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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

MIKKO J. ANTTONEN,*,† KALLE I. HOPPULA,‡ ROLF NESTBY,§ MICHÉL J. VERHEUL,# AND REIJO O. KARJALAINEN†,⊥

Institute of Applied Biotechnology, University of Kuopio, P.O. Box 1627, FI-70211 Kuopio, Finland; MTT Agrifood Research Finland, Horticulture, Toivonlinnantie 518, FI-21500 Piikkiö, Finland; The Norwegian Crop Research Institute, Kvithamar Research Centre, N-7500 Stjørdal, Norway; The Norwegian Crop Research Institute, Særheim Research Centre, N-4353 Klepp st., Norway; and MTT Agrifood Research Finland, FI-31600 Jokioinen, Finland

The influence of agricultural practices (fertilization, mulch color, early forcing, and planting date), environment (light and growing area), cultivar, and fruit order on the selected phenolic content and antioxidant activity in strawberry (*Fragaria* × *ananassa* Duch.) fruits was studied. Three different levels of fertilization were given to plants in the fertilization experiment. The lowest fertilization level increased the contents of flavonols and ellagic acid from 19 to 57%. Between cultivars, up to 4-fold differences were found in the flavonol content, and it also varied according to growing environment. Planting date in glasshouse production was important for the phenolic content, and a statistically significant interaction was found between planting date and fruit order. Fruit order caused at highest 1.5−2.0-fold differences in the contents of phenolics. Interestingly, compared with other phenolics, anthocyanins were affected differently by many factors. Thus, the findings show that minor cultivation changes can increase the content of phenolics, especially in under-glass production where conditions can be easily manipulated.

KEYWORDS: Strawberry; *Fragaria* × *ananassa* Duch.; cultivation; cultivar; fertilization; phenolic compounds; flavonoids; ellagic acid; flavonols; anthocyanins

INTRODUCTION

Epidemiological data (*1*) suggest that a high intake of fruits and vegetables offers a number of health benefits against degenerative diseases and can promote longevity. It is widely accepted that a possible mechanism behind the protective effect is related to the bioactive compounds in fruits and vegetables that reduce oxidative stress symptoms.

Strawberry (*Fragaria* × *ananassa* Duch.) fruits contain a wide array of phenolic compounds including hydroxycinnamic acids, galloyl esters, ellagic acid, ellagitannins, flavan-3-ols, flavonols, and anthocyanins (*2*). Compared with other fruits, strawberries possess high antioxidant activity (*3*). This activity is mainly due to phenolic compounds, whereas only ∼10% of the antioxidant activity is due to ascorbic acid (*4*). Antioxidant activity of strawberry phenolics is important in the prevention of cardiovascular diseases and cancer (*5*). However, phenolic compounds may also have more specific mechanisms of action. Recently, antioxidant activity, independent of in vitro antiproliferative activity, of strawberry extracts has been shown using the HepG2 human liver cancer cells (6). In addition, the effects of individual compounds may be different. In an in vitro assay using PC12 cells, strawberry phenolics were found to have neuroprotective activity (*7*). This activity was related to anthocyanins. Ellagic acid derivates and ellagitannins in strawberry fruits may harbor chemopreventive properties, and also their antimicrobial activity against human pathogens has been shown (*5*).

Strawberries are widely and highly consumed fresh and in processed forms, and their increasing production under glass makes them available all year round. Thus, strawberries can be considered a very potent source of bioactive phenolics, and even small increases in the contents of these compounds may have important health benefits. Genetic engineering and breeding are

* Address correspondence to this author at the Institute of Applied Biotechnology, University of Kuopio, Savilahdentie 9, P.O. Box 1627, FI-70211 Kuopio, Finland [telephone +358-40-535 9029; fax +358-17-16 3148; e-mail mikko.anttonen@uku.fi).
† University of Kuopio.
‡ MTT Agrifood Research Finland, Piikkiö.
§ Kvithamar Research Center, The Norwegian Crop Research Institute.
# Særheim Research Center, The Norwegian Crop Research Institute.
⊥ MTT Agrifood Research Finland, Jokioinen.

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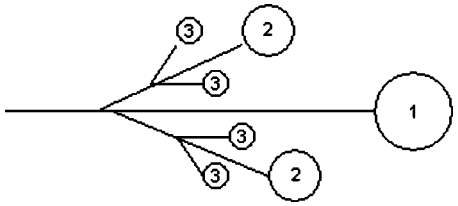

Figure 1. Fruit order in a strawberry inflorescence: (1) primary; (2) secondary; (3) tertiary.

both viable means to increase the phenolic content in plants. Several examples have shown the power of genetic engineering (*8*), and wide variation between genotypes in the contents of phenolics (*9*) offers a sound basis for breeding programs. However, optimizing the agricultural practices and the growing environments may also provide a rapid and efficient way to increase the phenolic content, especially under glasshouse environments where conditions can be easily manipulated. It has been previously shown that the contents of many phenolics increase in strawberry fruits when grown at higher temperatures (*10*) or when the carbon dioxide content is elevated (*11*). Soil composition and fertilization have also been shown to affect the phenolic content (*12*). In addition, the aroma content of strawberries grown on red plastic mulch has been shown to be higher than that when black mulch has been used (*13*).

The aim of this study was to evaluate the potential of agricultural regimens to increase the content of phenolic compounds in strawberry fruits and thus the consumption of these compounds. In this study the influence of agricultural practices (fertilization, mulch color, early forcing, and planting date), environment (light and growing area), cultivar, and fruit order (**Figure 1**) on the phenolic content (flavonols, ellagic acid, total phenolics, and anthocyanins) and antioxidant activity in strawberry fruits was evaluated.

MATERIALS AND METHODS

Fertilization. In this experiment the effect of fertilization amount on the content of ellagic acid and the flavonols quercetin and kaempferol was evaluated. The fertilization experiment was established in the summer of 2001 out on an open field in Finland at MTT Agrifood Research Finland/Horticulture (60° N, 22° E, 6 m above sea level). The soil was covered with a plastic foil over which beds of 20 cm height and 80 cm width were built of fertilized peat. The space between rows was 1.5 m. Peat beds were mulched with black plastic foil, and fresh plug plants of June-bearing strawberry (*Fragaria* × *ananassa* Duch., Rosaceae) cv. Bounty were planted in double rows with a 33 cm space between the plants (40000 plants per ha).

The fertilization experiment was executed during the summer of 2002. Plants were fertigated using an Agrojet injector and T-Tape TSX 508-30-340 drip tape (30 cm distance between nozzles; 3.4 L h^{-1} m^{-1}). The drip tape was installed under the plastic mulch ~5 cm below the soil surface. Soil moisture was monitored by means of tensiometers (TM-93, Nieuwkoop B.V.). Measuring tubes (30 cm) were installed 10 cm below the soil surface. Tensiometers were monitored manually three times a week, and −150 hPa was used as a threshold value for irrigation. Having reached the threshold, the soil was irrigated to approximately −30 hPa using fertilizer solutions with a conductivity of 0.6, 1.2, or 2.4 mS cm^{-1}, depending on the treatment. All fertilizers used in this trial were manufactured by Kemira Agro Oy (Helsinki, Finland). By the collection date at the fertilization level of 0.6 mS cm^{-1}, the seedlings had received (per plant) 339 μg of N, 82 μg of P, 645 μg of K, 65 μg of Ca, 39 μg of Mg, 51 μg of S, 3.7 μg of Fe, 514 ng of B, 308 ng of Cu, 2057 ng of Mn, 41 ng of Mo, 20 ng of Co, and 514 ng of Z. The amounts of nutrients were calculated according to the manufacturer's information. At the levels of 1.2 and 2.4 mS cm^{-1} the amounts were 2- and 4-fold higher, respectively.

The experimental design was a randomized block design with four replicates. Fruits (500 g) were collected at the end of the cropping season fully and evenly ripe according to surface color, immediately frozen in carbon ice, and stored at −20 °C until analyzed. Single analyses of each variable were made from each replicate.

Mulch Color, Early Forcing, and Fruit Order. In this experiment the effects of mulch color, early forcing, and fruit order (**Figure 1**) on the content of the total phenolics, anthocyanins, and ellagic acid and antioxidant activity were evaluated. The experiments were carried out in the field in Stjørdal, Norway (63° N, 11° E, 38 m above sea level). The soil was a poorly drained silt loam overlaying silty clay loam with an almost flat slope, containing 8.3% organic matter in the plow layer. Flower bud initiated Korona strawberries (*Fragaria* × *ananassa* Duch., Rosaceae), were planted on July 2, 2003, on 25 cm high beds mulched with polyethylene foil (Norfolier AS & CO, Icopal AS, Skårer, Norway). Centers of beds were 140 cm apart, with two plant rows on each bed. Rows were planted 20 cm apart with 25 cm plant spacing within each row, amounting to 5714 plants per 1000 m^2. A tunnel roofed with standard polyethylene foil (Haygrowe tunnels, Haygrove Ltd., Herefordshire, U.K.), was placed in the field immediately after planting. For the first 2 weeks after planting the plants were watered with overhead sprinklers. Later that year and in 2004, the plants were drip irrigated with a balanced macro- and micronutrient solution, automatically drip regulated by a Flori soil moisture sensor (Netafim USA, Fresno, CA). From October 10, 2003, to April 20, 2004, the field was covered with fleece [Agryl P30 vinter, BBA Fiberweb(tm) Global Headquarters, London, U.K.] for freezing protection. In 2004, the tunnel was roofed on April 20. Experiments in 2004 were undertaken in a field trial with two treatments in a split plot design of four replications. The treatments were as follows: (1) forcing with fleece on large plots randomized within replication, (a) from April 20, 2004, to 5% flowering, and (b) no fleece; (2) mulch randomized within large plots, (a) white polyethylene foil, and (b) brown polyethylene foil. Fruits (100 g) of different order (primary, secondary, and tertiary) were harvested in 2004 as midsummer crop fully and evenly ripe according to surface color and stored at −20 °C until analyzed. Single analyses of each variable were made from each replicate.

Planting Time, Shading, and Fruit Order. In this experiment the effects of planting time, shading, and fruit order (**Figure 1**) on the content of the total phenolics, anthocyanins, and ellagic acid and antioxidant activity were evaluated. The experiments were carried out in a glasshouse compartment at Særheim Research Station in southwestern Norway (58° N, 5° E, 80 m above sea level). Flower bud initiated Korona strawberries (*Fragaria* × *ananassa* Duch., Rosaceae) were planted on February 9 (week 6), February 25 (week 9), or March 15 (week 12) in standard peat bags (30 × 40 cm; BVB, Maasland, Netherlands) with a density of six plants per bag. Bags were placed in an unheated glasshouse on gutters with a plant density of 13.6 plants m^{-2}. Ventilation temperature was maintained at 20 °C. Plants were watered sufficiently using a complete nutrient solution with a conductivity level of 1.8 mS cm^{-1} containing the following elements (in mg L^{-1}): N, 205; P, 40; K, 208; Ca, 175; Mg, 17; S, 20; Fe, 1.4; Mn, 0.8; B, 0.2; Zn, 0.1; Cu, 0.05; Mo, 0,01; Co 0.01.

Half of the plants were shaded with a double standard polyethylene foil, providing a reduction of the incoming light by 32% when compared to nonshaded plants. Plant date was fully randomized with two replications, both consisting of 24 plants. Random samples (100 g) of different order fruits (primary, secondary, and tertiary) were harvested (**Figure 2**) fully and evenly ripe according to surface color and stored at −20 °C until analyzed. Single analyses of each variable were made from each replicate.

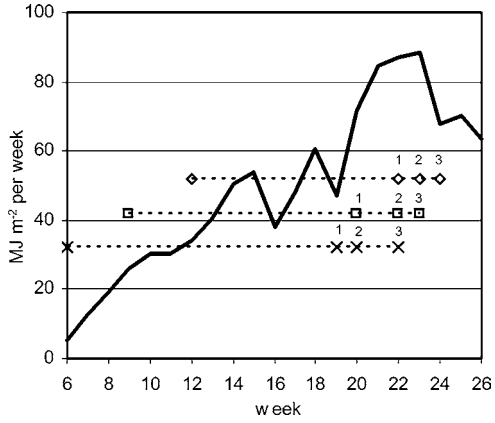

Figure 2. Incoming global radiation (solid line) and harvest of fruits of different order from different planting dates. Fruit order: (1) primary; (2) secondary; (3) tertiary. Planting dates: (×) week 6; (□) week 9; (◇) week 12.

Genetic and Environmental Variation. In this experiment the genetic and environmental variation of the flavonols quercetin and kaempferol was evaluated. The strawberries (*Fragaria* × *ananassa* Duch., Rosaceae) were grown by professional farmers in southern Finland. The fruit samples were collected at four farms at which either conventional (CONV1 and CONV2) or organic (ORG1 and ORG2) farming practices were used. Silt clay was a dominant soil type at all farms. At organic farms fields were previously used for growing legumes and cereals. At establishment, 25 tons/ha of cow manure compost was applied in the fields, and no additional fertilization or plant protection chemicals were used thereafter. Conventionally grown strawberries were established on fields previously growing cereals. Basic fertilization of 1000 kg/ha of NPK (25:20:100) was applied during the planting year, and additional surface fertilization was applied in the third year spring (NPK). During the growing season Decis (0.5 L.ha; Bayer CropScience, Monheim, Germany) was used once against insect pests. Euparen M (2.5 kg/ha; Bayer CropScience) and Switch (1 kg/ha; Syngenta Crop Protection A/S, Basel, Switzerland) were both used twice a season against gray mold. A composite sample (500 g) of fully and evenly ripe fruits according to surface color was collected during the summer of 2001 (third year from planting), from five randomly selected rows at the central area of the field. Fruits were collected from the same compass angle and at the same time of day. Fruits were frozen immediately in dry ice and stored at −20 °C until analyzed. Four separate flavonol content analyses were made from each composite sample.

Reagents. All reagents used were of analytical or higher grade. Standards of ellagic acid, gallic acid monohydrate, morin, quercetin, and kaempferol were purchased from Sigma Chemical Co. (St. Louis, MO). Folin−Ciocalteu's phenol reagent was purchased from Merck KGaA (Darmstadt, Germany).

Flavonols and Ellagic Acid. Flavonols and ellagic acid were analyzed from fruit extracts after acid hydrolysis using HPLC based methods as described previously (*9*). Aglycones released by acid hydrolysis were identified and quantified using pure standard compounds. Results are expressed as milligrams of measured compound in 100 g of fresh fruits (mg 100 g^{-1} of FW).

Total Phenolics, Total Anthocyanins, and Radical Scavenging Capacity. For the analysis of total phenolics, total anthocyanins, and antioxidant activity fruits were extracted with 70% acetone containing 0.01 M hydrochloric acid. The frozen fruits were first thawed in a microwave oven (650 W, ∼1 min) and homogenized using a food processor. Five grams of fruits was weighed and extracted three times with 15 mL of the solvent by shaking vigorously for 5 min. Between extractions solution was separated by centrifugation. Extracts were combined in a 50 mL volumetric flask.

For the analysis of the total anthocyanins the pH differential method was used (*14*). In the calculations a molar absorptivity of 22400 L mol^{-1} cm^{-1} was used. The results are expressed as milligrams per 100 g of FW as pelargonidin-3-glucoside.

The total phenolics were quantified using the Folin−Ciocalteu method (*15*) with minor modifications. Volumes of the sample, Folin−Ciocalteu's phenol reagent, and sodium carbonate were reduced to 1:10 compared with the original method with a final volume of 20 mL. Gallic acid was used for the quantification, and results are expressed as milligrams per 100 g of FW.

The free radical scavenging capacity of the fruit extracts was measured using the stable free radical α,α-diphenyl-β-picrylhydrazyl (DPPH). DPPH absorbs strongly at 517 nm (in ethanol), giving the solution a deep violet color (*16*). When the odd electron becomes paired, the absorption vanishes and the change is stoichiometric with respect to the number of electrons taken up.

Samples were diluted in 0.4 M acetate buffer (pH 5.0). The assay was performed in a microplate. To a well were added 50 μL of sample and 250 μL of DPPH solution (4.0 mg 100 mL^{-1} in methanol). Absorbance was read in a Victor 2 (Wallac/PerkinElmer, Wellesley, MA) multilabel counter using a 530 nm filter after 40 min, which was found to be sufficient time for the reaction to reach steady state. A calibration curve was used to calculate the amount of scavenged radical. From each extract three replicate measurements were made. Results are expressed as milligrams of consumed DPPH per gram of fruit FW.

The Folin−Ciocalteu and DPPH methods are electron transfer based methods, and thus they both measure the reducing capacity of the sample. Although hydrogen atom transfer based methods are considered to be biologically more relevant (*17*), these methods were chosen because of their simplicity and wide usage. In addition, regardless of the reaction mechanism the Folin−Ciocalteu method is considered to be a useful method for measuring antioxidant capacity.

Statistics. Statistical analyses were performed using SPSS for Windows version 11.5.1 (SPSS Inc., Chicago, IL). The effects of fertilization and genetic and environmental variation were evaluated using one-way ANOVA. Multiple comparison was done using either Tukey's or Dunnett's T3 test. Differences at $P < 0.05$ were considered to be significant. In other experiments the general linear model (GLM) univariate procedure was used to evaluate the effects of the main factors and their interactions. Differences at $P < 0.05$ were considered to be significant.

Table 1. Effects of Different Fertilization Levels on the Strawberry Fruit Phenolics ($n = 4$; ± SD)[a]

fertilization level (mS cm^{-1})	mg 100 g^{-1} of FW		
	quercetin	kaempferol	ellagic acid
0.6	0.52 ± 0.10 a	0.49 ± 0.06 a	29.29 ± 3.92 a
1.2	0.33 ± 0.07 b	0.44 ± 0.02 ab	24.20 ± 2.00 b
2.4	0.36 ± 0.13 ab	0.41 ± 0.02 b	24.79 ± 2.50 ab

[a] Statistically different ($P < 0.05$) results in columns are marked with different letters.

RESULTS

Fertilization. The effect of fertilization on the content of flavonols and ellagic acid was statistically significant (**Table 1**). The highest levels of these compounds were detected at the lowest fertilization level, and the differences between the two highest levels were nonsignificant. The ellagic acid content was 21% higher at the 0.6 mS cm^{-1} than at the 1.2 mS cm^{-1} level. The kaempferol content was 19% higher at the 0.6 mS cm^{-1} than at the 2.4 mS cm^{-1} level. The quercetin content was most affected by fertilization. It was 57% higher at the fertilization level of 0.6 mS cm^{-1} than at the 1.2 mS cm^{-1} level.

Table 2. Effect of Mulch Color on the Strawberry Fruit Phenolics and Antioxidant Activity as Scavenged DPPH ($n = 24$; ± SD)

mulch color	mg 100 g^{-1} of FW: total phenolics	antho-cyanins	ellagic acid	antioxidant activity (mg g^{-1} of FW)
brown	253.6 ± 19.1	28.4 ± 4.4	35.1 ± 4.8	13.5 ± 1.4
white	269.5 ± 19.6	25.8 ± 4.1	37.8 ± 4.5	14.1 ± 1.4
significance[a]	**	*	**	ns

[a] ns, nonsignificant; *, $P < 0.05$; **, $P < 0.01$.

Table 3. Effect of Fruit Order on the Strawberry Fruit Phenolics and Antioxidant Activity as Scavenged DPPH ($n = 16$; ± SD)

	mg 100 g^{-1} of FW: total phenolics	antho-cyanins	ellagic acid	antioxidant activity (mg g^{-1} of FW)
primary	251.7 ± 11.9	23.9 ± 4.8	32.8 ± 2.2	13.1 ± 1.0
secondary	256.0 ± 16.5	29.2 ± 3.1	35.5 ± 3.8	13.7 ± 1.5
tertiary	276.9 ± 23.5	28.2 ± 3.6	41.0 ± 3.9	14.6 ± 1.4
significance[a]	***	**	***	**

[a] ** $P < 0.01$, *** $P < 0.001$.

Mulch Color, Early Forcing, and Fruit Order. The effect of mulch color was statistically significant on parameters other than antioxidant activity (**Table 2**). Early forcing had no effects (data not shown), whereas the effect of fruit order was statistically significant on all measured parameters (**Table 3**). There were no statistically significant interactions between main factors.

Slightly higher amounts of the total phenolics and ellagic acid (6 and 7%) were found in fruits grown on white rather than brown mulch, whereas the total anthocyanin content was slightly higher (10%) in fruits grown on brown mulch (**Table 2**).

The contents of total phenolics and ellagic acid and the antioxidant activity were highest in tertiary fruits and lowest in primary fruits (**Table 3**). Tertiary fruits had 10, 25, and 11% higher contents of these compounds, respectively, than primary fruits. The content of the total anthocyanin was lowest in the primary fruits and 22 and 17% higher in secondary and tertiary fruits, respectively.

Planting Time, Shading, and Fruit Order. The effects of planting date and fruit order were statistically significant on all measured parameters (**Table 4**). In addition, a statistically significant interaction between these factors was found in all cases. However, except for the anthocyanin content, the effect of light was statistically nonsignificant (**Table 5**).

Table 5. Effect of Shading on the Strawberry Fruit Phenolics and Antioxidant Activity as Scavenged DPPH ($n = 17$; ± SD)

	mg 100 g^{-1} of FW: total phenolics	antho-cyanins	ellagic acid	antioxidant activity (mg g^{-1} of FW)
100% light	210.6 ± 28.8	29.1 ± 7.0	50.2 ± 13.7	11.8 ± 1.9
shading 32%	208.8 ± 31.1	26.6 ± 6.5	49.4 ± 14.5	11.7 ± 2.1
significance[a]	ns	*	ns	ns

[a] ns, nonsignificant; *, $P < 0.05$.

The variation between planting dates in the contents of measured phenolics and antioxidant activity was lowest in the primary fruits and highest in the tertiary fruits (**Table 4**). In the primary fruits, the ellagic acid and anthocyanin content decreased toward the latest planting date, whereas the total phenolic contents and antioxidant activity were similar in the primary fruits from all planting dates. In the secondary fruits, the highest levels of the total phenolics, ellagic acids, and antioxidant activity were found in fruits from the latest planting date, whereas the anthocyanin content was highest in fruits from the second planting date. In the tertiary fruits, the levels of the total phenolics, ellagic acid, and antioxidant activity were lowest in fruits from the first planting date and similar in fruits from the latest planting dates. The anthocyanin content was highest in tertiary fruits from the first planting date. In addition, there was a clear decreasing trend for the anthocyanin content toward the latest planting date.

The influence of the fruit order on the phenolics was planting-date dependent (**Table 4**). The contents of total phenolics and ellagic acid and antioxidant activity were lowest in primary fruits. In the fruits from the first planting, secondary and tertiary fruits had similar levels of these compounds, whereas in the fruits from other planting dates the contents were clearly highest in tertiary fruits. The differences between fruit orders in the contents of total phenolics and ellagic acid and antioxidant activity were more evident in the later planting dates, and 1.5−2.0-fold differences were observed. For the anthocyanin content the trend was not constant for fruits from different planting dates. The highest difference between lowest and highest anthocyanin content (76%) was in fruits from the latest planting date.

Shading slightly decreased the anthocyanin content (**Table 5**). In fruits grown at 100% light level the content was 9%

Table 4. Effects of Planting Date and Fruit Order on the Strawberry Fruit Phenolics as and Antioxidant Activity as Scavenged DPPH (± SD; $n = 4$)

planting	order	mg 100 g^{-1} of FW: total phenolics	anthocyanins	ellagic acid	antioxidant activity (mg g^{-1} of FW)
week 6	primary	190.6 ± 6.2	35.6 ± 3.2	40.5 ± 3.9	10.0 ± 0.7
	secondary	203.4 ± 6.3	28.7 ± 5.0	49.9 ± 4.3	11.5 ± 0.9
	tertiary	201.5 ± 4.6	30.9 ± 1.4	51.5 ± 3.0	11.2 ± 0.6
week 9	primary	180.6 ± 16.4	27.3 ± 6.5	37.7 ± 3.3	10.1 ± 1.3
	secondary	204.7 ± 9.0	34.7 ± 3.6	46.0 ± 4.8	11.9 ± 1.1
	tertiary	262.4 ± 7.9	25.6 ± 5.3	76.9 ± 10.5	15.5 ± 0.4
week 12	primary	187.6 ± 8.1	26.1 ± 2.4	35.2 ± 1.2	10.4 ± 1.0
	secondary	227.1 ± 10.3	22.8 ± 2.7	55.7 ± 4.0	13.0 ± 1.1
	tertiary	271.3 ± 13.9	14.8 ± 2.9	75.1 ± 13.4	15.2 ± 1.3
significance[a]					
planting		***	***	**	***
order		***	**	***	***
planting × order		***	**	***	**

[a] **, $P < 0.01$; ***, $P < 0.001$.

Table 6. Effects of Cultivar and Environment on the Strawberry Fruit Quercetin Content ($n = 4$; ± SD)[a]

	mg 100 g^{-1} of FW			
cultivar	CONV1[b]	CONV2	ORG1	ORG2
Honeoye	0.90 ± 0.13 ab2	na[c]	0.93 ± 0.14 a2	1.40 ± 0.07 a1
Jonsok	0.42 ± 0.04 c2	0.44 ± 0.03 ab2	0.62 ± 0.09 b1	0.76 ± 0.11 b1
Polka	0.57 ± 0.07 bc1	0.38 ± 0.02 b2	0.76 ± 0.14 ab12	0.58 ± 0.20 b12
Bounty	0.48 ± 0.07 c1	na	0.63 ± 0.09 b1	0.62 ± 0.07 b1
Korona	1.08 ± 0.14 a1	0.47 ± 0.06 a2	0.67 ± 0.11 ab2	na
Dania	0.76 ± 0.03 ab1	na	na	0.53 ± 0.03 b2

[a] Significantly different ($P < 0.05$) results are marked with different letters in columns and with different numbers in rows. [b] CONV, conventional cultivation; ORG, organic cultivation. [c] Not analyzed.

Table 7. Effects of Cultivar and Environment on the Strawberry Fruit Kaempferol Content ($n = 4$; ± SD)[a]

	mg 100 g^{-1} of FW			
cultivar	CONV1[b]	CONV2	ORG1	ORG2
Honeoye	1.37 ± 0.16 a2	na[c]	1.46 ± 0.03 a2	1.91 ± 0.19 a1
Jonsok	0.99 ± 0.07 a2	1.21 ± 0.03 a1	1.05 ± 0.10 b12	0.99 ± 0.15 b2
Polka	0.37 ± 0.03 bc2	0.43 ± 0.04 b12	0.49 ± 0.04 c1	0.45 ± 0.05 c12
Bounty	0.39 ± 0.06 bc2	na	0.62 ± 0.10 c1	0.46 ± 0.08 c12
Korona	0.34 ± 0.01 c1	0.32 ± 0.03 c1	0.30 ± 0.05 d1	na
Dania	0.43 ± 0.02 b1	na	na	0.43 ± 0.10 c12

[a] Significantly different ($P < 0.05$) results are marked with different letters in columns and with different numbers in rows. [b] CONV, conventional cultivation; ORG, organic cultivation. [c] Not analyzed.

higher. The effect was most evident for fruits from the first two planting dates, in which the contents were 15 and 16% higher when grown at the 100% light level (data not shown).

Genetic and Environmental Variation. The effects of cultivar and different growing environments on the flavonol (quercetin and kaempferol) content in strawberry fruits are shown in **Tables 6 and 7**. Quercetin and kaempferol were detected in all cultivars collected at different locations. The main flavonol was either quercetin or kaempferol, mostly depending on the cultivar. However, the relative contents of kaempferol and quercetin varied between cultivars and locations.

The influence of the cultivar on the flavonol levels was statistically significant. The highest kaempferol content was detected in Honeoye and Jonsok and the highest quercetin content in Honeoye. The ranking of the subsequent cultivars varied by location. Within the same location, the variation in the kaempferol content between cultivars was greater than that in the quercetin content. Within a single location (ORG2), the highest differences were >4-fold for the kaempferol and >2-fold for the quercetin.

The influence of the environment on the flavonol content was statistically significant (excluding the quercetin content in Bounty and the kaempferol content in Korona). Generally, the variation in the quercetin content between environments was higher than that in the kaempferol content, and variation due to the cultivar was more evident than that caused by the environment. The highest variation in the kaempferol content was found in Bounty, whereas the highest variation in the quercetin content was found in Polka (1.5- and 2.0-fold differences between the highest and lowest contents, respectively). The contents of flavonols were not systematically highest in fruits from a single location; however, in most cases the highest contents were found in organically grown fruits.

DISCUSSION

In this study the effects of different agricultural practices and environmental factors on the phenolic content in strawberry fruits were evaluated. Fertilization is of vital importance in modern glasshouse strawberry production, because balanced fertilization has an important effect on both the fruit yield and the fruit quality. The data presented in this study show that higher fertilization decreased the contents of flavonols and ellagic acid in strawberries. In grapevine, various combinations of nitrogen and potassium had no effect on yield, fruit size, or plant growth (*18*). However, the polyphenol content was affected by different fertilization combinations. In strawberry, the contents of various phenolics were increased in NPK fertilized plants compared to unfertilized ones (*12*). Compared with our study, the ratios of nitrogen and potassium were different, which may explain the observed differences (*18*). The influence of boron on phenol metabolism has also been well studied (*19*). Higher and lower levels of boron increased the contents of phenolics in tobacco leaves compared with the normal boron level, which was associated with the activity of phenylalanine ammonia-lyase, polyphenol oxidase, and peroxidase. Calcium applications have been used to increase the postharvest quality of strawberries (*20*). However, in tobacco it was found that application of higher calcium doses decreased the total phenolic content in leaves, which was associated with the higher activity of peroxidases and polyphenol oxidases (*21*).

In glasshouse production, where conditions are easily controlled, alterations in fertilization levels can be used for the modification of the phenolic content in fruits. However, before widescale applications are adopted, more detailed information is needed on the interaction of fertilization with other cultivation and environmental factors. In addition, biotic and abiotic stress factors may change the metabolism of phenolic compounds in different situations (*22*). For example, it was shown that the decrease in the phenolic content in bilberry leaves induced by nitrogen fertilization was reversed by a fungal infection (*23*).

Strawberries are commonly grown in a raised-bed culture using different polyethylene mulches. This system provides benefits for the grower, as it keeps the fruits clean, makes collecting easier, saves water, and lowers the need of herbicide use. Moreover, in Europe, strawberries grown on plastic mulches ripen a week earlier than those without mulch because the

temperature in the soil and leaf canopy becomes higher, and reflection caused by the mulch may improve light conditions. Our results show that white mulch increased the contents of the total phenolics and ellagic acid and the antioxidant activity in strawberry fruits more than brown mulch, whereas the total anthocyanin content was highest in fruits grown on brown mulch. Previously, significantly higher amounts of phenolics were found in strawberry fruits grown on plasticulture than in those grown in a matted row culture (*24*). In addition, higher anthocyanin content has been observed in strawberry fruits grown on plastic mulch compared to those grown on straw mulch (*25*). The elevated temperature inside the plastic may explain the higher contents of the phenolic compounds (*10*). Strawberries grown on red plastic mulch have been found to contain a significantly higher amount of aroma compounds than those grown on black mulch (*13*), which may be due to different light conditions caused by the mulch color.

Early forcing is used in strawberry production to speed maturation by raising the spring-time temperature. In this experiment early forcing did not influence the phenolic content or antioxidant activity. Fleece was used until 5% flowering took place, and thus temperature conditions during fruit maturation were quite similar with or without fleece.

Supplementary light and temperature increase the costs of glasshouse production. Light and temperature also have a significant impact on the fruit yield and the quality (*10*, *26*). Thus, optimization of planting date according to crop quality and production costs is essential for economically viable production. In this study significant interaction was found between the fruit order and the planting date. However, the trends for different parameters were not similar. The crop from the latest planting date seems to have the highest total phenolic content and antioxidant activity, whereas the anthocyanin content was lowest. In a related study (*27*), it was found that the harvest date affected the content of the ellagic acid in strawberries, and the prevailing temperature and rainfall during the harvesting time were assumed to cause the differences.

The effect of shading was nonsignificant for other measured parameters but not for the anthocyanins. Between the two light levels, slight differences in the anthocyanin levels were observed in fruits from the first two planting dates. Differences were reduced at later planting dates, which might be due to increased levels of natural light.

Fruit order significantly affected the phenolic content in fruits. In the two separate experiments, the levels of the total phenolics, ellagic acid, and antioxidant activity were found to increase from primary to tertiary fruits. The anthocyanins, however, performed differently. In the first experiment, the highest content of the anthocyanins was found in the secondary fruits, whereas in the second trial, planting-date dependent variation was observed. Primary fruits are often largest in size, and resources are allocated for the growth, which enhances the protein synthesis. This might lead to lower substrate availability for the phenylalanine ammonium-lyase and thus a reduction in the phenolic content. The observed decrease in the phenolic content might also be due to the dilution effect caused by increased biomass (*28*). However, the anthocyanin content in our experiments did not follow consistently these theories.

The flavonol content was more affected by the cultivar than the environment, showing the dominant role of cultivars for the determination of the basic levels of certain biochemical compounds over regions or seasons (*9*, *29*).

We found that in most cases the flavonol contents were higher in organically grown than in conventionally grown strawberry fruits. Differences in the fertilization practices might be the most plausible explanation for this (*30*). It has been shown that nitrogen can become a growth-limiting nutrient in organic production (*31*). As a consequence, this might lead to the allocation of resources to differentiation and thus enhance the metabolism of the phenolics according to the growth/differentiation balance hypothesis (*32*). Studies on the effects of cultivation techniques on the flavonol content have produced various results (*33*, *34*). It seems that different species and individual compounds are differently affected by the cultivation technique.

In conclusion, cultivation modifications may provide a potential means to increase the health-related value as well as the quality of fresh and processed forms of strawberry by altering the phenolic contents of the fruit. The most straightforward approach to influencing the phenolic content of fruits and end products is to select cultivars with the desired composition of these compounds or to adjusting the end use according to the fruit order. Agricultural factors can be easily manipulated especially in under-glass production. Fertilization could be optimized to the lowest level according to other quality components and yield. In addition, adjustment of planting date according to higher light and temperature conditions can be used to increase the total phenolic content and the antioxidant activity. In an open field production, the mulch color may be one of the factors that can be used to modify the composition of phenolics. However, in open field production it might be challenging to control interactions between different factors.

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