

## Ozone fumigation increases the abundance of nutrients in *Brassica* vegetables: broccoli (*Brassica oleracea* var. *italica*) and Chinese cabbage (*Brassica pekinensis*)

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**Abstract** *Brassicaceae* vegetables, among them broccoli and Chinese cabbage, are well recognized due to the nutritional properties. Four-week-old Chinese cabbage and broccoli seedlings were fumigated with O<sub>3</sub> for 3 days before being transplanted into the field. The effect of O<sub>3</sub> treatment was determined after reaching marketable quality (ca. 10 weeks). The inflorescences of O<sub>3</sub>-treated broccoli were enriched in vitamin E (α-tocopherol and γ-tocopherol), whereas Chinese cabbage heads had an increased content of anthocyanins and β-carotene. Ozone treatment did not significantly affect the productivity of both examined vegetables.

**Keywords** Antioxidants · Anthocyanin · Vitamin E · β-Carotene

### Introduction

Out of the numerous species and strains of the *Brassicaceae* family cultivated, broccoli (*Brassica oleracea* var. *italica*) and Chinese cabbage (*Brassica pekinensis*) are emerging as the most willingly consumed in Europe and North America. The rising interest of dietitians, food industry and consumers is drawn by their unique dietary properties, relatively low growth demands and low price. Both vegetables are abundant in protein, minerals (calcium, phosphorus, iron), sulfur-containing compounds [1] and vitamins, such as provitamin A (β-carotene), vitamin C (ascorbate) and vitamin E (tocopherol), with antioxidant properties [2]. The role of antioxidants in plant and animal physiology seems undisputable. In many cases, their abundance determines the plant's capacity to cope with unfavorable environmental conditions, whereas in humans, they are associated with chronic disease risk reduction [3, 4] including several types of cancer [5], cardio- and cerebro-vascular, ocular and many neurological diseases [2]. Increasing the abundance of antioxidants and other dietary compounds in plants from the *Brassicaceae* family seems to be of significant importance.

Ozone (O<sub>3</sub>) is a model abiotic elicitor of reactive oxygen species (ROS) in plant cells. It enters the leaves through open stomata and due to its high reactivity immediately reacts with components of the apoplastic space generating various ROS and activating detoxification, including enzymatic and non-enzymatic antioxidants and other defense mechanisms [6, 7]. Moderate doses of O<sub>3</sub> may enhance plant resistance, and thus, utilizing O<sub>3</sub> in improving the dietary quality of vegetables seems a promising perspective [8]. In the past, O<sub>3</sub> fumigation has been reported to have a positive impact on plant growth and attempts have been made to utilize O<sub>3</sub> in plant productivity improvement

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[9]. Most recently, our studies on white cabbage confirmed the possibility to improve marketable yield by fumigating seedlings with mild doses of O<sub>3</sub> [10].

## Materials and methods

### Plant material and ozone treatment

*Brassica oleracea* var. *italica* cultivar Monotop F1 and *Brassica pekinensis* cultivar Mirako F1 seeds were sown in a greenhouse to multipots filled with peat substrate. After 4 weeks, seedlings were transferred to closed top plexiglass chambers for ozone fumigation. Ozone (mixed with ambient air) was supplied by the Fischer type 500 M ozone generator (Germany). A constant concentration of 70 µg m<sup>-3</sup> of O<sub>3</sub> was controlled by the 49C UV photometric O<sub>3</sub> analyzer (Thermo Environmental Instruments Inc. USA). Plants were cultivated under a 12-h photoperiod at 400 µmol m<sup>-2</sup> s<sup>-1</sup>, with a constant day/night temperature of 17/13 °C. The humidity inside fumigation chambers was ca. 60 %. After fumigation, plant seedlings were transferred into the field in Mydlniki, Krakow (50°5'5"N 19°51'8"E). After 10 weeks in agriculture, plants were harvested for analysis. For one sample, two outermost leaves were harvested from three Chinese cabbage plants. For broccoli, three inflorescences were pooled together for one sample. All analyses were performed in three independent experiments.

### Tocopherol and β-carotene content determination

The abundance of tocopherols and β-carotene was measured with HPLC method according to the procedure described previously by Heudi et al. [11] with modifications according to Nosek et al. [12].

### Anthocyanin content determination

Anthocyanin content was determined spectrophotometrically according to the method described by Schmidt and Mohr [13]. Anthocyanins were extracted from 18 % (v/v) 2-propanol containing 1 % (v/v) HCl, and its abundance was determined as the difference between A<sub>650</sub> and A<sub>535</sub> per gram of fresh weight.

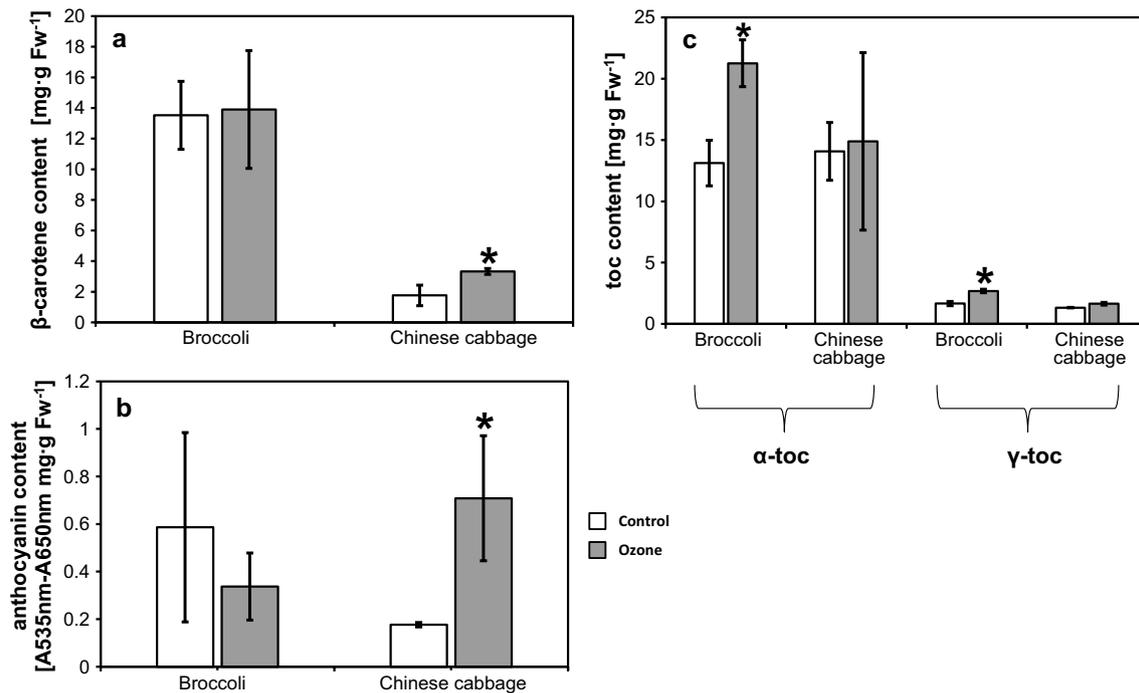
### Statistical analysis

Statistical analysis was performed by Statistica (Statsoft, USA) statistical software. One-way ANOVA followed by Tukey's HSD multiple range test was used to determine the individual treatment effects at using a significance level of 0.05.

## Results and discussion

Ozone is perceived as a dangerous gaseous pollutant responsible for plant growth limitation and yields loss [9]. Nevertheless, many reports indicate a positive role of episodes of near ambient concentrations of O<sub>3</sub> on plant growth. As previously reported, doses of up to 150 µg m<sup>-3</sup> had been utilized to enhance the productivity of plants such as *Phaseolus vulgaris*, natural grassland species, trees and white cabbage [9, 10, 14, 15]. According to the results presented in this communication, episodes of elevated O<sub>3</sub> during vegetation applied prior to transplantation affect the content of important dietary substances in Chinese cabbage and broccoli. O<sub>3</sub> treatment had no significant impact on plant productivity as size or marketable yield was unchanged in fumigated plants (data not shown). *B. oleracea* var. *italica* and *B. pekinensis* differ in their long-term response to O<sub>3</sub> treatment. In inflorescence of broccoli, the abundance of lipophilic, low molecular antioxidants: α-tocopherol and γ-tocopherol, was significantly increased 10 weeks after fumigation (Fig. 1c), but no changes in the content of β-carotene (Fig. 1a) and anthocyanins (Fig. 1b) were observed in O<sub>3</sub>-treated plants. As reported previously, the content of the tocopherols, β-carotene and anthocyanins varies among different cultivars of the same vegetable species. Cultivation conditions, e.g., light quality and quantity are a major factor determining the quantity and composition of important phytochemicals. According to previous reports, the concentration of α- and γ-tocopherol ranges from 22–429 to 2–6, 4 mg g Fw<sup>-1</sup> for the latter [16, 17]. In the present report, the concentration of α-tocopherol was lower (13 mg g Fw<sup>-1</sup>) compared with literature data; however, O<sub>3</sub> fumigation significantly increased its content (21, 25 mg g Fw<sup>-1</sup>). The content of γ-tocopherol was in the middle range of the reported concentrations (2–64 mg g Fw<sup>-1</sup>, Kurlich et al. [16]). O<sub>3</sub> fumigation had a similar impact on its concentration, allowing us to speculate that the abundance of this phytochemical is prone to manipulation. The concentration of other examined substances was in accordance with previously published results [2, 17].

On the contrary, in *B. pekinensis* heads, the content of β-carotene (Fig. 1a) and anthocyanins (Fig. 1b) was increased after fumigation. The relative abundance of anthocyanins was improved over threefold compared with control plants. The concentration of β-carotene after fumigation reached levels (10–40 mg g Fw<sup>-1</sup>) previously reported by Singh et al. [17]. In control plants, its concentration was in the middle range values (17, 6 mg g Fw<sup>-1</sup>) compared with previous reports. Fumigation had no effect on the concentration of tocopherol (Fig. 1c), and it was in accordance with previously reported results [17, 18].



**Fig. 1**  $\beta$ -carotene (a), anthocyanin (b) and -tocopherol  $\alpha$  and  $\gamma$  (c) contents in *Brassica oleracea* var. *italica* and *Brassica pekinensis* treated with  $70 \mu\text{g m}^{-3}$  of  $\text{O}_3$ . Bars represent mean  $\pm$  SD of three

independent series of measurements of (*white*) control plants and (*gray*) fumigated plants. Stars above bars indicate statistically significant differences

## Conclusions

Treatment of *B. pekinensis* with moderate doses of  $\text{O}_3$  may be utilized in enhancing abundance of both  $\beta$ -carotene and anthocyanins. On the other hand, similar treatment may induce accumulation of  $\alpha$ - and  $\gamma$ -tocopherols in *B. oleracea* var. *italica*.

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**Conflict of interest** None.

**Compliance with Ethics requirements** This article does not contain any studies with human or animal subjects.

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