

## Photoprotective Potential of Strawberry (*Fragaria × ananassa*) Extract against UV-A Irradiation Damage on Human Fibroblasts

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**ABSTRACT:** Exposure to UV-A radiation is known to induce discrete lesions in DNA and the generation of free radicals that lead to a wide array of skin diseases. Strawberry (*Fragaria × ananassa*) contains several polyphenols with strong antioxidant and anti-inflammatory activities. Because the major representative components of strawberry are anthocyanins, these may significantly contribute to its properties. To test this hypothesis, methanolic extracts from the Sveva cultivar were analyzed for anthocyanin content and for their ability to protect human dermal fibroblasts against UV-A radiation, as assayed using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide and Comet assays. Five anthocyanin pigments were identified using high-performance liquid chromatography–diode array detection–electrospray ionization/mass spectrometry. Moreover, the strawberry extract showed a photoprotective activity in fibroblasts exposed to UV-A radiation, increasing cellular viability, and diminishing DNA damage, as compared to control cells. Overall, our data show that strawberry contains compounds that confer photoprotective activity in human cell lines and may protect skin against the adverse effects of UV-A radiation.

**KEYWORDS:** *strawberry, anthocyanin, antioxidants, DNA damage, fibroblast*

### ■ INTRODUCTION

The exposure of skin to environmental insults such as smoke, microorganisms, or UV radiation can induce biological responses, including the development of hyperplasia, erythema, photoaging, and skin cancer.<sup>1–3</sup> In particular, UV-A radiation is able to penetrate through the dermis to subcutaneous tissue and may affect both epidermal and dermal skin components.<sup>4</sup> At the cellular level, this component of the solar UV spectrum may cause oxidative damage through the generation of reactive oxygen species (ROS), which can interact with cellular biomolecules such as nucleic acids, proteins, fatty acids, and saccharides,<sup>5</sup> altering the redox status of the intracellular milieu.

To mitigate such damage, the skin possesses extremely efficient defense mechanisms, including antioxidant enzymes (superoxide dismutase, catalase, and glutathione peroxidase) and nonenzymatic antioxidant molecules (vitamin C, vitamin E, glutathione, and ubiquinone). However, because of constant environmental exposure to physical and chemical agents, an oxidant/antioxidant imbalance results that may have a profound effect on well-being. Topical or systemic treatment of skin with products containing antioxidant components could be a useful strategy for the prevention of UV-mediated cutaneous damages.<sup>6</sup> Many studies have shown the efficacy of naturally occurring botanical antioxidants such as green tea polyphenols, silymarin, curcumin, apigenin, and resveratrol against UV radiation-induced inflammation and cancer.<sup>7–10</sup> Similarly, it has

been reported that topical application of plant-derived chemicals, such as caffeine or (–)-epigallocatechin gallate, inhibits carcinogenesis and selectively increases apoptosis in UV-B-treated mouse skin.<sup>11</sup>

Strawberry (*Fragaria × ananassa* Duch.) is an important dietary source of bioactive compounds, most of which are natural antioxidants that contribute to the high nutritional quality of the fruit. Its remarkable value has been correlated to the high content of vitamin C, folate, and, more recently, to the high levels and variety of phenolic constituents, especially anthocyanins, that are the quantitatively most important in strawberry.<sup>12–14</sup> More than 25 different anthocyanin pigments have been described in strawberry fruits from different varieties.<sup>15</sup> The major representative compounds show pelargonidin (Pg) as aglycone, although also some cyanidin (Cy) derivatives are generally observed. The presence of the two main anthocyanins (i.e., Pg-3-glc and in smaller proportion Cy-3-glc) seems constant in all varieties, but a qualitative and quantitative variability among cultivars is generally observed. They possess strong antiinflammatory, antioxidant, antimutagenic, anticarcinogenic, and photoprotective properties and are

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able to modulate enzymatic pathways;<sup>9,16</sup> thus, they play a role in preventing human diseases related to oxidative stress.<sup>7,17</sup> The aims of this study were to characterize the strawberry anthocyanin content and to evaluate the possible protective effect of strawberry extracts on UV-A-induced skin damage using human dermal fibroblasts.

## MATERIALS AND METHODS

**Strawberry Materials and Chemicals.** The strawberry cultivar Sveva was selected for the study. Ripe fruits were harvested from plants grown in an open experimental field for strawberry breeding and germplasm collection at the Azienda Agraria Didattico Sperimentale "P. Rosati" in Agugliano (Ancona, Italy). Within 2 h after harvest, whole fruits were stored at  $-20\text{ }^{\circ}\text{C}$  prior to analysis.

2,4,6-Tripyridyl-s-triazine (TPTZ) was purchased from Fluka Chemie (Buchs, Switzerland), and sodium chloride (NaCl) and agarose were from Fisher Scientific, while all other reagents and solvents were from Sigma-Aldrich Chemical Co. (Milan, Italy). Minisart filters were obtained from PBI International, while AQUA C 18 150 mm  $\times$  4.6 mm columns were obtained from Phenomenex (Torrance, CA). Essential modified Eagle's medium (EMEM) was purchased from Cambrex, as well as fetal bovine serum (FBS), glutamine, and antibiotics (100 IU/mL penicillin and 100  $\mu\text{g}/\text{mL}$  streptomycin).

**Sample Preparation and Anthocyanin Extraction.** Frozen strawberries were thawed for 60 min at  $4\text{ }^{\circ}\text{C}$ ; then, 10 g of the fruits was added to 100 mL (1:10 w/v) of extraction solution consisting of an 80% methanol solution acidified with 0.1% formic acid. Fruits were homogenized using an Ultraturax T25 homogenizer (Janke & Kunkel, IKA Labortechnik) at 12000 rpm for 2 min, and the extraction was maximized by stirring the suspension for 2 h at  $4\text{ }^{\circ}\text{C}$  in the dark. The tubes were then centrifuged at 1200g for 15 min (twice sequentially) to sediment solids, and supernatants were filtered through a 0.45  $\mu\text{m}$  Minisart filter (PBI International), transferred to 5 mL amber glass vials, and then stored at  $-20\text{ }^{\circ}\text{C}$  prior to analysis.

Anthocyanin extraction for high-performance liquid chromatography–mass spectrometry (HPLC-MS) analysis was performed as previously described.<sup>15</sup> Frozen strawberries (50 g) were homogenized in methanol containing 0.1% HCl, kept overnight ( $\sim 14\text{ h}$ ) at  $3\text{--}5\text{ }^{\circ}\text{C}$ , and then filtered through a funnel under vacuum. The solid residue was exhaustively washed with methanol (4–6 times). The filtrates obtained were centrifuged (4000g, 15 min,  $21\text{ }^{\circ}\text{C}$ ), and the solid residue was further subjected to the same process repeated until there was complete extraction of color. The aqueous extract obtained was washed with *n*-hexane to remove liposoluble substances, and then, 2 mL of the aqueous phase was carefully deposited onto a C-18 SepPaks Vac 6  $\text{cm}^3$  cartridge (Waters). Sugars and more polar substances were removed by rinsing with 15 mL of ultrapure water and anthocyanin pigments eluted with 5 mL of methanol:0.1% trifluoroacetic acid (95:5). The methanolic extract was concentrated under vacuum in a rotary evaporator at  $<30\text{ }^{\circ}\text{C}$ . Water was added to the extract to 2 mL and filtered through a 0.45 mm membrane filter (PBI international) prior to HPLC analysis.

**HPLC–Diode Array Detection (DAD)/Electrospray Ionization (ESI)-MS Analysis of the Anthocyanin Composition of the Extract.** Analyses were performed in a Hewlett-Packard 1100 series liquid chromatography. Separation was achieved on a 5  $\mu\text{m}$  AQUA C 18 150 mm  $\times$  4.6 mm column (Phenomenex, Torrance, CA) thermostatted at  $35\text{ }^{\circ}\text{C}$ . Solvents used were (A) 0.1% trifluoroacetic acid in water and (B) HPLC-grade acetonitrile, establishing the following gradient: isocratic 10% B for 5 min, 10–15% B over 15 min, isocratic 15% B for 5 min, 15–18% B over 5 min, and 18–35% B over 20 min, using a flow rate of  $0.5\text{ mL min}^{-1}$ . Double online detection was carried out in a diode array spectrophotometer (DAS), using 520 nm as the selected wavelength, and a mass spectrometer (MS) connected to the HPLC system via the DAS cell outlet.

The mass spectrometer was a API 3200 Qtrap (Applied Biosystems, Darmstadt, Germany) equipped with an ESI source and a triple quadrupole-ion trap mass analyzer that was controlled by the Analyst

5.1 software. Zero grade air served as the nebulizer gas (50 psi) and turbo gas for solvent drying ( $600\text{ }^{\circ}\text{C}$ , 40 psi). Nitrogen served as the curtain (10 psi) and collision gas (medium). The quadrupoles were set at unit resolution. The ion spray voltage was set at 5000 V in the positive mode. Enhanced MS (EMS) was employed to detect all ions. Settings used were as follows: declustering potential (DP), 41 V; entrance potential (EP), 7.5 V; and collision energy (CE), 10 V. Enhanced product ion (EPI) mode was further performed to obtain the fragmentation pattern of the parent ion(s) in the previous experiment using the following parameters: DP, 41 V; EP, 7.5 V; CE, 25 V; and collision energy spread (CES), 0 V.

Anthocyanins were quantified from the areas of chromatographic peaks recorded at 520 nm compared to calibration curves obtained with external standards of Cy-3-glucoside (for Cy-based anthocyanins) and of Pg 3-glucoside (for Pg-based anthocyanins). Strawberry extracts were analyzed in triplicate.

**Culture of Human Dermal Cell (HuDe) Line and Cell Treatment.** Primary cell cultures of HuDe were purchased from the Central Laboratory of Istituto Zooprofilattico Sperimentale (Brescia, Italy). Fibroblasts were cultured in 25  $\text{cm}^2$  flasks in EMEM (Cambrex) at  $37\text{ }^{\circ}\text{C}$  in a humidified atmosphere containing 5%  $\text{CO}_2$ . Cells were seeded at a density of  $0.5 \times 10^4$  cell/ $\text{cm}^2$ , and the medium was changed every 2–3 days. At confluence, fibroblasts were dissociated by trypsinization with a solution containing 0.5 mg/mL trypsin and 0.2 mg/mL EDTA and then placed in 96-well plates 24 h prior to the experiment at a density of  $2 \times 10^4$  cell/mL. Cells adhered to the 6 mm diameter well and reached over 75% confluence after 24 h.

To evaluate the potential cytotoxicity of the strawberry extracts, cells were incubated with different concentrations of strawberry extract, and cell viability was determined using the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay.<sup>18</sup> The MTT assay is based on the reduction of a tetrazolium salt, (MTT) by intracellular dehydrogenases of viable living cells, leading to the formation of purple formazan crystals. According to the results obtained by this test, strawberry extracts were serially diluted in EMEM to achieve three different final concentrations (0.05, 0.25, and 0.50 mg/mL of extract). The methanol concentration was always below 1% of the final volume. Treated cells were incubated for another 24 h with the three distinct concentrations of Sveva extracts, while the same quantity of methanol administered to the treated cells was added to control cells. After incubation with strawberry extracts, cells were exposed to UV-A light (see below).

**Treatment with UV-A Light.** Control and pretreated cells were washed twice with phosphate-buffered saline (PBS) and covered with a thin layer of PBS prior to exposure. The 96-well plate was then covered with the 2 mm thick quartz slab onto which a dark cardboard sheet was attached. The cells were then placed on a brass block embedded on ice to reduce the temperature and hence the evaporation during exposure, which could eventually dry out the medium. The UV-A irradiating source was a Philips Original Home Solarium (model HB 406/A; Philips, Groningen, Holland) commercial sun lamp equipped with a 400 w ozone-free Philips HPA lamp, UV type 3. The source delivered 23  $\text{mW}/\text{cm}^2$  between 300 and 400 nm at a distance of 20 cm from the cell cultures. It was always prerun for 10 min to allow the output to stabilize. The incident dose of UV-A received by the samples was 275  $\text{kJ}/\text{m}^2$ , that is, the dose approximately equivalent to about 90 min of sunshine at the French Riviera (Nice) in summer at noon<sup>19</sup> and measured with a UV Power Pack Radiometer (EIT Inc., Sterling, VA). The emission spectrum of the lamp was also verified using a StellarNet portable spectroradiometer (Tampa, FL).

Cells were exposed to the UV-A source for a maximum exposure time of 30 min because longer exposure times showed a significant loss of vitality and DNA damage (this time was determined in previous experiments, not shown).

### Evaluation of Protection Against UV-A-Induced Damage.

**Cell Viability.** After UV-A exposure, control and pretreated cells were washed twice with PBS and incubated with a salt solution of MTT at a concentration of 0.5 mg/mL for 2 h at  $37\text{ }^{\circ}\text{C}$ . Then, the medium was removed, and the crystals were dissolved in DMSO. The optical density of the suspension

**Table 1.** Retention Times ( $R_t$ ), Wavelengths of Maximum Absorption in the Visible Region ( $\lambda_{\max}$ ), Mass Spectral Data, Tentative Identification, and Quantification of Anthocyanin Pigments in Strawberry Sveva

peaks	$R_t$ (min)	$\lambda_{\max}$ (min)	(m/z)		tentative identification	quantification (mg/kg)
			molecular ion [ $M^+$ ]	$MS^2$		
1	23.2	515	449	287	Cy-3-glucoside	16.35
2	27.3	502	433	271	Pg-3-glucoside	611.18
3	29.3	503	579	433, 271	Pg-3-rutinoside	1.86
4	37.7	504	519	271	Pg-3-malonylglucoside	2.85
5	40.0	504	475	271	Pg-3-acetylglucoside	7.55
total content						639.79

was read at 550 nm using a microplate reader (Synergy HT, Biotek, Winooski, VT).<sup>18</sup> Cell viability was expressed as a percentage of live cells as compared to the control. The data reported represent average values from at least three independent experiments.

**Evaluation of DNA Damage by the Comet Assay.** The comet assay was performed as described by Singh et al.<sup>20</sup> with modifications by Tice et al.<sup>21</sup> The comet assay is a sensitive technique that allows visualization of DNA damage, essentially by electrophoresing the DNA from single cells from immobilized nuclei. Smaller fragments migrate faster, so highly damaged cells will produce "comets" that may be measured with ethidium bromide staining and fluorescent microscopy. Briefly, control and pretreated cells cultured in 12-well plates were washed twice with PBS and exposed to UV-A in 2 mL of PBS as described above for 12 min. PBS was then removed, cells were detached by trypsinization, EMEM was added, and cells were counted in a Kova Glasstic Slide 10 with grid chamber. Aliquots containing ~50 000 cells from each sample were transferred to Eppendorf tubes and centrifuged for 15 min at 1200 rpm at 4 °C. The supernatant was removed, and cells were resuspended in 110  $\mu$ L of 1% low melting agarose. Fifty microliters was then rapidly placed on a 1% agarose precoated microscope slide and immediately spread by covering with a coverslip. The microgel on the slides was then allowed to solidify at 4 °C. The cover glass was gently removed, and the slides were immersed in ice-cold, freshly prepared lysis solution (2.5 M NaCl, 100 mM Na<sub>2</sub>EDTA, 10 mM Tris-HCl, 1% Triton X-100, and 10% DMSO, adjusted to pH 10) for 15 h, at 4 °C in the dark. The nuclei of lysed cells were allowed to unwind in freshly prepared alkaline buffer (1 mM Na<sub>2</sub>EDTA, pH 13) for 20 min in a horizontal electrophoresis box and electrophoresed for 20 min at 1 V/cm. After electrophoresis, the slides were washed gently with 0.4 M Tris-HCl buffer (pH 7.5) to neutralize the excess alkali and to remove detergents. Slides were then dehydrated in 75% methanol for 5 min and dried at 60 °C for 15 min. They were then stained by adding 100  $\mu$ L of ethidium bromide and analyzed in fluorescence microscopy. For each sample, 50 comets on two different slides (i.e., 100 comets/sample) were evaluated. The slides were examined under a 20 $\times$  objective on a fluorescence microscope (Nikon Eclipse E600) using FITC filters (490 nm excitation/520 nm emission). The images of comets were analyzed using a CCD camera connected to HP computer. Computer image analysis was conducted with software developed in our laboratories where we have the advantage of acquiring images in semiautomated mode. DNA damage was quantified by determining the fluorescence intensity in the tail (% tail intensity). One hundred fifty randomly selected cells per slide were scored for each sample.

**Statistical Analysis.** All results are expressed as means  $\pm$  standard errors of the mean (SEMs). Statistical analysis was done using the one-way analysis of variance and Tukey's posthoc test;  $p \leq 0.05$  was considered significant.

## RESULTS AND DISCUSSION

**Anthocyanin Composition Determined by HPLC-DAD-ESI-MS/MS.** Anthocyanins in strawberry are the best known polyphenolic compounds and quantitatively the most important.<sup>13</sup> In the present work, five anthocyanin pigments were detected according to their UV-vis or mass spectral

characteristics. The HPLC anthocyanin profile of Sveva cultivar, obtained in positive mode analyses (retention time in the HPLC system,  $\lambda_{\max}$  in the visible region, molecular ion, main fragments observed in  $MS^2$ , and total content) is summarized in Table 1. Most of the compounds identified are derived from Pg. In addition to the compounds indicated in the table, other very minor pigments were also detected, although no good absorption or mass spectra could be obtained to allow speculation about their identity.

Pg shows a characteristic UV-vis spectrum shape with  $\lambda_{\max}$  in the visible region at lower wavelengths (about 500 nm) than other common anthocyanins and an additional maximum about at 430.<sup>15,22,23</sup> On the basis of this spectral profile, the peaks corresponding to Pg-derived anthocyanins could be easily assigned in the chromatograms. The presence of Pg as anthocyanin in those peaks was further confirmed by their mass spectra, which showed  $MS^2$  signal at  $m/z$  [ $M + H$ ]<sup>+</sup> 271. Up to four peaks could be assigned as Pg derivatives. Major peaks in HPLC chromatogram corresponded to Pg 3-gluc and Pg 3-malonylglucoside. Pg 3-gluc was first identified by Robinson and Robinson,<sup>24</sup> and since then, it has been the most known and reported anthocyanin in strawberry.<sup>15,22,23</sup> Moreover, another compound was relatively important in the sample. Its UV-vis ( $\lambda_{\max}$  at 504 nm) and mass spectra molecular ion at  $m/z$  519 (releasing a unique  $MS^2$  fragment at  $m/z$  271 [ $M + H$ ]<sup>+</sup> corresponding to Pg) allowed possible identity as Pg 3-malonylglucoside as previously reported in several strawberry varieties.<sup>15,22,23</sup> Pg 3-acetylglucoside has been also identified in the extract and showed a molecular ion [ $M + H$ ]<sup>+</sup> at  $m/z$  475, releasing a major  $MS^2$  fragment at  $m/z$  271, as previously identified.<sup>22</sup> Finally, only one peak was assigned to Cy derivatives. This compound showed a UV-vis spectrum characteristic of a Cy derivatives with a positive molecular ion at  $m/z$  449 [ $M + H$ ]<sup>+</sup>, releasing a  $MS^2$  fragment at  $m/z$  287 [ $M + H$ ]<sup>+</sup>. The loss of -162 amu is interpreted as corresponding to one hexose residue, which allowed its identification as Cy-3-glucoside as reported by several authors.<sup>15,22,25</sup> Moreover, the individual concentrations of the anthocyanin identified in the extract are shown in Table 1 with a total concentration of anthocyanin in samples of approximately 639.79 mg/kg FW.

**Cell Protection against UV-A Damage.** To analyze the photoprotective activity of strawberry extracts, we used primary cultures of fibroblasts from HuDe, in which an in vitro model of oxidative damage has been recreated. In particular, cells were exposed to ultraviolet radiation, specifically to UV-A. Several findings have demonstrated that exposure of skin to UV radiation results in various harmful biological effects that play important roles in the generation and maintenance of UV-induced skin damage.<sup>1-3</sup> One approach to protect skin against the dangerous effects of UV could be the use of phytochemicals

with antioxidant properties. In recent years, natural compounds such as vitamins and phenols have gained considerable attention as protective agents, which could be added in preparations for topical applications. Fruits and vegetables are rich in diverse nutrients, which can act as chemoprotectants. These structurally different components possess complementary and overlapping potential protective actions, including antioxidant and anti-inflammatory properties, as well as enhanced activity and expression of detoxification enzymes and strengthened immune system health.<sup>26</sup>

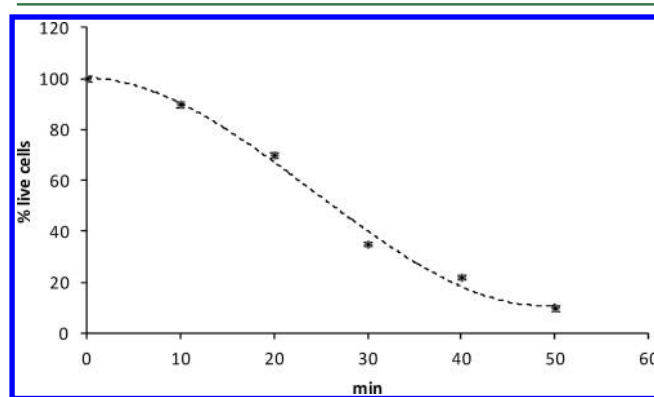
Various components of plant and fruit have been investigated for their potential protective capacity. Strawberry has attracted significant attention in recent years on micronutrient and beneficial phytochemicals contents. The major class of phenolic compounds in strawberry is represented by flavonoids (mainly anthocyanins, with flavonols and flavanols giving a minor contribution), followed by hydrolyzable tannins (ellagitannins and gallotannins) as the second most abundant class, and phenolic acids (hydroxybenzoic acids and hydroxycinnamic acids) together with condensed tannins (proanthocyanidins), being the minor constituents. Ellagitannins (ETs) are together with anthocyanins the most abundant phenolic compounds in strawberry.<sup>13</sup> They are different combinations of hexahydroxydiphenic acid with glucose, with a wide range of structures such as monomers (i.e., ellagic acid glycosides), oligomers (i.e., sanguin H-6, the most typical ET in strawberry), and complex polymers. Together with gallotannins, they are also called hydrolyzable tannins and, upon hydrolysis, release ellagic acid, although other metabolites can also be produced and are distinctive of individual ETs (i.e., gallic acid). The ETs content and composition in foodstuffs has been characterized only recently so that more studies are desirable in this field because of their important impact on human health. For example, ellagic acid is the phenolic component primarily associated with the chemopreventive effects, appearing to function as an anticarcinogen at the initiation and postinitiation stages of tumor development in *in vitro* and *in vivo* experiments.<sup>27</sup> Interestingly, in the past decade, the antitumorogenic properties of the distinct classes of phenolic components have been also demonstrated. In particular, the anthocyanin-rich<sup>25,28–31</sup> and tannin-rich fractions of different berry extracts, the latter mainly including ellagitannins<sup>32</sup> or proanthocyanidins<sup>33,34</sup> depending on the berry species, appear to contain key anticarcinogenic components of berries against multiple human cancer cell types *in vitro* and in *in vivo* animal model tumor systems.

Although their presence is minor in strawberry from a quantitative point of view, a relevant interest is focused on flavonols. They are found in strawberry in both monomeric (catechins) and polymeric form called condensed tannins or procyanidins, especially in flesh and achenes.<sup>12</sup> They have been reported to directly and indirectly possess antioxidant, antimicrobial, antiallergy, antihypertensive, and inhibition of the activities of some physiological enzyme and receptors.<sup>35</sup>

Finally, strawberries are important fruits also because of their extremely high content of vitamin C, even higher than citrus fruit, which makes them an important source of this vitamin for human nutrition. Vitamin C is an essential compound, with several biological functions in humans. In fact, the results of numerous prospective, observational as well as case-control studies, indicate that higher intakes of vitamin C from diet, from diet plus supplements, or from supplements use itself are often correlated with a lower incidence of cardio and

cerebrovascular diseases,<sup>36</sup> most types of cancers,<sup>37</sup> and other health problems such as lead toxicity. In addition to disease prevention, vitamin C supplementation seems to be a beneficial adjunct to conventional therapies for individuals with atherosclerosis,<sup>38</sup> hypertension, diabetes mellitus, and several types of cancers.<sup>39</sup>

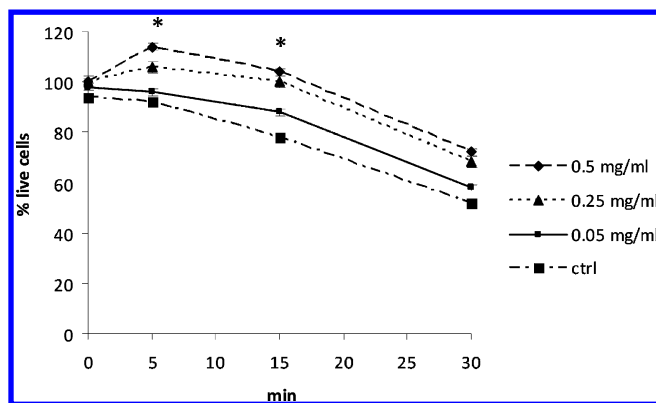
In the present work, we studied the potential photoprotective capacity of strawberry cultivar Sveva extract against UV-A damage on human fibroblasts. First, the possible toxic effect of the extracts in relation to their increasing concentration and exposure time was studied. Cell vitality did not vary with increases in strawberry extract concentration or the exposure time, thereby demonstrating that the extracts were not cytotoxic under the experimental conditions (data not shown). Next, control cells were subjected to various fluences of UV-A and immediately analyzed by MTT. For times more than 30 min, the treatment caused a significant loss of viability (Figure 1). Using this range of fluences, control cells and cells



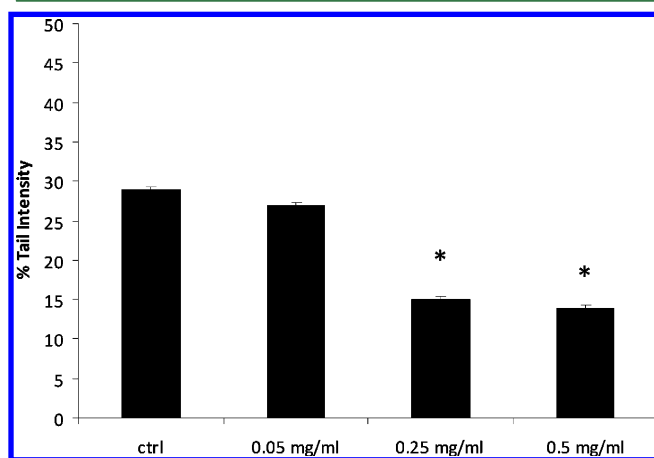
**Figure 1.** Viability of HuDe fibroblasts after exposure to UV-A as determined by MTT assay. Data are expressed as the percentage of live cells as compared to unexposed controls. Fifty percent of viability is reached after 30 min of exposure. Means and SEMs for eight replicates are reported.

pretreated with different concentrations of strawberry extract (0.05, 0.25, and 0.5 mg/mL) were exposed and analyzed with MTT to determine the possible photoprotective activity of Sveva extracts and if it was dose-dependent. Cells preincubated with the lower concentration of the extract exhibited vitality similar to the control, while the cells preincubated with higher concentrations of the extract showed higher survivability. In particular, cells treated with 0.5 mg/mL exhibited a significant difference in vitality ( $p < 0.05$ ), especially for exposure between 5 and 15 min (Figure 2). However, at higher fluence rates, the differences were not significant. This outcome is likely due to the depletion of the protective capacity of strawberry extracts after high UV treatments. Moreover, to assess whether Sveva extract could impart DNA protection, control cells and cells preincubated with strawberry were exposed to UV-A for 15 min and processed according the Comet Assay protocol.<sup>20,21</sup> Consistent with the vitality assay, DNA damage was observed in control cells and cells preincubated with the lower concentrations of the extract (0.05 mg/mL). Conversely, cells pretreated with 0.25 and 0.5 mg/mL of extracts showed a highly significant decrease ( $p < 0.05$ ) in DNA damage in comparison with the control (Figure 3).

Our results indicate that strawberry extracts provide photoprotective effects to dermal cells *in vitro*. It is exciting to speculate that the same compounds may be helpful in



**Figure 2.** Viability of HuDe fibroblast after exposure to UV-A determined by MTT assay. Control cells and cells preincubated with strawberry extracts were exposed to UV-A and analyzed to determine the percentage of viability. Cells preincubated with 0.5 mg/mL extract showed a significant increase in viability after exposure between 5 and 15 min than the controls. Means and SEMs for eight replicates are reported. \* $P < 0.05$  significantly different from control.



**Figure 3.** Effect of UV on DNA damage in HuDe fibroblasts as determined by Comet assays. Cells incubated with 0.25 and 0.5 mg/mL extract showed a significant decrease in DNA damage than the controls. Means and SEMs for eight replicates are reported. \* $P < 0.05$  significantly different from control.

protecting HuDe against UV radiation in general. This photoprotective potential may be based on the high levels of anthocyanins present in fruit. Recently, Bae et al.<sup>40</sup> found that bog blueberry (*Vaccinium uliginosum* L.) anthocyanins were able to prevent UV-B-induced skin photoaging by blocking collagen destruction and inflammatory responses via transcriptional mechanisms of NF- $\kappa$ B and MAPK signaling. Similarly, Cimino et al.<sup>6</sup> reported that Cy-3-glucoside (C3G) is potentially able to efficiently counteract UV-induced skin damage, very likely by blocking cellular oxidative stress-related events, in particular inflammation and apoptosis. In fact, C3G photoprotection is primarily associated with the down-regulation of NF- $\kappa$ B and AP-1 activation, inhibition of IL-8 overexpression, and prevention of pro-caspase-3 cleavage and DNA fragmentation. These findings are in agreement with reports indicating a good in vitro protective effect of anthocyanins against UV light-induced damage in human keratinocytes.<sup>41,42</sup> Furthermore, it has been suggested that the incorporation of C3G by the HaCaT cells reduces the damage elicited by oxidative stress following exposure to UV radiation.

Youdim and co-workers<sup>43</sup> reported that anthocyanins are incorporated into the membrane and cytosol of endothelial cells and significantly enhance their resistance to the damaging effects of several chemical oxidative stressors. Finally, Tarozzi and co-workers showed that C3G reduced DNA damage induced by H<sub>2</sub>O<sub>2</sub> and O<sub>2</sub> in HaCaT cells.<sup>41</sup>

This work provides a basis for more in-depth studies to examine the efficacy of strawberry fruit in the prevention of UV-induced stress. Future studies will examine the bioavailability of these molecules at dermal level when they are introduced through topical application.

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### Notes

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## ABBREVIATIONS USED

HuDe, human dermal cells; EMEM, essential modified Eagle medium; ROS, reactive oxygen species; Cy, cyanidin; Pg, pelargonidin; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide

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