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Original Article

Content of total phenolics and phenolic acids in tomato (*Lycopersicon esculentum* Mill.) fruits as influenced by cultivar and solar UV radiation

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Abstract

Two cultivars of fresh market tomato [Oregon Spring (OS) and Red Sun (RS)] were each grown in two high tunnels at Beltsville, MD covered with a contrasting material of similar thickness (0.152 mm) and durability (4-year polyethylene). One covering material (Tyco Tufflite IV) transmitted ambient solar UV radiation from 290 to 400 nm (designated + UV) while the other material (Dura-Film Super 4) blocked UV wavelengths below 380 nm (designated − UV). Both films transmitted comparable amounts of photosynthetically active radiation (PAR) from 400 to 700 nm. Ripe tomato fruits comparable in size and development were collected at maturity from plants of the two cultivars grown in each high tunnel under the contrasting covering materials. Four lots of tomatoes of each cultivar and each UV treatment were assayed for total phenolic (TP) content by a colorimetric Folin-Ciocalteu (FC) assay and for content of individual phenolic acids by a high performance liquid chromatography (HPLC)-diode array detection (DAD) procedure. The phenolic acids extracted from the base hydrolyzed fraction were identified as caffeic acid, *p*-coumaric acid, and ferulic acid. Caffeic acid was the predominant phenolic acid in both tomato cultivars (OS and RS) grown in the two high tunnels. The total concentration of these three phenolic acids measured by HPLC was approximately 20% higher under + UV than under − UV treatment; this was true for both cultivars. A similar trend in quantities of TP yield was also observed when tomato extracts were assayed by a FC method. These results indicate that the phenolic content of tomato fruits is significantly affected by the spectral quality of solar UV radiation. Since phenolic compounds are known to play a key role as antioxidants in human nutrition, subtle differences in phenolic composition between the two high tunnels as a result of differences in the UV transmission properties of these different covering materials may be of considerable importance.
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Keywords: Caffeic acid; *p*-Coumaric acid; Ferulic acid; HPLC; Folin-Ciocalteu assay; High tunnels; Phenolic acids; Tomatoes; *Lycopersicon esculentum*; Total phenolics; Ultraviolet radiation

1. Introduction

Recent epidemiological studies suggest a positive correlation between diets rich in vegetables and fruits and a reduced incidence of chronic diseases (Lasheras et al., 2000). This beneficial effect is primarily attributed to the occurrence of vitamins, minerals and secondary phytochemicals such as carotenoids, anthocyanins, flavonoids, and other phenolic compounds that are widely distributed throughout the plant kingdom (Anttonen and Karjalainenb, 2005; Giuntini et al., 2005; Lasheras et al., 2000). Over 8000 phenolic compounds have been identified from plant materials. These compounds have been categorized into different groups such as flavonoids, tannins, phenolic acids and coumarins. Phenolic acids can be further classified into two groups, namely, cinnamic acid derivatives and benzoic acid derivatives, based on their basic carbon skeleton.

The quantity and the composition of phenolic compounds present in foods are influenced by genotype

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(Howard et al., 2003), storage conditions (Asami et al., 2003), extraction procedure (Hinneburg and Neubert, 2005; Mukhopadhyay et al., 2006), and environmental conditions (Robinson and Britz, 2000; Caldwell et al., 2005). Asami et al. (2003) studied the influence of ivy peeling, storage temperature (4 and 30 °C) and three different time-temperature sterilization combinations on the level of total phenolics (TP) in Ross clingstone peaches. Ninfali and Bacchiocca (2003) determined the levels of phenolics and antioxidant capacity of six fresh and frozen vegetables. They observed that four of the six vegetables (beet green, spinach, broccoli and carrot) showed a decrease in TP and ORAC values in frozen samples. Onion showed an increase in both TP and ORAC values in frozen samples, but celery samples showed no significant change in phenolic content.

Tomatoes are the second most popular vegetable in the United States, next to potatoes, and are widely consumed throughout the world. They are an excellent source of vitamin C, vitamin E, folic acid, potassium, and secondary metabolites such as β-carotene, lycopene, and phenolic compounds. The quantity and quality of phytochemicals detected in tomato fruits is known to depend greatly on genotype and environmental condition (Crozier et al., 1997; Raffo et al., 2002; Gahler et al., 2003; Giuntini et al., 2005).

Since the early 1970s there has been keen interest in assessing the impact of stratospheric ozone reduction and the attendant increase in ultraviolet radiation on the growth and development of crop plants and natural ecosystems (Turunen et al., 1999; Hao et al., 2000; Buffoni-Hall et al., 2002; Krizek, 2004; Krizek and Gao, 2004; Krizek and Chalker-Scott 2005; Rozema et al., 2005; Sullivan, 2005). Numerous UV-enhancement studies have been conducted under controlled environments in greenhouses and plant growth chambers; however, the number of UV-B enhancement studies conducted under field conditions is relatively meager. Since the early 1990s, Krizek and his co-workers at Beltsville have studied the influence of UV exclusion of ambient solar UV-A and UV-B radiation on plant growth and development (Adamse et al., 1997; Krizek et al., 1997; Krizek et al., 1998; Krizek and Mirecki, 2004). The possibility of selecting greenhouse covering materials to block the transmission of certain UV wavelengths in high tunnels affords researchers an opportunity to test the hypothesis that such UV-blocking materials may be used to provide a means of integrated pest management (IPM) in high tunnels by preventing the sporulation of pathogenic fungi such as *Botrytis* and *Alternaria* and by reducing the prevalence of diseases caused by transmission of insects that require wavelengths below 380 nm to navigate (Krizek et al., 2005; Paul et al., 2005).

Plant productivity in a greenhouse or high tunnel is related to the amount of electromagnetic radiation received, which in turn is dependent on the amount of UV radiation, PAR and infrared (IR) radiation transmitted through the covering material of these structures (Krizek, 2004). The nutrient composition of tomato fruits obtained from plants grown under high tunnels is determined by both genetic and environmental factors. A high tunnel is a relatively simple, inexpensive enclosure similar to a greenhouse that provides farmers a means to extend the growing season, provide protection from natural climatic variations and reduce disease and pest pressures (Wittwer and Castilla, 1995; Butler and Ross, 1999; Bachmann and Earles, 2000; Byczynski and Nagengast, 2003; Lamont et al., 2003). Two high tunnels with contrasting covering materials of similar thickness (0.152 mm) and durability (4-year polyethylene) were constructed at Beltsville, Maryland for this study. One covering material allowed transmission of UV radiation from 290 to 400 nm (designated + UV). The other material blocked transmission of UV wavelengths at 380 nm and below (designated − UV). Fruits of tomato plants grown under the two high tunnels (+UV) and (−UV) were collected and assayed for their phenolic composition.

Earlier publications from our group dealt with development of a HPLC–DAD procedure for separation and quantitation of 16 phenolic acids (Robbins and Bean, 2004). This research was followed by a methods paper on the assay and detection of phenolic acids from representative foods [dry beans (Luthria and Pastor-Corrales, 2006) and eggplant (Luthria and Mukhopadhyay, 2006)] and a dietary supplement [(Black cohosh (Mukhopadhyay, et al., 2006)]. The present study was conducted to extend our work on phenolic acids to tomato fruits. Here we present results on total phenolic content and composition of individual phenolic acids in two fresh market tomato cultivars "Red Sun" (RS) and "Oregon Spring" (OS) grown at Beltsville in two high tunnels covered with plastic films having different transmission properties in the UV region.

2. Materials and methods

2.1. Experimental treatment

Two cultivars of tomato (OS and RS) were each grown in two 7 m wide × 32 m long high tunnels at Beltsville, MD that were covered with two contrasting covering materials of similar thickness (0.152 mm) and durability (4-year polyethylene). One covering material [Tyco Tufflite IV, Tyco Plastics and Adhesive Division of Tyco, Inc., Monroe, LA (obtained from Maryland Plants and Supplies, Inc., Baltimore, MD)] transmitted ambient solar UV radiation from 290 to 400 nm (+UV), while the other covering material [Dura-Film Super 4, AT Plastics (Brampton, Ont., Canada (obtained from Griffin Greenhouse Supplies, Leola, PA)] blocked UV wavelengths below 380 nm (−UV). Both films transmitted comparable amounts of PAR (400–700 nm). The spectral transmission characteristics of these two covering materials were described in a previous communication by Krizek et al. (2005).

2.2. Plant samples

Four sets of tomato fruits of the two cultivars (OS and RS) were collected at maturity from each high tunnel. The tomato fruits were comparable in size and development. Half of the samples were obtained from plants grown under UV transmitting covering material and half of the samples were obtained from plants grown under UV blocking covering material.

2.3. Sample preparation

All tomato fruits were chopped and stored under an inert nitrogen atmosphere at −70 °C. Prior to freeze-drying, each tomato sample (whole fruit) was cut into small pieces and freeze-dried (FreeZone 18 L Freeze Dry System with Purge Valve and Teflon-Coated Collector, Labconco Corporation, Kansa City, MO). The freeze-dried samples were ground in a coffee grinder and screened through a size-20 standard sieve to obtain uniform particle size (particle size <0.825 mm). All freeze-dried samples were stored under nitrogen at a temperature of −60 °C or below until analyzed. For estimation of free phenolic acids, saponified tomato extracts were analyzed by HPLC. However, for the determination of TP, unsaponified tomato extracts were assayed by colorimetric Folin-Ciocalteu (FC) procedure.

2.4. Chemical reagents

Phenolic acids and standards (caffeic, ferulic, and *p*-coumaric acids) were purchased from Sigma (St. Louis, MO). HPLC-grade solvents, methanol and analytical-grade ethyl acetate were obtained from Fisher Chemicals (Fair Lawn, NJ). HPLC-grade formic acid and sodium hydroxide were procured from Aldrich Chemical Company (Milwaukee, WI) and analytical grade hydrochloric acid was purchased from Fisher Chemicals (Fair Lawn, NJ). Deionized water (18 Ω) was prepared using a Barnstead NANO pure diamond ultrapure water purification system (Barnstead International, Dubuque, IA). Polyvinylidene difluoride (PVDF) syringe filters with a pore size of 0.45 μm were purchased from National Scientific Company (Duluth, GA).

2.5. Base hydrolysis

Approximately 200 mg of freeze-dried ground tomato sample were hydrolyzed by stirring the ground sample with 5 mL of a basic solution (2 N NaOH) containing 10 mM EDTA and 1% ascorbic acid for 30 min at 40–45 °C (Nardini et al., 2002). The reaction mixture was acidified by adding 1.4 mL of 7.2 N HCl. The mixture was placed on a vortex shaker for 5–10 s and free phenolic acids were extracted with ethyl acetate (2 × 6.4 mL). The combined organic layer was evaporated to dryness under a steady stream of nitrogen. The residue was re-dissolved in 2 mL 75:25 methanol:water (% v/v). The vial was placed in a sonicator for 5 min. The extract was filtered through PVDF syringe filters (0.45 μm) and analyzed by HPLC. Three replicate base hydrolysis experiments were carried out with each tomato sample.

2.6. Separation and analysis of phenolic acids by HPLC

As reported earlier (Robbins and Bean, 2004), phenolic acid mixtures were separated and quantified using a HPLC fitted with a DAD. Free phenolic acids were analyzed on a Beckman Coulter HPLC (System Gold) coupled to a programmable detector (System Gold, series 166) and an autosampler (System Gold, series 508) operated by a 32 karat software package. Separation of phenolic acids was achieved on a reversed phase C_{18} Luna column (Phenomenex, 150 × 4.6 mm; particle size 5 μm), preceded by a guard column (Phenomenex, 4 × 3.0 mm) of the same stationary phase. Both the column and the guard column were thermostatically controlled at 25 °C and the flow rate was set to 0.7 mL/min. The mobile phase consisted of two solvents: 0.1% formic acid (A) and methanol (B). The solvent gradient in volumetric ratios was as follows: 5–30% B over 50 min, held at 30% B for an additional 15 min; at 65 min the gradient was increased to 100% B and held at 100% B for an additional 10 min to clean up the column. Three wavelengths (270, 310 and 325 nm) were used to detect the eluent composition. HPLC analysis at 325 nm was used for quantification of ferulic acid and caffeic acid. Quantification of *p*-coumaric acid was performed at 310 nm as described earlier (Robbins and Bean, 2004). All three phenolic acids were quantified with external standards by using HPLC analysis (Robbins and Bean, 2004).

2.7. Extraction of samples for analysis of total phenolics

For each extraction, approximately 200 ± 1 mg of ground freeze-dried tomato sample was placed in a 15 mL centrifuge tube with 5 mL of the solvent mixture MeOH: H_2O (80:20, % v/v). The vials were then placed in a sonicator bath (Model 2510, Branson Ultrasonic Corporation, Danbury, CT) at ambient temperature for 30 min. The mixture was centrifuged and the supernant was transferred into a 10 mL volumetric flask. The residue was resuspended in 5 mL of MeOH:H_2O (80:20, %v/v), gently mixed manually and sonicated for an additional 30 min followed by centrifugation. The supernant was combined with the initial extract and the volume of combined supernant was made up to 10 mL with the extraction solvent and appropriate aliquots of extracts were filtered and assayed by a FC assay for TP content. For each sample, triplicate extractions and analyses were carried out.

2.8. Determination of total phenolics by the folin-ciocalteu assay

The content of TP was determined using the FC assay with gallic acid as a calibration standard using a Perkin-Elmer Lambda 25 spectrophotometer (Boston, MA, USA). The FC assay was carried out by pipetting 100 μL of tomato extract into a 12 mL amber vial. This was followed by addition of 7.9 mL of water. This mixture was vortexed for 10–20 s and 500 μL of FC reagent was added. The mixture was vortexed for an additional 20–30 s and 1.5 mL of filtered 20% sodium carbonate solution was added after 1 min and before 8 min of addition of the FC reagent. This was recorded as time zero; the mixture was then vortexed for 20–30 s after addition of sodium carbonate. After 2 h ± 3 min at ambient temperature, the absorbance of the colored reaction product was measured at 765 nm. A calibration curve was created using different concentrations of standard gallic acid solutions, each time an analysis was run. The level of TP in the extract was calculated from the standard calibration curve. Results were expressed on the basis of mg of Gallic Acid Equivalent per gram (mg GAE/g) of dried tomato powder (Singleton et al., 1974).

3. Results and discussion

In this communication, we studied the influence of cultivar and spectral transmission of the covering material of two high tunnels in the UV region on assay of TP and individual phenolic acid content of tomato fruits. Our findings indicate that there were significantly greater concentrations of both TP and total individual phenolic acids in the fruits of tomato plants grown under a UV transmitting film, Tyco Tufflite (+UV) than under a UV blocking film, Dura-Film Super 4 (−UV).

Fig. 1 shows a typical HPLC chromatogram of the base hydrolyzed phenolic acids separated by HPLC using a diode-array detector (325 nm). The three major phenolic acids extracted from the base hydrolyzed fractions of both cultivars (RS and OS) grown under the two conditions (+UV and −UV) were identified as caffeic, *p*-coumaric and ferulic acids. A similar HPLC profile for the phenolic acids was obtained for the RS cultivar. The structures of the three phenolic acids were confirmed by comparison of their UV spectra and retention time with authentic standards as described earlier (Robbins and Bean, 2004). Caffeic acid was the predominant phenolic acid in the base hydrolyzed extract of both tomato cultivars (RS and OS). The concentration of caffeic acid ranged from 13.9 to 24.1 mg per 100 g of dried tomato sample. The quantity of caffeic acid in tomato fruits of OS was higher than that for RS under both UV treatments.

Chlorogenic acid and caffeic acids have been previously identified and reported in tomato fruits (Minoggio et al., 2003). As base hydrolysis was carried out in the present study, chlorogenic acid was not detected during HPLC analysis due to its conversion to caffeic acid. The rationale behind base hydrolysis was to quantitatively determine total phenolic acids, both free and bound, as it is well documented that phenolic acids can occur in multiple conjugated forms with sugars, acids and other phenolic compounds (Robbins, 2003). In addition, phenolic compounds can also exist as complexes with other macromolecules such as proteins and cellular components (cell wall, lignin, etc.). Intermediate amounts of *p*-coumaric acid (3.5–5.5 mg per 100 g) were identified in all groups of tomatoes. The content of ferulic acid was the lowest of the three phenolic acids in all tomato extracts (0.9–1.5 mg per 100 g) as shown in Fig. 2.

When plants were grown in a high tunnel covered with Dura-Film Super 4 that blocked the transmission of UV

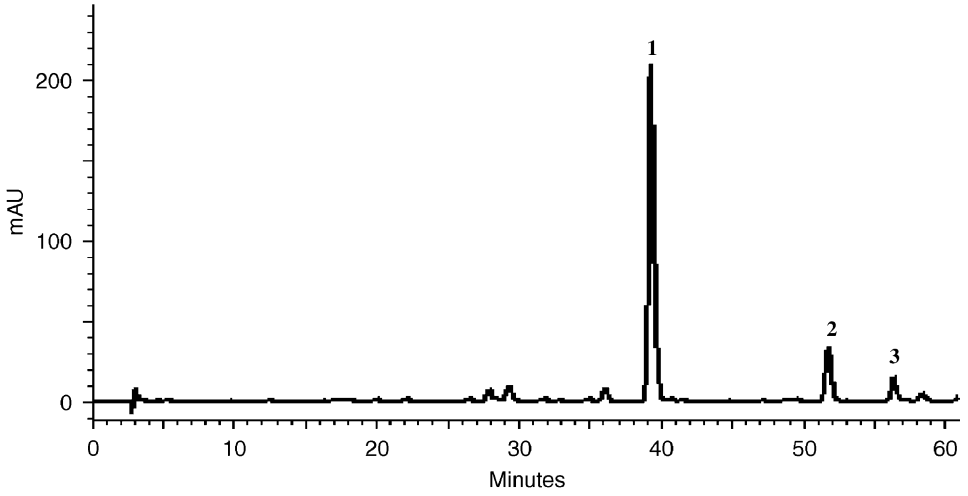

Fig. 1. A typical high performance liquid chromatogram (HPLC) profile of phenolic acids extracted from a base hydrolyzed extract of "Oregon Spring" tomato fruits collected from plants grown under ambient solar radiation in a high tunnel covered with UV-transmitting material. Peaks 1, 2 and 3 were identified as caffeic acid, *p*-coumaric acid, and ferulic acid, respectively. Fruits of the other cultivar ("Red Sun") grown under +UV and −UV conditions showed a similar HPLC profile of phenolic acids.

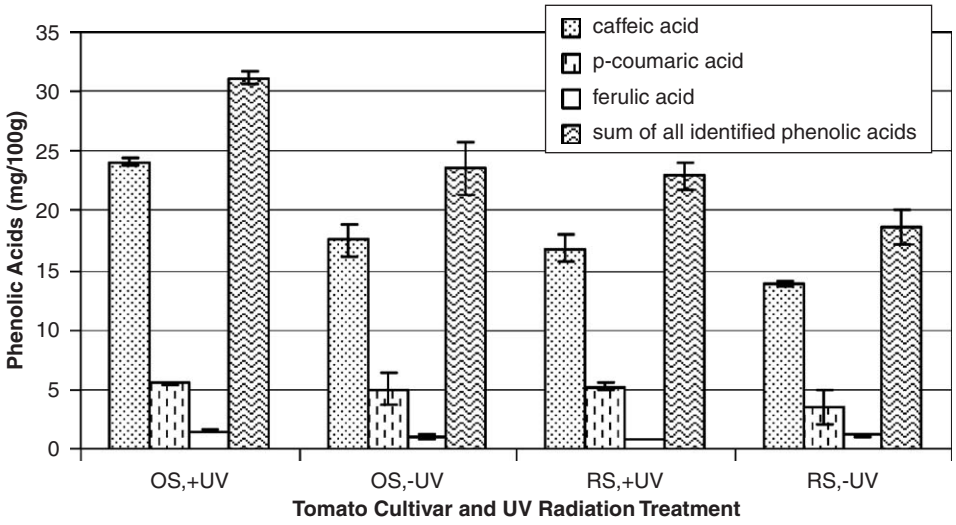

Fig. 2. Determination of phenolic acid content in "Oregon Spring" (OS) and "Red Sun" (RS) tomato fruits using a high performance liquid chromatography (HPLC) method with diode array detection of phenolic acids isolated from base hydrolyzed extracts. Fruits collected from plants grown under ambient solar radiation in high tunnels covered with a UV-transmitting (+UV) or a UV-blocking (−UV) material.

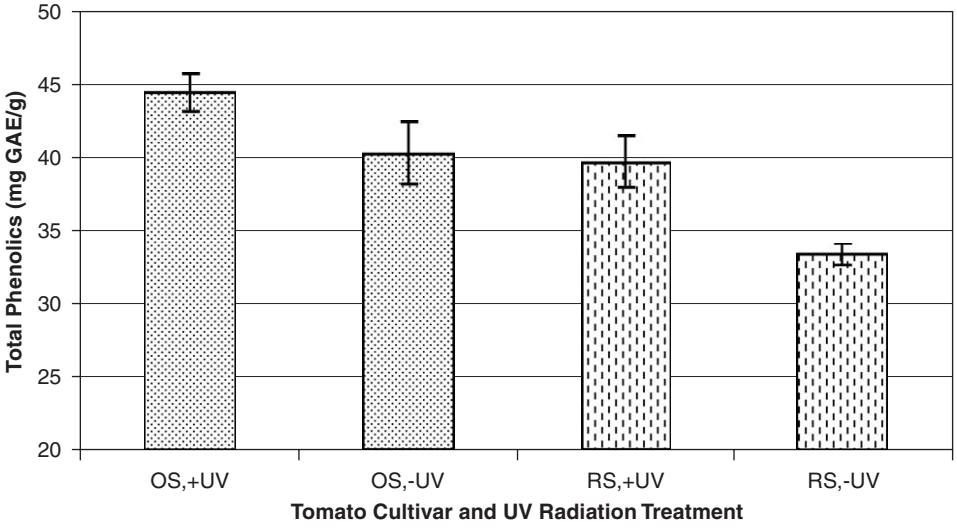

Fig. 3. Estimation of total phenolic acids in extracts of fruits of "Oregon Spring" (OS) and "Red Sun" (RS) tomato using a Folin–Ciocalteu procedure. Fruits collected from plants grown under ambient solar radiation in high tunnels covered with a UV-transmitting (+UV) or a UV-blocking (−UV) material.

radiation below 380 nm, the quantity of caffeic acid in OS and RS was reduced by 27.4% and 17.8%, respectively (Fig. 2). The combined yield of all identified phenolic acids also showed a similar reduction in percent phenolics under −UV treatment. Estimation of TP by the FC assay showed a similar trend in TP content in both cultivars (Fig. 3). When plants were grown under Tyco Tufflite IV covering material which allowed the transmission of solar UV radiation up to 400 nm, there was approximately a 10% higher content of TP in fruits of OS and a 16% higher content of TP in fruits of RS as compared to those grown under UV exclusion conditions. The small difference in the percentage of phenolic acids and TP observed in the present study between the two methods was due to differences in the sensitivity of the assay procedure. The HPLC method that was used for phenolic acid analysis is more accurate than the colorimetric FC method for estimation of TP. Estimation of TP by the FC procedure also lacks specificity and is influenced by interference from reducing sugars, sulfites, and amino acids (Singleton et al., 1974). Several additional phenolic compounds such as flavanones and flavonols have been also isolated from tomato fruits (Crozier et al., 1997; Raffo et al., 2002). These are detected by the FC assay but not the HPLC–DAD used in this study.

The results obtained from the two independent assay procedures (FC and phenolic acid estimation by HPLC–DAD procedure) clearly indicate that the TP and phenolic acid content of the two cultivars (OS and RS) are influenced by the spectral quality of solar UV radiation.

Both assays revealed an increase in TP and phenolic acid content in the two cultivars when grown in a high tunnel that transmitted the full range of ambient solar UV radiation from 290 to 400 nm as compared to samples from plants grown in a high-tunnel lacking UV wavelengths at 380 nm and below. In previous studies conducted by Krizek et al. (1993) in the growth chamber, they obtained a 27–83% increase in production of phenylalanine ammonia lyase (PAL) when cucumber plants were subjected to increased levels of biologically effective UV-B radiation. Since PAL is one of the key enzymes in the production of phenylpropanoid compounds, it is not surprising that higher concentrations of phenolic compounds were found in tomato fruits obtained from plants grown in a high tunnel receiving ambient UV from 290 to 400 nm as compared with those obtained from plants in a high-tunnel lacking wavelengths at 380 nm and below.

4. Conclusions

Three phenolic acids were extracted from tomato fruits in the base hydrolyzed fraction; these were identified as caffeic acid, *p*-coumaric acid, and ferulic acid. Caffeic acid was the predominant phenolic acid in both OS and RS tomatoes grown in the two high tunnels. The total concentration of these three phenolic acids determined by HPLC–DAD was approximately 20% higher under +UV than under −UV treatment; this was true for both cultivars. Similar trends were obtained in the concentration of TP in tomato fruits harvested from plants grown in the two contrasting high tunnels when the extracts were assayed using a FC assay. These results indicate that the phenolic content of tomato fruits is significantly affected by the spectral quality of ambient solar UV radiation available. Since phenolic compounds are known to play an important role as antioxidants in human nutrition, subtle differences in phenolic composition between the two high tunnels as a result of differences in the UV transmission properties of these different covering materials may be of considerable importance from a nutritional standpoint.

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