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Antioxidant enzyme activities in strawberry fruit exposed to high carbon dioxide atmospheres during cold storage

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Abstract

The effects of storage, in air or a 20% CO_2 in air (16.8% O_2) atmosphere for 12 d at 2 °C, on antioxidant enzymes of strawberry fruit (*Fragaria x ananassa* Duch. cv. 'Jewel') were investigated. The concentrations of acetaldehyde, ethanol and ethyl acetate associated with fermentation were measured, and the activities of peroxidase (POX), catalase (CAT) and superoxide dismutase (SOD) assayed. Ethanol, acetaldehyde and ethyl acetate concentrations increased in CO_2-treated fruit within a day of treatment, but more extensively after four days, while concentrations remained low in air-stored fruit. The total protein content extracted was not affected by CO_2 or storage time. Activities of POX were similar in air- and CO_2-treated fruit, with an increase occurring only in air-treated fruit on day-12. Neither CAT nor SOD activities were affected by CO_2 treatment. In summary, a 20% CO_2 storage treatment induced fermentation but did not significantly affect total antioxidant enzyme activities.

Keywords: Peroxidase; Catalase; Superoxide dismutase; Fermentative metabolism; Ethanol; Acetaldehyde; Ethyl acetate

1. Introduction

Exposure of strawberries to controlled atmospheres (CA) with elevated CO_2 (12–20%) can extend storage periods of this perishable fruit by maintaining firmness and other quality attributes and reducing decay (Almenar, Hernández-Muñoz, Lagarón, Catalá, & Gavara, 2006; Harker, Elgar, Watkins, Jackson, & Hallett, 2000). CO_2 treatments of fruit in pallet shrouds are also used during strawberry transport (Mitcham & Mitchell, 2002). Strawberry metabolism is also strongly affected by CO_2 in the storage atmosphere (Fernández-Trujillo, Nock, & Watkins, 1999; Holcroft & Kader, 1999a, 1999b; Zhang & Watkins, 2005), and cultivars vary greatly in tolerances to CO_2 treatment (Fernández-Trujillo et al., 1999; Pelayo, Ebeler, & Kader, 2003; Watkins, Manzano-Méndez, Nock, Zhang, & Maloney, 1999). One group, represented by 'Annapolis' and 'Cavendish', did not accumulate acetaldehyde and ethanol, while 'Honeoye', 'Jewel', 'Kent' and especially 'Governor Simcoe', accumulated high levels of acetaldehyde and ethanol after 3–9 d when treated with 20% CO_2 (Watkins et al., 1999).

Accumulation of fermentation products in fruit exposed to elevated CO_2 indicates that these treatments represent significant stresses to the tissues. However, no information exists about the effects of CO_2 on the antioxidative defence systems in strawberry fruit. Peroxidase (POX) activity decreases, particularly in the earlier stages of maturity, during strawberry ripening (Civello, Martinez, Chaves, & Añón, 1995). Exogenous ethylene treatment in strawberry increases ionic leakage, fruit water loss and POX activity (Tian et al., 2000). Better quality of heat-treated strawberry fruit than untreated fruit was associated with lower POX activity, and higher ascorbic acid peroxidase (APX) and superoxide dismutase (SOD) activities, although differences occurred later in storage (Vicente, Martínez, Chaves, &

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Civello, 2006). Other antioxidant enzymes, e.g., ascorbate peroxidase, slightly increased during cold storage and particularly after a subsequent shelf-life of 1–2 d at 20 °C in strawberry (Vicente et al., 2006).

Research with other fruits suggests an involvement of antioxidative enzymes in postharvest stress responses. In pear fruit, SOD activity increased with higher CO_2 treatments during storage, while increased APX and decreased CAT activities were associated with development of brown core (Pintó, Lentheric, Vendrell, & Larrigaudière, 2001). CA (2% O_2 and 5% CO_2)-stored pears had a sharp burst of APX and glutathione reductase activities immediately after storage, and later increases and decreases of SOD and CAT activity, respectively (Larrigaudière, Lentheric, Pintó, & Vendrell, 2001). In cherries, 12% CO_2 atmospheres show lower levels of POX activity during storage, irrespective of the O_2 concentration (Remón, Ferrer, López-Buesa, & Oria, 2004). In litchi, 70% O_2 treatments limited ethanol production of litchi flesh in the early period of storage and a slight decrease in POX activity was associated with pericarp browning (Tian, Li, & Xu, 2005). In peach, a storage treatment of 70% O_2/0% CO_2 for 15 d, then changed to 5% O_2/5% CO_2 at 0 °C, induced SOD and CAT activities (Wang, Tian, & Xu, 2005).

The objective of the current study was to investigate the activity of the antioxidative enzymes POX, CAT and SOD in response to elevated CO_2treatment of 'Jewel' strawberry fruit in relation to accumulation of fermentation products.

2. Materials and methods

2.1. Plant material and experimental design

Fruit of the strawberry cultivar 'Jewel' were harvested at the Cornell Orchard (Ithaca, NY) at the white tip/orange stage of maturity. Fruit were sorted quickly to remove any that were damaged or under- or over-ripe. 20-fruit samples were placed into 1–l glass jars at 2 °C for 3 h to cool the fruit. Jars, with lids fitted with inlet and outlet ports, were then attached to a flow-through system (50 ml min^{-1}) containing premixed atmospheres of air or 20% CO_2 in air. The gases were bubbled through water to humidify them, but the relative humidity was not measured. CO_2 and O_2 concentrations were measured by gas chromatography with a thermal conductivity detector equipped with a bridge current of 225 mA (Fisher Gas Partitioner, model 1200, Fisher Scientific, Springfield, NJ). The atmospheres at the inlet to jars were monitored at least once daily, and the outlet gas concentrations verified occasionally. The gas partitioner had a column temperature of 90 °C and injector temperature of 130 °C. Helium was used as the carrier gas with a flow rate of 30 ml min^{-1}. There were two columns arranged in series; 80/100 mesh Porapak Q (6′6″ × 1/8″ i.d. SS, Supelco, Inc, Bellefonte, PA) and 60/80 mesh Molecular Sieve 13X (11′ × 3/16″ i.d. SS, Supelco, Inc, Bellefonte, PA). The partitioner output was recorded on a Hewlett/Packard 3394 integrator (Agilent, Wilmington, DE). The gas chromatograph was calibrated with a standard mixture (4.97% CO_2 & 7.03% O_2 in a balance of N_2; Airgas, Inc, Radnor, PA). To obtain 20% CO_2, a constant stream of compressed CO_2 was added to a flow of compressed air through the use of needle valves. This resulted in an O_2 level of 16.8%. These concentrations were maintained within 1% of the desired levels throughout the experiment.

Jars with fruit were sampled in triplicate after the initial cooling period of 3 h, immediately after removal from storage, and after 1, 2, 4, 6, 8, 10 and 12 days. At each sampling time, calices were removed and approximately 100 g of berries were sliced immediately into liquid N_2. The frozen fruit were kept at −80 °C prior to analysis.

2.2. Antioxidant enzyme activities

Strawberry tissue (25 ± 0.1 g), 50 ± 1 ml of buffer, was ground in a Waring blender with a semi-micro stainless steel container (increasing the speed two or three times for 20 s maximum). The buffer (modified from Rao, Watkins, Brown, & Weeden, 1998) contained 0.2 M potassium phosphate buffer, pH 7.8, 2% PVP-40, 2 mM EDTA, 50 mM $CaCl_2$, and 1 mM phenylmethylsulfonyl fluoride (PMSF) dissolved in dimethyl sulfoxide (DMSO). The homogenate was transferred to a mortar kept in an ice bath for final grinding, then filtered through four layers of cheesecloth and collected. The pH was adjusted to 7.5 by adding HCl or NaOH solutions from 0.01 to 1 N while stirring in an ice bath, and then centrifuged at 25,000*g* for 30 min at 0–4 °C.

Acetone (−20 °C) was then added to the extracts in a ratio of 2:1 (v/w, ml acetone buffer per g of strawberry tissue). The extracts were kept at −20 °C for 10 min to precipitate proteins. Co-precipitation of pectin also occurred. The acetone extract was centrifuged at 10,000*g* for 10 min at 0–4 °C using Teflon tubes (Nalgene, Nalge Nunc International Corporation, Rochester, NY), and the supernatant discarded. The pellet was kept in an ice bath and dried using pure nitrogen flow. When no odour of acetone was detectable, the pellet was dissolved in a known volume of cold 0.1 M potassium phosphate buffer, pH 6.5 (usually 9 to 12 ml), using a vortex and an artist brush. This extract was stored in aliquots at −80 °C until used directly for enzymatic assays. Sometimes, Miracloth (Calbiochem, La Jolla, CA) was used to filter the final extracts and eliminate debris.

Protein content was determined according to Bradford (1976) using the standard assay of the BIORAD kit with BSA as a protein standard.

POX activity (EC 1.11.1.7) was assayed using guaiacol as the substrate (Rao et al., 1998). The assay mixture (3 ml) contained 100 mM phosphate buffer (pH 6.5), 13.3 mM guaiacol, and 500 µl of extract. A minimum of 25 µg of total protein was used per assay. The reaction was started by adding 10 µl of 30% H_2O_2 and mixing gently three times in each cuvette. The changes of absorbance at

470 nm were recorded for 5 min at 10 s intervals, using a Beckman DU-60 spectrophotometer (Fullerton, CA). Activity was usually measured at 60 min.

CAT activity (EC 1.11.1.6) was assayed, according to Aebi (1984), by measuring the decomposition of H_2O_2 at 240 nm. The assay mixture (1 ml in a quartz cuvette) contained 50 mM phosphate buffer (pH 7.0), and 3 mM H_2O_2, and 20 μg protein (about 40–100 μl of extract). Light yellow extracts are not useful for this assay, which requires really clear ones. Initial absorbance for the sample should be 0.5–0.9. CAT was usually calculated between 45 and 60 s.

SOD activity (EC 1.15.1.1) was assayed according to Asada, Takahashi, and Nagate (1974) and Rao, Paliyath, and Ormrod (1996) by measuring the reduction of ferricytochrome c. The following reagents, added sequentially, constituted the assay mixture of 3 ml: 50 mM sodium carbonate and sodium bicarbonate buffer (pH 10.2), 0.1 mM EDTA (pH 7.5), 0.1 mM xanthine, freshly prepared 0.015 mM ferricytochrome c (solution covered with aluminium foil) and 50 μl of crude strawberry extract. The assay was performed simultaneously in triplicate, using a blank containing 50 μl of 0.1 M potassium phosphate buffer at pH 7.5. The cuvettes were incubated at 25 °C for 3–4 min and the reaction initiated by adding 7.5–35 μl of fresh xanthine oxidase solution (Sigma grade 1). The volume was adjusted to obtain a change in absorbance for the control from 0.032–0.034 OD · min^{-1}, but changes of 0.029–0.040 OD · min^{-1} were found during the life of the fresh solution. The changes in absorbance were monitored at 550 nm for 5 min. One SOD unit was defined as the amount of enzyme that inhibited the rate of ferricytochrome c reduction by 50% (Asada et al., 1974).

2.3. Volatile analysis

Acetaldehyde, ethanol and ethyl acetate were determined by headspace analysis, as previously reported (Fernández-Trujillo et al., 1999).

2.4. Statistical analysis

Data were subjected to ANOVA using general linear model procedures for calculation of least squared means and LSDs by using Minitab software v 11.12 (Minitab, Inc., State College, PA). Factors considered were storage time and atmosphere. Data from enzyme activities were transformed to natural logarithms for ANOVA.

3. Results

Concentrations of the fermentation products, acetaldehyde, ethanol and ethyl acetate, remained low in fruit stored in air, but increased over time in CO_2-treated fruit (Fig. 1). Accumulations of these compounds were generally greater after day-4.

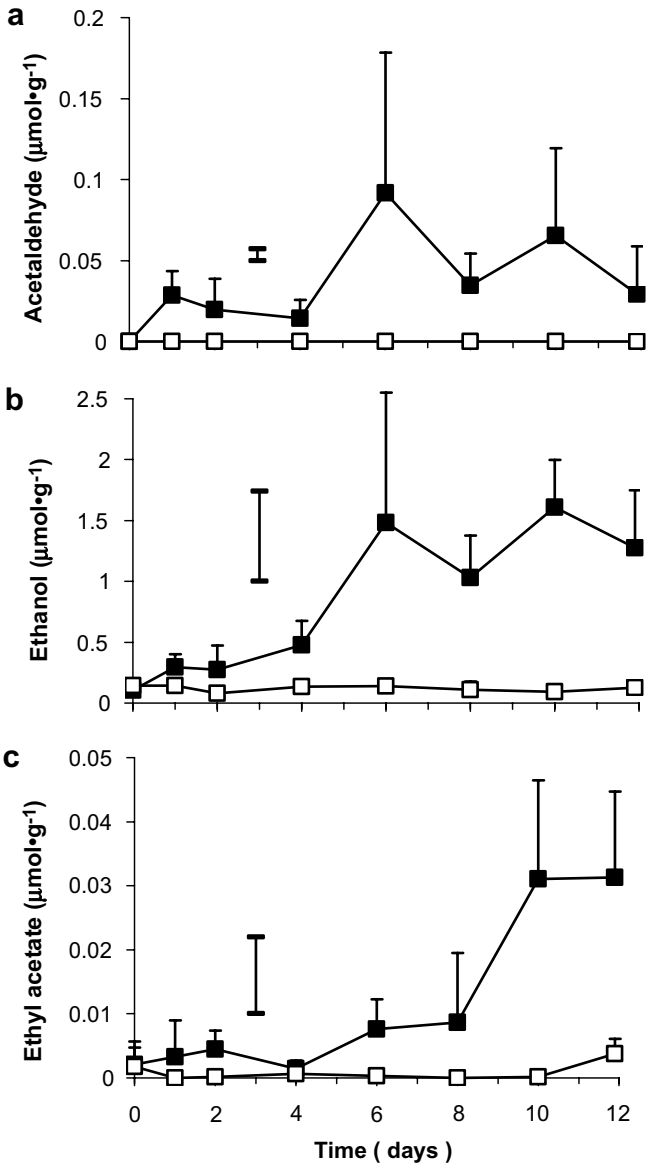

Fig. 1. Acetaldehyde (a), ethanol (b) and ethyl acetate (c) concentrations (μmol g^{-1} fresh weight) in strawberry cultivar 'Jewel' stored in air (open symbols) or 20% CO_2/16.8% O_2 (closed symbols) at 2 °C for up to 12 d. Vertical bars represent LSD values at $P = 0.01$ for the interaction time × atmosphere except in the case of acetaldehyde (LSD calculated for atmosphere).

Protein concentrations were not affected by atmosphere or storage time (data not shown), averaging 34 ± 10 μg/100 μl extract.

POX activity in strawberry was affected by time, atmosphere and the time × atmosphere interaction at $P<0.05$, but changes over time were inconsistent (Fig. 2a). However, activity of POX was much greater in air-stored fruit than in CO_2-treated fruit on day-12. CAT activity was not affected by CO_2 treatment (Fig. 2b), but declined to a minimum after 8 d of storage, confirmed by polynomial trend analysis (cubic and fourth-order storage time effects were significant at $P = 0.002$ and $P = 0.04$, respectively). No effects of time, atmosphere, or their interactions were

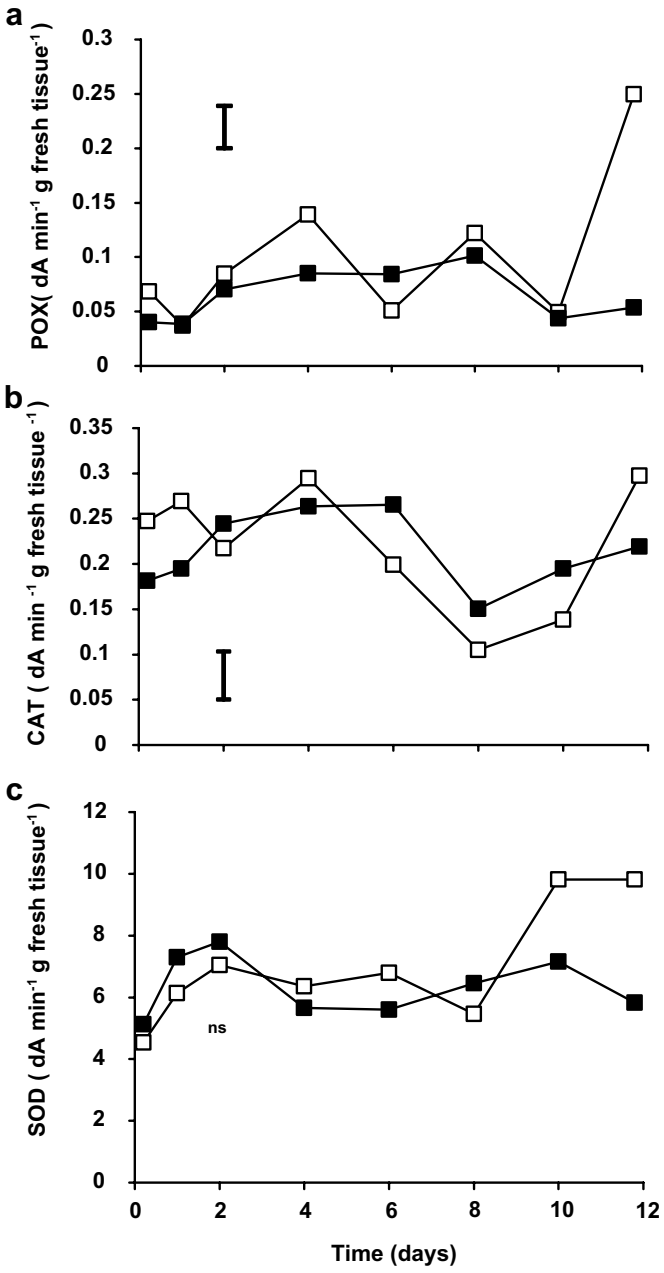

Fig. 2. Guaiacol-peroxidase (a), catalase (b), and superoxide dismutase (c) activities in fruit of strawberry cultivar 'Jewel' stored in air (open square) or 20% CO_2/16.8% O_2 (closed square) at 2 °C for up to 12 d. Vertical bars represent LSD values at $P = 0.01$ for the interaction time × atmosphere (peroxidase activity) or the time effect (catalase activity). Ns means no significant effects.

detected on SOD activity (Fig. 2c), the average activity being 6.7 ± 2.4 (SD) dA · g fresh tissue^{-1} min^{-1} overall.

4. Discussion

Accumulation of acetaldehyde, ethanol and ethyl acetate in response to CO_2 treatments is a common feature of strawberry fruit (Larsen & Watkins, 1995a, 1995b; Li & Kader, 1989), but the extent of accumulation is affected by cultivar, ripening stage and the period of exposure to the gas (Fernández-Trujillo et al., 1999; Pelayo et al., 2003; Watkins et al., 1999). The 'Jewel' strawberries are moderately resistant to CO_2 (Fernández-Trujillo et al., 1999; Watkins et al., 1999), as shown by major accumulations occurring only after 4 d of treatment (Fig. 1).

However, with one exception, we were not able to detect differences in activities of POX, CAT or SOD activities between treatments. Higher POX activity was measured on day-12 in air-treated fruit than in CO_2–treated fruit. This difference could be related to a delay in senescence by this treatment, as shown in apple fruit (Ingham, Parker, & Waldron, 1998). The possible role of isozymes that have been detected in strawberry (López-Serrano & Ros-Barceló, 1995) was not investigated in the current study, and such changes would not be detected by measurements of total activity. Civello et al. (1995) reported a decrease in POX activity during fruit ripening that was found primarily in a membrane-bound isoenzyme. Also, Holcroft et al. (1999a, 1999b) reported that inner and outer zones within the strawberry fruit exposed to high CO_2 may have different metabolisms that are not necessarily detectable by analyses of whole berries.

In conclusion, we have not been able to detect differences in antioxidant activities related to induction of fermentation in strawberry fruit due to high CO_2 atmospheres.

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References

Aebi, H. (1984). Catalase in vitro. *Methods in Enzymology, 105*, 121–126.

Almenar, E., Hernández-Muñoz, P., Lagarón, J. M., Catalá, R., & Gavara, R. (2006). Controlled atmosphere storage of wild strawberry fruit (*Fragaria vesca* L.). *Journal of Agricultural and Food Chemistry, 54*, 86–91.

Asada, K., Takahashi, M., & Nagate, M. (1974). Assay and inhibitors of spinach superoxide dismutase. *Agricultural and Biological Chemistry, 38*, 471–473.

Bradford, M. M. (1976). A rapid assay for the quantification of microgram quantities of protein utilizing the principle of protein-dye-binding. *Analytical Biochemistry, 72*, 248–254.

Civello, P. M., Martinez, G. A., Chaves, A. R., & Añón, M. C. (1995). Peroxidase from strawberry fruit *(Fragaria ananassa* Duch.): partial purification and determination of some properties. *Journal of Agricultural and Food Chemistry, 43*, 2596–2601.

Fernández-Trujillo, J. P., Nock, J. F., & Watkins, C. B. (1999). Fermentative metabolism and organic acid concentrations of strawberry fruit cultivars with different tolerances to carbon dioxide. *Journal of the American Society for Horticultural Science, 124*, 696–701.

Harker, F. R., Elgar, H. J., Watkins, C. B., Jackson, P. J., & Hallett, I. C. (2000). Physical and mechanical changes in strawberry fruit after high carbon dioxide treatments. *Postharvest Biology and Technology, 19*, 139–146.

Holcroft, D. M., & Kader, A. A. (1999a). Carbon dioxide-induced changes in color and anthocyanin synthesis of stored strawberry fruit. *HortScience, 34*, 1244–1248.

Holcroft, D. M., & Kader, A. A. (1999b). Controlled atmosphere-induced changes in pH and organic acid metabolism may affect color of stored strawberry fruit. *Postharvest Biology and Technology, 17*, 19–32.

Ingham, L. M., Parker, M. L., & Waldron, K. W. (1998). Peroxidase: changes in soluble and bound forms during maturation and ripening of apples. *Physiology Plantarum, 102*, 93–100.

Larrigaudière, C., Lentheric, I., Pintó, E., & Vendrell, M. (2001). Short-term effects of air and controlled atmosphere storage on antioxidant metabolism in Conference pears. *Journal of Plant Physiology, 158*, 1015–1022.

Larsen, M., & Watkins, C. B. (1995a). Firmness and concentrations of acetaldehyde, ethyl acetate and ethanol in strawberries stored in controlled and modified atmospheres. *Postharvest Biology and Technology, 5*, 39–50.

Larsen, M., & Watkins, C. B. (1995b). Firmness and aroma composition of strawberries following short-term high-carbon dioxide treatments. *HortScience, 30*, 303–305.

Li, C., & Kader, A. A. (1989). Residual effects of controlled atmospheres on postharvest physiology and quality of strawberries. *Journal of the American Society for Horticultural Science, 114*, 629–634.

López-Serrano, M., & Ros-Barceló, A. (1995). Peroxidase in unripe and processing-ripe strawberries. *Food Chemistry, 52*, 157–160.

Mitcham, E. J., & Mitchell, F. G. (2002). Postharvest handling systems: small fruits. Strawberries and cane berries. In A. A. Kader (Ed.), *Postharvest technology of horticultural crops* (3rd ed., pp. 364–370). Oakland: University of California, Publ. 3311.

Pelayo, C., Ebeler, S. E., & Kader, A. A. (2003). Postharvest life and flavor quality of three strawberry cultivars kept at 5 °C in air or air + 20 kPa CO_2. *Postharvest Biology and Technology, 27*, 171–183.

Pintó, E., Lentheric, I., Vendrell, M., & Larrigaudière, C. (2001). Role of fermentative and antioxidant metabolisms in the induction of core browning in controlled-atmosphere stored pears. *Journal of the Science of Food and Agriculture, 81*, 364–370.

Rao, M. V., Paliyath, G., & Ormrod, D. P. (1996). Ultraviolet-B- and ozone-induced biochemical changes in antioxidant enzymes of *Arabidopsis thaliana*. *Plant Physiology, 110*, 125–136.

Rao, M. V., Watkins, C. B., Brown, S. K., & Weeden, N. F. (1998). Active oxygen species metabolism in 'White Angel' x 'Rome Beauty' apple selections resistant and susceptible to superficial scald. *Journal of the American Society for Horticultural Science, 123*, 299–304.

Remón, S., Ferrer, A., López-Buesa, P., & Oria, R. (2004). Atmosphere composition effects on Burlat cherry colour during cold storage. *Journal of the Science of Food and Agriculture, 84*, 140–146.

Tian, S. P., Li, B. Q., & Xu, Y. (2005). Effects of O_2 and CO_2 concentrations on physiology and quality of litchi fruit in storage. *Food Chemistry, 91*, 659–663.

Tian, M. S., Prakash, S., Elgar, H. J., Young, H., Burmeister, D. M., & Ross, G. S. (2000). Responses of strawberry fruit to 1-methylcyclopropene (1-MCP) and ethylene. *Plant Growth Regulation, 32*, 83–90.

Vicente, A. R., Martínez, G. A., Chaves, A. R., & Civello, P. M. (2006). Effect of heat treatment on strawberry fruit damage and oxidative metabolism during storage. *Postharvest Biology and Technology, 40*, 116–122.

Wang, Y. S., Tian, S. P., & Xu, Y. (2005). Effects of high oxygen concentration on pro- and anti-oxidant enzymes in peach fruits during postharvest periods. *Food Chemistry, 91*, 99–104.

Watkins, C. B., Manzano-Méndez, J. E., Nock, J. F., Zhang, J., & Maloney, K. E. (1999). Cultivar variation in response of strawberry fruit to high carbon dioxide treatments. *Journal of the Science of Food and Agriculture, 79*, 886–890.

Zhang, J. Z. J., & Watkins, C. B. (2005). Fruit quality, fermentation products, and activities of associated enzymes during elevated CO_2 treatment of strawberry fruit at high and low temperatures. *Journal of the American Society for Horticultural Science, 130*, 124–130.