Respiratory parameters of onion bulbs (Allium cepa) during storage. Effects of ionising radiation and temperature

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Abstract: The O₂ and CO₂ respiration rates of untreated and irradiated onion bulbs (Allium cepa) at 0.15 and 0.30 kGy were measured at 4, 10 and 20 °C. The O₂ respiration rate increased for 24 h after treatment from 0.19 mmole kg⁻¹ h⁻¹ at 20 °C for control samples up to 0.26 and 0.39 mmole kg⁻¹ h⁻¹ for 0.15 and 0.3 kGy irradiated onions respectively. Respiratory quotient (RQ) increased with temperature. The Q₁₀ of the respiration of the control samples (1.61) was lower than that of any other plant tissue, but it increased with storage duration and irradiation dose. The respiration rate of control onions increased steadily over 25 weeks of storage at 4 °C, while that of the irradiated samples decreased during the same period after a peak observed after irradiation treatment. The apparent Kₘ for the Menten–Michaelis equation was determined on a new respirometer and averaged 1.6 kPa at 10 °C and 6.3 kPa at 20 °C. However, at this higher temperature (20 °C) apparent Kₘ varied with O₂ partial pressure, proving that the respiration of onion bulbs does not follow a Menten–Michaelis-like process. The Fermentative Index (FI) of onions was measured under anoxic conditions as CO₂ production rates in mmole kg⁻¹ h⁻¹ at 4, 10 and 20 °C.

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Keywords: onions; gamma radiation; respiration rate; activation energy; Q₁₀; respiratory quotient; sprouting

INTRODUCTION

Onion bulbs (Allium cepa) are one of the world’s oldest cultivated vegetables and are widely used for culinary purposes. Besides their remarkable medicinal powers, and their antifungal and antibacterial properties,¹ onions, as other Allium spp, are generally consumed for their flavour; their nutritive values have been appreciated only recently.² During their storage, bulbs are exposed to atmospheric conditions and temperatures which can affect their respiration rate. High catabolism is considered the main cause of the changes in quality.³ These changes, occurring during post-harvest storage, are principally heat production, transpiration, and mainly sprouting and rotting.²

Recent developments in storage technology, especially modified atmosphere packaging of onion bulbs (Allium cepa), require the determination of physiological parameters to predict gas exchanges of tissues and the behaviour of this vegetable during storage and to match the packaging film respiratory requirements of the commodity.

Values for physiological parameters of onions as well as for most vegetable crops, including Vᵐ (Menten–Michaelis equation) and their variations with temperature, sprouting and ionising radiation stresses are not readily available in the current literature.

Ionising treatments of bulbs, tubers and other vegetables offer many advantages to the producers and consumers, including extended shelf-life, replacement of unsafe chemical inhibitors (such as maleic hydrazide for onions) and improved quality of the commodities without apparent damage. The effect of ionising radiation on controlling onion sprouting associated with low temperatures is well documented.⁴–⁶ Gamma rays, when applied to whole plant organs, stimulate the respiration rate.⁷,⁸ Unfortunately, no data are available on the apparent Kₘ of onions and the effects of sprouting, irradiation and temperature on apparent Kₘ and other respiratory parameters including respiratory quotient (RQ) and fermentative index (FI). The objective of this study is to investigate the effects of temperature, ionising radiation stress and sprouting on the respiration of onion bulbs, including aerobic and anaerobic catabolism.

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MATERIAL AND METHODS

Onions
Onion bulbs, Allium cepa (organic product, free of any preharvest chemical treatments) cv Rouge AMPOSTA, were obtained from the local market (Avignon), sorted for uniformity and absence of defects, placed in commercial plastic (PVC) trays in batches of 12 kg and stored at 18°C for 48 h prior to ionising treatments.

Ionising treatments
The ionising treatment of onion bulbs was carried out at the Commissariat à l’Energie Atomique (CEA) of Cadarache (France) with 60Co source at doses of 0.15 kGy and 0.3 kGy at 20°C. The dose rate was 0.2 kGy min⁻¹ and dosimetry was controlled by a Gammachrome Yr dosimeter (Harwell, Oxfordshire, UK). Onions were ionised in the same PVC trays as described above.

Storage conditions
Immediately after ionising treatments, onions were stored at three sets of temperature and relative humidity: 4°C and 85% RH, 10°C and 80% RH and ambient conditions of 20°C and 65% RH.

Respiration rate (RR) assessment
The RR was determined by the glass jar technique. Plant tissues (350 ± 20 g) were placed in 1.5 litre glass jars previously equilibrated at the required temperature and stored in temperature controlled rooms. Jars were initially left open in the cold rooms. At time intervals, three jars per temperature setting were closed and gas samples (50 ml) were taken after 1, 2, 3, and 5 h through a silicone septum set with silicone glue in the jar lid and analysed by a MTI gas analyser (model M200, Fremont, USA). This instrument consisted of two manifolds: one fitted with an MS-5A, 4-m capillary column set at 80°C with argon as carrier gas at a pressure of 110 kPa; and the other fitted with a capillary Poraplot 4, 6-m column set at 110°C with helium as carrier gas at a pressure of 193 kPa. Both manifolds were fitted with katharometric detectors. These analytical conditions permitted the elimination of argon in the O₂ peaks. Rubber gaskets and silicone septa on the jars were changed after each experiment to prevent any air leak. Respiration rates (RR) were calculated by linear regression from O₂ or CO₂ depletion curves and expressed as mmole kg⁻¹ h⁻¹. Mean values and standard deviations were measured in triplicate.

Fermentative index (FI)
The FI was determined by the glass jar technique described above, but the head spaces of the jars were flushed with pure N₂ and the gas composition within the jars was controlled with a MTI gas chromatograph as described above. The final concentration of N₂ was at least 99.5%.

App Kₘ determination
App Kₘ was determined with a close-system respirometer (Fig 1). Two vessels were kept in a heating/cooling water bath whose temperature was regulated with an accuracy of ±0.1°C. About 2 kg of onions were placed into the sample vessel, the lid of which was tightly secured. These onions had been kept at the desired temperature for 12 h before use. After flushing with nitrogen to have 10–11% O₂ in the vessel, the experiment was run for 4 h prior to recording the gas exchange in order to have equilibrium between the onion internal atmosphere and the sample vessel head space. The gas composition within the respirometer was controlled with a gas chromatograph (MTI). As soon as the target atmosphere composition was reached, the valves were shut and the programme was run. Internal gas was pumped (pump 1, Watson Marlow, type 313, Falmouth, USA) at 300 ml min⁻¹ through an infrared transducer (Servomex, 1510 series, Saint Denis La Plaine, France) to measure CO₂ concentration. If this concentration was 0.1 kPa higher than the pre-set value of CO₂ partial pressure,

![Figure 1. Schematic manifold of the respirometer permitting the determination of O₂ and CO₂ respiration rates and app Kₘ.](image)
the computer activated an electrovalve that forced the gas flow from pump 2 (same type as pump 1) at a rate of 300 ml min$^{-1}$, through a CO$_2$ trap filled with about 215 g of 0.3 N sodium hydroxide until initial CO$_2$ concentration in the sample vessel was restored. The trapping of the excess CO$_2$ resulted in a proportional decrease in pressure, which was detected by a highly sensitive differential pressure probe (AS Technologies, series 600, Nimes, France). The pressures between the two vessels were balanced by injection of pure N$_2$ through a mass flow meter (MKS, type 117A, Le Bourget, France) into the vessel containing the onion samples. The CO$_2$ trapped in the sodium hydroxide was continuously measured with a conductivity probe (INRA, Montfavet, France). A zirconium O$_2$ probe (Arelco, type ZOA 100, Fontenay sous Bois, France) continuously monitored oxygen depletion in the respirometer with a sensitivity of 0.1 kPa. The computer recorded N$_2$ injection times and the changes in conductance of the carbonated sodium. At the end of the run, it calculated CO$_2$ production and O$_2$ partial pressure in the sample vessel along with the N$_2$ volume injected in order to balance O$_2$ consumption by the plant tissues. Oxygen partial pressures were plotted against time, and a regression curve, a second or third degree polynomial equation depending on the data was fitted through the data points. The respiratory quotient can be extrapolated to determine $V_m$ and $app K_m$.

**CO$_2$ measurement under anoxia**

Determination of CO$_2$ was performed 6 h after closing the jars flushed with N$_2$, by gas chromatography using the MTI described above.

**RQ, $E_a$ and $Q_{10}$ calculation**

The respiratory quotients ($RQ = RR_{CO_2} / RR_{O_2}$) for all temperatures were determined from raw data and calculated at any temperature from the following equation:

$$RQ(T) = RQ_0 \left[ \frac{Q_{10CO_2}}{Q_{10O_2}} \right]^{\frac{T}{10}}$$

$T =$ temperature (°C)

$Q_{10CO_2}$ and $Q_{10CO_2} = Q_{10}$ of $RR_{O_2}$ or $RR_{CO_2}$. $RQ_0 =$ respiratory quotient at 0°C. $Q_{10}$ was determined by plotting log($RR$) against temperature ($T$): $\log (RR) = aT + b$. The respiration rate at 0°C ($RR_0$) may be calculated from the $b$ coefficient ($RR_0 = 10^b$) and $Q_{10}$ from the $a$ coefficient ($Q_{10} = 10^{10a}$).

Activation energy ($E_a$) as described by Labuza$^{11}$ is based on an Arrhenius equation:

$$\ln (RR_T) = \frac{E_a}{R} \frac{1}{(T + 273)} + \ln (RR_0)$$

$RR_T =$ respiration rate at $T$ (mmolekg$^{-1}$ h$^{-1}$) $T =$ temperature (°C) $E_a =$ activation energy (J mole$^{-1}$) $R =$ gas constant (8.3 J mole$^{-1}$ K$^{-1}$)

**Statistical analysis**

Experiments were carried out for 2 years (1996 and 1997) and all experiments were done in triplicate except for the $app K_m$ determinations which were done in duplicate. Data analyses were performed using XLStat. Pro$^{13}$, and LSD calculated as described by Snedecor and Cochran.$^{12}$

**RESULTS AND DISCUSSION**

**Respiration rate**

The effect of temperature and treatment on O$_2$ and CO$_2$ respiration rates of onion measured under normal atmosphere is reported in Table 1. The respiratory quotient ($RQ$ = ratio between CO$_2$ production and O$_2$ consumption whatever the pre-treatment and temperature) of onions averaged 0.91 at 10°C with a standard deviation of 0.04. $RQ$ (data not shown) seemed to increase with temperature since $RQ$ at 4°C was 0.88 (sd 0.02) and reached 0.93 (sd 0.02) at 20°C, confirming the findings of Hagger et al$^{13}$ on broccoli. The $Q_{10}$ of untreated onion O$_2$ and CO$_2$ respiration rates were 1.67 and 1.84 respectively. This confirms that the increase in temperature will increase $RQ$.

Considering that the $Q_{10}$ of the respiration rate of most plant tissue ranges from 2 to 3,$^{14}$ corresponding to activation energies of 56 000 and 73 000 J mole$^{-1}$ in the range 0 to 10°C, temperature displays a rather

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Activation Energy (J mole$^{-1}$)</th>
<th>$Q_{10}$</th>
<th>$RR_0$ (mmolekg$^{-1}$ h$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$RR_{O_2}$</td>
<td>34 999</td>
<td>1.67</td>
<td>0.077</td>
</tr>
<tr>
<td>$RR_{CO_2}$</td>
<td>41 133</td>
<td>1.84</td>
<td>0.059</td>
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<tr>
<td>Irradiated 0.15kGy</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>$RR_{O_2}$</td>
<td>35 988</td>
<td>1.70</td>
<td>0.091</td>
</tr>
<tr>
<td>$RR_{CO_2}$</td>
<td>30 958</td>
<td>1.58</td>
<td>0.081</td>
</tr>
<tr>
<td>Irradiated 0.30kGy</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>$RR_{O_2}$</td>
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<td>1.89</td>
<td>0.109</td>
</tr>
<tr>
<td>$RR_{CO_2}$</td>
<td>46 080</td>
<td>1.98</td>
<td>0.093</td>
</tr>
</tbody>
</table>

$RR_0 =$ $RR$ extrapolated at 0°C.
moderate effect on onion respiration. Van den Berg and Lentz\textsuperscript{15} noted that $Q_{10}$ values of onion bulbs varied during storage; the $Q_{10}$ value was 2.5 after 1–2 months and 3.5 after 4–6 months of storage with a subsequent increase in respiration heat production which is significantly affected by temperature during storage. Irradiation at 0.30 kGy markedly increased the activation energy of the onion respiration rate. Irradiation would make onion catabolism more sensitive to temperature changes.

Ionisation increased both O$_2$ and CO$_2$ onion respiration rates measured 24h after the treatment at 20°C (Fig 2). Irradiation at 0.3 kGy almost doubled the respiration rate when compared with control.

After the initial burst in respiration caused by irradiation, the repair mechanisms resulted in a decrease in RR (Fig 3). After less than 2 weeks the initial RR was restored and the respiration rate of irradiated onions kept on decreasing but at a slower rate. The RR of irradiated onion bulbs at both 0.15 and 0.3 kGy showed a similar pattern of change with time. After approximately 7 weeks, the respiration rate of irradiated onions kept at 10°C increased slightly. This period corresponds to the break of dormancy and the onset of sprouting of untreated onions. It is likely that onions, or some irradiated onions, start the break of dormancy process, which fails due to genetic damage at the meristematic cells caused by ionisation. After this moderate peak, there was a continuous decline in respiration rates at all temperatures tested. This process led to progressive cell death and onion decay, which occurred between the 25th and the 30th week of storage. Storage temperature had a moderate effect on the rate of decay. The average rates of decrease in respiration between 10 and 25 weeks were determined by fitting linear regression lines to the respiration rate vs time data in Fig 3 from 10 to 25 weeks. The determination coefficients ($R^2$) of the regression ranged from 0.91 and 0.98. The decrease in respiration rate was 2.5, 4.6 and 7.7 μmole kg$^{-1}$ h$^{-1}$ per week at 4, 10 and 20°C respectively for 0.15 kGy and 2.6, 5.8 and 7.4 μmole kg$^{-1}$ h$^{-1}$ per week at 4, 10 and 20°C respectively for 0.3 kGy irradiated onions. From these data it was possible to calculate the $Q_{10}$ values for the two irradiation treatments, 1.98 and 1.85 for 0.15 and 0.3 kGy irradiated onions respectively.

The results of this work agree with several previous investigations. Thomas\textsuperscript{6} reported a temporary increase in the respiration rate of bulbs following irradiation with a subsequent decrease during storage. Metlitsky \textit{et al.}\textsuperscript{16} reported that ionisation treatment at a dose of 0.06 kGy increased the respiration rate of plant tissues, however, the respiration rate returned to the normal level after four weeks following the treatment. Park \textit{et al.}\textsuperscript{17} have found that respiration rates of onion bulbs irradiated at 0.05, 0.07 and 0.1 kGy were higher immediately after ionisation, but lower than the control 1 week after treatment; but the respiratory quotient (RQ) was little affected by irradiation. Similar results were reported by Ahmed \textit{et al.}\textsuperscript{17} on irradiated fruits (green mature tomato, strawberries, oranges and grapefruit) where CO$_2$ production was enhanced by approximately 75–90% with a treatment of 0.15 and 0.3 kGy, but no significant difference was observed in the respiratory quotient (RQ) of irradiated and control fruits. Using homogenates of meristem tissues of

![Figure 2](image-url)

**Figure 2.** Effect of ionisation treatment on respiration rates (RR) at 20°C and 24h after treatment.
irradiated garlic, Mettitsky et al. demonstrated that although a rise in respiratory rate occurred, irradiation caused uncoupling of the oxidation phosphorylation resulting in reduced synthesis of ATP which is required for the active growth process from a state of rest.

During storage the $RR_{O_2}$ of control samples increased with time at all tested temperatures. $RR_{O_2}$ doubled within about 15 weeks at 20 °C and 20 weeks at 10 °C; the influence of storage duration was much less marked at 4 °C (Fig 3c).

The increase in respiration rate of control onions is the consequence of physiological changes including break of dormancy and sprouting. Table 2 shows that after 8 weeks of storage at 4, 10 and 20 °C, the $RR_{O_2}$ values of sprouted onions are 50%, 72% and 52% higher respectively than those of unsprouted onions from the control samples taken from the same batch. The reported respiration rates (0.21 and 0.32 mmole kg$^{-1}$ h$^{-1}$ at 20 °C for unsprouted and sprouted onion bulbs respectively) are consistent with the findings of Loogheed and Franklin$^{21}$ who reported a value of 0.24 mmole kg$^{-1}$ h$^{-1}$ for fresh onions at 21 °C.

The effect of temperature on respiration rates of irradiated onions compared with untreated onions (Fig 3a, b, c) may be partly explained by the change in $Q_{10}$ of their catabolism. As shown in Tables 1 and 3, $Q_{10}$ for the respiration of the control samples increased from 1.67 ($Q_{O_2}$) and 1.84 ($Q_{CO_2}$) up to 2.4 ($Q_{O_2}$) and 2.89 ($Q_{CO_2}$) after 2 months of storage at 4 °C. This change in $Q_{10}$ could not have been due to sprouting or microbial growth since 4 °C was too low a temperature to favour these phenomena. The $Q_{10}$ of $O_2$ and $CO_2$ respiration rates of irradiated onions remained constant under these storage conditions (Tables 1 and 3).

**App $K_m$ calculation**

The apparent Michaelis constant for $O_2$ respiration rate of untreated onions after harvest was calculated from data for each of the 2 years (1997–1998). It is worthy to note that the $app K_m$ values were reproducible and were highly dependent on temperature (6.3 kPa at 20 °C for 1997 and 1998; and 1.6 kPa at 4 °C for both years). The temperature dependence of $app K_m$ was reported by Cameron et al.$^2$ Hagger et al.$^13$ and Ratti et al.$^{23}$ Based on a dynamic model of gas exchange of respiring produce, Hertog et al.$^{24}$ stated that $app K_m$ was independent of temperature. They postulated that the increase in $app K_m$ with temperature observed was probably due to interfering phenomena such as the onset of fermentation at high temperature or was within the margin of error of the experiment.

Values of $app K_m$ are usually measured by fitting Menten–Michaelis model to sets of data describing gas exchange of plant tissues as a function of $O_2$ partial pressure. Considering all possible errors due to intra- and intervariabilities of batches of fruits and vegetables, $app K_m$ cannot be accurately assessed using this approach. The respirometer used in this research measured the respiration rate under continuous decrease in $O_2$ in a closed system with a very low and constant $CO_2$ partial pressure. Varoquaux et al.$^{10}$ claimed that the respirometer used in this research could provide a rapid and direct estimation of $app K_m$ with an instrumental sensitivity of 0.1 kPa. Values of $app K_m$ for respiration rates of plant tissues range from 0.1 kPa for mushroom at 10 °C$^{10}$ to 23.2 kPa for tomato at the same temperature.$^{24}$ These authors$^{24}$ reported intermediary $app K_m$ values of 3.76 kPa for apple and 2.7 kPa for chicory. Song et al.$^{25}$ measuring the respiration rate of six blueberry cultivars at three temperatures, reported $app K_m$ values ranging from 0.78 to 0.104 kPa at 15 °C and from 0.1 to 5.2 kPa at 25 °C, confirming the very large variability in the determination of $app K_m$ with traditional techniques. Figure 4 shows that the respiration of onions (and most plant tissues) does not strictly follow the Menten–Michaelis equation. From the data presented in Fig 4, $app K_m$ varied from 14 kPa for high $O_2$ partial pressure (10 kPa and above) to 3 kPa for low $O_2$ partial pressure (below 7 kPa), with an average value of 6.3 kPa, which was the value previously reported. The fact that plant tissue does not strictly follow the Menten–Michaelis equation may account for the discrepancy in the literature about the temperature dependence of $app K_m$.

**Anaerobic catabolism**

The fermentative index of onion (FI), in mmole CO$_2$ kg$^{-1}$ h$^{-1}$, was measured within 6 h after closing the jars. Beyond 24 h at 20 °C, high CO$_2$ production was likely to be due to anaerobic or aero-anaerobic microorganisms such as yeast and lactic acid bacteria (Fig 5).

FI values were 0.048, 0.072 and 0.14 mmole CO$_2$ kg$^{-1}$ h$^{-1}$ at 4, 10 and 20 °C respectively. The fermentative process was temperature dependent and followed an Arrhenius-like equation ($R^2 = 0.992$). The $Q_{10}$ was 1.93 and $H_{10}$ (fermentative index at 0 °C) was 0.037 mmole CO$_2$ kg$^{-1}$ h$^{-1}$.

Onion anaerobic catabolism is rather moderate compared with other plant tissues since FI of asparagus is 1.08 mmole CO$_2$ kg$^{-1}$ h$^{-1}$ and 0.41 mmole CO$_2$ kg$^{-1}$ h$^{-1}$ for cut chicory at 18 °C.$^{26}$

The knowledge of physiological parameters permits
the prediction of gas changes in modified atmosphere packaging of onion bulbs.

O₂ respiration rate of the bulbs does not strictly follow a Menten–Michaelis process. It is possible that the diffusion of CO₂ and O₂ into the inner bulb tissues plays a role in onion respiration rate.²⁷,²⁸ Further investigations are necessary to determine the optimal gas concentration and RH to maintain onions commercial qualities including prevention of sprouting and rotting.

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Table 3. Respiratory parameters of onion bulbs after two months storage at 4°C.


