Glucosinolates in Broccoli Stored under Controlled Atmosphere

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Abstract. Content of total and individual glucosinolates were determined in, ‘Marathon’ broccoli florets (Brassica oleracea L. var. italica) stored 7 days at 10°C under air, 0.5% O2, 0.5% O2 + 20% CO2 or 20% CO2 atmosphere, followed by transfer to air for 2 days. ‘Marathon’ broccoli contained glucoraphanin, glucobrassicin, neoglucobrassicin, glucoseolin, 4-methoxyglucobrassicin, progoitrin, glucosylsulphide, and glucoamylase. The methylsulfonilylalkylglucosinolates (glucobrassicin and glucoraphanin) and the indol-3-ylmethylglucosinolates (glucobrassicin, neoglucobrassicin and 4-methoxyglucobrassicin) accounted for 78% and 20% of the total content, respectively, in freshly harvested broccoli. CA treatment and storage time had no significant effect on the relative content of these two groups of glucosinolates. Freshly harvested broccoli contained 47 μmol glucosinolates/g dry weight. The total glucosinolate content increased 42% and 21% during 7 days storage under air and 0.5% O2 + 20% CO2, respectively, as compared to freshly harvested broccoli, and decreased 15% in broccoli stored under 20% CO2. Treatment with 20% CO2 in the absence of O2, resulted in visible CO2 injury and water soaking of the tissue. Aeration had no significant effect on total glucosinolate content but reduced the glucobrassicin content 35% in broccoli stored 7 days under 0.5% O2 + 20% CO2 or 20% CO2 atmosphere. In contrast, the 4-methoxyglucobrassicin content increased during storage under low O2 atmosphere and increased further after transfer to air.

Plants belonging to the order Capparales including Brassicaceae, are characterized by their content of glucosinolates (Bjerg and Sørensen, 1987a). Glucosinolates and their breakdown products are important aroma and flavor compounds in Brassica vegetables (MacLeod, 1976), such as cabbage, Brussels sprouts, broccoli, cauliflower, and horseradish. The most notable example is allyl isothiocyanate in mustard and horseradish arising from enzymic breakdown of sinigrin. This compound causes a pungent and lacrymatory response upon cutting and chewing (Gilbert and Fenwick, 1983; Sørensen, 1990). The structural variability of glucosinolates is mainly in the R-group (Fenwick and Heaney, 1983; Sørensen, 1990). This is also the case for glucosinolates identified as constituents of Brassica vegetables (Table 1).

Total and individual glucosinolate contents vary among cultivars and plant parts (Lewis and Fenwick, 198; Olsen and Sørensen, 1981; Sang et al., 1984; Rahman et al., 1986; VanEitzen et al., 1976), but the concentration is also affected by nutrient level and cultivation practice (Heaney et al., 1983; Josefsson, 1970). During the plants growth and development, glucosinolates are synthesized from amino acids in a series of steps (Ettlinger and Kjær, 1968; Kjær, 1960; Kjær and Sørensen, 1982) found that the concentration of the thiocyanate ion, volatile isothiocyanates, and goitrin declined during storage and this was associated with decreasing quality of the cabbage. Similar results were observed in cabbage stored under controlled atmosphere (CA), except that the cabbage had more volatile isothiocyanates and goitrin during the early storage period and the content declined at a higher rate towards the end of storage (Bérard and Chong, 1983). Others (Hansen, 1979; Toivonen et al., 1982) found that white cabbage stored under CA increased in pungency, mustiness, and bitterness, but they did not study changes in glucosinolate content.

Broccoli is a commodity that benefits from storage under increased CO2 and reduced O2 concentrations (Lipton and Harris, 1974; Makhlouf et al., 1989). Short term storage of broccoli under CA or in film wraps was found to extend shelf life and maintain quality by delaying yellowing and reducing loss of chlorophyll and ascorbic acid (Forney and Rij, 1991; Wang, 1979). It is not known to what extent increased CO2 and reduced O2 concentrations may affect glucosinolate content and thus flavor and nutritional quality of broccoli during storage. The objective of the present study was to determine the total and individual glucosinolates in broccoli stored under low O2 and high CO2 to understand better glucosinolate metabolism in Brassica vegetables after harvest.

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Table 1. Numbers, structures and names of glucosinolates reported as constituents of *Brassica* vegetables.

| No | Structure of R-groups | Semisystematic names of R-groups<sup>x</sup> | Trivial names | Brassica spp. |
|----|----------------------|------------------------------------------|--------------|--------------|
| 1  | O<sub>2</sub> = O<sub>1</sub> - O<sub>1</sub> - | Allyl | Sinigrin | Cabbage, Brussels sprouts, cauliflower, broccoli |
| 2  | O<sub>2</sub> = O<sub>1</sub> - O<sub>1</sub> - O<sub>1</sub> - | But-3-enyl | Glucopropin | Cabbage, Brussels sprouts, cauliflower, broccoli, Chinese cabbage |
| 3  | O<sub>2</sub> = O<sub>1</sub> - O<sub>1</sub> - O<sub>1</sub> - | Pent-4-enyl | Glucobrassicanipinn | Cauliflower, broccoli, Chinese cabbage |
| 4  | O<sub>2</sub> = O<sub>1</sub> - O<sub>1</sub> - O<sub>1</sub> - | (2R)-2-Hydroxybut-3-enyl | Progoitrin | Cabbage, Brussels sprouts, cauliflower, broccoli, Chinese cabbage |
| 7  | O<sub>2</sub> = C<sub>6</sub>-O<sub>1</sub>-O<sub>1</sub>-C<sub>6</sub>-O<sub>1</sub>-C<sub>6</sub>-O<sub>1</sub>-C<sub>6</sub>- | 3-Methylpropyl | Glucosinovin | Cabbage, cauliflower |
| 8  | O<sub>2</sub> = C<sub>6</sub>-O<sub>1</sub>-O<sub>1</sub>-O<sub>1</sub>-C<sub>6</sub>-O<sub>1</sub>-C<sub>6</sub>- | 4-Methylthiobutyl | Glucoruxin | Cabbage, Brussels sprouts, cauliflower, broccoli |
| 10 | O<sub>2</sub> = C<sub>6</sub>-O<sub>1</sub>-O<sub>1</sub>-O<sub>1</sub>-C<sub>6</sub>-O<sub>1</sub>-C<sub>6</sub>- | 3-Methylsulphonylpropyl | Glucobrassin | Cabbage, Brussels sprouts, cauliflower, broccoli |
| 11 | O<sub>2</sub> = C<sub>6</sub>-O<sub>1</sub>-O<sub>1</sub>-O<sub>1</sub>-C<sub>6</sub>-O<sub>1</sub>-C<sub>6</sub>- | 4-Methylsulphobutyl | Glucoraphin | Cabbage, Brussels sprouts, cauliflower, broccoli, Chinese cabbage |
| 12 | O<sub>2</sub> = C<sub>6</sub>-O<sub>1</sub>-O<sub>1</sub>-O<sub>1</sub>-C<sub>6</sub>-O<sub>1</sub>-C<sub>6</sub>- | 5-Methylsulphinylpentyl | Glucalyssin | Chinese cabbage |
| 15 | O<sub>2</sub> = C<sub>6</sub>-O<sub>1</sub>-O<sub>1</sub>-O<sub>1</sub>-C<sub>6</sub>-O<sub>1</sub>-C<sub>6</sub>- | 4-Methylsulphinylbutyl | Glucorysidin | Cabbage |
| 16 | O<sub>2</sub> = C<sub>6</sub>-O<sub>1</sub>-O<sub>1</sub>-C<sub>6</sub>-O<sub>1</sub>-C<sub>6</sub>- | Benzy1 | Glucotropaeolin | Cabbage |
| 17 | O<sub>2</sub> = C<sub>6</sub>-O<sub>1</sub>-O<sub>1</sub>-C<sub>6</sub>-O<sub>1</sub>-C<sub>6</sub>- | Pheny1 | Glucosustarmin | Cabbage, Brussels sprouts, broccoli, Chinese cabbage |
| 21 | R<sub>1</sub> = H, R<sub>2</sub> = H | Indol-3-ylmethyl | Glucobrasscin | Cabbage, Brussels sprouts, cauliflower, broccoli, Chinese cabbage |
| 24 | R<sub>1</sub> = OCH<sub>3</sub>, R<sub>2</sub> = H | N-Methoxyindol-3-ylmethyl | Neoglucobrassin | Cabbage, Brussels sprouts, cauliflower, broccoli, Chinese cabbage |
| 26 | R<sub>1</sub> = H, R<sub>2</sub> = OH | 4-Hydroxyindol-3-ylmethyl | 4-Hydroxyglucobrassin | Cabbage, Brussels sprouts, cauliflower, broccoli, Chinese cabbage |
| 27 | R<sub>1</sub> = H, R<sub>2</sub> = OCH<sub>3</sub> | 4-Methoxyindol-3-ylmethyl | 4-Methoxyglucobrassin | Cabbage, Brussels sprouts, cauliflower, broccoli, Chinese cabbage |

Compiled from VanEtten et al. (1976), Heaney and Fenwick (1980), Lewis and Fenwick (1987,1988), Sones et al. (1984), Goodrich et al. (1989), and Lewis et al. (1991).

The semisystematic names of glucosinolates are composed of the name of the R-group followed by the word glucosinolate, e.g., allylglucosinolate for number-1 (Sorensen, 1990 and references cited therin).

### Materials and Methods

**Plant material.** ‘Marathon’ Broccoli heads (*Brassica oleracea* L. var. *italica*) were obtained on the day of harvest from Mann Packing Co., Salinas, Calif., top-iced and transported to the Mann Laboratory, Davis, Calif. where they were stored overnight at 0°C. Miniflorets, 25 mm long and 20 to 70 mm in diameter, were excised from uniform heads of prime quality, surface sterilized in distilled water containing 100 ppm NaOCl for 5 min, drained, and divided on the basis of floret diameter into small (<40 mm), medium (40 to 50 mm) and large (>50 mm) sizes.

**Storage under CA.** Samples of broccoli (650 g) in a 1:2:1 (by weight) ratio of each floret size were placed in a 3.8-liter glass jar as one replicate, closed with a neoprene rubber stopper fitted with inlet and outlet polyethylene tubes. The jars were placed in a room at 10°C for 7 days and ventilated with humidified gas at 10.1 ± 0.3 liters·h<sup>–1</sup>. The atmospheres were as follows: air, 0.5% O<sub>2</sub>, 0.5% O<sub>2</sub> + 20% CO<sub>2</sub>, or 20% CO<sub>2</sub> (all balanced with N<sub>2</sub>). After 7 days, the jars were transferred to air for 2 days at 10°C. Oxygen and CO<sub>2</sub> concentrations were verified daily by analyzing 0.5 to 3 ml gas samples by electrochemical (model S-3All; Applied Electrochemical, Sunnyvale, Calif.) and infrared analyzers (model PIR-2000; Horiba, Irvine, Calif.). The variation in O<sub>2</sub> and CO<sub>2</sub> concentrations was within ± 5%. After 2, 7, and 9 days of storage, two samples per treatment were removed for analysis except from the treatment with 20% CO<sub>2</sub> atmosphere. In this treatment, samples were only removed at days 7 and 9.

**Freeze-drying.** Freshly harvested and stored broccoli florets (50 g) were frozen in liquid N<sub>2</sub> and kept in polyethylene bags at −40°C until freeze-drying, usually within 1 month. Freeze-dried broccoli tissue was stored in sealed polyethylene bags at 4°C until analysis.

**Extraction and isolation of glucosinolates.** Glucosinolates were extracted from freeze-dried, finely ground broccoli powder by the method of Bjerg et al. (1984). The samples (0.2 g) were spiked with a 100 µmol internal standard solution containing 5.0 µmol·ml<sup>–1</sup> of sinigrin and glucobarbarin, and extracted three times with 5 ml boiling 70% methanol for 2 min using an Ultra-Turrax Homogenizer (Ika-Labortechnik, Staufen, Germany). The extract obtained after centrifugation was concentrated to dryness in vacuo, and the residue was dissolved in 2 ml deionized water. Desulfoglucosinolates were prepared and quantitatively determined by HPLC according to Bjerg and Sorensen (1987b) and Sorensen (1990). The glucosinolate concentration was calculated using glucobarbarin as internal standard.

**Statistical analysis.** Statistical significance was assessed for total and individual glucosinolates by one-way and two-way ANOVA for unbalanced data (SAS, Cary, N.C.). The sources of variation were treatment (air, 0.5% O<sub>2</sub>, 0.5% O<sub>2</sub> + 20% CO<sub>2</sub>, or 20% CO<sub>2</sub> (all balanced with N<sub>2</sub>)) and time (0, 2, 7, and 9 days). Duncan’s multiple range test and 95% confidence interval, respectively, were used to assess the location of the significant differences obtained.
Results and Discussion

Total and individual glucosinolates. HPLC separation of individual desulfoglucosinolates from freshly harvested ‘Marathon’ broccoli is shown in Fig. 1. The broccoli contained glucoraphanin (11), glucobrassicin (23), neoglucobrassicin (24), glucoiberin (10), 4-methoxyglucobrassicin (27), progoitrin (4), glucoraphanin (12) and gluconasturtiin (17). The major glucosinolates (found in concentrations >1 µmol·g⁻¹ dry weight) were glucoraphanin, glucobrassicin, glucoiberin, neoglucobrassicin, glucoiberin and 4-methoxyglucobrassicin. Others (Goodrich et al., 1989; Lewis et
Table 2. Average concentration of major glucosinolates (µmol·g⁻¹ dry weight) in ‘Marathon’ broccoli stored 7 days under CA and transferred to air for 2 days. The relative content of the total is indicated in parentheses.

| Glucosinolates  | Air            | 0.5% O₂ | 0.5% O₂ + 20% CO₂ | 20% CO₂ |
|-----------------|----------------|---------|-------------------|---------|
| Glucoiberin     | 3.2 a (5)      | 2.5 bc (5) | 2.8 ab (5)        | 2.0 c (5) |
| Glucoraphanin   | 46.6 a (71)    | 35.8 bc (73) | 39.2 ab (72)    | 29.0 c (76) |
| Glucobrassicin  | 10.6 a (16)    | 6.2 c (13) | 7.3 b (13)        | 4.5 d (12) |
| Neoglucobrassicin| 1.9 a (3)      | 1.7 a (3) | 1.8 a (3)         | 0.9 b (2)  |
| 4-Methoxyglucobrassicin | 1.7 a (3) | 1.4 b (3) | 1.7 a (3)        | 0.4 c (1)  |
| Methylsulphinylalkylglucosinolates | 49.8 c (76) | 38.3 bc (79) | 42.0 ab (78)    | 31.0 c (82) |
| Indol-3-ylmethylglucosinolates | 14.2 a (22) | 9.3 b (19) | 10.7 b (20)      | 5.8 c (15) |
| Total glucosinolates | 65.5 a (100) | 49.0 b (100) | 54.2 b (100)    | 37.9 c (100) |

* Chemical structures are shown in Table 1.
** Numbers within a row followed by different letters are significantly different at P = 0.05 by Duncan’s multiple range test.
† Interaction between CA treatments and storage time.
‡ Methylsulphinylalkylglucosinolates: glucoiberin and glucoraphanin.
§ Indol-3-ylmethylglucosinolates: glucobrassicin, neoglucobrassicin and 4-methoxyglucobrassicin.

al., 1991) reported a similar glucosinolate profile in broccoli. Of the major glucosinolates, the methylsulphinylalkylglucosinolates (glucoiberin and glucoraphanin) and the indol-3-ylmethylglucosinolates (glucoiberin, neoglucobrassicin and 4-methoxyglucobrassicin) accounted for 78% and 20% of the total content, respectively, in freshly harvested broccoli.

Storage under air. Total glucosinolate content increased from 47.1 µmol·g⁻¹ dry weight at day 0 to 67.0 µmol·g⁻¹ dry weight at day 7 followed by a slight decline at day 9 as the broccoli deteriorated (Fig. 2). Incipient yellowing of florets was observed on day 7 and at day 9 the flower buds were moderate yellow (Hansen, 1993). Although the total glucosinolate content differed during air storage, none of the contents were significantly different from the others, probably due to too few observations. Chong and Bérard (1983) demonstrated that the concentration of glucosinolate products from cabbage increased during cold storage until the cabbage began to senescence, then the content rapidly declined. Analysis of variance for the content of individual glucosinolates indicated significant differences (P = 0.05) for 4-methoxyglucobrassicin. The content increased from 0.4 µmol·g⁻¹ dry weight at day 0 to 1.8 µmol·g⁻¹ dry weight at day 9 in air stored broccoli.

Storage under CA. The green color of the florets was maintained under the three CA conditions used. The 20% CO₂ atmosphere resulted in severe off-odors. The total glucosinolate content increased 42% and 21% during 7 days storage under air and 0.5% O₂ + 20% CO₂, respectively, as compared to freshly harvested broccoli (Fig. 2). This increase could have been associated with enhanced synthesis or a release of bound compounds during storage. Total glucosinolate ‘content did not change for broccoli stored under 0.5% O₂, but decreased 15% in broccoli stored under 20% CO₂ in the absence of O₂. Exudation of cell sap, a symptom of physiological injury of the tissue, was visible in these latter samples. This symptom probably reflected membrane damage and cell rupture, conditions favorable for hydrolytic breakdown of glucosinolates by myrosinase catalyzed hydrolysis or autolysis (Olsen and Sorensen, 1981; Sorensen, 1990). In the intact cell, myrosinases are well separated from glucosinolates (Läthö and Matile, 1984). When glucosinolates and myrosinases are brought in contact, a number of volatile and nonvolatile degradation products are formed depending on the structures of the glucosinolates and myrosinases and the actual conditions for hydrolysis (Sorensen, 1990; VanEten and Tookey, 1983). During air and CA storage, the variation in the methylsulphinylalkylglucosinolate content (Fig. 3) was similar in both trend and magnitude to that of the total glucosinolates (Fig. 2). This result was in part due to the high relative content of methylsulphinylalkylglucosinolates (76% to 82%) in all samples (Table 2). The indol-3-ylmethylglucosinolate content (15% to 22% of total) increased 47% and 24% during 7 days storage under air and 0.5% O₂ + 20% CO₂ atmosphere, respectively, as compared to freshly harvested broccoli (Fig. 3). In contrast, the concentration did not change under 0.5% O₂ and decreased 28% following 7 days storage under 20% CO₂(Fig. 3). No significant differences were found between the relative content of methylsulphinylalkyl- and indol-3-ylmethylglucosinolates with regard to CA treatment and storage period.

The average concentrations of total and individual glucosinolates for day 7 to 9 are shown in Table 2. Broccoli stored under air had the highest content of glucosinolates followed by that stored under 0.5% O₂ + 20% CO₂, 0.5% O₂, and 20% CO₂(Table 2). There were

Table 3. Significance of CA treatments, storage time, and interactions for content of total and individual glucosinolates, methylsulphinylalkylglucosinolates' and, indol-3-ylmethylglucosinolates'

| Glucobrassicin | Glucoiberin | Glucoraphanin | Neoglucobrassicin | 4-Methoxyglucobrassicin | Methylsulphinylalkylglucosinolates | Indol-3-ylmethylglucosinolates | Total glucosinolates |
|---------------|-------------|---------------|------------------|------------------------|---------------------------------|-------------------------------|----------------------|
| **            | **          | ***           | **               | ***                    | *                              | ***                           | ***                  |

Day 7 and 9.
| Methylsulphinylalkylglucosinolates: glucoiberin and glucoraphanin.
| Indol-3-ylmethylglucosinolates: glucobrassicin, neoglucobrassicin and 4-methoxyglucobrassicin.

**NS**, ***Significant at P = 0.01, 0.001 and 0.0001, respectively.
significant differences among CA-treatments in the content of total and individual glucosinolates for day 7 to 9 (Table 3).

When the broccoli stored under CA for 7 days was transferred to air for 2 days, there was not a significant decrease in total glucosinolate content (Table 3). Storage period (transfer to air from day 7 to 9) only affected glucobrassicin and 4-methoxyglucobrassicin contents. Aeration reduced the glucobrassicin contents 35% in broccoli stored 7 days under either 0.5% O₂ + 20% CO₂ or 20% CO₂. Treatment with 0.5% O₂ + 20% CO₂ probably caused physiological stress in the tissue even though no symptoms of CO₂ injury were visible. This could result in an increase in the hydrolytic breakdown of glucosinolates upon aeration. On average, the glucobrassicin content decreased from 7.8 µmol·g⁻¹ dry weight at day 7 to 6.5 µmol·g⁻¹ dry weight at day 9. The opposite result was noted for 4-methoxyglucobrassicin (Fig. 3). The content increased during storage under low O₂, atmosphere and increased further after transfer to air. The average content of 4-methoxyglucobrassicin increased from 1.1 µmol·g⁻¹ dry weight at day 7 to 1.5 µmol·g⁻¹ dry weight at day 9. These results may indicate that storage could affect the nutritional value of broccoli since degradation products of indol-3-ylmethylglucosinolates, especially substituted indol-3-ylmethylglucosinolates, have been shown to have anticarcinogenic effects (Feldt et al., 1994 and references cited therein; Loft et al., 1992). In the present study, very low O₂ and very high CO₂ were imposed. Glucosinolate metabolism of broccoli stored at lower temperature and very extreme CA conditions should be investigated.

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