Storage technology of onion bulbs c.v. Rouge Amposta: effects of irradiation, maleic hydrazide and carbamate isopropyl, N-phenyl (CIP) on respiration rate and carbohydrates

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(Received 24 February 2000; Accepted in revised form 8 January 2001)

Summary The effects of γ-irradiation, maleic hydrazide (MH) and carbamate isopropyl, N-phenyl (CIP) on the carbohydrate content, respiration rate (RR) sprouting and rotting of red onion bulbs (Allium cepa L.) c.v. Rouge Amposta stored at 4, 10 and 20 °C were investigated. Between 6 and 8 weeks soluble sugars increased in concentration and varied between 6.0 and 6.5% fresh weight both at 10 and 20 °C. However, this peak did not exceed 6.0% fresh weight at 4 °C. The basal amount of soluble sugars was about 3% fresh weight. The content appeared to be influenced by temperature, with greater accumulation at 10 and 20 °C than at 4 °C. The RR increased when the bulbs sprouted, whereas it was reduced by the following treatments: irradiation, MH and CIP, the respiration of irradiated bulbs decreased during the final stage of storage because of the death of the sprouts. The chemical treatment used were effective in controlling sprouting of the bulbs during storage, although not as effective as irradiation and chilling.

Keywords Allium cepa, fructans, respiration, soluble sugars, sprout control.

Introduction

The commercial storage period for onion bulbs is often limited by the sprout elongation as well as initiation of root growth. Immediately after harvest, the bulbs are in a natural stage of dormancy, and re-growth of the shoot can be artificially delayed or controlled by physical influences such as temperature, γ-irradiation, or preharvest spraying with specific chemicals, i.e. maleic hydrazide (MH), carbamate isopropyl N-phenyl (CIP) or salicylic acid. The efficiency of sprout inhibition by γ-irradiation and chemical treatments has been investigated extensively (Isenberg & Ferguson, 1981; Matsuyama & Umeda, 1983; Sinha et al., 1994). However, despite this extensive data on the commercial quality of onions treated by these techniques, little is known about the changes in physiological and biochemical characteristics of the bulbs during long-term storage under different temperatures.

Normally sprouting of onion bulbs during storage is characterized by specific changes in development. It may be possible to extend the storage duration if the biochemical changes involved in the dormancy period could be determined. After break of dormancy, some major changes in carbohydrate catabolites (including soluble sugars and fructans) occurs, with the scales acting as the source of these compounds. Thus, in order to fully understand the metabolic changes in the dormant onion, assessment of carbohydrate content during storage may provide meaningful information (Benkeblia & Selselet-Attou, 1999). Carbohydrates constitute a high proportion of the

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Changes in the composition of these sugars may provide a biochemical indicator of the break of dormancy (Benkeblia & Selselet-Attou, 1999). Unfortunately very few data are available on the variation in carbohydrates, especially soluble sugars and fructans in the bulbs during storage, particularly when bulbs have been previously treated with γ-irradiation or with chemicals.

It has been found also that the respiration rate (RR) of MH-treated or untreated onion bulbs increases during the storage period at different storage temperatures (Ward & Tucker, 1976; Adamicki, 1977). However, there have not been any comparative studies on the respiration rate (RR) of γ-irradiated and CIP-treated onion bulbs during long-term storage.

The objective of this investigation was to determine the effects or γ-irradiation and chemical treatments (MH and CIP) on the variation in RR and oligosaccharide content (soluble sugars and fructans) of onion bulbs cv. Rouge Amposta during long-term storage.

The Materials and methods section outlines the procedures used for the experiments. The materials included onion bulbs of the cv. Rouge Amposta, which were freshly harvested and dried in the field. The bulbs were then treated with γ-irradiation or chemical treatments and stored at different temperatures.

MH and CIP treatments
Onion bulbs were dipped into 2200 ppm MH and 600 ppm CIP (Sigma, St Louis, MO) solutions for 15 min. Then, they were dried in a ventilated oven (Memmert, Schwabach, Germany) at 35 °C for 30 min. Chemicals were applied in the same period of time as ionizing treatment.

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

Respiration rate assessment
The RR was determined by the glass jar technique. Plant tissues (whole bulbs) (350 ± 20 g or 4–5 bulbs) were placed in 1.5 L glass jars previously equilibrated at the required temperature and stored in controlled temperature rooms. Jars were initially left open in the cold-rooms. At specific time intervals, three jars per temperature setting were closed and gas samples (50 L) 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; Microsensor Technology Inc., Fremont, CA, USA). This instrument consisted of two manifolds: one fitted with an MS-5A, 4-m capillary column set at 80 °C with argon as the 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 the carrier gas at a pressure of 193 kPa. Both manifolds were fitted with katharometric detectors. These analytical conditions permit the elimination of argon in the O2 peaks. The rubber gaskets and silicone septa on the jars were changed after each experiment to prevent any air leakage. RR was calculated by linear regression from O2 or CO2 depletion curves and expressed as mmol kg⁻¹ h⁻¹. The mean value of RR was determined from triplicate measurements.

Soluble sugars analysis
Glucose, fructose and sucrose content was determined by HPLC (Doyon et al., 1991). Samples of
5 g of freeze-dried tissues were homogenized in 50 mL of water using a Sorvall blender (Omni-mixer 17220; Newton, PA, USA). The homogenate was heated for 30 min in a boiling water bath (Haake Institute, Berlin, Germany). After cooling, the homogenate was centrifuged for 15 min at 25 000 g using a Heraeus centrifuge (Heraeus Sepatech GmbH, Osterode/Harz, Germany) and the supernatant was filtered on a 0.25-μm Millipore filter (Millipore S.A., Molsheim, France).

The sugars were separated by HPLC using a Varian 5000 model (Vista, 5000 series, Les Ulis, France) fitted with a Polyspher CH-CA column (300 × 7.8 mm, Merck, Darmstadt, Germany) set at 80 °C and a differential refractometer detector (Knauer GmbH, Hegaver, Berlin, Germany). The mobile phase was DDI water at a flow rate of 0.5 mL min⁻¹.

Sugars were identified and quantified by comparison with authentic samples (Sigma) and each determination was run in triplicate.

**Fructans analysis**

*Extraction of fructans*

Fructans were extracted using the method described by Shiomi (1993). Freeze-dried onion powder (1 g) was suspended in 70% ethanol (80 mL) containing small amounts of calcium carbonate, and was then immediately boiled in a water bath (Haake Institute, Berlin, Germany) for 30 min. The residue was extracted four times in 70% hot ethanol (80 mL) for 10 min and then in hot water (80 °C, 100 mL). Filtered extracts were combined and made up to 500 mL with distilled water. An aliquot of the extract (10 mL) was concentrated in vacuo at 30–33 °C to dryness using a Büchi rotavapor (Büchi labortechnik AG, Flawil, Switzerland). The concentrated sugar was passed through a filter (0.45 μm, Nalge Co., Rochester, NY). The solution or the dilute solution (3.5, 21 and 102 times) was analysed by high performance anion exchange chromatography (HPAEC; Dionex, Sunnyvale, CA). All the processes were duplicated.

*Analysis of fructans*

The synthesized or extracted saccharide mixtures were separated on an HPLC-carbohydrate column PA1, Carbo Pack (Sunnyvale, CA) with a Dionex Bio LC series HPLC (Sunnyvale, CA) and pulsed amperometric detection (PAD) (Rocklin & Pohl, 1983; Johnson, 1986). The gradient was established by mixing eluent A (150 mM NaOH) with eluent B (500 mM sodium acetate in 150 mM NaOH) in two ways. System I: 0–1 min, 25 mM; 1–2 min, 25–50 mM; 2–20 min, 50–200 mM; 20–22 min, 500 mM; 22–30 min, 25 mM. System II: 0–1 min, 5 mM; 1–2 min, 25–50 mM; 2–14 min, 50–500 mM; 14–22 min, 500 mM; 22–30 min, 25 mM. The flow rate through the column was 1.0 mL min⁻¹. The applied PAD potentials for E1 (500 ms), E2 (100 ms) and E3 (50 ms) were 0.01, 0.06 and –0.6 V, respectively, and the output range was 1 μC.

The standard saccharides were prepared according to the method described by Shiomi et al. (1976, 1979) and each analysis was duplicated.

**Statistical analysis**

The experiment was repeated in two successive harvesting seasons (1997 and 1998). The data were analysed statistically by determination of least significant difference (LSD at P < 0.01) using XLStat. Pro® statistical software (XLStat, Paris, France).

**Results and discussion**

*Variation of soluble sugars*

Changes in soluble sugars (glucose, fructose and sucrose) showed a similar pattern in control and treated (irradiated, MH or CIP) onion bulbs (Figs 1a–c). After a slight increase observed during the second and the third week of storage, we noted a ‘saccharidic peak’ between week 6 and week 8. This was an increase in soluble sugar concentration from c. 3 to 6.0–6.5% fresh matter at 10 and 20 °C (Fig. 1a and b), but at 4 °C the peak did not exceed 6% fresh weight (Fig. 1c). After 10 weeks of storage, sugar content in the onion had fallen to 2–3% of fresh weight. Closer observation of the peak at 10 °C every 3 days revealed an increase in sugar content between 30 and 60 days of storage (Fig. 2). This peak must logically result from hydrolytic activity of fructan depolymerases which hydrolyse fructans, found in the outer scales of the bulbs, into saccharides, which are in their turn hydrolysed into glucose and
fructose by invertase. Thus, catabolism of non-structural carbohydrates appears to represent a basic catabolism rather than a secondary catabolism.

The reduction in sugar concentration after fructan hydrolysis coincides with the appearance of the first sprouts at 10 and 20 °C. On the other hand, at 4 °C no sprouting was observed after the same period because the sprout expanded but remained atrophied in the bulb.

Changes in soluble sugars and nonstructural carbohydrates in the bulbs are not clearly understood, there are some reports of an increase in sugar concentration following 5–8 weeks of storage of untreated bulbs (Rutherford & Whittle, 1982; Suzuki & Cutcliffe, 1989; Böttcher, 1992), irradiated bulbs (Benkeblia & Selselet-Attou, 1994) and MH-treated bulbs (Gorin & Bőrcsök, 1980). The same change was noted by Salama et al. (1990) after 5 weeks followed by a fall in sugars.

Effect of temperature on fructans in bulbs during storage

We noted little change between the initial levels of 1-kestose and neokestose in the bulbs, and after 24 weeks of storage, except 1-kestose increased to 7.52 mg g⁻¹ dry weight at 20 °C and neokestose increased to 7.13 mg g⁻¹ dry weight at 10 °C (Table 1). However, the concentration of tetrasaccharides increased with temperature. In the same way, penta, hepta and other polymerized fructans increased with temperature.

Table 1 Effect of temperature on fructans variation of onion bulbs for stored 6 months (mg g⁻¹ dry matter)

<table>
<thead>
<tr>
<th>Fructans Type</th>
<th>Initial content 4 °C</th>
<th>10 °C</th>
<th>20 °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trisaccharides</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-Kestose</td>
<td>2.95</td>
<td>2.80</td>
<td>3.41</td>
</tr>
<tr>
<td>Neokestose</td>
<td>5.82</td>
<td>4.71</td>
<td>7.13</td>
</tr>
<tr>
<td>Tetrasaccharides</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nystose</td>
<td>0.74</td>
<td>0.96</td>
<td>1.64</td>
</tr>
<tr>
<td>Other tetra</td>
<td>3.36</td>
<td>6.54</td>
<td>8.29</td>
</tr>
<tr>
<td>Penta–hepta saccharides</td>
<td>12.94</td>
<td>10.56</td>
<td>34.67</td>
</tr>
<tr>
<td>Higher saccharides (DP*&gt;8)</td>
<td>6.71</td>
<td>6.33</td>
<td>14.91</td>
</tr>
<tr>
<td>Total fructans</td>
<td>32.52</td>
<td>25.59</td>
<td>61.47</td>
</tr>
</tbody>
</table>

* Degree of polymerization.
increased at 10 and 20 °C. The increased concentrations of these fructans were probably caused by enzymatic hydrolysis of fructan polymers by depolymerases.

Polymerized fructan (DP > 2) variation has not been studied extensively. However, Böttcher (1992) reported a decrease in total fructans during storage at 4 °C, similar to results reported by Suzuki & Cutcliffe (1989) in onions stored at 6–10 °C. On the other hand, Darbyshire & Henry (1978) claimed that low temperatures induced hydrolytic activity of fructan depolymerases, leading to an accumulation of fructose.

**Variation of respiration rate**

Ionization increased respiration rate, measured 24 h after the treatment, however, the initial RR was restored after less than 2 weeks (Fig. 3). RR of onion bulbs increase progressively during storage unless treated with ionizing radiation, but the increase depends on temperature and chemical treatments. The RR of control bulbs increased at 10 and 20 °C from 0.12 and 0.19 mmol kg\(^{-1}\) h\(^{-1}\) to 0.17 and 0.3 mmol kg\(^{-1}\) h\(^{-1}\), respectively, but remained stable at 4 °C after 8 weeks of storage (data not shown). After 24 weeks, the RR at 20 °C doubled (from 0.19 to 0.43 mmol kg\(^{-1}\) h\(^{-1}\)) (Fig. 3) and increased from 0.12 to 0.26 mmol kg\(^{-1}\) h\(^{-1}\) at 10 °C (data not shown). As would be expected the RR of control bulbs at 4 °C was only half (0.1 mmol kg\(^{-1}\) h\(^{-1}\)) of the control RR of dormant bulbs measured at 20 °C. The RR of MH- and CIP-treated bulbs varied in a similar pattern. An increase of 50% was observed after 24 weeks of storage for both treated bulbs at 10 and 20 °C but without any significant difference between MH and CIP samples. RR of MH- and CIP-treated bulbs were 0.2 and 0.21 mmol kg\(^{-1}\) h\(^{-1}\) at 10 °C and 0.32 and 0.3 mmol kg\(^{-1}\) h\(^{-1}\) at 20°C, respectively. However, at 4 °C the difference among control and treated bulbs did not exceed 25% and remained stable. RR of control and treated bulbs were 0.1 and 0.07 mmol kg\(^{-1}\) h\(^{-1}\), respectively. The RR of ionized bulbs, after the ionizing stress observed in the beginning, slightly increased after 10 weeks at 10 °C but decreased at 4 and 20 °C. Thereafter, a decrease was noted at 4, 10 and 20 °C and RR were 0.01, 0.06 and 0.05 mmol kg\(^{-1}\) h\(^{-1}\), respectively, after 24 weeks. This decrease can be attributed to degeneration of meristematic cells and death of the sprout caused by the radiation which slows down the complete respiratory pathway including glycolysis.

The average rates of increase in respiration of control, MH and CIP treated bulbs during 24 weeks were determined by fitting linear regression lines to the respiration rates vs. time data in Fig. 3. The determination of coefficient ($R^2$) of the regression were 0.97, 0.97 and 0.92, respectively. The increase in respiration rate was 10, 5.9 and 5.3 μmol kg\(^{-1}\) h\(^{-1}\) per week, respectively, at

![Figure 3](image3.png) **Figure 3** Respiration rates (oxygen consumption) of onion bulbs stored at 20 °C (LSD at $P < 0.01$).

![Figure 4](image4.png) **Figure 4** Sprouting of onion bulbs stored at 20 °C (a), and 10 °C (b) (LSD at $P < 0.01$).
20 °C. On the other hand, the average rate of decrease in respiration rate of ionized bulbs was 5.3 μmol kg⁻¹ h⁻¹ per week and coefficient $R^2 = 0.92$.

Previous workers reported that the inhibition of catabolism by MH reportedly induced a reduction in the RR but did not prevent its rise (Masters et al., 1984; Salama & Hicks, 1987). However, the difference between control and MH treated bulbs was not significant after only 12 weeks of storage at 10 and 15 °C. Ionizing radiation induced a respiratory stress causing an increase in RR which returned to steady-state values after 1 week (Ahmed et al., 1972; Benkeblia et al., 2000). Larrigaudière et al. (1987) and Strydiom & Witehead (1990) further reported a decrease in the RR of several ionized fruits which occasionally led to an abrupt acceleration of the senescence of the tissues.

**Sprouting**

During storage at 10 and 20 °C, sprouting of bulbs was visible after 6 and 8 weeks (Fig. 4a and b), whereas at 4 °C, sprouting was visible only after 16 weeks. However, chemical treatment (MH and CIP) and irradiation were as effective as chilling on the inhibition of sprouting. After 24 weeks, sprouting of the control bulbs was 50 and 75% at 10 and 20 °C, respectively (Fig. 4a and b). At 10 °C sprouting level of treated bulbs (ionization or chemicals) was more apparent than at 4 and 20 °C, ranging from 10 to 20%. This indicates that intermediary temperatures seemed more favourable to the sprouting of bulbs in spite of the treatment applied, e.g. sprouting of 0.15 kGy ionized bulbs after 24 weeks of storage reached 17% at 10 °C, although at 4 and 20 °C the level of sprouting was below 5%.

After 24 weeks of storage at 4 °C, sprouting level of control and treated bulbs was very low, ranging from 3% (ionized bulbs) and 7% (control) without significant difference.

Effects of ionizing radiation, chilling and MH have been reported by several authors (Isenberg & Ferguson, 1981; Matsuyama & Umeda, 1983; Sinha et al., 1994). However, other factors can affect or favour the sprouting of the bulbs in store, e.g. environmental conditions during bulbing (Tucker & Morris, 1984), maturity and harvesting mode of the bulbs (Smittle & Maw, 1988) and postharvest handling (Ryall & Lipton, 1972).

**Conclusion**

Temperature, sprout control treatment and storage duration showed marked effects on the RR, particularly during the last 3 months of storage when various physiological activities are believed to occur. However, the catabolism of carbohydrates appears to be more dependent on the physiological stage (dormancy and sprouting) than on temperature, which seems to have an activating effect on the variation of these compounds. Further investigation into the variation in fructans in different parts of the bulb tissue during storage under variable temperatures is necessary to determine the degree of variation in relation to the enzymes involved in the hydrolytic process of fructans (fructan depolymerases) and saccharides (invertase).

**Acknowledgments**

The authors thank Mr Siadoux, R (CEA Cadarache) for his help with irradiation treatment.

**References**


