Effect of post-harvest treatments on the level of glucosinolates in broccoli

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Abstract: Broccoli is a very perishable vegetable with a high water content (around 88%) which leads to rapid dehydration and probably to an alteration in composition if conditions after harvest are not controlled. This study evaluates the glucosinolate pattern and glucosinolate levels in the principal and secondary inflorescences of fresh broccoli cv ‘Tokyodome’, and after being submitted to some situations which are likely to occur during or after harvest: room temperature (±20°C) for 5 days, kept in the fridge at 4°C for 5 days, and frozen after blanching. Another set of material was harvested 5 days later, simulating a post-maturation stage, and analysed. The highest total glucosinolate content was found at commercial maturation with 20888 and 20355 μmoles kg⁻¹ DW in the principal and secondary inflorescences, respectively. Keeping the inflorescences at room temperature caused the most significant (P<0.05) reductions in total and individual glucosinolates, except for 4-hydroxyindol-3-ylmethyl-, 2-hydroxy-2-phenylethyl- and 2-phenylethyl-, when compared to the other situations. The highest levels (10925 μmoles kg⁻¹ DW) of 4-methylsulphinylbutyl-, the precursor of the anti-cancer isothiocyanate sulphoraphane, were found in the inflorescences freshly harvested at commercial stage. Refrigeration at 4°C and freezing were shown to be the best preservation processes for maintaining high levels of these and other glucosinolates in contrast with the other situations.

INTRODUCTION

Broccoli, a member of the Brassicaceae, has been associated with a decreased risk of cancer based on several beneficial properties such as the levels of vitamin C, fibre and glucosinolates, a group of secondary plant metabolites.1-4 These compounds, upon hydrolysis by the enzyme myrosinase (thioglucose glucohydrolase EC 3.2.3.1), yield a variety of bioactive products, including isothiocyanates, thiocyanates, nitriles and oxazolidine-2-thiones depending on the chemical structure and the conditions during enzymic cleavage. Glucosinolates and their breakdown products are important aroma and flavour compounds in Brassica vegetables.

Broccoli is a crop which has found increasing popularity. In the USA, production and consumption has increased dramatically5-7 since it was demonstrated that the crop contained a glucosinolate precursor of the isothiocyanate sulphoraphane (1-isothiocyanate-(4R)-(methylsulphinyl)butane), a compound with the ability to induce enzymes protective against cancer.8 Other glucosinolates, particularly those with indolyl groups, have also been associated with induction of protective mechanisms.9 Broccoli is often submitted to long transportation periods during which compositional alteration is likely to occur, such as reduction of beneficial glucosinolates. Senescence has also been referred as having a similar effect due to transportation of glucosinolates out of plant tissue or to degradation by myrosinases and release as volatiles.10 Thus, conditions at harvest and post-harvest must be under control to avoid this degradation process, since it is known that the hydrolytic activity of myrosinase differs within plant tissue and is higher in young tissues of the plant.11 Refrigerated storage and controlled atmosphere have been shown to increase levels of thiocyanate ion, volatile isothiocyanates and goitrin12,13 in cabbage, declining after senescence of the inflorescence. An increase in pungency, mustiness and bitterness was shown in white cabbage stored in controlled atmosphere, however, this was not related to glucosinolate levels.14,15 An increase in total glucosinolate content was reported in broccoli when stored under air or under controlled atmosphere for 7 days, until the beginning of deterioration, while the absence of O₂ with a 20% CO₂ concentration resulted in total glucosinolate decrease.16

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In this study we compared several situations to which broccoli might be submitted, either during or after harvest, and their effect on glucosinolate levels.

MATERIALS AND METHODS

Plant

Seeds of broccoli (Brassica oleracea var italica) cv ‘Tokyodome’ were sown at a 2.5 cm depth in polystyrene trays with alveoles of 65 cm³ filled with a peat compost (Humobact Terreau) and river sand in a proportion v/v of 3:1. At 28 days after seeding, transplants were set in the experimental field of the University of Trás-os-Montes e Alto Douro, at an altitude of 453 m, 41°17’N and 7°44’W in a randomized complete block with three replicates. At commercial maturation stage, which occurred 57 days after planting, the main inflorescences of the plants of each replicate were harvested and combined to make a composite sample. Each lot was divided in four as follows: one was used immediately for analysis; another was left at room temperature (20°C) for 5 days, another was blanched for 1 min and immediately frozen and kept at -20°C, and in the fourth treatment, the inflorescences were kept in the refrigerator at 4°C for 5 days. A fifth situation was leaving inflorescences on the plant and harvesting 5 days after the first harvest, corresponding to a post-maturation stage. The inflorescences from two plants were used per replicate and situation for glucosinolate analysis and the inflorescences from a third plant were dried in a forced-air oven (Memmert UL-80) at 65°C until constant weight was achieved, to determine the dry weight. When the secondary inflorescences reached commercial maturation, they were harvested and submitted to the same situations described above for the main inflorescences.

Analysis

The extraction and analysis of the plant material was according to the current methods used in our laboratory17 of which the main steps are as follows. Inflorescences were freeze-dried and reduced to a fine powder, and 2 g of material were extracted in 90% boiling methanol for 2 min using a small centrifuge tube to which was added 0.2 ml of benzyl glucosinolate (glucotropaeolin) (1 mg ml⁻¹) as an internal standard. After centrifugation the supernatant was transferred to a 10 ml flask. The residue was extracted twice in 70% boiling methanol for 1 min, centrifuged and supernatant added to the same flask. The final volume was made to 10 ml with water. A 2.5 ml aliquot was evaporated to dryness and resuspended in a similar volume of pure water. A 2 ml aliquot was added to a small Sephadex A25 column and desulphoglucosinolates were obtained after treatment of the column with sulphatase.18 A final volume of 1.5 ml was recovered for HPLC analysis according to the method described by Spinks et al.19 An analysis of variance was performed to compare differences between treatments using a SuperAnova program. Levels of glucosinolates are expressed in µmoles kg⁻¹ DW.

RESULTS AND DISCUSSION

The glucosinolate pattern found in a fresh harvested broccoli inflorescence, which is shown in Table 1, is in agreement with other reports,16,20 however, there are differences in the relative content of glucosinolates probably due to the cultivar and growing conditions. Generally the five treatments have a significant effect on total and individual glucosinolates (Table 1). Similarly most of the individual glucosinolates and totals vary between inflorescence types (Table 1).

The major glucosinolates in the principal inflorescences of fresh harvested broccoli were 4-methylsulphinylbutyl- and indol-3-ylmethyl-, representing 52 and 27% of the total glucosinolate content, respectively. In the secondary inflorescences, the same glucosinolates accounted for 75% of the total content with 36 and 39%, respectively. Large proportions of these glucosinolates were also reported by Hansen et al.16 and Lewis et al.20 Other glucosinolates such as 3-methylsulphinylpropyl-, 2-hydroxybut-3-enyl-, but-3-enyl-, 4-hydroxyindol-3-ylmethyl- and 2-phenylethyl- were observed in negligible amounts (less than 100 µmoles kg⁻¹ DW).

<table>
<thead>
<tr>
<th>Source</th>
<th>1</th>
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<th>3</th>
<th>4</th>
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<td>ns</td>
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</tr>
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<td>Treatment x Inflorescence</td>
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<td>ns</td>
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<td>ns</td>
<td>***</td>
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<td>***</td>
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</tr>
</tbody>
</table>

Glucosinolates are expressed in µmoles kg⁻¹ DW.

Glucosinolates in post-harvested broccoli

Table 1. Significance for the total and individual glucosinolates of the five treatments in the main and secondary inflorescences

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Table 2. Glucosinolate levels (μmoles kg⁻¹ DW) in the principal (P) and secondary (S) inflorescences of broccoli 'Tokyodome' submitted to different treatments during 5 days

<table>
<thead>
<tr>
<th>Glucosinolate</th>
<th>Inflorescence</th>
<th>Fresh material</th>
<th>Post-maturation</th>
<th>Room temperature (20°C)</th>
<th>Fridge (4°C)</th>
<th>Blanching and frozen (−20°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P</td>
<td>0±0aA</td>
<td>0±0aA</td>
<td>0±0aA</td>
<td>0±0aA</td>
<td>22±5aA</td>
</tr>
<tr>
<td></td>
<td>S</td>
<td>0±0aA</td>
<td>0±0aA</td>
<td>24±24aA</td>
<td>12±12aA</td>
<td>0±0aA</td>
</tr>
<tr>
<td>2-hydroxybut-3-enyl-</td>
<td>P</td>
<td>95±14bA</td>
<td>9.6±2.3bA</td>
<td>55±9bA</td>
<td>60±7bA</td>
<td>169±57a</td>
</tr>
<tr>
<td></td>
<td>S</td>
<td>33±17bA</td>
<td>0±0bA</td>
<td>60±7aA</td>
<td>20±10bA</td>
<td>109±14a</td>
</tr>
<tr>
<td>4-methylsulphinylbutyl-</td>
<td>P</td>
<td>10925±830aA</td>
<td>8307±1781bA</td>
<td>1948±336cA</td>
<td>7539±223bA</td>
<td>10101±130aA</td>
</tr>
<tr>
<td></td>
<td>S</td>
<td>7299±552aB</td>
<td>4922±1163abB</td>
<td>786±174cA</td>
<td>6566±202aB</td>
<td>3781±465bB</td>
</tr>
<tr>
<td>5-methylsulphinylpentyl-</td>
<td>P</td>
<td>230±7aA</td>
<td>146±25aA</td>
<td>191±109aA</td>
<td>150±10aA</td>
<td>249±20aA</td>
</tr>
<tr>
<td></td>
<td>S</td>
<td>223±6aA</td>
<td>109±22bA</td>
<td>76±19bB</td>
<td>137±7aA</td>
<td>162±16a</td>
</tr>
<tr>
<td>But-3-enyl-</td>
<td>P</td>
<td>50±8aA</td>
<td>36±12bA</td>
<td>66±3aA</td>
<td>41±2bA</td>
<td>32±8bA</td>
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<tr>
<td></td>
<td>S</td>
<td>13±13aB</td>
<td>0±0bA</td>
<td>0±0bA</td>
<td>0±0bA</td>
<td></td>
</tr>
<tr>
<td>4-hydroxyindol-3-ylmethyl-</td>
<td>P</td>
<td>57±3aA</td>
<td>44±11aA</td>
<td>124±12aA</td>
<td>38±1aA</td>
<td>61±17a</td>
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<tr>
<td></td>
<td>S</td>
<td>107±11cA</td>
<td>176±46cB</td>
<td>492±37aB</td>
<td>351±89bB</td>
<td>60±31dA</td>
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<tr>
<td>2-hydroxy-2-phenylethyl-</td>
<td>P</td>
<td>111±15bA</td>
<td>46±23cA</td>
<td>201±9aA</td>
<td>0±0aA</td>
<td>27±27cA</td>
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<td></td>
<td>S</td>
<td>18±18bB</td>
<td>0±0aA</td>
<td>127±16aB</td>
<td>18±18bA</td>
<td>0±0bA</td>
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<tr>
<td>Indol-3-ylmethyl-</td>
<td>P</td>
<td>5573±438aA</td>
<td>5134±765aA</td>
<td>457±137bA</td>
<td>5695±432aA</td>
<td>5264±167a</td>
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<td>S</td>
<td>7967±987aB</td>
<td>3388±547cB</td>
<td>893±270aA</td>
<td>6189±358bA</td>
<td>5151±635b</td>
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<td>2-phenylethyl-</td>
<td>P</td>
<td>78±8bA</td>
<td>71±4bA</td>
<td>393±96aA</td>
<td>69±3bA</td>
<td>60±2bA</td>
</tr>
<tr>
<td></td>
<td>S</td>
<td>46±23bA</td>
<td>0±0bA</td>
<td>719±163bA</td>
<td>22±22bA</td>
<td>0±0bA</td>
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<tr>
<td>4-methoxyindol-3-ylmethyl-</td>
<td>P</td>
<td>1396±78aA</td>
<td>1479±184aA</td>
<td>782±107bA</td>
<td>1403±126aA</td>
<td>1244±92a</td>
</tr>
<tr>
<td></td>
<td>S</td>
<td>1756±159cA</td>
<td>1414±349cA</td>
<td>3237±422aB</td>
<td>2582±55bB</td>
<td>1331±135c</td>
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<tr>
<td>1-methoxyindol-3-ylmethyl-</td>
<td>P</td>
<td>2473±338aA</td>
<td>3015±308aA</td>
<td>129±33bA</td>
<td>2612±413aA</td>
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<td>2893±707aA</td>
<td>1437±272bB</td>
<td>1016±316aB</td>
<td>3662±378aB</td>
<td>2652±522a</td>
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<td>Total</td>
<td>P</td>
<td>29588±971aA</td>
<td>18372±823aA</td>
<td>4347±457bA</td>
<td>17606±573aA</td>
<td>20255±1250a</td>
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<tr>
<td></td>
<td>S</td>
<td>20355±1390aA</td>
<td>11445±2243bB</td>
<td>7428±1327cA</td>
<td>19579±498aA</td>
<td>13446±1383b</td>
</tr>
</tbody>
</table>

Lower case letters represent comparisons between the 5 treatments (compare within row); capitals represent comparisons between the 2 inflorescences (compare pair of values within each column)

Means with the same letters did not show significant differences (P<0.05)

type of inflorescences (Table 2). When inflorescences were kept refrigerated at 4°C for 5 days the decrease in total glucosinolates was 16 and 4%, respectively, for the principal and secondary inflorescences, when compared to the fresh harvested material at the commercial maturation stage. Freezing did significantly reduce the levels of the total glucosinolates in the principal inflorescences whilst, in the secondary inflorescences, the decrease was significant (P<0.05) (Table 2).

The highest decrease in total glucosinolate content was observed when the fresh material was left at room temperature (20°C) for 5 days (Table 2). Thus it is likely that myrosinase was active during that time reducing glucosinolate levels with production of volatile and non-volatile degradation products. Other studies revealed the increased production of thiocyanate ion, volatile isothiocyanates and goitrin in cabbage even when stored under refrigerated storage and controlled atmosphere.12,13 Thus, in our study, conditions for endogenous hydrolysis might have been more favourable either due to the onset of senescence of the inflorescence or to more mild temperatures after harvest.

Although there was a reference16 describing an increase in total glucosinolate content explained by enhanced synthesis or a release of bound compounds during storage, in our study it seemed that the plant material is on the verge of senescence, characterised by dehydration or by yellosing of the florets.

Individual glucosinolates

There were significant differences (P<0.001) in the levels of 4-methylsulphinylbutyl glucosinolate between treatments in the principal and in the second inflorescences. The levels in the principal inflorescences were generally higher (P<0.05) than in the secondary inflorescences, the smaller differences being noted when the plant material was kept refrigerated, while the largest was noted for the frozen material (Table 2). No significant differences were noted in 4-methylsulphinylbutyl glucosinolate content between the fresh harvested material and frozen material for the principal inflorescence. Keeping the principal inflo-
escences at 4°C reduced 4-methylsulphinylbutyl-
 content by 31%, while at room temperature the
 reduction was 82%. In the secondary inflorescences
 the largest reduction was also at room temperature
 (89%), followed by the frozen material (48%).
 Levels of indol-3-ylmethyl glucosinolate tended to
 be higher in the secondary inflorescences except when
 inflorescences were harvested at post-maturation stage
 and when frozen (Table 2). Apart from the extremely
 low levels of indol-3-ylmethyl glucosinolate in the
 inflorescences at room temperature, no significant
 differences (P<0.05) were noted in the levels for the
 main inflorescence for the other treatments. On the
 other hand, and in the secondary inflorescences,
treatments differ significantly (P<0.001) with large
differences in the post-matured material (57%) and
 room temperature (89%); the decrease in indol-3-
 ylmethyl glucosinolate in the inflorescences kept in the
 fridge was lower (22%) than when frozen (35%). This
difference could be due to blanching prior to freezing
 which might leach out this thermolabile glucosino-
late.21 The loose structure of the broccoli stalk and
 flower head, which appears to be very susceptible to
 the leaching effects,22 and the water blanching used in
 our study might have contributed to higher losses.

 Frozen broccoli is expected to have a lower effect on
 the metabolism of foreign compounds due to a
 reduction in the activity of the mixed-function oxidase
 (MFO) enzyme system by blanching, a process which
 inactivates myrosinase23 and consequently reduces
 the release of the beneficial hydrolysis products of the
 indole glucosinolates.24 A similar effect was reported
 for cooked cabbage.24 The presence of active myr-
 osinase has been claimed to be essential for the
 capacity of glucosinolates from broccoli to induce the
 activity of several cytochrome P-450 isoenzymes.25
 Thus, despite inactivation of myrosinase by blanching
 the release of those beneficial hydrolysis products
 might be guaranted by myrosinase activity found in
 certain intestinal microflora which could be important
 when intact glucosinolates are digested.26

 The other indole glucosinolate, 1-methoxyindol-3-
 ylmethyl-, followed the same trend as the indol-3-
 ylmethyl glucosinolate between the two inflorescence
types, despite relative differences in levels (Table 2).
In the principal inflorescences, there were no significant
 differences between treatments except when exposed
to room temperature (95% reduction). Levels in the
 secondary inflorescences kept at 4°C were higher (but
 not significantly) than the fresh material and levels
 were unchanged by freezing. There was a 65% loss of
 1-methoxyindol-3-ylmethyl- on storage at room tem-
 perature and post-mature material also had signifi-
cantly lower levels.
 The 4-methoxyindol-3-ylmethyl glucosinolate also
 followed the trend of the previous indoles with respect
to relative differences between principal and second
 inflorescences. In the principal inflorescences, the
 lowest levels were noted when material was exposed to
 room temperature, which differs significantly
 (P<0.05) from the other treatments which did not
 show significant differences (Table 2). On the other
 hand, and in the second inflorescences, levels of 4-
methoxyindol-3-ylmethyl glucosinolate were the high-
est when kept at room temperature followed by the
 situation when stored the refrigerator.

 CONCLUSIONS
 The main source of glucosinolates in broccoli is the
 principal inflorescence followed by the secondary
 inflorescences, except for the indole group. The
 harvest of broccoli must be done at the full commercial
 stage to reach the highest levels of glucosinolates, and,
 from that developmental stage onwards, the inflores-
cence is under critical situations with respect to
 quality. These results indicate that the levels of
 glucosinolates and the beneficial properties of broccoli
 might be lost if not stored under suitable conditions.
 Freezing is the best method for preserving the
 glucosinolate content although, to avoid degradation
 of indole glucosinolates during blanching, refrigera-
tion at 4°C is the best procedure.

 ACKNOWLEDGEMENTS
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 of this work.

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 crop development in single- and double-low genotypes of
 winter oilseed rape (Brassica napus): II. Profiles and tissue-
 water concentrations in vegetative tissues and developing pods.