

Original Research Paper

Effects of elevated atmospheric CO₂ and higher temperature on folate and b-carotene content in common vegetables

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ABSTRACT

Atmospheric carbon dioxide (CO₂) and temperature have steadily increased since the industrial revolution. While the effects of elevated CO₂ and higher temperature on crop production and mineral content have been extensively studied and thus well-documented, how global environmental changes affect indicators of important nutritional values is much less known. In this study, we examined how folate and b-carotene were affected by higher CO₂ and temperature in five common vegetables i.e. lettuce, mustard green, radish, spinach and turnip green. Vegetables were grown in environmentally-controlled growth chambers at two levels of CO₂ (380 or 760 μmol mol⁻¹) and two levels of day-time temperature (24 or 26°C) for eight weeks. Folate and b-carotene were measured using high-performance liquid chromatography coupled with ultra-violet spectroscopy and mass spectroscopy (HPLC-UV and MS). We found elevated CO₂ mostly had no effect on folate content at lower temperature, but generally increased its content at higher temperature. β-carotene content was lower in lettuce, mustard green and radish, but higher in spinach at elevated CO₂, regardless of growing temperature. Our results demonstrated that elevated CO₂ and higher temperature will affect nutritional values of common vegetables in the future, but the direction and magnitude of the effect are species-specific and dependent on other environmental factors.

Keywords: β-carotene, elevated CO₂, folate, global warming, vegetables

INTRODUCTION

Vegetables are universally considered healthy food and vegetable consumption is encouraged by health professionals worldwide (van Duyn & Pivonka 2000; Vainio & Weiderpass, 2006; Das & Maitra, 2021). There are a number of substances found in vegetables that are beneficial to human health in multiple ways. For instance, folate (vitamin B9) is an essential nutrient required for DNA replication and as a substrate for a range of enzymatic reactions involved in amino acid synthesis and vitamin metabolism (Greenberg et al., 2011; Bailey et al., 2015). Folates thus play an important role in growth and development throughout human life, especially during the embryonic stage (Schwarzenberg & Georgieff, 2018). Carotenoids, such as b-carotene, have been shown to reduce the risk of macular degeneration, cardiovascular diseases and several forms of cancer (Eggersdorfer & Wyss, 2018; Elvira-Torales et al., 2019). It is a known fact that many people in the world, especially those in the less developed regions, rely heavily on the intake of these substances from

vegetables for well-balanced nutrition (Muller & Krawinkel, 2005; Kiani et al., 2022).

The growing conditions for plants have changed significantly since the industrial revolution. Two important environmental factors that have changed the most are carbon dioxide (CO₂) level in the atmosphere and temperature (IPCC, 2023). Atmospheric CO₂ has risen from 280 ppm to a current level of 425 ppm, which is 50% higher than before the industrial revolution. Concurrently, global air temperature has been gradually increasing over the last century and will likely continue to rise in the coming decades (IPCC 2023). It has been well-documented that production of plant biomass and chemical composition will be affected by rising CO₂ and temperature in the atmosphere (Curtis & Wang, 1998; Lefsrud et al., 2005; Ziska & Bunce, 2007). Much less, however, is known about the effects of elevated CO₂ and higher temperature on essential phytochemicals such as folates and carotenoids. Any change in the content of these phytochemical in response to environmental changes will affect the availability of these chemical



compounds and thus the health of the humans worldwide, particularly those who depend on vegetables as the main source for these nutrients.

The main objective of this study was to examine how fresh biomass and content of folates and β -carotene in common vegetables would be affected by elevated CO_2 and higher temperature. Because fresh vegetables are important sources for folate and β -carotene in human diet, results from this study would offer insight into the nutritional quality of vegetables to be produced in the future when atmospheric CO_2 and temperature are projected to be much higher than today's level.

MATERIALS AND METHODS

Study species and growing conditions

Five vegetables were chosen for their commonality in grocery stores in the US and presence of folate and β -carotene in sufficient content for detection. They were lettuce (*Lactuca sativa* L.), mustard green (*Brassica juncea* (L.) Czern.), radish (*Raphanus sativus* (L.) Domin), spinach (*Spinacea oleracea* L.) and turnip green (*Brassica rapa* L.). The study was conducted in two environmentally controlled growth chambers (Conviron, Winnipeg, MB, Canada) at Indiana University Indianapolis, Indiana, USA. Four pots for each species were placed in each growth chamber. The 20 pots in each chamber were placed randomly and re-distributed weekly. Pots were rotated between chambers every two weeks with corresponding switching of environmental conditions. Ambient and elevated CO_2 levels were set at 380 (AC) and 760 $\mu\text{mol mol}^{-1}$ (EC), respectively. Treatments started immediately after seed sowing and lasted till harvest. Lower and higher temperature was set at 24°C (AT) and 26°C (ET), respectively, during the light period, and at 10°C (AT) and 12°C (ET), respectively, during the dark period. We ran the experiment three times in order to produce replicates for statistical analysis. Each experiment run lasted up to eight weeks. Vegetables were harvested when they had grown to approximately marketable size.

Chemical analysis

Folates

Folates were extracted using the trienzyme method described by Pandrangi & LaBorde (2004). Standards were prepared in phosphate buffers (Thermo Fischer Scientific, Waltham, MA). Folic acid (Fischer

Scientific, Chicago, IL) was used as the external standard and was prepared at different concentrations in phosphate buffer. Standards were prepared at concentrations of 10, 25, 50 and 100 $\mu\text{g mL}^{-1}$ from a pre-mixed folic acid stock solution of 200 $\mu\text{g mL}^{-1}$. Solution was prepared and incubated at 37°C overnight as described in Pandrangi & LaBorde (2004). HPLC-UV-MS method was chosen for compatibility with the apparatus being used for analysis (Rychlik, 2004). HPLC was performed with an Agilent 1100 series reverse phase high performance LC using a reverse phase C18 column. Four standard concentrations were run through the apparatus to obtain values to form a concentration calibration curve for quantifying the extracts. Calibration curves were used to calculate the concentration of folates in the samples.

β -carotene

β -carotene was extracted using the method as described by Singh et al. (2007). Five mL of homogenate containing one gram of fresh vegetable material was placed into a 15 mL centrifuge tube. An equal amount of ethanol (Pharmco, Brookfield, CT) with 1% BTH was added. The resulting mixture was vortexed and placed in a 100°C hot water bath for 15 min for homogenization. The mixture was filtered and 180 μL of KOH was added. The mixture was saponified for 30 min to remove chlorophylls and lipids. Prepared samples were then frozen in 2 mL HPLC vials. Standards were prepared and run through the HPLC with the extracts. Extracts were run overnight (14 h) after the instrument was set up. HPLC was done on an Agilent 1100 series reverse phase high performance LC using a reverse phase C18 column that is 2.1 X 50 mm with 3.5 μm particle size. The mobile phase consisted of acetonitrile: methanol: chloroform (47:47:6) as described by Schmitz et al. (1989). The UV detector had a 16 nm band width and was set to detect absorbance at 450-470 nm. Mass spectroscopy was done with positive ion mode with a drying gas temperature of 350°C at 10 L min^{-1} and had a capillary voltage of 4000. Five standard concentrations (0, 0.03, 0.06, 0.125, and 0.25 mg mL^{-1}) of β -carotene were prepared in methanol and run through the HPLC-UV instrument. Concentrations of the individual samples were determined using the equation for the best fit line of the graph.

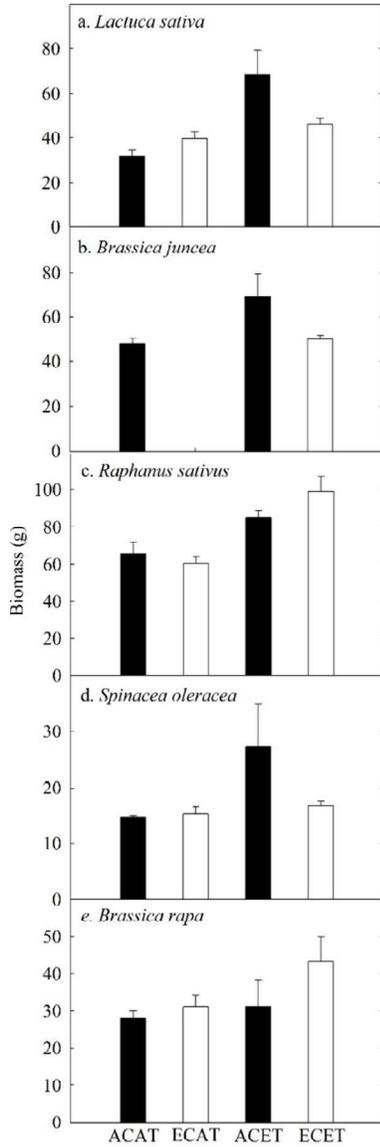


Fig. 1 : Biomass production of *Lactuca sativa*, *Brassica juncea*, *Raphanus sativus*, *Spinacea oleracea* and *Brassica rapa* grown under ambient CO₂ and ambient temperature (ACAT), elevated CO₂ and ambient temperature (ECAT), ambient CO₂ and elevated temperature (ACET) or elevated CO₂ and elevated temperature (ECET). Values are means±1 SEM (n=12 for ACAT and n=4 for all other treatments)

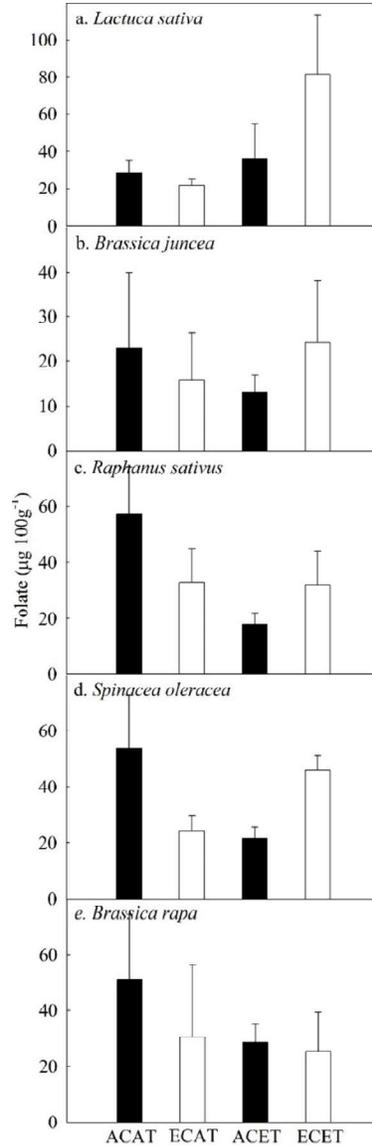


Fig. 2 : Folate content (mg 100 g⁻¹) in harvestable parts of *Lactuca sativa*, *Brassica juncea*, *Raphanus sativus*, *Spinacea oleracea* and *Brassica rapa* grown under ambient CO₂ and ambient temperature (ACAT), elevated CO₂ and ambient temperature (ECAT), ambient CO₂ and elevated temperature (ACET) or elevated CO₂ and elevated temperature (ECET). Values are means±1 SEM (n=12 for ACAT and n=4 for all other treatments)

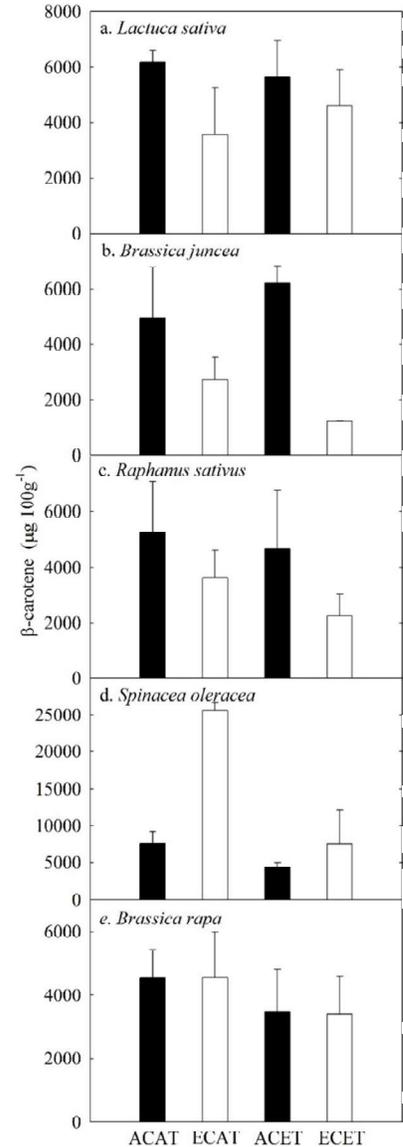


Fig. 3 : Content of β-carotene (mg 100 g⁻¹) in harvestable parts of *Lactuca sativa*, *Brassica juncea*, *Raphanus sativus*, *Spinacea oleracea* and *Brassica rapa* grown under ambient CO₂ and ambient temperature (ACAT), elevated CO₂ and ambient temperature (ECAT), ambient CO₂ and elevated temperature (ACET) or elevated CO₂ and elevated temperature (ECET). Values are means±1 SEM (n=12 for ACAT and n=4 for all other treatments)

Statistical analysis

Effects of CO₂, temperature and CO₂ x temperature interactions were examined using ANOVA for a randomized complete block design, where the chamber was the experimental unit. Comparisons among treatment means were made by least significant difference. Difference between treatments was considered significant if $P < 0.05$.

RESULTS AND DISCUSSION

Fresh biomass

Fresh biomass generally increased for all vegetable species when atmospheric CO₂ and temperature increased concurrently (Fig. 1). Fresh biomass, however, was mostly unaffected by CO₂ level at ambient temperature in the five vegetable species. At elevated temperature, biomass responses to elevated CO₂ varied greatly among species. While fresh biomass increased significantly for radish and turnip green, it decreased in lettuce, mustard green and spinach at elevated CO₂.

Folates

There was a clear pattern in the response of folate content to CO₂ level and growth temperature in lettuce, mustard green, radish and spinach. At ambient temperature, folate content was usually lower at elevated than at ambient CO₂ (Fig. 2). The opposite was true for folate content in these four vegetables at higher temperature. Folate content was usually higher at elevated than at ambient CO₂ (Fig. 2). Folate content in turnip green was unaffected by CO₂ level, regardless of growth temperature (Fig. 2).

β-carotene

Elevated CO₂ mostly reduced β-carotene content in lettuce, mustard green and radish but increased its content in spinach at both lower and higher growth temperature (Fig. 3). β-carotene content in turnip green was unaffected by CO₂ level or temperature (Fig. 3). β-carotene content was generally lower at higher temperature and elevated CO₂ for all five vegetable species studied in our experiment.

How future growing conditions affects yield of vegetables has much implication for vegetable producers and consumers alike as vegetables are mostly marketed and consumed in fresh form. It is interesting to find from our study that elevated CO₂ had no enhancing effect on fresh biomass production

at ambient temperature, whereas elevated CO₂ only increased fresh biomass production at higher temperature in two of the five species studied. These results did not seem to be in complete agreement with those from most previous studies on effects of elevated CO₂ on dry biomass production (Mortensen, 1994; Curtis & Wang, 1998). While response of biomass production to elevated CO₂ has been shown to be species-specific and variable in magnitude under different growing conditions, biomass production always increases with increasing CO₂ in the atmosphere. One likely reason for the disagreement may be the varying water content in fresh biomass of different vegetables, which have water content ranging from 90 to 99% (Popkin et al. 2010). The vast majority of previous studies measured dry biomass of plants grown under simulated environmental changes, whereas our study focused on fresh biomass of vegetables, hence the apparent nonconsistency in biomass production in response to elevated CO₂ at different temperatures.

Crops, particularly fresh vegetables, are important sources of dietary folate for people worldwide (Hanson & Gregory 2011). Any change in folate content in common vegetables in future will have significant health implications for humans. The varying response of folate to higher temperature and elevated CO₂ demonstrates the complex nature of the effect of environmental changes on nutritional quality of vegetables. At ambient temperature, folate was either reduced or unaffected by CO₂ enrichment. At higher temperature, folate increased in most vegetables in response to elevated CO₂. Whereas the exact mechanism why folate content was mostly higher at elevated CO₂ is not well understood, greater supply of intermediates for folate biosynthesis may be a contributing factor. Because of concurrent increase in CO₂ level in the atmosphere and temperature projected for the end of the 21st century (IPCC, 2023), nutritional value of common vegetables as indicated by folate content may be higher in the future. Increasing CO₂ and temperature could thus ameliorate nutritional deficiency to some extent in the less developed regions of the world where dependency on vegetables for folate intake is greater than in the more industrialized countries.

β-carotene has been shown to have numerous health benefits to humans (Kopsell & Kopsell, 2006). It is thus imperative to understand how its content in

vegetables will be affected by global environmental changes. Although responses of β -carotene to changes in growth conditions were variable, it is obvious that β -carotene content in common vegetables will decrease as CO₂ and temperature increase in the coming decades. Our findings are consistent with earlier results that showed a decrease in β -carotene in plants grown at higher temperature and CO₂ and these changes are species-specific (Agar et al., 1997; Lefsrud et al., 2005). As a result, a number of common vegetables grown in a future environment will have a lower level of vitamin precursor and vitamins, which are the major reasons why many people purchase and consume fresh vegetables. Lower nutritional quality in vegetables as indicated by lower level of β -carotene suggests consumers in the future may need to consume a greater amount of fresh vegetables to obtain an equivalent amount of vitamin precursor and vitamins as they currently do in order to meet nutritional requirements. This will apparently have a negative impact on human health as CO₂ and temperature continue to increase despite the zero-carbon efforts under way to slow down carbon emission (IPCC, 2023).

One noteworthy finding in our study was that different vegetable species responded to changing environmental conditions in both carotenoids and β -carotene levels. While the precise mechanism of species-specific responses is unclear, we hypothesize that it is related to their different optimal temperature in photosynthesis and biomass production. More detailed studies on how a wider range of temperature regimes, CO₂ level and other environmental factors will be needed to have a more complete understanding of nutritional changes in common vegetables.

CONCLUSION

Results demonstrated that yield and nutritional quality of common vegetables would be affected by global environmental changes, such as elevated CO₂ in the atmosphere and rising temperature. The direction and magnitude of these effects, however, are non-uniform across vegetable species and nutrition-quality indicators. Folate will mostly increase, but β -carotene will mostly decrease in a future environment when atmospheric CO₂ and temperature are higher than today's level. The findings from our study have important implication for the nutritional well-being of people in the future, particularly for those in the less

developed regions of the world, who may be less able to afford nutritional supplements and have to depend primarily on fresh vegetables for nutrient uptake.

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