DOI:10.1021/jf102937e

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

Solar UVB Response of Bioactives in Strawberry (*Fragaria* × *ananassa* Duch. L.): A Comparison of Protected and Open-Field Cultivation

Melanie Josuttis,*[,†,‡] Helmut Dietrich,[§] Dieter Treutter,[∥] Frank Will,[§] Ludger Linnemannstöns,[⊥] and Erika Krüger[‡]

[†]Hochschule RheinMain—University of Applied Sciences, 65366 Geisenheim, Germany, [§]Department of Wine Analysis and Beverage Research, Geisenheim Research Center, 65366 Geisenheim, Germany, [∥]Institute of Fruit Science, Technische Universität München, Weihenstephan, 85350 Freising, Germany, [⊥]Landwirtschaftskammer Nordrhein-Westfalen, Gartenbauzentrum Straelen/Köln-Auweiler, 50765 Köln-Auweiler, Germany, and [‡]Department of Pomology, Geisenheim Research Center, 65366 Geisenheim, Germany

Strawberries (*Fragaria* × *ananassa* Duch. cvs. Everest, Elsanta) were grown in a tunnel covered with two films, which were distinguished in their ultraviolet transparency, as well as under open-field conditions. One applied film was not transparent for UVB radiation, and the second film transmitted 70% of UVB radiation. During the present study, the nutritional value and quality parameters of the fruits were evaluated. Strawberries were UV-unresponsive in view of the content of ascorbic acid and sum parameters like total anthocyanins and antioxidant capacity measured with TEAC (trolox equivalent antioxidant capacity), ORAC (oxygen radical absorbance capacity) and total phenols. These parameters were mainly affected by sampling date and cultivar. However, HPLC analysis showed that individual phenolics were affected in the absence of UV radiation. The content of the anthocyanin cyanidin 3-glucoside and the flavonols quercetin 3-glucuronide and kaempferol 3-glucoside was decreased in the fruits grown under UV blocking film compared to open-field grown strawberries. By means of the UV transparent film the content of the mentioned flavonoids could be enhanced up to similar amounts like in open-field grown strawberries. All other phenolics were not consistently affected by UV radiation. This result was independent of cultivar.

KEYWORDS: Strawberries; antioxidant; cultivation; anthocyanins; flavonoids; ascorbic acid; TEAC; ORAC; UV radiation; total phenols

INTRODUCTION

During the past decade health-promoting components of plant products have received increasing attention. Particularly berry fruits are assumed to play an essential role in the prevention of cardiovascular diseases or cancer (*1*, *2*). In strawberries, phenolic compounds and ascorbic acid are main contributors to the antioxidant capacity of the fruits and their nutritional value (*3*). More than 40 phenolic compounds including anthocyanins, proanthocyanidins, ellagitannins, phenolic acids or flavonols have been identified in these fruits until now (*4*). However, cultivars differ in their phenolic composition (*5*, *6*). In addition, environmental effects like temperature, altitude, salt stress or deficit irrigation influence the formation of strawberry bioactives (*7*−*10*). Several studies were directed to the effect of cultural systems e.g. mulch color, high beds or planting date, which may interact with the synthesis of phytochemicals in strawberry (*11*−*13*).

Nowadays, the use of polyethylene tunnels for out-of-season production in Europe is common to produce strawberries (*14*). Additionally, this method offers advantages like increased plant density, protection from hail and rain or reduction of certain fungal diseases (*15*). However, less is known about changes of nutritional ingredients in fruits grown in plastic tunnels. The main used plastics are standard polyethylene foils, which are not transparent for UVB radiation.

However, different materials are available, which vary in their transparency for UV radiation (*16*). Plants often respond to UV light, which affects flavonoid biosynthesis genes (*17*). Phenolics can act as UV-protectors e.g. because of their absorption of light between 270 and 290 nm. Detailed investigations of different UV transparent plastic foils were focused on bioactives in lettuce (*18*, *19*). Furthermore, research on ultraviolet radiation and phenolic substances was performed with grapes, tomatoes and apples (*20*−*22*). The treatments with reduced UVB irradiation generally resulted in lower contents of secondary plant metabolites in the crops. In contrast, also increases of individual phenolics were observed during UVB absence in grapes (*20*). Recently, studies with strawberries grown under plastic films, which differ in their UV transparency, affected neither the content of anthocyanins nor the antioxidant capacity (*23*).

*Corresponding author. Mailing address: Hochschule RheinMain—University of Applied Sciences, Wiesbaden Rüsselsheim Geisenheim and Geisenheim Research Center—Department of Pomology, Von-Lade Str. 1, 65366 Geisenheim, Germany. Tel: +49 6722-502573. Fax: +49 6722-502560. E-mail: m.josuttis@fa-gm.de.

 Published on Web 11/18/2010

In the present study, the influence of tunnel cultivation with two different films compared to open-field grown strawberries was investigated. The aim was to analyze the impact of UV radiation on bioactives in strawberries with respect to agronomical and quality parameters like leaf area:yield ratio, soluble solids content and titratable acidity. Therefore the content of ascorbic acid, total anthocyanins and antioxidant capacity was determined. High performance liquid chromatography (HPLC) analysis was performed to detect alteration of the major individual polyphenols. In a subtrial the external ("peel") and internal tissues ("flesh") of strawberries cv. Everest were analyzed separately to determine the distribution of phenols in strawberry and to evaluate putative UV-protectors. To avoid influences of the sampling date fruits from the three treatments; blocking film, window film and open-field were sampled and compared on the same day with consideration of the fruit size.

MATERIALS AND METHODS

Chemicals. Folin−Ciocalteu's phenol reagent was purchased from VWR (Darmstadt, Germany); L-(+)-ascorbic acid from Carl Roth GmbH (Karlsruhe, Germany); 2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), (±)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox), 2,2′-azobis(2-methylpropionamidine)dihydrochloride (AAPH), gallic acid monohydrate (3,4,5-trihydroxybenzoic acid) and fluorescein sodium salt was from Sigma Aldrich (Steinheim, Germany). The standards for HPLC analysis were ellagic acid and coumaric acid obtained from Sigma Aldrich, cyanidin 3-*O*-glucoside, kaempferol 3-*O*-glucuronide and pelargonidin 3-*O*-glucoside from Extrasynthèse (Genay Cedex, France) and quercetin 3-D-glucuronide from Carbosynth (Berkshire, U.K.).

Plant Material and Field Experiments. In 2008, ever-bearing strawberry plants (*Fragaria* × *ananassa* Duch. L. cv. Everest) were grown at Landwirtschaftskammer Köln-Auweiler (Germany) soilless in peat bags under a four span plastic tunnel (Haygrove, length 34 m, width of each span 5.5 m) with automatic control of irrigation and fertilization. Two spans were covered with a standard film (blocking film) and the other two with a UV transparent film (window film). Plastic films were purchased from folitec Agrarfolien-Vertriebs GmbH (Westerburg, Germany). The blocking film was UV M 42 (clear, thickness 190 μm, transmittance 89/90%, UVB transparency 0%) and window film was UV B window (clear, thickness 190 μm, transmittance 89/90%, UVB transparency 70%). The assured transparency of the films was confirmed by photometric measurements (**Figure 1**). A control was cultivated under open-field conditions and covered with a net for crop protection against birds. The harvest started for the open-field treatment June 29 and was completed September 28. Strawberry plants in the high-tunnel were harvested from May 25 to November 2. Protected cultivation extended the harvest period of the ever-bearer cv. Everest because of increased temperatures. In 2009, the trial was repeated with the same film types at the Geisenheim Research Center (Germany). In addition, the June-bearer cv. Elsanta was planted. Both cultivars were set in the middle of May in bare soil, irrigated and covered with an open-sided tunnel (Haygrove, length 30 m, width 8 m) June 10. The harvest period for cv. Elsanta was from June 29 to July 27 outside and in the tunnel from June 25 to July 27. The first inflorescences of cv. Everest were removed as is common for ever-bearers, and the harvest began for all treatments on July 16 and was stopped on August 20.

For the period of the trials, air temperature and photosynthetically active radiation (PAR) were measured every 10 min for all treatments and collected using a data-logger in both years. The two plastic films transmitted similar amounts of PAR, which was comparable to the outside treatment with net in 2008 (around 98% of natural light under the net). In 2009, PAR radiation in the tunnel was 87% compared to the open-field only, because of the missing net. All trials were carried out in three repetitions with a minimum of 40 plants per plot and harvested in a three/four day rhythm. 500 g of healthy, nondamaged fruits from each treatment and replication were collected twice: for cv. Everest at the beginning of the harvest and two weeks later and for cv. Elsanta at harvest beginning and already one week later because of its shorter harvest period, respectively.

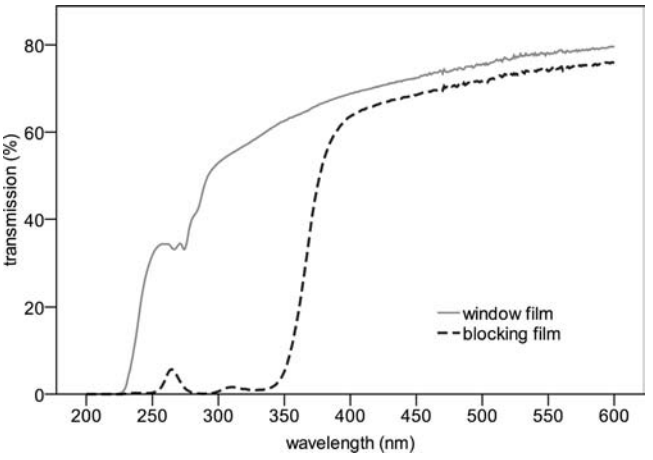

Figure 1. Spectral transmission of the blocking film in comparison to the window film used in this study.

Leaf Area. In 2009, the leaf area was analyzed; five complete plants per treatment and repetition were measured directly after the harvest period with a leaf area meter (LI-3100C, Licore, Lincoln, NE, USA).

Sample Preparation. The fruits were quartered and, to avoid bias, the counterparts of the fruits were immediately snap frozen in liquid nitrogen at sampling date, stored at −28 °C and milled to powder later on. The other two quarters of the fruits were homogenized with a hand blender, and the resulting puree was used for determinations of soluble solids content, titratable acidity and dry matter. Each biological replicate was analyzed separately.

To evaluate the distribution of phenolics in strawberry fruits, an additional sampling date of the cv. Everest in 2009 was fixed for a tissue trial. Therefore the sliced fruits were lyophilized, and the external tissue (peel, 2.5 mm) was separated from the internal tissue (flesh) and milled to a fine powder, which was used for determination of total phenol content and HPLC analysis.

Soluble Solids Content (SSC), Dry Matter and Titratable Acidity. The puree was directly measured with a digital refractometer (A. Krüss Optotronic GmbH, Hamburg, Germany) to determine SSC in %. For total dry matter content five grams of puree per sample was mixed with sea sand and dried for approximately four hours at 103 °C until the last weighing step was stable. The dry matter content was expressed in % of fresh weight. Titratable acidity was determined by resuspending five grams of strawberry puree with 50 mL of water. This mixture was automatically titrated (Schott-Titrator, Titrinoline Alpha; software, Titrisoft) with 0.33 mol·L^{-1} sodium hydroxide. Results were expressed as citric acid in % of fresh weight.

Ascorbic Acid. The ascorbic acid content was determined by iodometric titration. Five grams of frozen strawberry powder was extracted twice with 10 mL of oxalic acid (2%, w/v) and centrifuged. The supernatants were collected, acidified with sulfuric acid (10%) and determined with automatic potentiometric titration (Schott-Titrator, Titrinoline Alpha Plus; software, Titrisoft) with a 1/128 mol·L^{-1} iodide−iodate solution. Results were expressed as ascorbic acid in μg per g fresh weight.

Total Anthocyanins and Total Phenols Content. Five grams of frozen strawberry powder was extracted twice ultrasound-assisted for 30 min with 10 mL of 80% methanol. For the tissue trial 500 mg of lyophilized powder was used for the extraction. Supernatants were collected and the methanol was added to a volume of 25 mL for the following analysis as previously described (*24*). Briefly, total anthocyanins were determined by the pH differential method (*25*) and the extracts were diluted with two buffer solutions at pH 1 and 4.5. The absorbance was measured at 500 and 700 nm, and the results were calculated as μg of pelargonidin 3-glucoside per g fresh weight with a molar absorption coefficient of 15,600 L·mol^{-1}·cm^{-1} for pelargonidin 3-glucoside. Total phenols were determined by the Folin−Ciocalteu method (*26*) using gallic acid as reference compound. The absorbance was measured after 60 min at 760 nm, and the total phenol content was expressed as μg of gallic acid equivalents per g fresh weight.

Trolox Equivalent Antioxidant Capacity (TEAC) Assay. The TEAC assay was carried out as previously described (*24*). In brief,

Table 1. Effect of Cultural System on Agronomical Parameters in 2009[a]

cultivar	treatment	single leaf area (cm^2)	leaf area per plant (cm^2)	leaf area:yield ratio (cm^2/g)	yield (g/plant)	mean fruit wt (g)
'Everest'	blocking	36 ± 3 c	2210 ± 616 c	5.8 ± 1.0 b	390 ± 90 b	14.2 ± 0.8 b
	window	37 ± 4 bc	2451 ± 591 c	7.6 ± 1.1 b	321 ± 8 bc	14.5 ± 0.4 b
	open-field	41 ± 4 a	3790 ± 982 b	7.5 ± 0.6 b	505 ± 35 a	17.8 ± 0.2 a
'Elsanta'	blocking	27 ± 7 e	2399 ± 549 c	14.3 ± 1.1 a	168 ± 13 d	11.8 ± 0.4 c
	window	24 ± 4 e	2181 ± 675 c	15.2 ± 1.5 a	143 ± 15 d	11.6 ± 0.2 c
	open-field	32 ± 5 d	4622 ± 1150 a	15.1 ± 1.1 a	305 ± 25 c	11.9 ± 0.6 c
			Significance[b]			
treatment		*	**	ns	**	**
cultivar		**	ns	**	**	**
treatment x cultivar		ns	ns	ns	ns	**

[a] Data are expressed as the mean ± standard deviation ($n = 3$). Means within the same column followed by different letters were significantly different at $p < 0.05$. [b] ns = nonsignificant. *Significant at $p < 0.05$. **Significant at $p < 0.01$.

45 mmol·L^{-1} potassium persulfate was added to a 7 mmol·L^{-1} ABTS solution in 50 mmol·L^{-1} phosphate buffer (pH 7.4) to generate a stable ABTS radical cation solution overnight. Before measuring, the $ABTS^{\bullet+}$ solution was diluted with phosphate buffer to an absorbance of 0.800 ± 0.050. This solution was mixed with the buffer diluted supernatants from the extracts described above in a ratio of 1:20. The absorbance was measured after 6 min at 734 nm. Trolox was used as a reference compound, and the antioxidant capacity was expressed in μmol of Trolox equivalents per g of fresh weight.

Oxygen Radical Absorbance Capacity (ORAC) Assay. For the measurement of the antioxidant capacity by a hydrogen atom transfer, the ORAC assay was applied after Huang et al. (*27*). The analysis was carried out on a black 96-well plate with a microplate reader (Infinite M200, Tecan). Each well was filled with a 20 μL sample (diluted supernatants from the methanolic extracts described above), blank or Trolox-standard (12.5 μmol·L^{-1} to 100 μmol·L^{-1}) and 120 μL of fluorescein (120 nmol·L^{-1}). After 5 min incubation 60 μL of AAPH-solution (40 mmol·L^{-1}) was added. The fluorescence was recorded every minute for 90 min at 37 °C with an excitation wavelength of 485 nm and an emission wavelength of 520 nm. The ORAC values refer to the net area under the quenching curve (AUC). The final ORAC values were calculated by linear regression of the Trolox standards with the Magellan software and expressed as μmol per g of fresh weight.

HPLC Analysis of Phenolic Compounds. The methanolic extracts described above were directly analyzed using a ThermoFinnigan Surveyor HPLC system coupled with a photodiode array detector (PDAD), pump, and autosampler to separate and quantify individual phenolic compounds, which was controlled with Chromquest software 4.1. A 3 μL filtrated sample was injected using a Reprosil-PurC18AQ (Dr. Maisch GmbH, Ammerbuch-Entringen, Germany) column (150 × 2 mm, 3 μm) with a guard column. The mobile phase was acidified water containing 2% acetic acid (A) and acetonitrile/water/acetic acid (50/49.5/0.5) (B) with the following gradient: 0−31.5 min, 55% B; 31.5−37.5 min, 100% B; 37.5−41 min, 10% B with a flow of 200 μL·min^{-1} and an equilibration time of 10 min. The PDAD scanned 250 to 600 nm with three discrete channels at 280, 360, and 500 nm. The main peaks were analyzed and calculated with available standards. The results were given in μg per g fresh weight. Peaks were identified by HPLC ESI-MS on an Accela HPLC system coupled to a LXQ mass spectrometer (Thermo Scientific, Dreieich, Germany) equipped with an ESI source and a linear ion trap mass analyzer. HPLC conditions were the same as described above. The whole system was controlled by the Xcalibur software. For polyphenols, the mass spectrometer was operated in the positive and the negative mode with the following conditions: source voltage 4.5 kV; capillary voltage 26 V; capillary temperature 275 °C. Dependent scans were carried out on target peaks for MS^n scans with a collision energy of 35%.

Analysis of Conjugated Ellagic Acid and Ellagitannins after Hydrolysis. Because of nonavailable standards ellagitannins were analyzed after hydrolysis as ellagic acid equivalents. 1.5 g of strawberry powder and 150 mg of lyophilized strawberry powder for the tissue trial, respectively, was mixed with 5 mL of 50% methanol with 1.2 mol·L^{-1} hydrochloric acid and incubated at 80 °C for 8 h according to Häkkinen et al. (*28*). The hydrolyzed suspension was filled to 10 mL, filtrated and analyzed with the same HPLC system as described above on an ODS-A (Dr. Maisch GmbH, Ammerbuch-Entringen, Germany) column (125 × 2 mm, 5 μm). The mobile phase was 5% formic acid (A) and methanol (B) according to the following gradient: 0−25 min, 50% B; 25−37 min, 90% B; 37−39 min; 10% B; 39−46 min, 10% B with a flow of 200 μL·min^{-1}. The amount of ellagic acid was calculated at a wavelength of 255 nm and given in μg per g fresh weight.

Statistical Analysis. All data were analyzed statistically with analysis of variance using SPSS (version 17). The means of all parameters were compared with Duncan's test. Differences of $P < 0.05$ were considered as significant. Results were given as a mean ± standard deviation (SD). A principal component analysis (PCA) was performed with XLSTAT 2010 for the bioactive components to get an overview of the relationship between the analyzed data. PCA is an effective tool for reducing dimensions of a data set by creating a matrix and describing major trends. The score plot visualizes the distribution of the sample, which can be use for grouping the observations. The loading plot shows the importance and interactions of the variables correlating with the distribution of the samples. To suppress the effect of sampling, each value was divided with the mean of the open-field value per each sampling date. The data were autoscaled to supply comparable weights for all parameters. Accordingly, the four sampling dates for cv. Everest in 2008 and 2009 were compared and also the two sampling dates for cv. Elsanta in 2009.

RESULTS

Agronomical Parameters: Not Affected by UV Radiation. The three different treatments blocking film, window film and open-field were distinguished mainly in temperature and the films in UV transparency (**Figure 1**). The mean temperature outside was 19.5 °C ± 1.0 °C for each date calculated for four weeks prior to sampling. In 2008, the average daily mean temperature under the films was +2.0 °C (blocking film) or +2.5 °C (window film) and the average daily maximum temperature +6.5 °C (blocking film) to +8.0 °C (window film) higher than the open-field treatment. In 2009, the temperature differences were lower than in 2008 due to the use of an open-sided tunnel and better air exchange (mean temperature: +1.1 °C to +1.4 °C and maximum temperature +2.6 °C to +3.4 °C, respectively) Despite the fact that a single tunnel was used in both experimental years, temperature under the window film was higher than under the blocking film. The window film was transparent for radiation from 230 nm and the blocking film from 340 nm (**Figure 1**).

The environmental parameters affected the plant performance, i.e., leaf area, yield or fruit size (**Table 1**). In the first experimental year the yields of the two tunnel treatments (blocking film 1152 g/plant, window film 1138 g/plant) were significantly enhanced compared to the open-field yield (850 g/plant) because of an extended harvest period of the ever-bearer cv. Everest. In the second year, a higher yield of around 150 g more per plant was observed under open-field conditions for both cultivars compared to tunnel cultivation (**Table 1**). In addition, open-field grown plants of each cultivar had the highest total and single leaf area.

Table 2. Effects of Cultural System on Quality Parameters Like Dry Matter, Titratable Acidity and Soluble Solids[a]

cultivar/year	sampling date	treatment	mean fruit wt of the sample (g)	dry matter (%)	titratable acidity (%)	soluble solids content (% Brix)
'Everest' 2008	T1	blocking	13.7 ± 2.0 d	7.3 ± 0.1 h	0.77 ± 0.05 bcd	6.3 ± 0.1 h
		window	13.8 ± 1.8 d	7.8 ± 0.2 hg	0.83 ± 0.03 b	6.3 ± 0.1 h
		open-field	15.4 ± 2.1 bc	9.2 ± 0.3 f	0.72 ± 0.02 def	8.0 ± 0.1 de
	T2	blocking	15.0 ± 0.7 bc	8.2 ± 0.5 g	0.67 ± 0.03 f	7.0 ± 0.2 g
		window	17.7 ± 0.8 b	8.1 ± 0.5 g	0.66 ± 0.01 f	7.0 ± 0.1 g
		open-field	14.6 ± 0.9 cd	9.1 ± 0.5 f	0.81 ± 0.01 bc	7.7 ± 0.3 ef
'Everest' 2009	T1	blocking	17.7 ± 1.0 b	10.5 ± 0.5 bcde	0.93 ± 0.04 a	9.2 ± 0.6 b
		window	15.3 ± 1.2 bc	10.6 ± 0.1 bcde	0.97 ± 0.03 a	9.5 ± 0.1 b
		open-field	17.6 ± 2.1 b	10.3 ± 0.2 cde	0.88 ± 0.01 a	9.1 ± 0.1 b
	T2	blocking	13.8 ± 0.8 d	10.0 ± 0.4 de	0.76 ± 0.01 cde	7.4 ± 0.6 fg
		window	13.7 ± 0.8 d	10.6 ± 0.4 bcde	0.84 ± 0.01 bc	8.0 ± 0.3 cde
		open-field	21.3 ± 3.5 a	10.4 ± 0.1 bcde	0.83 ± 0.02 bc	8.3 ± 0.3 cd
'Elsanta' 2009	T1	blocking	13.0 ± 0.4 d	10.7 ± 0.4 bcd	0.78 ± 0.05 bc	8.4 ± 0.2 cd
		window	12.8 ± 0.6 d	11.6 ± 0.3 b	0.76 ± 0.03 bc	8.5 ± 0.3 c
		open-field	16.6 ± 1.3 bc	10.5 ± 0.3 bcde	0.79 ± 0.03 bc	8.3 ± 0.2 cd
	T2	blocking	7.6 ± 0.7 f	11.3 ± 0.8 b	0.71 ± 0.04 ef	10.3 ± 0.3 a
		window	7.6 ± 0.2 f	12.5 ± 0.6 a	0.72 ± 0.05 def	10.5 ± 0.3 a
		open-field	10.2 ± 0.2 e	10.9 ± 0.2 bc	0.70 ± 0.04 f	9.1 ± 0.3 b
				Significance[b]		
treatment			**	ns	ns	ns
cultivar			**	**	**	**
sampling			**	ns	**	*
treatment × cultivar			ns	ns	ns	*
treatment × sampling			ns	ns	ns	ns
treatment × cultivar × sampling			ns	ns	ns	ns

[a] Data are expressed as the mean ± standard deviation ($n = 3$). Means within the same column followed by different letters were significantly different at $p < 0.05$. [b] ns = nonsignificant. *Significant at $p < 0.05$. **Significant at $p < 0.01$.

For both cultivars none of the parameters were affected by the different film types and were therefore UV independent (**Table 1**). Furthermore, the leaf area:yield ratio was not significantly affected by the treatments. However, plants under the blocking film showed a tendency toward a closer leaf area:yield ratio than plants of the other two treatments. The mean fruit weight was increased for fruits of the cv. Everest in both years. In 2008, the mean fruit size of tunnel grown fruits was 15.5 g and significantly different for open-field fruits with 17.4 g. This effect was not observed for cv. Elsanta when grown under open-field conditions.

Quality Parameters. For both cultivars UV radiation did not affect the fruit size. In general, the fruit size of ever-bearers like cv. Everest alternates during their long harvest period. This was confirmed in our study. In contrast, the fruit size of June-bearers decreases continuously during the harvest period. The beginning of the June-bearer cv. Elsanta, harvested in the open-field, was four days later than in the tunnel, consequently the tunnel-grown fruits cv. Elsanta were smaller than open-field fruits at the same sampling date (**Table 2**).

Dry matter, titratable acidity and SSC were only inconsistently affected by the different treatments. Only in 2008, the open-field treatment increased significantly the dry matter and SSC in the fruits at both sampling dates, but no influence of UV radiation was observed (**Table 2**).

Overview UV-Effects on Bioactive Compounds: Characterization with Principal Component Analysis (PCA). For PCA only the 17 health-related parameters ascorbic acid, antioxidant capacity, colorless phenols and anthocyanins were considered. The result was displayed for principal component (PC) 1 and PC2, which explained for cv. Everest 70.9% of the data and for cv. Elsanta 56.5% (**Figures 2** and **3**). The 17 variables discriminated for cv. Everest mainly the treatment blocking film while the treatments window film and open-field were not clearly separated. Separation of the UV treatments was mainly caused by the content of anthocyanin cyanidin 3-glucoside, the flavonols quercetin 3-glucuronide, kaempferol 3-glucoside and the TEAC value. All other analyzed anthocyanins were correlated with the total anthocyanin content and split window film and blocking film of the first cultivation year (2008) from all other samples. Antioxidant capacity measured by ORAC, TEAC and total phenols was not correlated with anthocyanins except for cyanidin 3-glucoside, but with the ellagic acid content after hydrolysis, quercetin 3-glucuronide and kaempferol 3-glucoside. *p*-Coumaroyl glucose was for this cultivar negatively correlated to the anthocyanins and not correlated to all other parameters (**Figure 2**). For cv. Elsanta all three treatments were clearly clustered (**Figure 3**). The three flavonoids quercetin 3-glucuronide, kaempferol 3-glucoside including kaempferol 3-glucuronide and cyanidin 3-glucuronide separated UV-treated and UV-untreated fruits correlating negatively with *p*-coumaroyl glucose. All other health-related parameters were independent of these two groups with strong interactions (**Figure 3**).

Ascorbic Acid, Total Anthocyanins and Antioxidant Capacity. Overall, the treatments did not affect the content of ascorbic acid and total anthocyanins in fruits of both cultivars. Ascorbic acid was significantly affected by cultivar only and total anthocyanins by cultivar and the sampling date (**Table 3**). However, in 2008 the content of total anthocyanins was enhanced in strawberries of cv. Everest growing under plastic, but no effect was observed among the treatments in 2009 (**Table 3**). The antioxidant capacity of both cultivars measured with the three assays total phenols, TEAC and ORAC was significantly affected by the treatment, but no clear UV-effects were observed (**Table 3**). In general, significant differences among the treatments were lower than 15% and the variation among the sampling dates was partially higher than between the three treatments. The cultivars were distinguished only in antioxidant capacity measured by the ORAC-assay (**Table 3**).

Composition of Individual Anthocyanins. At all sampling dates significant effects for both cultivars were observed only for

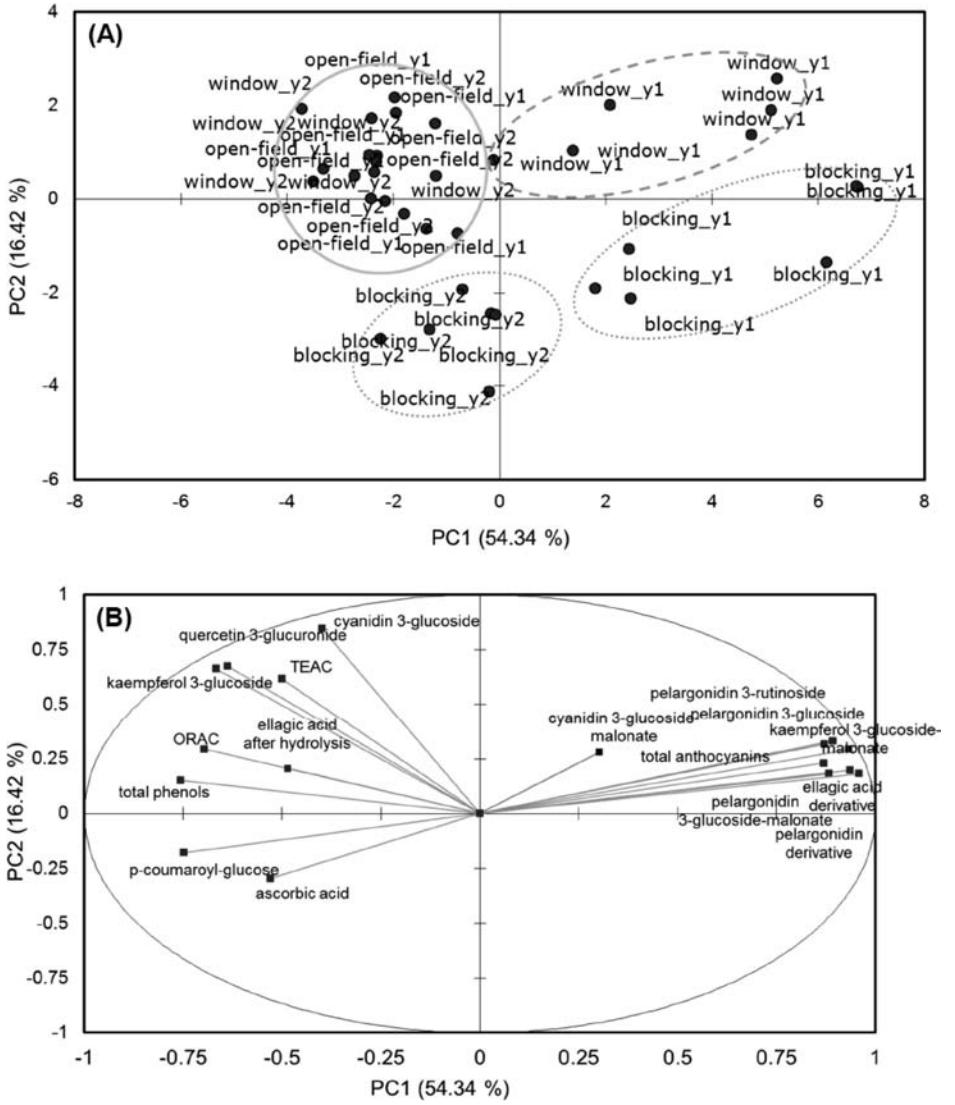

Figure 2. Principal component analysis (PC1 and PC2) of the different treatments blocking film, window film and open-field on cv. Everest score plot (**A**) and loading plot of the bioactives (**B**). The ending _y1 indicates the samples of the first experimental year (2008) and _y2 of the second experimental year (2009). Kaempferol 3-glucoside includes kaempferol 3-glucuronide.

cyanidin 3-glucoside and pelargonidin 3-glucoside-malonate content (**Table 4**). For each sampling date the content of cyanidin 3-glucoside was significantly reduced in fruits grown under blocking film compared to those grown under the window film or under open-field conditions, respectively (**Table 4**). The content ranged in fruits of cv. Everest (cv. Elsanta) treated with blocking film from 11.1 μg to 18.9 μg (1.9 μg to 4.9 μg) and in fruits treated with window film or open-field fruits from 16.6 μg to 25.7 μg (5.7 μg to 15.0 μg) per g fresh weight. As a result the formation of cyanidin 3-glucoside was influenced by UV radiation predicted by the PCA (**Figures 2** and **3**). The pelargonidin 3-glucoside-malonate content was decreased in open-field grown fruits of cv. Everest only in 2008, but in this year all other pelargonidin related anthocyanins were decreased, too (**Table 4**).

Concerning cv. Elsanta the amount of pelargonidin 3-glucoside-malonate was decreased in open-field fruits only at the first sampling date. The PCA results showed that only cyanidin 3-glucoside was independent from all other anthocyanins while pelargonidin 3-glucoside-malonate was correlated with them (**Figure 2**). Consequently, this anthocyanin was not affected by UV radiation. Both cultivars were significantly different for all analyzed anthocyanins. Sampling date influenced all anthocyanins except for pelargonidin 3-rutinoside in cv. Everest, which was not detected in cv. Elsanta.

Composition of the Major Colorless Polyphenols. The main effect on colorless phenols was observed on the content of quercetin 3-glucuronide and kaempferols in both cultivars (**Figures 2** and **3**, **Table 5**). The absence of UV radiation decreased strongly the content of quercetin 3-glucuronide at each sampling date. Fruits of cv. Everest and cv. Elsanta produced under blocking film contained only approximately 85% and 75% less of this flavonol, respectively, compared to open-field grown fruits, whereas the strawberries treated with window film contained approximately 40% less quercetin 3-glucuronide compared to open-field fruits in 2008. In 2009, they reached up to similar amounts as the open-field grown strawberries in both cultivars (**Table 5**). The impact on kaempferol-derivatives was not as strong as on quercetins and dependent on glycosylation. Kaempferol 3-glucoside and kaempferol 3-glucuronide, respectively, were for both cultivars negatively affected by missing UV radiation and correlated with the quercetin content (**Figures 2** and **3**). In cv. Everest approximately 35% less kaempferol 3-glucoside under blocking film and 15% under window film compared to the open-field fruits were found. For cv. Elsanta this kaempferol content was

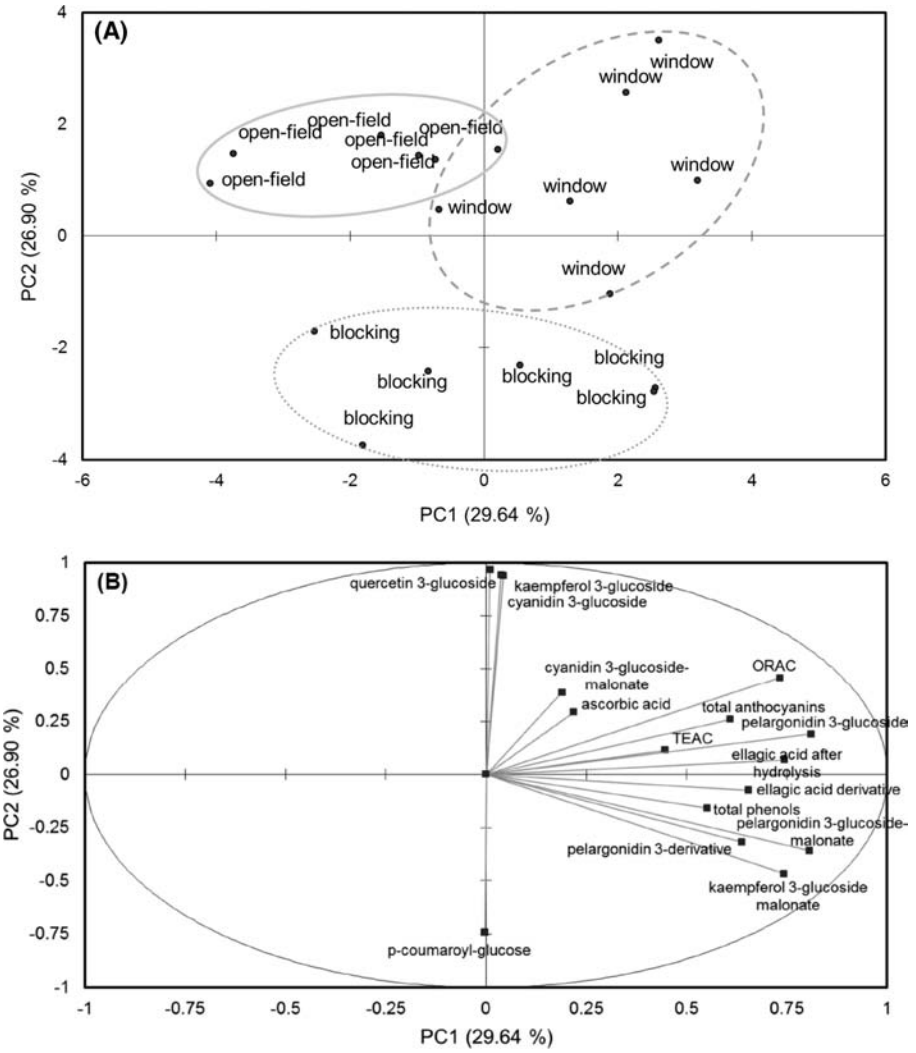

Figure 3. Principal component analysis (PC 1 and PC 2) of the different treatments blocking film, window film and open-field on cv. Elsanta score plot (**A**) and loading plot of the bioactives (**B**). Kaempferol 3-glucoside includes kaempferol 3-glucuronide.

decreased in fruits produced under the blocking film, too, but no differences were observed between the other two treatments, window film and open-field (**Table 5**). The formation of kaempferol 3-glucoside-malonate was not obviously affected by UV radiation (**Table 5**). PCA results showed that this kaempferol was for both cultivars correlated with all pelargonidin related anthocyanins (**Figures 2** and **3**).

The content of ellagic acid after hydrolysis of the ellagitannins and the ellagic acid derivative was significantly influenced by the treatment and by the sampling date, but no effects of UV radiation could be ascertained (**Table 5**). Ellagic acid after hydrolysis was correlated with antioxidant capacity for both cultivars. However, for cv. Everest no interaction of the ellagic acid after hydrolysis with the ellagic acid derivative was observed.

Overall, the content of *p*-coumaroyl glucose was not affected by the treatment or by the sampling date but by the cultivar (**Table 5**). Nevertheless, there was no or a negative correlation with this phenolic acid and all other bioactive compounds. For cv. Everest, *p*-coumaroyl glucose correlated negatively with pelargonidin related anthocyanins, kaempferol 3-glucoside-malonate and ellagic acid derivative but not with the UV affected parameters like quercetin 3-glucuronide, kaempferol 3-glucoside and cyanidin 3-glucoside, whereas for cv. Elsanta, *p*-coumaroyl glucose was negatively correlated with these three flavonoids, consequently the content was enhanced in UV-untreated fruits. However, no correlation of *p*-coumaroyl glucose with all other bioactives was observed (**Figures 2** and **3**).

Distribution of Polyphenols in Strawberry Flesh and Peel. To work out if the determined effects are limited to the surface of strawberry fruits, external and internal tissues were analyzed separately. The peel contained higher amounts of total phenols (3800 mg per 100 g dry weight) than the flesh (2300 mg per 100 g dry weight) (**Table 6**). All detected phenols except for *p*-coumaroyl glucose were up to 10 times higher in the peel than in the flesh (**Tables 6** and **7**). The content for *p*-coumaroyl glucose was similar in both tissues and enhanced in the peel of UV-untreated fruits as observed in cv. Elsanta. The previous result, which was determined in the whole fruits, was mainly confirmed by this additional sampling. However, significant effects for quercetin 3-glucuronide, kaempferol derivatives (**Table 6**) and cyanidin 3-glucoside (**Table 7**) were only observed in the peel of the fruits. The results for the flesh showed the same tendency as the external tissue.

DISCUSSION

The main objectives when applying different cultural systems are out-of-season production or high yields with high quality fruits. However, it is important that the nutritional value is not disregarded. In order to estimate the effect of UV radiation on

Table 3. Effects of Cultural System on Ascorbic Acid, Total Anthocyanins and Antioxidant Capacity[a]

cultivar/year	sampling date	treatment	ascorbic acid[b]	total anthocyanins[c]	total phenols[d]	TEAC[e]	ORAC[e]
'Everest' 2008	d1	blocking	551 ± 33 f	647 ± 26 a	2097 ± 23 bcde	23.0 ± 0.5 ij	32.4 ± 2.4 ghi
		window	564 ± 30 f	641 ± 36 a	2341 ± 85 ab	26.5 ± 0.9 defgh	37.1 ± 1.4 def
		open-field	609 ± 20 ef	553 ± 41 b	2254 ± 195 abc	24.4 ± 2.7 hi	36.8 ± 2.7 def
	d2	blocking	563 ± 15 f	692 ± 30 a	1822 ± 71 f	20.7 ± 0.8 jk	29.1 ± 1.3 i
		window	559 ± 16 f	692 ± 19 a	1869 ± 60 ef	20.2 ± 0.5 k	35.2 ± 1.5 efg
		open-field	614 ± 15 ef	484 ± 21 de	2254 ± 202 abc	24.0 ± 1.3 hi	34.4 ± 2.1 fgh
'Everest' 2009	d1	blocking	753 ± 31 c	383 ± 36 ghi	2327 ± 121 ab	28.9 ± 1.5 bcd	37.0 ± 0.1 def
		window	675 ± 14 de	382 ± 9 ghi	2460 ± 234 a	31.6 ± 0.9 a	39.0 ± 0.1 cde
		open-field	574 ± 9 f	418 ± 33 fgh	2224 ± 106 abc	28.6 ± 0.1 bcd	36.4 ± 1.2 def
	d2	blocking	730 ± 23 cd	426 ± 33 fg	1897 ± 177 def	25.2 ± 1.6 efghi	31.4 ± 2.3 hi
		window	740 ± 29 cd	409 ± 38 fgh	2423 ± 94 a	29.8 ± 2.1 bc	38.4 ± 2.5 cde
		open-field	740 ± 33 cd	409 ± 42 fgh	1937 ± 109 def	27.3 ± 2.0 cdefg	37.4 ± 0.4 def
'Elsanta' 2009	d1	blocking	893 ± 30 a	362 ± 24 hi	2195 ± 30 abc	28.6 ± 0.1 bcde	39.6 ± 0.6 bcd
		window	855 ± 30 ab	369 ± 12 hi	2416 ± 228 a	26.8 ± 0.8 defg	43.3 ± 3.1 ab
		open-field	896 ± 15 a	343 ± 22 i	2196 ± 138 bcd	27.7 ± 1.4 cdefg	41.2 ± 1.8 bc
	d2	blocking	768 ± 24 c	497 ± 14 d	2277 ± 89 ab	25.8 ± 0.3 efghi	41.1 ± 2.6 bc
		window	796 ± 69 bc	573 ± 25 b	2275 ± 164 ab	30.0 ± 2.4 ab	45.6 ± 2.2 a
		open-field	866 ± 45 a	540 ± 48 bc	2052 ± 140 cdef	25.0 ± 1.5 fghi	32.1 ± 1.5 ghi
				Significance[f]			
treatment			ns	ns	*	*	**
cultivar			**	*	ns	ns	**
sampling			ns	**	**	ns	**
treatment × cultivar			ns	ns	*	ns	**
treatment × sampling			ns	ns	ns	ns	**
treatment × cultivar × sampling			ns	ns	ns	ns	**

[a] Data are expressed as the mean ± standard deviation ($n = 3$). Means within the same column followed by different letters were significantly different at $p < 0.05$. [b] Data are expressed in micrograms per gram of fresh weight. [c] Data are expressed as micrograms of pelargonidin 3-glucoside equivalents per gram of fresh weight. [d] Data are expressed as micrograms of gallic acid (GAE) equivalents per gram of fresh weight. [e] Data are expressed as micromoles of Trolox equivalents per gram of fresh weight. [f] ns = nonsignificant.

bioactives in this study, two films differing in UV transparency (**Figure 1**) were used. Moreover, tunnel-grown strawberries and open-field grown fruits were compared focusing on health-beneficial components, for the first time to our knowledge.

Strawberry fruits contain a complex mixture of polyphenols, which contribute, together with ascorbic acid, to their antioxidant capacity in different ways, depending on the used assay, chemical constitution, content or synergistic effects of individual components. Considering these bioactives, PCA was able to discriminate among the fruits of the three treatments; blocking film, window film and open-field. The main characterization of both cultivars was caused by the content of the flavonols quercetin 3-glucoside, kaempferol 3-glucoside including kaempferol 3-glucuronide and the anthocyanin cyanidin 3-glucoside and separated UV-treated and UV-untreated fruits. All other health-related parameters did not explain a UV effect.

At one point in the sampling dates we could observe significant effects of the treatments in antioxidant capacity, but the differences were generally lower than 15% (**Table 3**). Fruits grown under blocking film had tended to a lower antioxidant capacity than under the window film, but also open-field grown fruits often showed the lowest values. This result was not sufficient for a certain conclusion of UV-effects on antioxidant capacity and agrees with the published research of soft fruits (*23*). The main research on tunnel cultivation and health-related compounds focused on leafy vegetables (*18*, *19*) and tomato fruits (*21*). In these studies the content of total phenols was enhanced with UV treatment.

Regarding the total anthocyanin result, UV radiation did not affect the content in our study. Other investigations observed positive UV effects for lettuce on the total anthocyanin content (*18*, *19*, *23*) but not for strawberries and other soft fruits (*23*). However, in 2008 we could observe less total anthocyanins (**Table 3**) and pelargonidin related anthocyanins (**Table 4**) in open-field fruits at both sampling dates. This observation could be explained by higher temperature differences measured during the first experimental year compared to the second. Wang et al. (*10*) found a strong correlation between temperature and anthocyanin content. In the face of the enhancement of total anthocyanins in 2008, it is astonishing that the formation of cyanidin 3-glucoside was inhibited in the absence of UV radiation for all sampling dates. In 2009, this result was confirmed, and a higher cyanidin 3-glucoside content was observed in both cultivars. Nevertheless, cyanidin 3-glucoside-malonate and all other pelargonidin related compounds were not affected by UV radiation. In strawberries, cyanidins are minor pigments, but they occur as major anthocyanins in the lettuce Lollo Rosso and therefore explain the detected enhancement of the total anthocyanins in these studies. The skin of several apple varieties contains cyanidin-related anthocyanins, which are mainly formed on the sun exposed side (*22*). Postharvest treatments with UVB radiation could enhance the total anthocyanin content, too (*29*).

During this study the main effect of UV radiation on strawberries was observed in the enlarged formation of quercetin derivatives. Strawberries grown without UV radiation contain only 15% to 25% of the amount compared to open-field grown fruits. By means of a UV transparent film, quercetin contents were increased from 50% up to similar contents as in open-field grown fruits (**Table 5**). Kaempferol 3-glucoside and kaempferol 3-glucuronide were decreased in the absence of UV radiation but less than quercetin, while kaempferol 3-glucoside-malonate was not affected. In addition, the lack of UV radiation caused by the blocking film increased the content of *p*-coumaroyl glucose in the whole fruits of cv. Elsanta and also in the peel of cv. Everest, while their flesh was affected in tendency, only. More research in this context is required to clarify if this result is a cultivar effect or an

Table 4. Effects of Cultural System on Individual Anthocyanins[a]

cultivar/year	sampling date	treatment	cyanidin 3-glucoside[b]	pelargonidin 3-glucoside[c]	pelargonidin 3-rutinoside[c, d]	pelargonidin derivative[c]	cyanidin 3-glucoside-malonate[b]	pelargonidin 3-glucoside-malonate[c]
'Everest' 2008	d1	blocking	12.8 ± 1.4 ef	357.9 ± 25.4 a	32.7 ± 2.3 a	2.1 ± 0.1 de	5.3 ± 0.5 bcd	75.8 ± 4.6 de
		window	21.0 ± 1.6 bc	377.0 ± 40.7 a	34.6 ± 3.0 a	2.6 ± 0.9 cd	6.8 ± 0.6 ab	74.2 ± 12.6 e
		open-field	23.8 ± 3.5 ab	307.6 ± 29.6 b	26.1 ± 2.9 b	2.0 ± 0.3 defg	5.3 ± 0.6 bcd	56.5 ± 5.2 fg
	d2	blocking	18.9 ± 2.5 cd	372.9 ± 16.3 a	36.0 ± 2.6 a	3.3 ± 0.4 b	8.0 ± 0.4 a	87.7 ± 4.5 c
		window	24.7 ± 1.9 ab	376.3 ± 12.6 a	33.3 ± 1.0 a	3.1 ± 0.1 bc	7.9 ± 0.7 a	84.7 ± 1.3 cd
		open-field	25.7 ± 3.9 a	271.4 ± 9.0 bc	25.1 ± 0.8 b	1.4 ± 0.2 fgh	6.2 ± 0.7 abc	47.4 ± 1.2 gh
'Everest' 2009	d1	blocking	11.1 ± 0.7 fg	219.5 ± 22.8 defg	25.2 ± 1.5 b	1.2 ± 0.3 h	3.4 ± 0.8 defg	42.6 ± 6.0 h
		window	17.9 ± 2.4 cd	201.6 ± 23.5 fg	21.6 ± 2.5 c	1.3 ± 0.4 gh	4.3 ± 0.1 cde	39.0 ± 4.3 h
		open-field	16.6 ± 2.0 de	229.8 ± 18.7 defg	24.7 ± 2.4 bc	1.4 ± 0.3 efgh	3.0 ± 0.2 efg	40.5 ± 3.2 h
	d2	blocking	11.8 ± 2.9 f	236.5 ± 15.4 cdefg	27.6 ± 1.9 b	1.4 ± 0.1 fgh	4.0 ± 0.1 de	46.8 ± 3.0 gh
		window	19.1 ± 0.8 cd	243.8 ± 29.7 cde	27.6 ± 4.1 b	1.2 ± 0.1 h	3.6 ± 0.7 def	47.4 ± 6.2 gh
		open-field	18.3 ± 3.4 cd	240.2 ± 21.1 cdef	27.5 ± 2.4 b	1.6 ± 0.2 efgh	4.9 ± 0.5 bcde	41.9 ± 2.3 h
'Elsanta' 2009	d1	blocking	1.9 ± 0.2 i	203.5 ± 10.2 efg	nd	2.1 ± 0.4 def	1.4 ± 0.7 g	61.2 ± 3.5 f
		window	5.7 ± 1.6 hi	200.6 ± 4.9 fg	nd	1.9 ± 0.2 defg	1.8 ± 0.5 fg	59.6 ± 4.9 f
		open-field	7.8 ± 0.7 gh	195.7 ± 13.3 g	nd	1.6 ± 0.2 efgh	1.7 ± 0.9 fg	47.5 ± 3.9 gh
	d2	blocking	4.9 ± 0.4 hi	247.6 ± 9.8 cd	nd	4.1 ± 0.3 a	4.2 ± 0.7 cde	100.9 ± 4.0 b
		window	15.0 ± 2.7 def	292.4 ± 26.4 b	nd	4.4 ± 0.6 a	6.3 ± 1.2 abc	111.8 ± 9.2 a
		open-field	12.7 ± 1.2 ef	289.5 ± 26.5 b	nd	4.0 ± 0.5 a	5.1 ± 0.7 bcde	99.9 ± 9.0 b
					Significance[f]			
treatment			**	ns	ns	ns	ns	*
cultivar			**	**		**	**	**
sampling			**	*	ns	**	**	**
treatment × cultivar			ns	ns		ns	ns	ns
treatment × sampling			ns	ns	ns	ns	ns	ns
treatment × cultivar × sampling			ns	ns		ns	ns	ns

[a] Data are expressed as the mean ± standard deviation ($n = 3$). Means within the same column followed by different letters were significantly different at $p < 0.05$. [b] Data are expressed as cyanidin 3-glucoside in micrograms per gram of fresh weight. [c] Data are expressed as pelargonidin 3-glucoside in micrograms per gram of fresh weight. [d] nd = not detected. [f] ns = nonsignificant. *Significant at $p < 0.05$. Significant at $p < 0.01$.

effect of distribution between peel and flesh tissue. Other studies showed a decrease in phenolic acids in tomatoes when UV untreated (*21*).

The content of polyphenols in strawberries was increased in the external tissue except for *p*-coumaroyl glucose, agreeing with other studies (*30*). This observation approves the UV-protection function of flavonoids. Especially quercetins in the strawberry external tissue seem to play a key role in the context with ultraviolet radiation agreeing with other studies (*18*, *19*, *22*). The main part of total phenol content was detected in the external tissue. Thus, the occasional above-mentioned low antioxidant capacity of open-field grown fruits compared to tunnel grown fruits can be explained by the fruit weight: A higher berry size results in a lower epidermal tissue:fruit size ratio; consequently larger fruits must have lower total phenol contents than smaller fruits. Anttonen et al. (*11*) also described that primary fruits, which have the highest berry size, contain a lower antioxidant capacity than secondary or tertiary fruits, respectively.

Quercetins are besides kaempferols one of the major flavonols occurring in strawberries, but compared to the complex antioxidant mixture of anthocyanins, ellagitannins, proanthocyanidins or ascorbic acid the content is relatively low. That is one reason why the intense changes of quercetins by the treatments were not detected by the antioxidant assays like TEAC or ORAC. Borges et al. (*31*) analyzed soft fruits by HPLC-PDA with online antioxidant detection (TEAC). They showed that quercetins in raspberries did not provide antioxidant capacity and also that the contribution of quercetins detected in blackcurrant, redcurrant and cranberry was low. Recently, Ordidge et al. (*23*) postulated that UV radiation does not affect bioactives in soft fruits, but in this study individual components were not regarded. Hence, present quercetin derivatives in blueberries or raspberries are supposedly influenced by UV radiation, too.

The flavonoid biosynthesis pathway (*32*) comprehends that eriodyctyol and dihydroquercetin, respectively, are precursors of quercetin and cyanidin, whereas kaempferol and pelargonidin result from naringenin and dihydrokaempferol. For this reason the main effect was found on the linked components cyanidin and quercetin derivatives. Accordingly, the expression of genes which, are involved in quercetin synthesis are inducible by UV radiation. In the case of kaempferol, our assumption is that the synthesis is little affected by UV, but one part of the glycosylation because kaempferol 3-glucoside-malonate was not affected by UV radiation. Results of 2008 and other investigation (*10*) indicated that especially the content of kaempferol 3-glucoside-malonate could be temperature sensitive like anthocyanins. Supplementary research, which includes the responsible genes is required. Already published metabolomic (*33*) or proteomic (*34*) work during fruit ripening should be estimated with caution because the used strawberries were produced in tunnels. The "original" flavonoid biosynthesis pathway for the mentioned studies is probably affected by the absence of UV radiation. Faith et al. (*33*) showed the highest quercetin 3-glucuronide content through the big green stage of unripe strawberry fruits, followed by a decline until full ripeness of the fruits. It is possible that this development is not in agreement with the quercetin accumulation in open-field grown fruits. Evidence for this hypothesis in our study is provided by the first and second sampling results of cv. Elsanta: Although the plants had been covered with the film at the first sampling for a shorter time (less than three weeks) than for the second, where already small green fruits were observable, we found the same relationship for the three treatments for both sampling dates. Consequently, the major formation of quercetins has to be at a later fruit stage agreeing with other publications (*35*, *36*).

Further investigations of individual phenolics in berries grown in tunnels are necessary, especially because the protected production

Table 5. Effects of Cultural System on Main Colorless Polyphenols[a]

cultivar/year	sampling	treatment	ellagic acid after hydrolysis[b]	ellagic acid derivative[b]	*p*-coumaroyl glucose[c]	quercetin 3-glucuronide[d]	kaempferol 3-glucoside + kaempferol 3-glucuronide[e]	kaempferol 3-glucoside-malonate[e]
'Everest' 2008	T1	blocking	360 ± 35 fg	5.8 ± 0.1 def	28.8 ± 3.7 fg	6.1 ± 0.6 g	4.7 ± 0.3 ghij	3.8 ± 0.2 d
		window	415 ± 11 def	5.7 ± 1.1 defg	28.5 ± 4.7 fg	19.5 ± 0.2 e	6.0 ± 0.4 def	4.1 ± 0.4 d
		open-field	481 ± 26 cd	4.5 ± 0.3 h	40.9 ± 4.7 ab	33.9 ± 2.4 c	7.5 ± 0.8 b	2.7 ± 0.2 e
	T2	blocking	480 ± 41 cd	6.7 ± 0.5 cd	20.7 ± 1.4 h	4.8 ± 1.1 g	5.1 ± 0.2 fghi	4.5 ± 0.1 d
		window	479 ± 13 cd	6.1 ± 0.3 def	17.4 ± 0.7 h	22.3 ± 0.9 de	7.2 ± 0.3 bc	4.2 ± 0.1 d
		open-field	481 ± 26 cd	3.3 ± 0.1 i	27.7 ± 1.9 g	40.5 ± 0.9 b	10.0 ± 1.6 a	2.5 ± 0.2 e
'Everest' 2009	T1	blocking	454 ± 39 cde	5.6 ± 0.6 efgh	41.9 ± 2.1 a	3.5 ± 0.4 g	3.7 ± 0.1 j	2.5 ± 0.1 e
		window	577 ± 39 ab	6.3 ± 0.4 cde	36.2 ± 3.4 bcd	24.8 ± 3.3 d	5.9 ± 0.8 ef	2.3 ± 0.2 e
		open-field	590 ± 74 ab	5.5 ± 0.4 efgh	34.6 ± 3.0 cde	24.1 ± 1.7 d	6.1 ± 0.4 def	2.3 ± 0.1 e
	T2	blocking	536 ± 48 abc	5.1 ± 1.0 fgh	37.1 ± 1.0 abcd	5.2 ± 0.8 g	4.2 ± 0.4 ij	2.4 ± 0.3 e
		window	520 ± 84 bc	6.1 ± 0.6 def	37.7 ± 4.4 abc	26.1 ± 3.5 d	6.1 ± 0.5 def	2.5 ± 0.2 e
		open-field	532 ± 20 abc	4.6 ± 0.8 gh	38.7 ± 2.7 abc	34.6 ± 2.1 c	7.6 ± 0.9 b	2.3 ± 0.2 e
'Elsanta' 2009	T1	blocking	427 ± 50 def	3.2 ± 0.1 i	31.8 ± 1.2 defg	10.3 ± 1.4 f	4.5 ± 0.2 hij	6.8 ± 0.4 ab
		window	383 ± 32 efg	3.3 ± 0.9 i	30.5 ± 0.5 efg	41.0 ± 0.7 b	5.4 ± 0.1 efgh	5.9 ± 0.2 c
		open-field	313 ± 12 g	3.0 ± 0.2 i	27.4 ± 1.6 g	48.8 ± 2.0 a	5.7 ± 0.3 efg	5.8 ± 0.3 c
	T2	blocking	425 ± 30 def	8.0 ± 0.2 ab	33.3 ± 1.6 cdef	11.0 ± 1.7 f	5.1 ± 0.1 fghi	6.9 ± 0.4 ab
		window	603 ± 71 a	8.7 ± 0.6 a	30.4 ± 1.6 efg	45.3 ± 1.7 a	7.0 ± 0.6 bcd	7.2 ± 0.2 a
		open-field	415 ± 45 def	7.3 ± 1.0 bc	30.6 ± 1.0 efg	40.4 ± 4.6 b	6.4 ± 0.3 cde	6.5 ± 0.2 b
					Significance[f]			
treatment			*	**	ns	**	**	**
cultivar			**	ns	ns	**	*	**
sampling			*	**	ns	ns	**	ns
treatment × cultivar			**	ns	ns	**	**	ns
treatment × sampling			ns	ns	ns	ns	ns	ns
treatment × cultivar × sampling			*	ns	ns	**	ns	ns

[a] Data are expressed as the mean ± standard deviation ($n = 3$). Means within the same column followed by different letters were significantly different at $p < 0.05$. [b] Data are expressed as ellagic acid equivalents in micrograms per gram of fresh weight. [c] Data are expressed as coumaric acid in micrograms per gram of fresh weight. [d] Data are expressed as quercetin 3-glucuronide in micrograms per gram of fresh weight. [e] Data are expressed as kaempferol 3-glucuronide in micrograms per gram of fresh weight. [f] ns = nonsignificant. *Significant at $p < 0.05$. **Significant at $p < 0.01$.

Table 6. Distribution of Polyphenols in Strawberry Flesh and Peel[a]

tissue	treatment	ellagic acid after hydrolysis[b]	ellagic acid derivative[b]	*p*-coumaroyl glucose[c]	quercetin 3-glucuronide[d]	kaempferol 3-glucoside + kaempferol 3-glucuronide[e]	kaempferol 3-glucoside-malonate[e]	total phenols[f]
flesh	blocking	241 ± 22 a	2.3 ± 0.6 c	55.6 ± 2.0 a	2.5 ± 0.6 d	1.7 ± 0.1 d	1.9 ± 0.1c	2248 ± 217 b
	window	292 ± 35 a	2.1 ± 0.2 c	49.6 ± 1.4 a	2.4 ± 0.2 d	1.9 ± 0.2 d	1.6 ± 0.2 c	2205 ± 55 b
	open-field	318 ± 15 a	2.1 ± 0.7 c	51.2 ± 2.9 a	7.7 ± 1.0 cd	2.2 ± 0.1 d	1.6 ± 0.2 c	2329 ± 121 b
peel	blocking	680 ± 65 b	9.3 ± 1.7 a	54.0 ± 2.8 a	10 ± 1.9 c	4.7 ± 0.3 c	3.6 ± 0.4 a	3865 ± 242 a
	window	677 ± 133 b	6.8 ± 0.6 b	39.9 ± 3.8 b	35.9 ± 2.5 b	6.8 ± 0.7 b	2.5 ± 0.4 b	3899 ± 262 a
	open-field	683 ± 10 b	6.7 ± 0.2 b	40.7 ± 5.0 b	55.2 ± 7.3 a	8.5 ± 1.0 a	2.8 ± 0.1 b	3748 ± 123 a
					Significance[g]			
treatment		ns	*	*	**	**	**	ns
tissue		**	**	**	**	**	**	**
treatment × tissue		ns	*	ns	**	**	*	ns

[a] Data are expressed as the mean ± standard deviation ($n = 3$). Means within the same column followed by different letters were significantly different at $p < 0.05$. [b] Data are expressed as ellagic acid equivalents in milligrams per 100 grams of dry weight. [c] Data are expressed as coumaric acid in milligrams per 100 grams of dry weight. [d] Data are expressed as quercetin 3-glucuronide in milligrams per 100 grams of dry weight. [e] Data are expressed as kaempferol 3-glucuronide in milligrams per 100 grams of dry weight. [f] Data are expressed as GAE in milligrams per 100 grams of dry weight. [g] ns = nonsignificant. * Significant at $p < 0.05$, ** Significant at $p < 0.01$.

of soft fruits is increasing and fruits do not react like leafy vegetables, e.g., lettuce, or other crops like apple or tomato.

The reduced content of quercetin 3-glucuronide of UV-untreated strawberries could impair the nutritional value of the fruits regarding that this flavonol has putative health effects (*37*). Quercetin is one of the most predominant flavonols occurring ubiquitously in fruits and vegetables. The most famous source for quercetins is onions, with a content of 284−486 μg of quercetin per g fresh weight (*38*) and accordingly more than 10-fold higher than in strawberries grown under open-field conditions. In Germany, the per capita consumption of onions per year was in 2008 around 7.1 kg and that of strawberries 3.3 kg. The strawberry is a seasonal fruit whereas onions are consumed constantly all over the year. Even in this short season strawberry consumption is only 2-fold higher compared to onions (*39*, *40*). Regarding this information the quercetin content in strawberries compared to onions seems to be less important. However, the only benefit of strawberries is that the main occurrence of this flavonol is quercetin 3-glucuronide as quercetin is present in plasma (*41*). Quercetins are available in onions only as glucosides, which could be a disadvantage in bioavailability.

Considering the agronomical and quality parameters, temperature effects covered possible UV effects. Tunnel-grown plants tended to lower yields for the same harvest period (**Table 1**),

Table 7. Distribution of Anthocyanins in Strawberry Flesh and Peel[a]

tissue	treatment	cyanidin 3-glucoside[b]	pelargonidin 3-glucoside[c]	pelargonidin 3-rutinoside[c]	pelargonidin derivative[c]	cyanidin 3-glucoside-malonate[b]	pelargonidin 3-glucoside-malonate[c]
flesh	blocking	2.6 ± 0.5 c	164.2 ± 6.8 c	14.4 ± 0.8 c	0.6 ± 0.1 b	1.8 ± 0.3 b	40.8 ± 1.5 d
	window	3.5 ± 0.1 c	141.0 ± 13.9 c	13.8 ± 0.9 c	0.4 ± 0.1 b	1.6 ± 0.2 b	34.7 ± 3.6 d
	open-field	3.1 ± 0.7 c	148.1 ± 13.5 c	16.0 ± 0.9 c	0.5 ± 0.2 b	1.5 ± 0.2 b	35.7 ± 3.9 d
peel	blocking	20.1 ± 1.1 b	310.8 ± 16.1 a	31.5 ± 2.5 a	1.1 ± 0.4 a	7.5 ± 1.0 a	69.8 ± 3.6 a
	window	24.0 ± 3.4 ab	252.4 ± 21.9 b	24.3 ± 1.3 b	1.2 ± 0.1 a	8.5 ± 1.3 a	53.1 ± 4.8 c
	open-field	26.6 ± 4.9 a	302.7 ± 10.4 a	31.5 ± 2.2 a	1.3 ± 0.3 a	7.8 ± 1.2 a	60.3 ± 1.7 b
				Significance[d]			
treatment		ns	**	**	ns	ns	**
tissue		**	**	**	**	**	**
treatment × tissue		ns	ns	**	ns	ns	ns

[a] Data are expressed as the mean ± standard deviation ($n = 3$). Means within the same column followed by different letters were significantly different at $p < 0.05$. [b] Data are expressed as cyanidin 3-glucoside in milligrams per 100 grams of dry weight. [c] Data are expressed as pelargonidin 3-glucoside in milligrams per 100 grams of dry weight. [d] ns = nonsignificant, ** significant at $p < 0.01$.

which may be an effect of temperature (*42*). However, protected cultivation can extend the season, which resulted particularly for ever-bearer in higher total yields as shown in 2008. Higher temperature decreases the average fruit weight, but cv. Elsanta showed only marginal reduction by mean temperatures higher than 20 °C (*42*). During this trial the temperature outside was on average 20 °C, and 1 °C higher under the standard film, not affecting the fruit size of cv. Elsanta Elsanta but of cv. Everest (**Table 1**). The ever-bearer cultivar seemed to be more temperature sensitive; lower fruit weights of tunnel-grown strawberries were observed in both experimental years. The window film inclined to slightly higher temperatures. However, no significant effects were observed by application of the window film for single leaf area, total leaf area per plant, yield or mean fruit weight compared to the blocking film (**Table 1**), although lettuce decreased leaf area with UV radiation (*18*). The plant performance of strawberries seems to be mainly UV unresponsive and more temperature dependent. Over all sampling dates, the open-field fruits had the highest average fruit weight (**Table 2**).

For most parameters, the sampling dates had a stronger impact on the fruits than the treatment. In principle, one should bear in mind that the growth stage of field-grown plants is delayed to that of tunnel-grown plants; consequently the comparability of the fruits on the same sampling date is only restrictedly possible. Generally, the treatments did not differ in quality parameters like titratable acidity, SSC or dry matter and consequently the irrigation in the tunnel was not deficit.

In summary, we could estimate losses of several flavonols in the absence of UV radiation by the use of standard UVB blocking films in strawberry fruits, although open-field grown strawberries are delayed compared to tunnel-grown fruits and the sampling date strongly affects the content of bioactives. The main observations caused by UV radiation were not cultivar dependent. The deficits can be partially avoided by the application of UVB transparent films. Most bioactives, which contribute to the antioxidant capacity, were not affected by UV radiation. In our study we could not provide any disadvantages of the use of UV transparent films in plant performance or yield compared to standard blocking films.

ACKNOWLEDGMENT

The authors appreciate Gerhard Müller and collaborators for excellent coordination and assistance of the field experiments, Gerold Schmidt for temperature and PAR measurements and Anna Gresch and Jeannette Zacharias for analytical support.

LITERATURE CITED

(1) Seeram, N. P. Berry fruits for cancer prevention: Current status and future prospects. *J. Agric. Food Chem.* **2008**, *56*, 630−635.

(2) Zafra-Stone, S.; Taharat, Y.; Bagchi, M.; Chatterjee, A.; Vinson, J. A.; Bagchi, D. Berry anthocyanins as novel antioxidants in human health and disease prevention. *Mol. Nutr. Food Res.* **2007**, *51*, 675−683.

(3) Meyers, K. J.; Watkins, C. B.; Pritts, M. P.; Liu, R. H. Antioxidant and antiproliferative activities of strawberries. *J. Agric. Food Chem.* **2003**, *51*, 6887−6892.

(4) Aaby, K.; Ekeberg, D.; Skrede, G. Characterization of phenolic compounds in strawberry (*Fragaria* × *ananassa*) fruits by different HPLC detectors and contribution of individual compounds to total antioxidant capacity. *J. Agric. Food Chem.* **2007**, *55*, 4395−4406.

(5) Buendía, B.; Gil, M. I.; Tudela, J. A.; Gady, A. L.; Medina, J. J.; Soria, C.; López, J. M.; Tomás-Barberán, F. A. HPLC-MS analysis of proanthocyanidin oligomers and other phenolics in 15 strawberry cultivars. *J. Agric. Food Chem.* **2010**, *58*, 3916−3926.

(6) Tulipani, S.; Mezzetti, B.; Capocasa, F.; Bompadre, S.; Beekwilder, J.; de Vos, C. H. R.; Capanoglu, E.; Bovy, A. G.; Battino, M. Antioxidants, phenolic compounds, and nutritional quality of different strawberry genotypes. *J. Agric. Food Chem.* **2008**, *56*, 696−704.

(7) Crespo, P.; Bordonaba, J. G.; Terry, L. A.; Carlen, C. Characterisation of major taste and health-related compounds of four strawberry genotypes grown at different Swiss production sites. *Food Chem.* **2010**, *122*, 16−24.

(8) Keutgen, A. J.; Pawelzik, E. Quality and nutritional value of strawberry fruit under long term salt stress. *Food Chem.* **2008**, *107*, 1413−1420.

(9) Terry, L. A.; Chope, G. A.; Bordonaba, J. G. Effect of water deficit irrigation and inoculation with Botrytis cinerea on strawberry (*Fragaria* × *ananassa*) fruit quality. *J. Agric. Food Chem.* **2007**, *55*, 10812−10819.

(10) Wang, S. Y.; Zheng, W. Effect of plant growth temperature on antioxidant capacity in strawberry. *J. Agric. Food Chem.* **2001**, *49*, 4977−4982.

(11) Anttonen, M. J.; Hoppula, K. I.; Nestby, R.; Verheul, M. J.; Karjalainen, R. O. Influence of fertilization, mulch color, early forcing, fruit order, planting date, shading, growing environment, and genotype on the contents of selected phenolics in strawberry (*Fragaria* × *ananassa* duch.) fruits. *J. Agric. Food Chem.* **2006**, *54*, 2614−2620.

(12) Atkinson, C. J.; Dodds, P. A. A.; Ford, Y. Y.; Le Miere, J.; Taylor, J. M.; Blake, P. S.; Paul, N. Effects of cultivar, fruit number and reflected photosynthetically active radiation on *Fragaria* × *ananassa* productivity and fruit ellagic acid and ascorbic acid concentrations. *Ann. Bot.* **2006**, *97*, 429−441.

(13) Wang, S. Y.; Millner, P. Effect of different cultural systems on antioxidant capacity, phenolic content, and fruit quality of strawberries (*Fragaria* × *ananassa* Duch.). *J. Agric. Food Chem.* **2009**, *57*, 9651−9657.

(14) Lieten, P. Strawberry production in central europe. *Int. J. Fruit Sci.* **2006**, *5*, 91−105.
(15) Daugovish, O.; Larson, K. D. Strawberry production with protected culture in southern California. *Acta Hortic.* **2009**, *842*, 163−166.
(16) Krizek, D. T.; Clark, H. D.; Mirecki, R. M. Spectral properties of selected UV-blocking and UV-transmitting covering materials with application for production of high-value crops in high tunnels. *Photochem. Photobiol.* **2005**, *81*, 1047−1051.
(17) Shirley, B. W. Flavonoid biosynthesis: 'new' functions for an 'old' pathway. *Trends Plant Sci.* **1996**, *1*, 377−382.
(18) Tsormpatsidis, E.; Henbest, R. G. C.; Davis, F. J.; Battey, N. H.; Hadley, P.; Wagstaffe, A. UV irradiance as a major influence on growth, development and secondary products of commercial importance in Lollo Rosso lettuce 'Revolution' grown under polyethylene films. *Environ. Exp. Bot.* **2008**, *63*, 232−239.
(19) García-Macías, P.; Ordidge, M.; Vysini, E.; Waroonphan, S.; Battey, N. H.; Gordon, M. H.; Hadley, P.; John, P.; Lovegove, J. A.; Wagstaffe, A. Changes in the flavonoid and phenolic acid contents and antioxidant activity of red leaf lettuce (Lollo Rosso) due to cultivation under plastic films varying in ultraviolet transparency *J. Agric. Food Chem.* **2007**, *55*, 10168−10172.
(20) Kolb, C. A.; Kopecký, J.; Riederer, M.; Pfündel, E. E. UV screening by phenolics in berries of grapevine (*Vitis vinifera*). *Funct. Plant Biol.* **2003**, *30*, 1177−1186.
(21) Luthria, D. L.; Mukhopadhyay, S.; Krizek, D. T. Content of total phenolics and phenolic acids in tomato (*Lycopersicon esculentum* Mill.) fruits as influenced by cultivar and solar UV radiation. *J. Food Compos. Anal.* **2006**, *19*, 771−777.
(22) Solovchenko, A.; Schmitz-Eiberger, M. Significance of skin flavonoids for UV-B-protection in apple fruits. *J. Exp. Bot.* **2003**, *54*, 1977−1984.
(23) Ordidge, M.; García-Marcías, P.; Battey, N. H.; Gordon, M. H.; Hadley, P.; John, P.; Lovegove, J. A.; Vysini, E.; Wagstaffe, A. Phenolic contents of lettuce, strawberry, raspberry, and blueberry crops cultivated under plastic films varying in ultraviolet transparency. *Food Chem.* **2010**, *119*, 1224−1227.
(24) Hartmann, A.; Patz, C. D.; Andlauer, W.; Dietrich, H.; Ludwig, M. Influence of processing on quality parameters of strawberries *J. Agric. Food Chem.* **2008**, *56*, 9484−9489.
(25) Giusti, M. M.; Wrolstad, R. E. Characterization and measurement of anthocyanins by UV-visible spectroscopy. In *Current Protocols in Food Analytical Chemistry*; Wrolstad, R. E., Acree, T. E., An, H.; Decker, E. A., Penner, M. H., Reid, D. S., Schwartz, S. J., Shoemaker, C. F., Sporns, P., Eds.; John Wiley & Sons, Inc.: New York, 2001; p 1.
(26) Singleton, V. L.; Orthofer, R.; Lamuela-Raventós, R. M. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. In *Methods in Enzymology Oxidants and Antioxidants Part A*; Lester, P., Ed.; Academic Press: 1999; Vol. 299, pp 152−178.
(27) Ou, B.; Hampsch-Woodill, M.; Prior, R. L. R. Development and validation of an improved oxygen radical absorbance capacity assay using fluorescein as the fluorescent probe. *J. Agric. Food Chem.* **2001**, *49*, 4619−4626.
(28) Häkkinen, S. H.; Mykkanen, H. M.; Heinonen, I. M.; Törrönen, A. R. Ellagic acid content in berries: Influence of domestic processing and storage. *Eur. Food Res. Technol.* **2000**, *212*, 75−80.
(29) Ubi, B. E.; Honda, C.; Bessho, H.; Kondo, S.; Wada, M.; Kobayashi, S.; Moriguchi, T. Expression analysis of anthocyanin biosynthetic genes in apple skin: Effect of UV-B and temperature. *Plant Sci.* **2006**, *170*, 571−578.
(30) Gil, M. I.; Holcroft, D. M.; Kader, A. A. Changes in strawberry anthocyanins and other polyphenols in response to carbon dioxide treatments. *J. Agric. Food Chem.* **1997**, *45*, 1662−1667.
(31) Borges, G.; Degeneve, A.; Mullen, W.; Crozier, A. Identification of flavonoid and phenolic antioxidants in black currants, blueberries, raspberries, red currants, and cranberries. *J. Agric. Food Chem.* **2009**, *58*, 3901−3909.
(32) Almeida, J. R. M.; D'Amico, E.; Preuss, A.; Carbone, F.; de Vos, C. H. R.; Deiml, B.; Mourgues, F.; Perrotta, G.; Fischer, T. C.; Bovy, A. G.; Martens, S.; Rosati, C. Characterization of major enzymes and genes involved in flavonoid and proanthocyanidin biosynthesis during fruit development in strawberry (*Fragaria* × *ananassa*). *Arch. Biochem. Biophys.* **2007**, *465*, 61−71.
(33) Fait, A.; Hanhineva, K.; Beleggia, R.; Dai, N.; Rogachev, I.; Nikiforova, V. J.; Fernie, A. R.; Aharoni, A. Reconfiguration of the Achene and Receptacle Metabolic Networks during Strawberry Fruit Development. *Plant Physiol.* **2008**, *148*, 730−750.
(34) Bianco, L.; Lopez, L.; Scalone, A. G.; Di Carli, M.; Desiderio, A.; Benvenuto, E.; Perrotta, G. Strawberry proteome characterization and its regulation during fruit ripening and in different genotypes *J. Proteomics* **2009**, *72*, 586−607.
(35) Wulf, J. S.; Rühmann, S.; Rego, I.; Puhl, I.; Treutter, D.; Zude, M. Nondestructive application of laser-induced fluorescence spectroscopy for quantitative analyses of phenolic compounds in strawberry fruits (*Fragaria* × *ananassa*). *J. Agric. Food Chem.* **2008**, *56*, 2875−2882.
(36) Halbwirth, H.; Puhl, I.; Haas, U.; Jezik, K.; Treutter, D.; Stich, K. Two-phase flavonoid formation in developing strawberry (*Fragaria* ×*ananassa*) fruit. *J. Agric. Food Chem.* **2006**, *54*, 1479−1485.
(37) Boots, A. W.; Haenen, G. R. M. M.; Bast, A. Health effects of quercetin: From antioxidant to nutraceutical. *Eur. J. Pharmacol.* **2008**, *585*, 325−337.
(38) Nemeth, K.; Piskula, M. K. Food content, processing, absorption and metabolism of onion flavonoids. *Crit. Rev. Food Sci.* **2007**, *47*, 397−409.
(39) Behr, H.-P. *AMI-Marktbilanz Gemüse 2010: Daten, Fakten, Entwicklungen, Deutschland, EU, Welt*; 2010.
(40) Würtenberger, E.; Schockemöhle, U. *AMI-Marktbilanz Obst 2010: Daten, Fakten, Entwicklungen, Deutschland, EU, Welt*; 2010.
(41) Wittig, J.; Herderich, M.; Graefe, E. U.; Veit, M. Identification of quercetin glucuronides in human plasma by high-performance liquid chromatography-tandem mass spectrometry. *J. Chromatogr., B* **2001**, *753*, 237−243.
(42) Le Mière, J.; Hadley, P.; Darby, J.; Battey, N. H. The effect of thermal environment, planting date and crown size on growth, development and yield of *Fragaria* × *ananassa* Duch. cv. Elsanta. *J. Hortic. Sci. Biotechnol.* **1998**, *73*, 786−795.

Received for review July 28, 2010. Revised manuscript received October 22, 2010. Accepted October 22, 2010. We thank Stiftung Gisela for financial assistance.