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Modified-atmosphere Packaging of Blueberry Fruit: Effect of Temperature on Package O_2 and CO_2

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Abstract. **Highbush blueberry *(Vaccinium corymbosum* L. 'Bluecrop') fruit sealed in low-density polyethylene packages were incubated at 0, 5, 10, 15, 20, or 25C until O_2 and CO_2 levels in the package reached a steady state. A range of steady-state O_2 partial pressures (1 to 18 kPa) was created by placing a range of fruit weights within packages having a constant surface area and film thickness. The steady-state O_2 partial pressure in packages containing the same weight of fruit decreased as temperature increased, indicating the respiratory rate rose more rapidly (i.e., had a greater sensitivity to temperature) than O_2 transmission through the film. Steady-state O_2 and CO_2 partial pressures were used to calculate rates of O_2 uptake. CO_2 Production. and the respiratory quotient (RO). The effects of temperature and 0_2 partial pressure on O_2 uptake and CO_2 production and the RQ were characte~zed. The steady-state O, partial pressure at which the fruit began to exhibit anaerobic CO_2 production (the RQ breakpoint) increased with increasing temperature, which implies that blueberry fruit can be stored at lower O_2 partial pressures when stored at lower temperatures.**

Studies using controlled-atmosphere (CA) storage techniques have indicated that shelf-life extension can be obtained for blueberry ***(Vaccinium*** spp.) fruit using combinations of elevated CO_2 and reduced O_2 in the storage environment (Ceponis and Cappellini, 1985; Smittle and Miller, 1988). CA storage of highbush blueberry fruit is now a commercial reality and current conditions range from 1.5 to 2.5 kpa O_2 (1% O_2 = 1.013 kPa O_2 at 1 atm) and 5 to 12 kPa CO_2 at 0C.

Present-day commercial techniques for packaging blueberry fruit do not modify O_2 and CO_2 to levels that would enhance storage. Modified-atmosphere packaging (MAP) has the potential to provide low O_2/high CO_2 regimes similar to those of CA storage, but throughout the marketing chain. Ideally, a package should maintain the appropriate atmospheric composition over the range of temperatures commonly encountered between harvest and consumption. Poor temperature control, however, can cause package O_2 levels to drop low enough to induce anaerobic respiration (Kader et al., 1989).

The purpose of our work was to investigate the influence of temperature on package O_2 and CO_2 partial pressures, on the rates of 0_2 uptake and CO_2 production as functions of package 0_2 partial pressure, and on the O_2 partial pressure at the RQ breakpoint. Blueberry fruit were chosen for investigation due to their relatively long shelf life, their minimal changes in respiration associated with ripening, and their relative insensitivity to CO_2 levels over the range of those expected to be encountered in the packaging system used.

Materials and Methods

Plant material

Fruit of 'Bluecrop' were hand-harvested into 46 × 61 × 20-cm plastic field lugs (≈ 8 kg of fruit) on 17 Aug. 1989 (second harvest of the season) in the West Olive area of Michigan. Fruit were transported immediately to East Lansing in ice chests. Fruit were held overnight at 5C, sorted for obvious defects, and packaged the next morning.

Film permeability

The permeability of 0.00495 cm (2 roil) low-density polyethylene (LDPE) film to O_2 and CO_2 was determined three to 15 times for each of three random samples at temperatures ranging from 0 to 35C at 5C intervals. It was important to obtain our own permeability coefficients, since values supplied by manufacturers are often inaccurate. Film permeability values vary with each production run for a particular film type, and, in fact, we have commonly found a 10% to 15% variation in permeability along the length of a single roll of film.

The various temperatures were established by submersing a specially built aluminum permeability cell in a water bath (Lauda RC20; Brinkman Instrument Co., West Bury, N.Y.), and temperature (± 0. 1C) was verified using mercury and thermocouple thermometers. The permeability cell contained two circular (8 cm diameter x 0.5 cm deep) 25-ml chambers separated by the film sample, of which 50 cm^2 was exposed to both chambers and sealed in place by an O-ring. The permeability cell was fitted with a 3.5-m coil of 0.64-cm (id.) copper tubing on the gas inlets to permit the inlet gas to reach the temperature of the water bath before entering the cell. Pure O_2, pure CO_2, or a mixture of both gases was introduced to one chamber of the cell and an N_2 carrier gas was introduced to the other chamber. The rate of O_2 and CO_2 diffusion through the film was calculated from the steady-state partial pressure of the sample gases diffusing through the film and into the carrier gas stream. The partial pressure of O_2 and/or CO_2 in the carrier gas stream was determined using a sequential combination of O_2 (Ametek S-3A/II with a calcia-zirconia electrochemical detection cell; Ametek Co., Thermox Instrument Div., Pittsburgh) and CO_2 (ADC 225-MK3 analytical infrared gas analyzer; Analytical Development Co., Hertfordshire, England) analyzers. Gas concentrations in

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Abbreviations: CA, controlled atmosphere; LDPE, low-density polyethylene; MAP, modified-atmosphere packaging; RQ, respiratory quotient.

the carrier gas ranged from 2.5 to 150 µl O_2/liter and 12.5 to 750 µl CO_2/liter, which were well within the limits of detectability. Concentrations were calculated relative to a certified standard gas mixture (109 µl O_2/liter and 94.3 µl CO_2/liter in N_2 gas). Flow rates were maintained at 100 ml·min^{-1} for all gases, and the chamber pressures were equalized and maintained at ≈ 6 cm H_2O above atmospheric.

Calculated concentrations were converted to partial pressures for determination of permeability coefficients. A best-fit equation of an Arrhenius plot of the data was used to determine permeability coefficients for packages at each storage temperature.

Packaging

Blueberry fruit were sealed into 10 × 20-cm (400 cm^2 total surface area) pouches comprised of 0.00495-cm-thick LDPE film (DOW Chemical Co., Midland, Mich.) for which O_2 and CO_2 permeability was measured as described above. Each package was equipped with a gas-sampling septum constructed of a short strip of electrical tape with a dab of DuPont Silicone II tub/tiling glue and sealant (Boylan-Pett, 1986). Fruit were weighed before being packaged, and weight per package was targeted at the following: 10, 20, 30, 40, 50, 60, 70, 80, 90, 110, 130, and 150 g. This range of 12 target weights was designed to generate a nearly continuous range of steady-state O_2 and CO_2 partial pressures within the package (Cameron et al., 1989). This range of fruit weights was placed at 0, 5, 10, 15,20, and 25C with four packages at each target weight/temperature combination.

Steady-state O_2 and CO_2 levels and respiratory rates

Package gas composition was determined daily by withdrawing a l-ml gas sample from the package with an insulin-type plastic syringe and analyzing the sample for O_2 and CO_2 using the above-noted O_2 and CO_2 analyzers connected in series with N_2 as the carrier gas (flow rate = 150 to 200 ml·min^{-1}. With this arrangement of detectors, O_2 and CO_2 concentrations could be determined for the same gas sample in ≈ 10 sec and there was no need to correct for argon as in conventional gas chromatography. As before, gas concentrations were converted to partial pressures. The gas composition of individual packages was monitored until steady-state conditions were reached, at which time data were collected and analyzed. The time needed to achieve steady-state conditions increased as the storage temperature decreased and ranged from 2 days at 25C to ≈14 days at 0. No fungicide treatment was used; data were not taken from packages having obvious holes or containing moldy berries.

The steady-state O_2 and CO_2 partial pressures of the packages and the permeability data were combined to ascertain the rates of respiration using the following formulae:

$$RR_{O_2} = \frac{\frac{P_{O_2} \cdot A}{x}[(O_2)_{atm} - (O_2)_{pkg}]}{W} \quad [1]$$

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

where RR_{O_2} and RR_{CO_2} are the rates of 0_2 uptake and CO_2 production (mmol·kg^{-1}·h^{-1}), respectively; P_{O_2} and P_{CO_2} are measured O_2 and CO_2 permeability coefficients (mmol·cm^{-1} per cm^2 per hour per kPa), respectively, for our LDPE at the storage temperature; A is film area (cm^2); x is film thickness (cm); $(O_2)_{,,m}$ and $(O_2)_{pkg}$ are atmospheric and package partial pressures of 0_2 (kPa), respectively; $(CO_2)_{pkg}$ and $(CO_2)_{atm}$ are the package and atmospheric CO_2 partial pressures (kPa), respectively, and W is fruit weight (kg). The RQ was calculated as RR_{CO_2}, divided by RR_{O_2}. Data were plotted and analyzed using a computer nonlinear regression analysis package (Eisensmith, 1987).

Results

Film permeability

P_{O_2} and P_{CO_2} increased exponentially with increasing temperature (Fig. 1). An Arrhenius plot of the data (Fig. 1, inset) indicated that the natural log of the permeability coefficient for both gases depended linearly on the reciprocal of temperature °K), and the relationship could be expressed with the equation:

$$\ln(P_i) = \frac{Ea}{RT} + \ln(A), \quad [3]$$

where Pi is P_{O_2} or P_{CO_2}; Ea is the energy of activation of 0_2 or CO_2 permeation (kJ·mol^{-1}); and R is the gas constant (0.0083144 kJ/mol per °K). The slope of the fitted line was Ea/R. Average Ea values were 39.72 kJ·mol^{-1} for O_2 (SD = 1.3) and 35.53 kJ·mol^{-1} for CO^2 (SD = 0.31). Average values for the Y intercept [in(A)] were 5.42 for O_2 (SD = 0.13) and 5.22 for CO_2 (SD = 0.027). The coefficient of determination (r^2) values for individual Es/R determinations ranged from 0.9951 to 0.9999 for O_2 and 0.9984 to 0.9999 for CO_2. Values obtained for Ea and permeability coefficients are similar to published values for LDPE (Yasuda and Stannett, 1975). Equations for predicting P_{O_2} and P_{CO_2} for the film at any T are as follows:

$$P_{O_2} = 0.2269 \times \exp(-4777/T) \text{ mmol·cm}^{-1} \text{ per cm}^2 \text{ per hour per kPa} \quad [4]$$

$$P_{CO_2} = 0.1858 \times \exp(-4273/T) \text{ mmol·cm}^{-1} \text{ per cm}^2 \text{ per hour per kPa}, \quad [5]$$

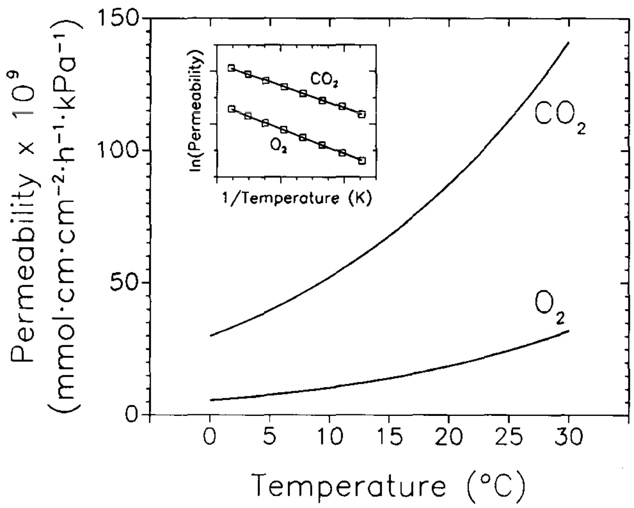

Fig. 1. Effect of temperature on film permeability constants for 0_2 and CO_2 (P_{O_2} and P_{CO_2}, respectively) for 0.00495-cm-thick LDPE film used in packaging experiments. Inset: Arrhenius plot of O_2 and CO_2 permeability for typical film sample.

where T is temperature in °K. Values of $P_{O_2}\cdot A/x$ and $P_{CO_2}\cdot A/x$ were generated using Eq. [4] and [5] and substituting 400 cm^2 and 0.00495 cm for A and x, respectively (Table 1). These values and weight vs. O_2 and CO_2 data from Fig. 2 can be substituted directly into Eq. [1] and [2] for calculation of RR_{O_2} and RR_{CO_2} for any one of the six temperatures studied. P_{CO_2} was ≈4.5 to 5 times P_{O_2} for this film over the temperature range (Table 1).

Steady-state O_2 and CO_2 levels and respiratory rates

Increasing the weight of fruit in the package caused a decrease in steady-state 0_2 at each temperature (Fig. 2). As the temperature increased, steady-state 0_2 tended to decrease for a given package fruit weight (Fig. 3). The data describing the relationship between fruit temperature and package 0_2 were fitted empirically with the simple exponential equation:

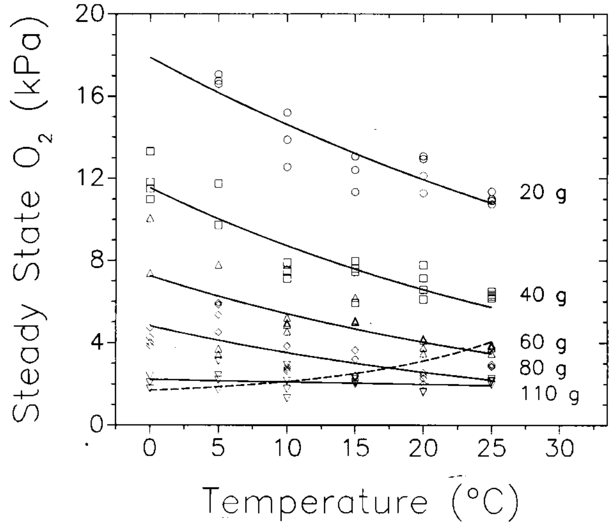

Fig. 3. Effect of temperature on: 1) steady-state 0_2 levels for various weights of blueberry fruit sealed in 400-cm^2, 0.00495-cm-thick LDPE packages (solid lines) containing 20, 40, 60, 80, or 110 g of fruit; and 2) the estimated lower O_2 limit for blueberry fruit (dashed line) based on Fig. 6. See Table 2 for equations describing curves.

Table 1. Whole package O_2 and CO_2 permeabilities (PO2·A/x and $P_{CO_2}\cdot A/x$, respectively) for the packages used in these experiments at 0, 5, 10, .15, 20, and 25C. Values were generated using Eqs. [4] and [5] for packages having a surface area (A) of 400 cm^2 and film thickness (x) of 0.00495 cm. P_{O_2} and P_{CO_2} are LDPE film permeability constants for O_2 and CO_2, respectively. Eq. [4]: P_{O_2} = 0.2269 × exp(–4777/T) mmol·cm^1 per cm^2 per hour per kPa; and Eq. [5]: P_{CO_2} = 0.1858 × exp(–4273/T) mmol·cm^{-1} per cm^2 per hour per kPa.

Temp (°C)	$P_{O_2}\cdot A/x$ (mmol·h^{-1}·kPa^{-1})	$P_{CO_2}\cdot A/x$ (mmol·h^{-1}·kPa^{-1})	P_{CO_2}/P_{O_2}
0	4.612×10^{-4}	2.393×10^{-3}	5.19
5	6.318×10^{-4}	3.171×10^{-3}	5.01
10	8.560×10^{-4}	4.160×10^{-3}	4.86
15	11.47×10^{-4}	5.407×10^{-3}	4.71
20	15.23×10^{-4}	6.965×10^{-3}	4.57
25	20.02×10^{-4}	8.896×10^{-3}	4.44

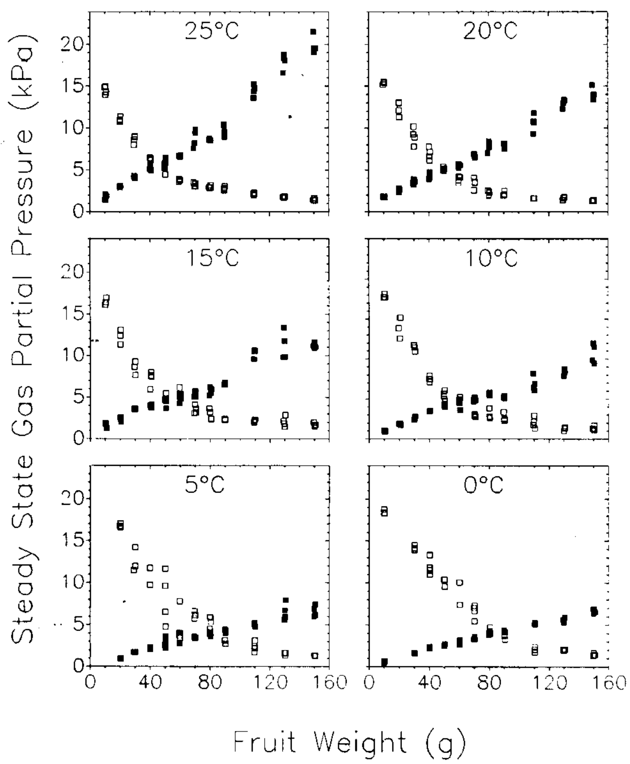

Fig. 2. Effect of blueberry fruit weight on steady-state O_2 (open symbols) and CO_2 (closed symbols) partial pressures in 400-cm^2, 0.00495 -cm-thick LDPE sealed packages held at 0, 5, 10, 15, 20, or 25C.

Table 2. General equation (Eq. [2]) and values of weight-dependent constants describing the relationship between temperature (°C) and steady-state O_2 partial pressures (kPa) for various weights of blueberry fruit sealed in LDPE pouches and stored at six temperatures. Eq. [2]: $(O_2)_{pkg} = x_1\cdot\exp(-x_2\cdot T)$.

Package wt (g)	x_1	x_2	R^2
20	17.89	2.02×10^{-2}	0.82
40	11.54	2.81×10^{-2}	0.82
60	7.248	2.94×10^{-2}	0.48
80	4.816	3.14×10^{-2}	0.52
110	2.218	5.66×10^{-3}	0.05

$$(O_2)_{pkg} = x_1\cdot\exp(x_2\cdot T), \quad [6]$$

where $(O_2)_{pkg}$ is the steady-state O_2 partial pressure and T is temperature in °C. Values for weight-dependent constants xl and x_2 and the R^2 of the fit are listed in Table 2.

The measured O_2 and CO_2 gradients across the film were used to calculate steady-state diffusion rates for both gases through the film, and the respiration rates were calculated (assuming diffusion rates through the package were equal to O_2 and CO_2 fluxes for the respiratory processes) on a per-package weight basis. For each temperature, the relationship between O_2 uptake and steady-state O_2 (Fig. 4) was empirically fitted with the exponential equation:

$$RR_{O_2}, = b_1\cdot\{1 - \exp[-b_2\cdot(O_2)_{pkg}\}^{b3} \quad [7]$$

(Cameron, 1990; Cameron et al., 1989), where RR_{O_2} is the rate of O_2 uptake (mmol·kg^{-1}·h^{-1}) and $(O_2)_{pkg}$ is the steady-state O_2 partial pressure (kPa). Values for constants b_1, b_2, and b_3 and the R^2 of the fit at each temperature are listed in Table 3.

Oxygen consumption decreased in response to decreasing temperature and decreasing steady-state O_2 (Fig. 4). Interestingly, the shape of the O_2-dependent respiratory curves changed with temperature. At the higher temperatures, O_2 uptake did not appear to approach saturation even at the highest levels of steady-

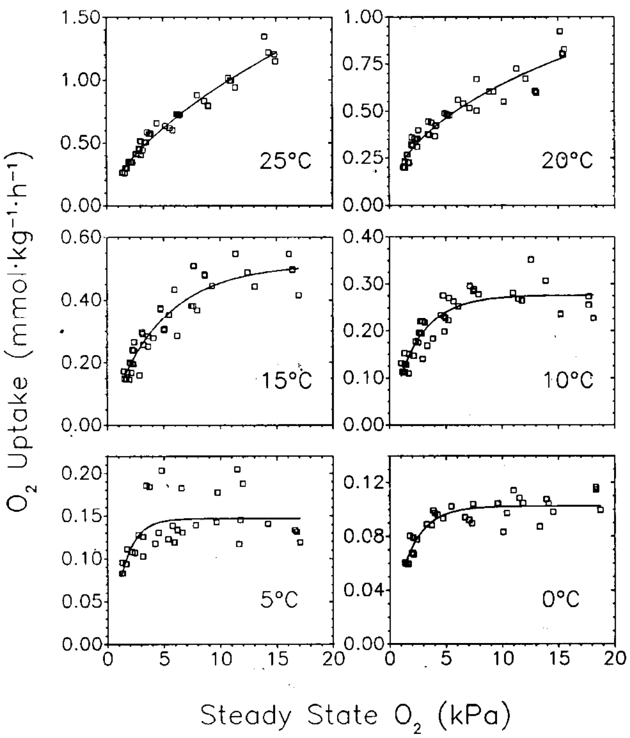

Fig.4. Interdependent effects of steady-state O_2 partial pressure and storage temperature on the calculated rate of O_2 uptake. of blueberry fruit in sealed LDPE packages. See Table 4 for equations describing curves.

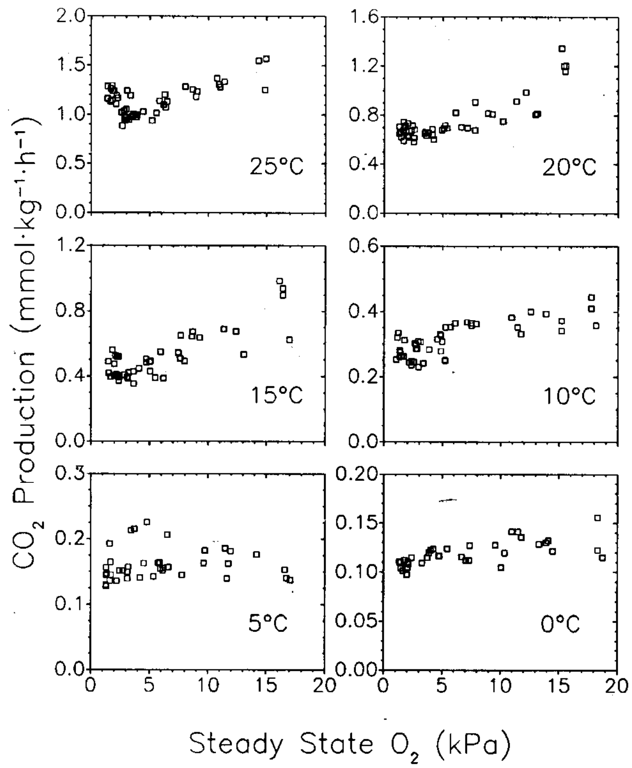

Fig. 5. Interdependent effects of steady-state O_2 partial pressure and storage temperature on calculated rate of CO_2 production of blueberry fruit in sealed LDPE packages.

Table 3. General equation (Eq. [7]) and values of temperature-dependent constants describing the relationship between steady-state 0_2 partial pressures (kPa) and O_2 uptake ($mmol \cdot kg^{-1} \cdot h^{-1}$) for blueberry fruit sealed in LDPE Douches and stored at various temperatures. Eq. [7]: $RRO_2 = b_1 \cdot \{1 - \exp[-b_2 \cdot (O_2)_{pkg}]\}^{b3}$.

Temp (°C)	b_1	b_2	b_3	R^2
25	4.561	9.111×10^{-3}	0.6428	0.97
20	1.871	1.235×10^{-2}	0.4968	0.92
15	0.514	2.067×10^{-1}	0.9205	0.88
10	0.2765	3.829×10^{-1}	0.8795	0.81
5	0.1469	8.461×10^{-1}	1.401	0.48
0	0.1024	5.427×10^{-1}	0.8506	0.80

state 0_2 generated. As temperature declined, however, O_2 uptake showed signs of saturation at ever decreasing O_2 partial pressures. As a result of this phenomenon, fruit were found to be more sensitive to restricted O_2 availability as temperatures increased. For instance, at 0C respiration at 1.5 kPa O_2 was about half that at 16 kPa O_2, while at 25C, respiration at 1.5 kPa O_2 was about one-fifth that at 16 kpa (Fig. 4).

Carbon dioxide production depended on temperature and O_2 partial pressure (Fig. 5). Carbon dioxide production declined with decreasing temperature and, in general, with decreasing O_2 levels. At the lower 0_2 partial pressures, however, the CO_2 respiratory rate did not approach zero, and at some temperatures, CO_2 production increased as the O_2 partial pressures decreased. The rate of CO_2 production was not fitted with an equation due to the difficulties presented by the inflection in the data occurring at the lower O_2 levels.

The RQ depended on both steady-state O_2 and storage temperature (Fig. 6). At all temperatures except 0C, the RQ increased as the steady-state O_2 approached zero; however, the RQ breakpoint occurred at higher levels of O_2 as temperature

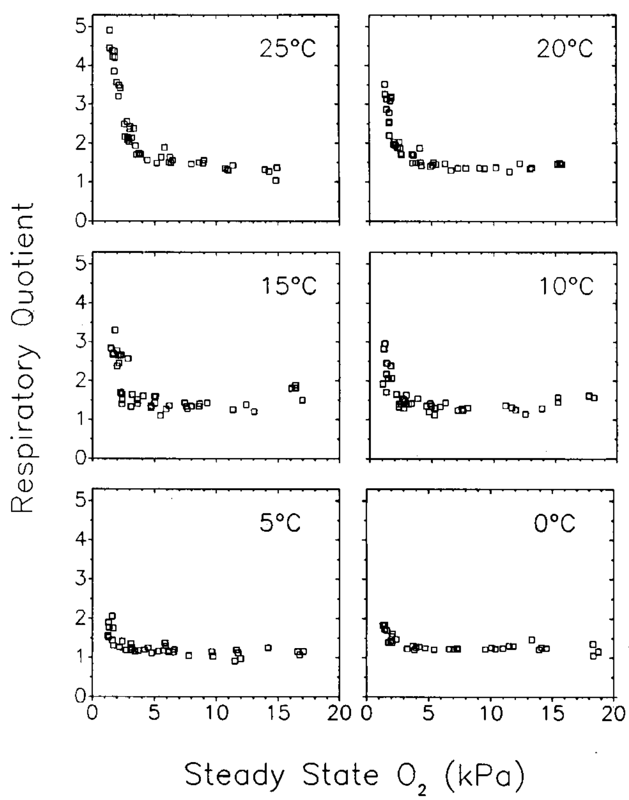

Fig. 6. Effect of steady-state O_2 partial pressure on the respiratory quotient of blueberry fruit in sealed LDPE packages held at 0, 5, 10, 15, 20, or 25C.

increased. Estimates of the O_2 partial pressure at which the RQ breakpoint occurred were made from the graphs: 1.8 kPa at 5C; 2.0 kPa at 10C; 2.5 kPa at 15G 3.0 kPa at 20C; and 4.0 kPa at 25C (Figs. 3 and 6). Insufficient data were available to estimate the RQ breakpoint at OC, although it did not appear to be higher than for 5C. The RQ at higher, aerobic 0_2 partial pressures was $\approx$1.3 for all temperatures.

Discussion

Storage temperature is known to affect the gaseous composition of MAP systems for various commodities (Kader et al., 1989), although in some cases the effect is rather minimal (Prince et al., 1986). It is recognized that steady-state O_2 and CO_2 levels depend on film permeability and product respiration and that the temperature dependence of these two processes is determined by film type, and commodity physiology, respectively. Additionally, suppression of respiration for some fruit takes place at CO_2 levels above 10 to 20 kpa, but to our knowledge, this has not been documented for blueberry fruit. We therefore assumed respiration was minimally affected by levels of CO_2 below the approximate 20 kPa that accumulated in packages under hypoxic conditions. This assumption has since been verified (R.M.B., unpublished).

The safe range of O_2 partial pressures needs to be identified for any product if a MAP system is to be used. One measure of the lower O_2 partial pressure limit is the lowest O_2 partial pressure that does not induce anaerobic CO_2 production. Anaerobic respiration can be detected by the upswing in the RQ (the RQ breakpoint) associated with the synthesis of acetaldehyde, ethanol, and CO_2. Although some degree of anaerobiosis can be tolerated (Cohen et al., 1990; Ke and Kader, 1990), there would be less risk if products were maintained at an O_2 level higher than that associated with the RQ breakpoint. The RQ is easily calculated for fruit in MAP systems (Cameron, 1990; Cameron et al., 1989) and its breakpoint can be correlated with the package 0_2 partial pressure. For blueberry fruit, we have assumed the lower limit for the storage O_2 partial pressure to be that partial pressure associated with the RQ breakpoint.

The rise in the lower O_2 limit with increasing temperature has not been previously reported. This observed change can be explained, at least in part, in terms of gas diffusion into the fruit. For many fruits, resistance to O_2 movement into the tissues is highest at the skin (Burg and Burg, 1965; Cameron and Yang, 1982). We hypothesize that as temperature increased, the rate of O_2 consumption by the tissue rose more rapidly than skin permeability to O_2. For fruit with similar internal 0_2 levels (e.g., that internal O_2 level which begins to induce fermentation), the O_2 gradient across the skin would have increased as temperature increased, leading to the observed rise in steady-state O_2 at the RQ breakpoint. The magnitude of the skin's permeability to either O_2 or CO_2 and the temperature sensitivity of these permeabilities are unknown.

The high RQ ($\approx$1.3) of aerobic fruit might be indicative of the oxidation of organic acids. Both organic acids (mostly citric acid) and sugars are plentiful in blueberry fruit, commonly reaching levels of 0.3% to 1.3% and 12% to 15%, respectively (Eck, 1988).

The possibility of creating hypoxic environments within MAP systems during handling, transit, and storage is a serious concern for commercial enterprises interested in using MAP techniques, especially with regard to temperature fluctuations. The data indicate that a MAP system for blueberry fruit, designed to develop and maintain aerobic steady-state O_2 partial pressures for storage temperatures ranging from 0 to 25C, should maintain an O_2 partial pressure at or above $\approx$4 kPa at 25C and at or above $\approx$1.8 kPa at 0C. If the goal of MAP of blueberry fruit were to maintain these minimal O_2 levels, then the LDPE film used here would not be appropriate. For example, when optimizing packages for storage at 0C, about 110 g fruit weight per package achieved an O_2 partial pressure near the lowest tolerable level of $\approx$1.8 kpa (Fig. 3). These same 110-g packages were clearly hypoxic at 25C, the fruit having an RQ of $\approx$3.

To maintain the lowest 0_2 partial pressures tolerable at all temperatures for blueberry fruit would require a film whose O_2 permeability would increase more rapidly with temperature than that of LDPE. In this experiment, the decline in steady-state O_2 for a given fruit weight with increasing temperature indicated that 0_2 consumption was more sensitive to temperature than O_2 permeation through the LDPE film. A film with a greater temperature sensitivity than LDPE would need to have an energy of activation for O_2 permeability higher than the 40 $kJ\cdot mol^{-1}$ for LDPE. To our knowledge, films with higher energies of activation for O_2 permeation all have very low O_2 permeability.

An enhancement of storage life of blueberry fruit by low O_2 is open to question in that decay is often the primary determinant of blueberry fruit shelf life. Ceponis and Cappellini (1985) found that CA storage of highbush blueberry fruit at 2 kPa O_2 and 2C did not effectively reduce the incidence of decay relative to air, nor did it enhance the effect of CO_2 treatment. However, the storage period was relatively short (7 to 14 days) in comparison to the potential storage life of blueberry fruit (35 to 40 days) (Bunemann et al., 1957; Hruschka and Kushman, 1963). In other studies, storage of highbush blueberry fruit under low O_2 (1.5 to 2 kPa) at nonfungistatic CO_2 partial pressures suggests that low 0_2 was helpful in reducing the incidence of runny and decayed berries during long-term storage (5 to 7 weeks) (Frisina et al., 1988; D.L.D. and R. M. B., unpublished data; Dilley, unpublished data).

The methodology outlined here would be useful in defining the lower 0_2 limits for numerous commodities. Furthermore, it can be used to generate the data necessary to establish film permeability characteristics needed for developing a working MAP system that would avoid anaerobic atmospheres. A model that can be used to specifically determine the needed temperature sensitivity of a film's 0_2 permeability has been developed for blueberry fruit using these respiration data (A.C.C. and R. M. B., unpublished).

Developing a functional and practical MAP system for any perishable commodity requires that we understand how temperature affects film permeability characteristics, the respiratory processes of O_2 consumption and CO_2 production, and the lower O_2 limit. Additional progress needs to be made in the areas of film manufacture, packing techniques, package construction, handling, and marketing to make MAP of blueberry or other commodities a viable alternative to either CA storage or the simple overwraps presently used.

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