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Antioxidant Levels and Inhibition of Cancer Cell Proliferation in Vitro by Extracts from Organically and Conventionally Cultivated Strawberries

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The effects of extracts from five cultivars of strawberries on the proliferation of colon cancer cells HT29 and breast cancer cells MCF-7 were investigated, and possible correlations with the levels of several antioxidants were analyzed. In addition, the effects of organic cultivation compared to conventional cultivation on the content of antioxidants in the strawberries and strawberry extracts on the cancer cell proliferation were investigated. The ratio of ascorbate to dehydroascorbate was significantly higher in the organically cultivated strawberries. The strawberry extracts decreased the proliferation of both HT29 cells and MCF-7 cells in a dose-dependent way. The inhibitory effect for the highest concentration of the extracts was in the range of 41−63% (average 53%) inhibition compared to controls for the HT29 cells and 26−56% (average 43%) for MCF-7 cells. The extracts from organically grown strawberries had a higher antiproliferative activity for both cell types at the highest concentration than the conventionally grown, and this might indicate a higher content of secondary metabolites with anticarcinogenic properties in the organically grown strawberries. For HT29 cells, there was a negative correlation at the highest extract concentration between the content of ascorbate or vitamin C and cancer cell proliferation, whereas for MCF-7 cells, a high ratio of ascorbate to dehydroascorbate correlated with a higher inhibition of cell proliferation at the second highest concentration. The significance of the effect of ascorbate on cancer cell proliferation might lie in a synergistic action with other compounds.

KEYWORDS: Ascorbate; dehydroascorbate; phenolics; hydroxycinnamic acids; ellagic acid; flavonol; anthocyanin; cancer cell proliferation; correlation

INTRODUCTION

Many epidemiological studies have shown that a higher consumption of fruit and vegetables is associated with the prevention of chronic diseases such as diabetes, heart disease, and certain cancers (*1*, *2*). There has been a growing interest in understanding the reason for the cancer-protective effect of fruit and vegetables and identifying the components with the anticancer effect. Apart from essential nutrients, fruit and vegetables also contain a variety of different phytochemicals that can act as antioxidants and prevent oxidation by reactive oxygen species on cell components and also exhibit other bioactive physiological properties. Different antioxidants, such as flavonoids, phenolic acids, carotenoids, and ascorbic acid, have been proposed to act anticarcinogenically (*3*−*5*). Oxidation of DNA is likely to be an important cause of mutations that can develop into cellular hyperproliferation and cancer. The anticarcinogenic effect of the phytochemicals might also be exerted by other mechanisms than by their antioxidant activity, such as induction of cell cycle arrest and apoptosis (*6*, *7*). Certain flavonoids have been shown to inhibit the potentially pro-oxidant enzymes lipoxygenase and xanthine oxidase, which can generate free radicals (*8*), whereas hydroxycinnamic acids exhibit antioxidant activity, preventing lipid oxidation in human systems (*9*) and anticancer effects on colon cancer (*10*).

Strawberries have been shown to exhibit high total antioxidant capacity in comparison to many other fruits (*11*), and this antioxidant activity has been shown to vary among different cultivars (*12*). There has been an increased interest in strawberries because of their relatively high content of ellagic acid, an antioxidant that has been proposed to exert antimutagenic and anticarcinogenic effects (*13*−*15*). Strawberries also contain other antioxidants with potential good health effects, such as vitamin C, hydroxycinnamic acids, anthocyanins, and other flavonoids (*16*−*18*).

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Proliferation of cancer cells has been shown to be inhibited by phytochemicals in extracts of strawberries. Among 11 common fruits, strawberries showed a relatively potent antiproliferative activity on the growth of human liver cancer cells HepG2 (*19*). Phytochemicals present in extracts of eight investigated strawberry cultivars all inhibited HepG2 cell growth in a dose-dependent way, and the inhibitory effects of the different cultivars were significantly different between those showing the highest effects compared to those with the lowest effects (*17*). However, different types of cancer cells might give different responses, and no correlation was found between the analyzed content of phenolic compounds or total antioxidant activity and the antiproliferative activity of the extracts of strawberries or other fruits and berries (*17*, *19*, *20*). Therefore, further investigations are needed to elucidate the antiproliferative roles of different phytochemicals in strawberries against cancer cell growth.

There is an increasing interest in a healthier and more environmentally friendly production method for strawberries. Today, organic production is not one cultivation method, but many, which are characterized by restrictions against the use of synthetic pesticides and synthetic fertilizers, although the detailed regulations of what can be called organically cultivated vary. Different organic fertilizers can be applied, and different methods can be used for removal of weeds and reduction of pathogenic attacks. Nevertheless, consumers today buy products that are labeled "organically cultivated" in stores, and many expect the quality to be superior to that of conventionally cultivated products in terms of contents that make the products healthier (*21*, *22*). Previous investigations have indicated that the content of ascorbic acid might be higher in organically cultivated fruit and vegetables (*23*, *24*). Results concerning the content of phenolic compounds in fruit and berries cultivated conventionally versus those cultivated organically have been conflicting. A higher content of total phenolics was found in marionberries and strawberries grown sustainably or organically than in those grown conventionally (*25*). Total phenolics and ellagic acid contents were higher in one of three investigated organically grown strawberry cultivars (*16*), whereas in yellow plums grown conventionally and organically, the results were conflicting concerning the content of the different phenolics (*26*). No consistent results were found when the flavonol content was compared in black currrants (*27*). Because the contents of antioxidants and other secondary metabolites have been shown to vary in response to different environmental conditions such as temperature, water availability, pathogenic attack, and nutrients (*28*, *29*) and because the different cultivation systems could result in different growth conditions, it would be expected that these parameters could vary and affect the results in the different investigations. Increasing numbers of investigations would show which responses are the most common in the two cultivation systems.

In the present investigation, the effects of extracts from different cultivars of strawberries on the proliferation of colon cancer cells HT29 and breast cancer cells MCF-7 were studied, and possible correlations with the different levels of several antioxidants in the cultivars were analyzed. In addition, the effects of cultivation system on the content of antioxidants in the strawberries as well as the effects of the strawberry extracts on cancer cell proliferation were investigated.

MATERIALS AND METHODS

Plant Material. All strawberries were grown at Rånna research station (58°27′ N, 13°51′ E), Swedish University of Agricultural Sciences, in Sweden. The selection of cultivars was determined from the availability at the research station. The strawberries, grown either organically or conventionally, were collected from fields situated near each other, but still far enough apart that pest control on the conventionally grown cultivars did not affect the organically grown cultivars. Five different strawberry cultivars were used in this experiment: Pavana, Cavendish, Dania, Korona, and Honeoye. Two of these, Honeoye and Cavendish, were grown both organically and conventionally. One cultivar, Dania, was grown organically, whereas Pavana and Korona were grown conventionally.

The organically grown strawberries were cultivated according to the following practices: Three weeks before planting, the soil was fertilized with fresh (turkey-chicks) and dried poultry manure (Binadan A/S, Nørre Snede, Denmark), in amounts equivalent to 50 kg of N per hectare. The manure was broadcasted and harrowed down into the soil. Further fertilization (0.5 g of N/plant) was supplied in April in the rows using the dried poultry manure and also later using Vinasse, a byproduct from the production of yeast (Jästbolaget AB, Rotebro, Sweden) and the dried poultry manure. The strawberry plants were planted either in holes in biodegradable plastic mulch or in bare soil. The weeds were removed by hand. The pest control was conducted by spraying with Bioruiskute S (Kemira Agro OY, Helsinki, Finland), at a concentration of 0.5% (v/v), in amounts of approximately 2 L per hectare, starting in May and also later during the season. The active substance in Bioruiskute S was pyrethrine, and the preparation did not contain piperonylbutoxide, which is not allowed in organic farming in Sweden.

The conventionally grown strawberries were fertilized with 400 kg of NPK fertilizer (11/5/18 plus micronutrients) per hectare before planting, equal to 44 kg of N per hectare, or 0.75 g per plant, as about approximately 50% of the area was covered with plants. An additional 300 kg of NPK fertilizer, equal to 33 kg of N per hectare, or 0.6 g per plant, was supplied later. The plants were planted in bare soil and in matted rows with a width of 30 cm. The weeds were removed by hoeing by hand, and the soil was then covered with straw. The pest control was conducted by spraying with Gusathion WP containing the active substance azinphosmethyl (Makhteshim-Agan Holland B.V.) at a dose of 1.0 kg per hectare for control of insects. For control of gray mold, the plants were first sprayed with Teldor WG 50 containing the active substance phenhexamide (Bayer AB, Bayer CropScience) at a dose of 1.6 kg per hectare, and the second time with Rovral containing the active substance iprodion (BASF AB) at a dose of 0.8 kg per hectare, followed later by an additional application of Teldor WG 50 at a dose of 1.6 kg per hectare.

The strawberries were harvested at commercial ripeness and, as soon as possible on the harvest day, were frozen at −20 °C and, shortly thereafter, stored at −80 °C, before use in an analysis of the antioxidant content or cancer cell proliferation experiments. The strawberries from the different cultivars and cultivation practices were collected at random in the fields. From each composite sample of each cultivar and cultivation method, four subsamples were extracted independently. All samples were treated consistently, independently of cultivar or cultivation method.

Preparation of Extracts. Each sample of berries was homogenized with an Ultraturrax apparatus (IKA T8) in ethanol−water with 50 mM H_3PO_4, so that the final proportion of ethanol and water was 1:1 including the freshwater content in the strawberries [approximately 20 g of fresh weight (FW) per 40 mL of solvent]. N_2 was blown through the extraction solution for 5 min, and the tubes were thereafter sealed and placed in darkness and shaking at 4 °C for 20 h for further extraction. The samples were centrifuged at 12 000*g*, 4 °C, for 10 min. Aliquots of the supernatants were stored at −80 °C before analysis by HPLC or use for cancer cell proliferation tests.

The extracts used for cell proliferation tests were evaporated to near dryness under N_2 and dissolved in 50% ethanol.

Cancer Cell Proliferation Studies. Human colon cancer cells HT29 and estrogen-receptor-positive breast cancer cells MCF-7 were obtained from the American Tissue Culture Collection (Rockville, MD). The cells were incubated for 24 h at 37 °C in an atmosphere of 95% air/5% CO_2. The cancer cells were cultured as in ref *30*. Effects on the cell proliferation rate were determined by the ability of the cells to cleave the tetrazolium salt WST-1 (Roche Diagnostics, Mannheim,

Germany) to formazan (*31*), a measure of mitochondrial activity. In brief, 2×10^4 cells in 0.2 mL of medium were plated in the wells of a 96-well microplate and incubated for 24 h for attachment. The medium was then replaced with 0.2 mL of new medium before the fruit or berry extracts were added. Four different concentrations of the extracts were used: 0.025%, 0.05%, 0.25%, 0.5% of plant dry matter of total weight in the wells (weight approximated to be equal to the volume in the wells, i.e., 200 μL). An equal amount of solvent (50% ethanol) was added in the control wells. After 24 h of incubation, 20 μL of the reagent WST-1 was added, and the samples were then incubated for 1 h for HT29 cells and for 3 h for MCF-7 cells. The formation of formazan was determined photometrically at 492 nm against 620 nm as the background by a microplate reader (Bio-Rad). Three replicates were used for each extract, and the proliferation tests were repeated on three different occasions.

Analysis of Antioxidants. The samples for ascorbate, about 1.0 g, were homogenized with an Ultraturrax apparatus (IKA T8) in dim green light in a darkroom and extracted in 10 mL of 1.5% *m*-phosphoric acid. The samples were centrifuged at 16 500*g* for 5 min at 4 °C. The supernatant was filtered through a C_{18} Sep-Pak column. The first 3 mL was discarded, and an aliquot of the following 1 mL was used for analysis by HPLC, as in ref *30*, using the method as in ref *32*, with minor modifications: the solvent concentration was changed to acetonitrile/15 mM $NH_4H_2PO_4$ 75:25, pH 3.9. The peak of ascorbate in the samples was identified by comparing the retention time and spectral data with an ascorbate standard. The dehydroascorbate concentration was determined by subtracting the ascorbate concentration from the total ascorbate concentration, obtained after the use of a reduction procedure (*33*).

The flavonols, non-cell-wall-bound hydroxycinnamic acids, and free ellagic acid were analyzed by modified methods of Madhavi et al. and Schieber et al. (*34*, *35*). The ethanol−water extracts were evaporated with N_2 until near dryness and redissolved in water before three additional extractions with ethyl acetate. The ethyl acetate extract was evaporated to dryness and redissolved in DMSO/MeOH/H_2O 25:10:15. Samples were then centrifuged at 10000*g* for 5 min before analysis on a Kontron HPLC system, equipped with a diode-array DAD440 detector. The mobile phase was a linear binary gradient with solvent A [1% (v/v) acetic acid and 5% (v/v) MeOH] and solvent B [acetonitrile/5% (v/v) MeOH] at a flow rate of 1 mL/min. A Phenomenex Luna 5 μm, 150 × 4.6 mm C18 column with a Phenomenex Security Guard C18 precolumn was used. Detection was carried out at 254, 320, and 370 nm. Evaluation was performed with a Kromasystem 2000, using retention times and spectral data as compared to standards.

In addition, to estimate the total amount of ellagic acid after hydrolysis, the strawberries were extracted (approximately 500 mg of freeze-dried strawberries in 20 mL of 50% methanol) as in ref *36*. The samples were heated to 85 °C for 2 h in 0.6 M HCl and 0.15% ascorbic acid. Samples were then centrifuged at 10000*g* for 5 min. The supernatant of each sample was diluted with distilled water to twice the volume, placed on a Seppak C18 column, and eluted with 1.4 mL of methanol before analysis on an Agilent 1100 HPLC system (Agilent Technology), equipped with a diode-array detector. The mobile phase was a linear binary gradient with solvent A [50 mM acetic acid and 5% (v/v) acetonitrile] and solvent B [acetonitrile/5% (v/v) MeOH] at a flow rate of 1 mL/min. A Phenomenex Luna 5 μm, 150 × 4.6 mm C18(2) column with a Phenomenex Security Guard C18 precolumn was used. Detection was carried out at 254 nm. Evaluation was performed with a Chemstation A09.03 software (Agilent Technology), using retention times and spectral data as compared to standards.

Anthocyanins were analyzed by HPLC as in ref *30*. Pelargonidin-3-glucoside was used as the standard. Total phenolics were quantified according to the Folin−Ciocalteu method: 0.1 mL of ethanol−water extract (0.5 mg of FW/mL) was mixed with 0.2 mL of Folin−Ciocalteu reagent, 2 mL of H_2O, and 1 mL of 15% Na_2CO_3 in a cuvette. After 2 h at room temperature, the samples with the reagents were measured in a spectrophotometer at 765 nm. Gallic acid was used as the standard, and total phenolics are expressed as milligrams per gram of gallic acid equivalents for triplicate samples.

Statistics. All values of antioxidants were based on three to four independent replicate samples for each strawberry cultivar. The results presented are the mean ± standard deviation (SD). All measurements of cancer cell proliferation were conducted with three independent samples for each concentration of extract and for each cell line, and the experiments were repeated on three occasions. The data were subjected to a one-way analysis of variance (ANOVA), and the level of significance used was $p < 0.05$. Results were analyzed with Origin Software (Microcal Software Inc., Northampton, MA). Correlations between the levels of the different antioxidants and the cancer cell proliferation inhibition by the extracts were calculated by Pearson correlation using Minitab Statistical Software (Minitab Inc., State College, PA).

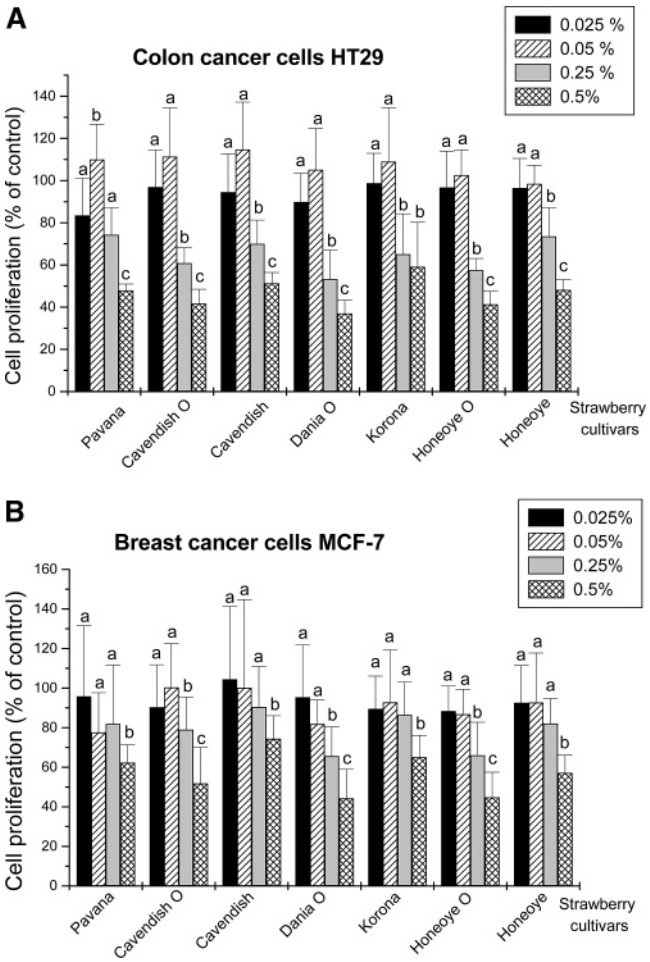

Figure 1. Effects of strawberry extracts on proliferation of cancer cells: (**A**) colon cancer cells HT29, (**B**) breast cancer cells MCF-7. Results are presented as means ± SD based on three independent replicates for each extract, and the proliferation tests were repeated on three different occasions ($n = 9$). Means marked with different letters, within each cultivar and cultivation method, were significantly different ($p < 0.05$).

RESULTS

Inhibition of Cancer Cell Proliferation. The strawberry extracts decreased the proliferation of both colon cancer cells HT29 and breast cancer cells MCF-7 (**Figure 1**). The different cultivars inhibited proliferation to different extents. The HT29 cells were, on average, inhibited to a higher degree than the MCF-7 cells. The inhibitory effect for the highest concentration of the extracts varied 2-fold among the cultivars, and it was in the range of 41−63% inhibition compared to controls (average 53%) for the HT29 cells and 26−56% inhibition (average 43%) for the MCF-7 cells. The inhibition of proliferation was concentration-dependent, although there was no significant

Table 1. Effect on Proliferation in Colon Cancer Cells HT29 and Breast Cancer Cells MCF-7 of Addition of Extracts from Organically and Conventionally Cultivated Strawberries

extract conc[a]	cultivar(s)[b]	HT29[c,d]	MCF-7[c,d]
0.5	all conv	50.3***	62.1***
0.5	all org	40.0	46.9
0.5	Cav conv	51.3**	74.4**
0.5	Cav org	41.7	51.8
0.5	Hon conv	48.1*	57.1*
0.5	Hon org	41.4	44.8
0.25	all conv	70.2***	81.8***
0.25	all org	57.2	70.2
0.25	Cav conv	70.0 ns	90.4 ns
0.25	Cav org	60.9	79.0
0.25	Hon conv	73.6**	82.0*
0.25	Hon org	57.5	66.0

[a] Concentration of the extracts used in the growth medium in terms of percent of plant dry matter of total weight in the wells (weight approximated to be equal to volume in the wells). [b] Investigated conventionally grown cultivars were Pavana, Korona, Cavendish, and Honeyoe, and organically grown cultivars were Cavendish, Honeyoe, and Dania. All conv = all conventionally grown cultivars, all org = all organically grown cultivars, Cav conv = Cavendish conventionally cultivated, Cav org = Cavendish organically cultivated, Hon conv = Honeyoe conventionally cultivated, Hon org = Honeyoe organically cultivated. [c] Values are expressed as percent of controls and are the means of three replicates that were repeated three times for each investigated cultivar ($n = 9$). [d] Significance: *, $p \leq 0.05$; **, $p \leq 0.01$; ***, $p \leq 0.005$; ns, not significantly different at $p \leq 0.05$. All conv were compared with all org, Cav conv with Cav org, and Hon conv with Hon org.

difference between the two lowest concentrations, 0.05% and 0.025%, with the exception of the extract from cv. Pavana for the HT29 cells. For the MCF-7 cells, there was a significant difference in the inhibition of the proliferation between the three highest concentrations (0.5%, 0.25%, and 0.05%) for all of the organically grown cultivars, whereas for the conventionally grown cultivars, there was only a significant difference between the two highest concentrations (0.5% and 0.25%). For the HT29 cells, there was a significant difference in the inhibition of the proliferation between the three highest concentrations (0.5%, 0.25%, and 0.05%) for all of the cultivars and cultivation systems, with the exception of cv. Korona (**Figure 1**).

The extracts from the organically grown strawberries inhibited cancer cell proliferation of both HT29 and MCF-7 to a significantly higher extent at the two highest concentrations, 0.5% and 0.25%, than the conventionally grown strawberries (**Table 1**). The growth inhibitory effect was significant at these concentrations when the inhibition effect of all organically grown cultivars was compared with that of all conventionally grown cultivars. The effect was also significant at these two concentrations of extracts when the organically grown cultivar Honeoye was compared with conventionally grown Honeoye and when the organically grown cultivar Cavendish was compared with conventionally grown Cavendish, with the exception of the concentration 0.25% for cv. Cavendish, where the significance was $p = 0.07$. At the two lower concentrations, 0.05% and 0.025%, no significant difference was found between the inhibitory effect on cancer cell proliferation of the extracts from the conventionally grown or organically grown strawberries (values not shown). The three cultivars that had been grown organically, Dania, Honeoye, and Cavendish, had the highest inhibition effect on cell proliferation of both HT29 and MCF-7 at the extract concentrations 0.5% and 0.25% dry weight (DW) (**Figure 1**).

Dry Weight Determinations. There were differences in the ratio of dry weight to fresh weight between cultivars, ranging from 9.0% to 15.0%, with an average of 11.9% for all cultivars.

Table 2. Contents of Ascorbate, Dehydroascorbate, and Vitamin C (Ascorbate Plus Dehydroascorbate) and Ratio of Ascorbate to Dehydroascorbate in the Investigated Strawberry Cultivars

cultivar[a]	AA[b,c]	DHA[b,c]	vit C[b]	AA/DHA
Pavana	4.56 ± 0.29	0.33 ± 0.10	4.89 ± 0.34	14.6
Korona	3.61 ± 0.21	0.52 ± 0.08	4.13 ± 0.27	7.0
Cavendish	4.82 ± 0.23 ns[d]	0.47 ± 0.18 ns[d]	5.29 ± 0.36 ns[d]	11.6 ns[d]
Honeoye	3.80 ± 0.19*** [d]	0.56 ± 0.14** [d]	4.36 ± 0.08*** [d]	7.0* [d]
Cavendish O	4.67 ± 0.20	0.22 ± 0.07	4.87 ± 0.17	23.3
Honeoye O	5.17 ± 0.22	0.11 ± 0.07	5.28 ± 0.22	57.0
Dania O	5.63 ± 0.26	0.34 ± 0.19	5.97 ± 0.19	21.8

[a] Strawberry cultivars followed by the letter O were organically grown, and all others were conventionally cultivated. [b] Results are expressed as means ± SD in mg/g of DW based on three to four independent replicate samples. [c] AA = ascorbic acid, DHA = dehydroascorbic acid. [d] Values from organically cultivated Cavendish and Honeyoe were compared with conventionally cultivated Cavendish and Honeyoe, and the significance is denoted as follows: *, $p \leq 0.05$; **, $p \leq 0.01$; ***, $p \leq 0.005$; ns, not significantly different at $p \leq 0.05$.

Table 3. Contents of Ellagic Acid, Hydroxycinnamic Acids, and Total Phenolics in the Investigated Strawberry Cultivars

cultivar[a]	ellagic acid[b]	HCA[b,c]	flavonols[b]	total phenolics[d,e]
Pavana	63.0 ± 0.5	34.8 ± 6.9	66.7 ± 1.8	11.7 ± 0.2
Korona	123.9 ± 0.8	54.0 ± 9.5	33.2 ± 5.7	13.6 ± 0.1
Cavendish	70.2 ± 1.0***	127.1 ± 49.4 ns	93.7 ± 14.9*	19.7 ± 0.4***
Honeoye	140.6 ± 2.9***	51.2 ± 6.6 ns	44.0 ± 1.4 ns	14.2 ± 0.1***
Cavendish O	109.0 ± 0.8	163.8 ± 31.9	55.1 ± 8.0	22.8 ± 0.1
Honeoye O	127.7 ± 1.0	57.3 ± 40.1	44.3 ± 2.6	12.5 ± 0.2
Dania O	121.5 ± 0.8	28.5 ± 2.1	85.9 ± 4.9	20.6 ± 1.0

[a] Strawberry cultivars followed by the letter O were organically grown, and all others were conventionally cultivated. [b] Values are expressed in μg/g of DW and are the means ± SD of three to four independent replicate samples. [c] HCA = hydroxycinnamic acids. [d] Values are expressed as means ± SD in mg of gallic acid equivalents/g of DW. [e] Values from organically cultivated Cavendish and Honeyoe were compared with conventionally cultivated Cavendish and Honeyoe, and the significance is denoted as follows: *, $p \leq 0.05$; **, $p \leq 0.01$; ***, $p \leq 0.005$; ns, not significantly different at $p \leq 0.05$.

The mean dry weight content of all of the organically grown cultivars (11.7%) compared to the mean dry weight content of all of the conventionally grown cultivars (12.1%) was not significantly different.

Antioxidants in the Strawberry Extracts. There were great differences in the contents of the analyzed antioxidants among the investigated cultivars and cultivation methods. The content of ascorbate was 48% higher in the highest ranking cultivar compared to the lowest, and the content of dehydroascorbate was 5-fold higher (**Table 2**). The ratio of ascorbate to dehydroascorbate varied 8-fold among the cultivars and cultivation methods.

The contents of total phenolics, ellagic acid, and flavonols varied 2-fold among the cultivars, whereas the content of hydroxycinnamic acids varied almost 6-fold (**Table 3**). The levels of total anthocyanins varied 16-fold among cultivars (**Figure 2**).

When the organically grown strawberry cultivars as a group were compared to the conventionally grown cultivars, the levels of each antioxidant in the group grown organically were higher in all of the investigated antioxidants, although not always significantly (**Table 4**). The contents of ascorbate and vitamin C (ascorbate plus dehydroascorbate) and the ratio of ascorbate to dehydroascorbate were significantly higher in all of the organically grown cultivars as a group compared to all of the conventionally grown cultivars. The same was true for cv.

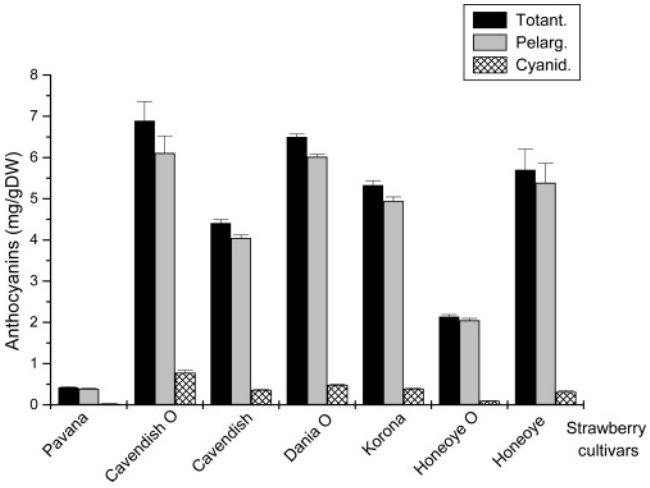

Figure 2. Contents of anthocyanins in the different strawberry cultivars and cultivation methods. Results are presented as means ± SD based on three to four independent replicate samples.

Table 4. Content of Antioxidants in Conventionally and Organically Grown Strawberry Cultivars

compound(s)	cultivars[a]	conc[b,c]
AA	all conv	4.19***
AA	all org	5.15
DHA	all conv	0.47***
DHA	all org	0.22
AA/DHA	all conv	10.0***
AA/DHA	all org	34.0
ellagic acid	all conv	99.4 ns
ellagic acid	all org	119.4
flavonols	all conv	59.4 ns
flavonols	all org	61.8
HCA	all conv	66.8 ns
HCA	all org	83.2
total anthocyanins[d]	all conv	3.96 ns
total anthocyanins[d]	all org	5.18
total phenolics[e]	all conv	14.8 *
total phenolics[e]	all org	18.6

[a] Investigated conventionally grown cultivars were Pavana, Korona, Cavendish, and Honeyoe, and organically grown cultivars were Cavendish, Honeyoe, and Dania. All conv = all conventionally grown cultivars, all org = all organically grown cultivars. [b] Values are expressed in mg/g of DW for AA (ascorbic acid) and DHA (dehydroascorbic acid) and in μg/g of DW for the others (except for AA/DHA and total phenolics), and the values are the means of three to four independent replicate samples for each cultivar. [c] Significance: *, $p \leq 0.05$; **, $p \leq 0.01$; ***, $p \leq 0.005$; ns, not significantly different at $p \leq 0.05$. All conv were compared with all org. [d] Pelargonidin-3-glycoside was used as the standard. [e] Values are expressed in μg of gallic acid equivalents/g of DW.

Honeoye, grown both organically and conventionally, but not for cv. Cavendish. Total phenolics were higher in all of the organically grown cultivars as a group compared to all of the conventionally grown cultivars. The same was true for cv. Cavendish, grown both organically and conventionally, but in cv. Honeoye, total phenolics were higher in the conventionally grown strawberries.

For cv. Cavendish, the levels of ellagic acid, HCA (nonsignificantly), total anthocyanins, cyanidin and pelargonidin, and total phenolics were higher, whereas flavonols were lower in the organically cultivated strawberries than in the conventionally cultivated strawberries. In cv. Honeoye, the level of HCA (nonsignificantly) was higher, and the levels of ellagic acid, total anthocyanin, cyanidin, pelargonidin, and total phenolics were lower in the organically grown strawberries than in the conventionally grown strawberries (**Table 3**; **Figure 2**).

Table 5. Correlations of Analyzed Compounds and Effects on Proliferation in Colon Cancer Cells HT29 and Breast Cancer Cells MCF-7

		HT 29		MCF-7	
compound(s)	extract conc[a]	r^b	p	r^b	p
AA[c]	0.5	–0.82*	0.025	–0.53	0.23
	0.25	–0.66	0.11	–0.70	0.08
DHA[d]	0.5	0.68	0.094	0.65	0.12
	0.25	0.59	0.15	0.68	0.09
vitamin C	0.5	–0.76*	0.046	–0.44	0.33
	0.25	–0.60	0.15	–0.62	0.14
AA/DHA	0.5	–0.59	0.16	–0.67	0.10
	0.25	–0.61	0.15	–0.75*	0.05
ellagic acid	0.5	–0.17	0.72	–0.60	0.16
	0.25	–0.44	0.33	–0.46	0.30
HCA	0.5	–0.003	1.0	0.26	0.57
	0.25	–0.004	0.99	0.37	0.41
flavonols	0.5	–0.33	0.46	0.19	0.68
	0.25	–0.068	0.88	0.013	0.98
total anthocyan	0.5	–0.11	0.81	–0.16	0.73
	0.25	–0.38	0.40	0.005	0.99
total phen	0.5	–0.41	0.36	–0.098	0.84
	0.25	–0.43	0.34	–0.035	0.94

[a] Concentration of the extracts used in the growth medium in percent of plant dry matter of total weight in the wells (weight approximated to be equal to volume in the wells). [b] Values denoted with * had $p \leq 0.05$. AA = ascorbic acid, DHA = dehydroascorbic acid.

When extracted and analyzed to maximize the content of total ellagic acid after hydrolysis, the levels in the strawberry extracts from the different cultivars were in the range of 1000 μg/g of DW.

Correlation between the Contents of the Different Antioxidants and Cancer Cell Proliferation. For colon cancer cells HT29, there was an inverse relationship between the contents of ascorbate and vitamin C (ascorbate and dehydroascorbate) and cancer cell proliferation, so the extracts of the cultivars with the highest concentration of vitamin C or ascorbate inhibited cell proliferation of HT29 to the highest extent, giving the lowest proliferation levels (**Table 5**). This effect was significant for the highest concentration of the extracts (0.5%). For breast cancer cells MCF-7, the extracts from the cultivars with a high ratio of ascorbate to dehydroascorbate inhibited cell proliferation significantly higher at concentration 0.25%, whereas a lower value of negative correlation with a lower significance level was found for ascorbate at the same concentration and for vitamin C at the concentration of 0.5%. For the other investigated antioxidants (including pelargonidin and cyanidin, values not shown), no other significant correlations could be found between the content of each antioxidant and the extent of inhibition of cancer cell proliferation, with two exceptions: For HT29 cells, a negative correlation was found at the extract concentration of 0.05 for the content of ellagic acid ($r = -0.78$, $p = 0.037$), and for MCF-7 cells, a positive correlation was found at the extract concentration of 0.05 for the concentration of HCA ($r = 0.84$, $p = 0.017$). In addition, for MCF-7 cells, there was a nonsignificant correlation between the content of ellagic acid and cell proliferation (concentration 0.5%: $r = -0.60$, $p = 0.16$).

DISCUSSION

The association between a higher intake of fruit and vegetables and a decreased risk of developing some types of cancer (*2*, *5*, *37*) has been suggested to be attributable to the content of antioxidants and other secondary metabolites present in plants.

However, because the number of secondary metabolites in plants has been estimated to be many thousands, it is difficult to evaluate which compounds or combinations of compounds exert health-promoting effects. The anticarcinogenic effects of fruit, berries, and vegetables might be exerted in several ways such as suppressing mutagenesis, inhibiting cell proliferation, or causing induction of apoptosis. In this investigation, the effects of extracts from several strawberry cultivars, grown in different cultivation systems, on cell proliferation in two cancer cell types were investigated. The contents of some major antioxidants were measured in order to analyze the possible correlation between the contents of the different antioxidants and the inhibition of cancer cell proliferation. The significantly higher inhibition effect of cancer cell proliferation shown by the organically cultivated strawberries as a group, but also for the two cultivars that were grown both organically and conventionally, might indicate that the content of secondary metabolites with anticarcinogenic properties was higher in the organically grown strawberries.

The levels of all of the analyzed antioxidants were higher, although not always significantly, in the organically cultivated strawberries compared to the conventionally cultivated strawberries, and this might be an explanation for the higher inhibition effect of cancer cell proliferation in the organically grown strawberries. A contrasting pattern was shown when the two cultivars that were grown both organically and conventionally were compared. In cv. Cavendish, the content of ascorbate was lower (although not significantly) in the organically grown strawberries, and the contents of ellagic acid, anthocyanins, and total phenolics were higher. The opposite was the case in cv. Honeoye, with a higher concentration of ascorbate in the organically grown strawberries and lower concentrations of ellagic acid, anthocyanins, and total phenolics. Nevertheless, the strawberries from both cultivars that had been grown organically inhibited cancer cell proliferation to a higher extent than those from conventionally grown strawberries, which might indicate that several antioxidants are involved in the antiproliferative effect and that the concentrations are exchangeable. It might also indicate that other substances apart from the investigated antioxidants might be important.

The levels of ellagic acid were somewhat lower than was found in a previous investigation (*18*). Variation in the contents between seasons as well by different analytical methods might have affected the results, but comparisons between the cultivars in this investigation should not be affected. When the content of ellagic acid after hydrolysis was analyzed as a comparison, the levels found were much higher. During digestion in the acid environment in the human stomach, a fraction of the ellagitannins is probably hydrolyzed to free ellagic acid, which is possibly absorbed (*38*). The bioavailability of ellagic acid in humans, however, is not known. Therefore, ellagic acid might have a larger impact on cancer cell proliferation than found in this investigation.

There was a large difference in the ratio of ascorbic acid to dehydroascorbic acid (AA/DHA) between the organically and conventionally grown cultivars: In all organically grown cultivars, the ratio was more than 3 times higher; in Cavendish, it was 2 times higher (although not significantly); and in Honeoye, it was 8 times higher in the organically grown strawberries. This fact is striking and might indicate that there is a more favorable antioxidant status in the organically grown strawberries, but the issue needs further investigation. These results are in accordance with a previous investigation where compost as a soil supplement was found to significantly enhance levels of ascorbate and the AA/DHA ratio in fruit of the strawberry cultivars Allstar and Honeoye. Further, the content of glutathione and the ratio of reduced to oxidized glutatione (GSH/GSSG) were also enhanced (*12*).

In this investigation, the strawberries were collected from field experiments on the same experimental station, so the strawberries were exposed to similar environmental conditions with respect to weather and soil type. In many crops, higher availability of nitrogen by fertilization has led to lower concentrations of ascorbic acid in the products (*39*), and this might have affected the content of ascorbic acid in the strawberries grown in the different cultivation systems in the present investigation. Apart from the actual amounts of nitrogen in a given fertilizer, the availability of nitrogen might also be affected by the presence of mycorrhizzal fungi, by the solubility of nitrogen from the fertilizer in the soil and its subsequent leakage from the soil compared to the rate of uptake by the plants, by soil pH, as well as by other factors (*40*). A difference in the availability of nitrogen, and also of other nutrients that have been shown to influence the contents of other substances in plants (*39*), between the organically and conventionally grown strawberries might therefore have affected the difference in the contents of antioxidants or other secondary metabolites. Factors other than the mineral nutrients might have also influenced the difference in the contents of antioxidants between the two cultivation systems, such as different responses to pathogen attacks, but further investigations are needed.

No correlation was found in the present investigation between the content of anthocyanins or ellagic acid in the strawberry extracts and inhibition of cancer cell proliferation, despite previous reports of antiproliferative activity of pure anthocyanin compounds (*41*) and pure ellagic acid (*42*). This is accordance with several other reported effects of extracts of strawberries, raspberries, and other fruits and berries, where no correlation has been found (*17*, *19*, *20*, *30*). This might be due to additive or synergistic effects of several compounds that might be interchangeable.

The correlation found between high contents of ascorbate and vitamin C and a high inhibition of proliferation of colon cancer cells HT 29 at the highest extract concentration of 0.5% and breast cancer cells MCF-7 at 0.25% (although not significantly; $r = -0.70$, $p = 0.08$ for AA) is in accordance with previous results when the effects of extracts of 10 different fruits and berries on the proliferation of the same types of cancer cells were investigated (*30*). When the effects of pure ascorbate at levels similar to those found in the fruit and berry extracts were investigated, the same effects as for the extracts were not found, so the effects could be exerted by synergistic action with other compounds (*30*), and this might also be the case in the present investigation. Vitamin C is present in relatively high levels in human tissues and blood plasma, and it might therefore be important in regulating the redox status of other antioxidants as well. Ascorbic acid in blood plasma has been shown to have the highest correlation with fruit and vegetable intake, and epidemiological investigations support a protective role of vitamin C against several cancers (*43*−*45*). However, lately, vitamin C has been reported to exhibit a pro-oxidant role in vivo. Markers of DNA damage mediated by the reactive oxygen species 8-oxoguanine (8OHG) and 8-oxoadenine (8OHA) were analyzed after supplementation of 500 mg of vitamin C to diets. An increase in the levels of 8OHA and a decrease in the levels of 8OHG were found (*46*, *47*), but the physiological significance of these contradictory changes is not understood (*48*). In vitro investigations of effects of ascorbic acid or derivatives of

ascorbic acid on cancer cell proliferation have shown various results or no effect (*49*, *50*). The significance of the effect of ascorbate on cancer cell proliferation might therefore involve a synergistic action with other compounds.

Different cultivars of strawberries have previously been shown to inhibit proliferation of another human cancer cell type, liver cancer cells HepG2 (*17*), and in the present investigation, the dose-dependent inhibition of cell proliferation was confirmed in two other cancer cell lines, colon cancer cells HT29 and breast cancer cells MCF-7. The varying magnitude of the inhibition effects of the extracts of the different strawberry cultivars on cancer cell proliferation indicates that there is a potential to select cultivars for health-promoting properties as components in food products and that factors in organic cultivation might be helpful in elucidating which compounds exhibit the antiproliferative activity against cancer cell growth.

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LITERATURE CITED

(1) Block, G.; Patterson, B.; Subar, A. Fruit, vegetables and cancer prevention: A review of the epidemiological evidence. *Nutr. Cancer* **1992**, *18*, 1−29.

(2) Kris-Etherton, P. M.; Hecker, K. D.; Bonanome, A.; Coval, S. M.; Binkoski, A. E.; Hilpert, K. F.; Griel, A. E.; Etherton, T. D. Bioactive compounds in foods: Their role in the prevention of cardiovascular disease and cancer. *Am. J. Med*. **2002**, *113*, 71S-88S.

(3) Parr, A. J.; Bolwell, G. P. Phenols in the plant and in man. The potential for possible nutritional enhancement of the diet by modifying the phenols content or profile. *J. Sci. Food Agric*. **2000**, *80*, 985−1012.

(4) Birt, D. B.; Hendrich, S.; Wang, W. Dietary agents in cancer prevention: Flavonoids and isoflavonoids. *Pharmacol. Therap*. **2001**, *90*, 157−177.

(5) Mullick, T.; Gasser, E. Nutrition in the chemoprevention of gastrointestinal cancer: Where are we in the new millennium. Nutrition issues in the gastroenterology 15. *Pract. Gastroenterol.* **2004**, 52−64.

(6) Yoshida, M.; Yamamoto, M.; Nikaido, T. Quercetin arrests human leukemic T-cells in late G1 phase of the cell cycle. *Cancer Res*. **1992**, *52*, 6676−6681.

(7) Wei, Y.; Zhao, X.; Kariya, Y.; Fukata, H.; Teshigawara, K.; Uchida, A. Induction of apoptosis by quercetin: Involvement of heat shock protein. *Cancer Res*. **1994**, *54*, 4952−4957.

(8) Fosslien, E. Molecular pathology of cyclooxygenase-2 in neoplasia. *Ann. Clin. Lab. Sci.* **2000**, *30*, 3-21.

(9) Meyer, A. S.; Donovan, J. L.; Pearson, D. A.; Waterhouse, A. L.; Frankel, E. N. Fruit hydroxycinnamic acids inhibit human low-density lipoprotein oxidation in vitro. *J. Agric. Food Chem*. **1998**, *46*, 1783−1787.

(10) Kawabata, K.; Yamamoto, T.; Hara A.; Shimizu, M.; Yamada, Y.; Matsunaga, K.; Tanaka, T., Mori, H. Modifying effects of ferulic acid on azoxymethane-induced colon carcinogenisis in F344 rats. *Cancer Lett.* **2000**, *157*, 15−21.

(11) Wang H.; Cao, G.; Prior, R. L. Total antioxidant capacity of fruits. *J. Agric. Food Chem*. **1996**, *44*, 701−705.

(12) Wang, S. Y.; Lin, H.-S. Compost as a soil supplement increases the level of antioxidant compounds and oxygen radical absorbance capacity in strawberries. *J. Agric. Food Chem.* **2003**, *51*, 6844−6850.

(13) Maas J. L.; Galletta, G. J.; Stoner, G. D. Ellagic acid, an anticarcinogen in fruits, especially in strawberries: A review. *Hort. Sci.* **1991**, *26*, 10−14.

(14) Clifford, M. N.; Scalbert, A. Ellagitannins—Nature, occurrence and dietary burden. *J. Sci. Food Agric.* **2000**, *80*, 1118−1125.

(15) Conney, A. H. Enzyme induction and dietary chemicals as approaches to cancer chemoprevention: The Seventh DeWitt S. Goodman Lecture. *Cancer Res.* **2003**, *63*, 7005−7031.

(16) Häkkinen, S. H.; Törrönen, A. R. Content of flavonols and selected phenolic acids in strawberries and Vaccinium species: Influence of cultivar, cultivation site and technique. *Food Res. Int.* **2000**, *33*, 517−524.

(17) 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.

(18) Olsson, M. E.; Gustavsson, K.-G.; Nilsson, J.; Ekvall, J.; Pillaua, D.; Sjöholm, I.; Svensson, U.; Åkesson, B.; Nyman, M. Antioxidants, low molecular weight carbohydrates and total antioxidative capacity in strawberries (*Fragaria* × *ananassa*)—effects of cultivar, ripening, and storage. *J. Agric. Food Chem*. **2004**, *52*, 2490−2498.

(19) Sun, J.; Chu, Y.-F.; Wu, X.; Liu, R. H. Antioxidant and antiproliferative activities of common fruits. *J. Agric. Food Chem*. **2002**, *50*, 7449−7454.

(20) Liu, M.; Li, X. Q.; Weber, C.; Lee, C. Y.; Brown, J.; Liu, R. H. Antioxidant and antiproliferative activities of raspberries. *J. Agric. Food Chem.* **2002**, *50*, 2926−2930.

(21) Magnusson, M. K.; Arvola, A.; Hursti, U. K. K.; Åberg, L.; Sjöden, P.-O. Attitudes towards organic foods among Swedish consumers. *Br. Food J*. **2001**, *103*, 209−226.

(22) Zanoli, R.; Naspetti, S. Consumer motivations in the purchase of organic food: A means−end approach. *Br. Food J.* **2002**, *104*, 643−653.

(23) Woese, K.; Lange, D.; Boess, C.; Bögl, K. W. A comparison of organically and conventionally grown foods—Results of a review of the relevant literature. *J. Sci. Food Agric*. **1997**, *74*, 281−293.

(24) Magos, F.; Arvaniti, F.; Zampelas, A. Organic food: Nutritious food or food for thought? A review of the evidence. *Int. J. Food Sci. Nutr*. **2003**, *54*, 357−371.

(25) Asami, D. K.; Hong, Y.-J.; Barrett, D. M.; Michell, A. E. Comparison of the total phenolic and ascorbic acid content of freeze-dried and air-dried marionberry, strawberry, and corn grown using conventional, organic, and sustainable agricultural practices *J. Agric. Food Chem.* **2003**, *51*, 1237−1241.

(26) Lombardi-Boccia, G.; Lucarni, M.; Lanzi S.; Aguzzi, A.; Cappelloni, M. Nutrients and antioxidant molecules in yellow plums (*Prunus domestica* L.) from conventional and organic productions: A comparative study. *J. Agric. Food Chem*. **2004**, *52*, 90−94.

(27) Mikkonen, T. P.; Määttä, K. R.; Hukkanan, A. T.; Kokko, H. I.; Törrönen, A. R.; Kärenlampi, S. O.; Karjalainen, R. O. Flavonol content varies among black currant cultivars. *J. Agric. Food Chem*. **2001**, *49*, 3274−3277.

(28) Noctor, G. Ascorbate and glutathione: Keeping active oxygen under control. *Annu. Rev. Plant Physiol. Plant Mol. Biol*. **1998**, *49*, 249−279.

(29) Mittler, R. Oxidative stress, antioxidants and stress tolerance. *Trends Plant Sci*. **2002**, *7*, 405−410.

(30) Olsson, M. E.; Gustavsson, K.-E.; Andersson, S.; Nilsson, Å.; Duan, R.-D. Inhibition of cancer cell proliferation in vitro by fruit and berry extracts and correlations with antioxidant levels. *J. Agric. Food Chem*. **2004**, *52*, 7264−7271.

(31) Liu, J.-J.; Nilsson, Å; Oredsson, S.; Badmaev, V.; Zhao, W.-Z.; Duan, R.-D. Boswellic acids trigger apoptosis via a pathway dependent on caspase-8 activation but independent of Fas/Fas ligand interaction in colon cancer HT-29 cells. *Carcinogenesis* **2002**, *23*, 2087−2093.

(32) Wimalasiri, P.; Wills, R. B. H. Simultaneous analysis of ascorbic acid and dehydroascorbic acid in fruit and vegetables by high-performance liquid chromatography. *J. Chromatogr. A* **1983**, *256*, 368−371.
(33) Esteve, M. J.; Farré, R.; Frigola, A.; Garcia-Cantabella, J. M. Determination of ascorbic and dehydroascorbic acids in blood plasma and serum by liquid chromatography. *J. Chromatogr. A* **1997**, *688*, 345−349.
(34) Madhavi, D. L.; Bomser J.; Smith, M. A. L.; Singletary, K. Isolation of bioactive constituents from *Vaccinium myrtillus* (bilberry) fruits and cell cultures *J. Plant Sci*. **1998**, *131*, 95−103.
(35) Schieber, A.; Keller, P.; Carle, R. Determination of phenolics of apple and pear by high-performance liquid chromatography. *J. Chromatogr. A* **2001**, *910*, 265−273.
(36) Häkkinen, S. H.; Kärenlampi, S. O.; Heinonen, I. M.; Mykkänen, H. M.; Törrönen, A. R. HPLC method for screening of flavonoids and phenolic acids in berries. *J. Sci. Food Agric*. **1998**, *77*, 543−551.
(37) Steinmetz, K. A.; Potter, J. D. Vegetables, fruit and cancer prevention: A review. *J. Am. Diet. Assoc*. **1996**, *96*, 1027−1039.
(38) Clifford, M. N.; Scalbert, A. Ellagitannins—Nature, occurrence and dietary burden. *J. Sci. Food Agric*. **2000**, *80*, 1118−1125.
(39) Mozafar, A. *Plant Vitamins: Agronomic, Physiological, and Nutritional Aspects*; CRC Press: Boca Raton, FL, 1994.
(40) Marschner, H. *Mineral Nutrition of Higher Plants*, 2nd ed.; Academic Press: London, 1995.
(41) Kobori, M. *In vitro* screening for cancer-suppressive effect of food components. *JARQ* **2003**, *37*, 159−165.
(42) Losso, J. N.; Bansode, R. R.; Trappey, A. 2nd, Bawadi, H. A.; Truax, R. *In vitro* anti-proliferative activities of ellagic acid. *J. Nutr. Biochem*. **2004**, *15*, 672−678.
(43) Block, G. Epidemiological evidence regarding vitamin C and cancer. *Am. J. Clin. Nutr*. **1991**, *54*, 1310S−1314S.
(44) Byers, T.; Guerrero, N. Epidemiological evidence for vitamin C and vitamin E in cancer prevention. *Am. J. Clin. Nutr*. **1995**, *62*, 1385S−1392S.
(45) Block, G.; Norkus, E.; Hudes, M.; Mandel, S.; Helzlsouer, K. Which plasma antioxidants are mostly related to fruit and vegetable consumption? *Am. J. Epidemiol*. **2001**, *154*, 1113−1118.
(46) Podmore, I. D.; Griffiths, H. R.; Herbert, K. E.; Mistry, N.; Mistry, P.; Lunec, J. Vitamin C exhibits pro-oxidant properties. *Nature* **1998**, *392*, 559.
(47) Griffiths, H. R.; Lunec, J. Ascorbic acid in the 21st century—More than a simple antioxidant. *Environ. Toxicol. Pharmacol*. **2001**, *10*, 173−182.
(48) Halliwell, B. Vitamin C: Poison, prophylactic or pancrea? *Trends Biochem. Sci*. **1999**, *24*, 255−259.
(49) Osmak, M.; Kovak, I.; Ljubenkov, I.; Spaventi, R.; Eckert-Maksic, M. Ascorbic acid and 6-deoxy-6-chloro-ascorbic acid: potential anticancer drugs. *Neoplasma* **1997**, *44*, 101−107.
(50) Kageyama, K.; Yamada, R.; Otani, S.; Hasuma, T.; Yoshimata, T.; Seto, C.; Takada, Y.; Yamaguchi, Y.; Kogawa, H.; Miwa, N. Abnormal cell morphology and cytotoxic effect are induced by 6-0-palmitoyl-ascorbate 2-0-phosphate, but not by ascorbic acid or hyperthermia alone. *Anticancer Res*. **1999**, *19*, 4321−4326.

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