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FRUIT RESPIRATION AND DETERMINATION OF LOW OXYGEN LIMITS
FOR APPLE (MALUS DOMESTICA, BORKH.) FRUIT

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Christopher David Gran

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FRUIT RESPIRATION AND DETERMINATION OF LOW OXYGEN LIMITS FOR APPLE (MALUS DOMESTICA, BORKH.) FRUIT

By

Christopher David Gran

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ABSTRACT

FRUIT RESPIRATION AND DETERMINATION OF LOW OXYGEN LIMITS FOR APPLE (MALUS DOMESTICA, BORKH.) FRUIT

By

Christopher David Gran

Low oxygen (O_2) storage of apple (*Malus domestica*, Borkh.) fruit is an important practice which slows respiration rates and maintains fruit quality longer than air storage. The experiments conducted employed modified atmosphere packaging (MAP) techniques to determine low O_2 limits of commercially stored apple cultivars over a range of temperatures. The low O_2 limit was determined from fruit respiratory quotient (RQ) breakpoint and level of ethanol (EtOH) in package headspace. Generally, as temperature increased, the low O_2 limit increased, however the extent of the effect was cultivar-dependent. CO_2 removal from package headspaces did not alter low O_2 limits. RQ breakpoint and elevated headspace EtOH were found to be useful tools for determining low O_2 limits. Low O_2 limits determined by elevated EtOH levels are in agreement with to recommended storage atmospheres, while values of low O_2 limits determined by the RQ breakpoint were slightly lower.

For Victor L. Leidel

1919-1988

If I had a choice
I'd break it
bury my memory
bones
wander off into the sea
and cast my lonely face
towards home
no crashing screams
no faded shore
I know too well the
nature balanced score

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Guidance Committee:

The journal paper format was chosen for this thesis in accordance with departmental and university regulations. The thesis is divided into 3 chapters in which the first has been accepted for publication in *Postharvest Biology and Technology*. The second and third chapters have been prepared according to format requirements for *Postharvest Biology and Technology* but have not been submitted.

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LIST OF SYMBOLS AND ABBREVIATIONS

A	surface area
atm	atmosphere
cm	centimeter
C	celsius
CA	controlled-atmosphere
C_2H_4	ethylene (ethene)
$Ca(OH)_2$	hydrated lime
CO_2	carbon dioxide
$[CO_2]_{atm}$	atmospheric carbon dioxide partial pressure
$[CO_2]_{pkg}$	package carbon dioxide partial pressure
EtOH	ethanol (ethyl alcohol)
$[EtOH]_{pkg}$	package ethanol level
g	gram
h	hour
H_2O	water
kg	kilogram
kPa	kilopascal
LDPE	low density polyethylene
MA	modified-atmosphere
MAP	modified-atmosphere packaging
min	minute
mmol	millimole
O_2	oxygen
$[O_2]_{atm}$	atmospheric oxygen partial pressure
$[O_2]_{pkg}$	package oxygen partial pressure
ppm	parts per million ($\mu l/l$)
P_{CO2}	partial pressure of carbon dioxide
P_{O2}	partial pressure of oxygen
P^{*}_{CO2}	carbon dioxide permeability coefficient
P^{*}_{O2}	oxygen permeability coefficient
P^{*}_{v}	water vapor permeability coefficient
RH	relative humidity
RQ	respiratory quotient
r	resistance ($s \cdot cm^{-1}$) of tissue to gas transport
r_v	resistance ($s \cdot cm^{-1}$) of tissue to H_2O vapor transport
r_{CO2}	resistance ($s \cdot cm^{-1}$) of tissue to CO_2 transport

LIST OF SYMBOLS AND ABBREVIATIONS (cont.)

r_{O2}	resistance ($s \cdot cm^{-1}$) of tissue to O_2 transport
r_{C2H4}	resistance ($s \cdot cm^{-1}$) of tissue to C_2H_4 transport
RR_{CO2}	rate of carbon dioxide production
RR_{O2}	rate of oxygen uptake
T	temperature (°C)
s	second
W	fruit weight
X	thickness of low density polyethylene
μl	microliter

LIST OF BOTANICAL NAMES

Common name	Botanical name
Apple	*Malus domestica*, Borkh.
Avocado	*Persea americana*, Mill.
Banana	*Musa*, L. sp.
Blueberry	*Vaccinium corymbosum* sp.
Cantaloupe	*Cucumis melo*, L.
Citrus	*Citrus*, L. sp.
Grapefruit	*Citrus paradisi*, Macf.
Mango	*Magifera indica*, L.
Onion	*Allium* sp.
Orange	*Citrus sinensis,* L. Osbeck
Peach	*Prunus persica*, L.
Pear	*Pyrus communicus*, L.
Pumpkin	*Cucurbita pepo*, L.
Strawberry	*Fragaria x ananassa*, Duch.
Tomato	*Lycopersicon esculentum*, Mill.

LITERATURE REVIEW

Fruit respiration, fruit storage, the internal atmospheres of fruit, and gas movement through bulky tissues have interested plant scientists for over 100 years. The movement of water vapor (H_2O), oxygen (O_2), carbon dioxide (CO_2), and ethylene (C_2H_4) through fruit cuticles has significant implications for the respiratory activity, ripening, and ultimately, the storability of bulky plant tissues. The purpose of this literature review is to investigate specific paths of gas movement in fruits and the physiology of low O_2 storage of fruits.

Transpirational vapor movement.

The characteristics of H_2O vapor movement through bulky plant tissues varies from the other biologically active gases (ie. O_2, CO_2, and C_2H_4). Resistance to H_2O transport (r_v) in 'Valencia' oranges (*Citrus sinensis*, L. Osbeck) has been measured at 110 $s \cdot cm^{-1}$, compared with transport resistance values of 5700, 6000, and 6900 $s \cdot cm^{-1}$ for CO_2 (r_{CO2}), O_2 (r_{O2}), and C_2H_4 (r_{C2H4}), respectively (Ben-Yehoshua, *et al.*, 1985).

Pieniazek (1944), examining apple fruit transpiration, found no correlation between cuticle thickness and rate of transpiration. Blocking of lenticels with paraffin reduced transpirational weight loss by 8 to 25%, while the removal of the wax coating from the cuticle using cheesecloth resulted in a 7% to 88% increase in transpiration rate. Increases in transpiration rates as low as 7% indicate incomplete removal of the wax coating.

Horrocks (1964), in his study of the waxes of apple fruits, concluded "...the cuticular wax is a prime factor determining the impedance to water vapour of an apple cuticle. It is possible that the density or geometry of the lenticels is important or it may be that the distribution or type of wax plays the dominant part". In addition, H_2O vapor permeability (P^*_v) of apple tissue was found to be similar to that for isolated peels.

Ben-Yehoshua *et al.*, (1985), using a scanning electron microscope (SEM), showed waxing to partially or completely plug open stomatal pores of 'Valencia' oranges, increasing the resistance to the transport of CO_2, O_2, and C_2H_4 by 140, 250, and 100%, respectively, while only increasing the r_v by 25%. Conversely, sealing individual fruits within high density polyethylene (HDPE) increased r_v by 1400%, while increasing r_{CO2}, r_{O2}, and r_{C2H4} by 72, 230, and 25%, respectively. Results indicate H_2O vapor moves through the cuticle by a pathway different from that of CO_2, O_2, and C_2H_4.

Schönherr (1976), examining the influence of cuticular waxes on P^*_v, found diffusion for H_2O through the cuticle membrane to be completely determined by the permeability coefficient of the cuticular waxes for citrus, pear (*Pyrus communicus*, L.) and onion (*Allium*, sp.). Schönherr and Schmidt (1979) found P^*_v to be independent of cuticle thickness. Further studies which consisted of the extraction of cuticular waxes increased P^*_v by 300- to 500-fold without cuticle thickness alterations of equal magnitude. Cuticular resistance is not a function of thickness, and therefore for isolated cuticles H_2O vapor movement does not obey Fick's Law of Diffusion, but is a function of the diffusional matrix and the diffusion coefficient resulting from cuticular composition.

Schönherr and Schmidt (1979) next tested the hypothesis that vapor pressure deficits alter cuticular permeability coefficients. Using isolated cuticles of citrus they determined P^{*}_{v}, transpiration (J_{ct}), and r_v, and found that each varied with both pH and H_2O vapor activity (a_{wv}), where a_{wv}=relative humidity (RH)/100. Based upon the observations of both citrus and artificial membranes, two models were developed for membrane transport (Schönherr and Schmidt, 1979).

Model I is a porous membrane, which consists of a lipid matrix containing H_2O-filled pores (Figure 1). These pores are such that pore length exceeds membrane thickness, and they form as a result of hydration of polar functional groups within the membrane matrix. Varying a_{wv} will both increase the driving force of diffusion and the flux of H_2O vapor, reducing the H_2O content of the

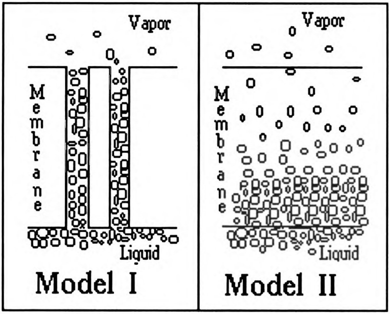

Figure 1. Transport models for lipid membranes. Model I. Porous membrane. Model II. Solubility membrane. Adapted from Schönherr and Schmidt, 1979.

membrane and, therefore, the P^*_v for the membrane. The effect of a_{wv} upon the diffusion coefficient can be described by:

$$P^*_v = \frac{D \bullet n \bullet \pi \bullet r^2}{\theta} \qquad (1)$$

where P^*_v is directly proportional to D, the diffusion coefficient (Schönherr and Schmidt, 1979). The relationship of the number of pores (n), and the square of the pore radius (r), are inversely proportional to diffusional path length (Θ). The driving force for diffusion is the sum of the vapor pressure gradients for each given gas. Experimental results indicated that for cuticles, pore number increased with increasing pH, while pore diameter was independent of pH (Schönherr, 1976). This model is believed to represent gas phase movement in apples of O_2, CO_2, and C_2H_4, but not H_2O vapor, with CO_2, O_2, and C_2H_4 gas movement facilitated by residually open lenticels.

Model II represents a solubility membrane, in which the molecules of the membrane are tightly packed and no pores develop which traverse the membrane. H_2O molecules move through the cuticle independently, and this movement is only accomplished with the breaking of all H bonds between neighboring H_2O molecules (Figure 2). Molecular movement through the membrane occurs in the gas phase, with transition to the gas phase occurring at the membrane/liquid phase interface, or the interior surface of the membrane structure. Because of the semi-closed nature of this system, diffusion and the permeability coefficient are constant and independent of a_{wv}, the H_2O vapor activity. The driving force for movement across the membrane is the partial pressure gradient for H_2O across the membrane.

The driving forces for H_2O vapor movement across the membrane in the two models differ in that Model I, the porous membrane, the driving force is the difference in H_2O potential between the inner and outer surface of the membrane, while in Model II the driving force is the partial pressure of H_2O vapor differences across the membrane. Data indicate that Model II is representative of H_2O vapor diffusion and transpirational resistance (Burg, 1990). This conclusion is based upon determinations of transpirational resistance for fruits ranging from 33 to 200 $s \cdot cm^{-1}$, compared with cuticular resistance to water of 30 to 290 $s \cdot cm^{-1}$ for the adaxial surfaces of hypostomatous leaves (Ben-Yehoshua *et al.*, 1985). Because r_v is not a function of cuticle thickness for isolated cuticles (Schönherr and Schmidt, 1979), a change in the resistance to gas exchange must occur with the isolation process.

In studies of transpirational H_2O loss and the openness of lenticels, apple (*Malus domestica*, Borkh,) peels stored under increased RH had less resistance to H_2O vapor loss (Anon., 1953). Models developed by Fockens and Meffert (1972) show loss of mass due to evaporation depends upon the cuticular resistance of the fruit, with cell shape and intercellular space affecting r_v. High RH results in H_2O uptake by the cell, circular shaped cells, increased intercellular spaces, and decreased diffusional resistance (r) of skin tissue, and visa versa. Schönherr and Schmidt (1979) have shown that transpirational H_2O loss, as well as gas phase diffusion, depended upon the barrier matrix and phase of transfer inherent for each tissue system.

The barriers to gas movement.

Maintaining O_2 levels sufficient for aerobic respiration in tissues at the center of fruit tissues, especially large fruits, poses an interesting problem. What are the driving forces for gas movement into and out of fruit tissues? What is the behavior of gas movement through fruit tissues, and what factors determine gas movement behavior? Are gas exchange characteristics static through the stages of maturation?

When examining a fruit, the most obvious barrier to gas exchange is the epidermis, or skin, of the fruit. Kidd and West (1934) hypothesized that cuticle permeability influenced fruit respiration during controlled-atmosphere (CA) storage. P_{CO2} and P_{O2} values were determined "...by dividing at corresponding times the rate of carbon dioxide-production by the concentration of carbon dioxide and by the oxygen deficit in the internal atmosphere respectively." (Kidd and West, 1949). Examination of the influence of apple skin permeability upon respiration determined low P_{CO2} decreased respiration rates. Apple cultivars varied in storage life, internal gas compositions, and susceptibility to storage damage. Time course data indicated cuticle P_{CO2} and P_{O2} did not change significantly through the climacteric, although after 60 days storage at 12° C cuticular r_{O2} increased more rapidly and to a greater extent than r_{CO2}. Similar increases in r_{O2} in comparison with r_{CO2} were noted by Trout *et al.*, (1942). For all cultivars examined, permeability to P^{*}_{O2} was less than P^{*}_{CO2}. Differences in P^{*}_{O2} and P^{*}_{CO2} within the fruit may directly influence storage disorders in CA storage.

Another factor thought to influence tissue permeabilities is tissue cell structure. Fockens and Meffert (1972), demonstrated that changes in cell structure were correlated with changes in RH. This underscores the importance of cell structure in relation to diffusional resistance. Increased RH results in H_2O uptake by the cell, swollen cells, and larger intercellular airspaces, and vise versa (Figure 2). Diffusional resistance is high when intercellular space is reduced and cells have a reduced cross-sectional width. Park (1990) has also shown a change in the gas exchange characteristics of fruit through development with variations thought to be directly influenced by the collapse of stomatal guard cells and the blocking of skin pores.

Trout *et al.*, (1942) tested the hypothesis that diffusional resistance of the fleshy tissues is negligible compared to cuticular resistance. Removal of the apple fruit's skin, O_2 levels increased from 6% to 20% while CO_2 levels decreased from 7.2% to 2.4%. Similar changes in O_2 and CO_2 concentrations were seen when small sections of the peel were removed. Based upon these results, Trout *et al.*, concluded that, for apples, determination of the cuticular resistance to diffusion is an accurate measure of a fruit's total diffusional resistance.

Biochemical analyses of cuticles have determined their structure to be a

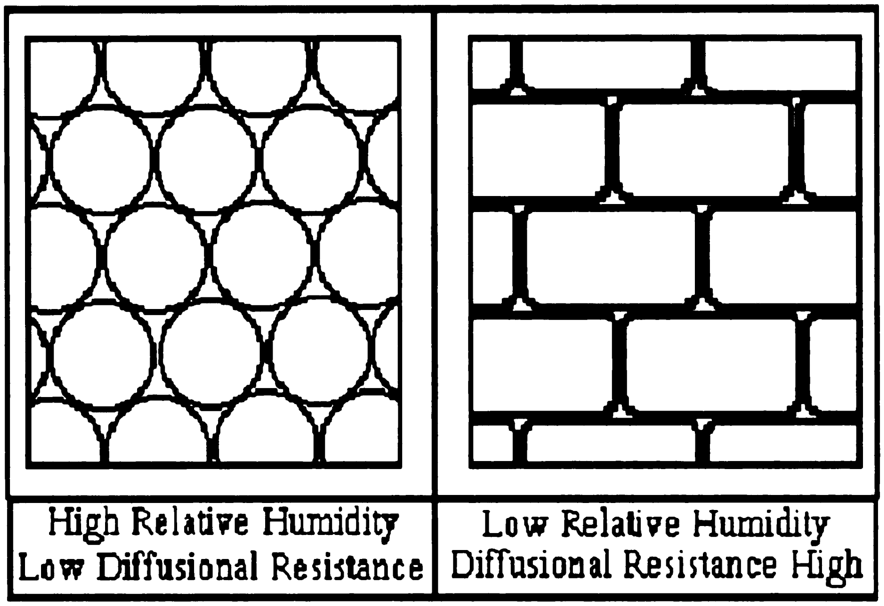

Figure 2. Structure of apple skin modeling the differences in cell structure and intercellular air spaces with changes in relative humidity. Adapted from Fockens and Meffert, 1972, J. Fd. Sci. Agric.

polymer membrane consisting of a cutin polymer matrix. This matrix contains imbedded lipids which are often referred to as waxes (Schönherr and Bukovac, 1973). These layers of lipids are oriented in a fashion parallel to the surface. P^*_{O2}, P^*_{CO2}, and P^*_{C2H4} may also change when the composition of the cuticle, or waxy layer, changes.

Gas movement through bulky fruit tissues.

Burg and Burg (1965) investigated gas movement and asked whether diffusion of gases through fruit tissues obeys Fick's First Law, which may be represented as:

$$\frac{ds}{dt} = \frac{-x \bullet \acute{A} \bullet D_i \bullet (C_{internal} - C_{external})}{T} \tag{2}$$

where ds/dt is the rate of transport, D is the diffusion coefficient, $C_{internal}$ and $C_{external}$ the concentrations of gas within and outside the fruit, respectively, T the effective thickness of the barrier to diffusion, 'A the surface area of the fruit, and x the fraction of the surface area through which gas exchange occurs (value from 0-1). This relationship will hold when the gases are at steady state. From this formula, a value *r*, the resistance coefficient for the barrier to a specific gas, can be generated. For a gas such a CO_2, which has an approximate $C_{external}$ value of 0, Fick's Law can be reduced to:

$$\frac{C_{internal}}{ds\ /\ dt} = \frac{-T}{x \bullet \acute{A} \bullet D} = r \tag{3}$$

Note that the resistance factor, *r*, when $C_{external}$ has a value of 0, divided by the rate of transport (ds/dt), is the inverse of the permeability factor used by Kidd and West (1934) in their determination of gas exchange rates, analogous to the relationship of resistance and conductance for the flow of current.

Diffusion of CO_2 and C_2H_4 for preclimacteric tissues of cantaloupe (*Cucumis melo*, L.) fruit (Lyons *et al.*, 1962), bananas and mangoes (*Magifera indica*, L.) were found to obey Fick's First Law (Burg and Burg, 1962). Climacteric avocado (*Persea americana*, Mill.) fruit (Ben-Yehoshua *et al.*, 1963) stored apples (Trout *et* *al.*, 1942), and post-climacteric bananas (Leonard and Wardlaw, 1941), however, do not. This indicates that a changed diffusional pathway or the barrier matrix to diffusion alters diffusional resistance during the course of the climacteric or aging. Cellular breakdown and the loss of cell turgor with cell senescence and the loss of water by transpiration, which then results in reduced intercellular airspace for the diffusional movement of gases into and out of the fruit, may be the cause of the deviation in results.

Studies using avocado and peach (*Prunus persica*, L.) by Rodriguez *et al.*, (1989) show the correlation between loss of flesh firmness and increased r_{O2}, r_{CO2}, and r_{C2H4}. The drastic changes in flesh texture and associated losses in unobstructed intercellular pathways have direct influences on gas exchange characteristics with fruit ripening. The dynamic nature of fruit maturation and ripening further complicate the mechanisms of fruit gas exchange and respiration.

Increases in the matrix barrier resistance, which would occur with cell wall softening, cellular membrane ruptures, and subsequent flooding of the intercellular airspaces of tissues and the pathways of gas diffusion, would result in an alteration in gas exchange properties. During flooding of intercellular air spaces, gas movement through this liquid matrix is substantially slower for O_2 and C_2H_4 when compared with CO_2 rates of diffusion. More importantly, flooding would reduce levels of O_2 available to internal cells because the diffusivity of O_2 in water is 10^6 less than in air (Burg and Burg, 1965). Examinations of the intercellular air space of apples have shown 30-35% of the total fruit volume consists of intercellular gas (Smith, 1947). Although a significant percent of the fruit's volume is open space, minor losses of intercellular space will reduce the continuous pathways for the unrestricted movement of gaseous O_2 and CO_2 through fruit tissues.

The rate limiting step in the outward passage of CO_2 and C_2H_4 gas occurs in a media where both possess nearly identical diffusivities (Burg and Burg, 1965; Ben-Yehoshua *et al.*, 1985). On the basis of solubilities of CO_2, C_2H_4, O_2, and water vapor in lipids, the rates of diffusion should be $H_2O > C_2H_4 > CO_2 > O_2$.

In an O_2 atmosphere enriched with N_2 or He diffusion of C_2H_4 through an apple peel mimics diffusion of C_2H_4 through pores in apple peels and paper of a known diameter. The dependance of CO_2 and C_2H_4 gas exchange upon atmospheric pressure and gas composition supports the theory that movement of CO_2 and C_2H_4 through apple peels occurs through air-filled pores, with open lenticels an obvious candidate for this passageway (Burg and Burg, 1965).

The driving force for gas movement of O_2, CO_2, C_2H_4, and N_2 would be partial pressure gradients, although the specific paths for gas movement differ. Mathematical models, based upon Fick's Law of Gas Diffusion, as well as other models related to gas phase movement of O_2, CO_2, H_2O, and C_2H_4 have indicated that water vapor moves preferentially through the cuticle of the fruit in the liquid state, while other gases move preferentially through available pores of the tissue. Partial pressure gradients for O_2, CO_2, and C_2H_4 gas allow for mass transfer of these molecules through the tissue.

Tissue gas gradients.

Rajapaske *et al.*, (1989) have found that O_2 concentration gradients exist from fruit centers to epidermal tissue. Gas gradients varied with cultivars and were dependant upon the respiration rate and volume of intercellular airspace. Solomos (1987) has also reported CO_2 gradients within apple tissues. The existence of O_2 and CO_2 gradients between tissue areas indicates that in certain cases tissue resistance to diffusion may not be negligible, especially under conditions of reduced atmospheric O_2 levels, when the existence of O_2 partial pressures 0.1% below that maintained in the atmosphere may result in fermentation and skin discoloration (Lau, 1989).

Pathways for gas movement.

Devaux (1891), after conducting experiments involving forcing gas through submerged tissues, postulated that gases pass through both pore openings and through the cuticle, with movement through the cuticle possible in both the free and dissolved state. Devaux suggested movement of gases depended on both the permeability and the porosity of the peridermic membrane, and that O_2 enters pumpkin (*Cucurbita pepo*, L.) fruit primarily through pores and CO_2 exits primarily through the membrane. Although these experiments were conducted well before determination of cuticle chemistry and the advent of gas chromatography, it is very interesting that data from recent experiments examining gas exchange support Devaux's conclusions.

Burg and Burg (1965) concluded that gas exchange in apples and other fruits was governed by Fick's law of diffusion, with the peel of apple fruit providing the primary resistance to gas exchange. CO_2 and C_2H_4 had similar diffusivities, with exchange dependent upon atmospheric pressure and gas composition. The diffusion barrier for CO_2 and C_2H_4 exchange was considered to consist of air filled pores, with indirect measurements of pore size in agreement with the expected size and number of lenticels present on the apple cuticle. Calculations of barrier thickness using Fick's Law generated estimated cuticle thicknesses similar with actual measurements.

Examinations of stomatal density and responsiveness for banana (*Musa* sp.) fruit have shown fruit stomatal density to be 30 times less than that for leaf tissues, averaging approximately 450 stomates/cm^2, with residual opening being influenced positively by high RH conditions and light exposure treatments (Johnson and Brun, 1966). Other anatomical studies indicate 1.8 lenticels/cm^2 of apple fruit cuticle (Clements, 1935), compared with 40,000 stomates/cm^2 for apple leaf tissue (Curtis and Clark, 1950). Consideration of these figures point to the reduced avenues of gas phase diffusion in fruits relative to leaves. For citrus (*Citrus*, L.) fruits, when diffusion coefficients are considered, residual stomatal opening of less than 0.4% initial open pore area is sufficient to account for the needed residual gas exchange through these means (Ben-Yehoshua *et al.*, 1985). Therefore, in certain fruits, gas exchange does not occur solely in the dissolved state through the cuticle. Some gases move through pores in the cuticle.

Ben-Yehoshua *et al.*, using oranges and grapefruit (*Citrus paradisi*, Macf.), examined the effect of waxing and sealing of fruits with high density polyethylene (HDPE) films and indicated mass transport of H_20 vapor and other gases occurs by different mechanisms. Values of r_{CO2}, r_{O2}, and r_{C2H4} are similar for untreated fruits, while the r_v is 60-100 times less (Ben-Yehoshua *et al.*, 1985). Waxing of fruits inhibits CO_2, O_2, and C_2H_4 transport but not H_2O vapor, while wrapping fruits with HDPE film mainly restricted H_2O transport. Waxing is thought to restrict non-H_2O vapor transport by blocking of open stomates and lenticels, while not effecting H_2O vapor transport due to the tendency of the wax layer to pit and crack. An increase of 1,400% in r_v was seen with HDPE wrapping compared with increases of 72, 230, and 25% for r_{CO2}, r_{O2}, and r_{C2H4}, respectively. These results arise from differences in the selective permeability of the fruit cuticle and the barrier to transpirational H_2O loss created by the film.

Cameron and Yang (1982) determined coefficients of diffusion for bulky plant tissues by means of loading of ethane (C_2H_6) gas, allowing this system to come to steady-state, and then measure gas efflux levels in order to determine specific paths of gas movement and each pathway's diffusion coefficient. Cameron and Yang determined 94, 81 and 67% of C_2H_4, CO_2, and H_2O vapor gas exchange in tomato (*Lycopersicon esculentum* Mill., Cv. 'Ace') fruit occurred through the stem scar, and that cuticular r_v was 1,000 times less than r_{CO2}, r_{O2}, and r_{C2H4}. The calyx end of 'Golden Delicious' apple fruit account for 29, 24 and 2% of C_2H_4, CO_2, and H_2O vapor exchange, respectively. On this basis Cameron and Yang concluded that lenticular gas exchange is insignificant for total fruit gas exchange.

It should be noted, however, that the physical structure of 'Golden Delicious' apple fruit, used by Cameron and Yang, vary significantly when compared with 'McIntosh' apples, which were used in the studies of Burg and Burg (1965). While the calyx end of 'Golden Delicious' is usually quite open and free to gas exchange, this is often not the case, especially with 'McIntosh'. It should also be noted that for 'Golden Delicious' lenticels are numerous and raised, and the fruit lack an apparent waxy bloom, which is quite opposite 'McIntosh' fruit.

Recent experiments by Park (1990), involving blocking of the lenticels of 'McIntosh' apples also support Burg and Burg's conclusion that for apple fruit, lenticels are the primary pathway for gas exchange, excluding water vapor exchange. Park also indicated that resistance to gas exchange is a factor of lenticel structure, not strictly related to lenticel density.

Respiration and low oxygen tolerance of apple fruits.

Rate of respiration affects storage life, value, and quality of stored fruits. The rate at which stored carbohydrates are converted by respiration to CO_2 may influence the length the stored fruits maintain marketable quality. In the study of low O_2 storage and the low O_2 limits of fruits, the influence of temperature, RH, and the influence of elevated CO_2 levels upon respiration are very important because of their abilities to alter respiratory activity and gas movement. Knowledge of these factors is vital to understanding those factors which affect respiration and the internal gas composition of the fruit present for respiration.

The fruit respiration studies initiated by Kidd and West, and continued by

Fidler and North, at the Low Temperature Research Station at Cambridge, England established many of the fundamental characteristics of climacteric fruit respiration, testing the influence of temperature, RH, and atmosphere composition on respiratory rates.

Decreased storage temperatures are known to lower respiration rates, slow metabolic processes in tissues, and maintain marketable value and edible qualities. With low temperature or high CO_2 damage (Fidler and North, 1964) respiration rates increase in comparison with apples at the same temperature. Respiratory quotients (RQ) increased with decreasing temperatures, from 1.3-1.4 at 38°C to 1.6-1.7 at 32°C (Fidler and North, 1966). Lower temperatures (0°-3.5°C) alter the respiratory substrate from the expected 1:1 ratio of carbon (C) lost to C evolved (Fidler and North, 1968).

Kidd and West (1933) have shown respiration rates of apple tissue can be increased with elevated O_2 partial pressures (P_{O2}) (50-100%) or decreased with lowered P_{O2} (5%). Elevated CO_2 partial pressures (P_{CO2})(5-10%) delayed the respiratory climacteric. With further P_{CO2} increases, continued decreases in respiration rates were observed.

Hardy (1949) found internal P_{CO2} concentrations in apple fruit vary with temperature, from 1.7% to 13.2% for 0° to 25°C respectively, and respiration rates varied as well. This reflects an increase in respiration rates relative to increased tissue permeability with increased temperatures.

The RQ for apple tissue is unaffected by fluctuations of P_{O2} from 2-21%, but is lowered with progressive increases in P_{CO2} (Fidler and North, 1964). When stored in CO_2-free atmospheres, the RQ can range from 1.5 to 1.8, with peaks of 3.0 at 0-2°C. Results using modified atmosphere packaging (MAP) follow these patterns, with RQ's ranging from 1.3 to 1.5 for the 9 cultivars tested at 0.1°C (Gran and Beaudry, 1993).

For apple fruit metabolism, malic acid synthesis occurs with exposure to high P_{CO2} concentrations, which may occur with climacteric respiration (Anon., 1950). Addition of malic acid to post-climacteric fruits will increase CO_2 production without an increase in O_2 uptake, but only in the presence of O_2, thus increasing the RQ (Anon., 1955). Pyruvic acid also has this effect in both pre- and post-climacteric fruit, when O_2 is present or absent. A saturation of enzymes converting pyruvate and malic acids into the Krebs cycle is indicated. Hulme (1961) hypothesized that the CO_2 climacteric results from increased activity of malic enzyme and pyruvic carboxylase, which should not result in an equivalent rise in O_2 uptake, thusly creating the observed increase in RQ during the climacteric. The RQ for apples through the climacteric, however, has been observed to remain rather constant at 1.1 to 1.3 at 7 to 12°C (Fidler and North, 1965).

Storage of fruits at P_{O2} levels below the low O_2 limit will lead to low O_2 damage, characterized by glycolytic conversion of pyruvate to acetaldehyde and ethanol, which can lead to the production of off-flavor compounds and visible tissue damage. Fruits with low O_2 damage have noticeable buildups of EtOH in the damaged areas, along with tissue browning.

Park (1990) has shown respiration is negatively correlated with resistance to gas exchange at picking. It may be the case that respirational gas exchange is restricted within those tissue areas highly susceptible to low O_2 damage, creating anaerobic conditions which lead to fermentation of tissues. We have observed that low O_2 damage of fruits generally occurs in areas of the fruit having smaller cell size and decreased intercellular air spaces. With extended storage under anaerobic conditions, the products of fermentation build up to levels toxic for normal cellular functions, with cell death and tissue browning the result.

Gas exchange characteristic are intricately related to physical characteristics of the tissue cell mass. Changes in physical characters have been seen to influence physiological processes during ripening. Maximum respiration of bananas is achieved only when RH levels exceed 80% (Grierson and Wardowski, 1978). Lowered RH is also known to decrease storage breakdown of 'Jonathan' apples, as well as decrease volatiles and flavor components of bananas and mangos and increase strawberry (*Fragaria x ananassa*, Duch.) and pear flavor development (Grierson and Wardowski, 1978).

The anaerobic extinction point.

The examination of low O_2 limits and the effects of limiting P_{O2} on O_2 uptake, CO_2 production, and the various indicators of increased tissue EtOH integrate those properties defined as the anaerobic extinction point (Blackman and Parija, 1928; Parija, 1928; Blackman, 1928). The anaerobic extinction point was defined as the theoretical "point ... such that just enough oxygen enters the cells to convert the whole product of D (terminal substrate of aerobic carbohydrate metabolism) to OA + OR (the final oxidation products) and there is no longer any NR (the product of nitrogen respiration) production. This marks a definite physiological state...(and we are conducting experiments in order to locate the) external concentration of oxygen that, for a given tissue, coincides with this ‘extinction point’ of NR." Blackman and Parija further define the anaerobic extinction point by stating "One physiological index of it, in a living apple, may be that this point there is a minimum production of CO_2. Incidentally it has the significance that at this value there is presumably no longer any accumulation of alcohol in the tissues." (Blackman, 1928). It is by the physiological indicators of reduced O_2 uptake, minimal CO_2 production, and tissue EtOH production that we may determine the anaerobic extinction point. Although Blackman and Parija could not specify the substrates and products of aerobic and anaerobic respiration, the definition is useful for a better understanding the physiological considerations of CA and MA storage.

The processes of fruit respiration, ripening, and senescence in storage are influenced by a complex interaction of physical properties and physiological processes. Low O_2 limits and the physiology of CA and MA stored fruits depend upon the interaction of tissue gas exchange characteristics, metabolic and cellular changes with maturation and ripening, and changes in tissue properties with maturation and ripening. The following studies examine low O_2 limits, considering the factors of gas exchange and physiological processes in MA storage, in order to better understand the requirements of low O_2 storage.

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Chapter 1

Determination of the Low Oxygen Limit for Several Commercial Apple Cultivars by Respiratory Quotient Breakpoint

ABSTRACT:

Low oxygen (O_2) limits for apple (*Malus domestica*, Borkh.) fruit were determined using modified-atmosphere packaging (MAP) techniques. Fruits were sealed in low density polyethylene (LDPE) packages and placed at 0.1°C until steady-state respiration was reached. Steady state O_2 and CO_2 partial pressures were varied by altering package thickness, package surface area, and the total fruit weight within the package. Based on measured permeabilities of the LDPE packages to O_2 and CO_2, package partial pressures were used to determine the gas flux for O_2 and CO_2 for each package, with gas flux rates representing respiratory rates. The effect of O_2 partial pressure on O_2 uptake, CO_2 production, and the respiratory quotient (RQ) was then determined. As the O_2 partial pressure decreased to approximately 2 kPa, RQ remained relatively constant. A marked increase in the RQ (the RQ breakpoint) occurred below 2 kPa O_2, which was associated with elevated EtOH concentrations. The low O_2 tolerance limit was then estimated for each cultivar as the O_2 level at the RQ breakpoint; these values ranged from 0.7 kPa O_2 for cultivars 'Red Delicious' and 0.8 kPa for 'Law Rome' to approximately 1.9 kPa for 'McIntosh'.

INTRODUCTION:

Low oxygen storage of apple fruit is an important commercial practice that slows respiration rates and maintains fruit quality longer than air storage. The storage experiments of Kidd and West (1927, 1933) established that both reduced O_2 (5 kPa) and elevated CO_2 concentrations (5-10 kPa) delayed and reduced the magnitude of the climacteric rise in respiration (1 %=1.0135 kPa @ 1 atm). Fidler and North (1966, 1967 and 1968) have shown that the respiratory quotient (RQ) for apple tissue is unaffected by variations in O_2 partial pressures ranging from 21 kPa down to 2 kPa, while being lowered with progressive increases in CO_2 partial pressures.

If O_2 levels fall below those supporting aerobic respiration, glycolytic conversion of pyruvate to acetaldehyde and EtOH occurs. The O_2 level at which tissue fermentation is induced may be taken as the lower O_2 limit. Although tissue tolerance to anaerobic conditions is variable, extended exposure to these conditions leads to tissue fermentation, browning, and a loss of economic value.

Commercial storage at O_2 levels below approximately 3 kPa was formerly limited by the technological limitations of controlled atmosphere facilities. Recent improvements in room construction, atmosphere generation and the control and sensing of O_2 and CO_2 partial pressures facilitates the regulation of gas levels to approximately ±0.1 kPa. As a result of these improvements, storage at partial pressures as low as 0.7 kPa for 'Red Delicious' for 7 or more months is being conducted with minimal fruit loss (Lau, 1989). Minimizing the O_2 partial pressures at which fruit are stored increases the necessity for an accurate determination of the lower O_2 limit.

Establishment of the lower O_2 limit for stored fruits has previously been accomplished empirically via a gradual decrease in the storage O_2 partial pressure until intolerable storage damage occurred. Each commodity and new cultivar required a large investment in time, equipment and materials to establish the lower O_2 limit for successful long term storage. We describe a method for determining the lower O_2 limit that is rapid, relatively simple, requires a minimal number of fruit and is based on the measurement of physiological responses, rather than empirical observations. This method measures the commodity's RQ and the dependence of RQ upon O_2 partial pressure. This information may then be used to establish the lower O_2 limit, as defined by the O_2 level at the upswing in the RQ (RQ breakpoint), as O_2 levels become limiting to aerobic respiration. This article reports upon the determination of low O_2 limits using this method for 9 commercially stored apple cultivars at 0°C.

MATERIALS AND METHODS:

Apple fruits of the cultivars 'Empire', 'Red Fuji', 'Golden Delicious', 'Ida Red', 'Jonathan', 'Law Rome', 'McIntosh' (strain MacSpur), 'Northern Spy', and 'Red Delicious' were harvested at the preclimacteric stage as monitored by internal ethylene levels and stored at 1°C under 1.5 kPa O_2 and 3.0 kPa CO_2 and packaged 1 to 2 weeks later.

As described by Cameron *et al*., (1989), a range of package headspace atmospheres with steady-state O_2 and CO_2 partial pressures was produced by varying film thickness, surface area and total fruit weight. Four thicknesses of low density polyethylene (LDPE) (DOW Chemical Co., Midland, MI) (0.00254, 0.00508, 0.00762, and 0.0116 cm) and two surface areas (450 and 1125 cm^2) were used. Total fruit weight ranged from approximately 100-250 g and 450-800 g for the 450 and 1125 cm^2 packages, respectively.

Fruits were removed from storage, weighed, and heat-sealed into LDPE packages. A septum consisting of cured silicone rubber (General Electric Clear Silicone II) dolloped on vinyl plastic electrical tape (Scotch, 3M Super 88) was attached to each package for gas sampling (Boylan-Pett, 1986). Packaged fruits were left at room temperature for approximately 1 day to maintain high respiratory rates and rapidly decrease O_2 levels towards the range of expected steady-state partial pressures. Fruits were then placed at 0.1°C and gas partial pressures were monitored until steady-state respiration was reached 50-70 days later. Gas samples (100 μl) were drawn from each package through the self-sealing silicone septum using a 0.5 ml insulin syringe. Gas samples were analyzed for O_2 (Servomex Paramagnetic O_2 Transducer, Series 1100, Servomex Co., Sussex, England) and CO_2 (ADC analytical infra red CO_2 Analyzer, 225-MK3, Analytical Development Co., Hoddesdon, England) in series, with N_2 as the carrier gas (flow rate = 100 ml • min^{-1}). Gas samples were drawn from 4 or less packages at a time and immediately analyzed, in order that samples not be contaminated by atmospheric gases. This was especially important for those packages with very low O_2 levels.

Respiratory rates were determined according to the following equations:

$$RR_{O_2} = \frac{\frac{P_{O_2} \bullet A}{X} \bullet ([O_2]_{atm} - [O_2]_{pkg})}{W} \tag{1}$$

$$RR_{CO_2} = \frac{\frac{P_{CO_2} \cdot A}{X} \cdot ([CO_2]_{pkg}-[CO_2]_{atm})}{W} \qquad (2)$$

where RR_{O_2} and RR_{CO_2} are the rates of O_2 uptake and CO_2 production ($mmol \cdot kg^{-1} \cdot h^{-1}$); P_{O_2} and P_{CO_2} are O_2 and CO_2 permeability coefficients measured for the LDPE at 0°C ($mmol \cdot cm \cdot cm^{-2} \cdot h^{-1} \cdot kPa^{-1}$); A is the surface area (cm^2); X is the thickness of LDPE (cm); $[O_2]_{atm}$ and $[O_2]_{pkg}$ are the external atmosphere and package O_2 partial pressures (kPa); $[CO_2]_{pkg}$ and $[CO_2]_{atm}$ are the package and external atmosphere CO_2 partial pressures; and W is fruit weight (kg). Permeabilities of the LDPE film packages to O_2 and CO_2 were determined as described previously (Beaudry *et al.*, 1992). The effect of O_2 partial pressure on O_2 uptake, CO_2 production, and the RQ was then determined using equations 1 and 2. Rates of CO_2 production as calculated using MAP techniques have been verified in a flow through system using carrot tissue (Lennington and Beaudry, unpublished data).

EtOH headspace concentrations were determined by gas chromatography (Carle GC with 45.7 cm column, .32 cm bore, Haysep N packing, at 120° C with gas flow rates of 40, 40 and 200 $ml \cdot min^{-1}$ for H_2, He and air, respectively).

RESULTS AND DISCUSSION:

Steady state headspace O_2 and CO_2 partial pressures ranging from 16.0 to 0.2 kPa O_2 (Figure 1) and 1.0 to 18.0 kPa CO_2 (data not shown) were generated. As O_2 partial pressure decreased to approximately 2 kPa, the RQ remained practically constant. Rapid increases in RQ were seen below 2.0 kPa O_2. Low O_2 limits were estimated for each cultivar at the RQ breakpoint, and ranged from 0.8 kPa for 'Northern Spy' to approximately 2.0 kPa for 'McIntosh'. CO_2 partial pressures ranged from 6.0 to 18.0 kPa for those packages with elevated RQ values.

Whereas the RQ remained rather constant from 16 to 2 kPa O_2 for all cultivars, the rate of O_2 uptake varied among cultivars as steady-state O_2 levels decreased (Figure 2). For instance, while O_2 uptake for 'Northern Spy' decreased slightly from 16 to 2 kPa O_2, O_2 uptake for 'Law Rome' decreased steadily.

Measurement of EtOH headspace levels for 'Law Rome' showed fruit with O_2 partial pressures below the RQ breakpoint exhibited elevated headspace EtOH concentrations (Figure 3), as well as detectable levels of acetaldehyde (data not shown). Packages with O_2 partial pressures above the RQ breakpoint had no detectable EtOH or acetaldehyde in the package headspace.

As O_2 partial pressures decreased to approximately 2 kPa, the RQ remained practically constant in agreement with the observations of Fidler and North (1967). Assuming the RQ breakpoint to indicate the lower O_2 limit, there is clearly variation between cultivars for the lower O_2 limit (Figure 1). RQ breakpoints for the 9 cultivars tested ranged from 0.7 kPa to 2.0 kPa. Assuming the cuticle provides the primary resistance to gas exchange (Burg and Burg, 1965; Cameron and Yang, 1982), we hypothesize that variation in the RQ breakpoint between cultivars largely reflects differences in cuticular resistance to diffusion,

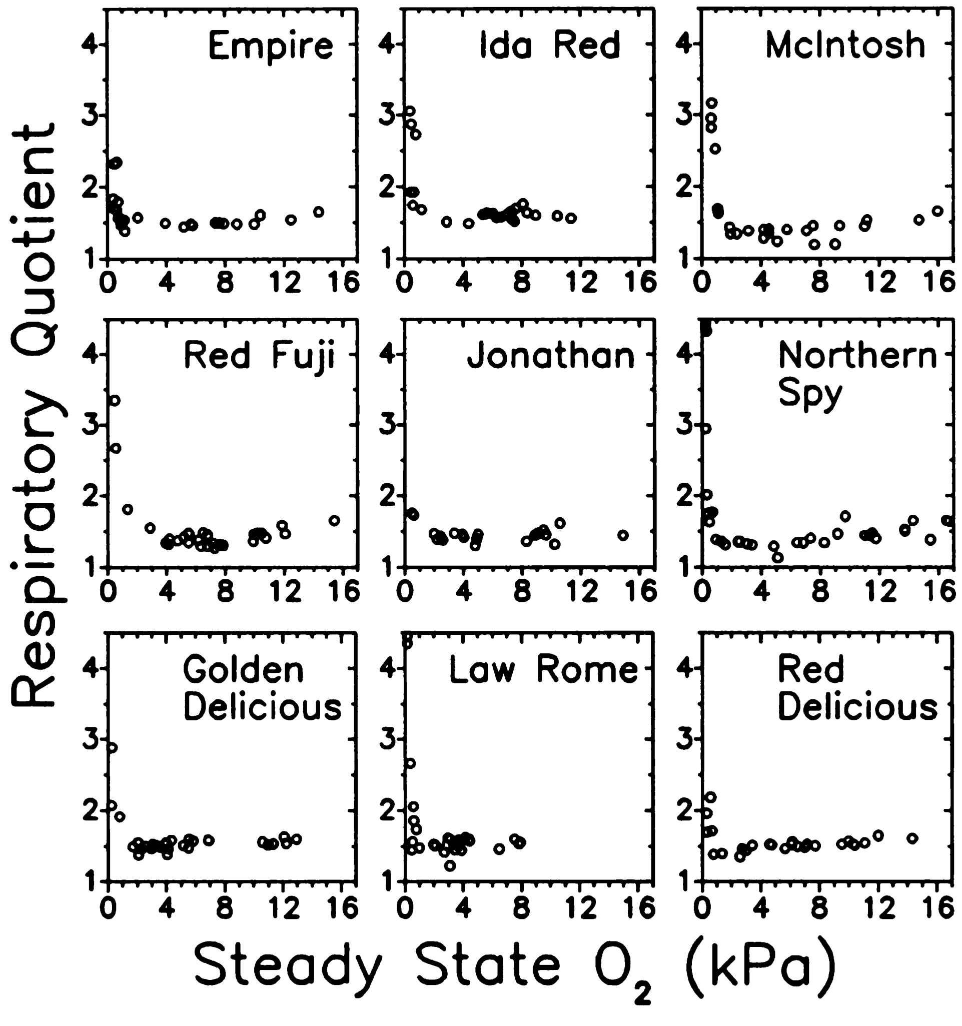

Figure 1. Effect of steady state O_2 partial pressures on the respiratory quotient (RQ) of 9 cultivars of apple fruit sealed in low density polyethylene and held at 0.1°C. Estimated lower O_2 limits as follows: Empire, 0.8; Red Fuji, 1.4; G. Del.,1.3; Ida Red, 1.1; Jonathan, <2.0 (limited data); Law Rome, 0.8; McIntosh, 1.9; Northern Spy, 0.9, and R. Del., 0.7. All values given in kPa.

and that with increasing resistance to diffusion, internal O_2 levels are more readily reduced to levels below the lower O_2 limit. Results from various studies indicate that, in fact, permeability coefficients for O_2, CO_2, and C_2H_4 gas varies between apple cultivars (Kidd and West, 1949; Burg and Burg, 1962; Cameron, 1982; and Rajapakse *et al.*, 1990). The RQ breakpoint at 0° C for 'McIntosh', a fruit with a notable heavy wax sheen, closed calyx end, and few visible lenticels, of 1.9 kPa O_2 compared with a RQ breakpoint of approximately 1.3 kPa O_2 for 'Golden Delicious', a fruit with less sheen, an often open calyx end, and noticeable lenticels, supports the hypothesis that the lower O_2 limits for fruits are in part a reflection of cuticular permeability.

Banks (1984), has observed increasing variation in internal O_2 concentrations with factors that increase O_2 gradients by reducing cuticular permeability. It would follow that fruit with lower cuticular permeabilities would tend to have more variable internal O_2 partial pressures relative to fruit with highly permeable skin. Increased variability in the internal atmospheres of fruits under low O_2 storage would likely result in an increased percentage of fruits with internal O_2 partial pressures below the lower O_2 limit, and a greater incidence of damage. For those varieties having relatively high skin permeabilities and, therefore, more homogeneous internal atmospheres, one may expect smaller numbers of fruits to exhibit damage symptoms at the lower O_2 limit. The influence of temperature upon the low O_2 limits for apple fruit has yet to be examined. At increased temperatures and correspondingly increased

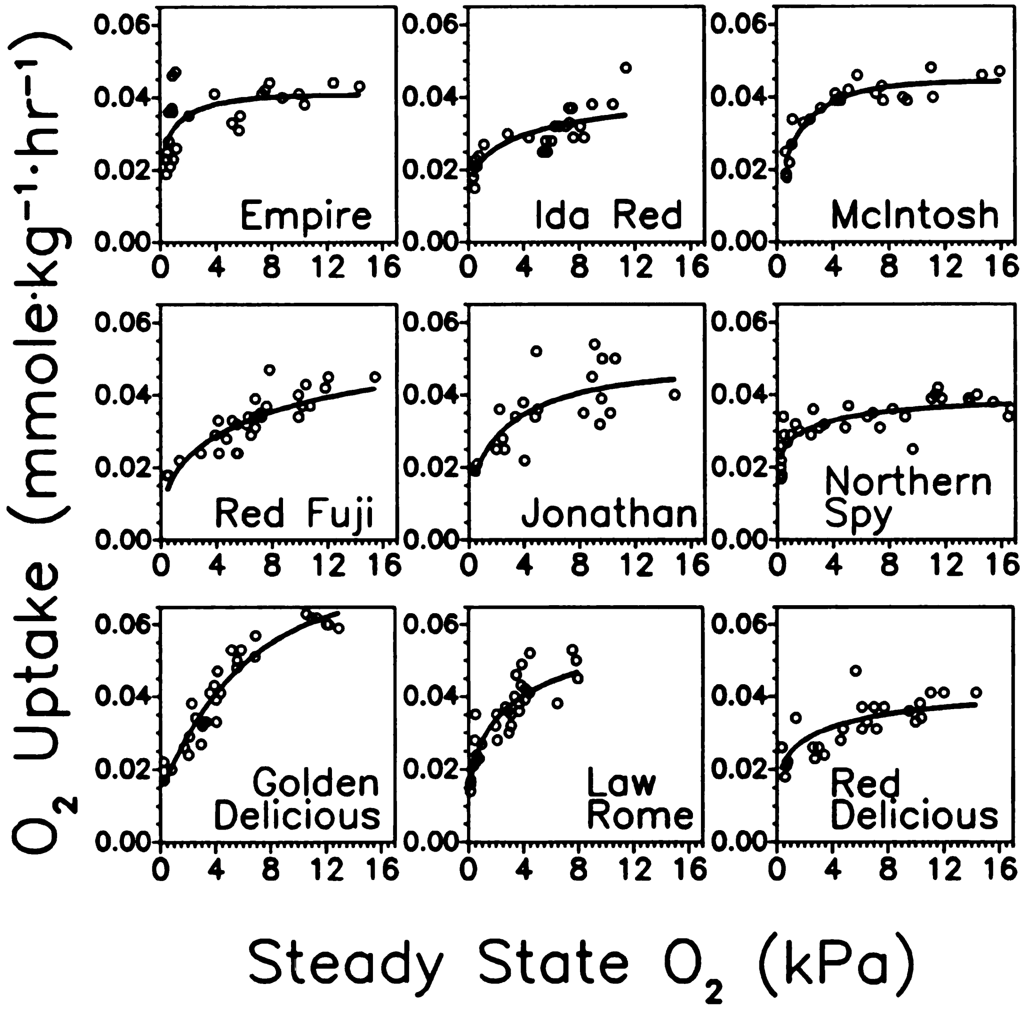

Figure 2. Effect of steady state O_2 partial pressures on the O_2 uptake of 9 cultivars of apple fruits sealed in low density polyethylene and held at 0.1°C. See Table 1 for equations describing curves.

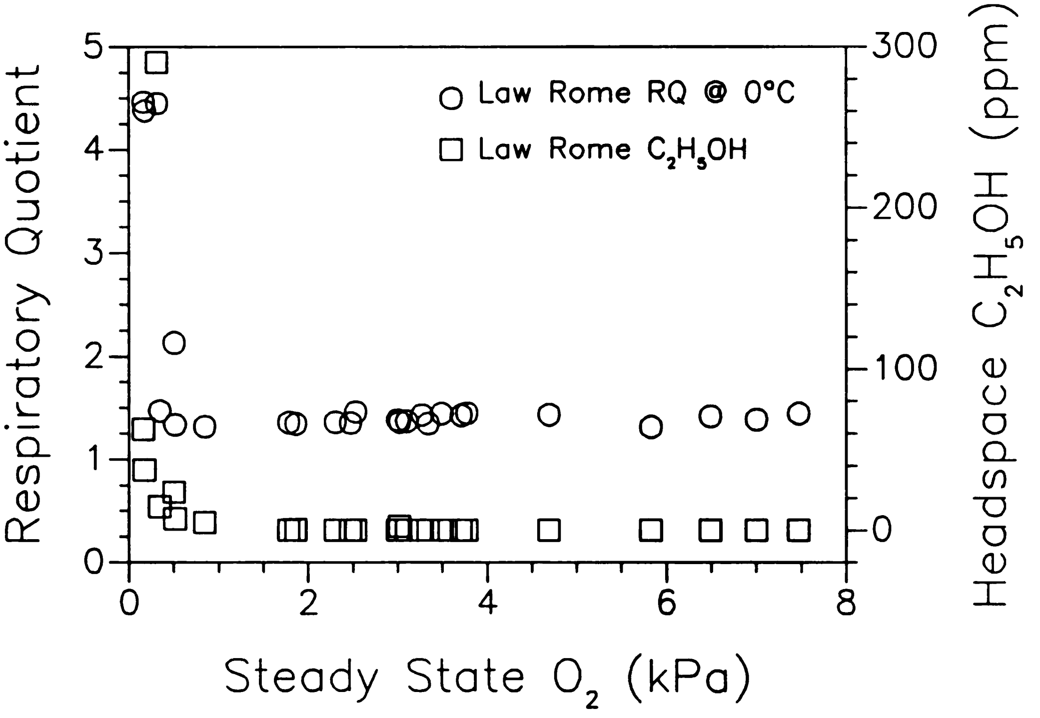

Figure 3. Effect of steady state O_2 partial pressure on the respiratory quotient (RQ) and headspace EtOH of 'Law Rome' apple fruit sealed in low density polyethylene and held at 0.1°C

Table 1. General equations (Eq. [1]) and values of constants describing the relationship between steady state O_2 partial pressures (kPa) and O_2 uptake ($mmol \cdot kg^{-1} \cdot h^{-1}$) for the given apple cultivars sealed in LDPE and stored at 0.1°C. Eq. [1]: $RR_{O2} = b_1 \cdot \{1 - \exp[-b_2 \cdot (O_2)_{pkg}]\}^{b_3}$

Cultivar	b_1	b_2	b_3	r^2
Empire	0.04074	0.30732	0.19208	0.408
Red Fuji	0.05048	0.05688	0.35304	0.721
Golden Delicious	0.07960	0.07300	0.48863	0.886
Ida Red	0.03972	0.07076	0.21003	0.655
Jonathan	0.04074	0.30732	0.19208	0.408
Law Rome	0.05637	0.09455	0.29418	0.794
McIntosh, (strain MacSpur)	0.04442	0.30241	0.39690	0.871
Northern Spy	0.03873	0.09112	0.13650	0.658
Red Delicious	0.04064	0.07697	0.18953	0.560

respiration rates, the influence of tissue permeability upon low O_2 damage would likely be accentuated. Beaudry *et al.*, (1992), have shown the O_2 level at the RQ breakpoint increased with increasing temperatures in blueberry (*Vaccinium corymbosum*) fruits. The authors suggest respiration rates increase at elevated temperatures more rapidly than skin permeability, creating an increased O_2 gradient across the skin.

Park (1990), using the ethane efflux method of Cameron and Yang (1982) to determine cuticular resistance to gas diffusion, showed considerable ($\pm 1 \times 10^4$ $s \cdot cm^{-1}$ at ripe) variability of cuticular resistance coefficients within apple cultivars from year to year. Variations in cuticular resistance also occurred through development, and these changes were largely correlated with stomatal and lenticular structure. O_2 gradients in the cortex of air-stored apple fruit have been reported recently by Dadzie *et al.*, (1990). In examination of 'Golden Delicious' apple fruit O_2 levels were consistently lower in the calyx end. This variation in O_2 levels within an individual fruit was hypothesized to result from tissue-specific differences in cortex resistance due to variation in intercellular airspace and cell size.

Headspace EtOH data show active fermentation within fruit tissues at O_2 levels at or below the RQ breakpoint, indicating anaerobic conditions. This does not, however, directly reflect tissue damage and the loss of value which may be expected when fruit are stored below the lower O_2 limit. Tolerance to anaerobic conditions appears to be quite variable (Lau, 1989), with 'Red Delicious' apples showing only 8% skin discoloration after 7 months at 0.5% O_2 versus 61% discoloration in 'Spartan' apples. The effects of long-term exposures to O_2 levels, at or below the RQ breakpoint, and the cause of cultivar variations are not clearly defined. Tolerance of 'Valencia' oranges (*Citrus sinensis*, L. Osbeck) to short-term anaerobic conditions for insect control has been shown (Ke and Kader, 1990). Numerous reports indicate a greater tolerance of low O_2 exposures by non-climacteric fruits versus climacteric fruits, although differentiation between fruit tolerance of the metabolic products of anaerobic respiration and the tolerance of off-flavor compounds by consumers is required. Further studies relating factors of gas movement through tissue to low O_2 damage may clarify cultivar tolerance variations and the effect of increased respiratory demands during the climacteric.

Rates of O_2 uptake with variations in O_2 partial pressure (Figure 2) show the reduction of respiration rates with decreased O_2 partial pressures. The decrease in O_2 uptake may also reflect the effects of elevated CO_2 levels, as CO_2 was not removed from the headspace of packages. Cultivar variations in the effect of decreased O_2 levels upon O_2 uptake, or respiration rates, shows reducing atmosphere O_2 levels does not always affect respiration to an equal extent. Whereas some cultivars (McIntosh, Red Fuji) showed decreases in rates of O_2 uptake below 4 kPa, other cultivars (Northern Spy) did not show a decrease in rate of O_2 uptake until O_2 levels fell below 1 to 1.5 kPa O_2. This may be influenced by both skin and tissue resistances to O_2 movement.

Additional examination of the effects of temperature and atmosphere composition on fruit respiration is required. Apple fruit respiration rates in initial experiments did not generate the full range of headspace atmospheres for each cultivar tested as hoped, and additional data will more definitively pinpoint the effect of O_2 partial pressure on O_2 uptake, CO_2 production and the RQ for apple fruits. Further studies of the physiology of modified-atmosphere fruit respiration, and the mechanisms of gas movement through bulky plant tissues should provide valuable information for optimizing low O_2 storage of apples and other fruits.

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Chapter 2

Modified Atmosphere Packaging of Apple: The Effect of Temperature on the Low Oxygen Limit as Determined by the Respiratory Quotient Breakpoint

ABSTRACT:

This study examines the interaction of temperature and cultivar on the low oxygen (O_2) limit for apple (*Malus domestica*, Borkh.) fruit. Low O_2 limits were determined by measuring the O_2 partial pressure at which the respiratory quotient (RQ) increased (RQ breakpoint), using modified atmosphere packaging (MAP) techniques. 'Golden Delicious', 'Early Red One Red Delicious', 'Redmax McIntosh', and 'Marshall McIntosh' apple fruits were sealed in low-density polyethylene (LDPE) packages and placed at 0.1, 5, 10, 15, 20, and 25°C, with 3°C substituted for 0.1°C for 'McIntosh' strains, until steady-state respiration was reached. For all apple cultivars tested, the package O_2 partial pressure ($[O_2]_{pkg}$) at which the RQ breakpoint occurred increased with increased temperature. From 3°C to 25°C, the RQ breakpoint increased from 0.75 to 2.5 and 1.2 to approximately 4.0 kPa (1%=1.0135 kPa @ 1 atm) O_2 for 'Redmax McIntosh' and 'Marshall McIntosh', respectively. From 0.1° to 25°C, the RQ breakpoint for 'Red Delicious' and 'Golden Delicious' increased from 0.5 to 0.8 and 0.6 to 1.0 kPa O_2, respectively. O_2 uptake (RR_{O2}) decreased with decreasing temperatures and decreasing $[O_2]_{pkg}$. CO_2 production (RR_{CO2}) rate decreased with decreasing temperatures; RR_{CO2} also decreased as $[O_2]_{pkg}$ fell below 6 to 8 kPa, and then increased at $[O_2]_{pkg}$ below the RQ breakpoint as anaerobic respiration began.

INTRODUCTION

Decreased O_2 partial pressures (P_{O2}), elevated CO_2 partial pressures (P_{CO2}), and low storage temperature extend the storage life of a wide range of horticultural commodities, including apple fruit (Kidd and West, 1927). Each factor may significantly inhibit rates of respiration. The minimum P_{O2} at which fruits may be stored without inducing anaerobic respiration may be influenced by both P_{CO2} and temperature. Normal storage strategies alter temperature and P_{O2} while maintaining P_{CO2} below levels that would limit aerobic O_2 uptake for fruit metabolism. Aerobic respiration decreases as P_{O2} decreases.

In addition to the influences of temperature and P_{CO2} on low O_2 limits, defined here as the minimal P_{O2} at which controlled-atmosphere (CA) or modified-atmosphere (MA) storage may be conducted without inducing anaerobic respiration, commodity-specific atmosphere requirements exist (Meheruik, 1989). Safe atmosphere compositions for CA or MA storage vary greatly among individual commodities as the result of inherent differences in organ respiration rates, ripening characteristics, physiological state, and gas exchange properties related to tissue and/or organ morphology and composition. In addition to specific atmospheric requirements, chilling-sensitive, tropical fruits such as mango (*Mangifera indica*, L.) and banana (*Musa*, L. sp.) require that storage temperatures not fall below critical temperatures, at which chilling injury may occur. Critical temperatures also vary by commodity and length of exposure. In the case of apple fruit, optimal storage temperatures and CA atmospheres vary with cultivar and strain (Meheruik, 1989).

Fruit quality must be retained and optimized to warrant the added expenses of CA and MA storage. Previous experiments have shown that the low O_2 limit determined by RQ breakpoint varies with temperature (Fidler and North, 1967). A modified-atmosphere packaging (MAP) approach and use of RQ breakpoint appear to be useful in determining the low O_2 limit for apple and blueberry (*Vaccinium corymbosum*) fruit (Gran and Beaudry, 1993; Beaudry *et al.*, 1992). In these studies the $[O_2]_{pkg}$ at which the RQ breakpoint occurred was taken to represent the low O_2 limit. Hypothesizing that low O_2 limits increase with temperature, and that increases in low O_2 limit vary with cultivar, experiments were conducted in order to determine the effect of temperature on RR_{O2}, RR_{CO2}, and low O_2 limits determined by RQ breakpoint for three cultivars of apple fruits.

MATERIALS AND METHODS:

Apple fruit of the cultivars 'McIntosh' (strains 'Redmax' and 'Marshall'), 'Golden Delicious', and 'Red Delicious' (strain 'Early Red One') were harvested at the preclimacteric stage as monitored by internal ethylene (C_2H_4) levels ($\leq$20% of fruit $\leq$0.2 μl/l) and stored at 3°C in air until packaged 2 to 6 days later.

As described by Cameron *et al.*, (1989), a range of steady-state $[O_2]_{pkg}$ and package CO_2 partial pressure ($[CO_2]_{pkg}$) were produced by varying film thickness and total fruit weight in the package. Four thicknesses (0.00254, 0.00508, 0.00762, and 0.0116 cm) of low density polyethylene (LDPE) (Dow Chemical Company, Midland, MI) were used. Package surface area was 960 cm^2; fruit numbered from 2 to 5 fruit and total fruit weights were approximately 250 to 800 g per package.

Fruits were packaged as previously described (Gran and Beaudry, 1993) and

30 or more packages of differing combinations of fruit weight and film thickness were placed at 0.1, 5, 10, 15, 20, and 25°C for 'Golden Delicious' and 'Red Delicious' cultivars, with 3°C substituted as the low temperature for 'McIntosh' strains. The lowest treatment temperatures used are those normally recommended for commercial CA storage (Dilley, 1989; Meheriuk, 1989). Over 30 additional packages for 'Marshall McIntosh' and 'Golden Delicious' fruits were prepared with 9 x 6 cm Tyvek™ pouches containing approximately 40 ml of hydrated lime [$Ca(OH)_2$], in order to reduce $[CO_2]_{pkg}$ to below ambient levels. Packages containing [$Ca(OH)_2$] pouches were stored over the same range of temperatures according to cultivar. $[O_2]_{pkg}$ and $[CO_2]_{pkg}$ were monitored until steady-state respiration was reached.

Duplicate gas samples (25 μl) were drawn from each package through a self-sealing silicone septum using a 50 μl glass syringe (Hamilton Co., Reno, NV). Samples were analyzed for O_2 (Servomex Paramagnetic O_2 Transducer, Series 1100, Servomex Co., Sussex, England) and CO_2 (ADC analytical infra red CO_2 Analyzer, 225-MK3, Analytical Development Co., Hoddesdon, England) in series, with N_2 as the carrier gas (flow rate = 100 ml$\cdot$min^{-1}).

RR_{O2} and RR_{CO2} were determined using measured permeabilities for O_2 (P^*_{O2}) and CO_2 (P^*_{CO2}) previously described (Beaudry *et al.*, 1992; Gran and Beaudry, 1993). The effect of $[O_2]_{pkg}$ on RR_{O2}, RR_{CO2}, and the RQ was determined at each storage temperature, and the effect of temperature on the low O_2 limit was determined.

RQ breakpoints and low O_2 limit boundaries were determined by marked increases (commonly 0.1-0.2 units) in RQ values above the basal aerobic RQ value which occurred between packages with contiguous $[O_2]_{pkg}$. Low O_2 limit boundaries were defined by the $[O_2]_{pkg}$ of the packages between which a change in RQ was observed.

Prediction of RR_{O2} over the range of temperature treatments was done using SAS® nonlinear regressions analysis. Data were fit to the Michaelis-Menten kinetic equation [Eq. (1)] given as:

$$RR_{O_2} = \frac{V_{max} \cdot [O_2]_{pkg}}{K_m O_2 + [O_2]_{pkg}} \qquad (1)$$

where V_{max} is the maximum initial velocity and K_mO_2 is the Michaelis-Menten constant for O_2 for whole fruit tissue. RR_{O2} curves were generated for 'Golden Delicious' and 'Marshall McIntosh' cultivars, for packages both with elevated $[CO_2]_{pkg}$ and packages scrubbed for CO_2.

RESULTS AND DISCUSSION:

A range of steady-state $[O_2]_{pkg}$, both aerobic and anaerobic, were generated for each apple cultivar at each treatment temperature. Steady-state $[CO_2]_{pkg}$ ranged from approximately 3 kPa for aerobic headspace atmosphere up to 15 kPa for anaerobic package atmospheres. For all cultivars examined, the $[O_2]_{pkg}$ at which the RQ breakpoint occurred increased with increased temperature. Low O_2 limits of 'Golden Delicious' and 'Red Delicious' fruits were affected to a lesser

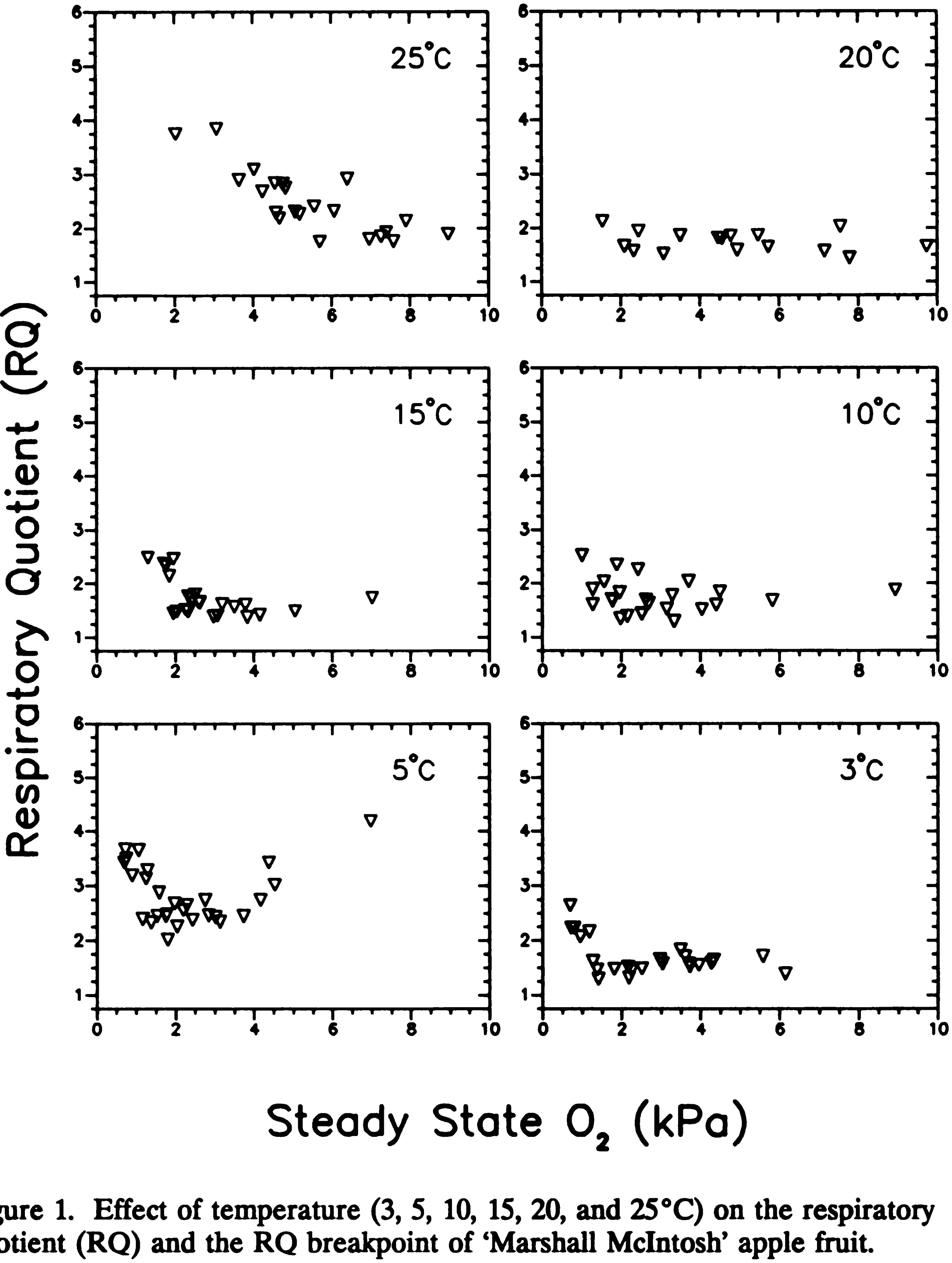

Figure 1. Effect of temperature (3, 5, 10, 15, 20, and 25°C) on the respiratory quotient (RQ) and the RQ breakpoint of 'Marshall McIntosh' apple fruit.

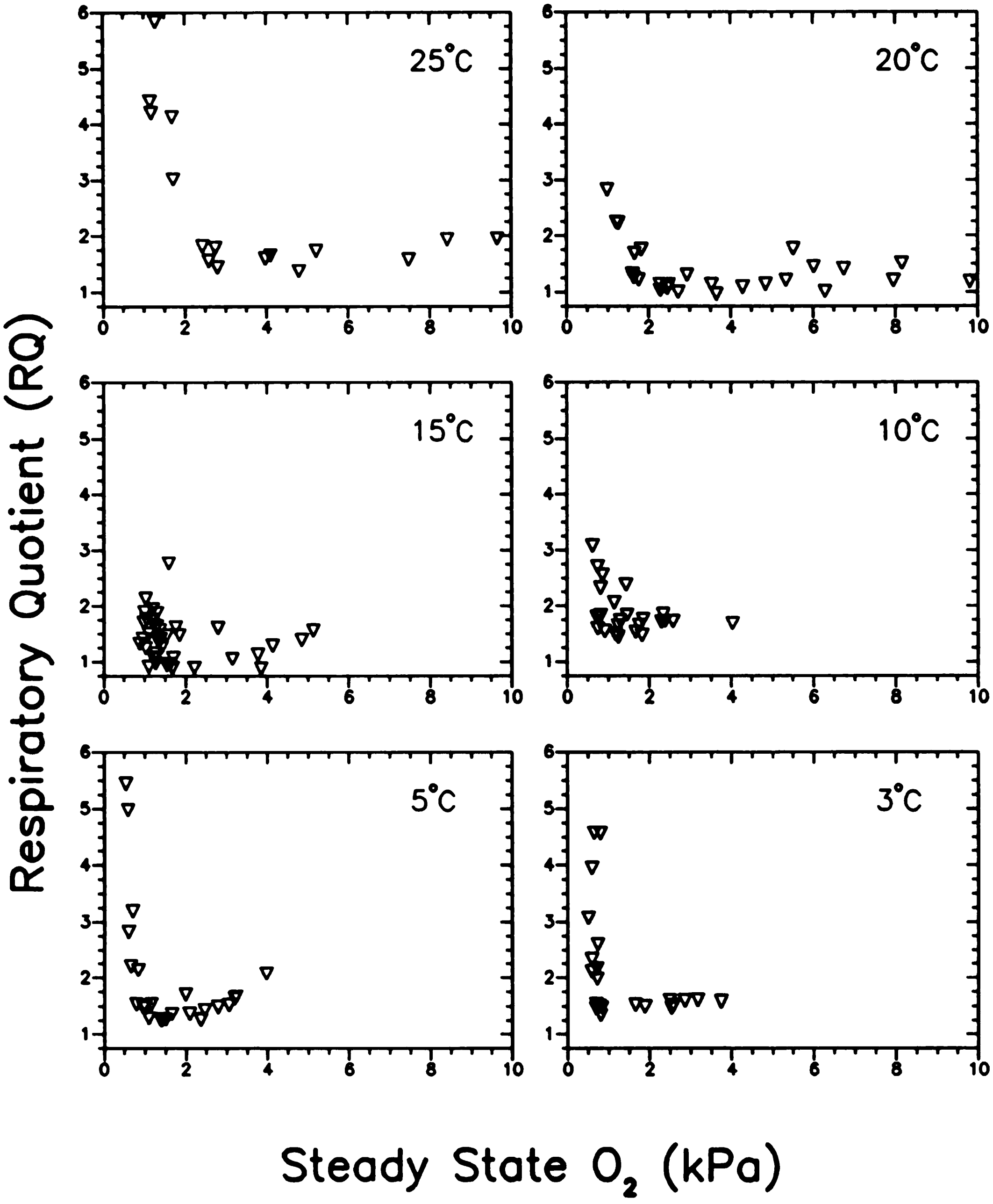

Figure 2. Effect of temperature (3, 5, 10, 15, 20, and 25°C) on the respiratory quotient (RQ) and RQ breakpoint of 'Redmax McIntosh' apple fruit.

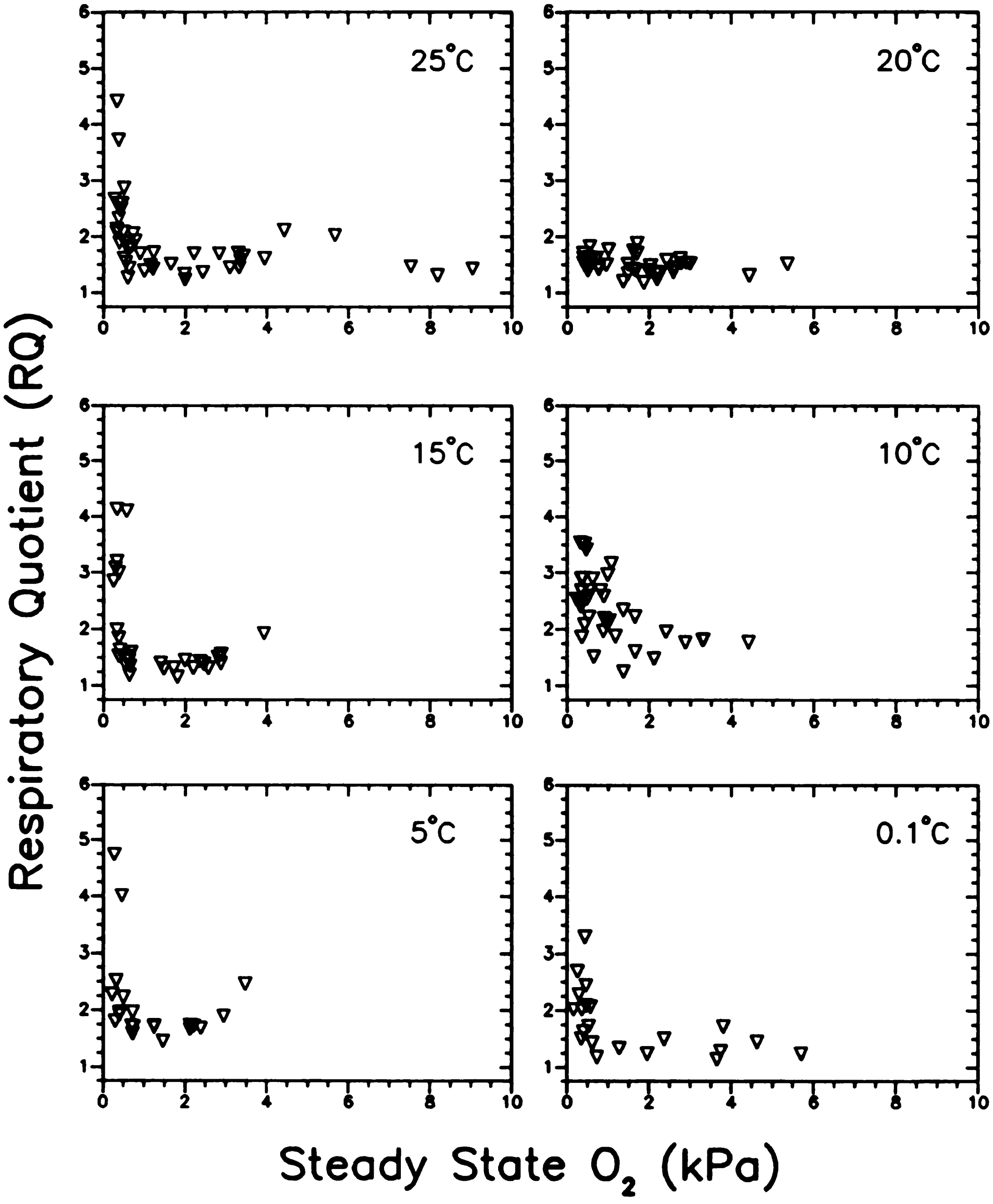

Figure 3. Effect of temperature (0.1, 5, 10, 15, 20, and 25°C) on the respiratory quotient (RQ) and RQ breakpoint of 'Golden Delicious' apple fruit.

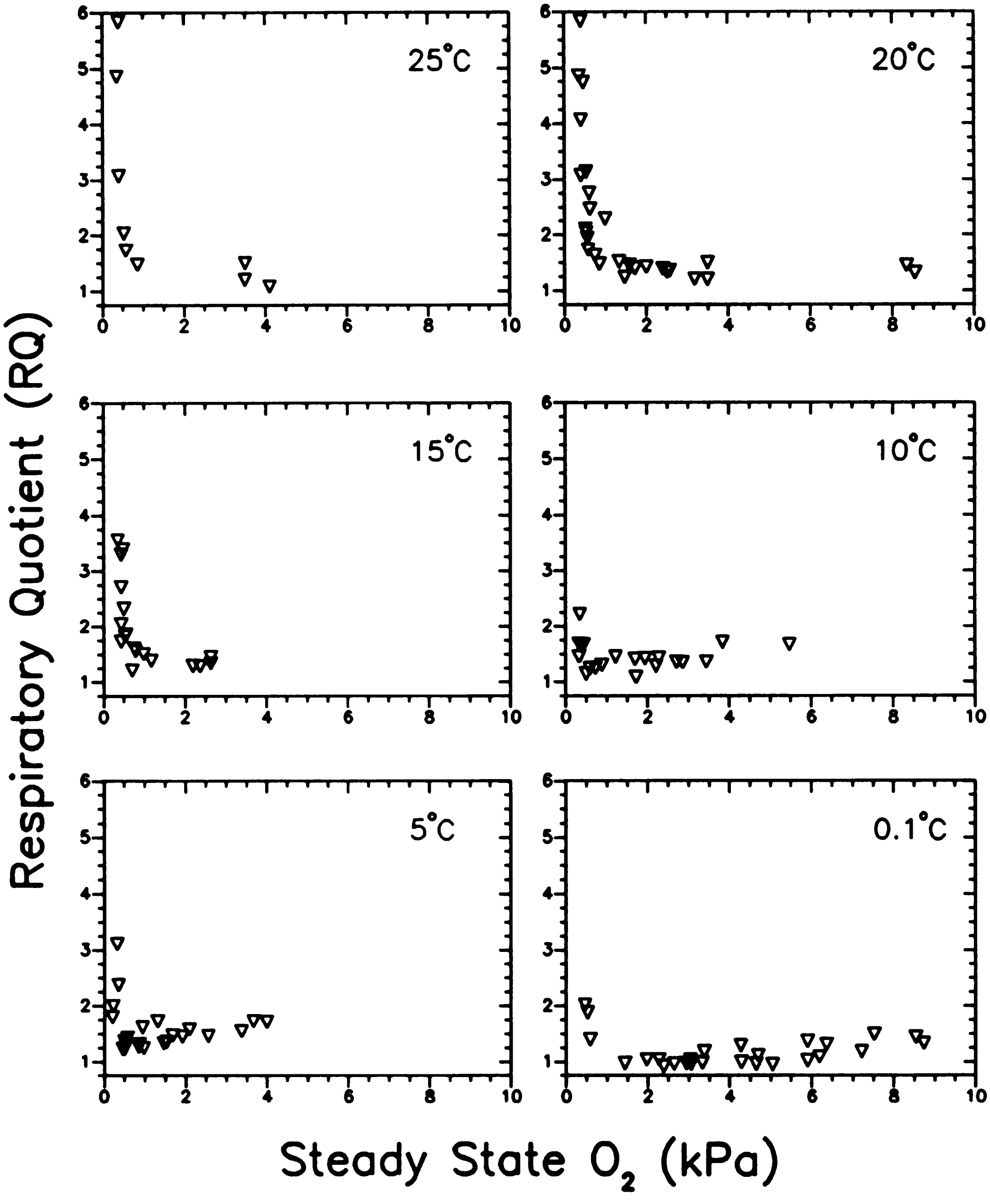

Figure 4. Effect of temperature (0.1, 5, 10, 15, 20, and 25°C) on the respiratory quotient (RQ) and RQ breakpoint or 'Early Red One Red Delicious' apple fruit.

Table 1. Estimated low O_2 limit ranges for apple fruit of 3 cultivars based upon RQ breakpoint (Figures 1-4).

	Low O_2 Limit			
	Cultivar			
Temperature (°C)	Golden Delicious	Red Delicious	Redmax McIntosh	Marshall McIntosh
0.1 / 3	0.55-0.60	0.45-0.60	0.75	1.20-1.25
5	0.50-0.70	0.35-0.45	0.85-0.95	1.25-1.60
10	0.45-0.65	0.45-0.50	1.15	1.85-2.00
15	0.40-0.60	0.55-0.70	1.75-1.85	2.50-3.00
20	0.70-0.95	0.60-0.75	1.90-2.15	4.00-4.10
25	0.80-1.00	0.55-0.85	2.45-2.50	3.80-4.90

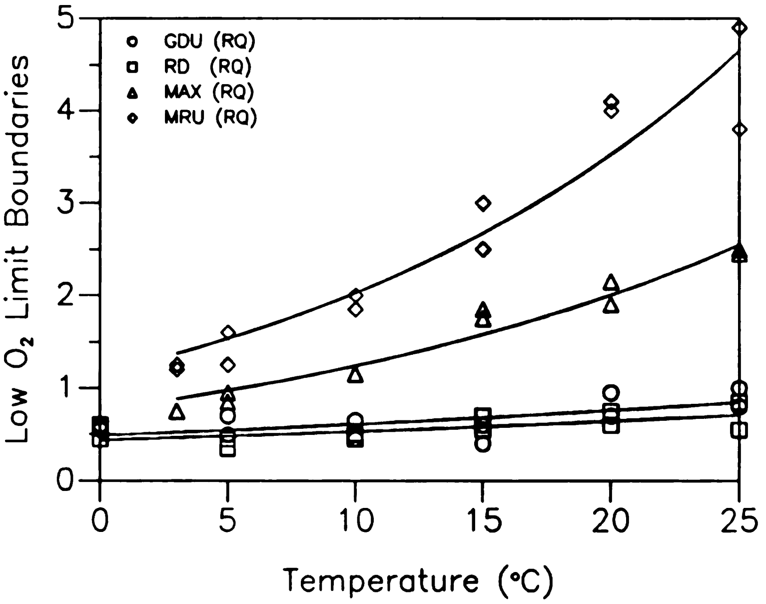

Figure 5. Low O_2 limit boundaries for 3 apple cultivars over a range of temperatures as determined by RQ breakpoint. (GDU, 'Golden Delicious' unscrubbed; RD, 'Red Delicious'; MAX, 'Redmax McIntosh'; MRU, 'Marshall McIntosh' unscrubbed)

extent by temperature than either strain of 'McIntosh' (Figures 1-4). Low O_2 limit boundaries increased from 0.6 to 1.0 kPa and 0.5 to 0.8 kPa O_2 for 'Golden Delicious' and 'Red Delicious' as temperature increased from 0.1°C to 25°C, while for 'Redmax McIntosh' and 'Marshall McIntosh' the low O_2 limit increased from 0.75 to 2.5 kPa and 1.2 to 4.0 kPa O_2, respectively, as temperature increased from 3 to 25°C. Estimated low O_2 limits and the specific $[O_2]_{pkg}$ at which the RQ breakpoint occurred for treatment temperatures are given for each cultivar in Table 1. Distinct differences in the low O_2 limits of different cultivars and strains of cultivars were observed. Changes in low O_2 limits occurred most markedly above 10°C for all cultivars.

The minimum P_{O2} determined to be safe for storage, at the lowest experimental temperature, was greater for both 'McIntosh' strains than either 'Golden Delicious' and 'Red Delicious' (Figure 5). Results reflect commercial CA storage practices, with O_2 atmosphere recommendations for CA storage of 'Marshall McIntosh' fruits being 1.0 kPa higher than either 'Red Delicious' or 'Golden Delicious'.

RQ levels for fruits under aerobic conditions decreased in most cases from 1.4 at 15 and 20°C to 1.0 at 0.1 or 3°C (Figures 1-4). This may indicate a loss of organic acids with time. CA storage, at a P_{O2} of 1 %, maintains organic acid content of stored 'Bartlett' pears (*Pyrus communis*, L.) at levels higher than for air stored fruits (Yoshida *et al.*, 1986).

For all cultivars examined, fruit RQ values were calculated between 1.0 to 1.4

at $[O_2]_{pkg}$ above the anaerobic extinction point (Figures 1-4). An exception was for the fruit stored at higher temperatures (20 and 25°C), where aerobic RQ levels ranged up to 1.8. The aerobic RQ for apple fruit has been determined to range from 1.5 to 1.8, with peaks of 3.0 at 0°C in CO_2-free atmospheres (Fidler and North, 1964). Time to steady-state respiration varied from 5 to 7 days at 25°C to 75 days or longer at 0.1°C. The occurrence of mold and general tissue breakdown at higher treatment temperatures required quick sampling and did not allow data collection beyond 15 days.

Differences in aerobic RQ values may be the result of chilling-induced ripening and an associated increased respiratory rate (Purvis, 1985) as observed with chilling of avocado (*Persea americana*, Mill.) fruit (Cooper *et al.*, 1969). Fruit ripening within packages may have occurred at lower treatment temperatures due to slow headspace $[O_2]_{pkg}$ reduction or large fluctuations in $[O_2]_{pkg}$ before steady-state respiration was established. In the case of 'Marshall McIntosh' at 3°C, $[O_2]_{pkg}$ fluctuated by 3.5 kPa between the 12th and 17th day following packaging, with $[O_2]_{pkg}$ traversing the subsequently determined low O_2 limit. Such fluctuations in $[O_2]_{pkg}$ would alter RR_{O2} and fruit ripening in storage.

A concurrent increase in the rate of respiration, as determined by RR_{CO2} (Figure 6-9) and predicted RR_{O2} (Figures 10-13), occurred above 10°C. RR_{O2} increased with increased temperatures for all cultivars examined. The effects of temperature on RR_{O2} uptake varied with cultivar (Figures 10-13). In the case of both 'McIntosh' strains, particularly 'Marshall McIntosh', respiration and the effect

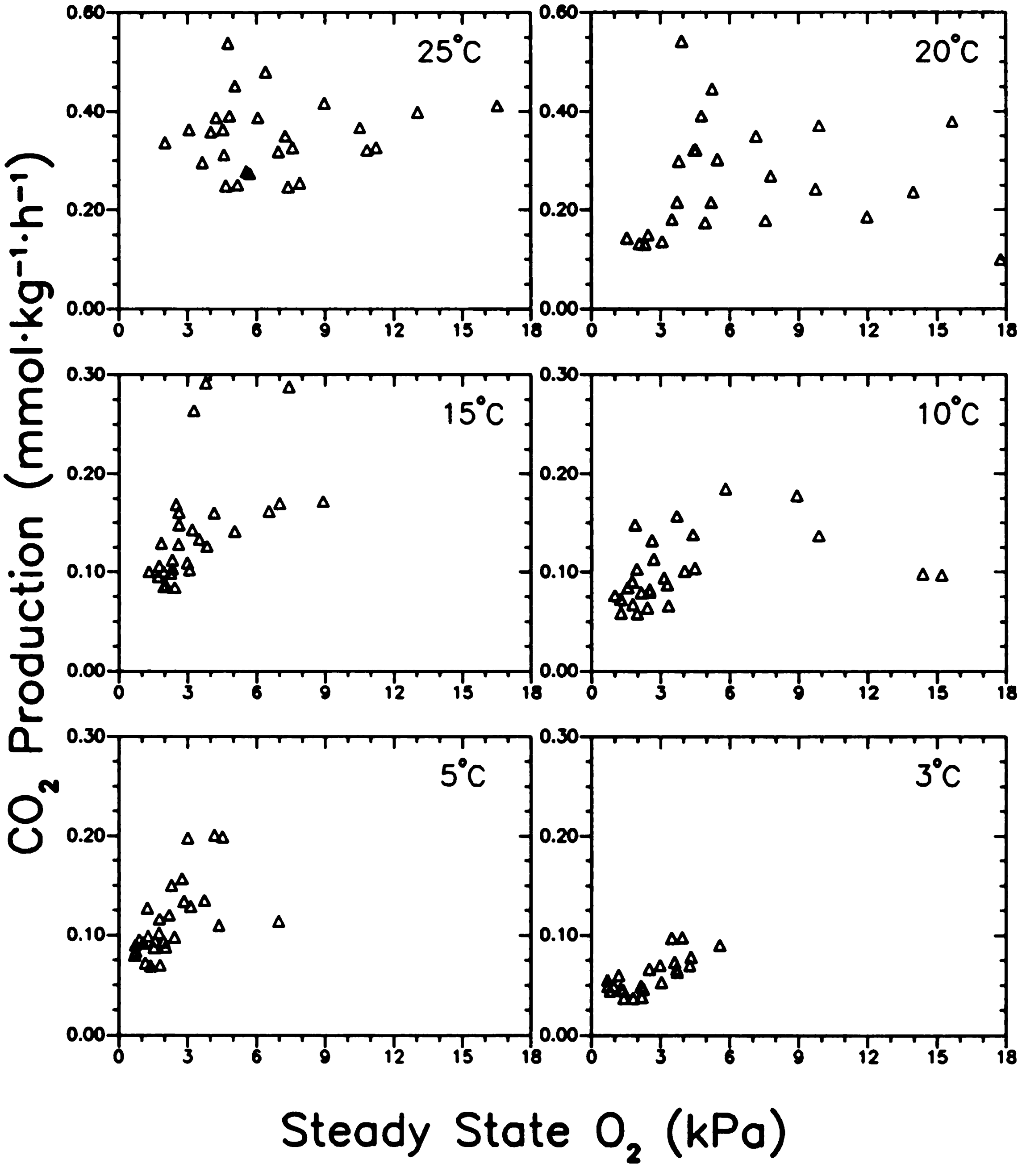

Figure 6. Effect of temperature (3, 5, 10, 15, 20, and 25°C) and $[O_2]_{pkg}$ on RR_{CO2} of 'Marshall McIntosh' apple fruit.

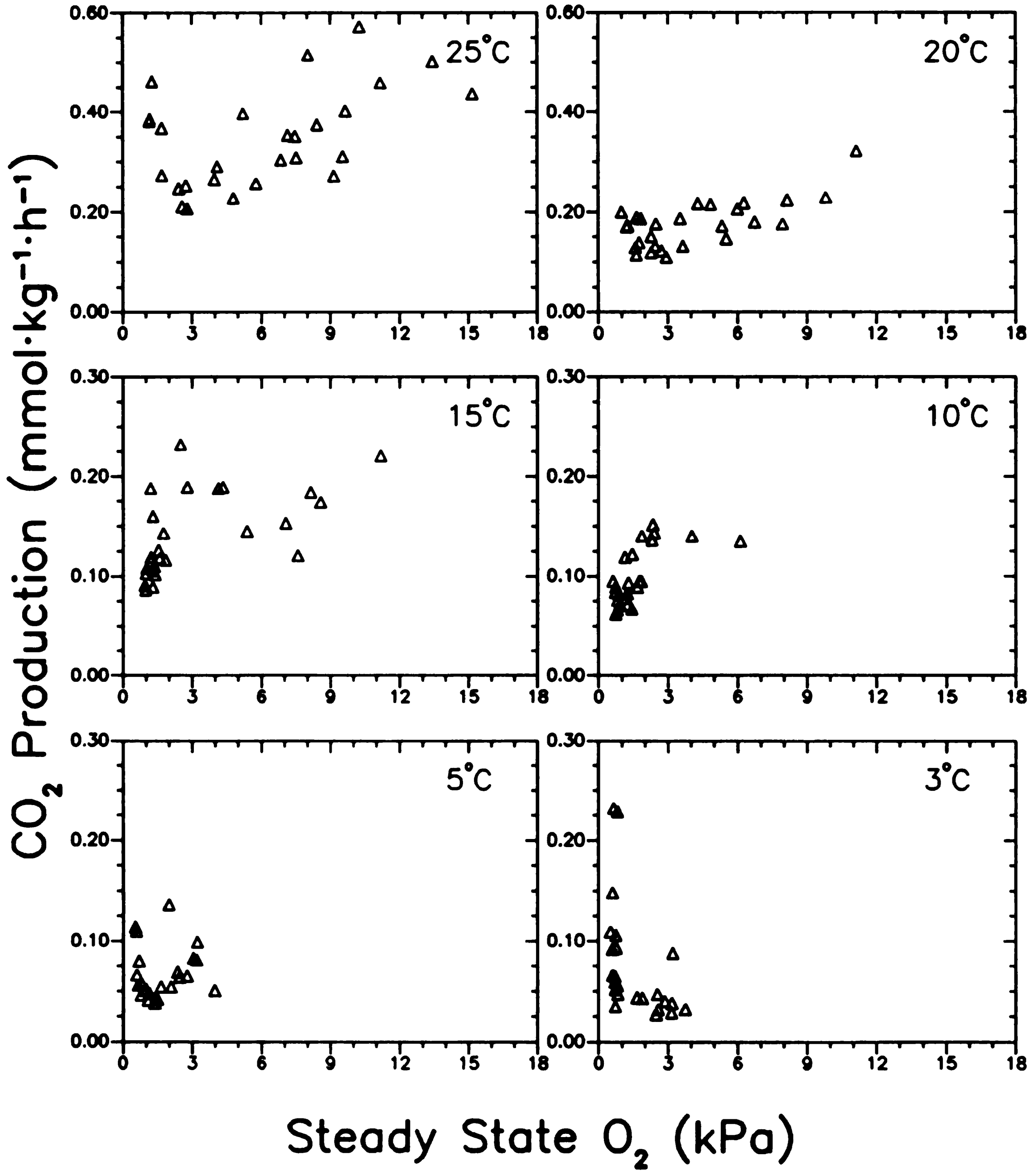

Figure 7. Effect of temperature (3, 5, 10, 15, 20, and 25°C) and $[O_2]_{pkg}$ on RR_{CO2} of 'Redmax McIntosh' apple fruit.

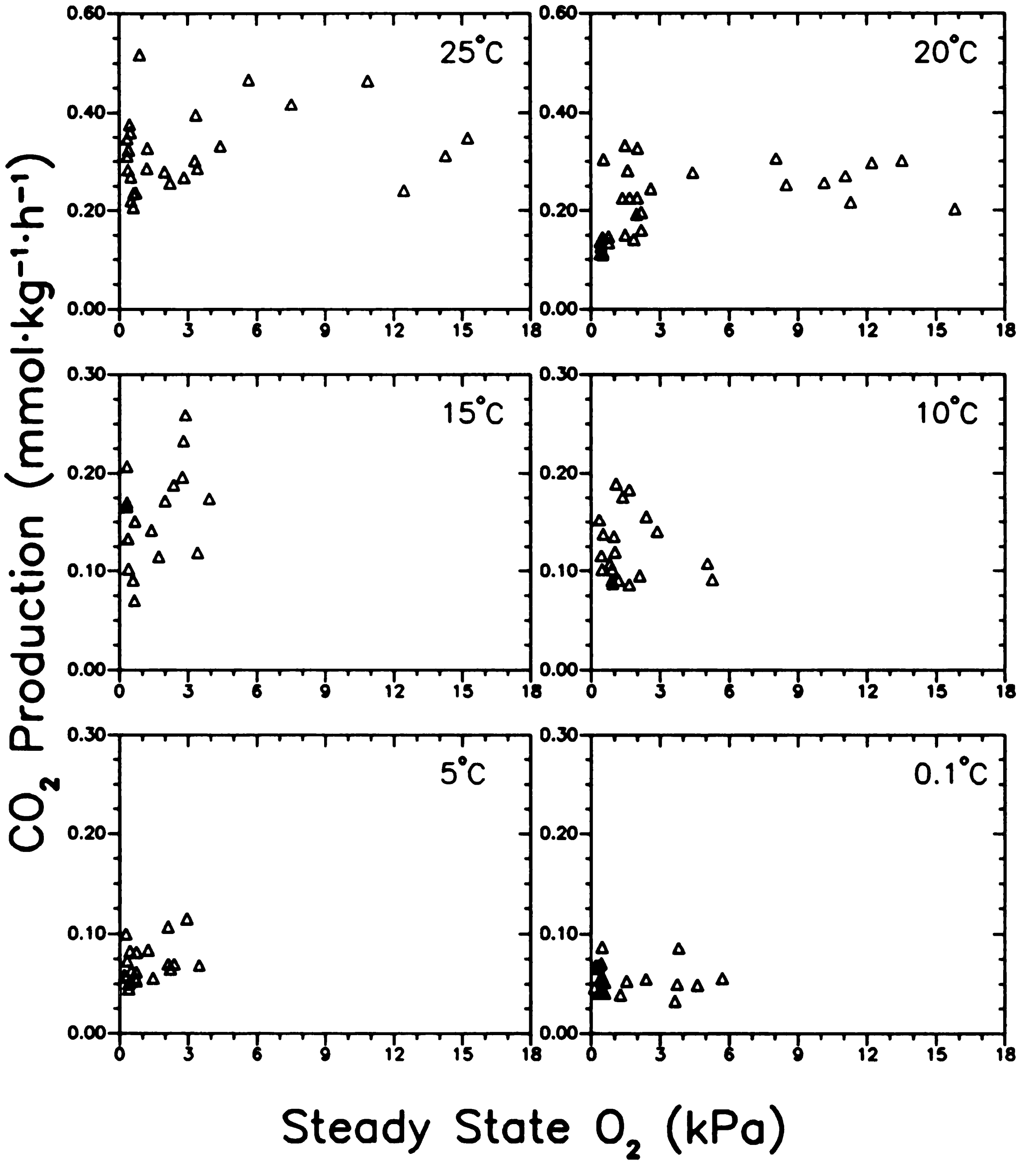

Figure 8. Effect of temperature (0.1, 5, 10, 15, 20, and 25°C) and $[O_2]_{pkg}$ on RR_{CO2} of 'Golden Delicious' apple fruit.

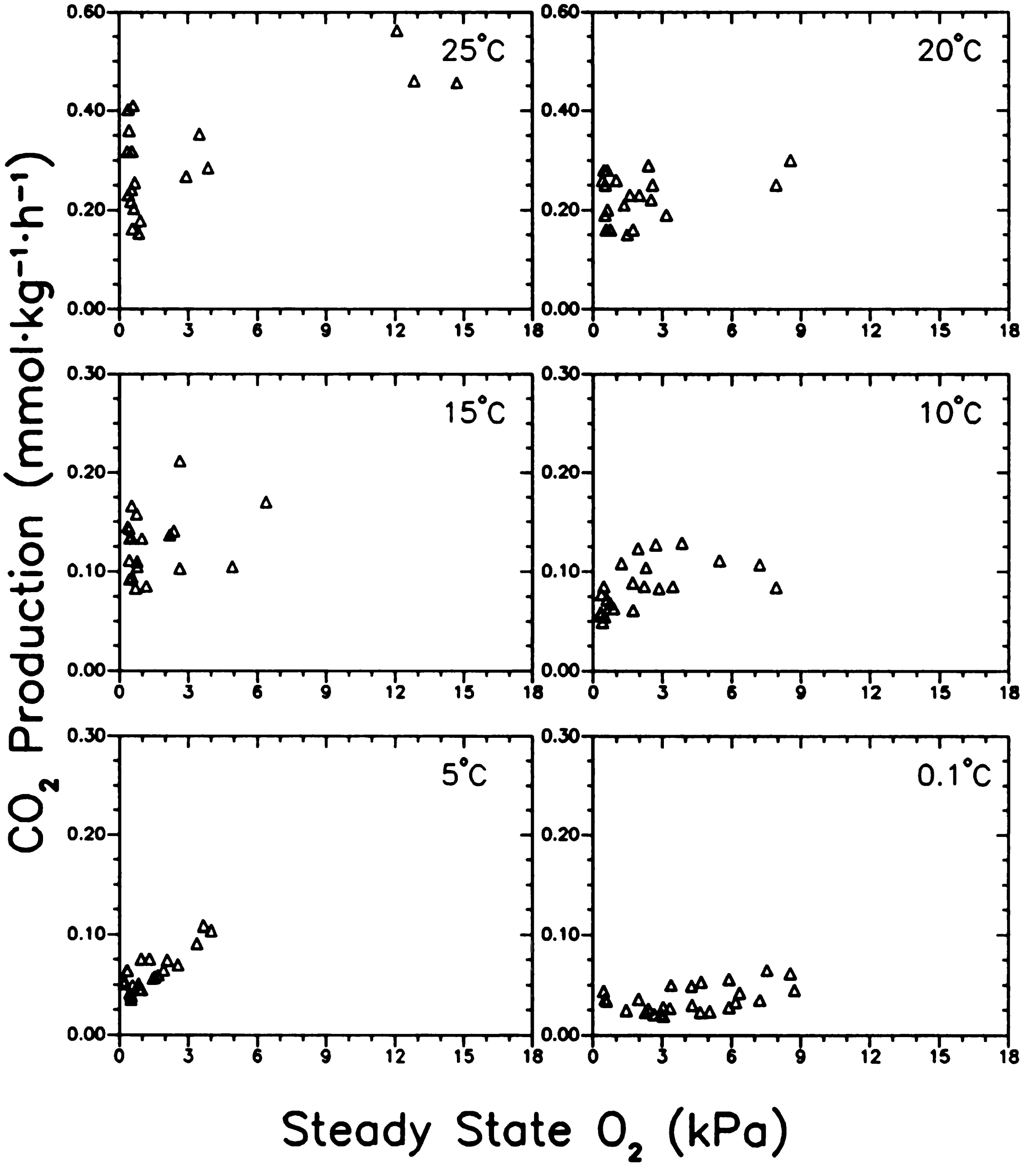

Figure 9. Effect of temperature (0.1, 5, 10, 15, 20, and 25°C) and $[O_2]_{pkg}$ on RR_{CO2} of 'Early Red One Red Delicious' apple fruit.

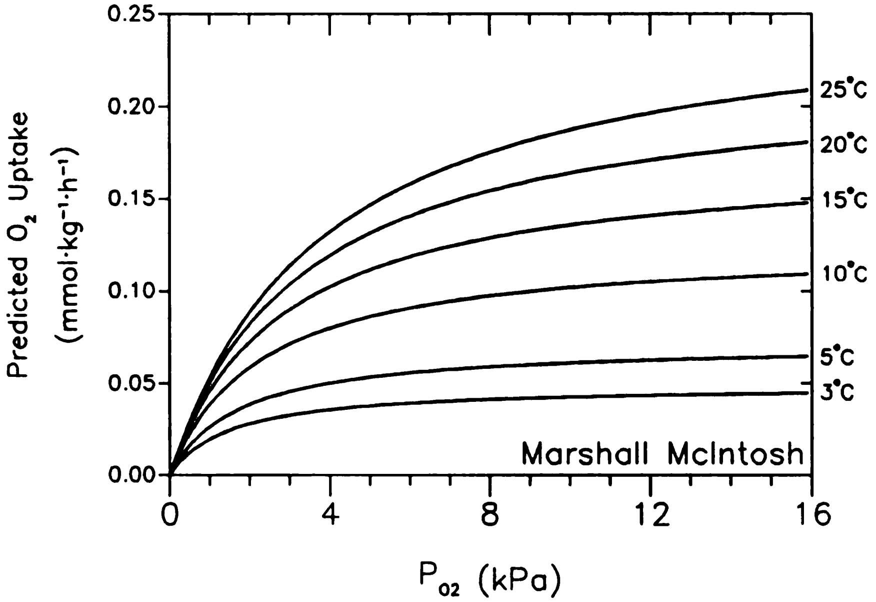

Figure 10. Comparison of O_2 uptake curves for 'Marshall McIntosh' apple fruit over a range of temperatures. See Table 2 for equations describing curves.

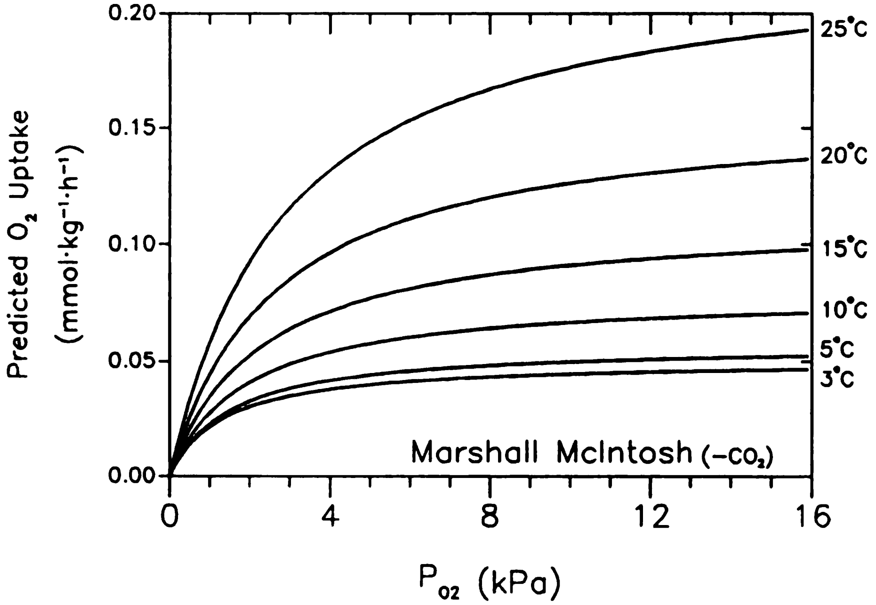

Figure 11. Comparison of O_2 uptake curves for 'Redmax McIntosh' apple fruit over a range of temperatures, with CO_2 removed from package headspaces. See Table 2 for equations describing curves.

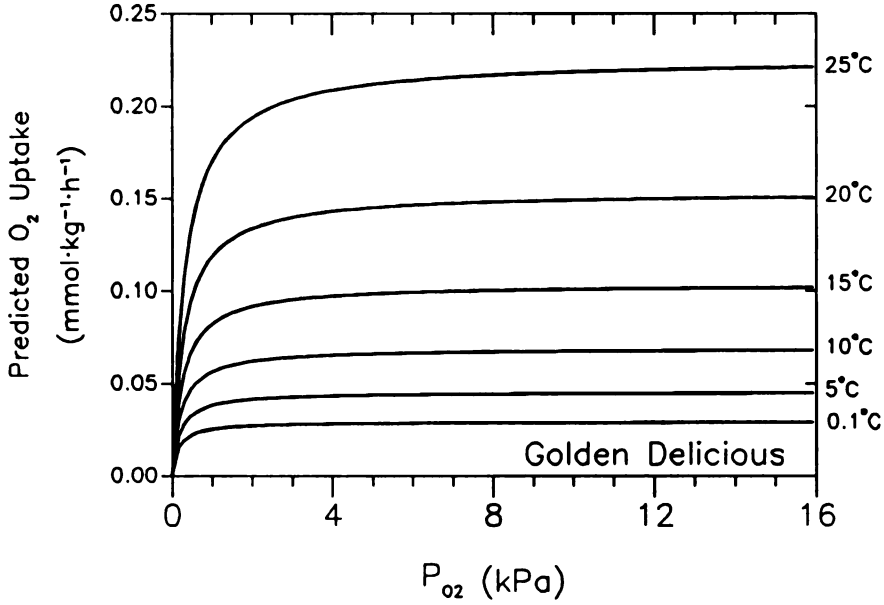

Figure 12. Comparison of O_2 uptake curves for 'Golden Delicious' apple fruit over a range of temperatures. See Table 2 for equations describing curves.

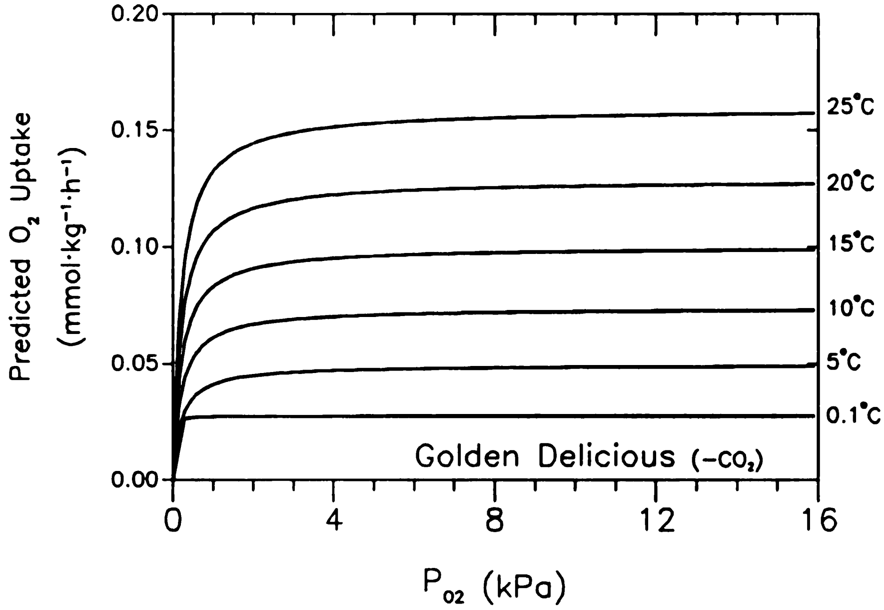

Figure 13. Comparison of O_2 uptake curves for 'Early Red One Red Delicious' apple fruit over a range of temperatures. See Table 2 for equations describing curves.

Table 2. General equations (Eq. (1) and Eq.(2)) and values of constants describing the relationship between steady state $[O_2]_{pkg}$ (kPa), RR_{O2} ($mmol \cdot kg^{-1} \cdot hr^{-1}$), and storage temperature (°C) for 3 apple cultivars in modified atmosphere packages.

Eq.(1) = $\{(a*\exp[b*\text{temperature}]c)*[O_2]_{pkg}\}/\{(d+e*\text{temperature})+[O_2]_{pkg}\}$

Eq.(2) = $\{(a*\exp[b*\text{temperature}]c)*[O_2]_{pkg}\}/\{(d*\exp[e*\text{temperature}]+f)+[O_2]_{pkg}\}$

Cultivar	a	b	c	d	e	f	r^2
Marshall McIntosh	-.7187	-.0167	.7325	1.1463	.1082	*	.7889
Marshall McIntosh ($-CO_2$)**	.0329	.0757	.0091	-9.176	-.0086	10.284	.8218
Golden Delicious	.0367	.0741	-.0079	.1514	.0071	*	.7893
Golden Delicious ($-CO_2$)**	.2721	.0158	-.2450	-.2128	-.9451	.2038	.7528

* Eq. (1)

** Eq.(2)

of temperature on RR_{O2} and RR_{CO2} appears variable with $[O_2]_{pkg}$, indicating variation in gas exchange characteristics and subsequently individual fruit respiration.

Respiration, as indicated by RR_{CO2}, was decreased with decreased temperatures similar to RR_{O2}. At $[O_2]_{pkg}$ below the anaerobic extinction point, where rapid decreases in RR_{O2} were seen, rates of RR_{CO2} increased rapidly (Figures 6-9), and RR_{CO2} curves often exhibited a characteristic 'checkmark' shape with decreasing $[O_2]_{pkg}$. This increase in RR_{CO2} is an indication of anaerobic respiration.

In addition to reducing rates of respiration, treatment temperature also affected the $[O_2]_{pkg}$ at which RR_{O2} was decreased, indicating a change in apparent K_m for O_2. Apparent K_m for O_2 for 'Marshall McIntosh' increased linearly with temperature, from approximately 1.0 kPa O_2 to 3.5 kPa O_2 from 3° to 25°C (Figure 14), while apparent K_m for O_2 for 'Golden Delicious' cultivars increased nonlinearly with temperature, increasing sharply from 0.05 kPa O_2 at 0.1° to approximately 0.25 kPa O_2 at 10°C were values remained relatively constant up to 25°C (Figure 15). K_m for O_2 values for 'Golden Delicious' and 'Red Delicious' cultivars ranged from 0.1 to 0.4 kPa O_2. Removal of CO_2 from package headspaces may have reduced apparent K_m for O_2. Previous MAP studies using blueberry fruits show reduction of RR_{O2} by decreased $[O_2]_{pkg}$ varies with temperature (Beaudry *et al.*, 1992). Whereas RR_{O2} decreased if $[O_2]_{pkg}$ fell below approximately 6 kPa at 0.1°C, RR_{O2} decreased continually up to 15 kPa O_2 at

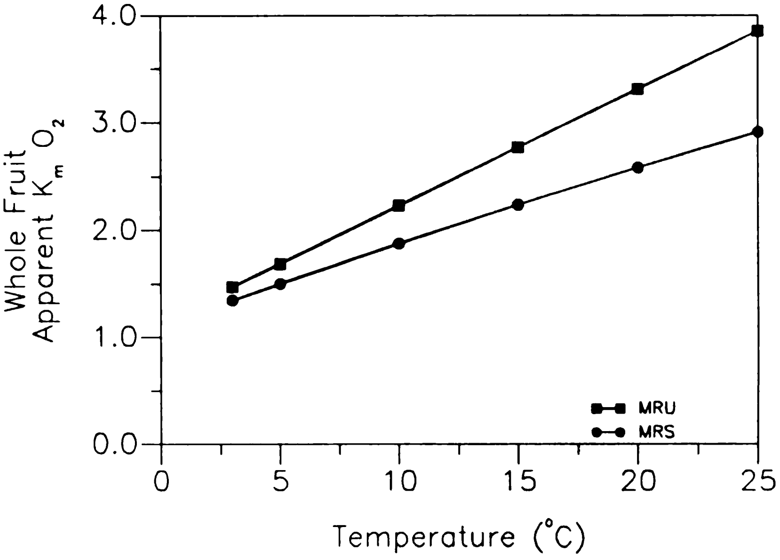

Figure 14. Apparent whole fruit K_m for O_2 of 'Marshall McIntosh' apple fruit stored over a range of temperatures, with and without scrubbing of CO_2 from package headspaces (MRU, 'Marshall McIntosh' unscrubbed; MRS, 'Marshall McIntosh' scrubbed).

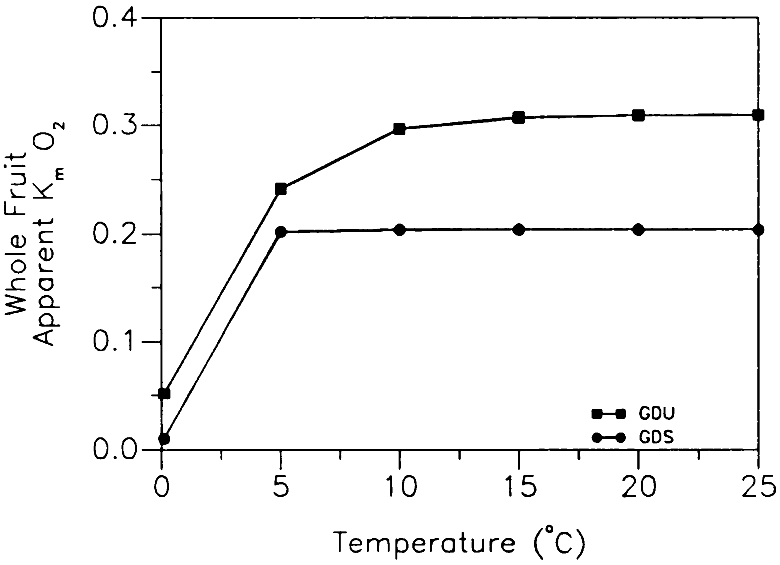

Figure 15. Apparent whole fruit K_m for O_2 of 'Golden Delicious' apple fruit stored over a range of temperatures, with and without scrubbing of CO_2 from package headspaces (GDU, 'Golden Delicious' unscrubbed; GDS 'Golden Delicious' scrubbed).

25°C. This indicates that at higher temperatures peel and tissue resistances to O_2 diffusion (r_{O2}) may limit RR_{O2} at a higher $[O_2]_{pkg}$ than at lower temperatures.

Removal of CO_2 from package headspaces may have produced noticeable differences in RR_{O2} for 'Marshall McIntosh' (Figures 1 and 2, APPENDIX B) or 'Golden Delicious' fruits (Figures 4 and 5, APPENDIX B) when compared with RR_{O2} curves for packages with $[CO_2]_{pkg}$ up to 12 kPa, although the removal of $[CO_2]_{pkg}$ which ranged up to 15 kPa unexpectantly did not increase respiration rates as determined by RR_{O2}, but rather decreased measured respiration rates.

Calculations of whole fruit apparent K_m for O_2 (Figures 14 and 15), using non-linear regressions of RR_{O2}, temperature, and $[O_2]_{pkg}$, allowed further indications of differences in gas exchange properties for the cultivars examined. Whereas K_m for O_2 for 'Marshall McIntosh' changed linearly with changes in temperature, indicating r_{O2} is limiting respiration, 'Golden Delicious' changed non-linearly, increasing quickly from 0.1 to 10°C and then remaining unchanged at temperatures above 10°C, indicating r_{O2} is not limiting respiration for this cultivar.

Determination of the low O_2 limit of 'Marshall McIntosh' was difficult due to variable respiration rates of fruits, with variability of RR_{O2} and RR_{CO2} increased with temperature. The RQ breakpoint at higher temperatures was poorly defined, which may indicate variable gas exchange characteristics between individual fruits. Variations in low O_2 limits with individual fruit and changes from year to year (Chapters 1 and 2) may result in storage problems, particularly in the case of storage at very low O_2 levels. Differences in low O_2 limits for different strains of apple cultivars, as indicated by comparison of ‘Marshall McIntosh’ and ‘Redmax McIntosh’ data, underscores the difficulties associated with CA and MA storage at low P_{O2} when cultivar-specific requirements of storage are not known. Problems in optimizing storage conditions also arise with variable low O_2 limits of strains when facilities or other limitations require storage of multiple strains of fruits with variable low O_2 limits in the same CA or MA storage unit.

The results show temperature control for low O_2 storage is important, especially in the case of cultivars with a propensity for low O_2 damage. Temperature control is particularly important for commercial MAP applications, with atmosphere maintenance dependent upon rates of respiration.

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Chapter 3

Modified Atmosphere Packaging of Apple: The Effect of Temperature on the Low Oxygen Limit as Determined by Headspace Ethanol Accumulation

ABSTRACT:

The purpose of this study was to determine the extent to which temperature alters the low O_2 limit for apple (*Malus domestica*, Borkh.) fruit of three cultivars, by measuring the accumulation of ethanol (EtOH) in the headspace of modified atmosphere packages. For the cultivars tested, the highest package O_2 partial pressure ($[O_2]_{pkg}$) inducing ethanol (EtOH) accumulation in package headspaces, termed here as the 'fermentation threshold', increased with increasing temperature. From 3° to 25°C the $[O_2]_{pkg}$ at which headspace EtOH levels $[EtOH]_{pkg}$ accumulated increased from 0.75 to 4.0 kPa and 2.0 to 6.6 kPa (1%=1.0135 kPa @ 1 atm) for 'Redmax McIntosh' and 'Marshall McIntosh', respectively, while increasing from 0.5 to greater than 1.0 kPa and 0.6 to 1.2 kPa from 0.1 to 25°C for 'Early Red One Red Delicious' and 'Golden Delicious', respectively. The accumulation of EtOH in the headspace of modified atmosphere packages at steady-state respiration appears to be a useful tool for quick, accurate determination of low O_2 limits of apple fruit.

INTRODUCTION:

Successful long term storage of pome fruits depends on optimized temperature and gas atmosphere compositions. Maintenance of the proper storage temperature is required in order to maintain reduced respiration rates and to maximize the retention of firmness and other quality characteristics, especially for those fruits to be stored greater than 6 months. The storage temperature must also be adjusted for various storage related disorders which may be attenuated or aggravated by temperature effects (Wills *et al.*, 1989). Needed adjustments of storage temperature may be related to cultural conditions and practices, environmental factors, mineral nutrition of the fruit, time from harvest to storage atmosphere establishment, and the predisposal of various varieties to develop storage disorders. A number of storage disorders can be characterized as chilling disorders, including superficial scald, core flush, Jonathan spot, and low temperature breakdown (Fidler *et al.*, 1973). Higher than optimal storage temperatures may also lead to the development of disorders such as senescent breakdown, especially in the case of fruit stored at an over-mature stage.

Storage O_2 and CO_2 partial pressures (P_{O2} and P_{CO2}) are known affect temperature-related disorders, as well as induce specific O_2- and CO_2-related storage disorders. Superficial scald was reduced or eliminated by decreased P_{O2} (Lau, 1989) or increased P_{CO2} (Tomkins, 1966). The effectiveness of elevated P_{CO2} to reduce the incidence of superficial scald may vary with storage ventilation, and was especially ineffective when elevated P_{CO2} is achieved by reduced venting (Meigh, 1970).

The induction of tissue fermentation by limiting P_{O2} or toxic P_{CO2} results, in part, in the production of EtOH and acetaldehyde. The buildup of these fermentation products may result in tissue browning, cell death, intercellular flooding, and production of off-flavor compounds. Tissue EtOH production is an indicator or cause of many controlled atmosphere storage disorders (Fidler *et al.*, 1973).

A primary deterrent to commercial applications of modified atmosphere packaging (MAP) technologies for storage and transit of fruits and vegetables is the likelihood that packages and the commodities they contain will be exposed to a wide range of temperatures throughout the transport chain. An increase in temperature will increase respiration rates to a greater extent relative to changes in film O_2 permeabilities (P^{*}_{O2}). With headspace atmosphere dependent upon the temperature effect on tissue respiration rates, temperature shifts will alter package atmosphere. In the case of blueberry (*Vaccinium corymbosum*) fruit, increased temperature lowered $[O_2]_{pkg}$ (Beaudry *et al.*, 1992). Temperature effects on packaged fruit physiology, with the disparity of changes in P^{*}_{O2} and RR_{O2}, can be marked when the low O_2 limit increases with temperature. Current research efforts center on the development of packages which would be able to alter atmospheres in response to deleterious storage atmospheres and temperatures.

It is generally accepted that respiration rates vary among individual fruits and throughout the various physiological states of maturation. Ripening and senescence processes, variation in physiological state, environmental conditions for growth, and environment after harvest affect respiration rates. Previous experiments have shown the low O_2 limit of a variety of commodities varies with temperature (Fidler and North, 1967). Studies in our laboratory have shown MAP systems well suited for determination of the low O_2 limit for apple fruit at 0.1°C (Gran and Beaudry, 1993) and blueberry fruit (Beaudry *et al.*, 1992). For apple fruit at 0.1°C, the $[O_2]_{pkg}$ at which a large increase in $[EtOH]_{pkg}$ occurred was taken to represent the low O_2 limit, and coincided with the RQ breakpoint (Gran and Beaudry, 1993).

Transport temperature shifts would change tissue respiration, potentially causing headspace atmosphere fluctuations, fermentation, and reducing tissue preservation with MAP transport and storage of apples. Hypothesizing that low O_2 limits increase with increasing temperatures, and that the extent of the increase varies with cultivar, experiments were conducted to determine the effects of temperature on low O_2 limits for three cultivars of apple fruit using $[EtOH]_{pkg}$ accumulation as the indicator.

MATERIALS AND METHODS:

Apple fruit of the cultivars 'McIntosh' (strains 'Redmax' and 'Marshall'), 'Golden Delicious', and 'Red Delicious' (strain 'Earli Red One') were harvested at the preclimacteric stage as determined by internal ethylene (C_2H_4) levels and stored at 3°C in air until packaged 2 to 6 days later.

As described by Cameron *et al.*, (1989), packages with a range of steady-state $[O_2]_{pkg}$ and CO_2 partial pressure ($[CO_2]_{pkg}$) were produced by varying film thickness and total fruit weight in the package. Four thicknesses (0.00254, 0.00508, 0.00762, and 0.0116 cm) of low density polyethylene (LDPE) (Dow Chemical Company, Midland MI) were used. Package surface area was 960 cm^2; fruit number from with a range of 2 to 5 fruit to generate fruit weights of approximately 250 to 800 g per package.

Fruits were packaged as previously described (Gran and Beaudry, 1993) and

30 or more packages of differing combinations of fruit weight and film thickness were placed at 0.1, 5, 10, 15, 20, and 25°C for 'Golden Delicious' and 'Early Red One Red Delicious', with 3°C substituted for the low temperature for 'McIntosh' strains. Over 30 additional packages of 'Marshall McIntosh' and 'Golden Delicious' fruits were prepared with 9 x 6 cm Tyvek™ pouches containing approximately 40 ml of hydrated lime [$Ca(OH)_2$], in order to reduce $[CO_2]_{pkg}$ to below ambient levels. Packages with $Ca(OH)_2$ were placed at the same range of temperatures according to cultivar. $[O_2]_{pkg}$ and $[CO_2]_{pkg}$ were monitored periodically for each cultivar stored at each treatment temperature until it was determined that fruits were at steady-state respiration.

Duplicate gas samples (25 μl) were drawn from each package through a self-sealing silicone septum using a 50 μl glass syringe (Hamilton Co., Reno, NV), and analyzed for O_2 (Servomex Paramagnetic O_2 Transducer, Series 1100, Servomex Co., Sussex, England) and CO_2 (ADC analytical infra red CO_2 Analyzer, 225-MK3, Analytical Development Co., Hoddesdon, England) in series, with N_2 as the carrier gas (flow rate = 100 $ml \cdot min^{-1}$).

Gas samples (250 μl) were drawn from packages using a 500 μl gastight glass syringe (Hamilton Co., Reno, NV) and $[EtOH]_{pkg}$ were determined by gas chromatography (Carle GC with 45.7 cm column, .32 cm bore, Haysep N packing, at 120°C with gas flow rates of 40, 40, and 200 $ml \cdot min^{-1}$ for H_2, He and air, respectively). EtOH levels were determined by comparison with standard solutions consisting of 100 $\mu l \cdot 100\ ml^{-1}$ dH_2O placed at treatment temperatures, with adjustments for standard vapor pressure changes with temperature (Harger *et al.*, 1950).

$[EtOH]_{pkg}$ and tissue EtOH levels were measured and compared using tissue samples from packages having previously been sampled for $[EtOH]_{pkg}$. Tissue samples were taken from core and cortical tissues, pooling 2-3 g of tissue sliced from multiple fruit removed from packages. Samples of approximately 10 g were macerated, placed in a 25 ml flask, capped with a rubber septum, and placed at original treatment temperatures. Gas samples (250 μl) from the sealed flasks were analyzed after 2 hours (Pesis and Avissar, 1990). $[EtOH]_{pkg}$ were determined over a range of $[O_2]_{pkg}$ each storage temperature, and the effect of temperature on the low O_2 limit was determined.

EtOH analysis was limited to 10-15 packages at each temperature, with the sample population consisting primarily of packages with a range of $[O_2]_{pkg}$ ±3 kPa P_{O2} from the low O_2 limit determined by the RQ breakpoint (Chapter 2).

Low O_2 limit boundaries, indicated by elevated $[EtOH]_{pkg}$, were determined by marked increases (commonly 10-50 ppm) in $[EtOH]_{pkg}$ above the basal aerobic $[EtOH]_{pkg}$ values which occurred between packages with contiguous $[O_2]_{pkg}$. Low O_2 limit boundaries were defined by the $[O_2]_{pkg}$ for the packages between which an increase in $[EtOH]_{pkg}$ was observed.

RESULTS AND DISCUSSION:

A range of steady state $[O_2]_{pkg}$ were generated for each apple cultivar at each treatment temperature. $[O_2]_{pkg}$ ranged from 0.1 to 6 kPa O_2. Time until steady-state respiration varied from 5 to 7 days at 25°C to more than 75 days at 0.1°C. Aerobic $[EtOH]_{pkg}$ measured ≤ 10 to 50 ppm at 0.1 or 3 to 25° C, respectively, until reaching the anaerobic extinction point (Figures 1-4).

For all cultivars examined, the $[O_2]_{pkg}$ at which elevated $[EtOH]_{pkg}$ were observed increased with temperature (Figures 1-5). Low O_2 limits are expressed as a range (Table 1), representing $[O_2]_{pkg}$ between which an increase in $[EtOH]_{pkg}$ increased above aerobic levels was observed. In some instances the range is small, while for others the increment of $[O_2]_{pkg}$ was separated by larger amounts, which did not allow accurate determination of specific low O_2 limit values. Estimated low O_2 limits at lower treatment temperatures are comparable with recommended atmospheres for controlled atmosphere (CA) storage of apple fruits (Dilley, 1989; Lau, 1989; Meheriuk, 1989).

Low O_2 limits increased from approximately 2.0 to 6.6 kPa and 0.75 to 4.0 kPa for 'Marshall McIntosh' and 'Redmax McIntosh', respectively, as temperature increased from 3 to 25°C (Table 1). Low O_2 limits for 'Golden Delicious' and 'Red Delicious' increased from approximately 0.6 to 1.0 kPa and 0.5 to 1.2 kPa from 0.1 to 25°C. Low O_2 limits increased most dramatically from 15 to 25°C, while increasing only slightly from 0.1 or 3 to 10°C (Figure 5).

$[EtOH]_{pkg}$ was highly correlated with EtOH levels of flasks containing macerated tissue (Figure 6). Internal and external tissue samples exhibited EtOH levels which did not differ significantly with area of fruit sampled. Packages with $[EtOH]_{pkg}$ above aerobic $[EtOH]_{pkg}$ showed increased $[EtOH]_{pkg}$ with time,

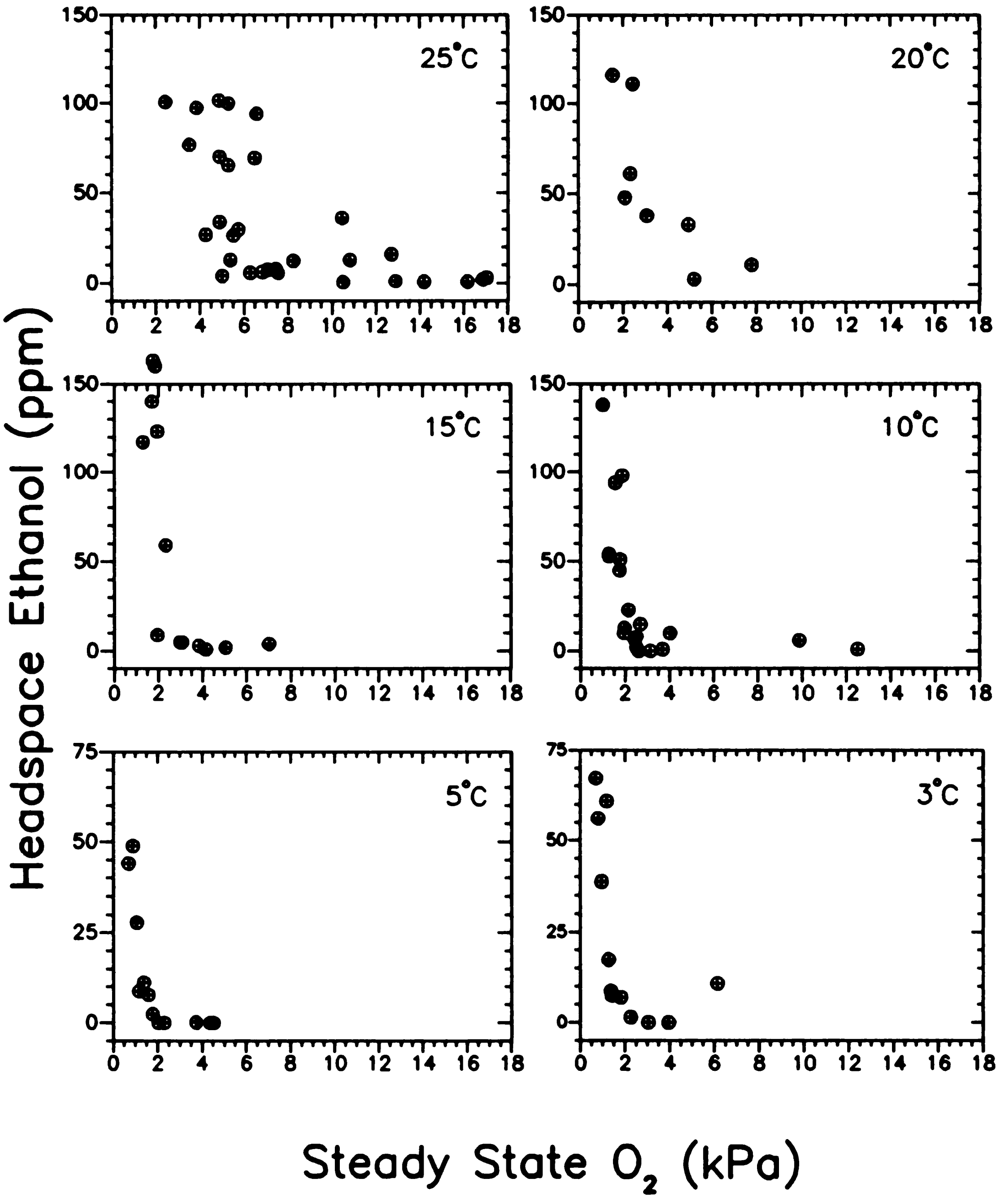

Figure 1. Effect of temperature (3, 5, 10, 15, 20 and 25°C) on headspace EtOH levels as an indication of the low O_2 limit of 'Marshall McIntosh' apple fruit.

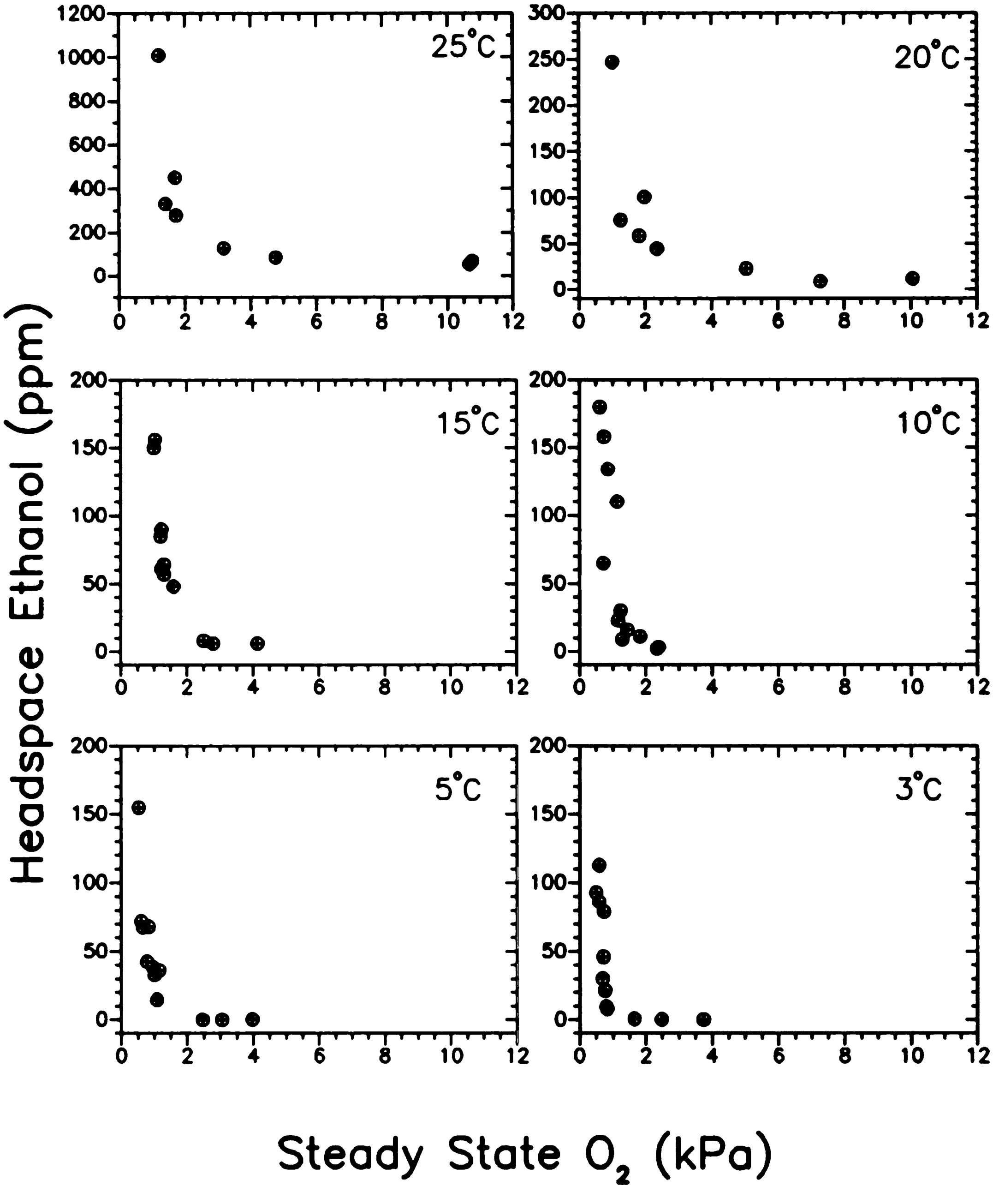

Figure 2. Effect of temperature (3, 5, 10, 15, 20, and 25°C) on headspace EtOH levels as an indication of the low O_2 limit of 'Redmax McIntosh' apple fruit.

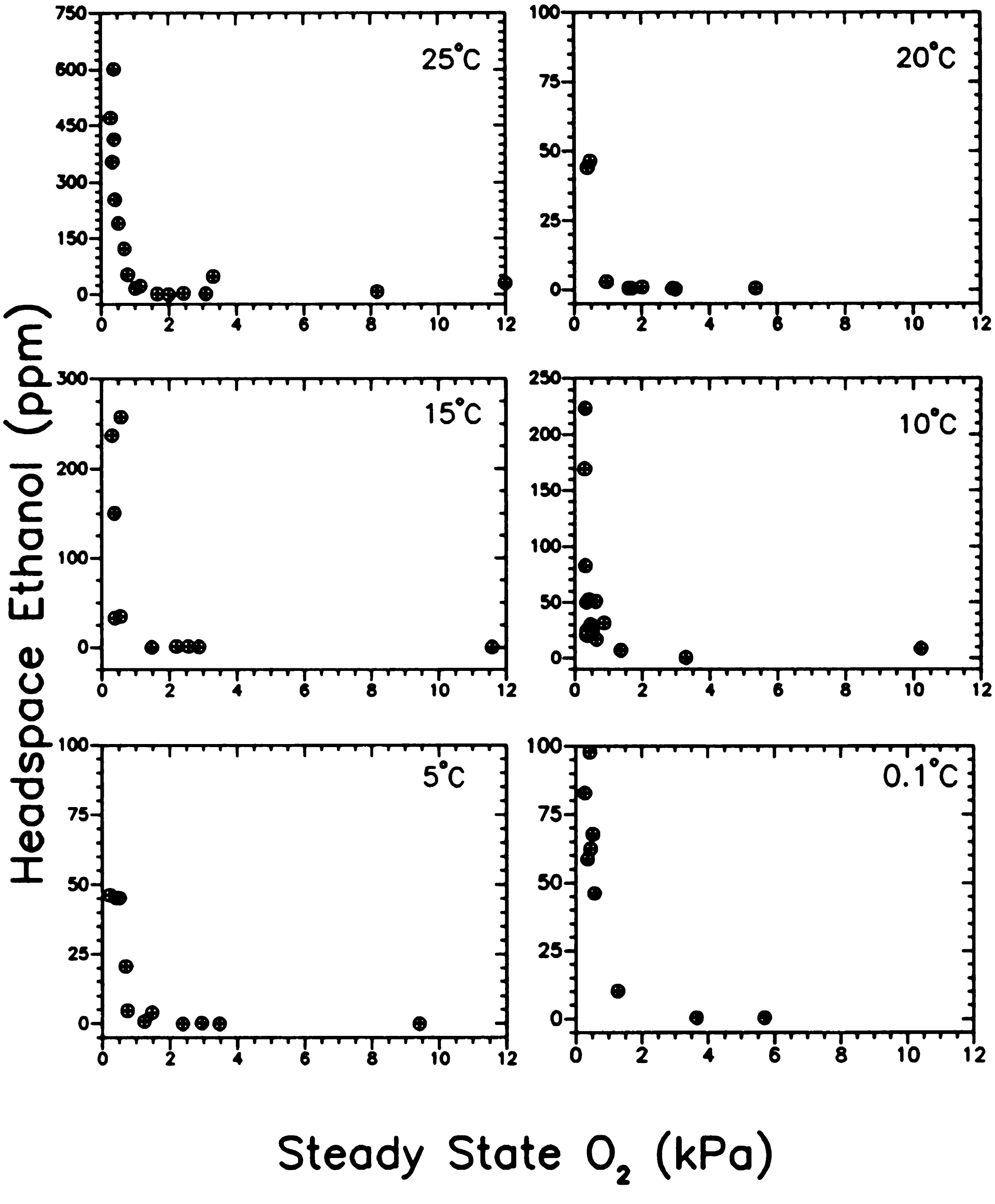

Figure 3. Effect of temperature (0.1, 5, 10, 15, 20, and 25°C) on headspace EtOH levels as an indication of the low O_2 limit of 'Golden Delicious' apple fruit.

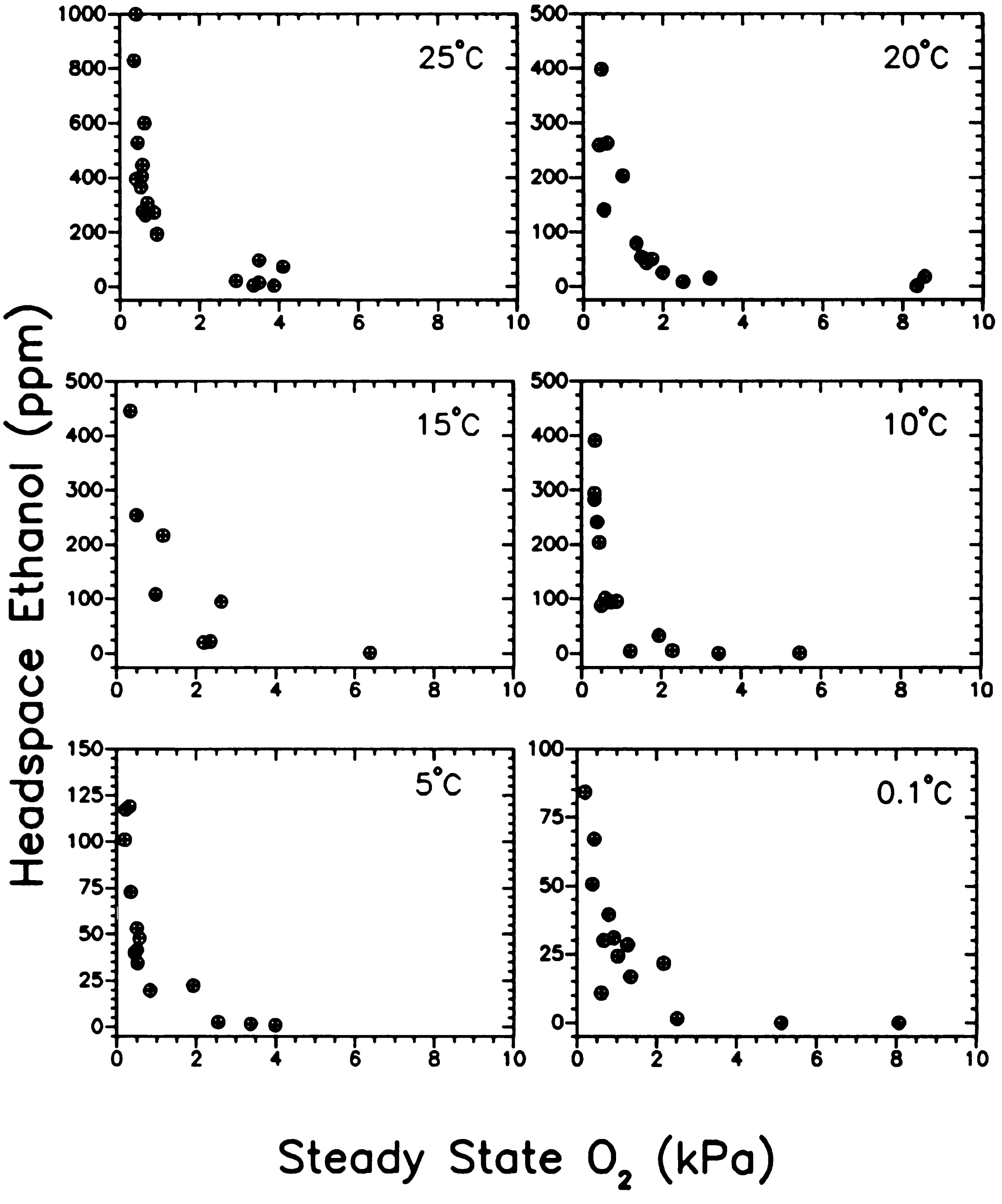

Figure 4. Effect of temperature (0.1, 5, 10, 15, 20, and 25°C) on headspace EtOH levels as an indication of the low O_2 limit of 'Early Red One Red Delicious' apple fruit.

Table 1. Estimated low O_2 limit ranges for 3 cultivars of apple fruit indicated by elevated headspace EtOH.

Temperature (°C)	Golden Delicious	Red Delicious	Redmax McIntosh	Marshall McIntosh
0.1/3	0.55-1.25	0.45-0.60	0.75-0.80	1.80-2.25
5	0.70-0.75	0.55-0.85	1.00-1.10	1.75-2.05
10	0.65	0.90-1.20	1.15	2.15-2.70
15	0.55-1.45	1.15-2.20	1.60-2.50	2.35-3.00
20	0.45-0.95	1.70-2.00	2.00-2.35	4.95-5.20
25	1.15-1.65	0.90-3.35	3.85-4.25	6.60-6.85

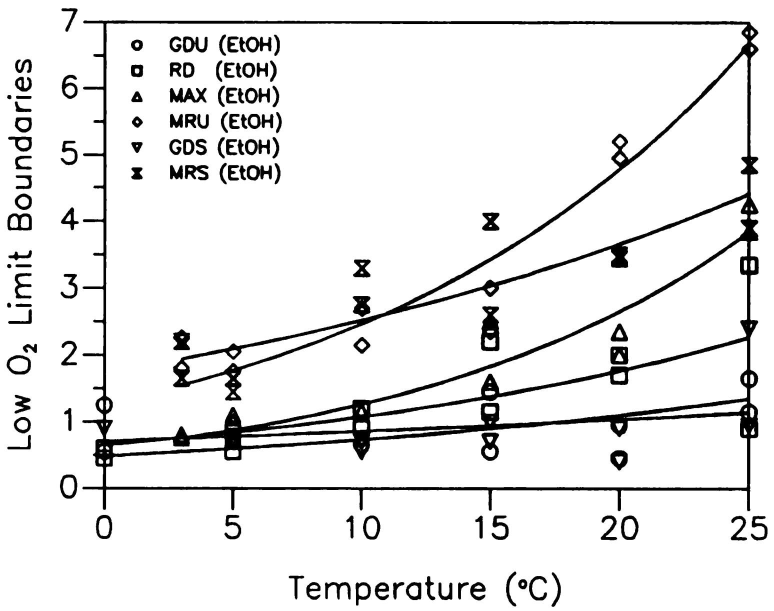

Figure 5. Low O_2 limit boundaries for 3 apple cultivars over a range of temperatures as determined by elevated $[EtOH]_{pkg}$. Treatments include packages with CO_2 removed from headspaces. All low O_2 limits determined by $[EtOH]_{pkg}$ accumulation.

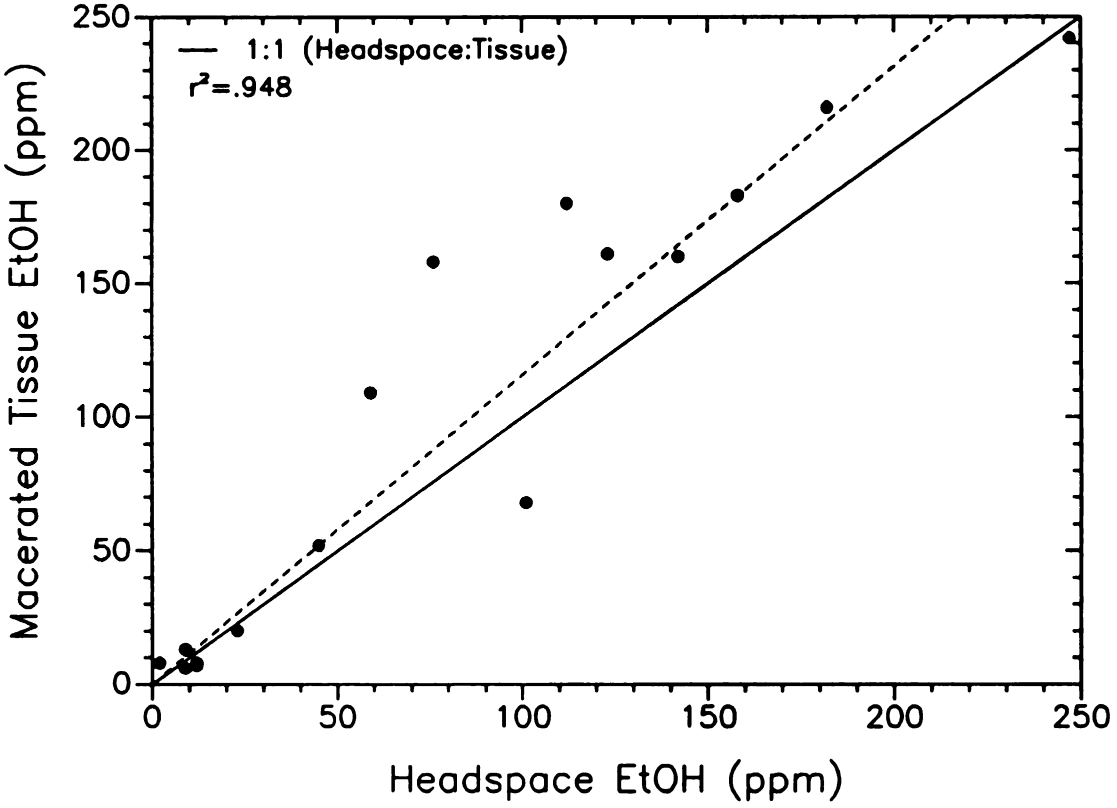

Figure 6. Correlation of headspace EtOH levels with macerated tissue EtOH levels. r^2 = 0.948.

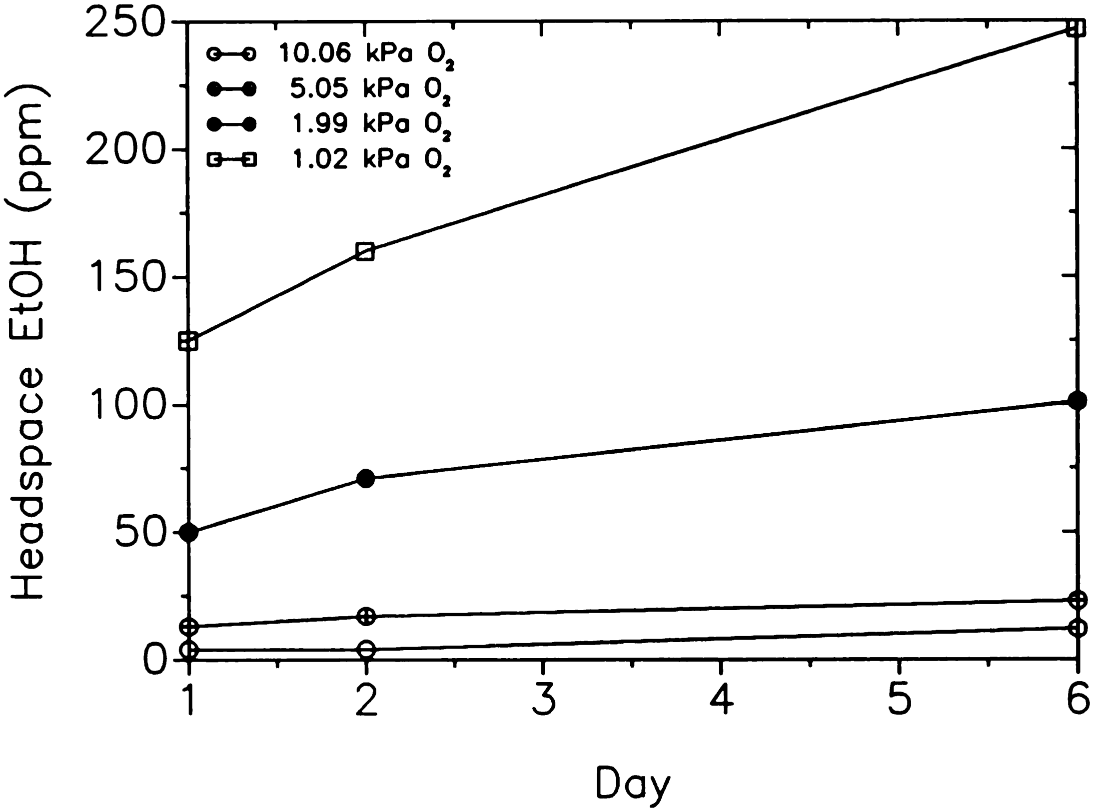

Figure 7. The accumulation with time of EtOH in the headspace of packages at steady-state respiration (Day 1), with a range of headspace O_2 partial pressures ('Redmax McIntosh', at 20° C, with a low O_2 limit of 2.00-2.35 kPa).

indicating continued fermentation of tissues (Figure 7), while packages with an aerobic $[EtOH]_{pkg}$ of approximately ≤ 10 and ≤ 50 ppm, at 0.1/3 and 25°C, respectively, did not show increased $[EtOH]_{pkg}$ during the same period.

$[EtOH]_{pkg}$ increased with the accumulation of EtOH in fermenting tissues. The usefulness of an observed breakpoint or increase in $[EtOH]_{pkg}$ to be an accurate indicator of the low O_2 limit was examined. Packages with anaerobic $[O_2]_{pkg}$ should exhibit elevated $[EtOH]_{pkg}$ with levels of EtOH increasing with time. $[EtOH]_{pkg}$ in packages possessing $[O_2]_{pkg}$ above the low O_2 limit would theoretically remain at a basal aerobic level. Observation of low level EtOH production by apples in storage is common (T. Solomos, personal communication). Packages were sampled at or shortly after it was determined that they had reached steady-state respiration. Although there were increases with time in $[EtOH]_{pkg}$ for fruits determined to have elevated $[EtOH]_{pkg}$ and assumed to be under anaerobic conditions (Figure 6), those fruits at higher $[O_2]_{pkg}$ did not exhibit increased $[EtOH]_{pkg}$. No indication of EtOH production as a result of tissue breakdown was observed for those packages at higher $[O_2]_{pkg}$ 6 days after steady-state respiration.

For 'Marshall McIntosh' fruit with CO_2 removed from headspaces, the $[O_2]_{pkg}$ at which elevated $[EtOH]_{pkg}$ were observed increased with increased temperatures (Figure 6, APPENDIX B). For 'Golden Delicious', the low O_2 limits did not increase noticeably from 0.1 to 20°C, increasing from 20 to 25°C (Figure 3, APPENDIX B). Low O_2 limits for fruit stored in packages with CO_2 removed from headspaces are given in Tables 2 and 4 (APPENDIX B), and did not vary from those with $[CO_2]_{pkg}$ ranging up to 12 kPa (data not shown).

Recent studies of Dadzie *et al.*, (1992) support the observation that the lowest P_{O2} at which fruits may be stored is not constant, but is temperature dependent, and the lowest P_{O2} safe for storage varies to the extent of changes in respiration rate, temperature quotient (Q_{10}) values (defined as the ratio of change in respiratory rate with a temperature change of 10°C and with a common value of approximately 2) and differences in surface resistance to gas diffusion. Differences in low O_2 limit for apple fruit with temperature may be directly related in part to variable tissue permeabilities and Q_{10} values, although tissue resistance to gas exchange has been shown to change significantly with ripening and aging (Rodriguez *et al.*, 1989), and Q_{10} values are not constant and may be as high as 7 with a change of temperature from 0 to 10°C (Wills *et al.*, 1989). Changes in Q_{10} values for respiration-linked reactions may vary among the cultivars examined.

The three cultivars examined possess different gas exchange characteristics, storage characteristics, and vary in susceptibility to storage disorders. 'McIntosh' cultivars are generally less permeable to gases and are especially prone to storage disorders, including low O_2 damage (Fidler *et al.*, 1973; Meheriuk, 1989). 'Marshall McIntosh' are predisposed to storage damage and are particularly problematic for storage. 'Golden Delicious' and 'Red Delicious' cultivars, in contrast, tend to be more permeable to gas exchange and less susceptible to atmosphere-related storage disorders. An interdependence of fruit morphology, gas exchange characteristics, lowest P_{O2} safe for storage, respiration rates, and susceptibility to low O_2 and high CO_2 storage disorders is evident with comparisons of low O_2 limits and fruit characteristics.

The determination of the low O_2 limit of 'Marshall McIntosh' was difficult due to variations in $[O_2]_{pkg}$ at which EtOH accumulated for individual fruits and packages. Variable $[O_2]_{pkg}$ for EtOH accumulation were accompanied by variable RQ breakpoints (Chapter 2), indicating fruit-to-fruit differences in gas exchange characteristics. Burg and Burg (1965) concluded that unblocked, open lenticels serve as a primary route for gas exchange of apple fruit. The differences in gas exchange characteristics of individual fruits may be the result of differences in the amount of cuticular waxes or the number of open lenticels present on individual fruit. It would also be expected that variations in gas exchange characteristics and low O_2 limits may be seen with variable growth conditions. Climate and environmental factors such as growing season moisture levels, humidity, and air pollution may alter the makeup and amount of cuticular waxes present, affecting the number of open lenticels closed by natural waxing. Observations of unwaxed orange (*Citrus sinensis* L. Osbeck) fruit and grapefruit (*Citrus paradisi* Macf.) fruit peels revealed lenticels were often clogged by natural waxing or foreign debris (Ben-Yehoshua *et al.*, 1985).

In the case of apple fruit with cuticles possessing few lenticels and heavy waxing, as is the case with 'Marshall McIntosh', the lenticels appear less likely to be the sole route of gas exchange. We cannot assume, for all cultivars of apple fruit, that the number of open lenticels, pore size, and the diffusive resistance of the cuticle serve as the sole determinants of gas exchange, thereby determining low O_2 limits for storage. The flooding of intercellular air space, measured at 30-35% of the total volume of apple fruit (Smith, 1947), would alter the diffusive resistance to gases, especially gases with low solubility in H_2O such as O_2. Watercore, a disorder seen especially in over-mature fruits, would alter gas exchange properties, increasing tissue resistance, and increase the low O_2 limit.

While accumulation of EtOH in the headspace of modified atmosphere packages appears to be a quick and accurate means to determine the low O_2 limit of apple fruit using a limited number of fruit, it does not provide a definite indication of actual fruit damage and propensity for the development of storage disorders. The studies of Lau (1989), examining fruit tolerance to anaerobic conditions, showed that while 61% of 'Spartan' apple fruit showed discoloration after 7 months at 0.5% O_2, 8% of 'Red Delicious' apples discolored. The development of storage disorders, including low O_2 damage, appears to be cultivar dependant with variations in tolerance to intermittent or continuous anaerobic conditions. While EtOH has not been determined to be the cause of any storage disorder, it may indicate tissue fermentation, which is a commonly associated symptom of many storage disorders.

The results of this study underscore the possible problems in developing and implementing commercial MAP of fruits and vegetables through the marketing chain, given the current packaging films available and their permeability characteristics. Data such as that generated herein may be used to calculate proper combinations of produce weight, package size, and film thickness to generate low O_2 and elevated CO_2 atmospheres, which in turn may extend storage life and maintain high fruit quality. For commodities exposed to a range of temperatures in transit and holding, rates of respiration would fluctuate, causing package $[O_2]_{pkg}$ to decline to P_{O2} below the anaerobic extinction point. Temperature increases would thereby induce tissue fermentation, while temperature decreases would elevate P_{O2}, such that shelflife would not be extended. Recent review articles by those working with the storage and transit of horticultural commodities reiterate these concerns for commercial applicability of MAP technology given the temperature exposures from harvest to consumer (Sharp *et al.*, 1993), the properties of films available for MAP, and the inability to control atmosphere compositions with changes in temperature (M.E. Saltveit, personal communication).

The determination of low O_2 limits by EtOH accumulation, compared with calculations of RQ values and RQ breakpoints (Chapter 2), another indicator of low O_2 limits, establish higher low O_2 limits, with disparity in the determined low O_2 limits increasing with increasing temperatures for both strains of 'McIntosh'. Previous experiments examining low O_2 limits of 'Law Rome' apple fruit indicated RQ breakpoints and $[EtOH]_{pkg}$ accumulation occurred at the same $[O_2]_{pkg}$ (Gran and Beaudry, 1993). The previous experiment, however, was conducted at 0.1° C and it appears that differences between the techniques in determining low O_2

limits increased with increased temperatures. Low O_2 limits determined by $[EtOH]_{pkg}$ accumulation are very close to the atmospheres recommended for commercial storage, while comparison of RQ-determined low O_2 values suggest slightly lower than recommended storage atmospheres at 0.1 and 3° C.

Using MAP to determine the low O_2 limits by means of $[EtOH]_{pkg}$ accumulation is a simple and accurate technique. Results are similar to previous large scale, commercial storage studies. The procedures do not require extensive facilities or the large quantity of fruit as is the case for CA storage experiments. While technical problems may plague the commercial application of MAP technologies, the system may prove a useful tool for applied studies of ripening and stored fruit physiology.

Differences in gas exchange characteristics must be considered for storage atmosphere recommendations and are likely to be helpful in establishing storage atmosphere regimes for new varieties. Gas exchange characteristics should not be overlooked as a factor affecting successful storage on a year to year basis. Commercial storage recommendations for P_{O2} continue to decrease, with ultra low O_2 storage (1 kPa or less P_{O2}) atmospheres increasingly used. Further reductions of P_{O2} for storage will accentuate the consequence of a variation in gas exchange characteristics. Cultivar-specific data relating fruit-to-fruit variability of gas exchange characteristics may prove important for successful storage in the future.

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Summary and Conclusions

Low O_2 Limits and Storage Studies

Results from two years of experimentation show low O_2 limits vary with temperature, cultivar, and slightly from year to year. Low O_2 limits increased with increasing temperature for all cultivars examined, using both RQ breakpoint and elevated EtOH levels as indicators. The extent to which increasing temperatures affected low O_2 limits varied highly between cultivars, with low O_2 limits for 'McIntosh' fruits, particularly the 'Marshall' strain, changing $\approx$4 kPa O_2 from 3 to 25°C.

Low O_2 limit values determined by RQ breakpoint were slightly lower than those indicated by elevated $[\mathrm{EtOH}]_{\mathrm{pkg}}$. Low O_2 limits indicated by elevated $[\mathrm{EtOH}]_{\mathrm{pkg}}$ compared closely with commercially recommended storage O_2 atmospheres. Differences in low O_2 limits among the various cultivars examined may be related to tissue gas exchange characteristics.

Variations in Low O_2 Limits Determined by RQ Breakpoint and Elevated Headspace EtOH Levels

The extensive storage studies initiated by Kidd and West and continued by Fidler and North at Ditton Laboratory in England established general physiological characteristics of fruit ripening and climacteric respiration. In these studies, RQ values associated with fruits in aerobic atmospheres were found to rise with increased temperature, be reduced with increased P_{CO2}, and normally ranged from 1.5 to 1.8, but up to 3.0 under certain conditions. The results of this study confirm many previous results and thereby demonstrate the usefulness of

MAP as a tool to examine the physiology of fruits in MA storage.

An interesting note from the study, particularly when examining the effects of temperature on low O_2 limits, is the apparent difference in low O_2 limit determined by RQ breakpoint and the low O_2 limit, or anaerobic extinction point, determined by headspace EtOH accumulation. In almost every instance, RQ breakpoint values determined and taken to represent low O_2 limits were slightly lower than those determined by elevated headspace EtOH levels.

Why is there an apparent difference in the determined low O_2 limit with alternate methods? Both methods rely upon physiological characteristics rather than empirical determination of threshold levels below which excessive damage occurs. One primary difference is the dependence of RQ breakpoints upon calculated flux levels, while headspace EtOH levels are measured directly. Differences in actual and measured film P^{*}_{CO2} would result in miscalculated RQ values. This error would primarily affect basal RQ values, while affecting RQ breakpoints to a lesser extent. Errors in calculated $[O_2]_{pkg}$, however, could effect both aerobic RQ levels and the determination of the $[O_2]_{pkg}$ at which the RQ breakpoint occurs.

A possible cause of variation in low O_2 limit determined by the two methods may be the differences in tissue permeability for the 'McIntosh' strains compared with 'Golden Delicious' and 'Red Delicious'. Comparisons of apparent whole fruit K_m for O_2 (Figures 14 and 15, Chapter 2) indicate relatively high and low tissue gas permeabilities for 'Golden Delicious' and 'Marshall McIntosh', respectively, over a range of temperatures. While the apparent K_m for O_2 of 'Marshall McIntosh' increased linearly with increasing temperature, the apparent K_m for O_2 for 'Golden Delicious' apple fruit increased rapidly from 0 to 10° C and remained constant at temperatures up to 25° C.

An additional indication of respiration being limited by tissue permeability for 'Marshall McIntosh' is the shape of predicted O_2 uptake curves (Figures 10 and 11, Chapter 2). With increased temperatures, predicted O_2 uptake curves approach linearity, indicating limited O_2 uptake with any decrease in $[O_2]_{pkg}$ at 25° C. Predicted O_2 uptake curves for 'Golden Delicious' (Figure 12 and 13, Chapter 2), in comparison, did not change markedly with increased temperature.

Variations in determined low O_2 limits may result from the indicated tissue permeability differences. Limited cuticle gas permeability may cause O_2 and CO_2 gradients within the fruit tissue. If the principal route of O_2 and CO_2 gas exchange into 'Marshall McIntosh' is through the calyx pore rather than lenticels, thought to be the principal route for gas exchange into apple fruits (Burg and Burg, 1965), those areas with the lowest level of P_{O2} would be directly below the cuticle rather than at the center of the fruit tissue, as is normally though to be the case for apple fruit and other bulky fruit tissues. Localized anaerobic areas created under the cuticle in an aerobic atmosphere for the whole fruit tissue may induce fermentation and EtOH production in portions of the 'Marshall McIntosh' fruit tissue at aerobic O_2 atmospheres, indicating low O_2 limits at higher a $[O_2]_{pkg}$.

Variations in O_2 Uptake Between Cultivars

Another issue for examination relates to variations in RR_{O2} between cultivars. Presentation of this data has brought differing opinions from fellow scientists, some believing this to be an actual phenomenon related to limiting RR_{O2} with variation in flesh permeability, others believing differences to be artifacts of the experimental methods. Further examination of this is required. Arguments that the apparent differences are actually artifacts include the heritable enzymatic systems involved in fruit respiration. Any differences in RR_{O2} indicate possible differences in K_m values for O_2. Apparent K_m for O_2 for whole fruit is not a function of cytochrome oxidase alone, but involves other oxidases, as well as whole tissue gas permeability to O_2. Differences in K_m for O_2 may be indicative of the extent to which tissue resistance to gas diffusion limits respiration of individual fruit or fruit cultivars.

Variations in tissue P^*_{O2}, P^*_{CO2}, P^*_{v}, P^*_{C2H4} (Burg and Burg, 1965; Cameron, 1982; Ben-Yehoshua *et al.*, 1985; Rodriguez *et al.*, 1989) have been shown for a number of fruit tissues throughout the ripening process. Variations for these tissue permeabilities have also been shown between individual cultivars of apple fruit (Park, 1990).

The internal atmospheres of fruits, both stored in air and under CA conditions, have been examined from the advent of CA storage (Kidd and West, 1949), with O_2 and CO_2 gradients found for various biological gases. The tissue gradients for various physiologic gases appear to be transient (Kidd and West, 1949), vary from year to year and between individual fruits and regions of each fruit (Dadzie *et al.*, 1992), and change significantly with maturation and ripening (Kidd and West, 1949). MAP techniques may be useful in the examination of gas exchange and internal gas gradients of fruits.

Problems of Modified Atmosphere Packaging Examination of Fruit Storage

One problem associated with the use of MAP techniques for examination of fruit physiology is the dependance of atmosphere establishment upon respiration, the long length of time for atmosphere pulldown at low temperatures, and changes in respiration with time. With delayed atmosphere establishment, ripening would progress in packages, allowing changes in carbohydrate and organic acid content. Such changes are detrimental for stored fruits, and would not be representative of those conditions desirable for commercial storage studies and applications.

The influence of CO_2 on fruit ripening, and the means by which elevated CO_2 partial pressures act to extend CA and MA storage life, are still unresolved. Further work must determine the specific mechanisms by which the pleiomorphic effects of CO_2 are induced. Use of MAP techniques to study these factors may be limited by the mechanical constraints of the methodology. While it is possible to completely remove CO_2 from package headspaces, establishment of precise CO_2 levels in combination with specific O_2 levels is more difficult, incorporating MAP and the methods of small scale CA studies. In this system, headspace CO_2 atmospheres depend upon film permeability, and with variation in film permeabilities of 10% on any given lot of film, additional experimental error may be introduced. The increased complexity of experiments and calculations with a possible decrease in accuracy using MAP techniques to study CO_2 effects are not warranted given the availability of CA techniques. Finally, the physiological responses of whole fruit to CO_2 are well studied and documented, with studies using MAP techniques unlikely to increase the information on specific CO_2 effects.

Further Applications of Modified Atmosphere Packaging Techniques

The experiments conducted demonstrate the usefulness of MAP techniques for quick and relatively simple determination of low O_2 limits. These techniques may be particularly useful when CA storage of newly introduced cultivars is desired. When CA storage facilities are not available for storage research, MAP techniques may serve as a useful substitute.

In addition to the study of MA storage of commodities, MAP techniques may prove useful for the study of changes in protein content and syntheses induced by CA and MA storage. Using MAP techniques it is possible to generate a range of O_2 atmospheres in small increments from which samples may be extracted and analyzed. Use of this tool may allow for quick, accurate generation of samples for the study of specific enzymes, proteins, or genes which are modified in transcription, translation, or activation under modified atmospheres.

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APPENDIX A

The following data constitute the CO_2 production rates for 8 cultivars of apple fruit packaged at 0.1° C for the first years experiments (1991). This data is presented as a supplement to data included in Chapter 1.

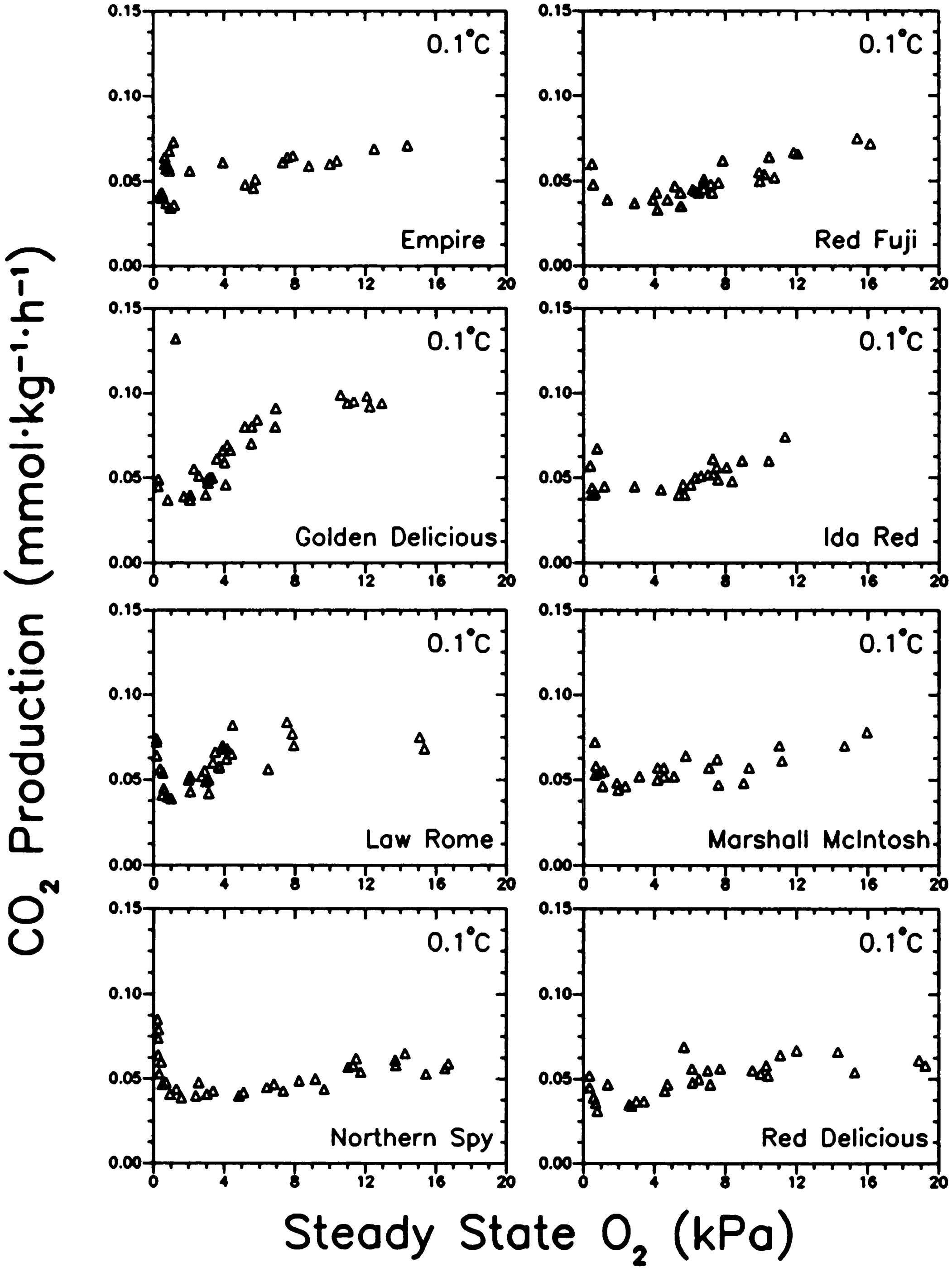

Figure 1. Effect of steady state O_2 partial pressure on CO_2 production of 8 cultivars of apple fruit at 0.1°C.

APPENDIX B

The following data are derived from the second year of experiments (1992). This material consists of raw data for O_2 production rates for the 3 cultivars of apple fruit examined with best fit curves and equations describing those curves, the raw data for SAS® predicted O_2 uptake analysis for 'Marshall McIntosh' and 'Golden Delicious' cultivars, the headspace $[EtOH]_{pkg}$ for packages of 'Marshall McIntosh' and 'Golden Delicious' with CO_2 removed by scrubbing with hydrated lime $[Ca(OH)_2]$, and the low O_2 limits estimated for these varieties using headspace EtOH as the indicator.

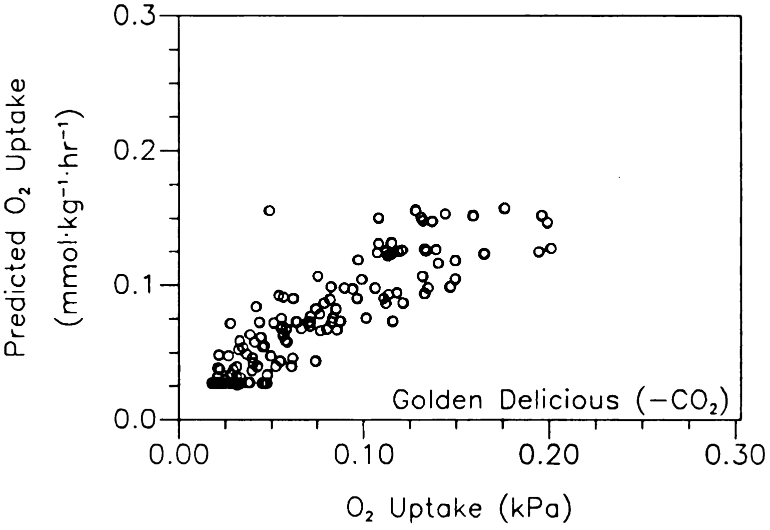

Figure 1. O_2 uptake and predicted O_2 uptake for 'Golden Delicious' apple fruit, with CO_2 removed from package headspaces, over a range of temperatures, using SAS® nonlinear regression fit.

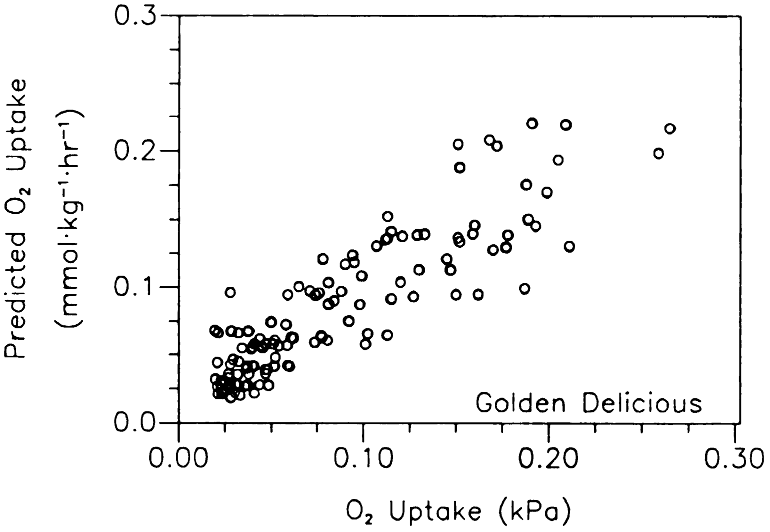

Figure 2. O_2 uptake and predicted O_2 uptake for 'Golden Delicious' apple fruit over a range of temperatures, using SAS® nonlinear regression fit.

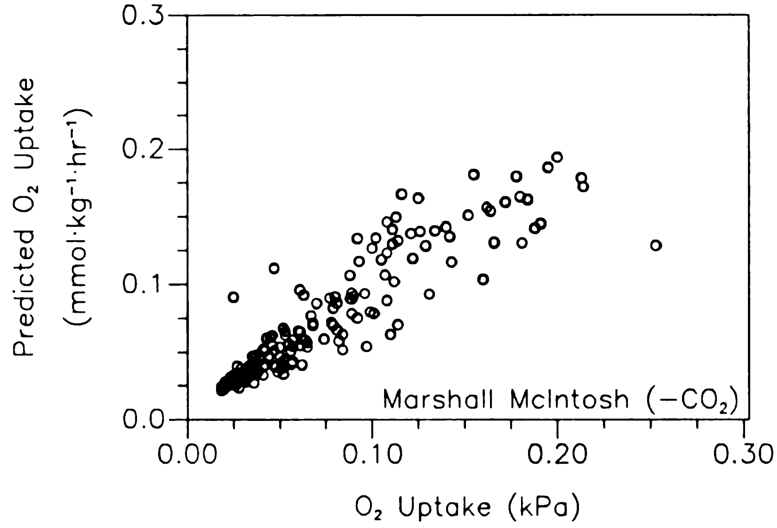

Figure 3. O_2 uptake and predicted O_2 uptake for 'Marshall McIntosh' apple fruit, with CO_2 removed from package headspaces, over at a range of temperatures, using SAS® nonlinear regression fit.

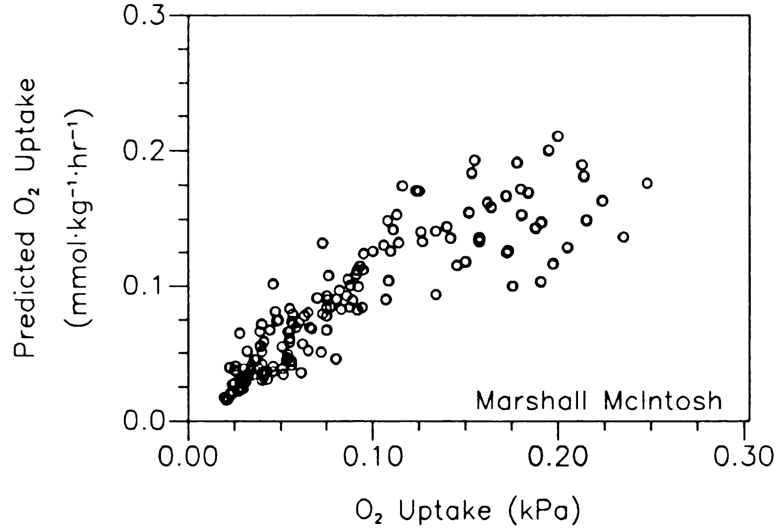

Figure 4. O_2 uptake and predicted O_2 uptake for 'Marshall McIntosh' apple fruit over a range of temperatures using SAS® nonlinear regression fit.

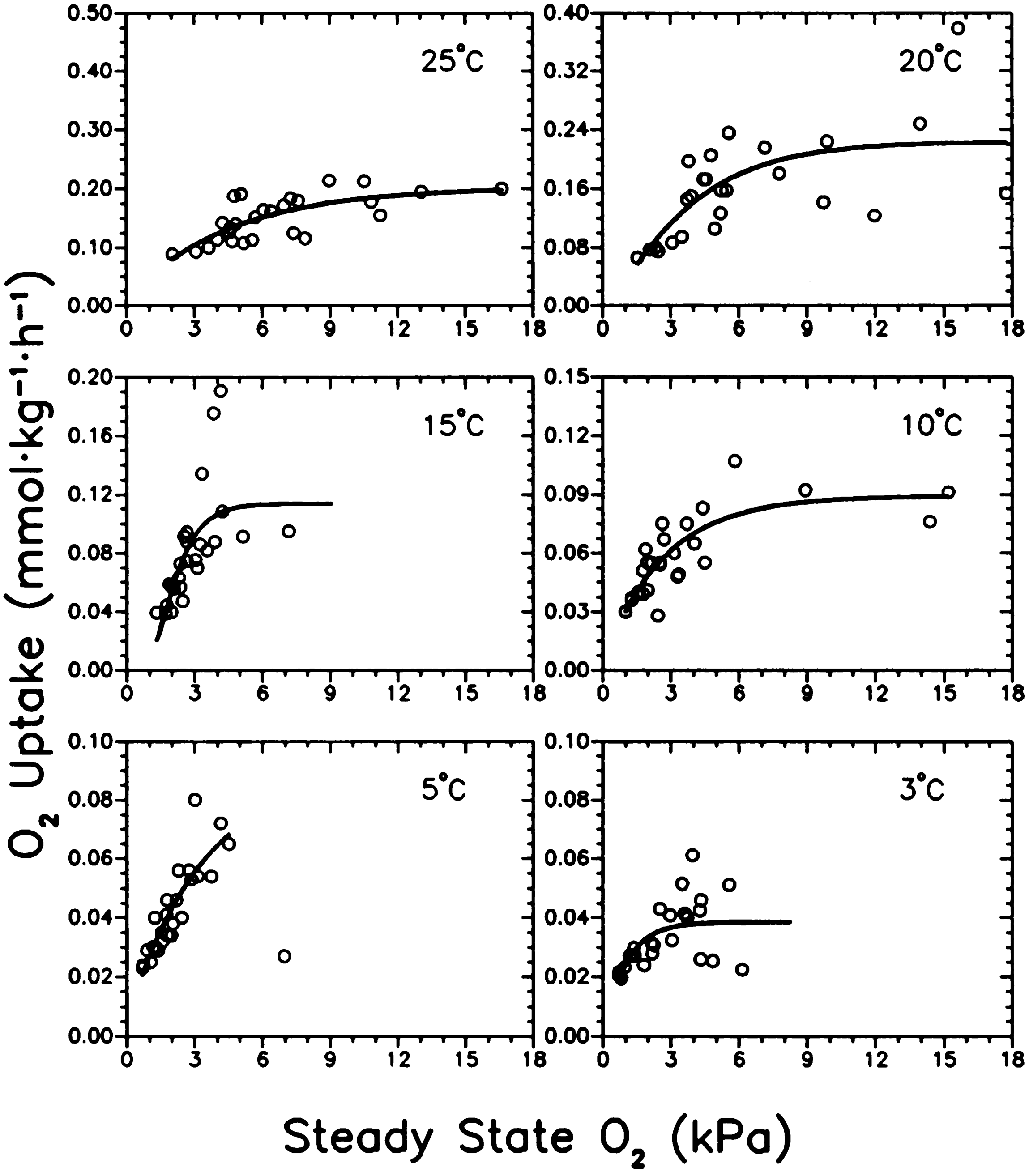

Figure 5. Effect of temperature (3, 5, 10, 15, 20, and 25°C) on RR_{O2} of 'Marshall McIntosh' apple fruit. See Table 1 for equations describing curves.

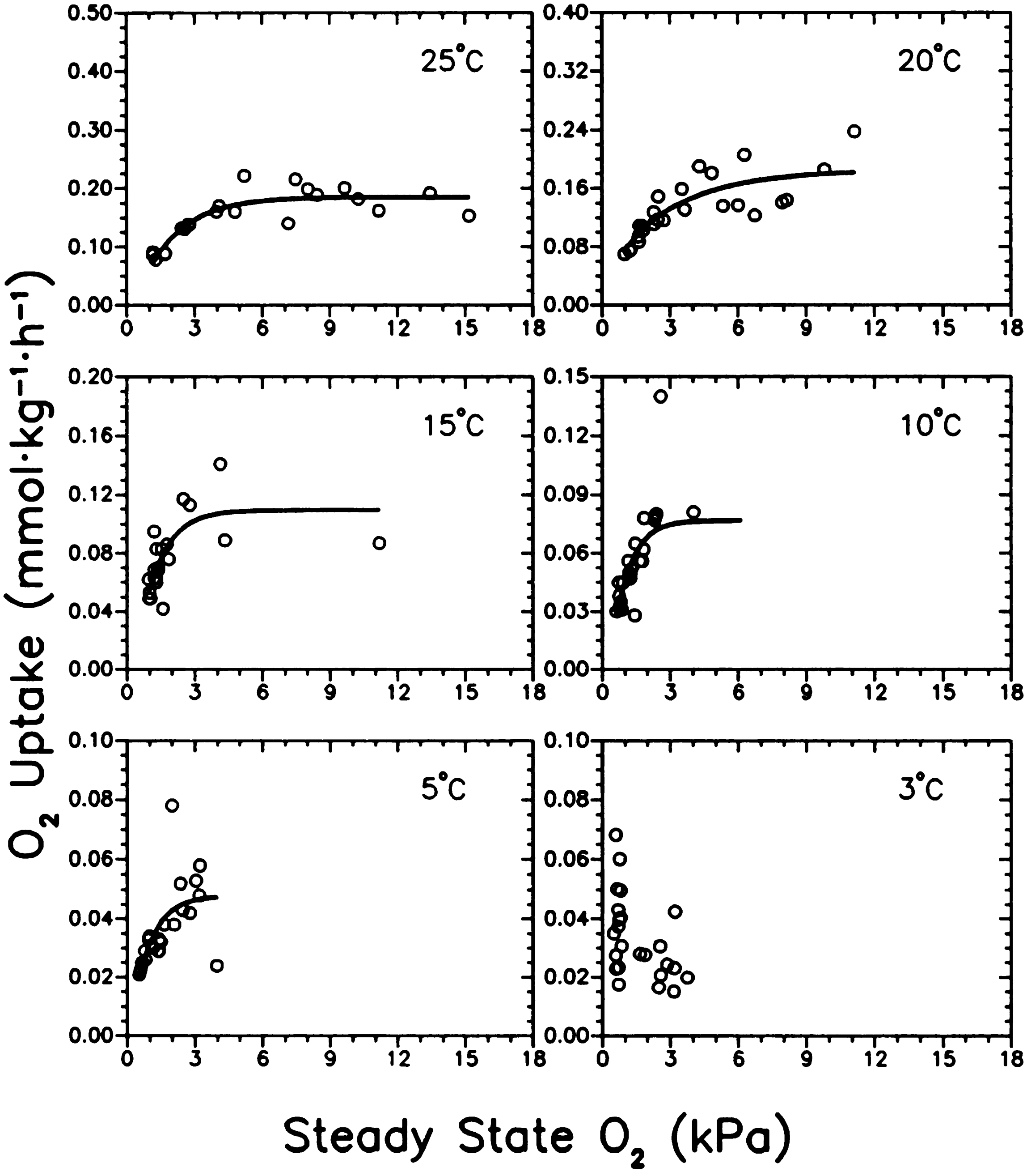

Figure 6. Effect of temperature (3, 5, 10, 15, 20, and 25°C) on RR_{O2} of 'Redmax McIntosh' apple fruit. See Table 1 for equations describing curves.

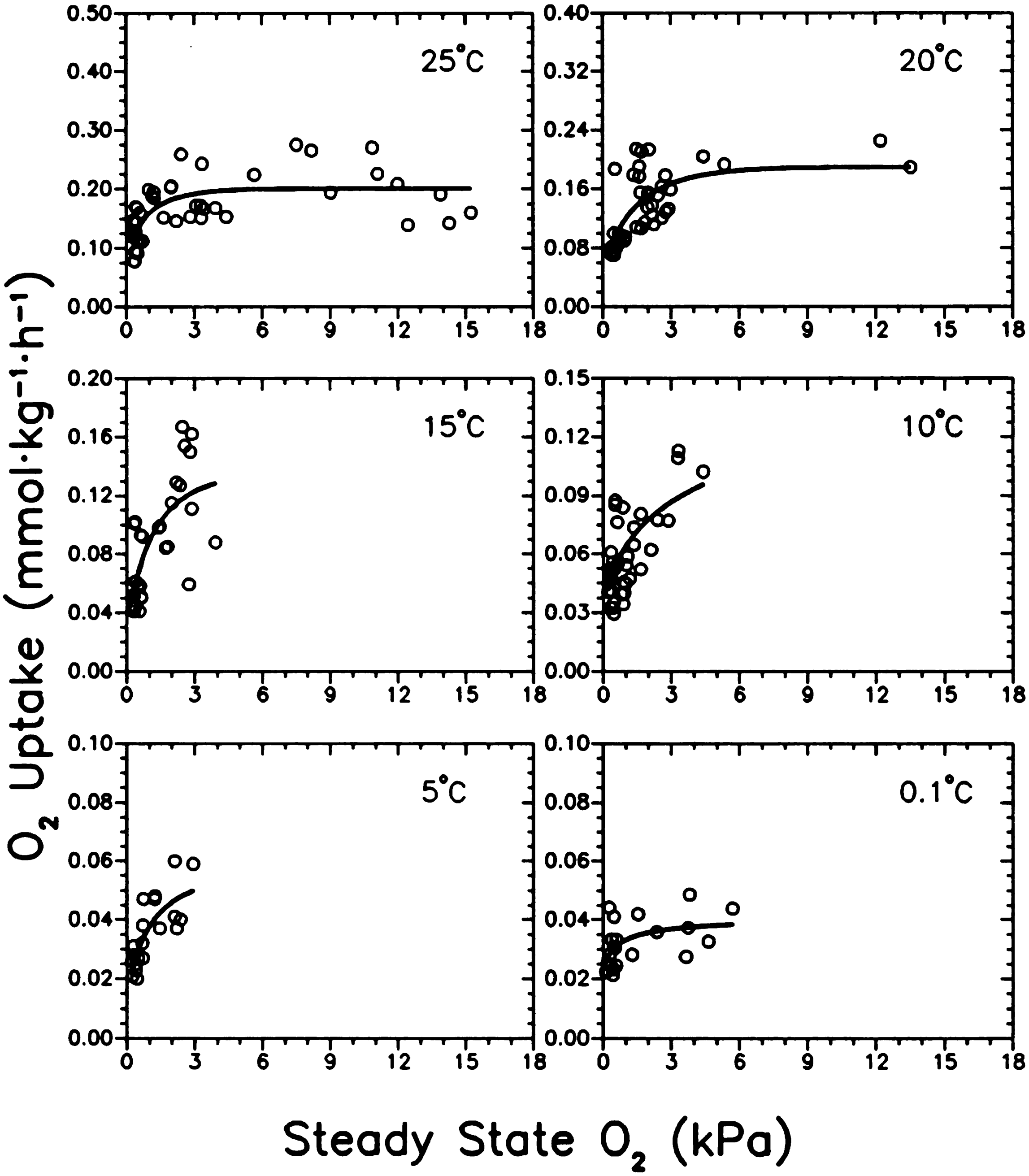

Figure 7. Effect of temperature (0.1, 5, 10, 15, 20, and 25°C) on RR_{O2} of 'Golden Delicious' apple fruit. See Table 1 for equations describing curves.

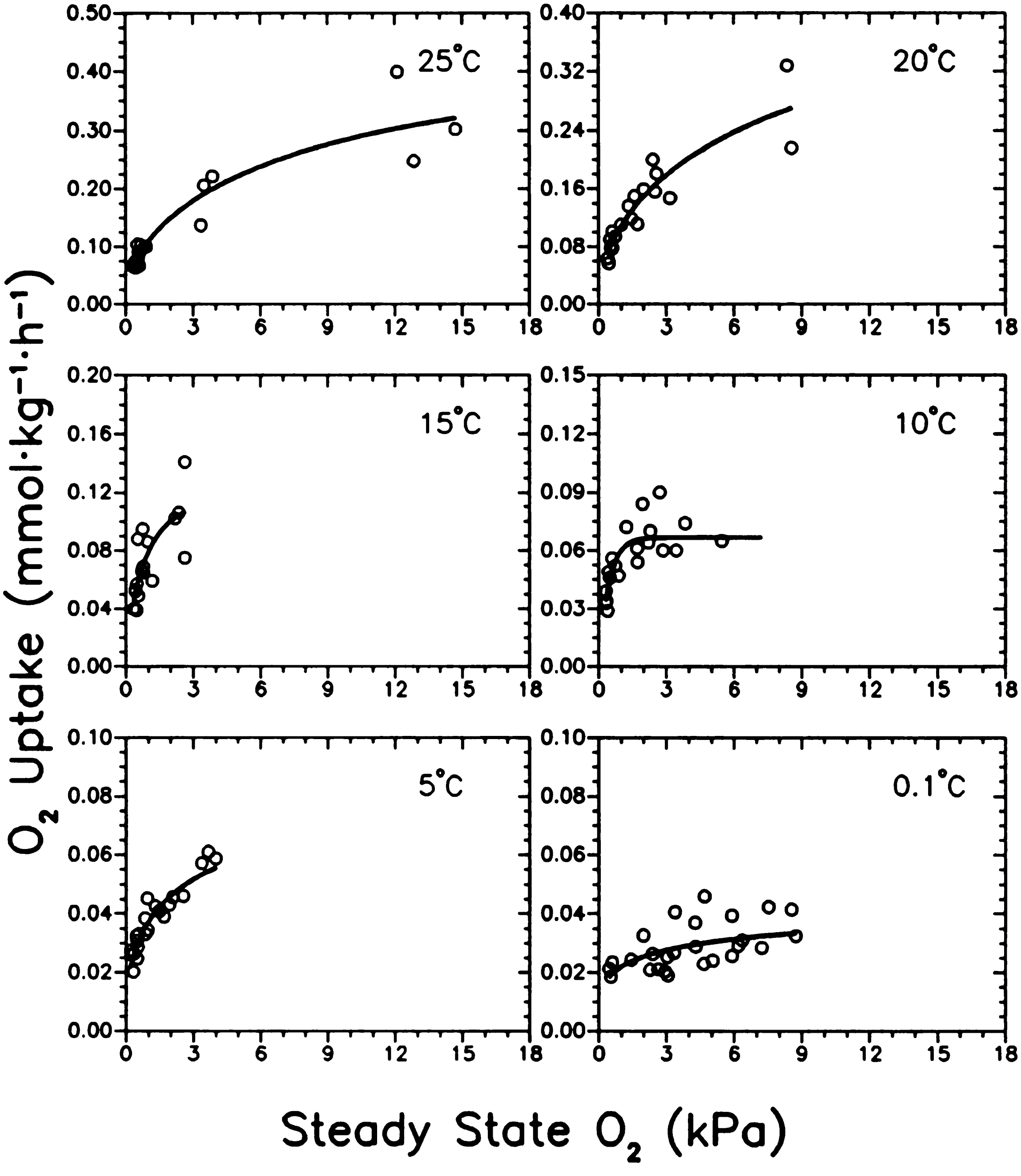

Figure 8. Effect of temperature (0.1, 5, 10, 15, 20, and 25°C) on RR_{O2} of 'Early Red One Red Delicious' apple fruit. See Table 1 for equations describing curves.

Table 1. General equations (Eq. (1)) and values of constants describing the relationship between steady state O_2 partial pressure (kPa) and O_2 uptake ($mmol \cdot kg^{-1} \cdot h^{-1}$) for 3 apple cultivars in modified atmosphere packages over a range of temperatures. Eq.(1): $RR_{O2} = b_1 \cdot \{1\text{-}\exp[-b_2 \cdot (O_2)_{pkg}]\}^{b_3}$

Cultivar	T (°C)	b_1	b_2	b_3	r^2
'Marshall McIntosh'	3	0.03858	1.04802	1.07847	0.357
	5	0.09628	0.23805	0.82028	0.787
	10	0.08918	0.36431	0.91880	0.652
	15	0.11381	1.16961	7.14997	0.469
	20	0.22397	0.32543	1.45153	0.465
	25	0.20351	0.20891	0.87544	0.531
'Redmax McIntosh'	5	0.04790	1.21530	1.19230	0.465
	10	0.07677	1.40427	1.84727	0.445
	15	0.10944	1.05992	1.56692	0.551
	20	0.18612	0.31237	0.69226	0.663
	25	0.18529	0.60942	1.34822	0.798
'Golden Delicious'	0.1	0.03927	0.32535	0.3136	0.259
	5	0.05529	0.51940	0.42338	0.629
	10	0.13979	0.07294	0.29358	0.436
	15	0.13571	0.58672	0.52379	0.521
	20	0.18988	0.48562	0.48583	0.545
	25	0.20109	0.69397	0.34256	0.461
'Early Red One Red Delicious'	0.1	0.03818	0.09332	0.22647	0.306
	5	0.06949	0.18928	0.35375	0.876
	10	0.06669	2.34255	10.3644	0.607
	15	0.11496	0.86286	0.69101	0.616
	20	0.38478	0.07736	0.48758	0.850
	25	0.38988	0.07546	0.48661	0.851

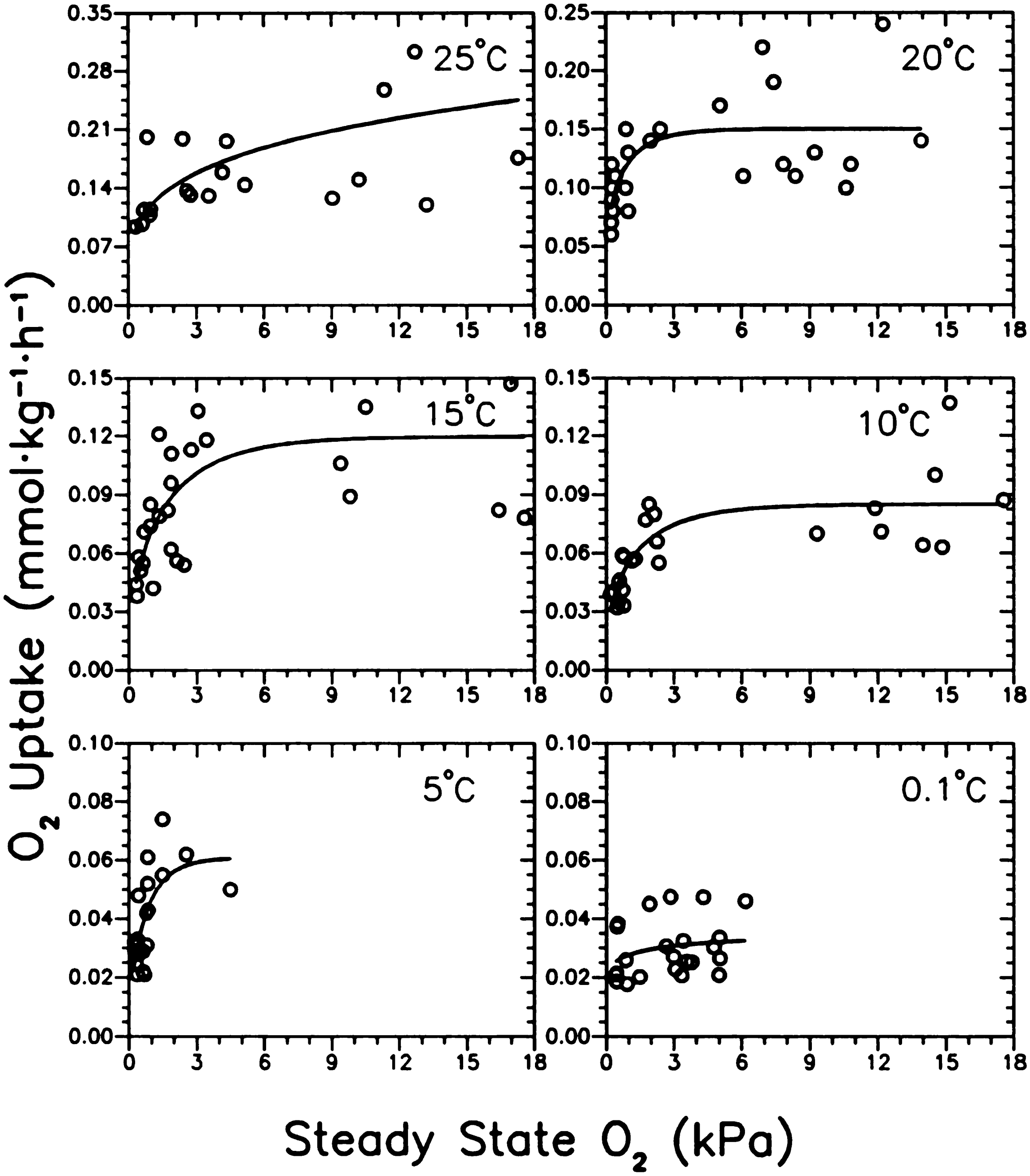

Figure 9. Effect of steady state O$_2$ partial pressure on the O$_2$ uptake of 'Golden Delicious' apple fruit over a range of temperatures with CO$_2$ removed from package headspaces. See Table 2 for equations describing O$_2$ uptake curves.

Table 2. General equations (Eq. (1)) and values of constants describing the relationship between steady state O_2 partial pressure (kPa) and O_2 uptake ($mmol \cdot kg^{-1} \cdot h^{-1}$) for 'Golden Delicious' apple fruit over a range of temperatures with CO_2 removed from package headspaces.

T (°C)	b_1	b_2	b_3	r^2
0.1	0.03499	0.11569	0.10414	0.070
5	0.06087	1.04650	0.61064	0.495
10	0.08503	0.43285	0.41455	0.588
15	0.11967	0.39001	0.45600	0.459
20	0.15026	0.74490	0.32932	0.398
25	0.52207	0.00292	0.25203	0.354

Table 3. Estimated low O_2 limits, as indicated by elevated headspace EtOH, of 'Golden Delicious' apple fruit sealed in packages with CO_2 removed from the headspaces, over a range of temperatures.

T (°C)	Estimated low O_2 limit (kPa)
0.1	≈0.90
5	0.70-0.75
10	0.55-0.70
15	0.70-1.00
20	0.40-0.90
25	0.95-2.40

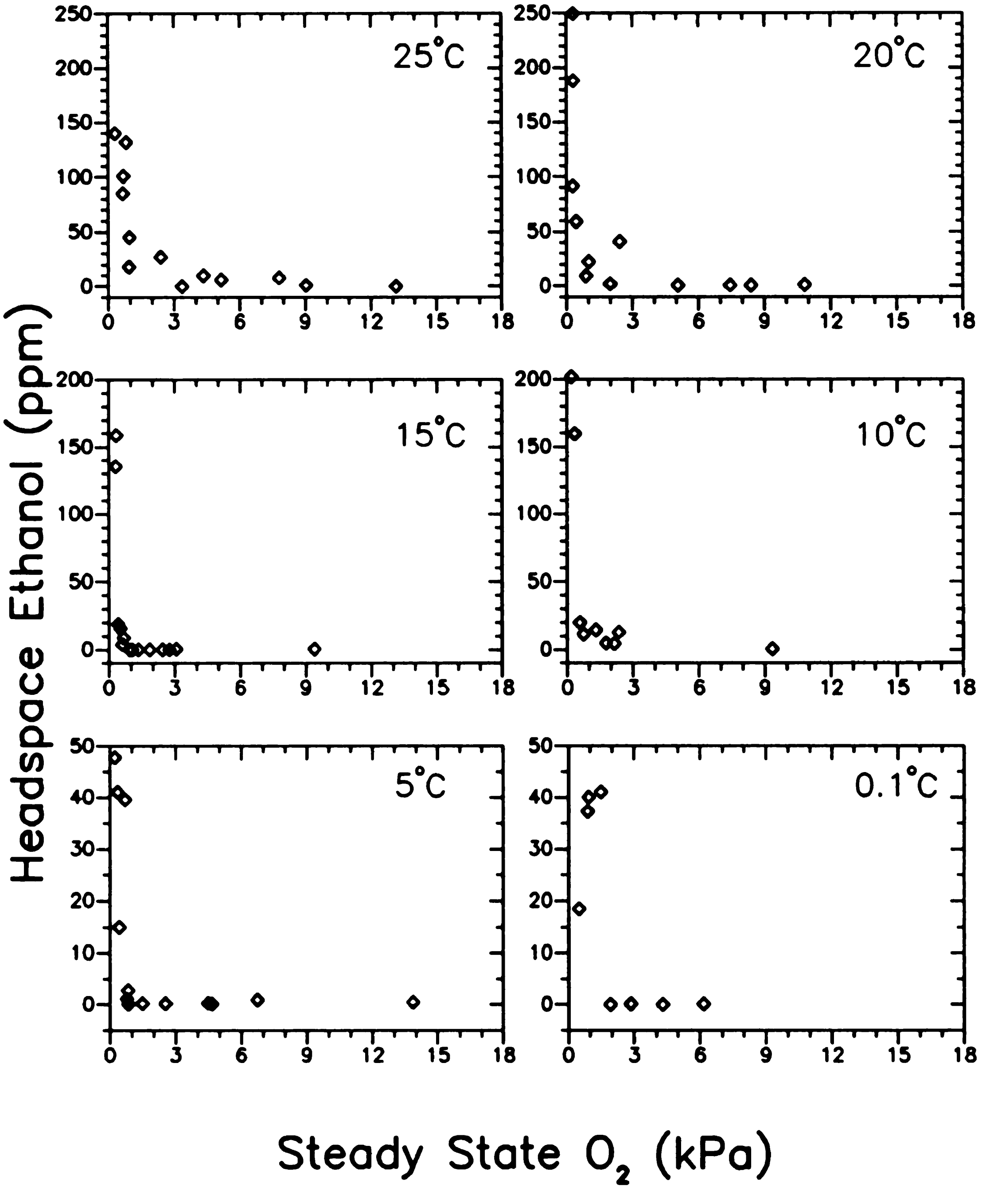

Figure 10. Effect of steady state O_2 partial pressure on the low O_2 limit, as indicated by elevated headspace EtOH levels, of ‘Golden Delicious’ apple fruit over a range of temperatures with CO_2 removed from the package headspaces. See Table 3 for estimated low O_2 limits.

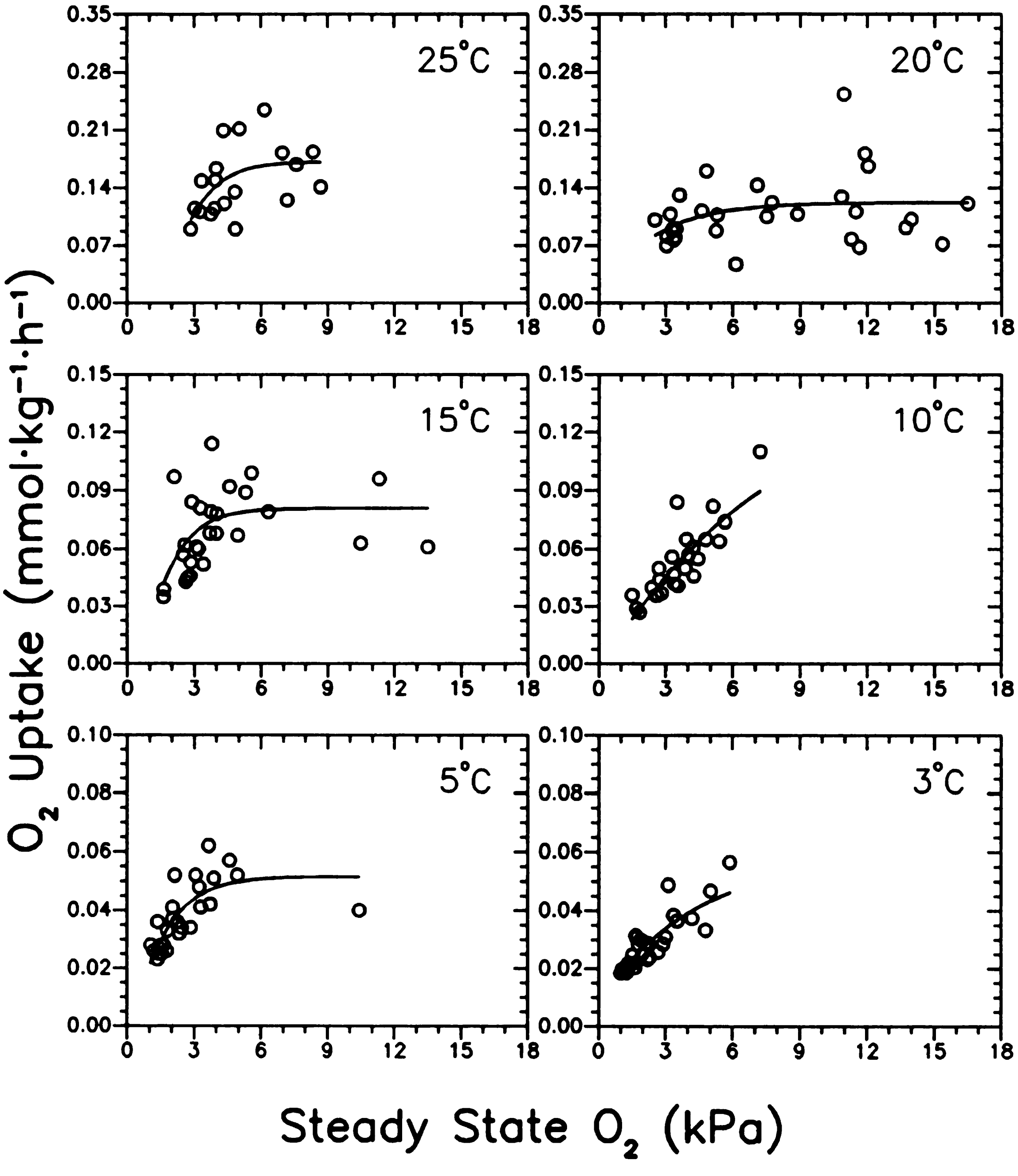

Figure 11. Effect of steady state O_2 partial pressures on O_2 uptake of 'Marshall McIntosh' apple fruit over a range of temperatures with CO_2 removed from package headspaces. See Table 4 for equations describing curves.

Table 4. General equations (Eq.(1)) and values of constants describing the relationship between steady state O_2 partial pressures (kPa) and O_2 uptake ($mmol \cdot kg^{-1} \cdot h^{-1}$) for 'Marshall McIntosh' apple fruit over a range of temperatures with CO_2 removed from package headspaces.

T (°C)	b_1	b_2	b_3	r^2
3	0.06152	0.18805	0.71192	0.730
5	0.05156	0.74187	1.41744	0.609
10	0.13914	0.15775	1.14515	0.712
15	0.08079	0.89873	2.46464	0.291
20	0.12236	0.42082	0.93988	0.104
25	0.17133	0.87569	6.08627	0.280

Table 5. Estimated low O_2 limits, as indicated by elevated headspace EtOH, for 'Marshall McIntosh' apple fruit sealed in packages with CO_2 removed from the package headspaces, stored over a range of temperatures.

T (°C)	Estimated low O_2 limit
3	1.65-2.20
5	1.45-1.65
10	2.75-3.30
15	2.60-4.00
20	3.45-3.50
25	3.90-4.85

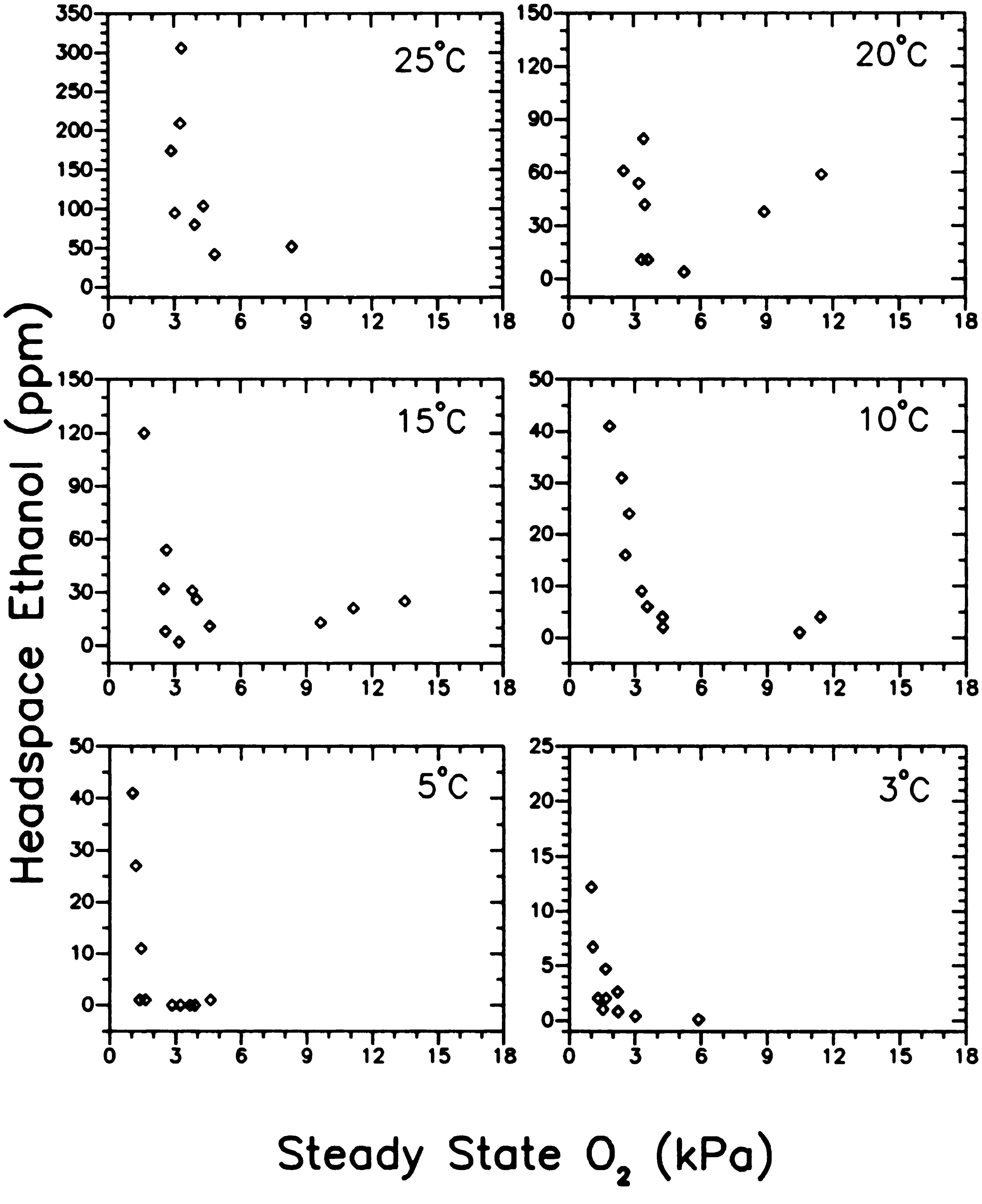

Figure 12. Effect of steady state O_2 partial pressure on the low O_2 limit, as indicated by elevated headspace EtOH levels, of 'Marshall McIntosh' apple fruit over a range of temperatures with CO_2 removed from package headspaces. See Table 5 for estimated low O_2 limits.