Effects of elevated CO_2 on vitamin E concentrations in grains of two wheat cultivars – A FACE study

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Abstract
Vitamin E is essential for the human diet. Wheat (*Triticum aestivum* L.), as a basic food staple for the human diet, supplies not only significant amounts of dietary carbohydrates and proteins, but is also abundant in structurally related vitamin E compounds called tocols. While it has been shown that rising atmospheric CO_2 [CO_2] decreases protein as well as macro- and micro nutrients in wheat grains, possible effects of elevated [CO_2] (e[CO_2]) on tocols have received little attention. This study compares the tocols (α–, γ–, δ–tocopherols and α–, β–, γ–tocotrienol) of two wheat cultivars (Janz and Zebu) under two CO_2 levels (ambient a[CO_2] ~390 ppm, e[CO_2] ~550 ppm) in order to study intra-specific variability of responses to e[CO_2]. Plants were grown within the Australian Grains Free Air Carbon Dioxide Enrichment (AGFACE) facility, Horsham (Victoria). Tocols of grains were analysed three weeks before and at maturity. Tocol concentrations were not affected by CO_2 treatment. Cultivar specific differences were only found for tocotrienols but not for tocopherols with Zebu having significantly greater α–, β–, γ–tocotrienol concentrations than Janz. Also, tocol concentrations decreased from early to late sampling date, except for α-tocotrienol, which increased, and γ-tocopherol, which did not change between sampling dates. Based on these findings our data suggest that rising CO_2 concentrations do not impact tocol concentrations in wheat grains, which has positive implications for the food quality of a future climate.

Key Words
Triticum aestivum, vitamin E, elevated CO_2, climate change, Free Air Carbon Dioxide Enrichment (FACE)

Introduction
Wheat is one of the three major cereal crops and provides one fifth of the total caloric input of the world's population. Wheat is not only is a source of carbohydrates but it is also a significant source of proteins and antioxidative compounds such as Vitamin E (Shewry 2009).

Vitamin E is a fat-soluble vitamin and is essential for human health (Colombo 2010). It is exclusively produced by photosynthetic organisms and is therefore an essential dietary component for humans and animals (Hidalgo et al. 2006). Vitamin E comprises a family of structurally related compounds, called tocols, which are α–, β–, γ– and δ–tocopherol and α–, β–, γ– and δ–tocotrienol. Out of the eight tocols, α-tocopherol has the highest vitamin E activity (Hidalgo et al. 2006). It has been shown that α-tocopherol from wheat bread and wheat germ has high bio-availability, indicating that wheat and wheat products are important sources of α-tocopherol (Lampi et al. 2008). In fact, Gao et al. (2004) suggested increasing the consumption of bread and other cereal servings to six a day to meet the intake recommendations of Vitamin E without increasing the amount of oils and fats in the diet.

The most certain factor of climate change is the rapid increase in atmospheric CO_2 concentration [CO_2]. Given that [CO_2] plays a significant role for the primary metabolism of plants, direct effects of elevated [CO_2] on plant performance have been reported. For example, it has been shown that elevated [CO_2] enhances growth and yield of crop plants (Ainsworth and Long 2005). However, increase in biomass production and yield is associated with changes in the chemical composition in plants. For example, wheat grown under elevated [CO_2], had significantly lower macro and micro nutrient concentrations as well as less protein concentrations in vegetative plant parts (Fangmeier et al. 1999) and in the grain (Fangmeier et al. 1999, Erbs et al. 2010) compared to their ambient [CO_2] grown counterparts. To our knowledge, there is no information available on how elevated CO_2 affects Vitamin E in wheat grains.

In order to study intra-specific variability of responses to elevated [CO_2] (e[CO_2]), we compared tocol concentrations (tocopherols and tocotrienols) of two wheat cultivars (‘Janz’ and ‘Zebu’) under two CO_2 levels (ambient a[CO_2] ~390 ppm, e[CO_2] ~550 ppm). A major focus was laid on α-tocopherol as it has the highest Vitamin E activity within the tocols. Plants were grown at the Australian Grains Free Air Carbon dioxide Enrichment (AGFACE) facility, Horsham (Victoria), within the Australian wheat belt. Tocols of grains were analysed three weeks before and at maturity. The aim of our work was to identify variation in Vitamin E concentrations (1) among wheat cultivars and (2) how they are affected by increased [CO_2].

Methods

Plant material and growth conditions

Janz and Zebu were grown within the 7.5 ha AGFACE facility 7 km west of Horsham, Victoria. A detailed description of the site and exposure set-up was provided by Mollah et al. (2009). In short, four ambient [CO_2] and four elevated e[CO_2] plots of 16 m diameter were used with elevated [CO_2] plots fumigated to an elevated target CO_2 of 550 μmol mol^{-1}. Within each plot, sub-plots (4 m x 1.5 m) of cultivars ‘Janz’ and ‘Zebu’ were randomly allocated and sown onto flat beds on 0.27 m row spacing. Sowing date was 27 May 2010. Grains were sampled on 19 November 2010 and at maturity on 9 December 2010.

Tocol analyses

Tocopherols and tocotrienols were measured by HPLC according to a modified method of Tausz et al. (2003). 0.5±0.01 g of lyophilised and ground grain was shaken in 1ml ice-cold acetone and centrifuged at 2°C for 2 minutes at maximum speed (10 000g; centrifuge 5415D; Eppendorf AG, Germany). The supernatant was collected in a cooled extra tube and the pellet was re-extracted twice for each sample. 20µl of the combined supernatant were subjected to an isocratic HPLC analysis using pure methanol as the solvent with 1ml min^{-1} flow. Tocopherols were detected directly by fluorometry (excitation 295 nm, emission 325 nm) using a Jasco FP-920 Intelligent Fluorescence Detector.

Statistical analyses

Data were analysed using SAS/STAT software. A split-plot ANOVA was conducted with CO_2 treatment as main-plot factor as well as cultivar as sub-plot factor and time of sampling as sub-sub-plot factor. Homogeneity of variances was checked with the Levene’s test. Distribution of residuals was graphically checked for deviations from normality.

Results and Discussion

α-Tocopherol, which has been related to the tocol with greatest vitamin E activity in humans (Hidalgo et al. 2006), ranged in concentration from 10 to 16 μg g^{-1} DW across treatments (Figure 1). This is within the reported range of 9.1 to 19.9 μg g^{-1} DW of Lampi et al. (2008) who compared α-tocopherol values of 130 winter wheat cultivars.

Sampling date had the greatest effect on α-tocopherol, with concentrations decreasing ~20% from early sampling to maturity (Figure 1). Similarly, sampling date significantly affected other tocols with β–,γ--tocotrienols decreasing from early sampling date to maturity by ~30% and α-tocotrienol increasing from early sampling date to maturity by ~50% (p-values in Table 1). These developmental-related changes were in good agreement with previous studies. For example, in barley, individual forms of tocopherols and tocotrienols accumulated with different dynamics during grain development (Falk et al. 2004) or Bramley et al. (2000) also reported of changing tocopherol concentrations up to 10fold for fruits and vegetables during the ripening process. These developmental related changes in tocols are related to their primary function to limit non-enzymatic lipid oxidation during seed storage, germination and early seedling development (Sattler et al 2004).

Tocols were not affected by CO_2 treatment or its interactions with cultivar or sampling date (except for a marginally significant CO_2 effect on α–tocotrienol (p=0.073, data not shown) with e[CO_2] grown plants having slightly decreased α-tocotrienol values). Similarly, Tausz et al (2004) reported that α-tocopherol concentrations in pine needles were not affected by increased [CO_2]. To our knowledge, there are no studies available on the effects of elevated [CO_2] on tocol concentrations in grains. These results indicate that vitamin E supply may be maintained in a future climate with elevated [CO_2].

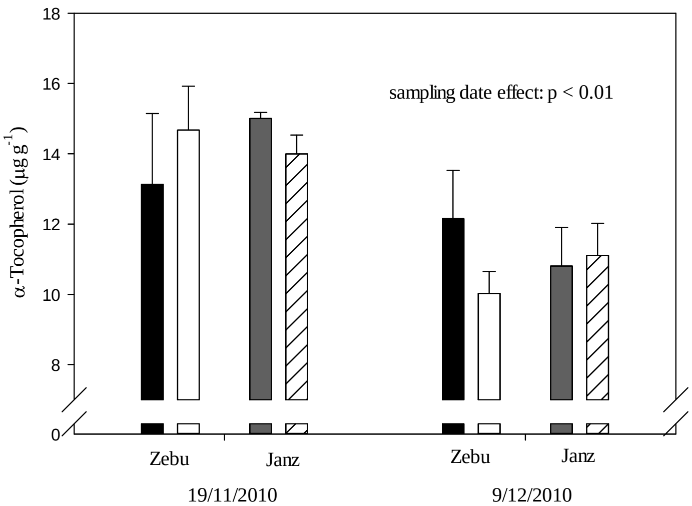

Figure 1. α-Tocopherol concentrations in grains of wheat cultivars 'Zebu' and 'Janz' when grown under elevated [CO_2] or ambient [CO_2]. Grains were harvested three weeks before (20/11/2010) and at maturity (9/12/2012). Black column: Zebu elevated [CO_2], white column: Zebu ambient [CO_2], grey column: Janz elevated [CO_2], dashed column: Janz ambient [CO_2]. Each column is the mean ± SE of 4 replicates.

Table 1. Various tocols measured in grains of two wheat cultivars when grown either at a[CO_2] or e[CO_2]. Grains were harvested three weeks prior to maturity and at maturity. Significance values for CO_2, cultivar and sampling date and their interactions are shown. Significant effects are in bold type, ns stands for not significant.

Tocols	CO_2	Cultivar	Sampling date	CO_2 x Cultivar	CO_2 x Date	Cultivar x Date	CO_2 x Cultivar x Date
α-Tocopherol	ns	ns	**0.007**	ns	ns	ns	ns
δ-Tocopherol	ns	ns	**<0.001**	ns	ns	ns	ns
γ-Tocopherol	ns	ns	ns	ns	ns	ns	ns
α–Tocotrienol	ns	**0.004**	**<0.001**	ns	ns	**0.039**	ns
β,γ–Tocotrienol	ns	**0.015**	**<0.001**	ns	ns	ns	ns

Significant cultivar-specific responses were found for tocotrienols but not for tocopherols. In detail, α, β, γ tocotrienols were 15-25% greater in Zebu compared to Janz, with α-tocotrienol being greatest in Zebu at maturity which resulted in a significant cultivar x sampling date interaction (Table 1). Large intra-specific variations in tocol concentrations were also found by Lampi et al. (2010) who suggested that tocopherol concentrations vary less among cultivars compared to tocotrienols.

Conclusion

Substantial differences in tocols between sampling dates and intra-specific variability between the studied cultivars were demonstrated. Elevated [CO_2] marginally affected α–tocotrienol, whereas other measured tocols did not respond to elevated [CO_2].

Acknowledgements

Research at the AGFACE facility is run by the Victorian State Department of Primary Industries (DPI) and The University of Melbourne, and receives crucial funding support by the Australian Commonwealth Department for Agriculture, Fisheries and Forestry (DAFF) and the Grains Research and Development Corporation (GRDC). ST-P gratefully acknowledges a University of Melbourne Early Career Researcher Grant. We wish to thank M. Mollah and R. Argall with the whole AGFACE support team for running the AGFACE facility.

References

Ainsworth EA and Long SP (2005). What have we learned from 15 years of free-air CO(2) enrichment (FACE)? A meta-analytic review of the responses of photosynthesis, canopyproperties and plant production to rising CO2. New Phytologist 165, 351-371.

Bramley PM, Elmadfa I, Kafatos A, Kelly FJ, Manios Y, Roxborough HE, Schuch W, Sheehy PJA and Wagner KH (2000). Vitamin E. Journal of the Science of Food and Agriculture 80, 913-938.

Colombo ML (2010). An Update on Vitamin E, Tocopherol and Tocotrienol-Perspectives. Molecules 15, 2103-2113.

Erbs M, Manderscheid R, Jansen G, Seddig S, Pacholski A and Weigel HJ (2010). Effects of free-air CO_2 enrichment and nitrogen supply on grain quality parameters and elemental composition of wheat and barley grown in a crop rotation. Agriculture Ecosystems & Environment 136, 59-68.

Falk J, Krahnstover A, van der Kooij TAW, Schlensog M and Krupinska K (2004). Tocopherol and tocotrienol accumulation during development of caryopses from barley (*Hordeum vulgare* L.). Phytochemistry 65, 2977-2985.

Fangmeier A, De Temmerman L, Mortensen L, Kemp K, Burke J, Mitchell R, van Oijen M and Weigel HJ (1999). Effects on nutrients and on grain quality in spring wheat crops grown under elevated CO_2 concentrations and stress conditions in the European, multiple-site experiment 'ESPACE-wheat'. European Journal of Agronomy 10, 215-229.

Gao X, Wilde PE, Maras JE, Bermudez OI and Tucker KL (2004). The maximal amount of alpha-tocopherol intake from foods alone in US adults (1994-1996 CSFII). An analysis by linear programming. In: Vitamin E and Health. Eds F Kelly, M Meydani, L Packer. pp. 385-386, New York Academic Sciences, New York.

Hidalgo A, Brandolini A, Pompei C and Piscozzi R (2006). Carotenoids and tocols of einkorn wheat (*Triticum monococcum* ssp *monococcum* L.). Journal of Cereal Science 44, 182-193.

Lampi AM, Nurmi T, Ollilainen V and Piironen V (2008). Tocopherols and tocotrienols in wheat genotypes in the HEALTHGRAIN diversity screen. Journal of Agricultural and Food Chemistry 56, 9716-9721.

Mollah M, Norton R and Huzzey J (2009). Australian grains free-air carbon dioxide enrichment (AGFACE) facility: design and performance. Crop & Pasture Science 60, 697-707.

Sattler SE, Gilliland LU, Magallanes-Lundback M, Pollard M and DellaPenna D (2004). Vitamin E is essential for seed longevity, and for preventing lipid peroxidation during germination. Plant Cell 16, 1419-1432.

Shewry PR (2009). Wheat. Journal of Experimental Botany 60, 1537-1553.

Tausz M, Wonisch A, Grill D, Morales D and Jimenez MS (2003). Measuring antioxidants in tree species in the natural environment: from sampling to data evaluation. Journal of Experimental Botany 54, 1505-1510.

Tausz M, Olszyk DM, Monschein S and Tingey DT (2004). Combined effects of CO_2 and O^{-3} on antioxidative and photoprotective defense systems in needles of ponderosa pine. Biologia Plantarum 48, 543-548.