Research Article

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Effects of short-term N_2 treatment on quality and antioxidant ability of loquat fruit during cold storage

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Abstract

BACKGROUND: Loquat fruit is susceptible to microbial decay and quality deterioration during postharvest distribution. In this study, in a search for effective alternatives to methods currently used for quality maintenance, the effects of short-term pre-storage N_2 treatment on the quality and antioxidant ability of loquat fruit were investigated.

RESULTS: Loquat fruits were exposed to 100% N_2 for 6 h at 20 °C and then stored at 5 °C for 35 days. Short-term N_2 treatment significantly delayed the increase in fruit decay rate and decreases in total soluble solid and titratable acidity contents, thereby maintaining better eating quality and extending the storage life of fruits. It also markedly delayed increases in membrane permeability, malondialdehyde content and superoxide anion production rate. In addition, N_2-treated fruits exhibited significantly higher superoxide dismutase and catalase activities and lower lipoxygenase activity than control fruits.

CONCLUSION: Short-term pre-storage N_2 treatment effectively reduced fruit decay and maintained quality in loquat fruits during cold storage. The reduction in fruit decay and quality deterioration by the treatment was correlated with enhanced antioxidant ability and reduced lipid peroxidation.

Keywords: loquat fruit; short-term N_2 treatment; quality; antioxidant enzymes; lipoxygenase

INTRODUCTION

Loquat is an evergreen tree native to China. The ripe fruits of loquat are spherical or oval in shape, orange/yellow or white in colour and have a soft and juicy flesh. They are highly favoured by consumers worldwide for their mild, subacid and sweet taste and nutritional value. Loquat fruits ripen in early summer in a hot and rainy climate. After harvest the fruits are susceptible to microbial decay and quality deterioration characterised by rapid decreases in total soluble solids and total titratable acidity, limiting their postharvest life to approximately 10 days at ambient temperature or 30 days at 1–5 °C.[1,2] Although various methods such as modified atmosphere packaging, low-temperature conditioning and application of polyamine, salicylic acid, methyl jasmonate or 1-methylcyclopropene have been demonstrated to reduce fruit decay and quality deterioration, there is still a need to develop more effective techniques for loquat fruit storage.[3]

Reactive oxygen species (ROS) such as superoxide radical, hydrogen peroxide (H_2O_2) and hydroxyl radical are involved in lipid peroxidation, membrane damage and consequently senescence in plants.[4] Furthermore, ROS reactions have been suggested to play an important role in the degradation process of membrane polar lipids in fruit senescence.[5] The metabolism of ROS is dependent on several functionally interrelated antioxidant enzymes such as superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT). SOD is believed to play a crucial role in antioxidant defence by catalysing the dismutation of $O_2^{\bullet -}$ into H_2O_2, whereas CAT and POD destroy H_2O_2.[6–8] Lipoxygenase (LOX) catalyses the peroxidation of polyunsaturated fatty acids and is believed to be a major contributor to senescence-related membrane damage in plant tissues.[9] An increase in LOX activity was observed in postharvest kiwifruit,[10] which suggested that LOX was also involved in fruit senescence. Although much research has been done on the production and regulation of oxidants and antioxidant activity during plant senescence,[6,11,12] the effect and mode of action of short-term N_2 treatment on fruit senescence have not been well elucidated.

Short-term N_2 treatment was reported to delay ripening of banana fruit,[13] inhibit browning of harvested litchi fruit and fresh-cut Chinese water chestnut,[14–16] reduce decay development of tomato fruit[17] and alleviate chilling disorders of cold-stored avocado fruit,[18] thereby maintaining the quality and extending the shelf life of these harvested fruits and vegetables. Recently, Liu *et al.*[15] reported that short-term pre-storage N_2 treatment

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maintained tissue energy levels and membrane integrity and effectively inhibited browning of pericarp tissues of harvested litchi fruit. This result suggests that reduced peroxidation of membrane lipids could be involved in the short-term N_2 treatment effects. However, there have been no reports on the effects of short-term N_2 treatment on antioxidant ability and quality in postharvest loquat fruit. The objective of this study was to investigate whether short-term N_2 treatment induces the activity of enzymatic antioxidant systems and maintains the quality of loquat fruit during cold storage.

MATERIALS AND METHODS

Plant material and treatment

Loquat (*Eriobotrya japonica* Lindl. cv. Dahongpao) fruits were harvested by hand at the ripe stage from an orchard in Zhejiang, China and transported within 2 h to the laboratory. Fruits were selected for uniform size and colour and the absence of visual defects, then randomly divided into two groups of 180 fruits each. Both groups of fruits were placed in 32 L jars for treatment. In a preliminary study we found that treatment with 100% N_2 for 6 h had the most beneficial effect on quality maintenance of loquat fruit, so this treatment time was used in the present work. The jars were placed at 20 °C and flushed with air (control) or 100% N_2 at a flow rate of 100 mL min^{-1} for 6 h. Both the air and N_2 were humidified to approximately 90% relative humidity (RH) by bubbling through water. Following treatment, the jars were opened and both groups of fruits were stored at 5 °C and approximately 95% RH for up to 35 days. There were three replicates per treatment and the experiment was conducted twice. Samples of 30 fruits were taken initially and at 7 day intervals during storage for measurements of total soluble solids (TSS), titratable acidity (TA), fruit decay rate, membrane permeability, malondialdehyde (MDA) content, superoxide anion rate and SOD, LOX and CAT activities.

Determinations of quality parameters and fruit decay

Flesh tissue from 15 fruits of each replicate was homogenised in a grinder, wrapped in cheesecloth and squeezed with a hand press. The resulting juice was analysed for TSS and TA. TSS content was determined at 20 °C using a Pocket refractometer (PAL-1, Tokyo, Japan). TA content was determined by titrating 20 mL of juice to pH 8.1 with 0.1 mol L^{-1} NaOH and expressed as % malic acid. The severity of fruit decay was evaluated visually during the course of the experiment. Any fruit with visible decay symptoms was considered to be decayed. Decay rate (%) was defined as (decayed fruits/total fruits) ×100.

Measurements of superoxide anion generation rate, membrane permeability and MDA content

Superoxide anion generation rate was measured by the method of Chaitanya and Naithani.[19] Approximately 1 g of flesh tissue was homogenised in 5 mL of 50 mmol L^{-1} sodium phosphate buffer (pH 7.8) containing 1 mmol L^{-1} diethyl dithiocarbamate to inhibit SOD activity. After centrifugation at 10 500 × *g* for 20 min, superoxide anion ($O_2^{\bullet -}$) in the supernatant was measured by the capacity to reduce nitroblue tetrazolium (NBT). The assay mixture in a total volume of 3 mL comprised 100 mmol L^{-1} sodium phosphate buffer (pH 7.8) containing 1 mmol L^{-1} diethyl thiocarbamate, 0.3 mL of 0.25 mmol L^{-1} NBT and the supernatant. The absorbance of the end product was measured at 540 nm in a Bausch and Lomb Spectronic-20 spectrophotometer. $O_2^{\bullet -}$ formation rate was expressed as ΔA_{540} min^{-1} mg^{-1} protein. Membrane permeability, expressed as relative electrolyte leakage rate, was determined by the method of Jiang and Chen.[20] Twenty discs (2–3 mm thick, 10 mm diameter) from the equatorial region (two discs per fruit from the opposite region) of ten fruits were rinsed and incubated in 25 mL of distilled water for 1 h and the initial conductivity was recorded using a conductivity meter (HI 9932, Villafranca Padovana, Italy). The discs were then boiled for 30 min, cooled to room temperature and the final conductivity was recorded. The level of lipid peroxidation products in loquat samples was determined by the method of Heath and Packer[21] and expressed as MDA content. Approximately 1 g of flesh tissue was ground in 2.5 g L^{-1} 2-thiobarbituric acid (TBA) in 100 g L^{-1} trichloroacetic acid (TCA) using a mortar and pestle. After being heated at 95 °C for 15 min, the mixture was quickly cooled in an ice bath and centrifuged at 10 000 × *g* for 10 min. The absorbance of the supernatant was read at 532 nm and corrected for unspecific turbidity by subtracting the absorbance of the same at 600 nm. The blank consisted of 2 mL of 2.5 g L^{-1} TBA in 100 g L^{-1} TCA. The concentrations of lipid peroxides together with oxidatively modified proteins of plants were thus quantified in terms of MDA level using an extinction coefficient of 155 L $mmol^{-1}$ cm^{-1} and expressed as μmol g^{-1} fresh weight (FW).

Measurements of SOD, CAT and LOX activities

For SOD activity measurement, 1 g of flesh tissue was ground in 10 mL of 50 mmol L^{-1} phosphate buffer (pH 7.8) containing 10 g L^{-1} polyvinyl polypyrrolidone (PVPP) at 4 °C and then centrifuged at 15 000 × *g* for 15 min at 4 °C. The supernatant was used as SOD extract. SOD activity was assayed by the method of Crosti *et al.*[22] One unit of SOD activity was defined as 50% inhibition of 6-hydroxydopamine (6-OHDA) autoxidation under the assay conditions. CAT activity was assayed by the method of Candan and Tarhan.[23] CAT was extracted from 2.5 g of flesh tissue ground in 25 mL of 50 mmol L^{-1} phosphate buffer (pH 7.8) at 4 °C and then centrifuged at 15 000 × *g* for 15 min at 4 °C. The supernatant was used to determine CAT activity. CAT activity was assayed in a reaction mixture comprising 50 mmol L^{-1} phosphate buffer (pH 7), 10 mmol L^{-1} H_2O_2 and the supernatant. One unit of CAT activity was defined as the amount of enzyme that decomposes 1 μmol H_2O_2 min^{-1} at 25 °C. LOX was extracted from 4 g of flesh tissue ground in 12 mL of 50 mmol L^{-1} phosphate buffer (pH 7) containing 10 g L^{-1} PVPP at 4 °C and then centrifuged at 15 000 × *g* for 15 min at 4 °C. The supernatant was used to determine LOX activity according to the method of Axelrod *et al.*[24] Briefly, 0.2 mL of the supernatant was mixed with 2.78 mL of 100 mmol L^{-1} sodium acetate buffer (pH 5.5) and 25 μL of sodium linoleate as substrate. The reaction was conducted at 30 °C and the absorbance at 234 nm was recorded. One unit of LOX activity was defined as the amount of enzyme that causes a change of 0.01 in absorbance at 234 nm in 1 min.

Protein content in all enzyme extracts was estimated by the Bradford[25] method using bovine serum albumin as a standard.

Data analysis

Experiments were performed using a completely randomised design. All statistical analyses were done with SPSS (SPSS Inc., Chicago, IL, USA). Data were subjected to one-way analysis of variance (ANOVA). Mean separations were performed by Duncan's multiple range test. Differences at $P < 0.05$ were considered significant.

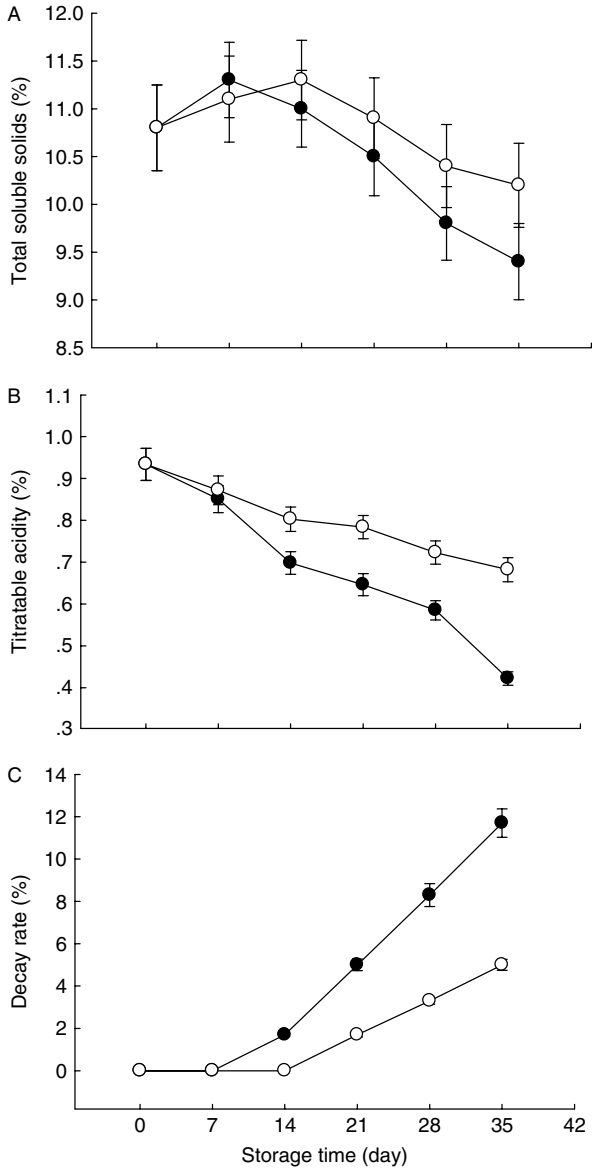

Figure 1. Effects of short-term N_2 treatment on (A) total soluble solids, (B) titratable acidity and (C) decay rate of loquat fruit during storage at 5 °C for 35 days: ●, control fruits; ○, N_2-treated fruits. Data are expressed as means of triplicate assays. Vertical bars represent standard errors of means.

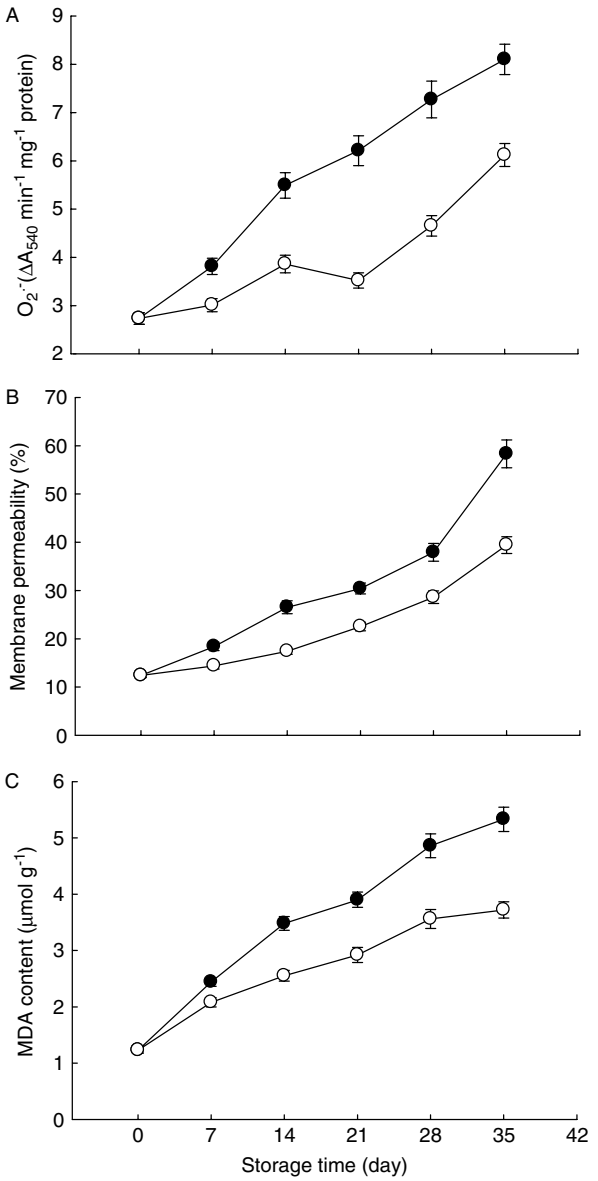

Figure 2. Effects of short-term N_2 treatment on (A) superoxide anion generation rate, (B) membrane permeability and (C) MDA content of loquat fruit during storage at 5 °C for 35 days: ●, control fruits; ○, N_2-treated fruits. Data are expressed as means of triplicate assays. Vertical bars represent standard errors of means.

RESULTS AND DISCUSSION

Effect of short-term N_2 treatment on fruit quality and decay

TSS, TA and fruit decay rate are important quality attributes of loquat fruit. Fruit TSS content showed a small increase in the first 7 days and then decreased during the remainder of storage. Short-term N_2 treatment significantly inhibited the decline in TSS content compared with control fruits (Fig. 1A). Fruit TA content decreased continuously during the whole storage time, but exposure of fruits to N_2 for 6 h resulted in a significantly slower decline in TA content (Fig. 1B). Therefore short-term N_2 treatment maintained better fruit taste by retaining higher levels of TSS and TA, possibly owing to reduced respiration and ethylene production rates as reported for banana fruit.[13] Control fruits showed decay incidence after 14 days of storage at 5 °C, after which the decay rate increased rapidly with storage time (Fig. 1C). Fruit decay rate was significantly delayed by short-term N_2 treatment. After 35 days of storage the decay rate of N_2-treated fruits was 57.3% lower than that of control fruits. Short-term N_2 treatments have also been shown to reduce the decay rate in tomato and litchi fruits.[14,17] However, the mode of action of N_2 treatment on disease is still unclear and needs further investigation.

Effect of short-term N_2 treatment on superoxide anion generation rate, membrane permeability and MDA content

The effect of short-term N_2 treatment on the superoxide anion generation rate of loquat fruit is shown in Fig. 2A. Superoxide anion generation rate increased with storage duration. Significantly higher superoxide anion generation rates were observed in control fruits than in short-term N_2-treated fruits throughout storage. Membrane permeability also increased with storage time, indicating that membrane systems became more vulnerable to leakage

(Fig. 2B). Short-term N_2 treatment significantly inhibited the increase in membrane permeability compared with control fruits. Throughout the storage period of 35 days, MDA content increased from an initial value of 1.23 μmol g^{-1} FW to 5.33 μmol g^{-1} FW for control fruits and 3.75 μmol g^{-1} FW for short-term N_2-treated fruits (Fig. 2C), indicating that short-term N_2 treatment significantly inhibited the peroxidation of membrane lipids. The changes in membrane permeability and MDA content found here provide evidence of membrane deterioration in loquat fruit during storage. Lipid peroxidation, membrane damage and consequently senescence often occur when ROS are present in excess of the scavenging capacity of the tissue, which results in reduced storage quality and marketability of fresh fruits and vegetables.[26] In the present study, pre-storage N_2 treatment for 6 h significantly inhibited increases in superoxide anion generation, membrane permeability and MDA content. These results suggest that short-term N_2 treatment might maintain membrane integrity by delaying superoxide anion generation, thereby inhibiting senescence and quality deterioration in cold-stored loquat fruit.

Effect of short-term N_2 treatment on SOD, CAT and LOX activities

SOD activity in both control and treated fruits increased initially with storage time, reaching a peak value on day 14, then decreased during the remainder of storage. Fruits exposed to short-term N_2 treatment maintained markedly higher SOD activity throughout storage (Fig. 3A). CAT activity in both control and treated fruits also increased initially during storage, reaching a peak value on days 7 and 14 respectively, then decreased afterwards. No significant differences in CAT activity were found between short-term N_2-treated and control fruits during the first 7 days of storage, but significantly higher CAT activity was observed in treated fruits compared with control fruits thereafter (Fig. 3B). The initial enhancement in CAT activity might be an adaptive response to cold storage or short-term N_2 treatment, while a more rapid increase in H_2O_2 content in the later storage phase might cause the decrease in CAT activity.

SOD and CAT are important ROS-scavenging enzymes. Superoxide can be converted into H_2O_2 by SOD, while H_2O_2 can be converted into water by CAT.[8] Oxidative stress, probably due to decreases in SOD and CAT activities and increase in LOX activity, might contribute to the development of senescence in loquat fruit in this study. Wang *et al.*[27] suggested that the effectiveness of high-oxygen treatment in delaying senescence of peach fruit may be due to a delay in the reduction of antioxidant enzymes during storage. Thus the delay in senescence of cold-stored loquat fruit by short-term N_2 treatment can involve alleviation of lipid peroxidation via enhancing antioxidant defence and suppressing oxidative damage.

LOX is a ubiquitous enzyme in eukaryotic organisms and results in the propagation of peroxidation.[28] It is considered to be partly responsible for the formation of superoxide ($O_2^{\bullet -}$) and singlet oxygen.[29] Therefore LOX is believed to be a major contributor to senescence-related membrane deterioration in a number of plant tissues.[5] As seen in Fig. 3C, control fruits exhibited a sharp increase in LOX activity over the first 14 days of storage, followed by a decrease, while short-term N_2-treated fruits showed a more stable and slower increase in LOX activity throughout storage. Increased LOX activity was found to be associated with enhanced lipid peroxidation in pepper fruit and kiwifruit.[10,30] In this study, short-term N_2 treatment significantly inhibited increases in LOX activity (Fig. 3C) and membrane permeability (Fig. 2B). These results suggest that short-term N_2 treatment might maintain membrane integrity by inhibiting LOX activity in loquat fruit.

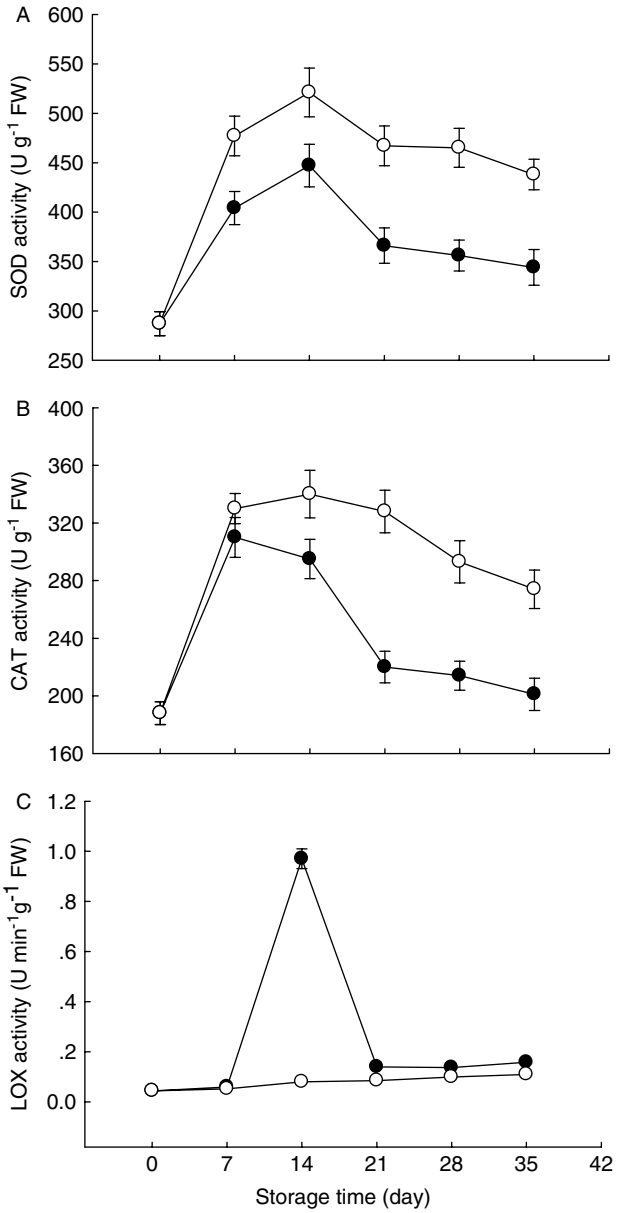

Figure 3. Effects of short-term N_2 treatment on (A) SOD, (B) CAT and (C) LOX activities of loquat fruit during storage at 5 °C for 35 days: ●, control fruits; ○, N_2-treated fruits. Data are expressed as means of triplicate assays. Vertical bars represent standard errors of means.

CONCLUSION

The results from our study suggest that pre-storage pure N_2 treatment of loquat fruit for 6 h can effectively reduce fruit decay and maintain taste quality. The reduction in fruit decay and quality deterioration by short-term N_2 treatment was correlated with enhanced antioxidant enzyme activities and delayed peroxidation of membrane lipids in cold-stored loquat fruit. As a non-chemical and inexpensive postharvest technology, this anoxia treatment deserves further development under commercial distribution conditions.

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