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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 olerucea* L. var. *italica* stored 7 days at 10C under air, 0.5% O_2, 0.5% O_2 + 20% CO_2 or 20% CO_2 atmosphere, followed by transfer to air for 2 days. 'Marathon' broccoli contained glucoraphanin, glucobrassicin, neoglucobrassicin, glucoiberin, 4-methoxyglucobrassicin, progoitrin, glucoalyssin, and gluconasturtiin. The methylssulfinylalkylglucosinolates (glucoiberin 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 glucosinolate/g dry weight. The total glucosinolate content increased 42% and 21% during 7 days storage under air and 0.5% O_2 + 20% CO_2, respectively, as compared to freshly harvested broccoli, and decreased 15% in broccoli stored under 20% CO_2. Treatment with 20% CO_2 in the absence of 0, resulted in visible CO, 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% O_2 + 20% CO_2 or 20% CO_2 atmosphere. In contrast, the 4-methoxyglucobrassicin content increased during storage under low O_2 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 Sorensen, 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 ally1 isothiocyanate in mustard and horseradish arising from enzymic breakdown of sinigrin. This compound causes a pungent and lachrymatory response upon cutting and chewing (Gilbert and Nursten, 1972). Indol-3-ylmethylglucosinolates, which occur in appreciable amounts in several *Brassica* vegetables, are of interest for their potential contribution of anticarcinogenic compounds to the diet (Loft et al., 1992; McDanell et al., 1988).

Glucosinolates have a well defined structure with a side chain (R-group) and *D*- glucopyranose as β– thioglucoside attached to carbon atom no. 0 in (Z)-N-hydroximine sulfate esters (Table 1) (Olsen and Sorensen, 1981; Sorensen, 1990). The structural variation of the more than 100 glucosinolates isolated from various plant sources 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 Sorensen, 1981; Sang et al., 1984; Rahman et al., 1986; VanEtten 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 Larsen, 1980; Underhill and Kirkland, 1980), where many details are still unknown (Bjerg et al., 1987; Sørensen, 1991).

At present, only limited information is available relating to glucosinolate metabolism in *Brassica* vegetables after harvest. Chong and Bérard (1983) reported the variation in glucosinolate breakdown products in three cabbage cultivars during refrigerated storage. They 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, 1985). 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 CO_2 and reduced O_2 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 CO_2 and reduced O_2 concentrations may affect glucosindlate 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 O_2 and high CO_2 to understand better glucosinolate metabolism in *Brassica* vegetables after harvest.

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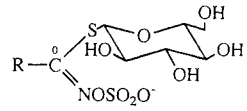
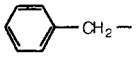
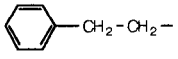
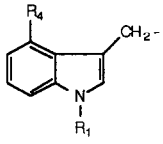
Table 1. Numbers, structures and names of glucosinolates reported as constituents of *Brassica* vegetables[z].

Glucosinolate skeleton 

No	Structure of R-groups	Semisystematic names of R-groups[x]	Trivial names	Brassica spp.
1	$CH_2=CH-CH_2-$	Allyl	Sinigrin	Cabbage, Brussels sprouts, cauliflower, broccoli
2	$CH_2=CH-CH_2-CH_2-$	But-3-enyl	Gluconapin	Cabbage, Brussels sprouts, cauliflower, broccoli, Chinese cabbage
3	$CH_2=CH-CH_2-CH_2-CH_2-$	Pent-4-enyl	Glucobrassicanapin	Cauliflower, broccoli, Chinese cabbage
4	$CH_2=CH-CH(OH)-CH_2-$	(2R)-2-Hydroxybut-3-enyl	Progoitrin	Cabbage, Brussels sprouts, cauliflower, broccoli, Chinese cabbage
7	$CH_3-S-CH_2-CH_2-CH_2-$	3-Methylthiopropyl	Glucoibervirin	Cabbage, cauliflower,
8	$CH_3-S-CH_2-CH_2-CH_2-CH_2-$	4-Methylthiobutyl	Glucoerucin	Cabbage, Brussels sprouts, cauliflower, broccoli
10	$CH_3-SO-CH_2-CH_2-CH_2-$	3-Methylsulphinylpropyl	Glucoiberin	Cabbage, Brussels sprouts, cauliflower, broccoli
11	$CH_3-SO-CH_2-CH_2-CH_2-CH_2-$	4-Methylsulphinylbutyl	Glucoraphanin	Cabbage, Brussels sprouts, cauliflower, broccoli, Chinese cabbage
12	$CH_3-SO-CH_2-CH_2-CH_2-CH_2-CH_2-$	5-Methylsulphinylpentyl	Glucoalyssin	Chinese cabbage
15	$CH_3-SO_2-CH_2-CH_2-CH_2-CH_2-$	4-Methylsulphonylbutyl	Glucoerysolin	Cabbage
16		Benzyl	Glucotropaeolin	Cabbage
17		Phenethyl	Gluconasturtiin	Cabbage, Brussels sprouts, broccoli, Chinese cabbage
23		$R_1=H$ $R_4=H$	Glucobrassicin	Cabbage, Brussels sprouts, cauliflower, broccoli, Chinese cabbage
24		$R_1=OCH_3$ $R_4=H$	Neoglucobrassicin	Cabbage, Brussels sprouts, cauliflower, broccoli, Chinese cabbage
26		$R_1=H$ $R_4=OH$	4-Hydroxyindol-3-ylmethyl	4-Hydroxyglucobrassicin Cabbage, Brussels sprouts, cauliflower, broccoli, Chinese cabbage
27		$R_1=H$ $R_4=OCH_3$	4-Methoxyindol-3-ylmethyl	4-Methoxyglucobrassicin Cabbage, Brussels sprouts, cauliflower, brocccoli, Chinese cabbage

[z]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).
[x]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 0C. 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 10C for 7 days and ventilated with humidified gas at 10.1 ± 0.3 liters·h^{-1}. The atmospheres were as follows: air, 0.5% O_2, 0.5% O_2 + 20% CO_2, or 20% CO_2 (all balanced with N_2). After 7 days, the jars were transferred to air for 2 days at 10C. Oxygen and CO_2 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_2 and CO_2 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_2 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_2 and kept in polyethylene bags at –40C until freeze-drying, usually within 1 month. Freeze-dried broccoli tissue was stored in sealed polyethylene bags at 4C 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 µl internal standard solution containing 5.0 µmol·ml^{-1} 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_2, 0.5% O_2 + 20% CO_2, and 20% CO_2) 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.

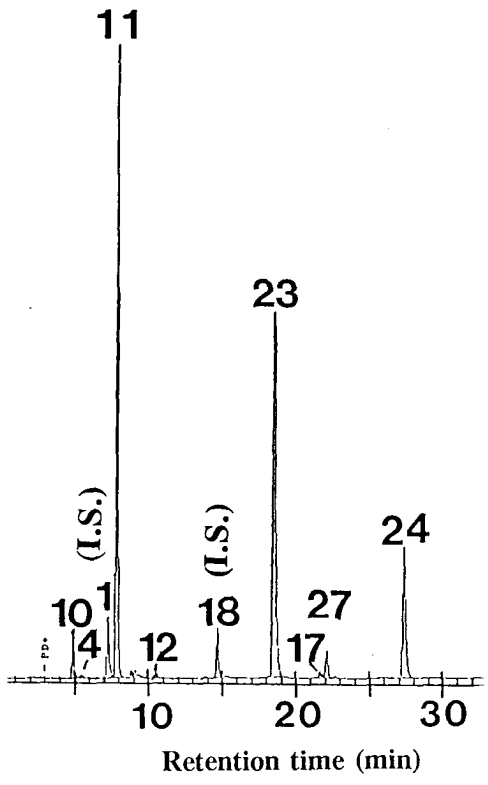


Fig. 1. Chromatogram of desulfoglucosinolates identified in freshly harvested 'Marathon' broccoli. The numbers refer to Table 1. No. 1 (sinigrin) and 18 (glucobarbarin) are internal standards (I.S.).

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), glucoalyssin (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

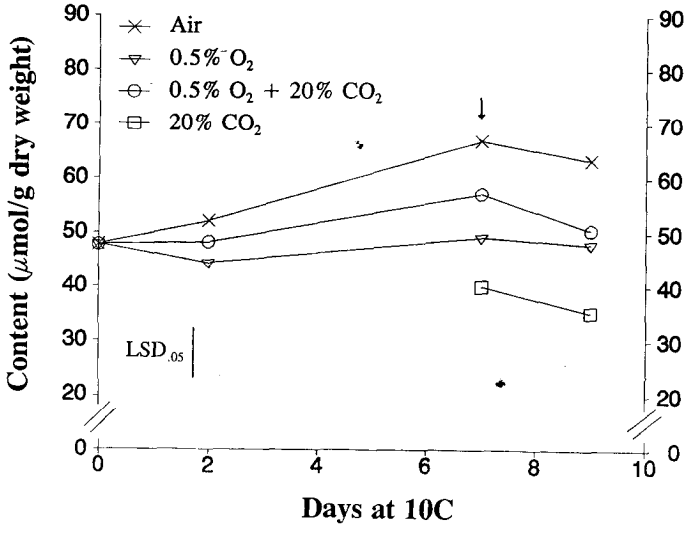


Fig. 2. Total glucosinolate content in broccoli stored 7 days under CA followed by 2 days aeration (⇓ transfer to air). Data are means of two replicates.

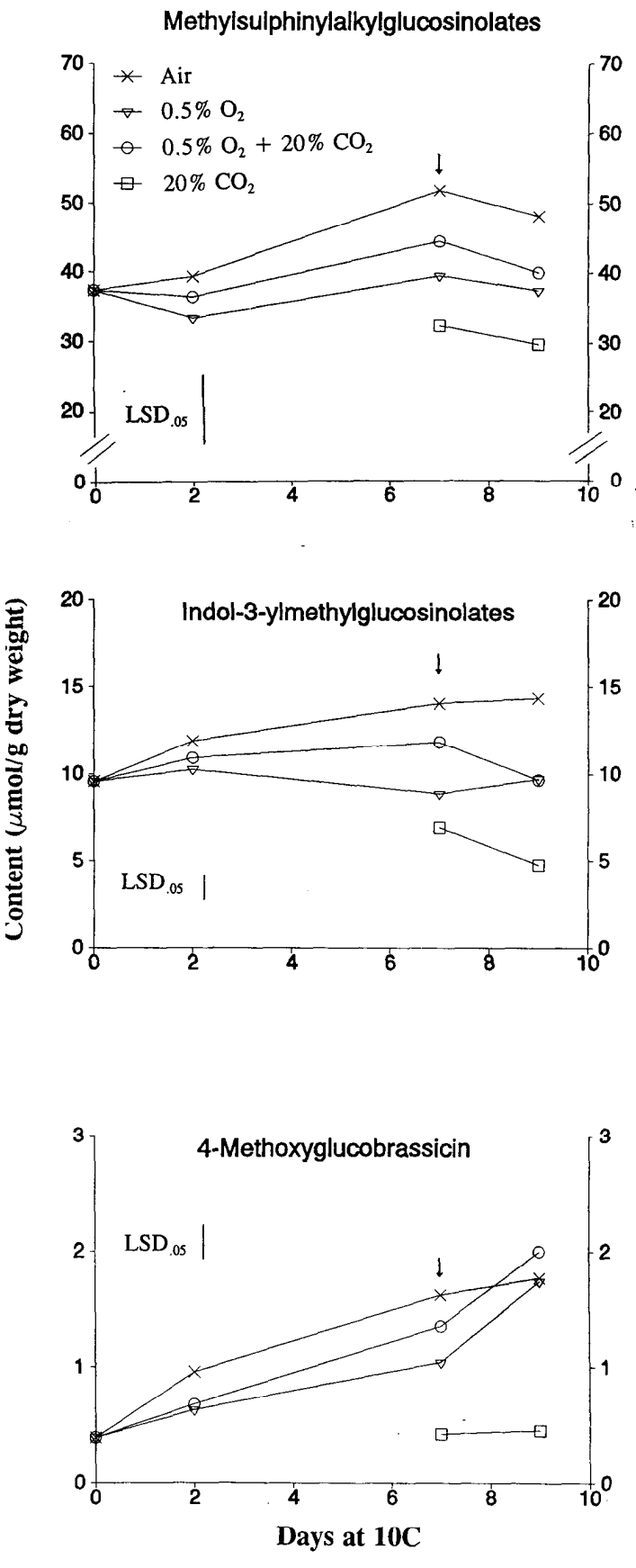


Fig. 3. Content of methylsulfinylalkylglucosinolates (glucoiberin and glucoraphanin), indol-3-ylmethylglucosinolates (glucobrassicin, neoglucobrassicin and 4-methoxyglucobrassicin) and 4-methoxyglucobrassicin in broccoli stoned 7 days under CA followed by 2 days aeration (⇓ transfer to air). Data are means of 2 replicates.

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 [z]	Air	0.5% O_2	0.5% O_2 + 20% CO_2	20% CO_2
Glucoiberin	3.2 a[x](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 [y]	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 [y]	1.7 a (3)	1.4 b (3)	1.7 a (3)	0.4 c (1)
Methylsulphinylalkylglucosinolates [w]	49.8 c (76)	38.3 bc (79)	42.0 ab (78)	31.0 c (82)
Indol-3-ylmethylglucosinolates [v]	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)

[z]Chemical structures are shown in Table 1.
[x]Numbers within a row followed by different letters are significantly different at $P = 0.05$ by Duncan's multiple range test.
[y]Interaction between CA treatments and storage time.
[w]Methylsulphinylalkylglucosinolates: glucoiberin and glucoraphanin.
[v]Indol-3-ylmethylglucosinolates: glucobrassicin, neoglucobrassicin and 4-methoxyglucobrassicin.

al., 1991) reported a similar glucosinolate profile in broccoli. Of the major glucosinolates, the methylsulfinylalkylglucosinolates (glucoiberin 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.

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% CO_2 atmosphere resulted in severe off-odors. The total glucosinolate content increased 42% and 21% during 7 days storage under air and 0.5% O_2 + 20% CO_2, 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% O_2, but decreased 15% in broccoli stored under 20% CO_2 in the absence of O_2. 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 (Lüthy 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; VanEtten 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% O_2 + 20% CO_2 atmosphere, respectively, as compared to freshly harvested broccoli (Fig. 3). In contrast, the concentration did not change under 0.5% O_2 and decreased 28% following 7 days storage under 20% CO_2 (Fig. 3). No significant differences were found between the relative content of methylsulfinylalkyl- 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% O_2 + 20% CO_2, 0.5% O_2, and 20% CO_2 (Table 2). There were

Table 3. Significance of CA treatments, storage time[z], and interactions for content of total and individual glucosinolates, methylsulphinylalkylglucosinolates[x] and, indol-3-yhnethylglucosinolates[y].

	Gluco-iberin	Gluco-raphanin	Gluco-brassicin	Neo-glucobrassicin	4-Methoxy-glucobrassicin	Methylsulphinyl-alkyl-glucosinolates	Indol-3-yl-methyl-glucosinolates	Total glucosinolates
CA-treatment	**	**	***	**	***	*	***	***
Storage time	NS	NS	**	NS	**	NS	NS	NS
Interaction	NS	NS	*	NS	*	NS	NS	NS

[z]Day 7 and 9.
[x]Methylsulphinylalkylglucosinolates: glucoiberin and glucoraphanin.
[y]Indol-3-ylmethylglucosinolates: glucobrassicin, neoglucobrassicin and 4-methoxyglucobrassicin.
[NS]*,**,***Nonsignificant or 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% O_2 + 20% CO_2 or 20% CO_2. Treatment with 0.5% O_2 + 20% CO_2 probably caused physiological stress in the tissue even though no symptoms of CO_2 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 $\mu mol \cdot g^{-1}$ dry weight at day 7 to 6.5 $\mu mol \cdot g^{-1}$ dry weight at day 9. The opposite result was noted for 4-methoxyglucobrassicin (Fig. 3). The content increased during storage under low 0, atmosphere and increased further after transfer to air. The average content of 4-methoxyglucobrassicin increased from 1.1 $\mu mol \cdot g^{-1}$ dry weight at day 7 to 1.5 $\mu mol \cdot g^{-1}$ 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 O_2 and very high CO_2 were imposed. Glucosinolate metabolism of broccoli stored at lower temperature and very extreme CA conditions should be investigated.

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