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ARTICLE

Atmospheric Carbon Dioxide Changes Photochemical Activity, Soluble Sugars and Volatile Levels in Broccoli (*Brassica oleracea* var. *italica*)

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Atmospheric carbon dioxide (CO_2) concentration is an environmental factor currently undergoing dramatic changes. The objective of the present study was to determine the effect of doubling the ambient CO_2 concentration on plant photochemistry as measured by photochemical quenching coefficient (qP), soluble sugars and volatiles in broccoli. Elevated CO_2 concentration increased qP values in leaves by up to 100% and 89% in heads, while glucose and sucrose in leaves increased by about 60%. Furthermore, in broccoli heads elevated CO_2 concentration induced approximately a 2-fold increase in concentrations of three fatty acid-derived C_7 aldehydes ((*E*)-2-heptenal, (*E,Z*)-2,4-heptadienal, (*E,E*)-2,4-heptadienal), two fatty acid-derived C_5 alcohols (1-penten-3-ol, (*Z*)-2-pentenol), and two amino acid-derived nitriles (phenyl propanenitrile, 3-methyl butanenitrile). In contrast, concentrations of the sulfur-containing compound 2-ethylthiophene and C_6 alcohol (*E*)-2-hexenol decreased. Finally, elevated CO_2 concentration increased soluble sugar concentrations due to enhanced photochemical activity in leaves and heads, which may account for the increased synthesis of volatiles.

KEYWORDS: Brassicaceae; effect of CO_2; photochemical activity; glucose; fructose; sucrose; volatiles

INTRODUCTION

The carbon dioxide (CO_2) concentration in the atmosphere has significantly increased since the start of the industrial revolution. For example, atmospheric CO_2 concentration has risen from 280 μmol mol^{-1} in preindustrial times to approximately 380 μmol mol^{-1} at present (*1*). Moreover, climate simulations predict a further ongoing increase in atmospheric CO_2 of up to twice current concentrations (*2*).

A key effect of elevated CO_2 levels is enhanced photosynthesis leading to more vigorous plant growth, greater biomass production, and higher yields (*3, 4*). Several hypotheses suggest that environmental conditions promoting photosynthesis should lead to production of excess nonstructural carbohydrates, resulting in higher levels of more carbon-based secondary compounds (*5, 6*). However, studies on nonstructural carbohydrate levels such as soluble sugars in vegetables grown at elevated CO_2 concentrations are not consistent. For example, growth chamber-grown tomato fruits have increased sucrose, fructose, glucose, and total soluble solids concentrations at elevated CO_2 concentrations (*7*); whereas, in greenhouse-grown tomato fruits, elevated CO_2 concentrations showed no effect on soluble sugar concentrations, although photosynthesis was increased (*8*).

Literature on how CO_2 concentration affects levels of key plant compounds in vegetables, other than for dry matter content or nonstructural carbohydrates, is scarce, although there is great interest in volatile composition. In grapevine, elevated CO_2 concentration (500 μmol mol^{-1}) increased esters such as ethyl 2-methylbutyrate, isoamyl acetate, and ethyl hexanoate, as well as acids such as butyric acid and isovaleric acid, and the terpenol linalool, whereas higher alcohols such as 1-octanol and methionol, or the phenol 4-ethylguaiacol decreased (*9*). In the strawberry fruit Wang and Bunch (*10*) found that high CO_2 concentration (600 μmol mol^{-1}) enhanced concentrations of several esters such as ethyl hexanoate, ethyl butanoate, methyl hexanoate, methyl butanoate, hexyl acetate, hexyl hexanoate, as well as furaneol and linalool. In contrast, the esters butyl acetate and methyl methanoate decreased. Furthermore, elevating ambient CO_2 concentrations caused enhanced fructose, glucose, and total soluble sugar concentrations in strawberry fruits, which the authors suggested was an increase in precursors able to produce aromatic compounds.

Volatile organic compounds including oxygenated green leaf volatiles (C_6 and C_5 alcohols) may be involved in protecting plants against environmental factors. However, the exact underlying mechanism mediating such plant protection is still unknown (*11*). Schonhof et al. (*12*) found in broccoli (*Brassica oleracea* var. *italica*) that elevated atmospheric CO_2 in comparison to ambient CO_2 concentration increased aliphatic glucosinolate (3-methylsulfinylpropyl and 4-methylsulfinylbutyl glucosinolate) levels, while indole glucosinolate (3-indolylmethyl, 4-methoxy-3-indolylmethyl, and 1-methoxy-3-indolyl glucosinolate) levels decreased. Changing nitrogen content and nitrogen/sulfur ratios in the plants under different CO_2 concentrations, as

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well as alterations in photochemical processes within the plant's photosynthetic system, were proposed to be the reason for these changes. Upon tissue disruption the glucosinolates are brought into contact with the plant enzyme myrosinase (β-thioglucosidase), releasing aglycone, and depending on reaction conditions (pH, Fe^{2+}, epithiospecifier protein) generating the corresponding isothiocyanates, nitriles or thiocyanates (*13*). Certain degradation products of glucosinolates are volatile. These volatiles are responsible for the toxicity and deterrence of herbivores and pathogens (*14*), and can also contribute to the flavor in Brassicaceae in addition to other aroma volatiles (fatty acid-derived volatiles and sulfur compounds) (*15−18*). Currently, consumers rejected raw florets of broccoli cultivars with higher levels of bitter tasting glucosinolates such as allyl, 3-butenyl, 3-indolylmethyl, 4-methoxy-3-indolylmethyl, and 1-methoxy-3-indolyl glucosinolates (*19*).

At present, no information is available on the effect of elevated atmospheric CO_2 on *Brassica* vegetable volatiles. For this reason, the objective of the present study was to determine the effect of ambient and elevated atmospheric CO_2 concentrations on volatiles in Brassicaceae using broccoli as an example. We hypothesized that higher CO_2 concentration should increase photochemical activity and thus soluble sugars, which in turn could increase potential precursor levels for volatiles and subsequently the volatile concentration. Furthermore, soluble sugars are also relevant as important sensory compounds since higher sugar concentrations in broccoli heads seem to mask bitter tasting glucosinolates, thus having a positive influence on consumer acceptance (*19*). Therefore, we examined the photochemical quenching coefficient (qP), the major soluble sugars found in broccoli leaves and heads—glucose, fructose, and sucrose—and volatiles.

MATERIALS AND METHODS

Plant Material. Broccoli (*Brassica oleracea* var. *italica*) cv Marathon was grown in a controlled greenhouse environment in a block design with three replicates. Each treatment and replicate used a total of 54 broccoli plants planted in soil-filled 40 L containers, arranged at a density of 2 plants m^{-2}. Starting at the three-leaf stage, commercially pure CO_2 was supplied during the daytime to three of six greenhouse compartments aiming at 1,000 μmol mol^{-1} and resulting in an average atmospheric CO_2 concentration of 880 μmol mol^{-1} due to CO_2 losses during ventilation of the greenhouse. In the unsupplied compartments the average atmospheric CO_2 concentration was 450 μmol mol^{-1}. Plants were grown at a daily mean temperature of 15.3 °C and at a mean daily photosynthetic active radiation (PAR) of 10.04 mol m^{-2}. A mixed sample of heads and leaves from each replicate, consisting of five fully developed broccoli plants, was taken for analyses.

Chemicals. Calcium dihydrate was obtained from Merck (Darmstadt, Germany). Pentanal, 1-penten-3-one, dimethyl disulfide, hexanal, (*E*)-2-pentenal, 2-ethylthiophene, 1-penten-3-ol, heptanal, (*E*)-2-hexenal, hexyl acetate, (*E*)-2-heptenal, (*Z*)-3-hexenylacetate, (*Z*)-2-penten-1-ol, dimethyl trisulfide, hexanol, (*E*)-3-hexen-1-ol, (*Z*)-3-hexen-1-ol, (*E*)-2-octenal, (*E,E*)-2,4-heptadienal and 2-octanone were purchased from Sigma-Aldrich (Taufkirchen, Germany). 2,3-Pentanedione were purchased from Alfa Aesar GmbH & Co KG (Karlsruhe, Germany).

Sample Preparation for Compound Measurement. Fresh material from the heads (florets, flower buds, and second order-branches) was used for volatile analysis. For sugar analyses, leaf and head samples were dried at 80 °C in a ventilated oven for two days, and then the material was finely ground.

Dynamic Headspace Sampling and GC−MS. Volatiles of broccoli were isolated by a modification of the dynamic headspace method of Buttery et al. (1987) (*20*). Fresh samples (300 g) were blended with 250 mL of distilled water for 30 s and left for a further 180 s, after which 400 mL of a saturated calcium chloride solution was added to deactivate enzymes of the lipid oxidation pathway, and then the mixture was blended for 10 s. A volume (1 mL) of a stock solution of internal standard (containing 10 μL

2-octanone in 100 mL of water) was added, and the mixture was blended for another 10 s. While stirring in a 3 L flask, purified air (150 mL min^{-1}) was passed through the mixture and into a trap (200 mg Tenax TA, 60−80 mesh, Machery-Nagel, Düren, Germany) for 150 min, after which trapped volatiles were extracted with 3 mL of acetone and concentrated with nitrogen flux to a volume of 50 μL. The whole sample preparation procedure was performed in duplicate for each greenhouse replicate.

Volatiles were determined by GC−MS using an Agilent 6890 gas chromatograph equipped with a 5973N mass selective detector (Agilent Technologies, Inc., Santa Clara, CA) and a Supelcowax 10 column (30 m × 0.25 mm i.d./0.25 μm, Supelco, Inc., Bellefonte, PA). Analyses were performed in the splitless mode with an injector temperature of 250 °C, a helium flow of 1 mL min^{-1}, and an oven temperature programmed at 40 °C for 3 min, then up to 60 at 1 °C min^{-1}, and held for 2 min, then up to 180 at 5 °C min^{-1} and held for 20 min. The GC−MS interface was heated at 280 °C with the actual temperature in the MS source reaching 230 °C. The electron impact energy was set at 70 eV, and data were collected using full scan mode in the range of 35−250 atomic mass units. Compound identification was based on mass spectra (Wiley6 and NIST98 library), and when possible, identification was confirmed by comparing mass spectra and retention time with those of authentic standards. The relative peak areas of the total ion chromatogram were normalized with the peak area of the internal standard (peak area of measured compound was divided by peak area of the internal standard).

Soluble Sugar Analyses. Sugar (fructose, glucose, and sucrose) concentrations of finely ground dried material (0.2 g) were analyzed enzymatically using a test kit (R-Biopharm, Mannheim, Germany), where reduced nicotinamide adenine dinucleotide phosphate (NADPH) was formed during the reaction of the reducing sugars (glucose, fructose) with hexokinase, adenosine-5′-triphosphate, nicotinamide adenine dinucleotide phosphate (NADP), glucose 6-phosphate-dehydrogenase, and phosphoglucose-isomerase (*21*). The absorbance of NADPH was measured spectrophotometrically at 340 nm. The sucrose content was determined from the difference of the glucose concentrations before and after enzymatic inversion by the enzyme β-fructosidase. Chemical analyses of each greenhouse replicate were performed in duplicate.

Photochemical Activity. To determine changes in plant photochemistry, which could finally lead to changes in soluble sugars and volatile precursors, photochemical activity was determined by chlorophyll fluorescence. Chlorophyll fluorescence was measured for six plants from each of the six greenhouse compartments. After 20 min of dark adaptation, chlorophyll fluorescence was measured for the two fullest developed leaves (four measurements per leaf from the upper part of the leaf area) nearest to the broccoli head and for the broccoli inflorescences (six measurements per head) using a Mini-Pam (Walz, Effeltrich, Germany). To assess the minimal fluorescence (F_o), a pulse-modulated actinic light source of about 0.02 μmol m^{-2} s^{-1} was used (*22*). For the maximal fluorescence (F_m), a saturating light pulse (duration, 30 s; intensity, 3000 μmol m^{-2} s^{-1}) was applied. This procedure was repeated after light adaptation, and the current fluorescence (F) and maximal fluorescence (F_m') were then measured. The following derived function was used to calculate the photochemical quenching coefficient qP (*23*):

$$qP = (F_m' - F)/(F_m' - F_o)$$

Statistical Analysis. The means of the treatments were compared using Student's *t* test at a significance level of $p \leq 0.05$. Relationships between plant compounds were established by correlation analysis, and the correlation coefficients (r^2) were evaluated using Student's *t* test ($p \leq 0.05$).

RESULTS AND DISCUSSION

Using the dynamic headspace sampling technique in conjunction with GC−MS, a total of 26 volatiles were identified (**Table 1**). All compounds identified were previously reported for *Brassica* species (*15−17*, *24−27*). Fresh broccoli florets were found to have relatively high concentrations of C_5-C_7 aldehydes, C_5-C_6 alcohols, C_5 ketones and nitriles. Elevated atmospheric CO_2 concentration significantly changed the concentrations of several fatty acid-derived aldehydes and alcohols, as well as amino acid-derived nitriles and a sulfur-containing compound. Chromatograms of

Table 1. Relative Peak Areas (Area Units $\times$ 10^3) of Volatiles in Broccoli Heads Grown at Ambient CO_2 Concentration (450 μmol mol^{-1}) and Elevated CO_2 Concentration (880 μmol mol^{-1})[a]

no.[b]	compound[c]	ambient CO_2	elevated CO_2	significance	refs[e]
1	3-pentanone/pentanal	93.1 ± 6.3	149.9 ± 58.5	ns	*17*
2	1-penten-3-one	393.6 ± 23.0	442.0 ± 53.3	ns	*17*
3	2,3-pentanedione	40.6 ± 8.0	53.0 ± 11.8	ns	*17, 27*
4	dimethyl disulfide	7.7 ± 1.8	7.5 ± 2.2	ns	*15−17, 24, 25, 27*
5	hexanal	4156.8 ± 488.2	3553.5 ± 1083.1	ns	*16, 17, 24−26*
6	(*E*)-2-pentenal	98.0 ± 21.2	133.6 ± 18.3	ns	*17*
7	(*Z*)-3-hexenal[d]	5489.1 ± 498.3	3979.3 ± 688.0	ns	*16*
8	1-penten-3-ol	605.9 ± 92.6	1175.9 ± 128.2	*	*17, 27*
9	2-ethylthiophene	54.6 ± 6.8	28.6 ± 3.4	*	*24*
10	heptanal	9.8 ± 1.5	14.3 ± 2.0	ns	*17, 25, 27*
11	(*E*)-2-hexenal	6067.0 ± 522.1	7501.0 ± 1884.8	ns	*15−17, 25−27*
12	3-methyl butanenitrile[d]	53.6 ± 6.5	82.4 ± 7.6	*	*24*
13	methyl thiocyanate[d]	88.6 ± 9.8	78.9 ± 7.1	ns	*16, 17, 27*
14	hexanenitrile[d]	39.6 ± 2.6	39.6 ± 3.0	ns	*24*
15	(*E*)-2-heptenal	21.2 ± 3.6	75.6 ± 8.1	*	*17*
16	(*Z*)-3-hexenyl acetate	377.0 ± 15.2	414.4 ± 35.0	ns	*25, 26*
17	(*Z*)-2-penten-1-ol	408.3 ± 77.2	848.3 ± 130.6	*	*25, 27*
18	dimethyl trisulfide	19.7 ± 0.5	25.1 ± 7.6	ns	*15−17, 25, 27*
19	hexanol	846.6 ± 135.9	591.2 ± 61.2	ns	*15, 16, 25−27*
20	(*E*)-3-hexenol	54.9 ± 7.5	62.2 ± 4.1	ns	*16*
21	(*Z*)-3-hexenol	6575.0 ± 1146.7	8280.8 ± 1047.7	ns	*15, 16, 24−27*
22	(*E*)-2-hexenol	98.6 ± 29.6	34.7 ± 1.2	*	*24−26*
23	(*E*)-2-octenal	3.6 ± 0.7	4.8 ± 0.6	ns	*15, 17*
24	(*E,Z*)-2,4-heptadienal[d]	76.5 ± 11.2	143.9 ± 8.5	*	*15, 16*
25	(*E,E*)-2,4-heptadienal	87.7 ± 16.4	177.0 ± 12.6	*	*15, 16, 25, 26*
26	phenyl propanenitrile[d]	24.9 ± 4.3	76.6 ± 17.1	ns	*15, 24, 25*

[a] Data expressed as means ± standard error (*n* = 3); *, significant at *p* ≤ 0.05; ns, not significant. [b] Peak numbers correspond to the peaks in **Figure 1**. [c] Tentative identification, mass spectra were consistent with those of an authentic compound unless otherwise noted. [d] Tentative identification, no authentic compound available. [e] The compound was earlier reported in the given reference.
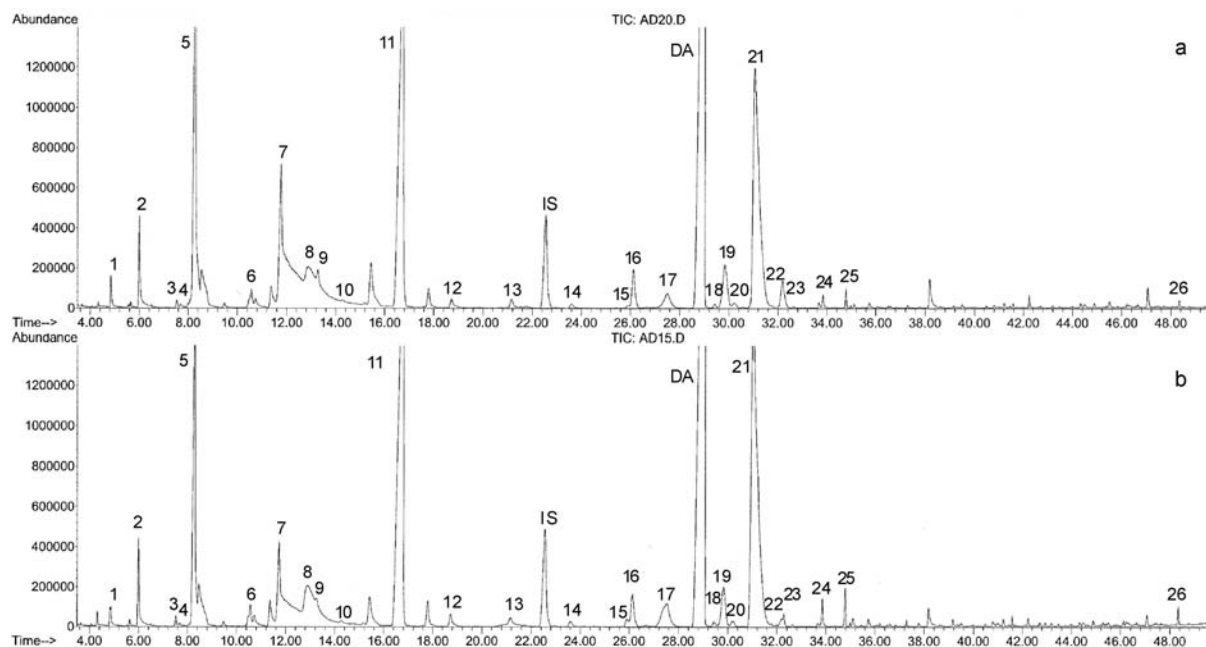
Figure 1. GC−MS chromatograms of volatiles in broccoli: (**a**) broccoli grown at ambient CO_2 concentration (450 μmol mol^{-1}); (**b**) broccoli grown at elevated CO_2 concentration (880 μmol mol^{-1}). IS interner standard; DA diacetone alcohol (degradation product of acetone).

volatiles in broccoli grown at ambient and elevated CO_2 are shown in **Figure 1**. More specifically, the elevated atmospheric CO_2 concentration led to approximately 2-fold increases in the C_7 aldehydes (*E*)-2-heptenal (**15**), (*E,Z*)-2,4-heptadienal (**24**), and (*E,E*)-2,4-heptadienal (**25**), C_5 alcohols 1-penten-3-ol (**8**) and (*Z*)-2-pentenol (**17**), as well as the two amino acid-derived nitriles, 3-methyl butanenitrile (**12**) and phenyl propanenitrile (**26**) (**Table 1**).

Phenyl propanenitrile is a degradation product of the well-known aromatic phenylethyl glucosinolate, which in part is derived from the amino acid phenylalanine (*28*). The corresponding isothiocyanate was not detected. 3-Methyl butanenitrile, a degradation product of the methionine-derived aliphatic 2-methylpropyl glucosinolate, is not very common in *Brassica* vegetables, but was identified in kohlrabi (*Brassica oleracea* var. *gongylodes*) (*24*). Furthermore,
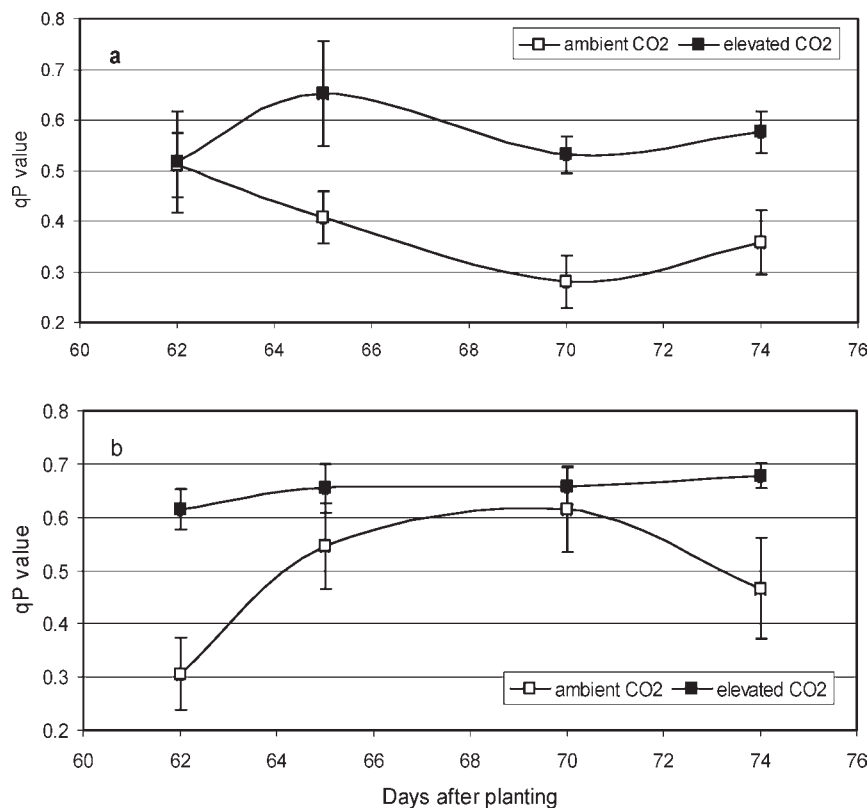
Figure 2. Photochemical quenching coefficient (qP) of broccoli heads (**a**) and leaves (**b**) grown at ambient CO_2 concentration (450 μmol mol^{-1}) and elevated CO_2 concentration (880 μmol mol^{-1}). Values are the means of 36 measurements. The bars represent the standard error.

2-methylpropyl glucosinolate was identified in the *Brassica* species *Arabidopsis thaliana* (*29*). While 3-methyl butanenitrile and phenyl propanenitrile are degradation products from minor glucosinolates in cv Marathon, degradation products of the major methylsulfinyl and indole glucosinolates are undetectable by dynamic headspace GC−MS. Methionine-derived aliphatic methylsulfinyl glucosinolate levels analyzed with HPLC increased while tryptophan-derived indole glucosinolate levels decreased with elevated atmospheric CO_2 in comparison to ambient CO_2 concentration in cv Marathon (*12*). In contrast to nitriles, C_7 aldehydes and C_5 alcohols that increased by about 2-fold, the C_6 alcohol (*E*)-2-hexenol (**22**) as well as the sulfur-containing compound 2-ethylthiophene (**9**) decreased (**Table 1**). Elevated atmospheric CO_2 concentration did not significantly influence the major C_6 alcohols hexanol (**19**) and (*Z*)-3-hexenol (**21**) or the C_6 aldehydes hexanal (**5**), (*Z*)-3-hexenal (**7**) and (*E*)-2-hexenal (**11**) in broccoli, although we used three replications per treatment (each replicate grown in one separate greenhouse compartment), which we assume is due to naturally occurring biological variation.

The accumulation of amino acid- and fatty acid-derived volatiles at elevated atmospheric CO_2 concentration could be caused by stimulating broccoli photochemistry. Enhanced photochemical activity can result in increased formation of ATP (adenosine triphosphate) and reduction equivalents necessary for fixing and assimilating CO_2 in the Calvin cycle, leading to the formation of a carbohydrate pool that forms the basis for biosynthesis of further compounds (*30*). Carbon dioxide is bound to pentose phosphate ribulose-1,5 biphosphate, which then undergoes carboxylation and further reduction to finally form glyceraldehyde-3-phosphate (GAP). This GAP is then added to a pool of carbohydrates with different carbon chain lengths (C_3−C_7) that act as precursors for the synthesis of further compounds, such as amino acids or fatty acids (*30*). In our experiments with elevated CO_2 concentration, we found that both broccoli heads and leaves used a greater

portion of the absorbed light for photochemical purposes, as indicated by the enhanced photochemical quenching coefficient (qP) (**Figure 2**). During their entire development (62 to 74 days after planting) starting from 5 cm up to 19−20 cm head diameter, the qP of the broccoli heads under elevated atmospheric CO_2 concentrations was 60−89% higher than that of corresponding heads at ambient atmospheric CO_2 concentrations (**Figure 2a**). Starting with head formation, vegetative growth is terminated. However, also the fully developed leaves showed a pronounced increase in photochemical activity during head formation at elevated atmospheric CO_2 concentration by up to 100% (**Figure 2b**). This systemic plant response to rising atmospheric CO_2 levels suggests an improved plant status in phytochemical activity leading to amplified biosynthesis of soluble sugars, which were subsequently involved in forming amino and fatty acids. This assumption is supported by the significant correlation between qP of the leaves and total C_7 aldehydes ($r^2 = 0.66$), (*E,E*)-2,4-heptadienal ($r^2 = 0.67$) and (*E*)-2-heptenal ($r^2 = 0.74$).

To test that higher CO_2 concentration increases not only photochemical activity but also nonstructural carbohydrates, the soluble sugar concentrations in broccoli heads and leaves were determined. In broccoli heads, fructose was found to be the most abundant soluble sugar, followed by glucose and sucrose (**Table 2**). Indeed, fructose concentrations increased by 12% under elevated atmospheric CO_2 (**Table 2**). Furthermore, we found significant linear relationships between fructose and total C_7 aldehydes ($r^2 = 0.76$), as well as between fructose and the individual C_7 aldehydes, (*E*)-2-heptenal ($r^2 = 0.86$), (*E,Z*)-2,4-heptadienal ($r^2 = 0.71$), and (*E,E*)-2,4-heptadienal ($r^2 = 0.69$). In leaves, the effect of elevated CO_2 concentrations was even more pronounced. For example, glucose and sucrose concentrations were significantly increased by 65% and 60%, respectively (**Table 2**). Furthermore, significant linear relationships were found between glucose and total C_5 aldehydes ($r^2 = 0.66$) as well

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Table 2. Soluble Sugar Concentration in mg per 100 g of Fresh Matter in Broccoli Heads and Broccoli Leaves Grown at Ambient CO_2 Concentration (450 μmol mol^{-1}) and Elevated CO_2 Concentration (880 μmol mol^{-1})[a]

| | CO_2 | | |
compound	ambient	elevated	significance
		Heads	
fructose	516.7 ± 11.8	580.6 ± 22.9	*
glucose	390.2 ± 34.2	449.3 ± 85.8	ns
sucrose	300.8 ± 35.6	313.4 ± 39.8	ns
total sugars	1207.7 ± 80.2	1343.3 ± 137.1	ns
		Leaves	
fructose	324.9 ± 95.4	396.6 ± 26.8	ns
glucose	370.1 ± 136.7	611.7 ± 66.0	*
sucrose	270.9 ± 68.9	433.3 ± 69.9	*
total sugars	965.9 ± 300.6	1441.5 ± 121.3	ns

[a] Data expressed as means ± standard error ($n = 3$); *, significant at $p \leq 0.05$; ns, not significant.

as between sucrose and total C_7 alcohols ($r^2 = 0.63$). These relationships between soluble sugars and several volatiles suggest that soluble sugars in both broccoli heads and leaves play a decisive role in generating volatiles.

In general, plants grown under elevated CO_2 concentration show increased concentrations of nonstructural carbohydrates in leaves of C_3 plants (*31*), which is in agreement with our data (**Table 2**). In the present study, heads and leaves of broccoli under elevated atmospheric CO_2 concentration demonstrated a distinct increase in photochemical activity (**Figure 2**) suggesting an enhanced assimilate level (*30*). In addition, during head development no further leaves were formed resulting in limited assimilate requirements for vegetative growth. Thus, the soluble sugar accumulation observed at elevated atmospheric CO_2 concentration (**Table 2**) presumably leads to higher availability of volatile precursors. Wang and Bunce (*10*) also suggested that the increase in soluble sugars they observed in strawberry fruits due to elevated CO_2 treatment may result in increased availability of precursors for esters and furanones, since they also found increased concentrations of furaneol, linalool and seven important esters, e.g. ethyl hexanoate and ethyl butanoate.

Goncalves et al. (*9*) found that different esters in grapevine increased under elevated CO_2 concentrations, but C_6 alcohol concentrations were not affected. In our study, we found that of the investigated C_6 alcohols in broccoli only (*E*)-2-hexenol decreased whereas (*Z*)-2-pentenol increased. Both (*E*)-2-hexenol and (*Z*)-2-pentenol belong to the so-called green leaf volatiles (*32*). In response to various environmental stimuli, green plants emit these substances to communicate with or attack other species as well as to attract or repel insects (*33*). Green odor compounds are synthesized via the lipoxygenase pathway where unsaturated fatty acids, including linolenic and linoleic acids, are converted into the corresponding 13-(*S*)-hydroperoxides by the enzyme lipoxygenase during cell disruption (*33*) and ripening when cell walls and membranes become more permeable (*34*). Changes in the levels of the two green leaf volatiles ((*E*)-2-hexenol, (*Z*)-2-pentenol) may be due to enzyme-specific responses to elevated CO_2 concentrations. Loreto et al (*35*) discussed in a study of Mediterranean evergreen oak (*Quercus ilex*) seedlings grown at ambient and elevated CO_2 concentrations that both the different response of relevant enzyme activity and the higher carbon availability due to enhanced photosynthetic activity at elevated CO_2 concentrations may stimulate emission.

Only odor-active volatiles can contribute to the flavor. In this study, the major odor-active volatiles that changed under elevated CO_2 concentration conditions were (*E,Z*)-2,4-heptadienal, 1-penten-3-ol, and 2-ethylthiophene. These aroma volatiles have herbaceous, grassy, and moldy odors, respectively (*18*). While Buttery et al. (*15*) described relatively low odor thresholds in water for phenyl propanenitrile (15 μg L^{-1}), we did not find any odor for this compound in GC eluates of broccoli cv Marathon, probably due to low concentrations of this compound in the investigated cultivar (*18*). Quantitative descriptive sensory analysis of different broccoli and cauliflower cultivars, combined with multivariate statistical analysis, showed that (*E,Z*)-2,4-heptadienal and 1-penten-3-ol are closely associated with the sensory attribute "broccoli" in both odor and flavor, whereas 2-ethylthiophene is associated with the flavor attribute for both "leek" and "green/grassy" (*18*). Furthermore, it is expected that the sensory relevance of the increased soluble sugar concentrations measured in broccoli heads grown at elevated CO_2 concentrations is low, since sensory analyses of different broccoli and cauliflower cultivars showed that sugar concentrations alone do not significantly influence the taste attribute "sweet" (*19*). The bitter tasting glucosinolates also affect the taste attribute "sweet" (*19*). Additionally, Schonhof et al. (*19*) found concentrations of individual sugars in broccoli and cauliflower heads of up to 1 g per 100 g fresh matter depending on the cultivar, whereas the CO_2-induced increase in sugars in our experiment was distinctly lower (**Table 2**). Finally, we assume that the reduced concentrations of the bitter tasting indole glucosinolates known to be found in broccoli grown at elevated atmospheric CO_2 concentrations (*12*) along with changes in odor-active volatiles as found in the present study may contribute toward improved flavor. However, this assumption needs further detailed sensory analysis combined with consumer acceptance evaluation.

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