A METHOD FOR THE SIMULTANEOUS MEASUREMENT OF GAS EXCHANGE AND DIFFUSION RESISTANCE UNDER VARIOUS GAS CONDITIONS

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Abstract

To measure the relationship between gas exchange rates and diffusion resistance, and their changes in time, both should be measured on the same fruit or vegetable, because diffusion resistance shows large variations between individuals of the same species and cultivar. The method described enables this simultaneous measurement, under various gas conditions, without influencing the product. Neon (Ne) was used as a tracker. Its concentration was brought to 110 Pa after closing a flask containing an apple kept at a specific gas condition. Changes in oxygen and carbon dioxide concentration were used to calculate gas diffusion resistance. The method enables assessment of important physiological data by a simple extension of standard gas exchange measurements. One benefit of using Ne instead of ethane, in addition to a possible influence of ethylene concentrations on ethane measurements, is that ethane production is found in ageing plant tissues.

1. Introduction.

Use of Modified Atmosphere (MA) and CA storage for fruits and vegetables is focused on creating optimum gas concentrations around the fruit. However fruits can be considered as MA packages themselves, with an internal atmosphere different in composition from the external one (Dadzie et al., 1993). Internal gas concentrations are determined by gas exchange rates and the resistance to gas diffusion. Although optimal gas concentrations are related to the external atmosphere, the actual metabolic processes taking place are related to internal concentrations. Changes in gas exchange rates and/or resistance to gas diffusion will influence internal concentrations. For future applications in CA storage, like dynamic control systems where storage conditions interact with product physiology, knowledge about such changes is needed. The knowledge will also be used to develop MA packages designed to provide optimal concentrations, and also to keep the gas concentrations within certain limits. To find the relationship between the time change of gas exchange rates and diffusion resistance, both should be measured on the same fruit or vegetable, because diffusion resistance varies greatly between individual fruits (Banks, 1985; Rodriguez et al., 1989). The method should allow simultaneous measurement under various gas conditions without influencing the product so that they can be repeated over time. Changes of both gas exchange and diffusion resistance, and their influence on optimal storage conditions, can then be monitored. A good method of determining diffusion resistance is to measure efflux of a trace gas (ethane) from the fruit once the fruit has been loaded with the gas (Cameron and Yang, 1982; Knee, 1991). This method has limitations, because of the time needed to first let the product adjust to the gas conditions. One option to avoid preloading is to start with products already adjusted to the storage atmosphere, and then add a trace gas to the atmosphere in order to measure the flux of the gas into the product. We address this option.

2. Material and Methods

Produce information and storage conditions - For measurement of gas flux into the product, neon (Ne) was selected, as it was easy to detect by gas chromatography and does not interfere with ethylene detection. Ne is an inert gas, present in the atmosphere at 18.18 ppm (Greenwood and Earnshaw, 1984). Three cultivars of apples (*Malus domestica*

Borkh.) were used: 'Golden Delicious', 'Elstar' and 'Cox's Orange Pippin'. 'Golden Delicious' was harvested on 27 September 1993 and stored in air for 4 weeks at 1°C. 'Elstar' was harvested on 31 August 1993 and stored in air for 9 weeks at 1°C. 'Cox's Orange Pippin' was harvested on 8 September 1993 and stored in air for 9 weeks at 1°C. A complete factorial design was used with the following gas concentrations: 0, 0.5, 1, 2, 6 and 21 % O_2 by 0 and 5% CO_2 in two replicates, resulting in 24 apples per cultivar. After 4 and 5 days under the mentioned gas conditions, gas exchange and Ne flux were measured.

Measurements of gas exchange and neon flux - The method of Baumann and Henze (1983) was used to measure apple weight, total volume and internal gas volume (V_i) of each individual fruit. A small amount of grease was put on the calyx end to avoid entry of water (during underwater measurements) or air (during measurements of diffusion resistance) into the core cavity. The apples were placed in 1.5 L flasks containing small ventilators in a piece of PVC pipe that supported the apple. The flasks were connected to a flow-through system to achieve various gas conditions. Temperature was controlled and recorded every 15 minutes (Vaisala HMP 31 UT); and in all experiments was 18-19°C ± 0.4°C. The gas entering each flask was humidified to an RH near saturation. After closing the flasks, 1.5 ml Ne was added to each flask, resulting in an initial partial pressure close to 110 Pa. The air in the flasks was mixed by the ventilators for 30 s. Then the ventilators were stopped (to prevent a local increase in temperature), and the first measurement on gas concentrations was carried out. In total, 5 measurements were made 1, 19, 37, 127 and 289 minutes after closing the flask; each measurement consisting of three samples. After the last measurement the flasks were reconnected to the flow-through system.

Table 1. Characteristics of apples used in gas exchange experiments: weight = average apple weight (± S.D.), volume = average total apple volume, and internal volume is the calculated volume of air inside the fruit.

Cultivar	**temperature, °C**	**weight, grams**	**apple volume, ml**	**internal volume, ml**	**resistance for neon, $s.mm^{-1}$**
'Golden Delicious'	19.0	168.4 ± 13.1	216.0 ± 16.9	56.7 ± 5.8	408 ± 136
'Elstar'	19.6	147.1 ± 10.1	186.0 ± 12.5	46.3 ± 3.3	780 ± 167
'Cox's Orange Pippin'	19.5	165.4 ± 13.2	199.6 ± 16.9	42.6 ± 4.9	665 ± 240

The sampled gas was led directly from the flasks to a gas chromatograph (Chrompack CP 2001). Ne, O_2 and N_2 were measured on a Molsieve A column (T = 60°C, p = 110.3 kPa), and CO_2 and ethane were measured on a Hayesep A column (T = 60°C, p = 81.4 kPa). The carrier gas was He. For every sample 1.2 ml was taken from the flasks, so the total gas volume taken from the flasks was 18 ml, resulting in a pressure drop of 1.38 kPa. Concentrations were corrected for pressure loss. For calculations of the gas concentrations the second and third sample were used. Gas exchange rates were calculated using the concentration differences between measurements 1 and 4 (time difference 127 min). The free volume of the flasks (V_o) was calculated by subtracting the calculated apple volume plus the measured volumes of the ventilator and the PVC pipe from the measured flask volume. The atmospheric pressure was measured (Druck PDCR 930) to correct for changes in atmospheric pressure, and to convert gas concentration (% or ppm) to Pa.

Calculation of diffusion resistance - The process described is the diffusion of trace gas from the free volume in the flask outside the apple (V_o) to the free gas volume inside the apple (V_i). The resistance value obtained is an estimate of the overall resistance to gas diffusion of skin and flesh of an apple. Diffusion can be described with Fick's first law (Burg and Burg, 1965; Cameron and Yang, 1982):

$$\frac{ds}{dt} = \frac{(C_o^t - C_i^t) * A}{R} \qquad [1]$$

where ds/dt is the rate of diffusion ($ml \cdot s^{-1}$), R is the resistance coefficient ($s \cdot cm^{-1}$), A is the surface area of the tissue (cm^2), C_o^t is the concentration outside the tissue ($ml \cdot ml^{-1}$) and C_i^t the concentration inside at time t. The apple surface A was calculated from the apple volume, assuming the apple to be a perfect sphere (after Knee, 1991). The diffusion rate can also be described by (after Cameron and Yang, 1982):

$$\frac{ds}{dt} = V_o * \left(\frac{dC_o}{dt}\right) \qquad [2]$$

where V_o is the free volume of the flask and dC_o the change in concentration in the free volume. When the total amount of trace gas in the flask (a closed system) is constant, and V_i is not neglected, the following is true:

$$V_i * C_i^{t=t} + V_o * C_o^{t=t} = C_o^{t=\infty} * (V_i + V_o) \qquad [3]$$

where V_i = the internal volume of an apple and $C_o^{t=\infty}$ = concentration of Ne at time = ∞ (equilibrium concentration). Equations [2] and [3] can be substituted into equation [1] resulting in:

$$\frac{dC_o}{dt} = -\frac{A * (V_i + V_o)}{R * V_i * V_o} * (C_o^{t=t} - C_o^{t=\infty}) \qquad [4]$$

When equation 4 is integrated from time 0 to time t, then:

$$C_o^{t=t} = C_o^{t=\infty} + (C_o^{t=0} - C_o^{t=\infty}) * e^{-t * \frac{A * (V_i + V_o)}{R * V_i * V_o}} \qquad [5]$$

with t = the time period after start of the experiment (s). With the concentration measurements available, R can be calculated as:

$$R = \frac{-t * A * (V_i + V_o)}{V_i * V_o * \mathrm{Ln}\,\frac{C_o^{t=t} - C_o^{t=\infty}}{C_o^{t=0} - C_o^{t=\infty}}} \qquad [6]$$

3. Results and Discussion

The exact Ne concentrations applied and the external and internal volumes differed per apple. Resistance values found differed widely between individual apples: minimum and maximum values were 1.93×10^3 and 7.06×10^3 $s \cdot cm^{-1}$ for 'Golden Delicious', 4.96×10^3 and 11.9×10^3 $s \cdot cm^{-1}$ for 'Elstar', and 2.75×10^3 and 12.0×10^3 $s \cdot cm^{-1}$ for 'Cox'. When the average resistances of the three apple cultivars were compared, the value for 'Golden Delicious' apples was lowest and for 'Elstar' apples highest (Table 1). O_2 uptake rates (Figure 1) and CO_2 production rates (not shown) were also established. Highest O_2 uptake rates were found for 'Golden Delicious' and the lowest for 'Elstar' (Figure 1). Resistance values were averaged per apple cultivar (Table 2). The total change in Ne concentration found in the experiments was about 4 Pa, which is about 4% of the initial concentration (Figure 2), and 12 times larger than the measurement error. For future use of this method, accuracy will be improved by increasing the initial concentrations, or reducing the volume outside the apple (V_o). With the measured external volume (V_o), the calculated internal volume (V_i) and the measured Ne concentration at the start of the experiment (Ne_{start}), the expected Ne concentration at the end of the measurement (Ne_{end}) can be calculated. The calculated value was higher than the measured Ne_{end} for all three apple cultivars (Table 2). Three factors could cause this result: diffusion of Ne into the water phase of the apples, leakage of Ne out of the flasks and/or a systematic experimental error. Diffusion of Ne into the aqueous phase was calculated. With a Ne solubility in water of 10.5 $ml \cdot l^{-1}$ (Greenwood and Earnshaw, 1984), apple weight used and the percentage of water in apples (Knee, 1991), the maximum amount of Ne that can dissolve in the aqueous phase under the experimental conditions, Ne_{aq}, can be calculated.

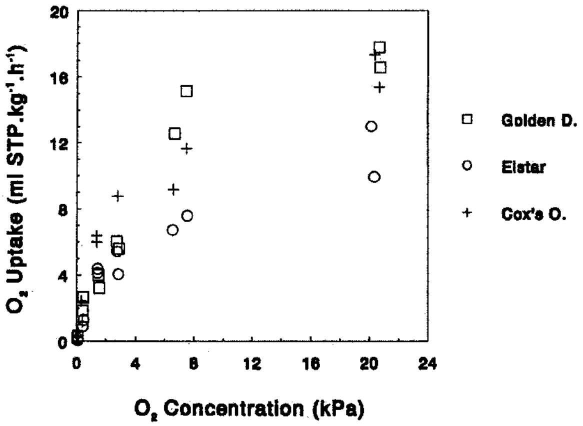

Figure 1. Oxygen uptake rates (ml STP.kg^{-1}·h^{-1}) of 'Golden Delicious', 'Elstar' and 'Cox's Orange Pippin' apples at several O_2 concentrations (kPa). CO_2 concentration is ≈ 40 Pa).

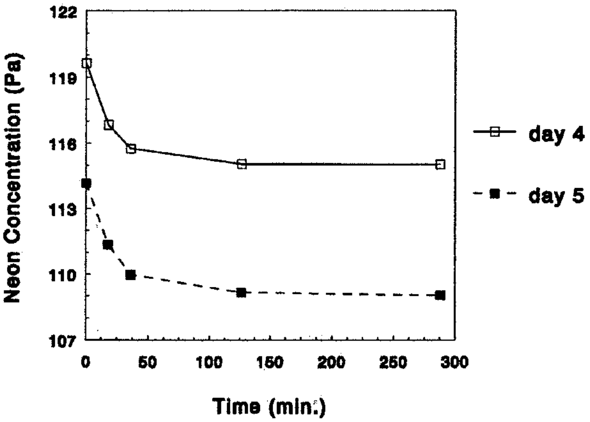

Figure 2. Typical decrease of the Ne concentration in the flask over time (measurements on both days on the same apple, cv. 'Golden Delicious').

Table 2. Summary of the measurements on Ne diffusion into the products. V_o = product volume (ml), V_i = internal gas volume (ml), 'Ne' refers to Ne concentrations (Pa).

	Cultivar		
Variable	*'Golden Delicious'*	*'Elstar'*	*'Cox's Orange Pippin'*
V_o	1309	1339	1325
V_i	56.7	46.3	42.6
neon$_{start}$ measured (a)	118.1	109.4	109.8
neon$_{end}$ measured (b)	112.4	105.4	106.0
neon$_{end}$ calculated (c)	113.2	105.7	106.4
neon$_{aq}$ (d)	0.14	0.11	0.13
c-d-b	0.66	0.19	0.27
accuracy	0.42	0.42	0.42

This Ne_{aq} was only a minor contributor to the decrease of Ne concentration in the volume around the apple (Table 2). After correcting the calculated Ne_{end} for Ne_{aq} it still exceeded the measured Ne_{end}. For two apple cultivars, 'Elstar' and 'Cox', this difference was less than the GC accuracy, and can be ignored. For the experiment on 'Golden Delicious', leakage probably contributed to the differences found. In equation [3] the total amount of gas in the system is assumed constant. When leakage occurs, the assumption is no longer true. However the calculated leakage in the experiments is considered too small to influence the resistance values found, and can therefore be neglected. It remains important, however, for application of equation [3], to eliminate all leakages in closed systems.

These data on gas exchange (Figure 1) are comparable to those obtained earlier (Peppelenbos and van't Leven, 1996), implying that the measurement of respiratory gas exchange has not been influenced by the method of measuring the Ne flux. Our resistance values can be compared with data from the literature (Table 3).

Table 3. Comparison of literature and experimental data on diffusion resistance of apples. Method: 1 = Ethane flux outwards from the apple, 2 = gas sampling of a tube glued on the surface, 3 = Ne flux inwards to the apple. R is ethane and Ne resistance values.

Apple cultivar	**Source**	**Method**	**Age** (days)	**Temp.** (°C)	**R. H.** (%)	**Resistance** ($s.mm^{-1}$) low	high
'Golden Delicious'	Banks (1985)	1	± 7	?	?	970	205
	Knee (1991)	1	?	?	?	560	760
	This study	3	31	19.0	>95%	193	706
'Cox's Orange Pippin'	Rajapakse et al. (1990)	2	3	20	?	594	
	Knee et al. (1990)	1	35-80	3.3	?	980	151
	This study	3	66	19.5	>95%	275	120

Our highest resistance values found for 'Golden Delicious' and 'Cox's Orange Pippin' are comparable to those given in the literature. The lowest values, however, are considerably different. The significance is unclear, since the measurements have been made under a range of conditions. The temperature has been lower (Knee et al., 1990) or not been given (Banks, 1985; Knee, 1991): the relative humidity, which can influence the values found for diffusion resistance (Lidster, 1990), has also not been given. Because the apples used also have different ages, and because diffusion resistance of apples can change over time (Solomos, 1987; Park et al., 1993), it is clear that only a comparison of the order of magnitude is meaningful, which is the case here. The Ne flux method seems a good one for measuring diffusion resistances. Its advantage is that it can easily be combined with gas exchange measurements. One has to take into account that the produce to be measured should have a considerable internal volume (V_i) compared to the volume surrounding the product (V_o) for a large enough decrease in Ne concentration to be measurable.

Table 4. Diffusion rate of metabolic gases related to ethane and Ne using Graham's Law

	Ethane	**Neon**
Carbon dioxide	0.799	0.679
Oxygen	0.937	0.796
Ethylene	0.966	0.821
Ethane	1	0.850
Neon	1.176	1

One disadvantage of using ethane instead of Ne for the measurement of diffusion resistances, in addition to a possible influence of ethylene concentrations on the ethane measurements, is that ethane production is found in ageing plant tissues. Ethane production is commonly associated with membrane damage and cell death, and is increases with increasing injury of plant tissues (Abeles et al., 1992). Particularly when research is focussed on a relation between changes in diffusion resistance in fruits during storage and the onset of storage disorders, this ethane production may influence the measurements.

After deriving resistance values for Ne diffusion in plant tissues, and for ethane diffusion as well, it is important to know the relation to resistance values for O_2 and CO_2. This issue was not addressed by Cameron and Yang (1982) or Knee (1991). Banks (1985) suggests that ethane diffusion is probably similar to O_2 and ethylene diffusion. Using Graham's Law predictions on the relationship between the diffusion of the various gases can be made: one would expect diffusion rates of O_2, ethane and ethylene to be comparable, but Ne diffusion rate to be 18% higher and CO_2 diffusion rate to be 20% lower (Table 4). The diffusion routes, however, of Ne, ethane, O_2 and CO_2 are not necessarily equal (Banks, 1985), which makes the use of only Graham's Law suspect. A real comparison of resistance values is necessary, and for this purpose one might combine the method of Rajapakse et al. (1990) and the ethane (Cameron and Yang, 1982) or Ne method.

References

Abeles, F.B., Morgan, P.W. and Saltveit, M.E., 1992. Ethylene in Plant Biology. Academic Press Inc., San Diego, USA: pp. 53-55.

Banks, N.H., 1985. Estimating skin resistance to gas diffusion in apples and potatoes. J. Exp. Bot. 36: 1842-1850.

Baumann, H. and Henze, J., 1983. Intercellular space volume of fruit. Acta Hortic. 138: 107-111.

Burg, S.A. and Burg, E.A., 1965. Gas exchange in fruits. Physiol. Plant. 18: 870-884.

Cameron, A.C. and Yang, S.F., 1982. A simple method for the determination of resistance to gas diffusion in plant organs. Plant Physiol. 70: 21-23.

Dadzie, B.K., Banks, N.H., Cleland, D.J. and Hewett, E.W., 1993. Role of skin resistance to gas diffusion in the response of fruits to modified atmospheres. Acta Hortic. 343: 129-134.

Greenwood, N.N. and Earnshaw, A., 1984. Chemistry of the elements. Pergamon Press.

Knee, M., Hatfield, S.G.S. and Farman, D., 1990. Sources of variation and ester content of 'Cox' apples stored in 2% oxygen. Ann. Appl. Biol. 116: 617-623.

Knee, M., 1991. Rapid measurement of diffusion of gas through the skin of apple fruits. HortSci. 26: 885-887.

Lidster, P.D., 1990. Storage humidity influences fruit quality and permeability to ethane in 'McIntosh' apples stored in diverse controlled atmospheres. J. Am. Soc. Hort. Sci. 115: 94-96.

Park, Y.M., Blanpied, G.D., Jowziak, Z. and Liu, F.W., 1993. Postharvest studies of resistance to gas diffusion in 'McIntosh' apples. Postharvest Biol. Technol. 2: 329-339.

Peppelenbos, H.W. and van't Leven, J., 1996. Evaluation of four types of inhibition for modelling the influence of carbon dioxide on oxygen consumption of fruits and vegetables. Postharvest Biol. Technol. 7: 27-40.

Rajapakse, N.C., Banks, N.H., Hewett, E.W. and Cleland, D.J., 1990. Development of oxygen concentration gradients in flesh tissues of bulky plant organs. J. Am. Soc. Hort. Sci. 115: 793-797.

Rodriguez, L., Zagory, D. and Kader, A.A., 1989. Relation between gas diffusion resistance and ripening in fruits. Proc. Fifth Int. Contr. Atm. Res. Conf., June 14-16, Wenatchee, Washington. Pp. 1-7

Solomos, T., 1987. Principles of gas exchange in bulky plant tissues. HortSci. 22: 766-771.