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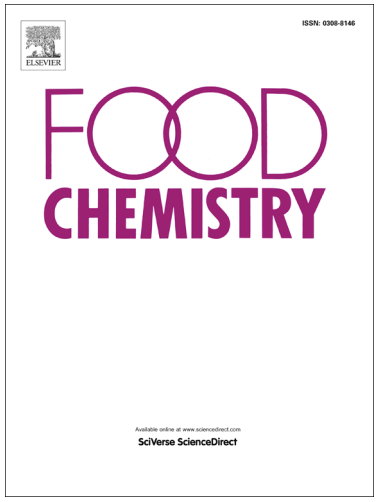

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CO_2 enrichment can enhance the nutritional and health benefits of parsley (*Petroselinum crispum* L.) and dill (*Anethum graveolens* L.)

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Running title:

eCO_2 improves the nutritional and health benefits of herbs

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CO_2, Parsley, Dill, Metabolites, Nutritional value, Medicinal value, Biological activity

Abstract

The functional food value of herbal plants is greatly related to their contents of valuable phytochemicals. Regarding its impact on primary and secondary plant metabolism, CO_2 enrichment could be a candidate strategy to modulate the levels of nutritionally and medicinally interesting phytochemicals in herbal plants. Herein, the concentrations of 98 metabolites and minerals were evaluated in shoot tissues of parsley and dill grown under two levels of CO_2, ambient (378±25 µmole CO_2 $mole^{-1}$ air, aCO_2) and elevated (627±24 µmole CO_2 $mole^{-1}$ air, eCO_2). Regardless of the plant species, eCO_2 improved the levels of soluble sugars, starch, organic acids, some EAAs, most of USFA, total phenolics, total flavonoids and vitamins A and E. However, notable variations in the metabolites responsiveness to eCO_2 were recorded among the tested plant species. Moreover, considerable improvements in the total antioxidant capacity, antiprotozoal, antibacterial and anticancer activities were recorded for parsley and dill in response to eCO2.

1. Introduction

Herbs are plants that are utilized both for their nutritional and health-promoting values (Liu, 2010; Opara & Chohan, 2014). Different parts of herbal plants are used in cooking to improve the flavor and aroma of foods (Peter, 2006). Moreover, herbs are used as natural alternatives to synthetic food preservatives (Badei, Faheid, El-Akel, & Mahmoud, 2002). Regarding their use in traditional medicine, herbs are well recognized for their antioxidant, antimicrobial, antiprotozoal, anti-inflammatory, antidiabetic and anticancer properties (Kurian, 2012). Generally, the nutritional and medicinal properties of medicinal herbs are ascribed to their content of minerals, vitamins, unsaturated fatty acids (USFA), essential amino acids (EAAs) and secondary metabolites such as phenolic acids and flavonoids (Bidlack, Omaye, Meskin, & Topham, 2000). Therefore, much attention has been devoted to improve the levels of these valuable phytochemicals in herbs and other medicinal plants. Indeed, plant tissue chemistry is greatly influenced by the growing conditions and agronomic practices (Björkman et al., 2011). For example, environmental stress and climate change have been reported to affect the accumulation of phytochemicals in plants (Oh, Trick, & Rajashekar, 2009). A similar effect is achieved by application of elicitors, which are substances or growth factors that can trigger metabolic events in plants (Baenas, García-Viguera, & Moreno, 2014). Under these circumstances, plants modulate their metabolism in order to accumulate specific compounds that help in coping with the imposed stress, climate change or elicitor. Although the accumulation of these bioactive compounds could improve nutritional and health-promoting values of plants, however it may be accompanied by reduced growth due to shift in allocation of resources, such as non-structural carbohydrates (NSC) and other intermediate compounds, to secondary metabolism (Matyssek et al., 2014). Therefore, improvement of carbon (C) metabolism could

help in adjusting the resource allocation balance between growth and synthesis of secondary metabolites by improving the availability of precursor compounds and metabolic energy (Ballare, 2009).

Elevated CO_2 (eCO_2) has been investigated as an inducer for growth and as a modulator of metabolism in crop and model plants (Azam, Khan, Mahmood, & Hameed, 2013; Noguchi, Watanabe, & Terashima, 2015). As a substrate of photosynthesis, elevated CO_2, upto a physiological limit, has been reported to enhance the photosynthetic C assimilation, therefore improve the accumulation of NSC and their breakdown via dark respiration (Leakey et al., 2009; Li et al., 2017). This can provide the required precursors and metabolic energy for the synthesis of the different classes of phytochemicals. Therefore, CO_2 fertilization can be used as a strategy to improve the nutritional and health-promoting values of herbal and medicinal plants. In this context, there are some evidences for the fertilization effect of eCO_2 treatment on the growth of several medicinal herbs such as *Isatis indigotica* (Li et al., 2017) and *Hymenocallis littoralis* (Idso et al., 2000). Moreover, some investigations have pointed to the positive impact of eCO_2 on the accumulation of bioactive phytochemicals in herbal and medicinal plants with a subsequent influence on their biological activities (Ghasemzadeh & Jaafar, 2011; Ghasemzadeh, Jaafar, & Rahmat, 2010; Ibrahim & Jaafar, 2011; Idso et al., 2000; Jaafar, Ibrahim, & Karimi, 2012; Wang, Bunce, & Maas, 2003). However, the current understanding for eCO_2-induced changes in the metabolism of herbal and medicinal plants is still fragmentary, as compared with crop or model plants. Accordingly, more efforts are needed to explore the global metabolic changes triggered by eCO_2 in this important group of plants and to assess the accompanying change in their nutritional and medicinal properties.

Among the commonly utilized culinary and medicinal herbs worldwide, both as fresh materials or essential oils, are the Umbelliferae members parsley (*Petroselinum crispum* L.) and dill (*Anethum graveolens* L.). Parsley and dill are valuable sources of minerals, vitamins, USFA, phenolic acids, flavonoids and other bioactive compounds that support their nutritional and medicinal benefits (Karkleliene et al., 2014; Lisiewska, Kmiecik, & Korus, 2006; Parry et al., 2006; Słupski, Lisiewska, & Kmiecik, 2005). They are recognized for their antioxidant, antibacterial, antifungal, antiinflammatory, antidiabetic and anticancer activities (Chahal, Kumar, Bhardwaj, & Kaur, 2017; Farshori, Al-Sheddi, Al-Oqail, & Siddiqui, 2013; Wong & Kitts, 2006). Growth, phytochemicals accumulation and oil composition of these two herbs are known to be influenced by several stress agents and conditions (Frąszczak, 2009; Hashem & Sahab, 1999; Petropoulos, Daferera, Polissiou, & Passam, 2009) . However, to our knowledge, the impact of eCO_2 on the nutritional and health-promoting values of parsley and dill has not been investigated. Therefore, the current study was conducted to elucidate the impact of eCO_2 treatment (627±24 µmole CO_2 $mole^{-1}$ air) on the concentrations of metabolites (sugars, amino acids, fatty acids, vitamins, phenolic acids and flavonoids) and minerals in parsley and dill as well as the associated changes in their biological activities. Moreover, to explore the correlation between different classes of phytochemicals and to understand the global metabolic response of the two herbal plants to eCO_2, Hierarchical Clustering Analysis (HCA) was performed.

2. Materials and methods

2.1. Plant materials, growth microclimate and eCO_2 treatment

Parsley (*Petroselinum crispum* L.) and dill (*Anethum graveolens* L.) seeds, purchased from online supplier (https://www.seeds4garden.com/; codes SL2150 and SL2070, respectively), were sown in plastic pots filled with a mixture of loamy soil and organic compost (1:1, v/v). Pots were maintained in controlled-growth cabinet under 21/18 °C air temperature, 16/8 h day/night photoperiod and two mole fractions of atmospheric CO_2, ambient (378±25 µmole CO_2 $mole^{-1}$ air, aCO_2) and elevated (627±24 µmole CO_2 $mole^{-1}$ air, eCO_2). eCO_2 scenario was chosen according to the IPCC-SRES B2-scenario prediction of moderate change for the year 2100 (Murray & Ebi, 2012). Two independent growth cabinets were used for the two climate scenarios. For each treatment, twenty pots, six plants each, were distributed in the growth cabinet. All pots were irrigated to maintain soil humidity of 65%. After four weeks, some shoots were harvested, thoroughly washed with double distilled water, gently blotted, dried at 75 °C for 72 h and then used for dry mass determination and elemental analysis. The residual shoot material was immediately frozen in liquid nitrogen and stored at -20 °C until processing. In order to minimize any bias among the cabinets, the experiment had been replicated once again with swapping the two CO_2 levels among the cabinets. CO_2 was supplied in the airflow of the cabinet and its concentration was continuously monitored with a CO_2 analyser (WMA-4, PP Systems, Hitchin, UK).

2.2. Identification and quantification of individual metabolites and their totals

Chromatographic techniques, HPLC or GC/MS, were employed to determine the levels of individual sugars, organic acids, amino acids, fatty acids, phenolic acids, flavonoids and vitamins in plant samples (Hamad et al., 2015; Torras-Claveria, Berkov, Codina, Viladomat, & Bastida, 2014). Fatty acids were identified using NIST 05 database and Golm Metabolome

Database (http://gmd.mpimp-golm.mpg.de). The rest of compounds were identified by comparing their relative retention time with those of the standard mixture. The concentration of each compound was calculated based on peak area and comparison with a calibration curve of the corresponding standard. Nelson's, Folin–Ciocalteu and aluminum chloride colorimetric assays were employed to determine the total content of soluble sugars, total phenolics and flavonoids, respectively (Saleh, Madany, & González, 2015).

2.3. Determination of individual Minerals

For extraction of minerals, 200 mg of powdered-oven dried shoot materials were digested in 10 ml of 30% nitric acid for 48 h. The concentrations of macro and micro-elements were determined using inductively coupled plasma mass spectrometry (ICP-MS, Finnigan Element XR, Scientific, Bremen, Germany).

2.4. Biological activity assessments

Total antioxidant capacity was determined according to the commonly utilized ferric reducing antioxidant power (FRAP) and diphenylpicrylhydrazyl (DPPH) assays and expressed as µmole Trolox equivalent g^{-1} dry weight or as % inhibition of DPPH radical, respectively. Antibacterial activity of the plant extracts was determined using the disc diffusion method (7.5 mg/disc) on Muller Hinton agar (MHA) and expressed as diameter of inhibition zone, mm (Selim, Aziz, Mashait, & Warrad, 2013). In vitro assessment of the antiprotozoal activity of the plant extracts (5 mg/ml) against *Trypanosoma cruzi* was conducted according to the protocol described by Räz, Iten, Grether-Bühler, Kaminsky and Brun (1997) and expressed as percentage reduction in parasite load relative to the control. Moreover, plant extracts (2 mg/ml) were tested for their anticancer activities against four human cell lines, hepatocellular carcinoma (HepG2), colon carcinoma (Colo205), embryonic kidney adenocarcinoma (293) and urinary bladder

carcinoma (T24P). Cell viability was determined by CellTiter-Blue reagent (Promega, Madison, WI, USA) as described by Solowey et al (2014) and the results were expressed as % dead cells.

2.5. Statistical analyses

Experiments were carried out following a completely randomized block design. Data analyses were performed using the Statistical Analysis System (SPSS Inc., Chicago, IL, USA). Data normality and the homogeneity of variances were checked using the Kolmogorov– Smirnov test and Levene´s test, respectively. All the data were subjected to one-way analysis of variance (ANOVA). Tukey's Test ($p \leq 0.05$) was conducted as the post hoc test for separation of means. Number of replicates for each experiment were three (n = 3). Cluster analysis was performed by using Pearson distance metric of the MultiExperiment Viewer (MeV)™ 4 software package (version 4.5, Dana-Farber Cancer Institute, Boston, MA, USA).

3. Results

3.1. Biomass, sugars, organic acids and EAAs are enhanced in response to eCO_2

Elevated CO_2 treatment (627±24 µmole CO_2 $mole^{-1}$ air) resulted in significant improvements in the biomass production in parsley and dill, about 1.5 fold relative to aCO_2 conditions (378±25 µmole CO_2 $mole^{-1}$ air) (Figure 1). The accumulation of individual soluble and insoluble sugars and organic acids were assessed in both plants under the two CO_2 scenarios. Under aCO_2 conditions, parsley shoot tissues contained higher levels of glucose, fructose, sucrose, total soluble sugars, starch and total carbohydrates as compared to dill (Table 1). However, both plants contained comparable levels of organic acids, except for succinic acid that was significantly higher in parsley. eCO_2 treatment improved the accumulation of all sugar fractions in both plants, whereas glucose was the most accumulated soluble sugar in parsley

(0.68) and fructose was the most accumulated one in dill (0.61), as indicated by fold change calculations (Figure 2). Starch showed comparable fold changes (0.29) in both plants as affected by eCO_2. Regarding organic acids, citric, isobutyric, fumaric and malic acids showed similar response to eCO_2 in both plants, whereas the levels of citric and isobutyric were significantly increased and those for fumaric and malic were not significantly affected (Table 1). On the other hand, the level of succinic acid significantly improved in parsley but not affected in dill, and that for oxalic acid significantly reduced in dill but improved in parsley, as affected by eCO_2. On fold change basis, citric, isobutyric and succinic acids showed higher improvement in parsley than in dill, in response to eCO_2 (Figure 2).

Essential (EAAs) and non-essential amino acids (NEAAs) were quantified in shoots of parsley and dill grown under both aCO_2 and eCO_2 scenarios (Table 1). In both plants, lysine was the major EAAs followed by histidine, while glutamine was the abundant NEAAs followed by glutamate. The levels of all the EAAs and most of the detected NEAAs were higher in parsley as compared with their respective levels in dill. Moreover, parsley shoot contained about 4.75 and 1.13 fold amounts of the total essential and non-essential amino acids, respectively, as compared with dill. Under eCO_2, the levels of isoleucine, methionine, cystine, glutamine, ornithine and proline were significantly improved in both plants, when compared with their corresponding levels in aCO_2 plants. Some amino acids (leucine, lysine, phenylalanine and serine) were significantly induced in parsley but not dill, while others (valine and aspartate) were significantly induced in dill only, as affected by eCO_2. Moreover, total content of EAAs showed 0.51 and 0.10 fold increase in parsley and dill, respectively, in response to eCO_2 (Figure 2). The highest fold changes in amino acids levels as affected by eCO_2 were recorded for phenylalanine (1.36) and

lysine (0.66) in parsley and for methionine (1.50) aspartate (0.96), isoleucine (0.93) and cystine (0.90) in dill.

3.2. Levels and composition of fatty acids are altered by eCO_2 treatment

Parsley and dill contained comparable levels of most of the detected saturated fatty acids (SFA), while the contents of the majority of the detected USFA and their total were significantly higher in parsley (Table 1). Hexadecanoic (C16:0) was the dominant SFA, where it accounted for about 25 and 22% of the total SFA in parsley and dill, respectively. However, in both plants, octadecatrienoic and hexadecadienoic acids were the most abundant USFA. CO_2 enrichment did not significantly affect the accumulation of individual SFA or their total content in parsley, but significantly increased their levels in dill. On the other hand, eCO_2 treatment resulted in 0.24 and 0.45 fold increase in the total content of USFA in parsley and dill, respectively, (Figure 2). Such accumulation in total USFA was mainly due to accumulation of hexadecadienoic (C16:2), hexadecatrienoic (C16:3) and octadecatrienoic (C18:3) in parsley and most of the detected USFA in dill. The higher fold changes in the accumulation of USFA was observed for octadecatrienoic (C18:3) (0.60) in parsley and for hexadecanoic (C16:1) (0.91) in dill. Moreover, in both plants, eCO_2 resulted in lower SFA/USFA ratios as compared with aCO_2 conditions, whereas the lowest value (0.83) was detected in the treated parsley.

3.3. Levels of antioxidant metabolites as affected by eCO_2

Parsley and dill possessed similar phenolic acids, flavonoids and vitamins profiles with comparable levels of the detected compounds except for phylloquinone (vitamin K1) that was significantly higher in parsley (Table 2). In both plants, gallic acid, quercetin and α-carotene (vitamin A) were the most abundant phenolic acid, flavonoid and vitamin, respectively. eCO_2

treatment significantly increased the levels of most of the detected individual phenolic acids and flavonoids in parsley but not in dill. However, the total contents of flavonoids and phenolics were significantly enhanced in both plants as affected by eCO_2. Moreover, eCO_2 treatment resulted in significant increase in the levels of β-carotene, β-tocopherol and γ-tocopherol in parsley but not in dill and those for α-tocopherol and δ-tocopherol in dill only, meanwhile the β-cryptoxanthin and total tocopherol content was improved in both plants. On fold change basis, resorcinol was the most enhanced phenolic acid (15.17) in parsley and quercetrin was the most accumulated flavonoid (7.93) in dill, as affected by eCO_2 (Figure 2). Moreover, β-tocopherol and γ-tocopherol showed the highest fold changes (2.38 and 3.71, respectively) in parsley, while δ-tocopherol accounted for the highest fold change (0.64) in dill, in response to eCO_2 treatment.

3.4. Accumulation of minerals in parsley and dill is not affected by eCO_2

The levels of four macronutrients (K, Ca, Mg and P) and four micronutrients (Zn, Cu, Fe and Mn) were assessed in shoot tissues of parsley and dill under both aCO_2 and eCO_2 conditions. Parsley contained higher levels of K, Mg, Zn and Mn, however dill possessed higher values of Ca, P and Fe. The eCO_2 treatment did not exert a significant impact on concentrations of minerals in both plants, but for Ca and Zn there were significantly improvements in response to CO_2 enrichment.

3.5. Elevated CO_2 improves the biological activities of parsley and dill

Under aCO_2 conditions, parsley and dill showed comparable values of total antioxidant capacity, both FRAP and DPPH, antiprotozoal (*Trypanosoma cruzi*), anti-bacterial (*Escherichia coli* and *Streptococcus* sp.) and anticancer (HepG2 and Colo205) activities (Table2). On the other hand, parsley extract showed higher activities against embryonic kidney adenocarcinoma

(293) and urinary bladder carcinoma (T24P) than that of dill. CO_2 enrichment significantly improved all the measured biological activities for both plants, except for antihepatocellular carcinoma (HepG2) in dill. The fold change calculations revealed that the eCO_2-induced improvement in FRAP and DPPH scavenging activities was more obvious in dill than in parsley, however similar fold change was observed for antiprotozoal activity in both plants (Figure 2). Moreover, the highest fold change in antibacterial and anticancer activities was accounted for *Streptococcus* sp. (0.74) and HepG2 cell line (0.54), respectively, in parsley.

4. Discussion

The nutritional and medicinal values of herbs are strongly correlated with their content of minerals, vitamins, USFA, EAAs and secondary metabolites such as phenolic acids and flavonoids. In the current research, the impact of eCO_2 (627±24 µmole CO_2 $mole^{-1}$ air) on the concentrations of 98 metabolites and minerals in shoot of parsley and dill were evaluated as compared to their aCO_2 conditions. Hierarchical clustering analysis revealed that, under aCO_2 atmosphere, the two plants had qualitatively similar but somewhat quantitatively different metabolic profiles (Figure 3). Moreover, notable variations in the metabolites responsiveness to eCO_2 were recorded among the tested plant species.

CO_2 enrichment is known to improve the rate of photosynthesis by suppressing the oxygenation reaction of rubisco, the key enzyme in photosyhthetic C fixation, where CO_2 and O_2 act competitively for this enzyme (Pérez-López et al., 2009). Accordingly, such upregulation of photosynthesis will improve the accumulation of sugars and also enhance its breakdown through dark respiration leading to accumulation of respiration intermediates such as organic acids (Li et al., 2013; Watanabe et al., 2014). Beside their diverse role in plant growth and metabolism, the significant accumulation of soluble sugars, starch and organic acids, such as citric and isobutyric, reported herein under eCO_2 treatment could improve the culinary value of parsley and dill as sugars and organic acids are determinants for taste and flavor (Malundo, Shewfelt, Ware, & Baldwin, 2001). Due to its antimicrobial properties, some organic acids are known to improve preservation of fruits and vegetables and to restrict the harmful microflora in human's digestive system (Nawirska-Olszanska, Biesiada, Soktowska, & Kucharska, 2014). Similar to our results, Moore, Palmquist, and Seemann (1997) had reported significant increases in the levels of sucrose and starch in parsley leaves subjected to 1000 µmole CO_2 $mole^{-1}$ air. Moreover,

accumulation of soluble sugars and starch in response to eCO_2 had been reported in crop plants such as tomato and wheat (Aranjuelo et al., 2011; Li et al., 2013). Organic acids such as fumarate and malate had also been reported to increase in the model plant *Arabidopsis thaliana* subjected to 780 µmole CO_2 $mole^{-1}$ air (Watanabe et al., 2014).

Among the factors that determine the nutritional value of plants are EAAs, specially lysine, tryptophan, and methionine that are constrained in cereals and legumes (Ufaz & Galili, 2008). Thus, from commercial and health value perspectives, it is important to enrich the content of EAAs in plant-based food. In the current study, the eCO_2-induced accumulation of isoleucine, leucine, lysine, methionine, phenylalanine, valine and their total in parsley and/or dill suggests a positive impact of eCO_2 on the nutritional value of herbal plants. In this regard, eCO_2 has been reported to affect the amino acid profile in model and crop plant species by improving, through its positive impact on the processes of photosynthesis and dark respiration, the availability of C skeleton and metabolic energy required for biosynthesis of amino acids (Noguchi et al., 2015; Nunes-Nesi, Fernie, & Stitt, 2010).

Fatty acid composition of plant-based foods is of a great health value importance where higher proportions of SFA are linked to cardiovascular diseases (Livingstone, Lovegrove, & Givens, 2012). By its positive impact on dark respiration, eCO_2 is expected to alter fatty acids profile as the precursor of their biosynthesis, malonyl-CoA, is produced from ATP-dependent carboxylation of acetyl-CoA that is an intermediate of the glycolytic pathway (Brown, Slabas, & Rafferty, 2010). Interestingly, the present results revealed that eCO_2 treatment resulted in higher levels of USFA accompanied with lower SFA/USFA ratios in both plants. Thus, parsley and dill produced in eCO_2 atmosphere are healthier than those grown under aCO_2. Unfortunately, little is known about the impact of eCO_2 on fatty acids metabolism, however, dissimilar to the present

results, two earlier investigations had reported a negative impact of eCO_2 on the biosynthesis of fatty acids in silver birch seedlings (Huang, Eglinton, Ineson, Bol, & Harkness, 1999) and root tubers of carrot, radish and turnip (Azam et al., 2013). Therefore, the impact of eCO_2 on the fatty acids biosynthesis and composition seems to be species dependent.

Biological activities of medicinal plants rely on the presence of bioactive phytochemicals, such as vitamins and secondary metabolites, that exert antioxidant, antibacterial, antiprotozoal and anticancer activities (Idso et al., 2002; Kumar, Tirpude, Maheshwari, Bansal, & Misra, 2013; Mohamed, Saleh, Abdel-Farid, & El-Naggar, 2017; Roleira et al., 2015; Sun et al., 2016). Moreover, the role of some micro-elements such as Cu, Zn, Se and Mn in free radical scavenging mechanisms has been reported (Fedor et al., 2017). In the current study, eCO_2 resulted in improved levels of total phenolics and flavonoids, β-Cryptoxanthin (vitamin A) and total tocopherol (vitamin E) in both parsley and dill, but mostly did not affect their mineral composition, except for Ca and Zn. Moreover, some individual flavonoids such as quercetrin and ellagic acid were accumulated in one plant species but not in the other. Such eCO_2-induced accumulation of these vitamins and secondary metabolites could be ascribed to the excess availability of C and N intermediates that are diverted for the biosynthesis of these bioactive phytochemicals (Herms & Mattson, 1992). In this regard, eCO_2 has been reported to affect C and N metabolism as well as nutrient uptake (Asif, Yilmaz, & Ozturk, 2017; Noguchi et al., 2015; Watanabe et al., 2014). As a logical consequence for its impact on the accumulation of bioactive compounds, eCO_2 treatment improved total antioxidant, antiprotozoal, anti-bacterial and anticancer activities of parsley and dill. Similarly, improved antioxidant activity accompanied with accumulation of anthocyanins, pelargonidin, quercetin and kaempferol, has been reported in fruits of strawberry treated with eCO_2 (Wang et al., 2003). Moreover, Ghasemzadeh et al. (2010)

had ascribed the eCO_2-induced improvement in the antioxidant capacity of ginger to the enhanced levels of quercetin, kaempferol, catechin and fisetin. Not only flavonoids, but also some phenolic acids (gallic and caffeic acids) and vitamin C (ascorbic acid) were reported to accumulate in *Labisia pumila* and *Citrus aurantium*, respectively, in response to eCO_2 (Idso et al., 2002; Jaafar et al., 2012). In addition, a similar improvement in the antioxidant and anticancer, against human cell lines MCF–7 and MDA–MB–231, activities has been observed in two varieties of ginger grown under 800 µmol/mol CO_2 (Ghasemzadeh & Jaafar, 2011).

5. Conclusion

Based on the results presented in this research it could be concluded that manipulation of eCO_2 as a strategy to improve the growth and the nutritional and health benefits of herbal plants is worthwhile. eCO_2 (627±24 µmole CO_2 $mole^{-1}$ air) improved the accumulation of nutritionally and medicinally valuable phytochemicals in both parsley and dill. However, there was obvious difference between the two plants in the degree by which their individual metabolites respond to eCO_2 treatment. Regardless of the plant species, eCO_2 triggered improvements in the levels of soluble sugars, starch, organic acids, some EAAs, most of USFA, total phenolics, total flavonoids and vitamins A and E. Moreover, parallel improvements in the total antioxidant capacity, antiprotozoal, antibacterial and anticancer activities were recorded for parsley and dill in response to eCO_2.

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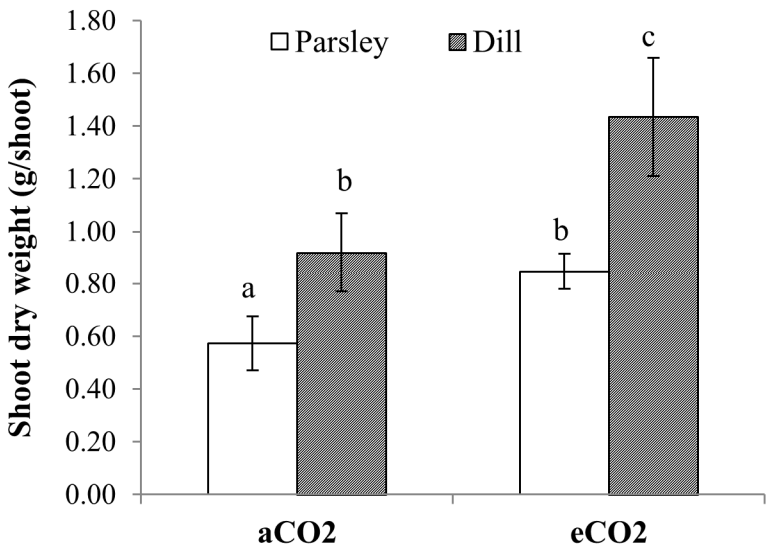
Figure Captions

Figure 1. Shoot biomass of parsley and dill grown under two levels of CO_2, ambient (378±25 µmole CO_2 $mole^{-1}$ air, aCO_2) and elevated (627±24 µmole CO_2 $mole^{-1}$ air, eCO_2). Values are mean ± standard error of three independent replicates. Same lower-case letters on bars indicate no significant difference at the 0.05 probability level**.**

Figure 2. Heatmap of fold change ratios in metabolite, vitamins, minerals and biological activities of parsley and dill in response to eCO_2 (627±24 µmole CO_2 $mole^{-1}$ air). Fold change in each metabolite was calculated relative to its corresponding mean (n=3) in the aCO_2 (378±25 µmole CO_2 $mole^{-1}$ air) grown plant. As shown in the color scale, red indicates inhibition, white no change and blue improvement in metabolite levels as affected by eCO_2.

Figure 3. Heatmap of metabolite accumulation in shoot tissues of parsley and dill grown under two levels of CO_2, ambient (aCO_2, 378±25 µmole CO_2 $mole^{-1}$ air) and elevated (eCO_2, 627±24 µmole CO_2 $mole^{-1}$ air). The relative accumulation patterns are shown in the heatmap based on the average value (n=3) for each metabolite. Red and blue colors indicate lower and higher concentrations, respectively.



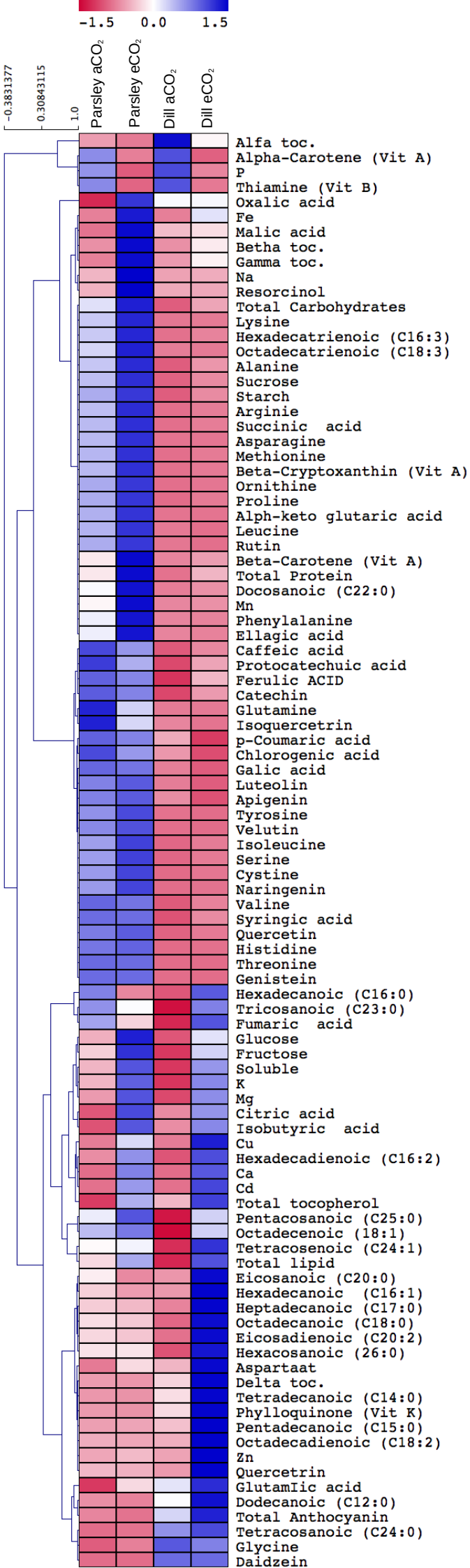
	Parsley	Dill
Sugars		
Glucose	0.69	0.56
Fructose	0.48	0.61
Sucrose	0.44	0.33
Total soluble sugars	0.49	0.82
Starch	0.29	0.29
Total carbohydrate	0.51	0.60
Organic acids		
Citric acid	0.45	0.27
Fumaric acid	-0.04	0.13
Isobutyric acid	0.45	0.28
Malic acid	0.11	0.01
Oxalic acid	0.31	-0.21
Succinic acid	0.45	0.14
Essential amino acids (EAAs)		
Histidine	0.06	0.08
Isoleucine	0.28	0.93
Leucine	0.43	0.00
Lysine	0.66	0.09
Methionine	0.50	1.50
Phenylalanine	1.36	-0.08
Valine	-0.03	0.45
Threonine	-0.02	0.10
Total EAAs	0.51	0.10
Non-essential amino acids (NEAAs)		
Aspartate	0.38	0.96
Cystine	0.33	0.90
Glutamic acid	0.44	0.32
Glutamine	0.19	0.00
Glycine	0.13	-0.07

	Parsley	Dill
Ornithine	0.47	0.60
Proline	0.43	0.33
Serine	0.28	0.26
Tyrosine	0.23	-0.06
alanine	0.12	0.05
Asparagine	0.40	0.04
Total NEAAs	0.24	0.15
Saturated Fatty acids (SFA)		
Tetradecanoic (C14:0)	-0.01	0.44
Hexadecanoic (C16:0)	-0.14	0.23
Heptadecanoic (C17:0)	-0.01	0.31
Octadecanoic (C18:0)	-0.02	0.39
Eicosanoic (C20:0)	-0.11	0.42
Docosanoic (C22:0)	0.39	0.04
Tricosanoic (C23:0)	-0.09	0.41
Tetracosanoic (C24:0)	0.18	0.47
Pentacosanoic (C25:0)	0.00	0.31
Total SFA	0.01	0.32
Unsaturated fatty acids (USFA)		
Hexadecanoic (C16:1)	-0.12	0.91
Octadecenoic (C18:1)	0.07	0.43
Tetracosenoic (C24:1)	0.02	0.87
Hexadecadienoic (C16:2)	0.31	0.52
Octadecadienoic (C18:2)	-0.01	0.37
Eicosadienoic (C20:2)	-0.02	0.44
Hexadecatrienoic (C16:3)	0.23	0.03
Octadecatrienoic (C18:3)	0.60	0.05
Total USFA	0.24	0.45

	Parsley	Dill
Phenolic acids		
Caffeic acid	0.33	0.33
Ferulic acid	0.55	0.34
Protocatechuic acid	0.42	0.29
Catechin	0.54	0.26
Gallic acid	0.58	0.03
p-Coumaric acid	0.52	-0.19
Resorcinol	15.17	0.17
Chlorogenic acid	0.44	-0.05
Syringic acid	0.61	0.23
Flavonoids		
Quercetin	0.67	0.16
Quercetrin	0.42	7.93
Luteolin	0.63	0.00
Apigenin	0.68	-0.06
Isoquercetrin	0.28	0.07
Rutin	0.92	0.08
Ellagic acid	1.23	0.02
Velutin	0.53	0.04
Total flavonods	0.37	0.60
Total Phenols	0.96	0.32
Vitamin A		
α-Carotene	-0.27	-0.10
β-Carotene	0.56	-0.13
β-Cryptoxanthin	0.58	0.00
Vitamin B		
Thiamine	-0.36	-0.10
Vitamin E		
α-tocopherol	-0.07	-0.14
β-tocopherol	2.38	0.06

	Parsley	Dill
γ-tocopherol	3.71	0.06
δ-tocopherol	-0.08	0.64
Total tocopherol	1.35	0.25
Vitamin K1		
Phylloquinone	-0.38	0.18
Macro-elements		
K	0.04	-0.08
Ca	0.88	0.61
Mg	0.38	-0.12
P	0.04	-0.06
Micro-elements		
Zn	1.18	0.57
Cu	0.00	1.00
Fe	0.33	-0.03
Mn	0.07	0.19
Total antioxidant activity		
FRAP	0.46	0.59
DPPH	0.37	0.60
Antiprotozoal		
Trypanosoma cruzi	0.53	0.58
Antibacterial		
Streptococcus sp.	0.74	0.24
Escherichia coli	0.32	0.42
Anticancer		
HepG2	0.54	0.00
Colo205	0.20	0.17
293	0.06	0.16
T24P	0.13	0.28

≤-1.5 0.0 ≥1.5


-1.5
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1.5
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1.0
Parsley aCO2
Parsley eCO2
Dill aCO2
Dill eCO2
Alfa toc.
Alpha-Carotene (Vit A)
P
Thiamine (Vit B)
Oxalic acid
Fe
Malic acid
Betha toc.
Gamma toc.
Na
Resorcinol
Total Carbohydrates
Lysine
Hexadecatrienoic (C16:3)
Octadecatrienoic (C18:3)
Alanine
Sucrose
Starch
Arginie
Succinic acid
Asparagine
Methionine
Beta-Cryptoxanthin (Vit A)
Ornithine
Proline
Alph-keto glutaric acid
Leucine
Rutin
Beta-Carotene (Vit A)
Total Protein
Docosanoic (C22:0)
Mn
Phenylalanine
Ellagic acid
Caffeic acid
Protocatechuic acid
Ferulic ACID
Catechin
Glutamine
Isoquercetrin
p-Coumaric acid
Chlorogenic acid
Galic acid
Luteolin
Apigenin
Tyrosine
Velutin
Isoleucine
Serine
Cystine
Naringenin
Valine
Syringic acid
Quercetin
Histidine
Threonine
Genistein
Hexadecanoic (C16:0)
Tricosanoic (C23:0)
Fumaric acid
Glucose
Fructose
Soluble
K
Mg
Citric acid
Isobutyric acid
Cu
Hexadecadienoic (C16:2)
Ca
Cd
Total tocopherol
Pentacosanoic (C25:0)
Octadecenoic (18:1)
Tetracosenoic (C24:1)
Total lipid
Eicosanoic (C20:0)
Hexadecanoic (C16:1)
Heptadecanoic (C17:0)
Octadecanoic (C18:0)
Eicosadienoic (C20:2)
Hexacosanoic (26:0)
Aspartaat
Delta toc.
Tetradecanoic (C14:0)
Phylloquinone (Vit K)
Pentadecanoic (C15:0)
Octadecadienoic (C18:2)
Zn
Quercetrin
GlutamIic acid
Dodecanoic (C12:0)
Total Anthocyanin
Tetracosanoic (C24:0)
Glycine
Daidzein

Table 1. Levels of sugars, organic acids (mg g^{-1} dry weight), amino acids and fatty acids (µg g^{-1} dry weight) in shoot tissues of parsley and dill grown under two concentrations of CO_2, ambient (378±25 µmole CO_2 $mole^{-1}$ air, aCO_2) and elevated (627±24 µmole CO_2 $mole^{-1}$ air, eCO_2). Values are mean ± standard error of three independent replicates. Means followed by the same lower-case letter in a row do not differ significantly at the 0.05 probability level.

	aCO2		eCO2			aCO2		eCO2	
Metabolite	**Parsley**	**Dill**	**Parsley**	**Dill**	**Metabolite**	**Parsley**	**Dill**	**Parsley**	**Dill**
Sugars									
Glucose	0.62±0.01b	0.50±0.00a	1.05±0.02d	0.78±0.00c	Asparagine	4.34±0.78b	1.05±0.11a	6.06±1.17b	1.09±0.06a
Fructose	0.81±0.05b	0.59±0.02a	1.20±0.08c	0.95±0.03b	Glycine	0.64±0.06a	1.41±0.24b	0.72±0.03a	1.31±0.17b
Sucrose	0.77±0.03c	0.27±0.00a	1.11±0.04d	0.36±0.00b	Ornithine	1.05±0.10c	0.05±0.01a	1.54±0.15d	0.08±0.01b
Total soluble sugars	1.74±0.18b	1.30±0.10a	2.59±0.27c	2.36±0.18c	Proline	3.53±0.37c	0.58±0.06a	5.04±0.53d	0.77±0.08b
Starch	12.28±0.45c	4.81±0.02a	15.80±0.58d	6.21±0.02b	Serine	0.83±0.08b	0.19±0.02a	1.06±0.11c	0.24±0.02a
Total sugars	24.58±1.02c	10.73±0.14a	37.13±1.55d	17.19±0.23b	Tyrosine	4.94±4.94b	0.81±0.12a	6.10±0.69b	0.76±0.13a
Organic acids					Total NEAAs	267.48±13.4a	236.52±13.1a	331.34±12.6b	271.71±17.1a
Citric acid	1.98±0.10a	2.11±0.11a	2.87±0.15b	2.68±0.14b	**Saturated Fatty acids (SFA)**				
Fumaric acid	0.25±0.01a	0.23±0.01a	0.24±0.02a	0.26±0.02a	Tetradecanoic (C14:0)	112.0±3.7a	125.7±4.6a	111.3±2.8a	181.0±7.3b
Isobutyric acid	1.70±0.09a	1.83±0.09a	2.46±0.13b	2.35±0.12b	Hexadecanoic (C16:0)	319.3±37.3ab	264.9±17.6a	273.6±32.5ab	326.7±15.2b
Malic acid	7.39±0.45a	7.54±0.46a	8.17±0.37a	7.60±0.29a	Heptadecanoic (C17:0)	245.9±15.4a	231.3±6.3a	242.4±5.0a	303.2±22.9b
Oxalic acid	1.29±0.08ab	1.50±0.08b	1.69±0.10c	1.19±0.03a	Octadecanoic (C18:0)	37.1±1.7a	33.2±1.6a	36.5±1.8a	46.3±1.9b
Succinic acid	3.99±0.24b	1.24±0.06a	5.79±0.35c	1.41±0.07a	Eicosanoic (C20:0)	134.7±8.7a	121.9±8.2a	119.9±4.0a	173.6±17.7b
Essential amino acids (EAAs)					Docosanoic (C22:0)	72.1±1.9b	55.5±0.5a	100.4±4.0c	57.9±3.5a
Histidine	10.21±1.20b	1.61±0.25a	10.80±1.18b	1.74±0.10a	Tricosanoic (C23:0)	8.2±0.3b	5.9±0.5a	7.5±0.3ab	8.3±0.7b
Isoleucine	0.67±0.07b	0.15±0.01a	0.86±0.09c	0.29±0.02b	Tetracosanoic (C24:0)	228.9±0.7b	160.8±13.5a	270.0±47.4b	236.4±17.7b
Leucine	0.14±0.01b	0.01±0.00a	0.20±0.02c	0.01±0.00a	Pentacosanoic (C25:0)	22.4±0.7a	23.2±1.7a	22.5±2.0a	30.4±1.2b
Lysine	23.14±0.22b	5.03±0.53a	38.44±4.52c	5.46±0.37a	Total SFA	1270.2±35.6a	1186.1±116.1a	1278.1±56.3a	1562.6±150.2b
Methionine	0.12±0.01b	0.02±0.00a	0.18±0.02c	0.05±0.01b	**Unsaturated fatty acids (USFA)**				
Phenylalanine	4.79±0.54b	0.53±0.08a	11.32±1.26c	0.49±0.02a	Hexadecanoic (C16:1)	121.0±0.75a	105.9±2.3a	107.0±1.0a	201.9±19.0b
Valine	5.09±0.44b	1.94±0.20a	4.95±0.43b	2.82±0.35b	Octadecenoic (C18:1)	41.6±1.80b	28.7±1.7a	44.4±4.6b	40.9±1.6b
Threonine	0.48±0.01b	0.10±0.01a	0.47±0.04b	0.11±0.01a	Tetracosenoic (C24:1)	146.5±0.43b	102.4±0.7a	150.1±10.0b	191.7±7.7c
Total EAAs	44.62±1.31b	9.40±0.61a	67.22±5.8c	10.35±0.51a	Hexadecadienoic (C16:2)	279.4±50.5ab	257.9±31.8a	366.2±21.7c	392.4±26.1c
Non-essential amino acids (NEAAs)					Octadecadienoic (C18:2)	16.3±0.18a	16.3±0.2a	16.2±1.9a	22.3±0.9b

Aspartate	0.21±0.02a	0.26±0.07a	0.29±0.03a	0.51±0.03b	Eicosadienoic (C20:2)	153.4x12.07b	126.1±13.9a	150.4±6.0b	181.4±8.1c
Cystine	4.07±0.41c	0.20±0.01a	5.43±0.55d	0.38±0.03b	Hexadecatrienoic (C16:3)	164.1±3.70b	117.7±7.0a	201.8±8.1c	121.77±9.4a
GlutamIic acid	65.31±4.0a	106.1±16.5b	94.23±9.03b	139.6±16.55	Octadecatrienoic (C18:3)	310.3±11.47b	143.9±20.5a	495.1±19.9c	150.5±14.0a
Glutamine	150.4±4.8c	99.2±2.52a	178.44±5.10d	98.89±5.85b	Total USFA	1232.7±42.2b	898.9±33.a	1530.9±48.2c	1302.9±32.6b
Alanine	32.16±5.34a	26.67±5.11a	36.03±4.09a	28.08±7.30a	**SFA/USFA**	1.03	1.32	0.83	1.20

Table 2. Concentrations of individual phenolic acids, flavonoids, their totals, vitamins, minerals (mg g^{-1} dry weight) and biological activities of parsley and dill grown under two concentrations of CO_2, ambient (378±25 µmole CO_2 $mole^{-1}$ air, aCO_2) and elevated (627±24 µmole CO_2 $mole^{-1}$ air, eCO_2). Values are mean ± standard error of three independent replicates. Means followed by the same lower-case letter in a row do not differ significantly at the 0.05 probability level**.**

	aCO2		eCO2			aCO2		eCO2	
Metabolite	**Parsley**	**Dill**	**Parsley**	**Dill**	**Metabolite**	**Parsley**	**Dill**	**Parsley**	**Dill**
Phenolic acids									
Caffeic acid	0.03±0.00a	0.03±0.00a	0.04±0.00b	0.04±0.00a	γ-tocopherol	0.024±0.00a	0.031±0.00	0.113±0.01	0.033±0.00
Ferulic acid	3.26±0.46a	3.58±0.39a	5.04±0.50b	4.79±0.47a	δ-tocopherol	0.012±0.00a	0.022±0.01	0.011±0.00	0.036±0.00
Protocatechuic acid	0.26±0.04a	0.28±0.03a	0.37±0.04a	0.36±0.04a	Total tocopherol	0.211±0.09a	0.338±0.06	0.496±0.02	0.421±0.02
Catechin	0.98±0.14a	1.06±0.12a	1.51±0.15b	1.34±0.13a	**Vitamin K1**				
Gallic acid	25.99±3.70a	28.02±3.0a	40.94±4.08b	28.73±2.83	Phylloquinone	0.121±0.01a	0.496±0.04	0.075±0.03	0.585±0.00
p-Coumaric acid	5.59±0.38a	6.02±0.66a	8.51±0.85b	4.87±0.48a	**Macronutrients**				
Resorcinol	0.06±0.01a	0.06±0.01a	0.97±0.86b	0.07±0.01a	K	31.59±1.22a	29.20±2.30	32.7±1.30a	26.88±1.12
Chlorogenic acid	0.34±0.05a	0.37±0.04a	0.49±0.05a	0.35±0.03a	Ca	7.75±0.29a	11.94±0.80	14.25±0.52	19.17±0.95
Syringic acid	1.53±0.22a	1.65±0.18a	2.46±0.24b	2.03±0.20a	Mg	4.94±0.52b	2.93±0.26a	6.81±0.53b	2.57±0.06a
Flavonoids					P	5.69±0.22a	7.40±0.58a	5.89±0.34a	6.97±0.48a
Quercetin	2.30±0.33a	2.48±0.27a	3.85±0.38b	2.88±0.28a	**Micronutrients**				
Quercetrin	0.26±0.04a	0.28±0.03a	0.37±0.04a	2.50±0.25b	Zn	0.50±0.09b	0.21±0.05a	1.09±0.17c	0.33±0.02b
Luteolin	0.08±0.01a	0.08±0.01a	0.13±0.01b	0.08±0.01a	Cu	0.01±0.00a	0.01±0.00a	0.01±0.00a	0.02±0.00a
Apigenin	0.50±0.07a	0.53±0.06a	0.84±0.08b	0.50±0.05a	Fe	0.21±0.01a	0.34±0.03a	0.28±0.02a	0.33±0.03a
Isoquercetrin	1.24±0.18a	1.34±0.15a	1.59±0.16a	1.44±0.14a	Mn	0.042±0.00b	0.026±0.00	0.045±0.00	0.031±0.00
Rutin	1.61±0.23a	1.74±0.19a	3.09±0.31b	1.88±0.19a	**Total antioxidant activity** (FRAP, µmole Trolox g^{-1} DW; DPPH, %				

Ellagic acid	0.40±0.06a	0.40±0.02a	0.89±0.04b	0.41±0.02a	FRAP	4.01±0.04a	4.20±0.67a	5.85±0.07b	6.67±0.44b
Velutin	0.47±0.07a	0.47±0.02a	0.72±0.03b	0.49±0.02a	DPPH	18.56±0.16a	21.56±3.56	25.41±0.33	34.53±3.24
Total flavonods	72.75±0.67a	64.74±2.33	99.58±2.78b	103.49±6.0	**Antiprotozoal** (% reduction in parasite)				
Total Phenols	55.02±1.29a	53.10±4.89	107.70±4.40	70.29±2.52	*Trypanosoma*	21.56±0.20a	20.41±3.04	33.07±0.73	32.18±4.34
Vitamin A					**Antibacterial** (diameter of inhibition zone, mm)				
α-Carotene	0.473±0.03a	0.505±0.03	0.346±0.07a	0.452±0.03	*Streptococcus* sp.	6.17±0.26a	12.22±1.17	10.74±0.14	15.17±1.65
β-Carotene	0.393±0.02b	0.313±0.03a	0.613±0.04c	0.271±0.00	*Escherichia coli*	18.22±1.48a	17.69±1.24	23.99±0.93	25.08±1.45
β-Cryptoxanthin	0.310±0.02a	0.026±0.00	0.490±0.22b	0.026±0.00	**Anticancer** (% dead cells)				
Vitamin B					HepG2	47.34±1.11a	56.38±6.82	72.92±4.12	56.53±1.91
Thiamine	0.348±0.02a	0.373±0.03	0.221±0.09a	0.335±0.01	Colo205	66.23±0.61a	60.75±3.00	80.06±2.24	70.93±1.75
Vitamin E					293	73.69±0.65c	53.97±2.59	77.99±1.01	62.56±1.15
α-tocopherol	0.150±0.01a	0.264±0.10	0.139±0.01a	0.228±0.09	T24P	76.96±0.73b	55.15±4.49	87.21±2.69	70.40±3.90
β -tocopherol	0.032±0.00a	0.032±0.00	0.108±0.02b	0.034±0.00					

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Highlights

- CO_2 enrichment improves the levels of flavor-related compounds such as sugars and organic acids
- Elevated CO_2 enhances the nutritive value through higher EAAs content and lower SFA/USFA ratio
- Levels of antioxidant metabolites and antioxidant activities are improved by elevated CO_2
- CO_2 enrichment supports the value of parsley and dill as functional foods