SafePod: a respiration chamber to characterise apple fruit response to storage atmospheres

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Abstract

During long-term storage of apples, detection of low O_2 stress is used to optimise low O_2 storage regimes for dynamic controlled atmosphere (DCA) storage. Monitoring respiratory characteristics, specifically the respiratory quotient (RQ), provides a tool to achieve this. The objectives of this study were to evaluate protocols to monitor response of apple consignments to low O_2 using a respiration chamber, the SafePod, designed for use in commercial stores and research laboratories, and to compare the RQ response to changes in chlorophyll fluorescence (CF) yield from the fruit skin as used in DCA-CF.

Protocols to identify the lowest oxygen limit (LOL), the O_2 concentration below which RQ rises, were tested using 'Braeburn' (sensitive to low O2) and 'Gala' (less sensitive to low O2). A protocol that allows fruit to acclimatise at each O_2 concentration takes several weeks and is therefore not practical for commercial use. A rapid profile without fruit acclimatisation can be completed in 2-3 days. Although this underestimates RQ values, and results in an increase in RQ at a higher O_2 concentration than observed for acclimatised fruit, the rapid RQ protocol provides a practical method to compare response of apple consignments between cultivars, orchards and seasons. By the rapid protocol, the LOL of 'Braeburn' consignments was near 0.6 kPa and of 'Gala' consignments was near 0.2 kPa, consistent with detection of alcoholic taints below the LOL in each case. The RQ response using the SafePod was consistent with increase in CF yield using HarvestWatch^{TM}.

Fruit respiration rates change through the storage season, including a substantial decrease over the first 2 months after harvest. As RQ response is affected by respiration rate, accurate comparison of consignments depends on profiles being measured at the same stage in the storage season. It is more difficult to determine the LOL by RQ profiling later in the season when respiration rates are lower.

Keywords

dynamic controlled atmosphere, apple (*Malus domestica*), respiratory quotient, respiration rate, SafePod

1. Introduction

Long-term apple storage relies on the use of low temperatures and controlled atmospheres (CA) (Beaudry, 1999; Dilley, 2010). Apples exhibit significant cultivar differences in tolerance to the combination of low O_2, and/or elevated CO_2 leading to a range of CA storage recommendations over a range of temperatures, impacted by seasonal and regional climatic differences. For example, in the UK, ‘Gala’ benefits from storage in 3-5 kPa CO_2, whereas ‘Braeburn’ is stored in <1.0 kPa CO_2 (Dadzie, 1992, AHDB Apple Best Practice Guide, https://apples.ahdb.org.uk/). The response of fruit respiration rates (CO_2 evolution and O_2 consumption) to different atmospheres can be used to help identification of optimal storage conditions. In particular, the respiratory quotient (RQ = rate of CO_2 evolution/rate of O_2 consumption) increases above its normal value (near to unity when sugars are used as respiratory substrate) when tissues switch to anaerobic respiration, and can therefore be used to identify the lowest safe O_2 concentration for storage (Gran and Beaudry, 1993; Yearsley et al., 1997). Monitoring rates of apple respiration through storage, can also help to optimise storage conditions by providing a measure of apple quality (Argenta et al., 2000). Until recently this usually involved removing fruit samples from the store, however with development of chambers that can be placed within stores (Keshri et al., 2020), measurements of respiration *in situ* will allow changes in the physiology of produce to be monitored in real-time providing an early indication of the onset of physiological disorders such as senescence.

For some apple cultivars the use of O_2 concentrations below 1 kPa has been shown to allow longer storage, reducing physiological disorders such as superficial scald (Colgan et al., 1999; Sabban-Amin et al., 2011; Zanella, 2003; Zanella et al., 2008) and promoting the retention of fruit quality attributes (Colgan et al., 1999; DeEll and Lum, 2017; Thewes et al., 2015; Zanella et al., 2008). It is reported that low O_2 storage is a more cost effective way to avoid storage disorders and to retain fruit quality than treatments such as 1-methylcyclopropene (DeEll and Lum, 2017; Prange et al., 2013; Weber et al., 2020, 2017; Zanella, 2003). However, given that low O_2 can cause damage, including that associated with anaerobic respiration, strategies have been developed to detect low O_2 stress in apples in order to determine the optimum O_2 concentration for long-term storage. These technologies theoretically allow the atmosphere to be adapted through the storage season as fruit acclimatises to low O_2 (de Oliveira Anese et al., 2019; Wright et al., 2010). For this reason the term dynamic controlled atmosphere (DCA) is used.

A number of systems, both research and commercial, are based on RQ measurement. These include systems based on sealable chambers, such as the SafePod system discussed in this paper, that can be used within a commercial store or a laboratory (Schaefer and Bishop, 2014) or purely laboratory based systems (Bessemans et al., 2016; Brackmann et al., 2015; Thewes et al., 2015; Weber et al., 2015). An alternative approach is to measure respiratory characteristics within a whole commercial CA store by temporarily interrupting CA control and sealing the store. This requires a well-sealed store so that gas leakage does not introduce significant error into calculations. Given the challenges of eliminating leakage both in whole stores and when using chambers, especially with changes in atmospheric pressure, a model-based leak correction measurement, allowing real-time correction of measured RQ values for leakage of the storage environment, has been developed (Bessemans et al., 2018).

An alternative to the RQ uses the fact that the rate of CO_2 evolution is at a minimum when the O_2 concentration is at the anaerobic compensation point (ACP), below which anaerobic respiration is triggered and the RQ starts to rise (Thewes et al., 2020). Where a store is leaky, given that for most conditions used for apple CA storage the difference between the concentration inside and outside a store is less for CO_2 than for O_2, the respiration rate measured by CO_2 evolution is likely to be less prone to leakage error. Commercial application of the Fruit Atmo technology appears to aim to keep the fruit at the ACP on the assumption that this provides optimal storage conditions.

Researchers testing DCA-RQ have investigated storing fruit at or below the ACP by maintaining RQ at specific levels at or above 1. This requires frequent measurements of RQ, and continuous adjustment of the storage atmosphere, tending to result in rapid fluctuations in storage atmosphere (Bessemans et al., 2016a; Thewes et al., 2017b). Several studies have explored the concept of storing fruit at O_2 concentrations at which anaerobic respiration has been initiated so that the fruit tissues are exposed to the products of anaerobic respiration (RQ of 1.5 or 2), and have reported that this can reduce disorders (Thewes et al., 2019; Weber et al., 2020, 2019). The products of anaerobic respiration that accumulate in the fruit stored under these conditions have been shown to inhibit ethylene response and to have a direct inhibitory effect on enzymes involved in fruit softening.

DCA technologies based on chlorophyll fluorescence (DCA-CF) rely on the observation that chlorophyll fluorescence (CF) yield from chloroplasts in fruit skin increases when subjected to low O_2 stress (Prange et al., 2013, 2012; Wright et al., 2012). While the direct metabolic response of chloroplasts to low O_2 is well documented (Harris and Heber, 1993), it has been postulated that it is the redox state of distant tissues leading to over-reduction of the plastoquinone pool in the chloroplasts that leads to a CF response indicative of whole fruit stress (Wright et al., 2012). In practical application of DCA-CF the O_2 concentration is decreased until the point at which CF responds, and then O_2 is set at a safe level (usually 0.2 kPa) above this point.

The objectives of this study were to evaluate protocols to monitor the response of apple consignments to low O_2 using a respiration chamber, the SafePod designed for use in commercial stores and research laboratories, and to compare the RQ response to changes in CF yield from the fruit skin as used in DCA-CF.

2. Materials and Methods

2.1 Integrity of the SafePod chamber design

Figure 1: SafePod chamber consisting of a stainless steel base and a clear moulded lid sitting in a water trough. Internal volume was approximately 0.43 m^3. The chamber was configured for locating within a commercial apple store, with a valve and fan in the base to enable the internal atmosphere to be shared with the external environment.

Trials were carried out with SafePods (SCS Inc. USA) located in commercial stores and LabPods located in temperature controlled rooms at the facilities of the Produce Quality Centre, of the Natural Resources Institute, in the UK.

The SafePod (Fig. 1) was a sealed chamber with a volume of approximately 0.43 m^3, with a base constructed of stainless steel and a clear moulded cover sealed to the base in a water trough. Approximately 75 kg of produce was placed in four standard produce crates. Motorised valves and a circulation fan at the base of the SafePod allowed equilibration with the store atmosphere. O_2 and

CO_2 sensors were fitted within the SafePod to allow real-time monitoring of O_2 and CO_2 concentrations.

The SafePod was designed to be placed within a controlled atmosphere (CA) storage room. Periodically, under the control of a built-in processor (XV-102-D6, EATON, USA) and on command from the external computer, the valves were closed and the fan stopped. In this isolated mode, the SafePod was sealed so that respiration could be measured from the rate of increase in CO_2 and decrease in O_2. The LabPod had the same design as the SafePod but had additional atmospheric control so that it could be used in a temperature controlled room rather than within a CA storage room. Most of the time the LabPod was in control mode, so that a preset CA atmosphere was maintained by the injection of nitrogen, air and CO_2 under the automatic control of the built-in gas analysers and PCL as shown in Fig. 2.

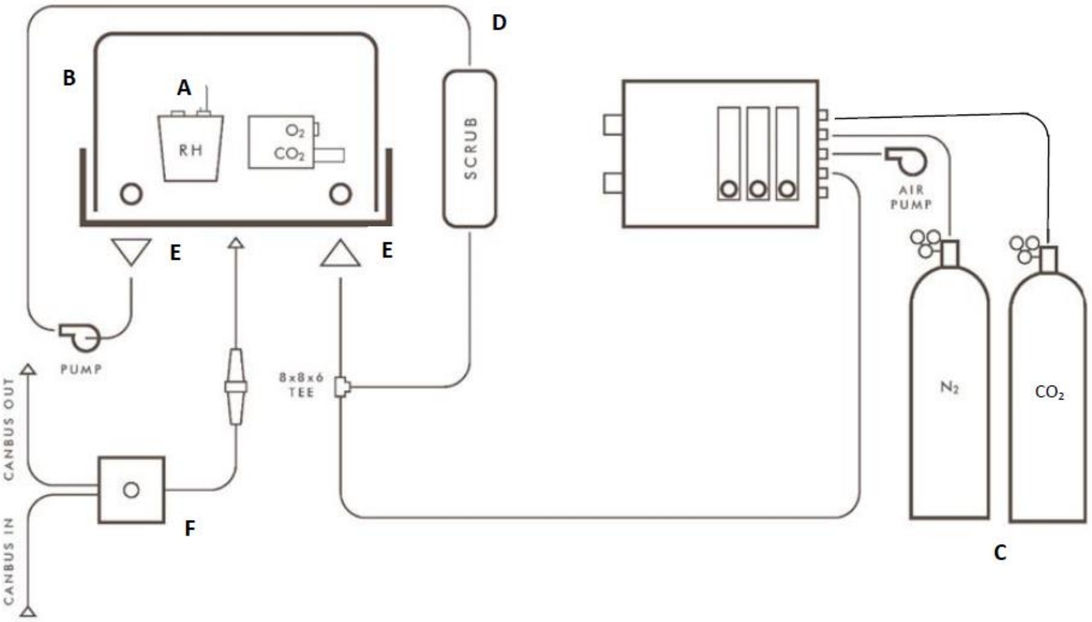


Figure 2: Design of the LabPod configured for use in a temperature controlled laboratory. In common with the SafePod, sensors (A) inside the Pod (B) monitor relative humidity, O_2 and CO_2. Unlike the SafePod the LabPod system has no valve to the external environment, but includes (C) a

supply of N_2 and CO_2 (from gas cylinders) and air from a compressor, as well as (D) an additional circulating system to remove CO_2. Valves (E) are closed during respiration measurements. The whole system is under computerised control (F).

In the LabPod the excessive CO_2 produced by product respiration was usually removed by the purging flow of N_2, but where this was not possible the CO_2 was removed using a chemical scrubber (D in Fig. 2) containing soda lime (75 % w/w {$Ca(OH)_2$} and 3 % w/w sodium hydroxide {NaOH}). A pump was used to circulate the Pod's atmosphere through the scrubber with a flow rate sufficient to remove excess CO_2 at a rate greater than the produce respiration. The gas analysers and control system controlled the pump operation to maintain the required level of CO_2 in the Pod. For the LabPod a fan situated in the centre of the chamber circulated air in both control mode and during respiratory measurement.

To measure the respiratory characteristics of the produce, periodically the atmosphere control action was stopped and, as in the SafePod, the analysers measured the increase in CO_2 and decrease in O_2 over a selected period of time. Once this measurement was complete the atmospheric control was resumed, and the required atmosphere re-established.

The O_2 sensor (I-103 ITG, Germany) was an electrochemical sensor connected locally to anelectronic amplifier and gave a measurement resolution of 0.002 kPa O_2. The CO_2 sensor was a dual beam infrared absorption sensor (type GMP251, Vaisala, Finland) with a range of 0-10 % CO_2 and gave a resolution of 0.002 kPa CO_2.

The gas entered the sensors by diffusion and no sampling tubes were needed. With no sampling required and as the analysers were continually exposed to the measured gas there were minimum errors due to sensor response and settling time.

To accommodate stresses due to changes in atmospheric pressure (of several percent) the lid of the pod floated on a water seal, thereby allowing the chamber volume to expand and contract, without gas leakage, as atmospheric pressure decreased and increased respectively. Rate of gas leakage through the water seal depends on gas solubility and the concentration difference between the chamber and external environment. CO_2 is 40 – 50 times more soluble than O_2 over the storage temperature range and for both gases solubility increases at low temperatures. In terms of concentration difference the rate of leakage was expected to be greater for LabPods, where the difference between the internal and external atmosphere was greater compared to SafePods situated in CA stores, in which case the internal (in Pod) and external (in store) atmospheres differed by only a small amount during the measurements of respiratory characteristics.

To determine magnitude of errors due to gas diffusion, rate of leakage was measured over a week from empty LabPods with an internal atmosphere of 3 kPa CO_2 and 1 kPa O_2 and at two temperatures; 9°C and 0.7°C.

2.2 Measurement of fruit respiration and compensation for atmospheric pressure changes

2.2.1 Atmospheric pressure compensation

The SafePod gas sensors respond to partial pressure of the measured gas, so that for the accurate calculation of respiration rates, any variation in pressure that occurs during measurement should be compensated for. The predominant pressure change was the atmospheric barometric pressure variation which typically varies over the range 95 to 105 kPa, with a nominal sea-level value of 101.3 kPa. Atmospheric pressure was measured at the store or laboratory and was used to adjust the gas reading to the value corresponding to 101.3 kPa.

Compensated gas value = $G_{meas} \times 101.3/ P_{meas}$ (1)

Where G_{meas} is the measured gas reading and P_{meas} is measured atmospheric pressure in kPa at the time of the reading.

Unless indicated, gas concentrations shown in this paper are compensated values.

2.2.2 Calculation of respiration rate

The system recorded the gas concentration and atmospheric pressure every 15 minutes.

Rates of fruit respiration were calculated when SafePods were sealed and, in the case of the LabPod, atmospheric control was disabled.

Rate of change of O_2 and CO_2 partial pressure was calculated over the time for O_2 to decrease by 0.1 kPa, using the “slope” function in excel (slope of the linear regression line), to provide the best straight line fit for compensated values. Void volume in the Pod was calculated assuming a Pod space volume of 354 L and subtracting fruit volume assuming a specific density of 1.15.

$$\text{Respiration rate [nmol kg-1 s-1]} = ((\text{slope} \times (V-1.15 \times W) \times 1000 \times 12.4)/(W \times 101.3) \qquad (2)$$

‘slope’ is rate of CO_2 rise or the rate of O_2 decrease [kPa h^{-1}], V is pod volume [L] and W is weight of fruit [kg], 12.4 is the correction factor to convert respiration rate from ml h^{-1} kg^{-1} to nmol kg^{-1} s^{-1}

The time taken for a decrease in O_2 of 0.1 kPa depended on the respiration rate of the fruit. For these studies this typically took 7.5 – 10 h (30 – 40 data points) for ‘Gala’ apples at 0.7°C and 7 - 7.5 h (28 – 30 data points) for ‘Braeburn’ apples at 1.6°C.

2.2.3. Demonstration of the impact of atmospheric pressure changes on RQ profile measurement

‘Gala’ apples from orchard G-A (see 2.7) were loaded into a LabPod on 5 September 2017 and stored under CA conditions; O_2 0.6 kPa, CO_2 3 kPa at 0.7°C. After 75 days of storage three repeat respiratory profiles were obtained with CA control disabled, each taking approximately 4 days and with a pause of 24 h between each during which CA control was re-established. For each profile O_2 was allowed to decrease through fruit respiration until it fell below 0.05 kPa. Using data with and without atmospheric pressure compensation, the rate of CO_2 rise and O_2 decrease was calculated for each O_2 concentration range of 0.1 kPa using the slope calculated over 60 data points (15 hours). For the first and last range, fewer data points were used, the precise number depending on the timing of valve closure and opening. Atmospheric pressure compensation and respiration rates (CO_2 evolution and O_2 consumption) were calculated as described above.

2.3 Comparing protocols for RQ profiling

2.3.1. RQ profiling protocols

Three protocols for RQ profiling were tested:

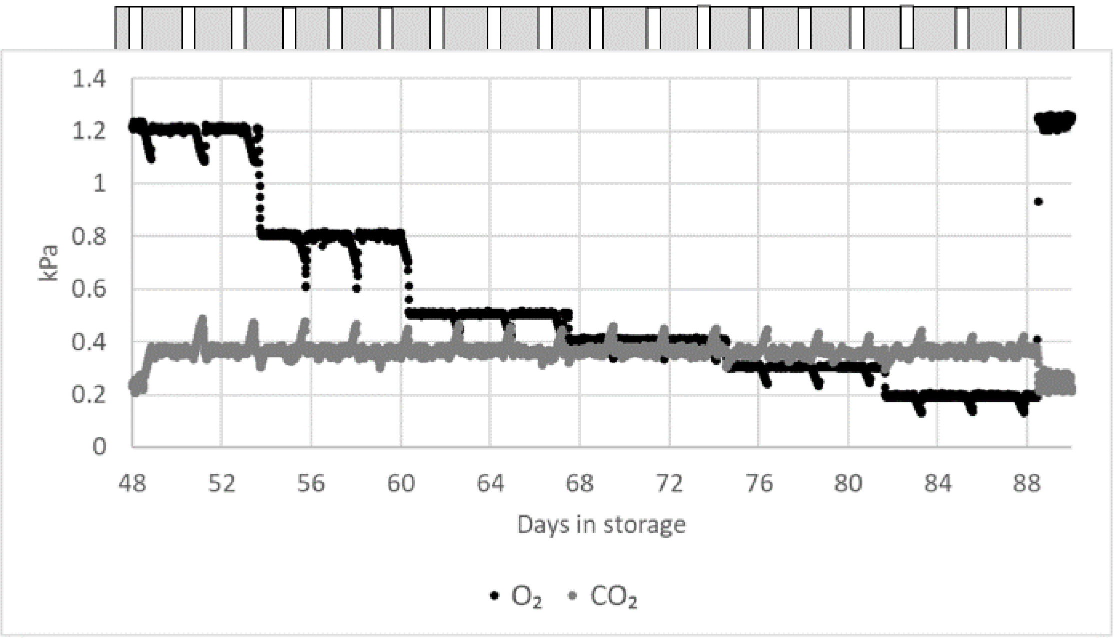
Slow protocol: Single step measurements were made with the SafePod isolated, or the LabPod CA control disabled, over the time period equivalent for fruit respiration to decrease O_2 concentration by 0.1 kPa. This was repeated three times at each O_2 concentration before O_2 concentration was progressively stepped down for the next measurement. The fruit was allowed to acclimatise for 48 hours before each measurement and between each repeat measurement. Typically measurements were made at 5 or 6 O_2 concentrations and the whole protocol typically took about 40 days. Fig. 3 (A) and (B) shows gas partial pressures during a slow protocol for 'Braeburn' apples.

Reverse protocol: This followed the same procedure as the slow protocol, except that for the first measurement, the O_2 concentration was taken down to a level expected to be at a stress level for the cultivar under test, and O_2 concentration was then progressively stepped up until the RQ decreased to normal levels. This protocol typically took 20 - 24 days.

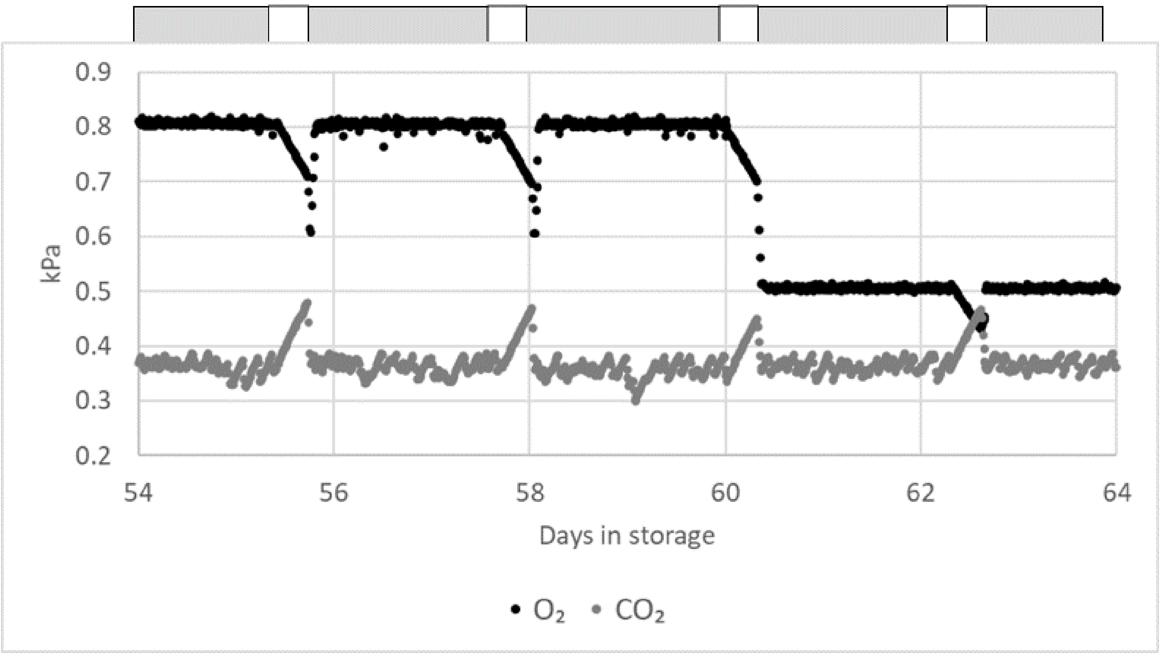
Rapid protocol: The Pod was isolated/CA control disabled to allow the O_2 concentration to decrease by fruit respiration until the fruit experienced low O_2 stress. The rate of CO_2 rise and the rate of O_2 decrease was calculated at intervals equivalent to a decrease in O_2 of 0.1 kPa as described in 2.2. This protocol typically took 3-4 days.

Fig. 3 (C) and (D) show the O_2 and CO_2 concentrations during a series of rapid RQ measurements for 'Braeburn' apples in a laboratory LabPod (C) and a series of rapid RQ measurements for 'Gala' apples in a commercial store (D). The noise shown in the gas concentration in the LabPod during CA control was an indication of the frequent adjustment to keep the CA within 0.1 kPa of the set point, whereas in the commercial CA store the atmosphere across the whole store was dependent on the room settings and in this case was subject to slower oscillations. A delay of at least 1 hour was used after putting the Pod into isolated mode before measurements were initiated to avoid errors due to transient changes in gas concentrations that were assumed to be due to equilibration of Pod atmosphere with internal fruit atmosphere (Fig. 3D).

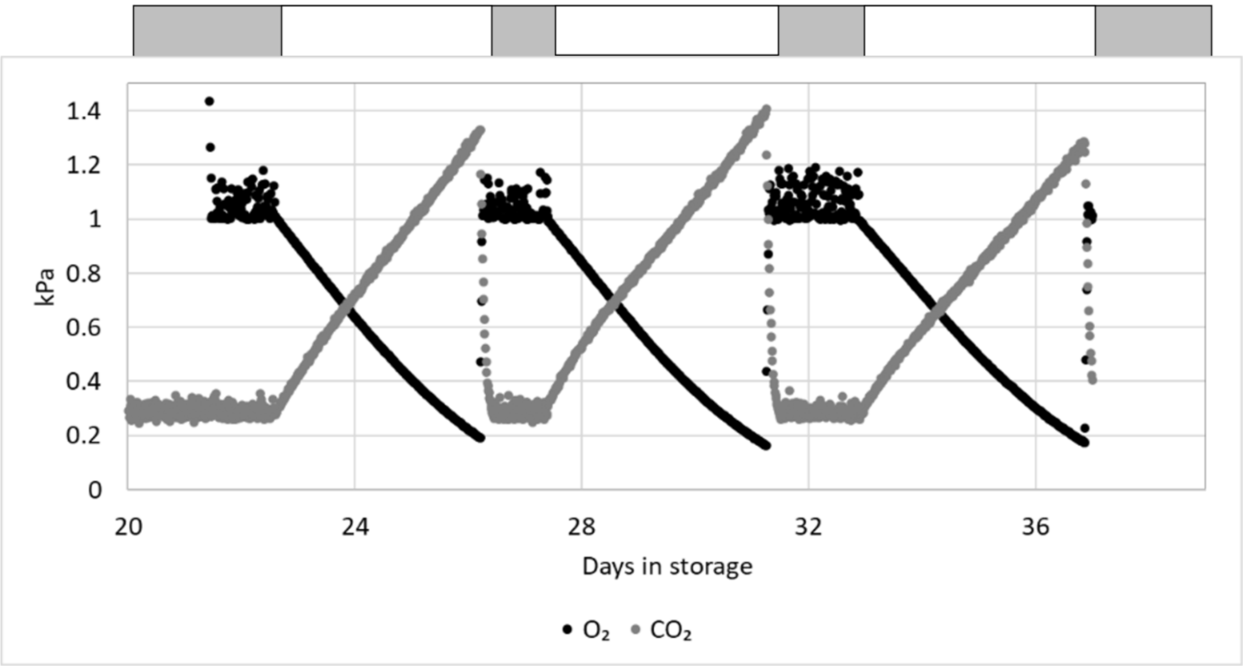
(A)


1.4
1.2
1
0.8
0.6
0.4
0.2
0
kPa
48
52
56
60
64
68
72
76
80
84
88
Days in storage
• O_2 • CO_2

(B)


0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
kPa
54
56
58
60
62
64
Days in storage
• O_2 • CO_2

(C)



(D)

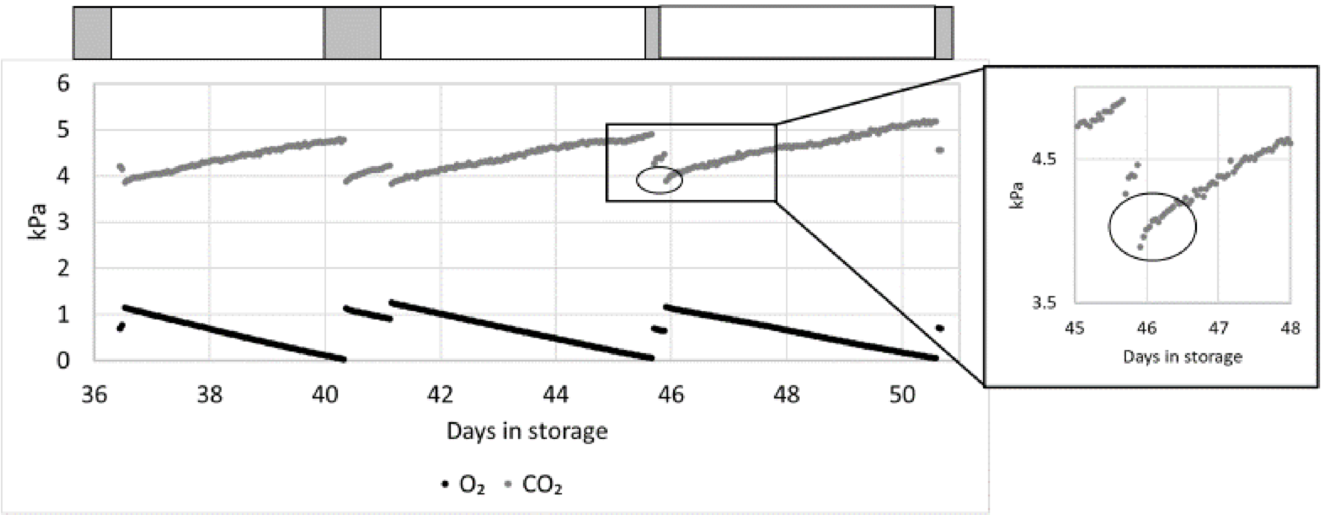


Figure 3: O_2 and CO_2 concentrations during RQ profiling (A) Slow protocol for 'Braeburn' apples (Orchard B-B) in a research LabPod in 2015. (B) An expansion of part of the Slow protocol as above. (C) Rapid protocol repeated 3 times for 'Braeburn' apples (Orchard B-A) in a research

LabPod in 2016. (D) Rapid protocol for 'Gala' apples in a commercial store in 2016, with a detail showing changes in CO_2 (circled) at the start of one of the isolated mode stages.

The bar at the top of each graph indicates when the LabPods were under CA control (with limits set to 0.1 kPa) (grey) or with control disabled (white) , and when the SafePod was in shared (grey) or isolated mode (white).

2.3.2. Trial design for protocol comparison

To test the consistency of the rapid RQ protocol, 60-70 kg 'Braeburn' apples from a commercial orchard B-A were loaded into a LabPod on 24 October 2016 at 1.6°C, and CA established over 3 weeks at O_2 1.0 kPa, CO_2 0.3 kPa. After 22 days of storage, with CA established, three repeat rapid RQ protocols were run each taking approximately 4 days and with a pause of 24 h between each, during which CA control was re-established. For each profile O_2 was allowed to decrease through fruit respiration until it fell below 0.15 kPa. Rates of respiration and RQs were calculated from partial pressures compensated for atmospheric pressure as described in 2.2 using 36 – 40 data points for each calculation, equivalent to 9-10 hours.

For comparison of rapid and slow RQ protocols 'Gala' and 'Braeburn' apples from commercial orchards G-C and B-A were each loaded into a LabPod, the day after harvest on 1 and 8 October 2020 respectively. 'Gala' fruit were stored at 0.7°C, CA set to O_2 0.8 kPa, CO_2 3 kPa and 'Braeburn' fruit were stored at 1.6 °C, CA set to O_2 1.0 kPa, CO_2 0.3 kPa following an establishment protocol. From 20 November two repeat rapid RQ protocols were run each taking approximately 4 days and with a pause of 24 h between each, during which CA control was re-established. This was followed by a slow RQ protocol starting on 6 and 1 December for 'Gala' and 'Braeburn' respectively, and a further two rapid RQ protocols starting 25 and 23 January respectively. Rates of respiration and RQs were calculated from partial pressures compensated for atmospheric pressure as described in 2.2 using 30 - 40 data points (7.5 – 10 h) and 28 – 30 data points (7 – 7.5 h) for each calculation for 'Gala' and 'Braeburn' respectively.

For comparison of slow and reverse RQ protocols ‘Braeburn’ apples from commercial orchard B-A were loaded into a LabPod the day after harvest on 12 October 2015 at 1.6 °C, and the CA set to O_2 1.0 kPa, CO_2 0.3 kPa following an establishment protocol. In November/December a slow RQ protocol was run followed by a reverse protocol in February and a further slow protocol in March/April. Rates of respiration and RQs were calculated from partial pressures compensated for atmospheric pressure as described in 2.2 using 28 - 30 data points (7- 7.5 h) for each calculation.

2.4 Comparing respiratory responses with chlorophyll fluorescence yield

Simultaneous measurements of chlorophyll fluorescence (CF) yield and respiration were made by placing a HarvestWatch^{TM} unit (Isolcell, Italy) into one of the upper crates in each LabPod, during RQ profiling as below. Each unit contained an excitation light and CF yield sensor positioned above 5-6 representative fruit. . Measurements of CF yield following an excitation light flash were recorded by the unit at hourly intervals. The values provided by the instrument indicated relative yield (no units). A reverse RQ profile was obtained for ‘Braeburn’ fruit from orchard B-A in Feb 2016 (4 months after harvest) and storage at 1.6 °C, O_2 1.0 kPa, CO_2 0.3 kPa. Rapid RQ profiles were obtained for two ’Gala’ orchards (orchard G-A and G-C) in November 2016 after 2 months storage at 0.7°C O_2 0.6 kPa, CO_2 3 kPa. Rates of respiration and RQs were calculated from partial pressures compensated for atmospheric pressure as described in 2.2 using 28 – 30 data points (7-7.5 h) and 30 - 40 data points (7.5 – 10 h) for ‘Braeburn’ and ‘Gala’ respectively.

2.5 Comparison of RQ profiles between years and cultivars.

In each of 2016 and 2017, 2 LabPods were loaded with duplicate consignments of ‘Gala’ fruit from commercial orchard G- A and stored at 0.7°C O_2 0.6 kPa, CO_2 3 kPa. In 2016 and 2017 4 and 6 LabPods were loaded with ‘Braeburn’ fruit from commercial orchard B-A and stored at 1.6 °C, O_2 1.0 kPa, CO_2 0.3 kPa. Three repeat rapid RQ profiles were carried out during October/November and November/December for ‘Gala’ and ‘Braeburn’ respectively corresponding to 1-2 months after harvest in both cases. Rates of respiration and RQs were calculated from partial pressures compensated for atmospheric pressure as described in 2.2 using 30 – 40 data points (7.5 – 10 h) and 28 - 30 data points (7- 7.5 h) for ‘Gala’ and ‘Braeburn’ respectively.

2.6 Changes in fruit response through the storage season

In 2016 fruit from two commercial ‘Braeburn’ orchards B-A and B-C were each loaded into a LabPod on 15 October, one day after harvest and stored at 1.6 °C, O_2 1.0 kPa, CO_2 0.3 kPa. Following an initial rapid RQ profile carried out 1 month after harvest (November 2016), the apples were stored at 0.6 kPa O_2, 0.3 kPa CO_2 except during RQ tests. All respiration measurements were made by measuring the rate of O_2 decrease from 0.6 to 0.5 kPa O2. In addition further rapid RQ profiles were carried out after 3, 5 and 6 months of storage.

2.7 Apple supply, pre-storage treatment, LabPod loading and storage initiation

‘Gala’ and ‘Braeburn’ apples were harvested from commercial orchards in Kent, UK over four harvest seasons; 2015, 2016, 2017 and 2020.

‘Gala’ apples were harvested from an orchard (referred to as ‘Gala’ Orchard G-A (51°22'39.6"N 0°35'56.5"E) over 2 pick dates in each season; 2015: 14 and 24 September. 2016: 14 and 20 September. 2017: 1 and 6 September. Data are also presented for orchard (G-B) located adjacent to G-A. In 2020 fruit from a third ‘Gala’ orchard (G-C) were harvested 30 September.

‘Braeburn’ apples were harvested from two orchards; B-A and B-B on the same farm (51°22'39.6"N 0°35'56.5"E) in 2015 on 11 October, at two maturity stages in 2016 (B-A: 14 and 24 October, B-B: 14 and 18 October) and two maturity stages in 2017 (B-A & B-B: 3 and 9 October) and for one orchard at one maturity stage in 2020 (B-A: 7 October). For the 2016/17 season data is also presented for a third ‘Braeburn’ orchard, B-C (51°13'22.8"N 0°31'24.5"E) which was harvested 19 and 25 October.

Fruit was transported to the storage facilities at the Produce Quality Centre of the Natural Resources Institute where they were randomised and damaged or misshapen fruits removed before loading into LabPod chambers situated within controlled temperature stores (each LabPod containing four crates each of 15-18 kg fruit). Crates were weighed, so that total fruit weight per Pod could be used for calculation of respiration rates. Fruit were cooled to storage temperature (0.7°C for ‘Gala’ and 1.6°C for ‘Braeburn’) over 24-48 hours. For ’Gala’, CA regimes were established immediately after fruit reached storage temperature by flushing with N_2 and addition of CO_2 (3 kPa CO_2, 1 kPa O_2). For ‘Braeburn’, fruit were exposed to one of two CA establishment regimes; Delayed and Rapid Seal. Delayed seal regime: fruit kept in refrigerated air storage for 14 days, CA manually established at 3.0 kPa O_2 and <0.5 kPa CO_2, held for 2 days, O_2 reduced manually to 1.0 kPa. Rapid seal regime: fruit kept refrigerated for 48 h to remove field heat, cabinet sealed, O_2 concentration dropped sequentially from 21 kPa to 2.0 kPa over 10 days, fruit held at 2.0 kPa O_2 for a further 10 days then O_2 reduced manually to 1.0 kPa.

Data are also presented (Fig. 3) for a SafePod installed in a commercial ’Gala’ apple store in Kent, UK in 2016. The store was run at 0.5 – 1.0°C at an atmosphere of 1.2 kPa O_2, 4 kPa CO_2. The SafePod contained fruit from a single commercial orchard. The store was loaded in the last week of September, and a rapid RQ profile run from day 36 of storage.

2.8 Statistical analysis

One way analysis of variance to compare data at single time points was carried out using Genstat 18th edition.

3. Results

3.1 Integrity of SafePod chamber design

To determine the likely magnitude of errors introduced by gas diffusion through the water seal, the rate of leakage was measured from empty LabPods with an internal atmosphere of 3 kPa CO_2 and 1 kPa O_2 and at two temperatures; 9°C and 0.7°C. Over one week, the rate of change in O_2 was approximately 0.01 kPa d^{-1} for O_2 at either temperature, and the rate of change in CO_2 was 0.02 kPa d^{-1} and 0.03 kPa d^{-1} at 9°C and 0.7°C respectively. Considering rates of O_2 and CO_2 changes of the order of 0.25 to 0.35 kPa d^{-1} during assessment of stored fruit respiration (Fig. 3), this represents maximum errors of up to 12 % for CO_2 and 4 % for O_2.

3.2 Measurement of fruit respiration and compensation for atmospheric pressure changes

Fig. 4 shows typical data obtained to measure the respiration characteristics within a LabPod. Three replicate runs followed 'Gala' apple response over a decrease of O_2 from 0.6 kPa to less than 0.1 kPa. Figs. 4 (A) and (B) show the increase in CO_2 and decrease in O_2 respectively, in each case with and without correction for changes in atmospheric pressure (Fig. 4 C). Changes in atmospheric pressure have the greatest impact for higher gas concentrations. In the example shown the measurement is made on 'Gala' apples stored at 0.6 kPa O_2 and 3 kPa CO_2 so that the compensation had a more notable effect on CO_2 concentration.

Fig. 5 shows the rates of respiration calculated from the data in Fig. 4. The rate of CO_2 evolution measured is very noisy without correction for atmospheric compensation, while O_2 consumption rate is hardly affected by application of compensation.

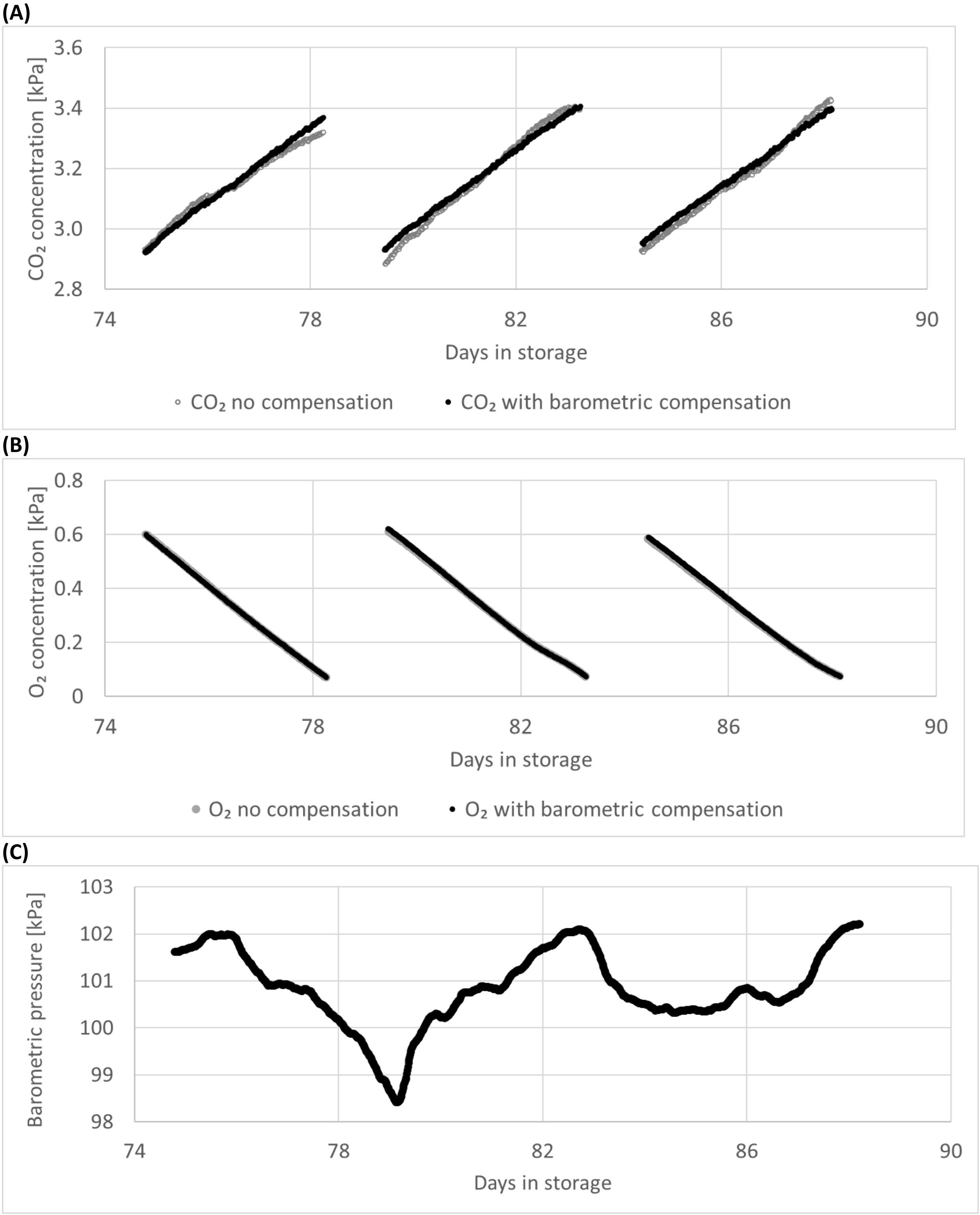


Figure 4. Changes in (A) CO_2 and (B) O_2 concentration in a LabPod loaded with 'Gala' apples (Orchard G-A) and with CA control disabled to measure the fruit respiration rate. of 'Gala' apples Data are shown with and without compensation for changes in barometric pressure (C).

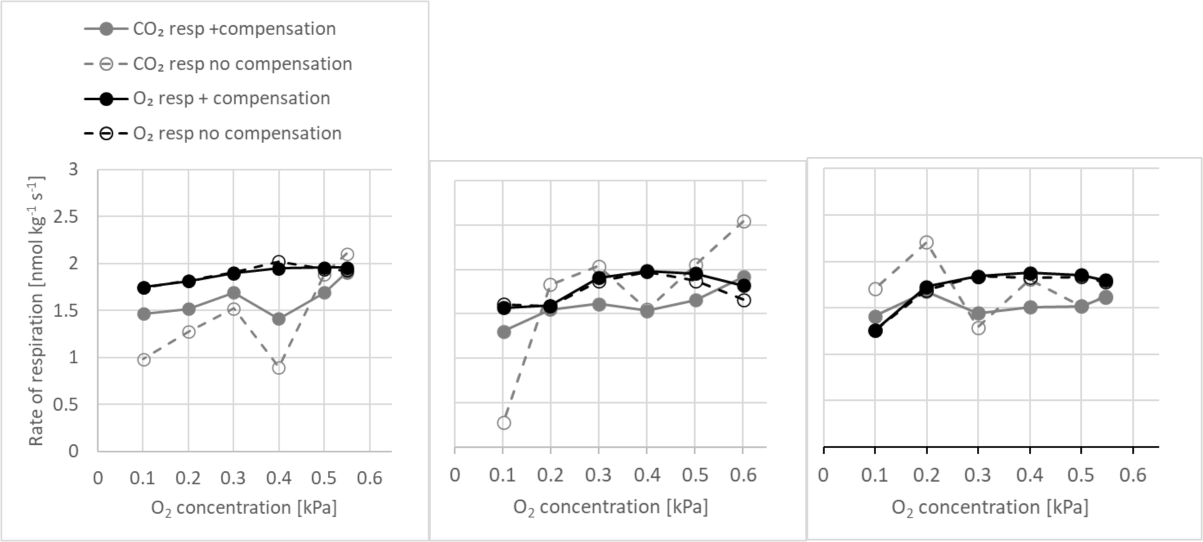


Figure 5. Respiration rates for three repeat respiratory profiles. Rates of respiration were calculated from the data shown in Figure 4.

3.3 Comparing protocols for RQ profiling

Fig. 6 shows the rates of O_2 consumption and CO_2 evolution and the RQ calculated for a 'Braeburn' consignment stored in a LabPod and assessed early in the storage season (Raw data shown in Fig. 3 C). As the O_2 concentration decreased the rate of O_2 consumption decreased as expected, while below 0.5 kPa the rate of CO_2 evolution started to rise. Consequently, the RQ rose as the O_2 concentration decreased below 0.6 kPa. The decrease in RQ as the O_2 concentration decreased from 0.9 kPa to 0.6 kPa resulting in a U shaped RQ profile was observed consistently for the rapid protocol. The data were consistent for the three replicate runs, except for a small decrease in respiration rates in sequential runs.

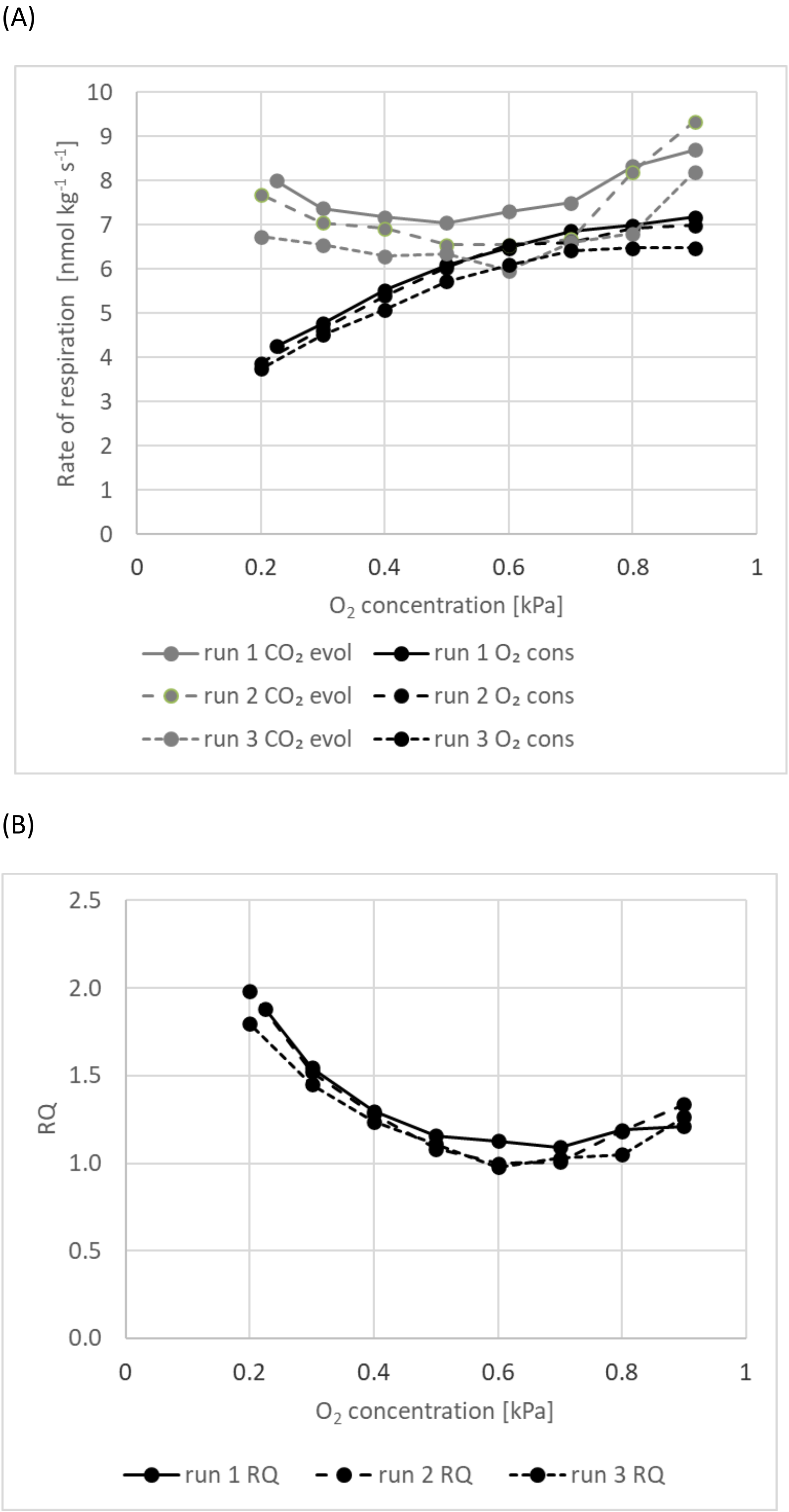


Figure 6: (A) Rates of respiration and (B) RQ profile calculated for 'Braeburn' apples (Orchard B-A) in November 2016 (1 month after harvest) stored in a LabPod at 1 kPa O_2 and 0.3 kPa CO_2 . Three replicate measurements are shown. The data were calculated from the gas partial pressures shown in Fig. 3C.

Fig. 7 compares the RQ profile for a consignment of ‘Braeburn’ and a consignment of ’Gala’ apples obtained using both the rapid and the slow protocols during the first 6 months of storage. For ‘Braeburn’ it is notable that the rapid protocol gives a lower RQ value (Fig 7A). For both cultivars the rapid protocol indicates a decrease in RQ through the protocol as the O_2 concentration decreases, followed by a subsequent rise as O_2 decreases further. This U shaped response is not observed for the slow protocol. Consistent with normal commercial practice ’Gala’ was stored at lower temperatures than ‘Braeburn’. The respiration rates were correspondingly lower and the data more variable. It was notable that the increase in RQ in ‘Braeburn’ below 1 kPa O_2 seen for the slow RQ occurred gradually over a relatively wide O_2 concentration. For a consignment of ‘Braeburn’ apples, a comparison of RQ profiles obtained using a slow and reverse protocol, both allowing fruit to acclimatise to O2 concentrations found similar profiles (Fig. 8A).

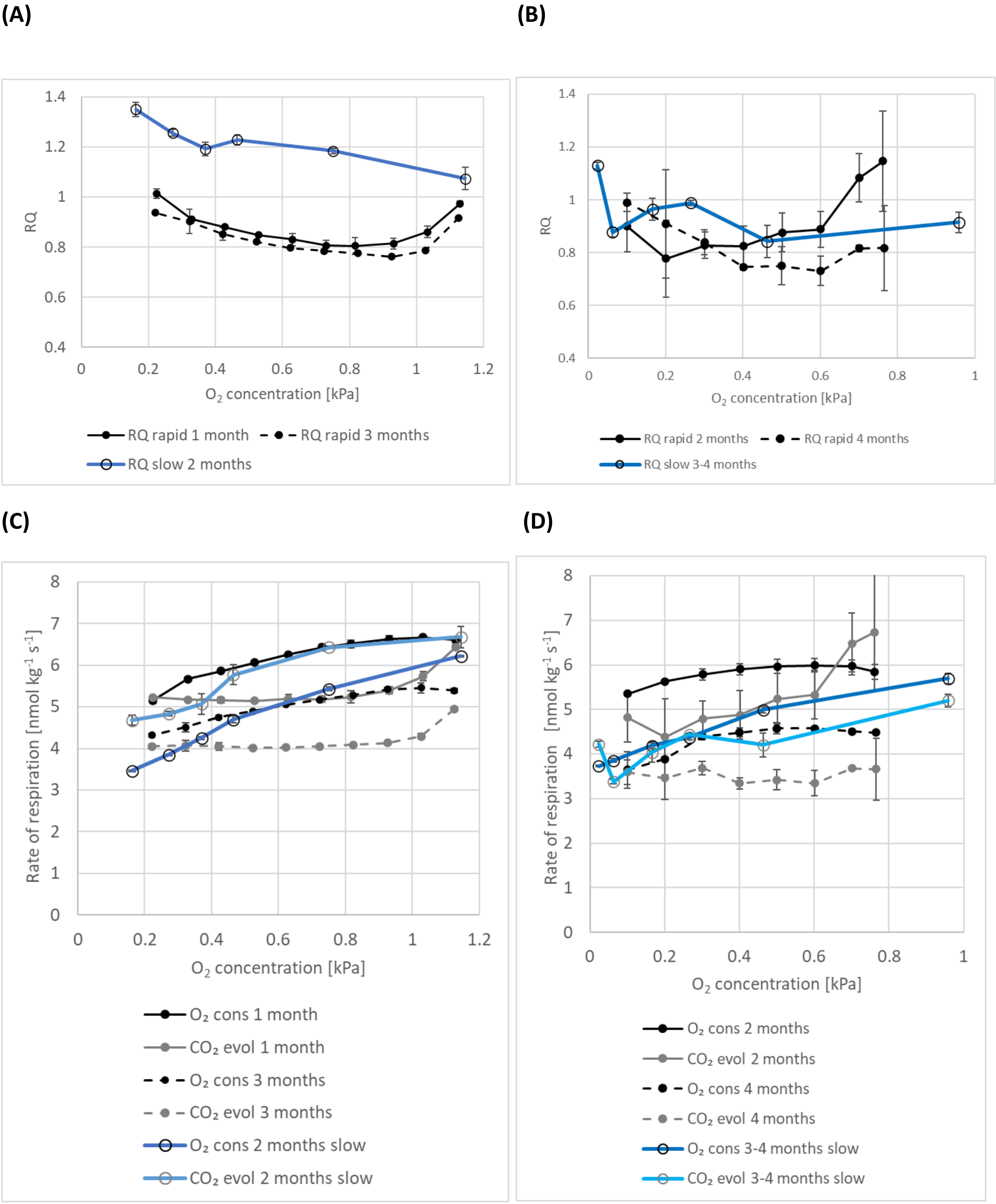


Figure 7: RQ profile and respiration rates for ‘Braeburn’ (A), (C) and ’Gala’ (B), (D) fruit obtained using both *slow* and *rapid* protocol, season 2020-21. Each point is the mean +/- se of 2 – 3 measurements. For each consignment 2 repeat rapid protocols were run starting in Nov 2020, (1, 2 months storage for ‘Braeburn’ and ’Gala’ respectively) followed by a slow protocol starting in Dec 2020 (3 months storage) and then two further repeats of the rapid protocol starting in Jan 2021

(3, 4 months storage for ‘Braeburn’ and ’Gala’ respectively). O_2 cons: rate of O_2 consumption. CO_2 evol: rate of CO_2 evolution.

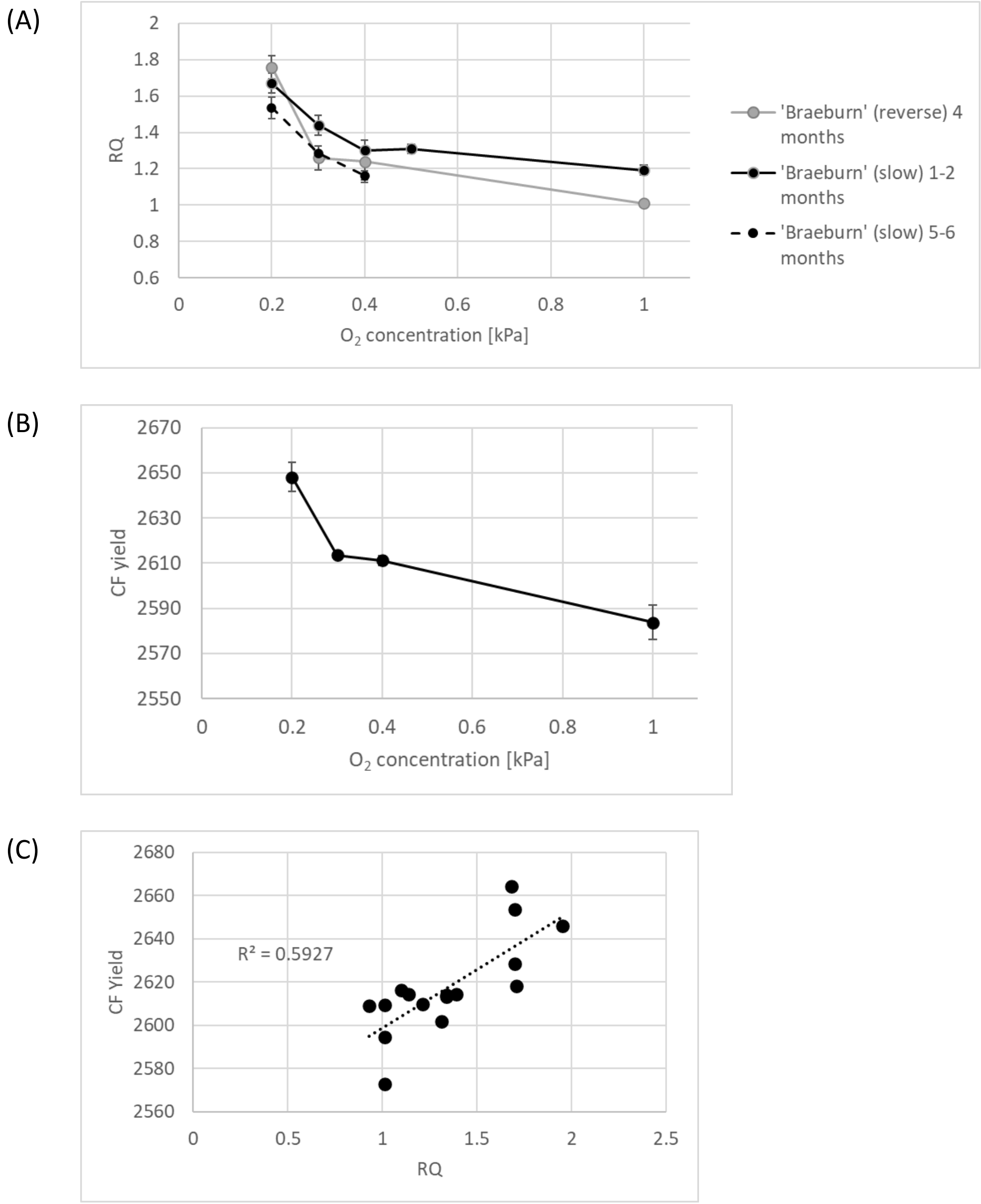


Figure 8: (A) RQ profile for ‘Braeburn’ fruit obtained using *slow* and *reverse* protocol, season 2015-16. Each point is the mean +/- se of 2 – 8 measurements. Measurements were made in Nov - Dec 2015 (1-2 months after harvest), and repeated in Mar – Apr 2016 (5-6 months after harvest), while for the reverse protocol measurements were made in Feb 2016 (4 months after harvest). Each point is the mean +/- se of 2-4 repeated measurements on a single consignment of fruit

('Braeburn' orchard B-A). (B) CF yield obtained using a HarvestWatch^{TM} sensor on 5 fruit in the same LabPod during the reverse protocol. (C) Relationship between fluorescence yield and RQ.

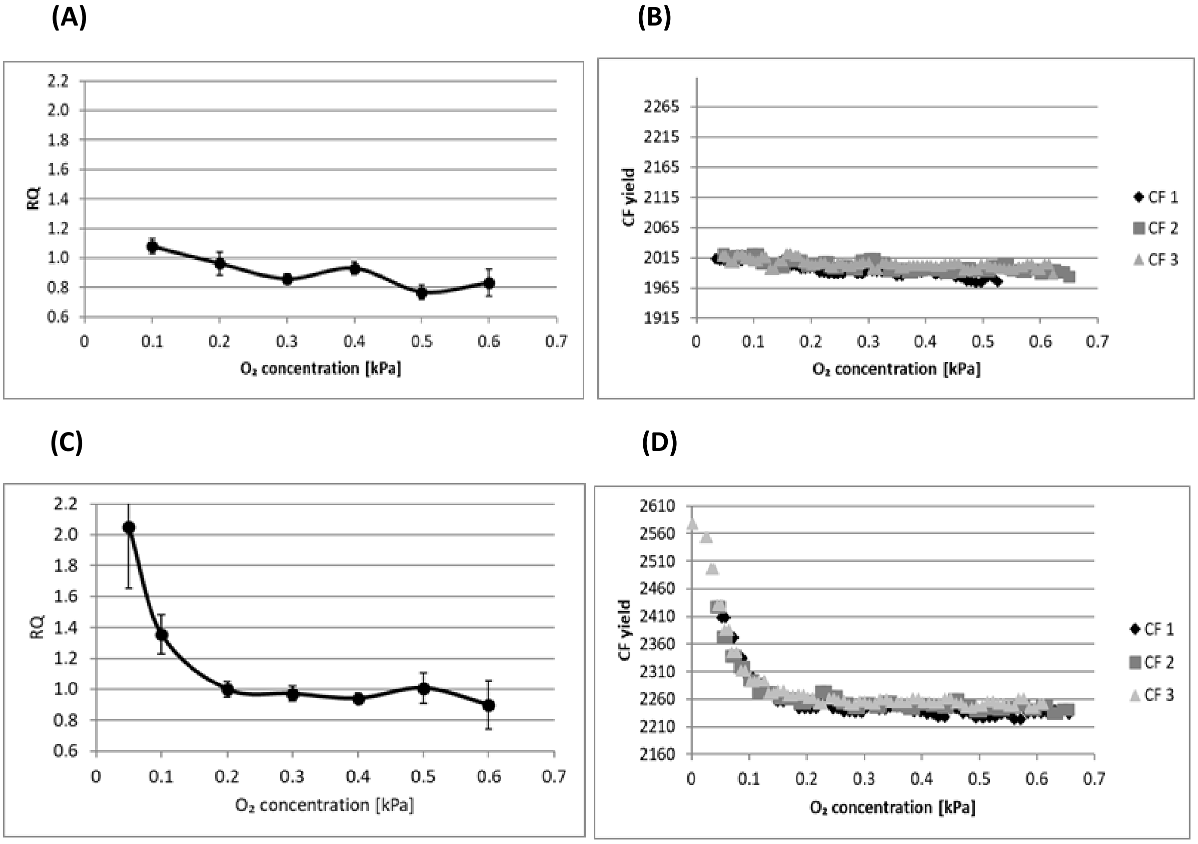


Figure 9: Comparison of rapid RQ profile (A,C) and chlorophyll fluorescence yield (B,D) obtained in November 2016 for two 'Gala' orchards; Orchard G-A (A,B), Orchard G-C (C,D). The y axis range for (B) and (D) is 20 % of average fluorescence yield over the O_2 range 0.6 – 0.65 ~~kPa~~kpa.

3.4 Comparing respiratory responses with chlorophyll fluorescence yield

The HarvestWatch^{TM} technology for DCA monitors an increase in CF associated with low O_2 stress. Figs 8 and 9 show a comparison of RQ response and CF yield during a reverse RQ profile carried out on 'Braeburn' apples and during rapid RQ profiles carried out on two consignments of 'Gala' apples, respectively. In all three cases an increase in CF yield was mirrored by an increase in RQ. The usual commercial practice for the HarvestWatch^{TM} technology is to reduce the O_2 concentration until a CF spike is observed and then increase O_2 until the spike is reversed (similar to the reverse RQ protocol presented in this paper). The CF yield recorded at the time of each RQ measurement was positively correlated with the RQ value (R=0.77 **, Fig. 8).

The two 'Gala' consignments illustrated in Fig. 9 had distinct responses to O_2 decrease. Orchard G-B, which had a respiration rate 25 % higher than orchard G-A at 0.6 kPa O_2, had a more distinct RQ response below 0.2 kPa O_2. The distinction between the two consignments was also observed for the CF yield response.

3.5 Comparison of RQ profiles between years and cultivars.

Fig. 10 shows the RQ profile for 'Braeburn' and 'Gala' apples harvested from the same orchards over successive seasons (2016-2017, 2017-2018) using the rapid RQ protocol; in both cases assessed approximately one month after harvest. There was no statistically significant difference between seasons (by ANOVA at each O_2 concentration), but as observed previously a very significant difference between the two cultivars; 'Braeburn' RQ increased markedly below 0.5 kPa O_2, whereas any increase in 'Gala' RQ was not significant until below 0.2 kPa. At steady state the RQs were higher for 'Braeburn' than for 'Gala' (consistent with Fig. 7). For 'Braeburn' the decrease in RQ as O_2 decreases from 1 kPa is very clear (consistent with Figs. 6 and 7).

The different RQ profiles for the 'Braeburn' and 'Gala' orchard imply that under these storage conditions ('Gala' 0.7°C, CO_2 3 kPa, 'Braeburn' 1.6°C, $CO_2 < 0.4$ kPa) the 'Braeburn' fruit switch to anaerobic respiration at a higher O_2 concentration than 'Gala' fruit. This is supported by the results of trials conducted on fruit from the same orchards in 2015/16, in which fruit were assessed for alcohol taint after 7-8 months storage over a range of O_2 concentrations. For 'Gala', alcohol taint was detected in 25 % of fruit stored at 0.1 kPa O_2, but was not detected for any fruit stored at 1 or 0.25 kPa O_2. However for 'Braeburn', alcohol taint was detected in 2 %, 10 % and 52 % fruit stored at 0.6, 0.4 and 0.3 kPa O_2 respectively.

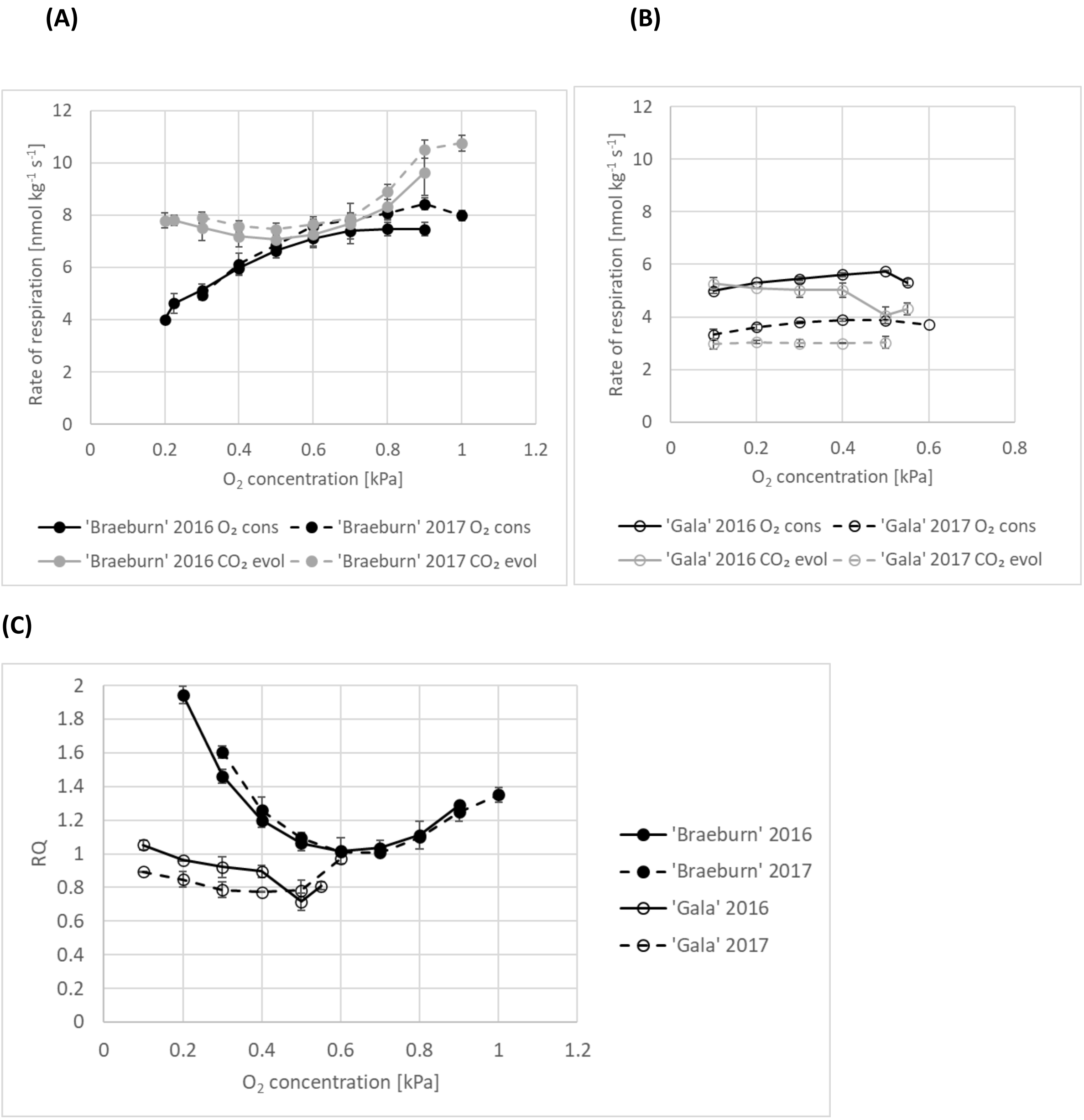


Figure 10: A) B) Respiration rates and C) RQ profiles for 'Gala' and 'Braeburn' fruit obtained using the rapid protocol in 2016 and 2017. For 'Gala' two duplicate consignments from the same orchard (G- A) were assessed in Oct – Nov (1-2 months after harvest) in both years. For 'Braeburn' four and six consignments were assessed in 2016 and 2017 respectively from the same orchard (B-A) in Nov – Dec (1-2 months after harvest).

3.6 Changes in fruit response through the storage season

Through the storage season a decrease in apple respiration rate was observed. ‘Braeburn’ apples from two orchards in 2016/17 (Fig. 11) display a marked decrease in the rate of respiration between November to January (1 to 3 months storage). For one (Orchard B-A) the respiration rate started to increase again between 6-8 months storage, while for the other (Orchard B-C) the respiration rate remained steady between 4-8 months storage. On the basis of the RQ profiles obtained within one month of harvest, the fruit from both orchards were stored at 0.6 kPa O_2. For this reason the later RQ profiles started at 0.6 kPa $O_{2.}$. For both orchards the decrease in respiration rate was associated with a less pronounced RQ response, while the increase in respiration rate later in the season for Orchard B-A was associated with a “recovery” of the RQ response at low O_2. On the other hand Orchard B-C maintained a more stable rate of respiration rate and no increase in RQ response at low $O_{2.}$. In all cases the U shaped RQ response was observed.

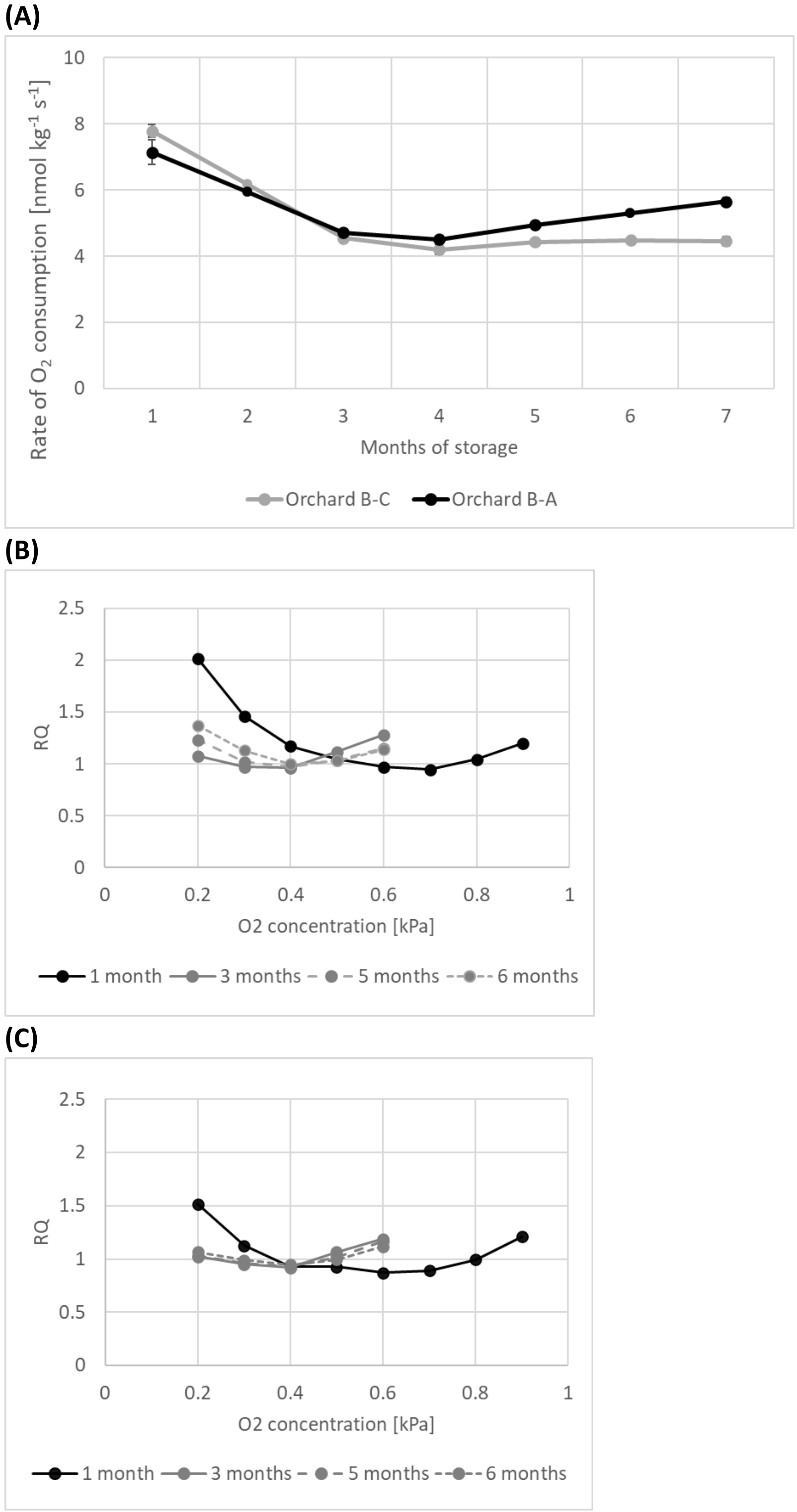


Figure 11: (A) Respiration rate (O_2 consumption) for 'Braeburn' apples from Orchard B-A and B-C. Following the initial RQ profile in 1 month after harvest (November 2016), the apples were stored at 0.6 kPa O_2 except during RQ tests. All respiration measurements for (A) were made by measuring the rate of O_2 decrease from 0.6 to 0.5 kPa O_2. Data are shown as mean +/- SE. (B) and (C) RQ profiles measured for Orchard B-A and B-C

respectively after 1, 3, 5 and 6 months of storage. RQ respiratory quotient = rate of CO_2 evolution/rate of O_2 consumption.

4. Discussion

4.1 Integrity of SafePod chamber design

This paper provides baseline information on data that can be obtained using the SafePod^{TM} technology. This technology has versatility as a tool both for long-term monitoring of produce metabolic status, and for providing the specific information on response to low O_2 concentrations for DCA storage of fruit. The potential of a technology that enables *in situ* monitoring of produce respiration during storage rather than relying on sample removal for subsequent respiratory analysis has been emphasised previously (Keshri et al., 2020). The approach of using a specially designed chamber rather than monitoring directly within a commercial store reduces confounding issues associated with gas leakage from the whole store environment. Data has been presented from SafePod chambers installed inside commercial stores as well as from laboratory trials using the LabPod. For correct interpretation of the data it is important to understand the limitations and experimental artefacts due both to equipment design, and the nature of the fruit (both metabolic and physical). It is particularly important to consider the implications when monitoring fruit in non-steady state situations.

The use of the water seal is important to reduce the physical stresses incurred due to changes in atmospheric pressure and the associated increase in incidence of leaks (Bessemans et al., 2016a). However, this also introduces the issue of gas diffusion through the water seal. The rate of CO_2 diffusion measured in tests is of the order of 8-12% of the rates of gas concentration changes due to respiration recorded in this study. However, it will be important to consider this source of error if the technology is applied to scenarios where even more accuracy is required.

4.2 Measurement of fruit respiration and compensation for atmospheric pressure changes

While the water seal allows changes in volume of the SafePod to overcome the physical stresses imposed by atmospheric pressure changes, it is important to compensate for resulting changes in chamber internal pressure by including atmospheric pressure compensation. The effectiveness of this compensation is demonstrated in Fig. 4. The lack of atmospheric compensation may be responsible for some of the variability evident in the data presented by previous authors (Keshri et al., 2020) who also used chambers with water seals. Bessemans et al (Bessemans et al., 2016a) noted inconsistent RQ measurements as atmospheric pressure changed, and employed the use of an error detection system to eliminate RQ values that were unrelealistic. The impact of atmospheric pressure changes on measurements of respiration rates depends on the absolute gas concentration; for CA storage of apples at O_2 concentrations below 1 kPa the effect is small, however where greater O_2 concentrations are used, or for apple cultivars stored at high CO_2 concentrations (such as 'Gala' in the UK at 3-5 kPa CO_2) the effect is more significant. Furthermore, if this technology is applied to commodities stored at higher O_2 or CO_2 concentrations then the use of atmospheric pressure compensation is increasingly important.

For most purposes, and as shown in Fig. 4 it is sufficiently accurate to use pressure compensation with a single sensor at each commercial site, positioned at a central location outside the stores. However the water seal will act to some extent as a buffer for pressure changes and therefore where more accurate data is needed it will be necessary to have pressure measurement inside each Pod.

4.3 Comparing protocols for RQ profiling

Several technologies have been tested for detecting the anaerobic compensation point (ACP) also referred to as the lowest oxygen limit (LOL); the point of lowest CO_2 evolution rate and the O_2 concentration below which fruit goes anaerobic. Several studies have been carried out suggesting that the ACP or even O_2 levels just below the ACP provides the optimum storage condition for the long-term storage of some apple cultivars (Bessemans et al., 2016b; Thewes et al., 2019, 2017a; Weber et al., 2020, 2019). While optimum storage conditions for many apple cultivars are likely to be above this O_2 concentration, it is still useful to be able to pinpoint the ACP.

In this study different protocols for profiling the RQ response have been tested using the SafePod technology; a slow protocol allowing fruit adaptation at each O_2 concentration takes too long (4-6 weeks) to be of practical commercial use, so a protocol allowing a continuous measurement of respiration rates from 1 or 0.6 kPa O_2 downwards has been tested. The rapid RQ profile presented in this paper is typically a U shaped curve. The initial decrease in RQ as O_2 decreases is considered to be an artefact arising from solubilisation of O_2 and CO_2 in apple tissues as described and modelled by Bessemans et al. (2020) with RQ being underestimated due to the higher solubility of CO_2 compared to O_2. Comparison of the rapid RQ profile with the slow RQ profile, during which fruit can acclimatise, (Fig. 7) suggests that the low point of the curve for the rapid RQ profile tends to be at an O_2 concentration higher than the ACP indicated by the slow protocol. However, using this rapid protocol is an effective way to compare consignments from different cultivars, orchards and seasons (Fig. 10). Commercial application of this technology requires rapid and accurate measurement of respiratory characteristics. Accurate measurement of respiration rate during long-term apple storage is particularly challenging given the low respiration rates of fruit stored at low temperatures under CA conditions, and ironically is likely to become more challenging as CA storage protocols improve. The approach of using Pods with integral sensors allows continuous monitoring of gas partial pressures so that respiration rates can be calculated using best line fits to a continuous data set, with reliable measurements in 7-10 hours.

4.4 Comparing respiratory responses with chlorophyll fluorescence yield

Commercially, chlorophyll fluorescence (CF) yield has been used as a DCA technology in several countries; notably in Italy. While the direct metabolic response of chloroplasts to low O_2 is well documented (Harris and Heber, 1993), it is not so clear how the CF signal relates to whole fruit response. However it has been postulated that it is the redox state of distant tissues leading to over-reduction of the plastoquinone pool in the chloroplasts that leads to a CF response indicative of whole fruit stress (Wright et al., 2012). In this study comparison of RQ response with CF response indicated that they were similar. Whenever apples were exhibiting an RQ rise indicating a switch to anaerobic respiration, there was an increase in CF yield (Figs 8 and 9). Respiratory responses are essentially a cumulative signal across all apple tissues whereas the CF signal arises from the chloroplasts in the periderm and their response to metabolic signals. We therefore expect the responses to be distinct under some circumstances and will examine that in future studies.

4.5 Comparison of RQ profiles between years and cultivars.

The two apple cultivars used to provide data in this paper, ’Gala’ and ‘Braeburn’, respond differently to low O_2 concentrations during storage. ’Gala’ stored at 0.7°C consistently exhibits an LOL near or below 0.2 kPa while ‘Braeburn’ stored at 1.6°C exhibits an LOL of 0.5-0.6 kPa. This distinct RQ response fits with observations of alcoholic taint; with ‘Braeburn’ showing alcoholic tainting below 0.6 kPa O_2 and ’Gala’ below 0.2 kPa. One hypothesis is that the relatively higher apparent susceptibility of ‘Braeburn’ to low O_2 stress compared to ‘Gala’ is related to tissue structure resulting in lower rates of gas diffusion in ‘Braeburn’. The slow rise in RQ observed for ‘Braeburn’ with decreasing O_2 concentration for the slow protocol (Fig. 7) is consistent with a cumulative response from tissues at different depths within the fruit exposed to a range of O_2 concentration.

Using the SafePod technology, and the rapid RQ protocol, RQ values observed for both ’Gala’ and ‘Braeburn’ are often below unity, under both laboratory and commercial storage conditions and especially for ’Gala’. Theoretically when hexoses are used as substrates for aerobic respiration, RQ is equal to 1, while when organic acids are used as substrate, they will be greater than 1.0. An RQ less than 1.0 occurs when lipids are used as a substrate (Cameron et al., 1994). However, Bessemans et al. (2020) have produced a model to demonstrate how gradients in gas concentrations between fruit flesh and chamber headspace affect the measurement of respiration rates and the RQ. Given the high solubility of CO_2 there is a tendency to underestimate RQ. We believe that this effect is responsible for the low RQs observed in 'Gala' in this study, but still need to carry out trials to confirm this.

4.6 Changes in fruit response through the storage season

For practical application of DCA technologies if the storage atmosphere is to be dynamically controlled through the season it is important to be able to compare response of consignments not only between seasons, but also changes through a storage season. The RQ profile changes as the rate of respiration changes through the season, with a stronger, more distinct RQ rise where respiration rates are higher (Fig. 11). This is particularly critical at the start of the the storage season at which point the respiration rates are decreasing susbstantially through storage. For reliable comparison of consignments, it will therefore be necessary to have a constant protocol for recording the profile i.e. at a fixed time after harvest. It is also notable that when the respiration rate is lower, it is harder to pinpoint the O_2 concentration at which the RQ starts to rise. Bessemans et al. (Bessemans et al., 2016a) noted this same issue.

Thewes et al (2020) are promoting the use of the profile of CO_2 evolution to pinpoint the LOL (Fruit Atmo technology), on the basis that where whole stores are used for the measurements, this is less prone to errors due to store leakage. The data from 'Braeburn' consignments shown in Fig. 6 and Fig. 10 indicate a minimum rate of CO_2 evolution at 0.5 kPa O_2, with the minimum RQ at 0.6. Thus in principle the SafePod technology could be used to obtain equivalent data to that obtained by Thewes *et al*.. For measurements made by LabPods, later in the storage season where lower respiration rates prevail, the lowest CO_2 evolution rate was difficult to determine accurately. Similarly for ’Gala’ with lower respiration rates under commercial conditions, we were unable to identify the point of lowest CO_2 evolution, underlining the technical challenge for this approach.

Fig. 11 illustrates the fact that apple fruit metabolic rate changes very significantly through the storage season. Typically there is a rapid decrease in rates of respiration over the first few weeks of storage. Fruit are probably less susceptible to low O_2 damage when the rate of respiration is lower. Using DCA we would therefore expect to store at lower concentrations later in the season. As discussed above, in practice this is challenging, given that the RQ response is less distinct and therefore it is more difficult to pinpoint the LOL later in the season. In practice the technology would be used to check that the steady state RQ remains constant. Fig. 11 also illustrates the fact that apple consignments can differ in their response, with some maintaining a constant low level of respiration over many months while others exhibit a rise. This has often been associated with loss in quality. We will address this in a later study.

5. Conclusions

Protocols to identify the LOL were tested using ‘Braeburn’ (sensitive to low O2) and ‘Gala’ (less sensitive to low O2). A protocol that allows fruit to acclimatise at each O_2 concentration takes several weeks and is therefore not practical for commercial use. A rapid profile without fruit acclimatisation can be completed in 2-3 days. Although this underestimates RQ values, and results in an increase in RQ at a higher O_2 concentration than observed for acclimatised fruit, the rapid RQ protocol provides a practical method to compare response of apple consignments between cultivars, orchards and seasons. As indicated by the rapid protocol, under commercial storage conditions, the LOL of ‘Braeburn’ consignments was higher (near 0.6 kPa) than that of ‘Gala’ consignments (near 0.2

kPa) and this was associated with a greater propensity for Braeburn to develop alcoholic taints. The RQ response using the SafePod was consistent with increase in CF yield using HarvestWatch^{TM}.

Fruit respiration rates change through the storage season, including a substantial decrease over the first 2 months after harvest. As RQ response is affected by respiration rate, with a smaller rise in RQ when respiration rates are lower, accurate comparison of consignments depends on profiles being measured at the same stage in the storage season. It is also more difficult to determine the LOL by RQ profiling later in the season when respiration rates are lower.

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