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Research note

Oxygen concentration affects chlorophyll fluorescence in chlorophyll-containing fruit☆

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Abstract

The effect of lowering O_2 concentration on chlorophyll fluorescence was continuously monitored in apple (*Malus x domestica* Borkh.), pear (*Pyrus communis* L.), banana (*Musa* L. Cavendish subgroup), kiwifruit (*Actinidia deliciosa* (A. Chev.) C.F. Liang et A.R. Ferguson), mango (*Mangifera indica* L.), and avocado (*Persea americana* Mill.) fruit, using a large surface-area sensor. In all of the six fruit, there were specific O_2 concentrations at which the F_o and F_v/F_m chlorophyll fluorescence values suddenly increased and decreased, respectively. When the O_2 concentrations were increased, the F_o and F_v/F_m signals returned to their previous values. Since the O_2 concentrations at which this phenomenon occurred were close to known low O_2 thresholds for these fruit, it may be that chlorophyll fluorescence can rapidly and non- destructively determine the lowest acceptable O_2 concentration for stored chlorophyll-containing plant products.

Keywords: Low-oxygen tolerance; Controlled atmosphere; *Malus x domestica* Borkh.; *Pyrus communis* L.; *Musa* L. Cavendish subgroup; *Actinidia deliciosa* (A. Chev.) C.F. Liang et A.R. Ferguson; *Mangifera indica* L.; *Persea americana* Mill.

1. Introduction

Applications of chlorophyll fluorescence techniques in postharvest physiology have been well documented (DeEll et al., 1999). One of the most interesting postharvest applications is the possible effect of low O_2 and/or high CO_2 storage atmospheres on chlorophyll fluorescence in stored apples (DeEll et al., 1995; Prange et al., 1997). Using the chlorophyll fluorescence terminology of van Kooten and Snel (1990), it has been reported that chlorophyll fluorescence (expressed as F_v/F_m or $((F_p - F_t)/F_p) \times 100$) can drop within 1–5 days in apples subjected to excessively low O_2 (1–2%) or high CO_2 (5% or greater) conditions, when measured in air immediately after treatment (DeEll et al., 1995; DeEll et al., 1998). Prange et al. (1997) changed the method of sampling by keeping fruit

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inside sealed glass jars and measuring both F_v/F_m and F_o through the glass while the fruit were under CA conditions. They observed that as the O_2 concentration inside the jars is reduced with N_2 flushing, the F_v/F_m and F_o signal of 'Elstar' apple, measured through the glass jar every 3–6 days, decreases and increases, respectively. Similar results were observed with 10% CO_2 (Prange, unpublished observations). These reports are limited by several factors: the small surface areas of the sensors could only measure a small portion of a single apple; the sensors could not measure repeatedly the exact same surface; and a lack of rapid hourly measurements to detect when F_v/F_m and F_o signals were first affected by the change in atmosphere. In order to overcome these problems a continuous chlorophyll fluorescence monitoring system which could measure several fruit, e.g. nine apples or a hand of bananas, was designed and constructed according to our specifications by Opti-Sciences Inc. (Tyngsboro, MA). This system also had a controlled-atmosphere (CA) capability as it could simultaneously monitor and control O_2 and CO_2 concentrations within each fruit chamber. The purpose of this study was to use this new system to determine the hourly changes in chlorophyll fluorescence in situ as oxygen concentration was lowered in six chlorophyll-containing fruits, i.e. apple, pear, banana, kiwifruit, mango and avocado.

2. Material and methods

2.1. Plant material

'McIntosh' apple and 'Bartlett' pear fruit were obtained from commercial orchards in the Annapolis Valley, NS. Green banana fruit, untreated with ethylene, were obtained from Atlantic Wholesalers, Moncton, NB. The kiwifruit, mango, and 'Hass' avocado fruit were obtained from the Atlantic Superstore, New Minas, NS. All fruit were firm, disease-free and of marketable quality.

2.2. Chlorophyll fluorescence

The chlorophyll fluorescence/CA system was connected to six chlorophyll fluorescence units and six fruit sample chambers, each consisting of a 4-l clear polyethylene sealable wide-mouth jar which could hold up to nine apples, eight pears, five bananas, 12 kiwifruit, three mangoes or three avocados (Fig. 1). The lid had a gas inlet and outlet connected to the CA sensors and computer. The sample jar was placed inside a triangular chlorophyll fluorescence unit, which had three fluorescence sensors, one at each internal angle of the triangle (Fig. 1). Initial trials showed that lowering the O_2 from 21 to 3.0% at 16 °C had little effect on chlorophyll fluorescence in all six fruit. Thus, the protocol for each fruit was designed to get the O_2 concentration lowered first to 3.0% and then begin fluorescence measurements. The method of lowering the O_2 concentration in the jars varied with each fruit type. Based on preliminary research and the limited availability of sensors and temperature-controlled rooms, O_2 concentration was lowered at 14–16 °C using fruit respiration in the banana, pear and kiwifruit trials with the CA system removing the CO_2 generated from respiration. For apple (3 °C), avocado (10 °C) and mango (14 °C), respiration did not rapidly lower the O_2 concentration so it was decreased to 3.0% with N_2 gas immediately after sealing. Then the O_2 was diminished at a programmed rate of 0.2% every 12 h for avocado and mango and 0.2% every 8 h for apple. Beginning in January of 1999, each fruit type was tested, one at a time. In each test, three jars were subjected to a lowering of the O_2 concentration as described above, and O_2 and chlorophyll fluorescence (F_o and F_v/F_m, where $F_v = F_m - F_o$) were recorded simultaneously every hour. The other three jars were air controls in which chlorophyll fluorescence was also measured.

3. Results and discussion

Since there was a consistent pattern in the chlorophyll fluorescence response to lowered O_2 concentration in all six fruit tested, only the pat-

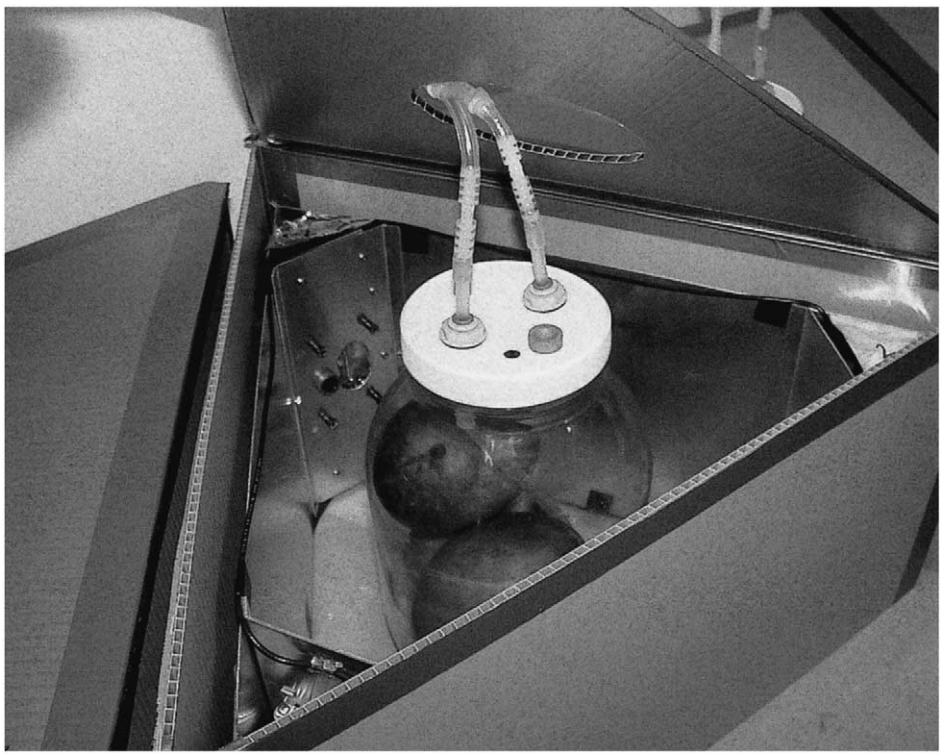

Fig. 1. One of the six triangular chlorophyll fluorescence sensor units with a sealed sample jar in the center containing mango fruit.

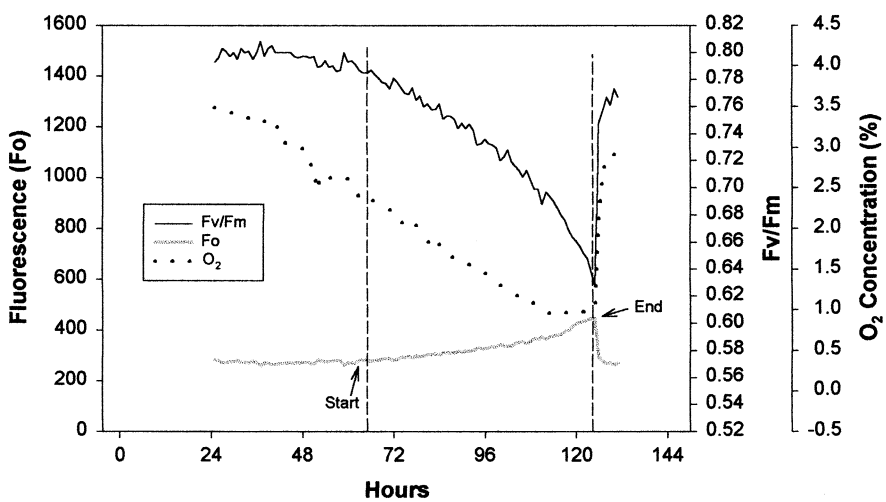

Fig. 2. Changes in apple fruit F_o and F_v/F_m chlorophyll fluorescence and O_2 concentration over time. 'Start' indicates a change in both F_o and F_v/F_m due to low O_2. 'End' indicates an increase in O_2 concentration and a corresponding reversal in F_o and F_v/F_m values.

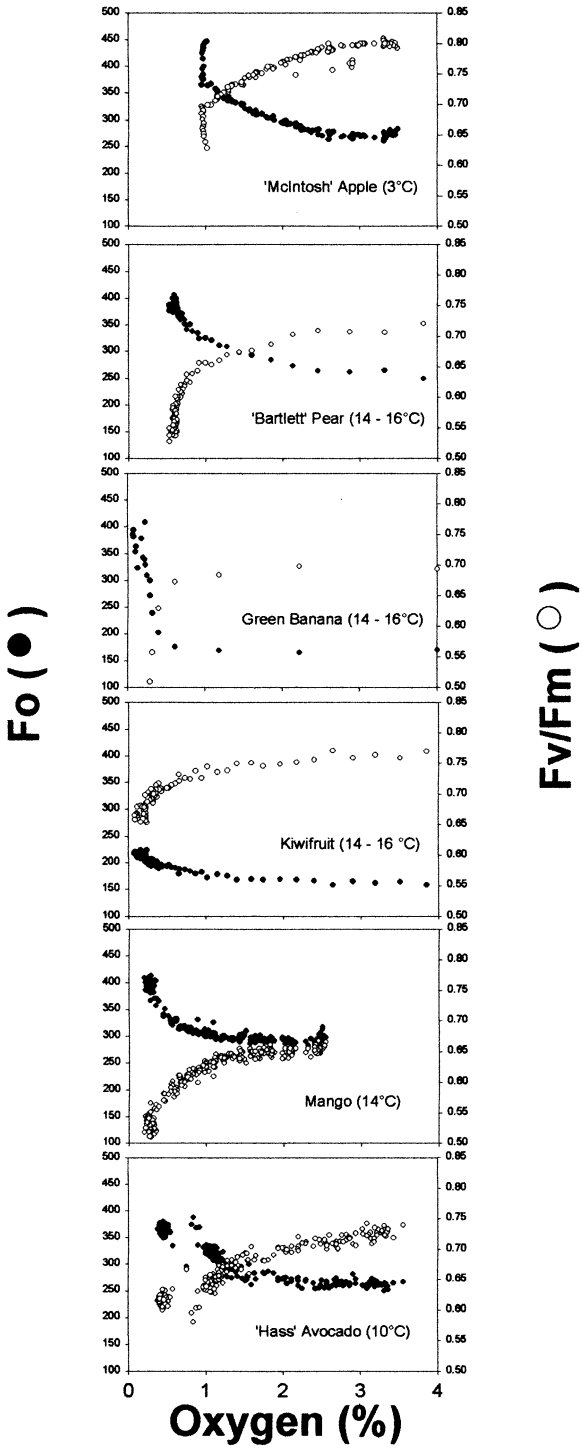

Fig. 3. Typical scatter plots of F_o (●) and F_v/F_m (○) vs. O_2 concentration using the data from a representative chamber of each fruit type as illustrated for apple (Fig. 2).

tern for apple is presented as an example (Fig. 2). As the O_2 concentration declined there was an increase in F_o with a concomitant decrease in F_v/F_m. Surprisingly, as the O_2 concentration was lowered for each fruit type, a unique concentration existed at which a rapid increase in F_o and a decline in F_v/F_m was observed. This previously unknown phenomenon, which is indicated as 'Start' in Fig. 2A, was not permanent, i.e. when the O_2 was increased, F_o and F_v/F_m returned to previous values, indicated as 'End' in Fig. 2. This phenomenon is more clearly seen in scatter plots of O_2 concentration versus F_o and F_v/F_m (Fig. 3). In Fig. 3, the data for each fruit type are from a single representative chamber as illustrated for apple (Fig. 2).

The results of this study indicate that there was a reversible change in F_o and F_v/F_m as O_2 concentration declined, confirming the previous report of Prange et al. (1997). However, as O_2 concentration declined, the increase in F_o and the decrease in F_v/F_m was not gradual. Surprisingly, F_o increased and F_v/F_m decreased suddenly at a particular low O_2 concentration. As previously suggested by Prange et al. (1997), the increase in F_o and decline in F_v/F_m with decreasing O_2 in this study could be due to one or both of the following: (1) the distance between the light harvesting complex (LHC) and the reaction center (RC) of photosystem II (PSII) in the thylakoid membrane increased as O_2 concentration decreased. As the distance increased, the probability of energy transfer decreased and the energy absorbed in the LHC has a higher probability of being fluoresced, increasing and decreasing the F_o value and the F_v/F_m value, respectively; or (2) reduction of Q_A, thus blocking electron flow through PSII, decreasing F_m and F_v/F_m, and subsequently increasing F_o. The second possibility seems less likely since F_m did not always decrease as O_2 concentration decreased (data not shown) and it does not explain the increase in F_o simultaneous with F_v/F_m.

The sudden change in F_o and F_v/F_m at a low O_2 concentration, as detected by the new continuous monitoring system, is a newly-observed low O_2 effect. Apparently, there is an O_2 threshold below which there is an increase in the separation of the LHC and PSII. The physicochemical explanation for this newly-observed phenomenon awaits further study. One important issue is to determine if

the low O_2 threshold for a sudden change in F_o and F_v/F_m is the threshold that is physiologically relevant to the retention of overall product quality. In other words, how similar is it to the minimum acceptable low O_2 reported in the CA literature, (Gorny, 1997; Kader, 1997; Kupferman, 1997; Reid, 1997; Richardson and Kupferman, 1997; Saltveit, 1997; Thompson, 1998). In four fruit types, the scatter plots of F_o and F_v/F_m versus O_2 (Fig. 3) suggest that the inflection points for both F_o and F_v/F_m occurred at O_2 concentrations similar to minimum acceptable low O_2 values reported in the literature for similar conditions, e.g. $<3\%$ at 3 °C ('McIntosh' apple), <1 to 1% at 15 °C (banana), <3 to 7% at 13 °C (mango), and <2 to 5% at 10 °C ('Hass' avocado). For 'Bartlett' pear and kiwifruit, the inflection points for F_o and F_v/F_m were at O_2 concentrations, between 2 and 3% and between 1 and 2.5%, respectively (Fig. 3), which may be higher than reported minimum acceptable low O_2 values, e.g. <1 to 2% at 0 °C ('Bartlett' pear) and <1 to 2% at 0 °C (kiwifruit). This may be due to the higher temperature (14–16 °C) used in this study for both the 'Bartlett' pear and kiwifruit, which could increase the respiration rate and demand for a higher O_2 concentration. With more sensors and replicated samples of each storage commodity, additional research could provide a predictive algorithm of F_o and/or F_v/F_m for maximum quality retention. Regardless of its biochemical cause, there is practical utility to this phenomenon. It may be a very sensitive, non-destructive method of dynamically controlling the O_2, and possibly the CO_2 environment, according to the unique requirements of each product. Thus, it offers a solution to a major constraint in improving the use of CA technology, i.e. assurance to the storage operator that the O_2, and possibly CO_2, concentrations are at the most appropriate levels for maximum quality retention.

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