Research Article

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Impact of elevated carbon dioxide and temperature on strawberry polyphenols

Himali N Balasooriya,[a*] Kithsiri B Dassanayake,[a,b] Saman Seneweera[a,c] and Said Ajlouni[a]

ABSTRACT

BACKGROUND: The strawberry cultivars 'Albion' and 'San Andreas' ('SA') were grown under various combinations of day temperature (25 and 30 °C) and carbon dioxide [CO_2] (400, 650 and 950 μmol mol^{-1}) conditions. The influence of different growth combinations on the polyphenol, flavonoid, anthocyanin, antioxidant, and individual phenolic compound content of fresh strawberry fruits was studied. The content of individual phenolic compounds of fresh strawberry fruits was quantified using high-performance liquid chromatography – ultraviolet (HPLC-UV).

RESULTS: Elevated [CO_2] and higher temperature caused significant increases in total polyphenol, flavonoid, anthocyanin and antioxidants in both strawberry cultivars when compared with plants grown under ambient conditions. Results of HPLC-UV analysis also revealed that individual phenolic compounds of fruits were also increased with increasing [CO_2] and temperature. However, the responses were significantly altered by the interaction of elevated [CO_2] and higher temperature. The individual and interaction effects of [CO_2] and temperature were also significantly cultivar dependent. The largest amounts of flavonoid (482 ± 68 mg kg^{-1} FW) and antioxidant (19.0 ± 2.1 μmol g^{-1} FW) were detected in 'Albion' grown at 30 °C and under 950 μmol mol^{-1}, and total polyphenol (3350 ± 104 mg GAE kg^{-1} FW) and anthocyanin (332 ± 16 mg kg^{-1} FW) in 'San Andreas' grown at 25 °C and 950 μmol mol^{-1}.

CONCLUSION: Strawberry fruit was rich with polyphenols and antioxidants when grown under elevated [CO_2] and higher temperature. There were also interactions between [CO_2] and temperature affecting the fruits' content. An increase in the polyphenol and antioxidant content in strawberry fruits would be highly beneficial to human health.

Keywords: carbon dioxide; HPLC; polyphenol; strawberry; temperature

INTRODUCTION

Horticultural plants are used as the one of the main sources of food, fiber, biofuel, medicine, and other products to sustain and enhance human life.[1–3] Strawberry (*Fragaria ananassa* Duch.) continues to experience greater consumer demand globally due to its excellent nutrition profile, including minerals, vitamin C, folates, and polyphenols.[4] Strawberry has been consistently appreciated for its health benefits and high dietary fiber content. Phytochemicals in strawberry display an immense biological potential in humans by providing protective effects against most diseases.[4–8] Ascorbic acid, ellagitannins and polyphenols are the major antioxidant compounds in strawberry that can prevent oxidative stresses and related diseases.[8] In addition to their antioxidant properties, strawberry polyphenols possess dietary phytochemicals with anti-inflammatory, antimicrobial, antiallergy, and antihypertensive properties.[6] More than 40 different compounds of polyphenols have been identified in strawberries[9] with various structures and functions.

Flavonoids, tannins, phenolic acids, stilbenes, and lignans are the major subgroups of polyphenols with the flavonoids being the most abundant subgroup in plants.[10] Anthocyanins are quantitatively the most important subclass within flavonoids. They are the naturally occurring water-soluble pigments, which give strawberry its unique bright red color, a critical factor in assessing the visual fruit quality of strawberries. The variability of anthocyanins is noteworthy, with more than 25 different anthocyanin compounds discovered in different strawberry cultivars.[11] Pelargonidin and cyanidin derivatives are the main categories in this group and pelargonidin-3-glucoside (pel-3-glu) is the most dominant anthocyanin in strawberry. Cyanidin-3-glucoside (cy-3-glu) and pelargonidin-3-rutinoside (pel-3-rut) are the other anthocyanins commonly found in smaller amounts than pel-3-glu. Quercetin and kaempferol derivatives, which belong to the subclass of flavonols in the flavonoids, also function as co-pigments contributing to the unique color of strawberry fruits. Bioavailability, absorption and bioactivity of these compounds in humans are well known to be relatively high,[4] despite being present in strawberry

* *Correspondence to: HN Balasooriya, School of Agriculture and Food, Faculty of Veterinary and Agricultural Sciences, The University of Melbourne, Parkville, VIC 3010 Australia. E-mail: hbalasooriya@student.unimelb.edu.au*

a *Faculty of Veterinary and Agricultural Sciences, School of Agriculture and Food, The University of Melbourne, Parkville, Victoria, Australia*

b *Department of Infrastructure Engineering, Faculty of Engineering, The University of Melbourne, Parkville, Victoria, Australia*

c *National Institute of Fundamental Studies, Kandy, Sri Lanka*

at very low concentrations in comparison to pelargonidin and cyanidin derivatives.

A range of other phenolic compounds such as phenolic acids (hydroxycinnamic acids, *p*-coumaroyl, ferulic acid derivatives), stilbenes (resveratrol), and coumarins (*p*-coumaric acid), which are beneficial to human nutrition and health, have also been identified in strawberry fruits.[9,11–13] However, the strawberry polyphenol profile can vary widely qualitatively and quantitatively as it is affected by various factors, including genotype, environmental factors, and their interactions.[14–21]

The genetic makeup of strawberries is the most critical contributory factor in the variability of the nutritional quality of fruits in terms of total polyphenol content (TPC), total antioxidant content (TAC) and levels of individual polyphenols; however, growing conditions or environmental factors also play a significant role in determining the nutritional value of strawberries.[14,16,18] Both genotype and environmental factors have been reported to influence the chemical composition of strawberry significantly.[12,14,22–25] In general, a range of abiotic factors (environmental influences including climate, soil, and water stress) and biotic factors (plant pest and diseases) are known to alter strawberry quality and antioxidant profiles. However, due to the anticipated climate changes, environmental factors such as air temperature and CO_2 concentration [CO_2] are important as they are detrimental to the quality and nutritional value of strawberries. Atmospheric [CO_2] may increase from the current levels of 390 µmol mol^{-1} to around 1000 µmol mol^{-1} and temperature by 2.5 to 7.8 °C at the end of this century.[26–29] These ongoing increases are predicted to cause significant changes in the quantity and quality of agricultural produce.[29] Furthermore, climate change could alter the water availability for crop production and increase the risks of drought and diseases, reducing food production and quality.[30,31] These factors, independently or interactively, are expected to cause a substantial influence on strawberry phytochemicals.

Among the abiotic factors, growing temperature significantly influences nutritional quality of strawberries.[21,32] For example, Wang and Zheng[21] demonstrated that increased polyphenol content and high antioxidant capacity occur with increased day / night growing temperature. Palmieri *et al.*[17] reported enhanced concentrations of flavonols and ellagitannins in strawberries when the plants were subjected to higher temperature, ultraviolet irradiation and longer sunshine duration. Higher levels of anthocyanins and phenolic compounds were also reported when strawberries were grown under elevated [CO_2] (e[CO_2]).[33] For example, resveratrol, an important polyphenol that provides several health benefits, was found in greater amounts in strawberries grown either at high temperatures or e[CO_2].[34] Sun *et al.*,[24] on the other hand, reported reductions in antioxidant activity and antioxidant compounds occurred in strawberries grown under e[CO_2].

In general, most studies in the past evaluated the effects of temperature and [CO_2] on the nutritional quality of strawberries separately. However, an understanding of the interactive effects of these two environmental factors (high temperature and [CO_2]) on phytochemicals is limited in the literature.[35] Thus, it is critical to investigate the interactive effects of e[CO_2] and temperature on nutritional quality, especially the antioxidant content, and polyphenols in particular, in strawberries.

The polyphenol profile of strawberries shows that they have a highly diverse composition and La Barbera *et al.*[13] have confirmed or tentatively identified over 130 different individual compounds. The diversity of these compounds must be taken into consideration in fruit nutrition as their biological and pharmacological activities are specific to their chemical structure.[7] Analyzing the amounts of individual compounds is therefore important to evaluate the effects of genetic or environmental factors on the nutritional profile of the fruit. Previous studies have mostly focused on total polyphenols as a group or a specific subgroup, i.e. anthocyanins. However, a broad spectrum of polyphenol compounds would be available in different quantities in strawberries and will be affected in varying degrees by environmental factors. This study examined the effect of e[CO_2] and high temperature, and their interactions, on various polyphenols in two different strawberry cultivars.

MATERIALS AND METHODS

Chemicals

High-performance liquid chromatography gradient grade methanol, acetonitrile, and formic acid were obtained from Thermo Fisher Scientific, Melbourne, Victoria, Australia. Hydrochloric acid, ethanol, Folin–Ciocalteu's reagent (FCR), gallic acid, sodium carbonate, 2, 2′-azinobis [3-ethylbenzo] thiazoline-6-sulfonic acid (ABTS), potassium persulfate, Trolox solution (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), potassium chloride, sodium acetate, potassium persulfate, and formic acid were purchased from Sigma-Aldrich Pty. Ltd., Sydney, Australia. For laboratory analysis, polyphenol extractions and the HPLC mobile phase, ultrapure water (Milli-Q® water) was used. Milli-Q® water was generated from a Millipore Milli-Q Ultrapure Water Purification System (ZMQP60001), Danvers, Massachusetts, United States. High-performance liquid chromatography grade (≥99.0%) reference standards, catechin, callistephin chloride, cyanidin chloride, *p*-coumaric, 6-*O*-*p*-coumaroyl-1,2-digalloylglucose, transferulic acid and resveratrol were also purchased from Sigma-Aldrich Co, Australia. High-performance liquid chromatography grade reference standards, pelargonidin-3-rutinoside chloride, kaempferol-3-*O*-glucoside, kaempferol-3-glucurnoride, quercertin-3,4-di-glucoside and quercertin-3-*O*-glucuronide were purchased from Extrasynthase, Genay Cedex, France.

Strawberry fruits

The strawberries used in this study were produced from strawberry plants grown in a controlled environment (CE), in chambers (Model: TPG-2400-TH-CO_2, Thermoline Scientific Equipment Pty Ltd, Wetherill Park, NSW, Australia) at the Parkville Campus of the University of Melbourne, Australia, as described by Balasooriya *et al.*[36] Briefly, two different day neutral strawberry cultivars, 'Albion' and 'San Andreas' ('SA') were grown under different [CO_2] and temperature combinations inside automated CE chambers. The six different treatment combinations were 400 µmol mol^{-1} × 25 °C, 400 µmol mol^{-1} × 30 °C, 650 µmol mol^{-1} × 25 °C, 650 µmol mol^{-1} × 30 °C, 950 µmol mol^{-1} × 25 °C and 950 µmol mol^{-1} × 30 °C. Each experiment had four replicates and there were three plants per replicate. The treatments were applied during plant growth and development until fruit harvesting. Finally, healthy fruits (including all primary and secondary fruits) with 90% red color were harvested separately from each chamber for each cultivar and for each replicate.

The antioxidant and polyphenol properties of strawberries were analyzed in fresh strawberry fruits. Fruits for fresh analysis were stored in a cold room at 4 °C until use.

Extraction of strawberry polyphenols

Polyphenol compounds in strawberry fruits were extracted using 70% methanol and 0.18 N HCl following the method described by Tow *et al.*[37] with some modifications. Triplicate samples (5 g each) of fresh strawberry were homogenized with 70% methanol (15 mL) and 0.18 N HCl (5 mL) in 50 mL polyethylene tube (Ultra Turrax homogenizer, Janke and Kunnel, IKA-Labortechnik Ultra-Turrax T25, Sigma Aldrich Pty. Ltd., Sydney, NSW, Australia). The homogenate was centrifuged at 8422*g* (Centrifuge, Thermoline Scientific Equipment Pvt Ltd, Wetherill Park, NSW, Australia) for 15 min at room temperature and the resultant supernatant was collected quantitatively. The residue was washed with an additional 5 mL of methanol and the supernatants were combined. Methanol was evaporated from the collected supernatant under vacuum in a rotary evaporator at 60 °C at 8 rpm. The final extract was redissolved in Milli-Q water and the final volume was adjusted to 25 mL using a volumetric flask. This final polyphenol extract was then used for analyzing the total antioxidants, polyphenols, flavonoid, and anthocyanin content in strawberries. An aliquot (2 mL) of the extract was filtered through 0.45 µm membrane when used for the analysis of individual polyphenol compounds using HPLC.

Total polyphenol content (TPC)

The TPC was measured following the method described by Tow *et al.*[37] In this method, a mixture of 20 µL of polyphenol sample extract, 100 µL of 0.2 N Folin–Ciocalteu's reagent (FCR), 200 µL of Milli-Q water and 80 µL of sodium carbonate (7.5% w/v) was incubated at room temperature for 1 h in a 96-well microplate. Absorption was then measured at 756 nm using a micro plate reader (Multiskan GO, Thermo Scientific, Australia) and the total polyphenol content was expressed as milligrams of gallic acid equivalent (GAE) per kilogram of fresh strawberry sample (mg kg^{-1} FW). The same procedures were repeated with the solvent blank and the gallic acid standards.

Total antioxidant capacity (TAC)

The TAC in strawberry samples was analyzed using ABTS assay by Re *et al.*[38] with slight modifications. The $ABTS^{\bullet+}$ radicals solution was prepared by reacting the stock solution of ABTS (7 mM) with 2.45 mM potassium per sulfate at the same ratio. The mixture was incubated overnight in the dark to generate the green ABTS• organic free radicals. It was then diluted with methanol to an absorbance of 0.7 at $\lambda = 734$ nm. Sample extract or standard Trolox solution (25 µL) was mixed with ABTS solution (250 µL) directly in a 96-well microplate and the absorbance was read at 734 nm after 6 min. The TAC was reported as µmol Trolox equivalents per gram of strawberry fresh weight (µmol g^{-1} FW).

Total monomeric anthocyanin content (TMAC)

The TMAC of strawberries was determined using the pH differential method as described by Giusti and Wrolstad.[39] This method is based on the significant reversible changes in absorption of the anthocyanin pigments when the pH changes between 1.0 (highest absorption) and 4.5 (lowest = colorless hemiketal). The strawberry samples were appropriately diluted in 0.025 mol L^{-1} potassium chloride (pH 1.0) and 0.4 M sodium acetate (pH 4.5). The mixtures were kept for 15 min at room temperature before the absorptions were measured at 496 and 700 nm using the microplate reader. The TMAC was calculated using molar absorptivity of 27 300 L cm mol^{-1} and molecular weight of 433.2 g mol^{-1} for the anthocyanin Pel-3-glu and expressed as mg kg^{-1} FW.

Total flavonoid content (TFC)

A slightly modified colorimetric aluminium chloride method[40] was used in the determination of the samples' TFC. In a microplate well, 125 µL of strawberry extract or solvent blank or quercetin standard was mixed with 25 µL of 1 M sodium acetate, 25 µL of 10% aluminium chloride, and 175 µL of Milli-Q water. The mixtures in the microplate were then left at room temperature for 1 h before measuring the absorbance at 415 nm using a microplate reader. The TFC was calculated using quercetin as a standard and expressed as mg of quercetin equivalents per kilogram of strawberry fresh weight (mg kg^{-1} FW).

HPLC-UV analysis for identification and quantification of individual polyphenols

Separation of polyphenols was performed using a Gemini C18 silica 250 × 4.6 mm, 5 µm column (Phenominex Inc., Lane Cove West, NSW, Australia) connected to a HPLC system equipped with Water 2690 Alliance Separation Module (Waters, Rydalmere NSW, Australia) and coupled with a Waters 2998 Photodiode Array (PDA) Detector. High-performance liquid chromatography analysis was performed following a method discussed by Kosar *et al.*[16] with slight modifications. The mobile phase was acidified with 0.2% (v/v) formic acid in Milli-Q water, (A) and acetonitrile (B). The gradient was 5% B for 1 min, 10% B at 10 min, 13% B at 15 min, 20% B at 20 min, 30% B at 25 min, 100% B at 35 min, and returned to 5% B at 40 min. The injection volume was 20 µL and the flow rate was maintained at 1 mL min^{-1}. Separation and quantification of polyphenols were performed at room temperature (25 °C). Identification of individual polyphenol peaks was based on both internal and external standards.[41] Identified peaks in strawberry extracts were quantified based on peak area and mass of the external calibration standard that generated the corresponding curve to the peak. During the quantification, the average peak area was calculated as the average of two targeted peaks from duplicated HPLC injections. Different polyphenol compounds were detected and quantified at optimum and different wavelengths as required. These included, pel-3-glu, pel-3-rut and cyanidin at 510 nm; kaempferol-3-glucoside and kaempferol-3-glucurnoride at 360 nm; resveratrol at 320 nm; and catechin, ferulic acid, quercertin-3,4-di glucoside, quercertin-3-*O*-glucuronide, *p*-coumaric and *p*-Coumaroyl at 280 nm. Results were expressed as milligrams or micrograms per kilogram of strawberry fresh weight (mg or µg kg^{-1} FW).[12]

Statistical analysis

Each laboratory experiment was performed in duplicate (two trials) and each sample was triplicated in each trial. The results were presented as mean ± standard deviation (SD). Data were statistically analyzed using Minitab® 17 Statistical Software following ANOVA procedures with a general linear model (Minitab 17 Statistical Software (2010). The differences between the means were determined using Tukey's multiple comparison method at a 95% confidence level.

RESULTS AND DISCUSSION

Effect of high temperature and e[CO_2] on total polyphenols, flavonoid, anthocyanin and antioxidant content in fresh strawberry

In general, an increase in [CO_2] from 400 to 950 µmol mol^{-1} tended to increase the TPC, TFC, TMAC, and TAC of strawberry fruit

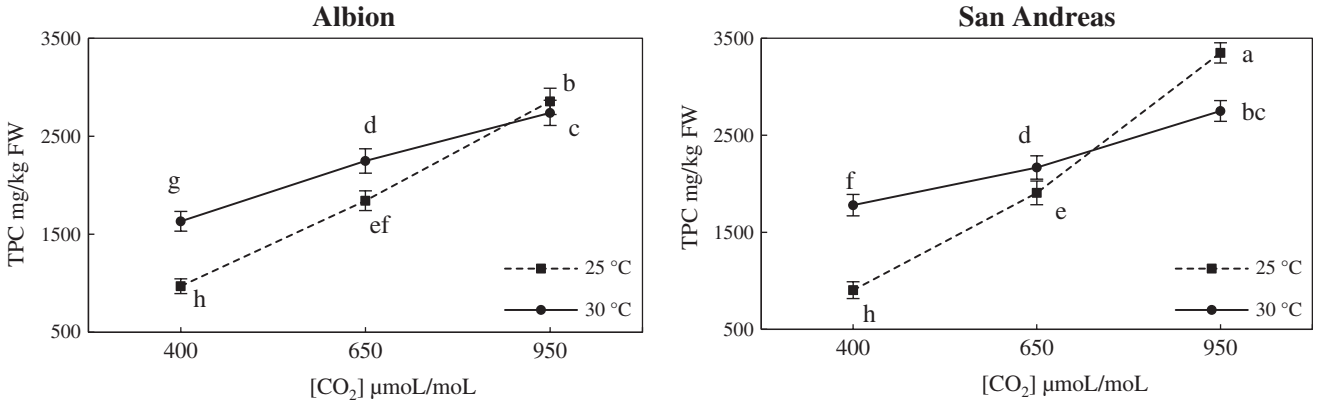

Figure 1. Total polyphenolic content (TPC) of two different strawberry cultivars ('Albion' and 'San Andreas') at different combinations of temperature and [CO_2]. Error bars refer to standard deviation of data (n = 12). Different letters in both cultivars and within each CO_2 concentration are significantly ($P \leq 0.05$) different.

significantly ($P < 0.05$) across cultivars. An increase of temperature by 5 °C significantly enhanced the TPC, TFC, TMAC, and TAC of strawberries irrespective of the cultivar. However, strawberry fruits in the 'Albion' cultivar contained significantly higher TAC than 'SA' grown at a higher temperature (30 °C). As a whole, considering the effect of strawberry cultivar, the 'SA' cultivar had remarkably higher total polyphenol and anthocyanin content; however, the TAC in the fruits was significantly higher in the 'Albion' cultivar. The interaction between [CO_2] and temperature had significant impacts on the TPC, TFC, TMAC, and TAC of strawberries and the responses were cultivar dependent.

The effects of temperature, [CO_2] and their interactions on the TPC of fruits in the 'Albion' and 'SA' cultivars are shown in Fig. 1. In general, the TPC of strawberries ranged from 970 ± 74 to 2856 ± 134 mg GAE kg^{-1} FW for the 'Albion' cultivar, and from 904 ± 87 to 3350 ± 104 mg GAE kg^{-1} FW for the 'SA' cultivar. Although higher temperature (30 °C) had significant positive impacts on the TPC of strawberry grown at ambient (400 µmol mol^{-1}) and moderately high (650 µmol mol^{-1}) [CO_2], the effects turned negative at very high [CO_2], indicating strong temperature and [CO_2] interaction effects on TPC. The highest TPCs were detected at a maximum level of [CO_2] and 25 °C temperature in both 'Albion' and 'SA' while the lowest TPCs were observed under 400 µmol mol^{-1} and 25 °C for both strawberry cultivars (Fig. 1).

It could therefore be concluded that, despite the increases in temperature, elevated [CO_2] positively affected on TPC in fruits of both cultivars. Higher temperature (30 °C) at 400 µmol mol^{-1} [CO_2] enhanced fruit polyphenol contents by 68% and 98% in 'Albion' and 'SA', respectively. Elevated temperature (30 °C) at lower [CO_2]; 400 µmol mol^{-1} and 650 µmol mol^{-1}, increased the TPC of fruits but, it was opposite at 950 µmol mol^{-1} in both cultivars. However, both elevated temperature (30 °C) and [CO_2] to 950 µmol mol^{-1} increased polyphenols by 182% in 'Albion' and 206% in 'SA'.

The TFC of fruits in both strawberry cultivars under different temperatures, [CO_2]s, and their interactions are shown in Fig. 2. The TFC of fruits ranged from 173 ± 48 to 482 ± 68 mg kg^{-1} FW for the 'Albion' cultivar and from 155 ± 30 to 405 ± 43 mg kg^{-1} FW for the 'SA' cultivar. Increased temperature (30 °C) under 400 µmol mol^{-1} [CO_2] significantly enhanced the strawberry flavonoid content by 65% and 113% respectively in 'Albion and 'SA'. The interaction of e[CO_2] and higher temperature also had a significant impact on the TFC of strawberries and varied across the cultivars. Interaction effect of, 950 µmol mol^{-1} [CO_2] and 30 °C enhanced the TFC by 183% and 173% respectively in 'Albion' and 'SA'. The interaction effect of e[CO_2] and temperature was stronger on the TFC of the fruits in the 'SA' cultivar than in the 'Albion' cultivar (Fig. 2). Significantly lower total flavonoid content was reported at 400 µmol mol^{-1} [CO_2] and 25 °C in both cultivars.

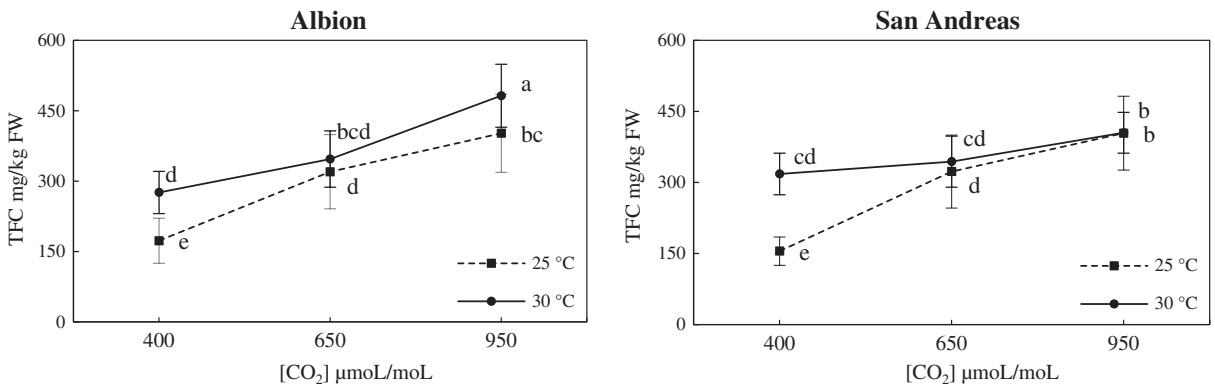

Figure 2. Total flavonoid contents (TFC) of two different strawberry cultivars ('Albion' and 'San Andreas') at different combinations of temperature and [CO_2]. Error bars refer to standard deviation of data (n = 12). Different letters in both cultivars and within each CO_2 concentration are significantly ($P \leq 0.05$) different.

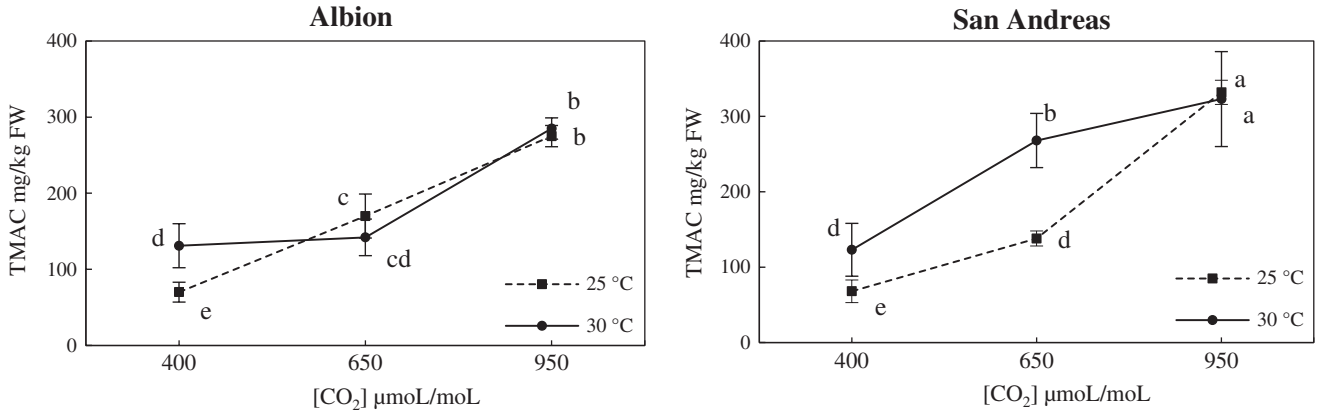

Figure 3. Total monomeric anthocyanin contents (TMAC) of two different strawberry cultivars ('Albion' and 'San Andreas') at different combinations of temperature and [CO_2]. Error bars refer to standard deviation of data (n = 12). Different letters in both graphs are significantly ($P \leq 0.05$) different.

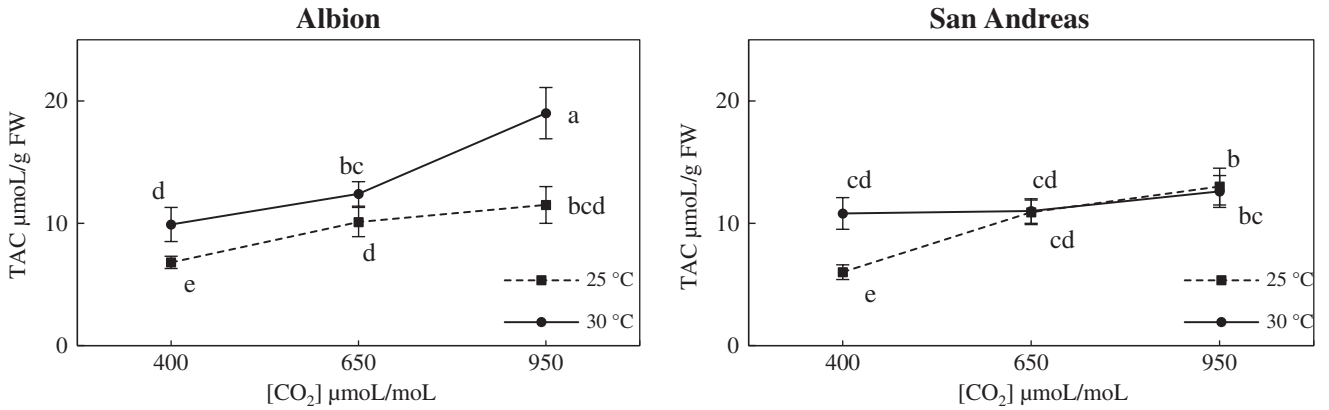

Figure 4. Total antioxidant contents (TAC) (µmol Trolox equivalents per g of FW) of two different strawberry cultivars ('Albion' and 'San Andreas') at different combinations of temperature and [CO_2]. Error bars refer to standard deviation of data (n = 12). Different letters in both graphs are significantly ($P \leq 0.05$) different.

Figure 3 illustrates the effects of temperature, [CO_2], and their interactions on the TMAC of strawberry fruits in the 'Albion' and 'SA' cultivars. The TMAC ranged from 70 ± 14 to 285 ± 14 mg kg^{-1} FW in the 'Albion' cultivar and from 67 ± 15 to 331 ± 16 mg kg^{-1} FW in 'SA'. A temperature increase of 5 °C in lower and moderately high [CO_2] positively affected the TMAC of fruits in the 'SA' cultivar; however, the effect became negative at 950 µmol mol^{-1} [CO_2], showing a stronger interaction between [CO_2] and temperature. Although a higher temperature showed a significantly positive effect on the TMAC of fruit in the 'Albion' cultivar under 400 and 950 µmol mol^{-1}, it had a negative effect at 650 µmol mol^{-1}. The results also revealed a significant interaction effect of [CO_2], temperature and cultivar on the TMAC of strawberry fruit (Fig. 3). Moderately high [CO_2] and 30 °C had a significant positive impact on the TMAC in the 'SA' cultivar; however, it was totally opposite for the 'Albion' cultivar. Such variations in the responses of the tested cultivars to elevated [CO_2] and temperature could be attributed to the influence of each cultivar's genotype on strawberry polyphenols. Increasing the temperature from 25 to 30 °C at 400 µmol mol^{-1} enhanced strawberry anthocyanins by only 80% and 70% in 'Albion' and 'SA', respectively. The increase in the TMAC of strawberries grown under 950 µmol mol^{-1} [CO_2] and 30 °C temperature was approximately 300% to 350% in comparison to the fruits grown under ambient growth conditions, which had the lowest TMAC.

Figure 4 shows the effects of [CO_2], temperature, and their interactions on the TAC of strawberry fruit. The interaction effect of [CO_2] and temperature on the TAC of strawberry was significant and cultivar dependent (Fig. 4). Increasing the temperature to 30 °C positively affected the content of strawberry antioxidants under all [CO_2] in the 'Albion' cultivar; however, it negatively affected the TAC of fruit in the 'SA' cultivar under moderate and highest [CO_2]. Elevated temperature (30 °C) at ambient [CO_2] enhanced TAC of strawberry fruits by 46% and 80% respectively in cultivar 'Albion' and 'SA'. However, the highest e[CO_2] and higher temperatures increased the TAC by 179% and 110%, respectively in 'Albion' and 'SA'. The results explained a stronger interaction of [CO_2], temperature and cultivar on the TAC of strawberries. The 'Albion' cultivar had the maximum Trolox equivalent TAC of 19.0 ± 2.1 µmol g^{-1} FW under 950 µmol mol^{-1} and 30 °C and 'SA' contained the maximum of 12.6 ± 1.3 µmol g^{-1} FW under 950 µmol mol^{-1} and 25 °C. Strawberry therefore contains greater fruit antioxidant content under elevated [CO_2] and high temperature. These findings were in agreement with results reported in this section regarding the effect of high temperature and elevated CO_2 on total polyphenol content, and the conclusion of Wang and Lin.[42] It has also been reported that the total antioxidants of fruits would increase significantly in the presence of higher contents of polyphenols,[43] flavonoids, and anthocyanins.[44]

The correlations between TAC and TPC, TFC, and TMAC are summarized in Table 1. The correlation values between the TAC and

Table 1. Pearson's correlation matrix between TPC, TFC and TMAC in strawberry cultivars

Variable	TPC	TFC	TMAC
TFC	0.919**		
TMAC	0.922**	0.851**	
TAC	0.789*	0.937**	0.729*

Values are Pearson correlation coefficients.
*Significant correlation at $P \leq 0.05$.
**Significant correlation at $P \leq 0.001$.

TPC, TFC, and TMAC were r = 0.94, $P \leq 0.001$, r = 0.79, $P \leq 0.05$, and r = 0.73, $P \leq 0.05$, respectively. Similarly, the results also reveal that TPC significantly correlated with both the TFC and TMAC (r = 0.92, $P \leq 0.001$) of strawberries. Further, TFC positively correlated with TMAC (r = 0.85, $P \leq 0.001$) in fresh strawberries.

The positive correlation between increment in antioxidant, phenolic, anthocyanin, and flavonoid compounds was also reported by Wang *et al.*[33] It is evident that flavonoids may increase in plants as a part of general stress response and provide beneficial antioxidant properties.[45] These antioxidant compounds are produced in plants to help to reduce oxidative stresses caused by free radicals.[46] The hydroxyl groups attached to the aromatic rings are responsible for the free radical scavenging properties of phenolic compounds. Besides the direct health benefits of polyphenols as antioxidants, strawberry polyphenols indirectly play a fundamental role against chronic metabolic disorders and cancers.[7] The same authors explained that strawberry polyphenols may be involved with cellular signaling, may control gene transcription and expression in cellular metabolisms, and may influence survival by preventing chronic disorders and cancers.

Previous studies have reported significantly higher polyphenol, flavonoid, anthocyanin, and antioxidant content under increased growth temperatures[21] or e[CO_2].[33] In this study, e[CO_2], increased temperature and their interactions enhanced the levels of polyphenols, flavonoids, anthocyanins, and antioxidants in strawberries; however, these responses varied significantly across the cultivars. The highest TPCs and TMACs were detected in the 'SA' cultivar. However, TFC and TAC were significantly ($P < 0.05$) higher in the 'Albion' cultivar. A previous study by Palmieri *et al.*[17] demonstrated the effect of genotype and environmental conditions on the nutrient content in nine strawberry cultivars. According to those results, 'Albion' was the most sensitive of the nine in responding to the different environmental conditions. Palmieri *et al.*[17] attributed the higher plant secondary product content under different growth conditions to the acclimatization imposed by environmental stresses. The same authors concluded that genotype was more influential on some strawberry cultivars over all the other parameters. A study by Ariza *et al.*[47] showed that genotype has a strong influence on strawberry phenolic and anthocyanin contents. The current study revealed that, similar to the genotype effect, e[CO_2] and / or higher temperature could significantly ($P < 0.05$) enhance the polyphenol and antioxidant content of strawberry fruit. As polyphenols, flavonoids, anthocyanins, and antioxidants are considered to be important fruit bioactive compounds, increasing the content of such fruit nutrients with e[CO_2], higher temperature and their interactions would improve strawberries' functional properties. Consequently, strawberries grown under higher temperatures (30 °C) and e[CO_2] (650 and 950 μmol mol^{-1}) could support better human health.

Table 2. Identification of strawberry polyphenols

Peak	Polyphenol compound	RT (minutes)	λ (nm)
01	Catechin	17.5 ± 0.03	280
02	Unknown	18.2 ± 0.30	280
03	Pelargonidin-3-glucoside (pel-3-glu)	20.0 ± 0.16	510
04	Pelargonidin-3-rutinoside (pel-3-rut)	20.8 ± 0.12	510
05	Cyanidin	23.5 ± 0.21	510
06	Quercetin-3,4-di glucoside (Q-3,4-diglu)	23.8 ± 0.26	280
07	p-Coumaric	24.2 ± 0.80	280
08	Ferulic acid	25.4 ± 0.21	280
09	Quercetin-3-*O*-glucuronide (Q-3-*O*-glu'nide)	26.8 ± 0.21	280
10	Coumaroyl	27.4 ± 0.22	280
11	Kaempferol-3-glucoside (K-3-glu)	28.3 ± 0.28	360
12	Kaempferol-3-glucuronide (K-3-glu'nide)	28.7 ± 0.26	360
13	Resveratrol	29.9 ± 0.31	320

Different polyphenol compounds were identified in a range of 200–600 nm wavelength. λ – specific wavelength of compound quantification, RT – retention time (mean ± SD).

Identification and quantitation of individual polyphenols in strawberry gown under high temperature and e[CO_2]

The main polyphenols that have been reported in strawberries belong to chemical classes, namely flavonoids (anthocyanins, flavanols, flavonols), phenolic acids, lignans, stilbens, tannins and coumarins.[48] In this investigation, 12 different phenolic compounds were identified in 'Albion' and 'SA' cultivars using external and internal standards at different wavelengths (Table 2). High-performance liquid chromatography chromatograms of polyphenol compounds in strawberries at different wavelengths are shown in Fig. 5**.**

Peak 2 detected at 280, 320, and 360 nm (Fig. 5) could not be identified with external or internal standards due to the inaccessibility of the reference standard. According to the literature,[11,49] this peak could be cyanidin-3-glucoside, and was reported to be constant in all strawberry varieties.[11] Most of the identified polyphenol compounds were quantified considering their peak areas at the maximum absorbance. Tables 3 and 4 show the concentrations of different polyphenol compounds of fresh fruits of the 'Albion' and 'SA' cultivars and illustrate the effects of different [CO_2], temperature, and their interactions on each compound.

Effect of e[CO_2] and high temperature on strawberry anthocyanins

Anthocyanins are very important in the strawberry polyphenol profile; specifically, they are the major group of pigments accounting for the strawberry's color. The aglycones of pelargonidin and cyanidin are the predominant anthocyanins[11] and are very much responsible for the red color, depending on cultivar.[22] Pel-3-glu revealed the highest contents followed by cyanidin and pel-3-rut in both 'Albion' and 'SA'. The impacts of [CO_2], temperature, and their interactions on different anthocyanins compounds of fruits in strawberry cultivars 'Albion' and 'San Andreas' are shown in Table 3.

The pel-3-glu content ranged from 115 ± 23 to 273 ± 30 mg kg^{-1} FW for the 'Albion' cultivar and from 114 ± 24 to 348 ± 33 mg kg^{-1} FW for the 'SA' cultivar. Elevated [CO_2] gradually increased the content of pel-3-glu in strawberry fruits in both cultivars. Elevating

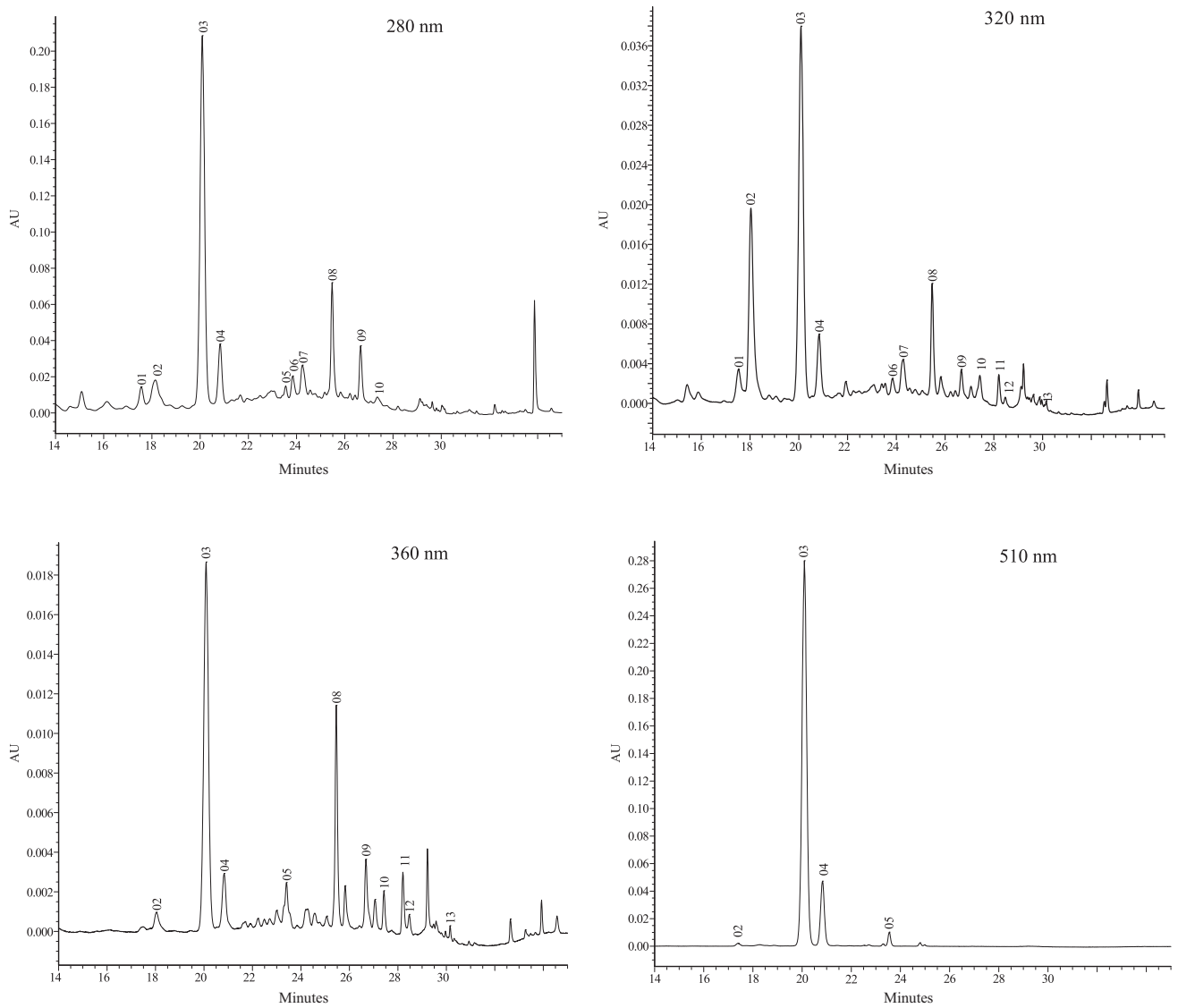

Figure 5. High-performance liquid chromatography chromatographs of polyphenols of strawberries obtained at 280, 320, 360, and 510 nm. 1. Catechin, 2. Unknown, 3. Pelargonidin-3-Glucoside (Pel-3-Glu), 4. Pelargonidin-3-Rutinoside (Pel-3-Rut), 5. Cyanidin, 6. Quercetin-3,4-di Glucoside (Q-3,4-diGlu), 7. p-Coumaric, 8. Ferulic acid, 9. Quercetin-3-*O*-Glucuronide (Q-3-*O*-Glu'nide), 10. Coumaroyl, 11. Kaempferol-3-Glucoside (K-3-Glu), 12. Kaempferol-3-Glucuronide (K-3-Glu'nide), and 13. Resveratrol.

$[CO_2]$ to 950 µmol mol^{-1} caused 137% and 205% increment in pel-3-glu content in 'Albion' and 'SA' respectively in comparison with the growth at 400 µmol mol^{-1} and 25 °C. A higher temperature had a positive impact on pel-3-glu only at 400 µmol mol^{-1} by increasing the pel-3-glu content by 15% and 51% in 'Albion' and 'SA' respectively. Under e$[CO_2]$ (650 and 950 µmol mol^{-1}), a 30 °C temperature had a negative impact on pel-3-glu, showing a significant interaction effect of $[CO_2]$ and temperature. The interaction of elevated $[CO_2]$ (950 µmol mol^{-1}) and higher temperature (30 °C) enhanced pel-3-glu content by 90% and 103% in 'Albion' and 'SA' respectively, in comparison with plants grown under 400 µmol mol^{-1} and 25 °C. The maximum pel-3-glu content was detected under maximum e$[CO_2]$ and 25 °C in both cultivars; however, the 'SA' cultivar contained significantly more pel-3-glu than 'Albion'.

Elevated $[CO_2]$ and higher temperature separately showed positive effects on pel-3-rut content in both cultivars (Table 3). The highest e$[CO_2]$ and higher temperature interactively enhanced the pel-3-rut content of fruit from 18 ± 3 to 41 ± 4 mg kg^{-1} FW for the 'Albion' cultivar and from 24 ± 4 to 58 ± 6 mg kg^{-1} FW for the 'SA' cultivar, respectively. The interaction effect of $[CO_2]$ and temperature on the pel-3-rut content was significantly dependent on the cultivar. Higher temperature and 950 µmol mol^{-1} $[CO_2]$ positively affected the pel-3-rut content of strawberries in the 'Albion' cultivar; however, the effect turned negative in the 'SA' cultivar (Table 3). Under all growth combinations, 'SA' contained comparatively and significantly higher pel-3-rut content than 'Albion'.

The cyanidin content of fruit in the 'Albion' and 'SA' cultivars was increased by e$[CO_2]$ from 400 to 950 µmol mol^{-1} (Table 3). However, the interaction of e$[CO_2]$ and temperature was stronger on the cyanidin content of strawberry than their individual impacts. Elevated temperature (30 °C) was positively affected on cyanidin contents of strawberry fruits in both cultivars only under 400 and 950 µmol mol^{-1} $[CO_2]$. Higher temperature had a negative impact on cyanidin contents under 650 µmol mol^{-1} in both cultivars. Elevating the $[CO_2]$ to 950 µmol mol^{-1} at 30 °C increased the cyanidin content from 27 ± 4 to 175 ± 32 mg kg^{-1} FW in the 'Albion' cultivar

Table 3. Effect of different temperature and [CO_2] on different anthocyanins and flavonols compounds in strawberry cultivars

			Anthocyanins			Flavonols			
Cultivar	T	[CO_2]	Pel-3-Glu [a]	Pel-3-Rut [b]	Cyanidin [c]	Q-3,4-di-*O*-Glu'side [d]	Q-3-*O*-Glu'nide [e]	K-3-Glu'side [f]	K-3-Glu'nide [g]
Albion	25 °C	400	115 ± 23 f	18 ± 3 g	27 ± 4 g	10.1 ± 1.0 e	18 ± 2 c	596 ± 81 e	512 ± 81 e
		650	177 ± 29 d	25 ± 3 ef	79 ± 13 d	19.3 ± 2.1 b	6.3 ± 0.8 g	508 ± 58 e	534 ± 58 e
		950	273 ± 30 b	34 ± 4 c	94 ± 9 d	24.6 ± 3.4 a	20 ± 3 c	1432 ± 111 c	733 ± 111 d
	30 °C	400	132 ± 42 ef	29 ± 4 cde	48 ± 6 fg	20.1 ± 1.5 b	39 ± 3 b	619 ± 69 de	624 ± 69 de
		650	165 ± 22 de	29 ± 4 d	61 ± 4 ef	24.8 ± 2.8 a	13 ± 3 fg	760 ± 87 d	572 ± 87 e
		950	219 ± 25 c	41 ± 4 b	175 ± 32 a	16.5 ± 3.3 c	15 ± 2 cde	168 ± 121b	1767 ± 121 a
San Andreas	25 °C	400	114 ± 24 f	24 ± 4 f	28 ± 3 g	9.8 ± 1.1 e	18 ± 3 cd	603 ± 85 e	591 ± 85 e
		650	154 ± 18 de	31 ± 4 de	66 ± 8 ef	20.1 ± 2.6 b	10 ± 2 efg	532 ± 65 e	595 ± 65 e
		950	348 ± 33 a	62 ± 6 a	128 ± 18 c	25.1 ± 3.3 a	52 ± 7 a	1849 ± 33 a	949 ± 33 c
	30 °C	400	172 ± 60 d	34 ± 3 cd	48 ± 5 fg	20.4 ± 2.4 b	45 ± 5 b	469 ± 49 b	1407 ± 49 b
		650	160 ± 28 de	34 ± 4 c	57 ± 4 ef	22.6 ± 3.8 ab	9.9 ± 2 def	626 ± 25 e	590 ± 25 e
		950	231 ± 26 c	58 ± 6 a	150 ± 22 b	13.9 ± 1.7 d	9.4 ± 2 fg	1439 ± 28 c	1527 ± 28 b
Temperature × [CO_2]			*	*	*	*	*	*	*
Temperature × [CO_2] × Cultivar			*	*	*	*	*	*	*

Values are mean ± SD (n = 12), different letters in each column are significantly ($P \leq 0.05$) different.
[a,b,c,d,e] expressed as mg kg^{-1} FW, [f,g] expressed as μg kg^{-1} FW.
*Significant ($P \leq 0.05$).
T – Temperature and [CO_2] = μmol mol^{-1}.

and from 28 ± 3 to 150 ± 22 mg kg^{-1} FW in the 'SA' cultivar. Elevating [CO_2] from 400 to 950 μmol mol^{-1} at 30 °C increased the cyanidin content by 600% and 435% in 'Albion' and 'SA', respectively.

Both pel-3-glu and pel-3-rut (pelargonidin derivatives) were detected in larger quantities in the 'SA' cultivar, while cyanidin was present in large quantities in the 'Albion' cultivar when grown under the highest e[CO_2] and at higher temperatures (950 μmol mol^{-1} and 30 °C). These results were in agreement with those reported by Wang and Zheng[21] and Wang *et al.*[33]

Effect of e[CO_2] and high temperature on strawberry flavonols

Flavonol compounds showed significant variations under increased [CO_2], temperature, and their combination (Table 3). The flavonol content was comparatively lower than the anthocyanin content in strawberries. The quercetin glucoside (Q-3, 4-di-*O*-Glu) content of fruit ranged from 10.1 ± 1.0 to 24.8 ± 2.8 mg kg^{-1} of FW and 9.8 ± 1.0 to 25.1 ± 3.0 mg kg^{-1} FW in the 'Albion' and 'SA' cultivars, respectively (Table 3). Elevated [CO_2] significantly increased the Q-3, 4-di-*O*-glu content at 25 °C in both strawberry cultivars. Higher temperatures enhanced the Q-3, 4-di-*O*-glu content of strawberries however, interaction effect of increased temperature (30 °C) and 950 μmol mol^{-1} affected negatively on Q-3, 4-di-*O*-Glu contents in both strawberry cultivars.

Kaempferol glucoside (K-3-glu'side) and glucuronide (K-3-glu'nide) were found in comparatively smaller quantities in strawberries compared to other flavonoids (Table 3). K-3-glu'side content varied from 596 ± 81 to 1648 ± 121 μg kg^{-1} FW and 603 ± 85 to 1849 ± 33 μg kg^{-1} FW in the 'Albion' and 'SA' cultivars, respectively. Elevated [CO_2], temperature, and their interactions, significantly increased the amount of kaempferol in strawberries; however, the amounts varied among cultivars. Unlike anthocyanins, which showed the greatest increment at 30 °C and 950 μmol mol^{-1} [CO_2], the maximum amounts of K-3-glu'side were detected under 950 μmol mol^{-1} and 25 °C growth conditions in both cultivars. K-3-glu'nide content ranged from 512 ± 81 to 1767 ± 121 μg kg^{-1} FW and from 591 ± 85 to 1527 ± 28 μg kg^{-1} FW in 'Albion' and 'SA', respectively. The lowest amounts were detected under 400 μmol mol^{-1} and 25 °C and the highest content under the highest e[CO_2] and higher temperatures. Like the results for anthocyanins, most flavonol content revealed the greatest amounts under extreme growth conditions of 30 °C and 950 μmol mol^{-1}. These findings are in agreement with those reported in the literature.[21,33] For example, Wang *et al.*[33] reported higher quercetin glucoside, quercetin glucuronide, and kaempferol glucoside concentrations in strawberry fruit under enriched (600 μmol mol^{-1} + ambient) [CO_2] conditions. These flavonoids contain hydroxyl or methoxy groups, which contribute to their biological and antioxidant properties. Increased amounts of these flavonoids play pivotal roles in preventing oxidative stresses.[50]

Effect of e[CO_2] and high temperature on other phenolic compounds

In addition to flavonols, which were discussed before, catechin, ferulic, coumaric, coumaroyl, and resveratrol were detected and quantified in strawberries (Table 4). All these compounds were found to vary significantly in strawberry cultivars under different [CO_2] and temperature conditions and their interactions. Catechin is a monomeric flavanol, generally, reported to be difficult to detect.[51] When [CO_2] was elevated from 400 to 950 μmol mol^{-1} in the growth environment, the catechin content of fruits increased gradually from 9 ± 1.6 to 43 ± 5.8 mg kg^{-1} FW and 8 ± 0.8 to 58 ± 4.3 mg kg^{-1} FW in 'Albion' and 'SA', respectively. Higher temperature also positively affected the catechin content of fruit in both strawberry cultivars only under elevated [CO_2]. The interaction of e[CO_2], temperature, and cultivar was statistically significant ($P < 0.05$). Interaction effects of elevated temperature (30 °C) and 650 and 950 μmol mol^{-1} [CO_2] enhanced catechin concentrations in the 'Albion' strawberry cultivar. However, higher temperature showed a negative impact

Table 4. Effect of different temperature and [CO_2] on other polyphenol compounds in strawberry varieties

Cultivar	Temperature	[CO_2]	Catechin[a]	Ferulic acid[b]	*p*-Coumaroyl[c]	*p*-Coumaric[d]	Resveratrol[e]
Albion	25 °C	400	9 ± 1.6 f	24 ± 0.4 ef	4.4 ± 0.4 c	1.07 ± 0.03 c	23 ± 1 f
		650	15 ± 2.4 ef	28 ± 1 cd	10.4 ± 0.8 b	1.35 ± 0.03 c	65 ± 8 cd
		950	32 ± 4.8 d	23 ± 1 e	10.7 ± 0.3 b	2.86 ± 0.02 a	59 ± 3 de
	30 °C	400	9 ± 0.7 f	63 ± 4 b	5.5 ± 0.8 c	3.23 ± 0.03 a	66 ± 2 cd
		650	21 ± 0.4 e	30 ± 2 b	9.4 ± 1.9 b	2.98 ± 0.03 a	84 ± 7 b
		950	43 ± 5.8 c	29 ± 1 cd	14.0 ± 1.6 a	2.39 ± 0.03 b	113 ± 5 a
San Andreas	25 °C	400	8 ± 0.8 f	24 ± 0.4 ef	4.4 ± 0.3 c	1.05 ± 0.02 c	23 ± 2 f
		650	20 ± 2.2 e	30 ± 0.9 cd	11.0 ± 0.9 b	2.15 ± 0.02 b	71 ± 6 c
		950	58 ± 4.3 a	25 ± 2 f	13.9 ± 0.9 a	2.79 ± 0.02 a	58 ± 5 e
	30 °C	400	8 ± 0.8 f	67 ± 3 a	5.6 ± 0.8 c	2.89 ± 0.03 a	71 ± 3 c
		650	30 ± 4.4 d	31 ± 2 c	10.2 ± 1.7 b	2.90 ± 0.03 a	82 ± 6 b
		950	50 ± 6.0 b	28 ± 0.4 cd	15.4 ± 0.7 a	2.96 ± 0.08 a	68 ± 2 c
Temperature× [CO_2]			*	*	*	*	*
Temperature× [CO_2] × Cultivar			*	*	*	*	*

Values are mean ± SD (n = 12), different letters in each column are significantly ($P \leq 0.05$) different.
[a,b,c,d]Expressed as mg kg^{-1} FW.
[e]Expressed as μg kg^{-1} FW.
[CO_2] = μmol mol^{-1}.
*Significant ($P \leq 0.05$).

on the catechin content of fruits in the 'SA' cultivar at 950 μmol mol^{-1}. The 'SA' cultivar contained comparatively higher catechin content than 'Albion' under all e[CO_2] and higher temperature conditions.

Increased temperature significantly influenced on the ferulic acid content of strawberries (Table 4). Ferulic acid content of strawberries showed changes in pattern compared with catechin, with the maximum detected quantities of 63 ± 4 and 67 ± 3 mg kg^{-1} FW in cultivar 'Albion' and 'SA' respectively, at 400 μmol mol^{-1} CO_2 and 30 °C. Consequently, increasing the [CO_2] above the 400 μmol mol^{-1} would not have any significant effect on ferulic acid. However, the interaction of [CO_2], temperature and cultivar was significant on ferulic acid contents of fruits. The same results revealed that e[CO_2] at higher temperature caused negative effects on strawberries. Coumaroyl content ranged from 4.4 ± 0.4 to 14 ± 1.6 mg kg^{-1} FW for the 'Albion' cultivar and from 4.4 ± 0.3 to 15.4 ± 0.7 mg kg^{-1} FW for 'SA'. The lowest coumaroyl content was detected under 400 μmol mol^{-1} and 25 °C and the highest under highest e[CO_2] and higher temperature. In previous studies, higher contents of *p*-coumaroyl glucose was reported in strawberry grown under high temperature (30 °C)[21] and enriched [CO_2].[33] Greater amounts of coumaroyl derivatives were also detected in grapes grown under high-growth temperatures.[52,53]

Resveratrol is a stilbene polyphenol and is available only in a few plant sources including strawberries in smaller quantities. As far as resveratrol is concerned, its synthesis can be induced successfully in plants using molecular engineering techniques.[54] From all the polyphenol compounds identified in this study, resveratrol had the lowest quantities in strawberry. The resveratrol content in strawberry varied from 23 ± 1 to 113 ± 5 μg kg^{-1} FW and from 23 ± 2 to 82 ± 6 μg kg^{-1} FW in 'Albion' and 'SA', respectively. Elevated [CO_2] and high temperature, and interactions between them, enhanced the resveratrol contents in strawberries. However, 'Albion' revealed the highest resveratrol content under 950 μmol mol^{-1} and at 30 °C and 'SA' under 650 μmol mol^{-1} and at 30 °C. Similar observation were reported by Wang *et al.*[34] who demonstrated an increase in resveratrol contents in strawberries under high growth temperatures (25 and 30 °C) or under elevated [CO_2] (ambient + 600 μmol mol^{-1}) in the growth environment. In the current study, the resveratrol content in strawberries was increased by both e[CO_2] and temperature compared to the strawberries grown under normal (400 μmol mol^{-1} and 25 °C temperature) growth conditions. Like other polyphenols, resveratrol can contribute significantly to the antioxidant content in strawberry fruits. Resveratrol has also been linked to many health benefits in humans including preventing and delaying cancers, cardiovascular diseases, heart diseases, pathological inflammations, viral infections, and tumors.[54] Therefore resveratrol produces no harm even when the intake is high.[55] All these phenolic compounds express antagonistic and synergistic activities interactively with other polyphenols and / or phytochemicals in fruits[40] and could increase the biological potential of strawberries.

The positive impacts of e[CO_2] 650 and 950 μmol mol^{-1} and higher temperature (30 °C) individually and interactively on the contents of individual polyphenols in strawberries were clearly established in this investigation. These findings agree with the literature, which indicates that, under optimal growth conditions, plants promote biomass production and reduce the biosynthesis of secondary metabolites.[56] However, plants stimulate the production of secondary metabolites as a defensive response to the environmental stresses.[46] The biosynthesis of polyphenols starts from phenylalanine to produce phenylpropanoid.[57] *P*-coumaroyl CoA and malonyl CoA are derived from phenylpropanoid and they are the precursors of flavonoid biosynthesis. Different enzymes are involved in synthesizing major flavonoid classes.[45] These key enzymes usually compete for the same substrate to produce different flavonoid compounds. Generally, the ultimate product of the phenylpropanoid pathway is anthocyanin; however, it branches in the middle and other polyphenols, including stilbenes and lignin, are synthesized.[58] The synthesis of these compounds was reported to be sensitive to the stresses in the growth environment.[57,59]

However, the degree of stress accounts for different secondary metabolites and their different quantities in plants.[58] In this study, higher temperature (30 °C) in the growth environment, on its own

and interacting with e[CO_2], encouraged the increase of individual polyphenols in different quantities in strawberry. Increasing the temperature in the growth environment would increase the synthesis and accumulation of polyphenolic compounds in response to the increased rate of metabolic processes in plant.[58] However, high temperature could also stop or significantly reduce the metabolic processes in some plants due to irreversible damage to the plant tissues. This irreversible damage to some plants by heat could be varied in different plant species and cultivars. For example, Downey[58] observed that some phenolic compounds were decreased or stable at a high temperature (30 °C) in grapes.

Similarly, high [CO_2] in the growth environment can increase the carbon supply where it promotes higher carbon availability in plants and accumulates more carbohydrates.[60] These carbohydrates are first used in plant growth and then the excess amounts are used in the synthesis of carbon-based secondary products, especially soluble phenols and condensed tannins.[61]

In summary, stress growth conditions, e[CO_2] (650 and 950 µmol mol^{-1}) and / or higher temperature (30 °C), can encourage the synthesis and accumulation of phenolic compounds and significantly enhance the corresponding antioxidant properties. Although a previous study in our lab[36] showed that strawberry fruit had lower yields and physical quality under stress growth conditions, results from the current investigation revealed that strawberry fruit was rich with polyphenols and antioxidants under e[CO_2] (950 µmol mol^{-1}) and higher temperature (30 °C) conditions. The increased content of total and individual polyphenols and antioxidants of strawberry fruits will be highly beneficial to human health.

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