standard errors of the means where they exceed the symbol size. ●, air; ○, pure oxygen

oxygen apparently contributed to a significantly higher level of energy charge, which was associated with reduced skin browning index, compared to those exposed to air (Figs. 1 and 3D). The study supports the hypothesis that skin browning of harvested longan fruit may be a consequence of membrane damage caused by a lack of maintenance energy.

MDA is formed by peroxidation of fatty acids with resulting free radicals during fruit senescence (*12*), as a major product of membrane fatty acid oxidation. MDA can act as a suitable index of membrane integrity (*11*, *34*). In this study, MDA concentration instead of membrane leakage rate was measured because preparation for skin disc of longan fruit, due to abrupt rupture, may influence the measurement of the leakage. The other advantage of MAD analysis is better understanding of the effect of pure oxygen treatment on the formation of fatty acid peroxidation. This investigation exhibited that the pure oxygen treatment evidently delayed the accumulation of MDA (Fig. 2B). As previous studies showed that exposure of plant tissues to high O_2 atmospheres may stimulate, inhibit or may not affect MDA formation (*13–15*), the role of high O_2 atmospheres in the MDA formation is still unclear and it requires further investigation.

Involvement of PPO and POD in enzymatic browning of longan fruit is generally accepted (*2*,*3*,*18*). PPO and POD catalyze the oxidation of phenolics to quinines and then condense tannins to brown polymers. The initiation of the enzymatic browning depends largely on the loss of compartmentation of enzymes and substrates. In this study, there were high activities of PPO and POD in longan fruit at harvest, but no skin browning occurred while high ATP production and low MDA content were observed, which further supports the hypothesis that the loss of compartmentation of enzymes and substrates was the key factor for the enzymatic browning reaction of plant tissues. Thus, reduced skin browning of longan fruit by pure oxygen treatment could be accounted for maintenance of compartmentation of enzymes and substrates by enhanced respiration and ATP production. Similar results were found in reduced browning associated with enhanced ATP production of litchi fruit exposed to pure oxygen atmosphere (*35*).

In conclusion, application of pure oxygen significantly prevented pericarp browning of harvested longan fruits. Exposure of longan fruits to pure oxygen enhanced respiration rate, maintained high levels of ATP and energy charge, reduced lipid peroxidation and delayed decompartmentation of PPO and POD, and their substrates.

Acknowledgements

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Utjecaj čistoga kisika na brzinu posmeđivanja kore voća longan i njegov energetski status

Sažetak

Posmeđivanje perikarpa je glavni problem pri skladištenju voća longan. Provedeni su pokusi kako bi se utvrdile promjene u koncentracijama adenozintrifosfata (ATP), adenozindifosfata (ADP) i adenozinmonofosfata (AMP), razine energetskoga naboja i aktivnosti polifenol oksidaze (PPO) i peroksidaze (POD) u usporedbi s posmeđivanjem perikarpa. Voće čuvano 6 dana u atmosferi čistoga kisika pri 28 °C pokazivalo je niže indekse posmeđivanja i veće koncentracije ATP, a niže AMP te veliku brzinu respiracije u usporedbi s voćem čuvanim na zraku. Tijekom skladištenja energetski se naboj snizivao, što je bilo znatno usporeno izlaganjem voća čistome kisiku. U posmeđenom voću bio je manji energetski naboj, što je povezano s brzim povećanjem koncentracije malondialdehida. Pojačana respiracija voća longan, izloženog čistome kisiku, može uzrokovati nastajanje ATP. Voće izloženo čistome kisiku imalo je veće aktivnosti PPO i POD, što nije povezano sa smanjenom inhibicijom posmeđivanja. Dobiveni rezultati podupiru hipotezu prema kojoj je posmeđivanje ovoga voća posljedica oštećenja membrana uzrokovanih manjkom energije potrebne za održavanje.