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Etiology of UV-C-Induced Browning in Var. Superior White Table Grapes

ROCÍO GONZÁLEZ-BARRIO,[†] MARJATTA SALMENKALLIO-MARTTILA,[§] FRANCISCO A. TOMÁS-BARBERÁN,[†] EMMA CANTOS,[†,#] AND JUAN CARLOS ESPÍN*,[†]

Research Group on Quality, Safety and Bioactivity of Plant Foods, Department of Food Science and Technology, CEBAS-CSIC. P.O. Box 164, 30100 Campus de Espinardo, Murcia, Spain, and VTT Biotechnology, P.O. Box 1500, Espoo, FI-02044, Finland

White table grapes, var. Superior, were treated with UV-C light after harvest to increase stilbenes concentration, especially *trans*-resveratrol (RES), because this may be of relevance to the health-promoting properties assigned to these compounds. However, irradiated grapes also developed some browning on the surface on the third day of storage at 22 °C, with the subsequent detriment in the sensorial quality of the fruit. Possible causes for browning development during storage were investigated. The phenolic-related oxidative enzymes, polyphenol oxidase (PPO) and peroxidase (POD), were not specifically activated, and no new isoforms appeared upon UV-C treatment. UV-treated grapes had lower content of chlorophyll *b* than control grapes on the fourth day of storage, concomitant with the increase of pheophytins (chlorophyll degradation derived compounds). Microscopy data showed lower fluorescence emission in chloroplasts from the UV-treated samples, which may explain the decrease of chlorophylls content in the corresponding grape berries extracts. In addition, microscopy images showed cell wall thickening in the skin tissue of UV-treated grapes which could be considered as a general wound response in plant tissues. These results suggest that the development of browning in Superior white grapes after UV-C treatment is not closely related with the evolution of oxidative enzymes during storage and may be mainly due to the decrease of chlorophylls content.

KEYWORDS: Browning; chlorophyll; polyphenol; pheophytin; polyphenol oxidase; peroxidase; stilbenes; table grape; UV-C; cell wall; microstructure

INTRODUCTION

Consumption of grapes as part of the diet has been associated with multiple beneficial health effects that have been mainly attributed to the polyphenol content of this fruit (*1*). Among grape polyphenols, the stilbene resveratrol (RES) has been acknowledged to have multiple biological activities including cardioprotective and anticancer properties (*2*). However, RES concentration in table grapes is very low. Taking into account that stilbenes are phytoalexins (stress-inducible molecules), a number of processes have been reported to increase stilbene concentration in table grapes. Among these treatments, post-harvest UV-C irradiation has been proven to increase significantly stilbenes concentration (*3*, *4*). However, UV-C treatment must be performed under controlled conditions because long UV-C treatments can accelerate the ripening and senescence of some fruits (*5*, *6*) and may produce undesirable effects such as the loss of potentially health-beneficial molecules and the decrease of sensorial properties (*3*).

Many phenolic compounds are well-known substrates for oxidative enzymes such as polyphenol oxidase (PPO; EC 1.14.18.1) and peroxidase (POD; EC 1.11.1.7). PPO is a copper-containing enzyme that catalyzes in the presence of molecular oxygen the hydroxylation of monophenols (monophenolase activity) and the oxidation of *o*-diphenols (diphenolase activity) to *o*-quinones. The latter chemically evolve to give rise to brown pigments (melanins) responsible for the loss of fruit quality (*7*). POD is a heme-containing enzyme that performs single-electron oxidation of phenolic compounds in the presence of hydrogen peroxide (*8*) with special relevance in wound-healing processes such as lignification (*9*). Whereas PPO is considered to be the key enzyme in the melanogenesis pathway (*10*), the possible role of POD in melanin formation has been questioned due to the low hydrogen peroxide content of vegetable tissues. However, the generation of hydrogen peroxide in the oxidation of some phenolics catalyzed by PPO could indicate a possible synergistic action between both PPO and POD, which suggests the involvement of POD in browning processes (*11*).

* Author to whom correspondence should be addressed (fax +34-968-396213; e-mail jcespin@cebas.csic.es).
[†] CEBAS-CSIC.
[§] VTT Biotechnology.
[#] Present address: CIFA, Rancho de la Merced, P.O. Box 589, 11471 Jerez de la Frontera, Cádiz, Spain.

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In grapes, PPO is mainly located in the cytoplasm of skin cells (*12*), spatially separated from the phenolic substrates that are located in the vacuoles. When berries are damaged, PPO and the phenolic compounds may come into contact and oxidative browning may occur (*12*, *13*). POD is a multifunctional enzyme in grapevines, and apart from participating in lignification processes, it could be also involved in both melanin (*14*) and viniferins formation (*15*).

Nonenzymatic browning, including Maillard reactions, may also occur in fruits and can lead to the formation of brown melanoidin pigments (*16*). The involvement of chlorophyll degradation in browning promotion has also been suggested (*17*). The synthesis of chlorophylls is induced by light (*18*), although extended illumination may produce a decrease in the chlorophyll content (*19*). Chlorophylls may lose the Mg^{2+} cation, transforming themselves into brownish pigments called pheophytins (*20*), and may also contribute to browning in the case of grape berries (*21*).

Despite the applicability of postharvest UV-C irradiation treatments to increase resveratrol concentration in table grapes, the browning appearance is undesirable. The aim of the present study is to identify the etiology of the UV-C-induced browning in white table grapes after harvest.

MATERIALS AND METHODS

Reagents. RES, chlorogenic acid, catechin, chlorophylls *a* and *b* from spinach, ascorbic acid (AA), 3-methyl-2-benzothiazolinone hydrazone (MBTH), 2,2′-azinobis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS), hydrogen peroxide (H_2O_2) (30%, v/v), serum bovine catalase (EC 1.11.1.6), 3,4-dihydroxyphenylpropionic acid (DHPPA), catechol, tropolone, *N*,*N*′-dimethylformamide (DMF), sodium dodecyl sulfate (SDS), and Triton X-114 (TX-114) were purchased from Sigma-Aldrich (St. Louis, MO). Quercetin 3-rutinoside, formic acid, and methanol (MeOH) of analytical grade were supplied by Merck (Darmstadt, Germany). Milli-Q system (Millipore Corp., Bedford, MA) ultrapure water was used throughout this research.

Grapes. White table seedless grapes of the variety Superior were harvested in July 2004, in Murcia (Spain), transported to the laboratory, and processed the same day as they were at mature ripening stage and ready to be commercialized.

UV-C Irradiation Treatment. Grape berries were UV-treated as previously described (*3*, *4*). Briefly, grape berries were detached from the clusters and mixed to obtain a homogeneous and representative sample. Then, the berries were individually placed in plastic wells and irradiated with 17 (Sylvania, G30T8) germicidal lamps (total power = 510 W, peak output at 254 nm) for 1 min. The distance between lamps and berries was 40 cm. Afterward, grape berries were turned and the other side was also UV-C-irradiated under the same conditions (both sides were UV-C exposed). Irradiation experiments were performed in triplicate. Both irradiated and control (nontreated) grape berries were stored at 22 °C for 6 days in perforated plastic bags and at a relative humidity of 90−95% to avoid water loss. Phenolic content, activity of oxidative enzymes, and chlorophyll and pheophytin content were determined after storage for 6 days; microstructure studies were also made at the same time.

Extraction of Phenolic Compounds. Grapes were peeled with a sharp knife, and the skins were stored at −20 °C until analyzed. Skin represented ∼10.5% of the total fresh weight (fw) of the grape berry. Phenolics were extracted using a solution of MeOH/formic acid (97:3) per gram of grape skin as previously reported (*4*). Extracts were prepared in triplicate.

HPLC Analysis of Phenolics. Samples (20 μL) of the clear filtered extract were analyzed using a Merck-Hitachi HPLC system according to the protocol of Cantos et al. (*4*). Phenolic content was expressed as milligrams per kilogram of fresh weight of berries. Hydroxycinnamic acid derivatives, flavonols, flavan-3-ols, and RES were identified by their UV spectra and by chromatographic comparisons with authentic standards as described elsewhere (*4*, *22*). Hydroxycinnamic acids were quantified as chlorogenic acid, flavan-3-ols as catechin, flavonols as quercetin-3-rutinoside, and stilbenes as RES (*4*, *22*).

Enzyme Extraction. PPO was extracted using the method of Cantos et al. (*23*) with some modifications. Briefly, 10 g of fresh grape skin was homogenized in a blender in 20 mL of cold 50 mM sodium phosphate buffer (pH 7.0) containing 40 mM AA. The homogenate was centrifuged at 5000*g* for 10 min, and the pellet was redissolved in 10 mL of phosphate buffer 50 mM (pH 7.0) containing 3% TX-114 and kept in ice for 15 min, followed by incubation at 35 °C for 15 min and further centrifugation at 15000*g* for 30 min at 25 °C. In the case of POD, the protocol was similar but in the presence of 1 M NaCl to extract the possible fraction of ionically bound peroxidase.

The corresponding pigments-free supernatants were dialyzed at 4 °C overnight against 10 mM sodium phosphate buffer containing 10 mM AA. Desalted extracts were used as the enzyme sources to perform isoelectric focusing and kinetic assays. Extractions were done in triplicate.

Spectrophotometric Assays. PPO activity was determined according to the method of Espín et al. (*24*, *25*). This assay measures the accumulation of a stable adduct formed between the enzymatically generated *o*-quinones and the nucleophile MBTH, which is reddish and could be followed at 500 nm ($\epsilon_{500} = 40000$ M^{-1} cm^{-1}, at pH 4.5). The standard reaction mixture for determining PPO activity contained 100 mM sodium acetate buffer (pH 4.5), 2% DMF, 2.5 mM MBTH, 2 mM DHPPA, 10 μg/mL of bovine catalase (to remove possible traces of hydrogen peroxide), and 5.0 μL of enzymatic extract. The final volume of the reaction mixture was 1 mL. To discriminate between latent and active PPO, 0.05% SDS was added to the reaction medium (*26*) at pH 6.5 ($\epsilon_{467} = 20000$ M^{-1} cm^{-1}, at pH 6.5) (*23*). One unit of PPO was defined as the amount of enzyme that produces 1 μmol of MBTH−DHPPA−*o*-quinone adduct per minute.

POD activity was determined by measuring the accumulation of the ABTS radical cation ($ABTS^{\bullet+}$) at 414 nm ($\epsilon_{414} = 31300$ M^{-1} cm^{-1}, at pH 4) according to the method of Rodríguez-López et al. (*27*) with some modifications. The standard reaction mixture for determining POD activity contained 100 mM sodium acetate buffer (pH 4), 2 mM ABTS, 2 mM H_2O_2, 0.2 mM tropolone, and 5.0 μL of enzymatic extract. The PPO inhibitor tropolone (*28*) was included in the medium to determine specifically POD activity. The final volume of the reaction mixture was 1.0 mL. One unit of POD was defined as the amount of enzyme that produces 1 μmol of $ABTS^{\bullet+}$ per minute.

Enzyme activities were recorded in a UV-1603 spectrophotometer (Shimadzu, Tokyo, Japan). Temperature was controlled at 25 °C using a CPS-240 temperature controller (Shimadzu).

Isoelectric Focusing (IEF) Experiments. Isoelectric points (IEP) of both PPO and POD were determined in the electrophoresis unit Phast System (Pharmacia, Uppsala, Sweden). Enzymatic extracts were prepared as described above (previously desalted), and a total of 4.0 μL of extract was applied to the gel. IEF gels (PhastGel from Pharmacia) with IEP ranges between 4 and 6.5 (for PPO) and between 3 and 9 (for POD) were used. IEP values were calculated by comparison to broad IEP (3.0−10.0 and 4.0−6.5) kit markers from Pharmacia. After focusing, the gels were rinsed in 50 mM sodium acetate buffer, pH 4.5 for PPO or pH 4 for POD, and transferred to a Petri dish for activity staining. Solutions to develop PPO activity contained 3 mM catechol, 3 mM MBTH, 40 mM sodium acetate buffer (pH 4.5), and 10.0 μg/mL catalase. To detect the latent form of enzyme, the gels were also rinsed in 50 mM sodium phosphate buffer (pH 6.5) containing 0.05% SDS and stained following the described protocol. To develop POD activity, the solution contained 3 mM catechol, 3 mM MBTH, 40 mM sodium acetate (pH 4), 0.5 mM tropolone, and 2.5 mM H_2O_2. Staining was stopped by washing the gel in water and drying it. IEF analyses were performed five times.

Determination of Chlorophylls and Pheophytins. Chlorophylls were extracted from the skin of fresh grapes (2 g) with 20 mL of acetone/water (80:20) for 24 h in the dark at 4 °C. The extraction protocol was repeated twice to ensure total recovery. Conversion of the chlorophylls to their corresponding pheophytins *a* and *b* was obtained by mixing saturated oxalic acid in acetone/water (80:20) and

allowing the mixture to stand for 3 h in the dark at room temperature (*29*). Chlorophyll and pheophytin contents were estimated following the method of Vernon (*29*).

Bright-Field and Epifluorescence Microscopy. The structure of skin grapes was analyzed by microscopic methods. Fresh grapes were cut into smaller pieces and fixed in 10 g/L glutaraldehyde in 100 mM phosphate buffer (pH 7.0) at 4 °C for 1 day. The samples were washed three times for 30 min with reverse osmosis water and dehydrated in a subsequent series of ethanol dilutions at 20 °C: 500 g/L ethanol for 30 min (three times), 700 g/L ethanol for 60 min (twice), and 950 g/L ethanol for 12 h (twice). Then the samples were mixed gently in an infiltration solution (absolute ethanol/infusion solution 1:1) for 2 days and in an infusion solution for 4 days, polymerized with a Historesin embedding kit (Leica Microsystems Nussloch GmbH, Heidelberg, Germany) at 20 °C for 24 h, fixed in an adapter for 15 min, and cut into 4.0 μm sections with a Microm HM355 microtome (Microm Laborgeräte GmbH, Walldorf, Germany). Sections were dried onto glass slides and stained with specific staining solutions. For the bright-field microscopic examinations, the sections were stained as follows: (a) Cellulose (red-violet), 2.5 g/L Thionin (Merck) in water for 1 min followed by washing three times for 1 min with 10 g/L $NaHCO_3$ solution; (b) cell wall polymers (blue), 10 g/L Toluidine blue in water for 1 min followed by rinsing with water. For the fluorescence microscopic examinations, grape sections were stained with 700 g/L Oil Red O (BDH Chemicals, Poole, U.K.) in ethanol for 2 min followed by rinsing with water and drying. The sections were examined under fluorescent light (excitation at 420−480 nm, fluorescence >515 nm). All dried samples were mounted in immersion oil (Olympus 96, Olympus Optical Co. Ltd., Tokyo, Japan) under cover slides. Chloroplasts and chlorophyll content were directly examined under fluorescence on unstained cryosections from fresh grapes. Sections from fresh grapes were rapidly frozen in a cryostat (Leitz kryostat 1720, Ernst Leitz, Wetzlar, Germany), and 25 μm sections were cut and collected on slides. The samples were examined with an Olympus BX-50 microscope (Tokyo, Japan). Micrographs were obtained using a SensiCam PCO CCD camera (Kelheim, Germany) and the AnalySIS 3.0 image analysis program (Soft Imaging System, Münster, Germany).

Protein Determination. Protein content was determined using the method of Bradford (*30*) with bovine serum albumin as standard.

Statistical Analysis. For comparison between control and UV-treated grapes, the results were submitted to a factorial analysis of variance with 95% confidence interval, and the mean values were compared using the least significant difference test (LSD) by using the SPSS 11.0 program (SPSS Inc., Chicago, IL). This program was also used to look for correlations between variables, which were determined by the Pearson coefficient (χ^2), with a p value of 5% set for significance.

RESULTS AND DISCUSSION

Postharvest UV-C Irradiation of Superior Grapes. After 3 days of storage at 22 °C, the level of RES in UV-treated grapes reached a maximum concentration of 10.8 mg/kg of fw of berries (∼10-fold RES induction of the control). Other stilbenes such as *trans*-piceid, *trans*-piceatannol, and viniferins, not detected initially in the control grapes, were also induced in UV-treated grapes with maximum concentrations around 0.35, 1.16, and 8.23 mg/kg of fw, respectively. These values were in the range of those previously reported (*4*) with some changes (lower piceid induction in the present study). This could be due to different ripening stage, agronomic factors, etc.

As a consequence of the UV-C treatment, irradiated grapes also developed browning on the surface. Browning became evident on the third day of storage and progressed in treated grapes throughout storage. During this period, samples of control and treated grapes were further investigated to determine the possible causes of the browning.

Polyphenol Oxidase and Peroxidase. PPO is a key factor in the development of browning in grape berries, raisins, grapevine, must, and wine (*13*, *16*, *31*). In fact, reduced capacity for browning has been described in grapevine mutants with decreased PPO activity (*12*). POD may also participate in enzymatic browning in a hydrogen peroxide-dependent manner (*32*). Hydrogen peroxide could be generated in a reaction catalyzed by PPO, which could imply a synergistic effect of both PPO and POD in enzymatic browning (*11*, *33*). The induction of metabolic enzymes by UV light has been described for enzymes such as phenylalanine ammonia lyase (PAL), cinnamate 4-hydroxylase, and stilbene synthase in leaves of Vitaceae (*34*) as well as for chitinase, β-1,3-endoglucanase, PAL, and POD in grapefruit peel (*35*, *36*). Therefore, it was possible that the UV-C treatment might have induced the levels and/or activity of the stress-inducible oxidative enzymes PPO and POD (*23*, *37*) in the grapes and thus triggered the development of browning.

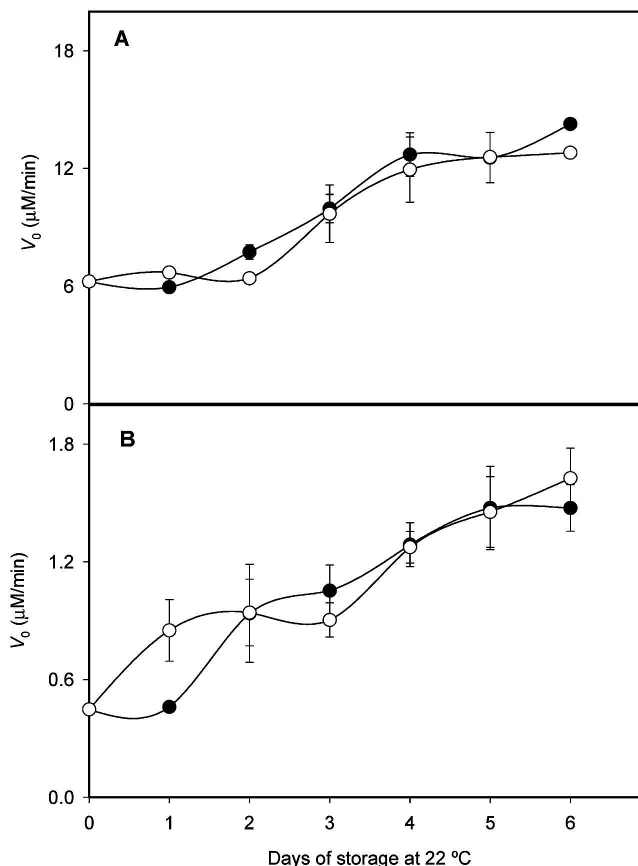

Figure 1. Polyphenol oxidase (PPO) activity from grape berries upon postharvest storage at 22 °C: (**A**) active PPO; (**B**) latent PPO; (●) control grapes; (○) irradiated grapes. Conditions are detailed under Materials and Methods. Values are the mean of three separate experiments. The coefficient of variation was always <10%.

PPO is often found in a latent form and can be activated by a number of treatments such as acid shock, detergents, fatty acids, or proteases (*26*). The degree of latency varies greatly between species (*38*, *39*). In our grape extracts, ∼10% of the PPO activity corresponded to its latent form, in agreement with Sapis et al. (*39*, *40*). PPO activity related to both active and latent forms increased over storage time in both UV-treated and untreated grape berries (**Figure 1**). After 6 days at 22 °C, PPO activity was ∼3-fold higher than on the first day of the experiment for both active and latent forms of PPO. Therefore, these results indicate that no activation from latent to active form occurred upon UV-C treatment as reported for other abiotic stress such as cutting in lettuce (*23*). Despite the increase in

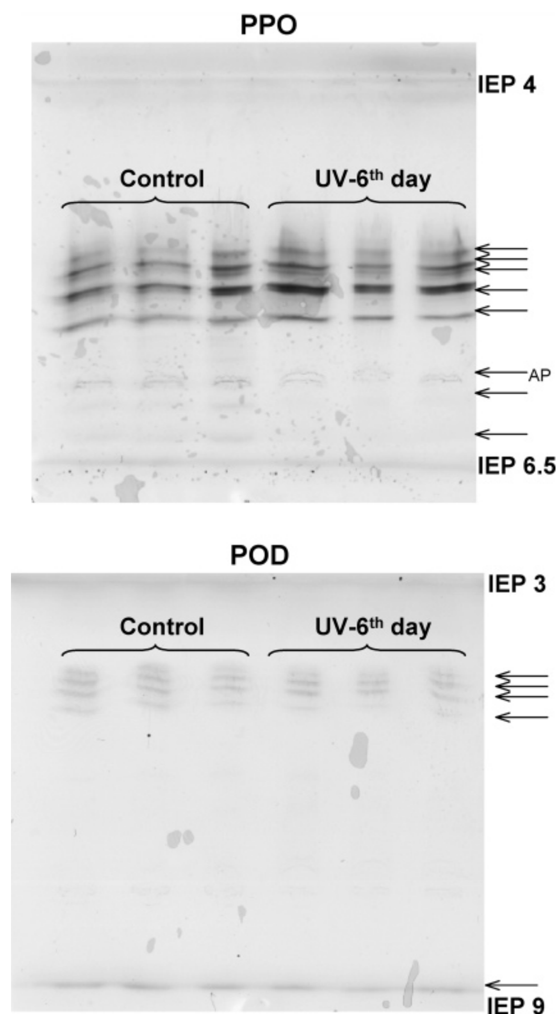

Figure 2. Isoelectric pattern of PPO (**A**) and POD (**B**) from skin of white grape variety Superior. Conditions are detailed under Materials and Methods. The arrows designate the different bands for which IEP values are given throughout the text. AP represents the application point. The protein loaded was 6 μg/5 μL. A representative gel from five replicates is shown.

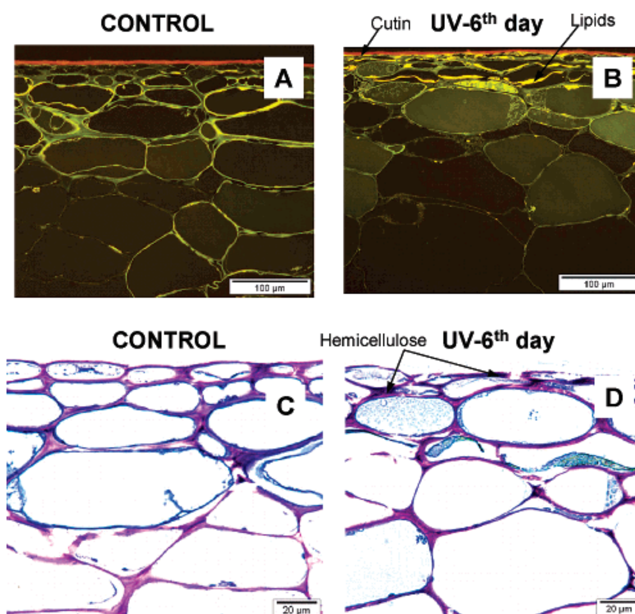

Figure 3. Cross-section of (**A**) control and (**B**) UV-treated white grapes, stained with Oil red O to show cutin (orange) and lipid material (yellow); cross-section of (**C**) control and (**D**) UV-treated white grape, stained with toluidine blue to show cell wall structure, especially hemicellulose (violet).

PPO activity upon storage, however, there was no difference between untreated and treated grapes, indicating no apparent direct association with the observed browning development in the treated samples.

Total POD activity was very low in grape berries and constant throughout the storage period for both untreated and irradiated grapes (data not shown), which was in accordance with previous reports (*32*, *36*, *41*).

We also investigated the presence and/or induction of different isoforms of these enzymes by the UV-C treatment. IEF gels developed for PPO activity showed the same eight separated bands with similar intensities in both control and treated grape extracts. Five major isoforms with IEP values of 5.14, 5.21, 5.28, 5.32, and 5.51 and two other minor isoforms with IEP values of 6.03 and 6.25 were detected (**Figure 2A**). The same band profile was detected when the gel was stained with SDS (at pH 6.5) (data not shown), indicating there was no activation of PPO from the latent to the active form (no new isoforms appeared upon UV-C treatment), which confirmed the kinetic values previously determined. No direct cause–effect relationship between browning and PPO activity could be inferred from these data, which was in agreement with previous studies (*39*).

IEF for POD also showed no differences between control and treated grapes (**Figure 2B**). One basic (IEP $\geq$ 9) and four different acid isoforms of POD (faint bands) were detected (IEPs 4.83, 4.58, 4.41, and 4.24).

Phenolic Compounds. Oxidation of phenolic compounds catalyzed by oxidative enzymes such as PPO or POD can lead to the formation of quinones that can polymerize to form melanins (*7*). Therefore, possible changes in the levels of phenolics upon UV-C treatment might be associated with the observed browning and, thus, we investigated the phenolic composition (flavonols, hydroxycinnamic acids derivatives, flavan-3-ols, and total phenols) of control and treated grapes throughout the storage period. Flavonol and flavan-3-ol concentrations remained unchanged (results not shown) upon UV-C treatment, in agreement with previous studies (*3*). However, the levels of hydroxycinnamic acid derivatives increased 3-fold over the control ($p < 0.001$) with storage time (results not shown), which was in accordance with the increase of these phenolics upon postharvest storage of other fruits and vegetables (*42*). In the present study, both treated and nontreated (control) grapes showed similar increases of hydroxycinnamic acid derivatives. Although the increase of hydroxycinnamic acids was evident on day 3 of storage (coincident with browning development), the increase was also the same in nontreated grapes (no browning development) and, thus, the browning observed in UV-C-treated grapes did not seem to be closely related to possible changes in the phenolic composition of grapes.

Microstructure Analysis of Grape Cell Wall. In normal healthy plant tissue, cytosolic PPO is separated from its substrates, which are mostly found within vacuoles. Damaging the tissue may cause the breakage of cellular membranes, allowing PPO to react with its phenolic substrates and lead to the formation of brown pigments (*12*, *16*). In principle, no clear damage was observed in cell walls from UV-C-treated grape skins. There were some noticeable differences between the structure of the cell wall of control samples and the UV-treated samples. No changes were observed in the cutin layer covering the grape skin in either control or irradiated grapes (**Figure

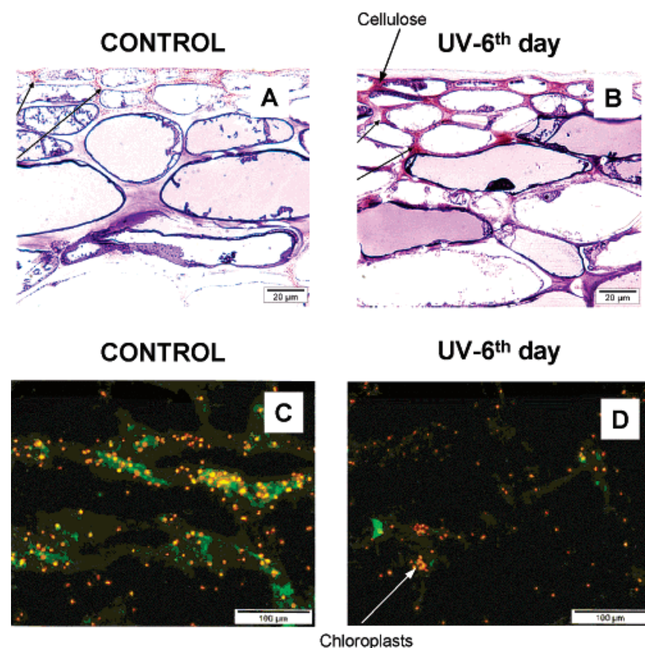

Figure 4. Cross-section of (**A**) control and (**B**) UV-treated white grape, stained with thionine to show cellulose (red); unstained cryosections of (**C**) control and (**D**) UV-treated white grape showing chloroplasts and chlorophyll content (orange) directly examined under fluorescence.

3A,**B**). However, lipidic material, hemicelluloses (**Figure 3A–D**), and cellulose (**Figure 4A**,**B**) were all detected in higher amounts in the UV-treated samples than in the control samples, suggesting a marked thickening of the plant cell wall (especially due to hemicellulose and cellulose accumulation) in treated grapes, which could be considered as a general wound response of plant tissues.

Chlorophyll and Pheophytin Contents. Pheophytins (*a* and *b*) can be formed from chlorophylls (*a* and *b*, respectively) by a loss of the Mg^{2+} cation. This process can take place spontaneously, for example, during freezing and thawing episodes or by the action of the enzyme Mg^{2+} dechelatase, causing the development of the brown color (*20*). It has been shown that the chlorophyll content in diatom can be strongly reduced by UV-B irradiation (*44*). In fact, the typical spectrum of chlorophyll *b* (commercial standard) was severely affected by UV-C irradiation for 1 min and completely disappeared upon UV-C irradiation for 3 min (results not shown). The analyses of chloroplasts and chlorophyll content under fluorescence microscopy revealed an evident loss of chlorophyll fluorescence in the UV-treated samples (**Figure 4C**,**D**), supported by the brownish color of these samples (*43*).

The concentrations of chlorophylls and pheophytins in acetone extracts from control and treated grapes throughout the storage period are presented in **Table 1**. The ranges of values were in good agreement with previously published data (*20*, *45*). The main difference between control and UV-C-treated grapes was the significantly lower chlorophyll *b* content in the UV-treated samples and the higher content in pheophytins ($p < 0.001$) on the third and fourth days of storage, approximately coincident with browning development (**Table 1**), which could explain in part the lower fluorescence observed in the UV-treated samples by microscopy.

UV-C treatment of grapes has been described as an alternative tool to increase the levels of health-beneficial stilbenes, especially resveratrol (*3*, *4*). However, UV-C irradiation can also promote browning in some white table grapes, such as in var. Superior. Nevertheless, it should be stressed that the browning does not occur in all of the UV-C-irradiated white grape varieties. For example, no browning (or very light browning) was detected in other varieties, such as Dominga and Moscatel Italica (*4*). In addition, the study reported here was also carried out in the white grape var. Airén (results not shown). In this case, the UV-C-induced browning was less evident than in the Superior grapes, although the same overall results were found (no effect on oxidative enzymes, some wall-thickening, and some chlorophyll degradation). Therefore, the UV-C-induced browning in white table grapes does not seem to be a “universal” undesirable side effect, but it seems to depend on the features of the variety irradiated (skin thickness, chlorophyll content, etc.). The results presented here show that the UV-C treatment appears to trigger some senescence-related processes such as chlorophyll degradation as well as wound responses such as cell wall thickening. Therefore, our results suggest that the development of browning after UV-C irradiation and throughout storage may be mainly due to the loss of chlorophylls with concomitant accumulation of the degradation-derived products (pheophytins). However, as decompartmentalization of cellular organelles was not explored, the enzyme-catalyzed browning with involvement of phenolics cannot be completely ruled out.

Table 1. Chlorophyll and Pheophytin Pigments in Skin of Var. Superior Grape Extracts[a]

	chlorophyll *a*		chlorophyll *b*		pheophytin *a*		pheophytin *b*	
days	CT	UV	CT	UV	CT	UV	CT	UV
0	2.2	2.4	1.01	1.25	0.45	0.45	0.00	0.00
1	2.2	2.4	1.01	1.25	0.43	0.35	0.00	0.00
2	2.09	2.16	0.98	1.08	0.24	0.02	0.31	0.29
3	2.72	2.91	0.81	0.89	0.94	0.77	0.00	0.08
4	2.40	2.01	1.13	1.02	0.58	0.00	0.29	0.60
5	2.65	2.09	1.35	0.75	0.80	0.00	0.04	0.83
6	2.92	2.72	0.95	0.75	1.13	0.55	0.00	0.14
factor treatment	NS		(0.05)***		(0.11)***		(0.09)***	
factor time	(0.19)***		(0.098)***		(0.20)***		(0.16)***	

[a] Mean values are shown ($n = 6$). Values are expressed in mg/100 g of fresh skin grapes. CT, control (nontreated grapes); UV, UV-C-treated grapes; LSD, values are given in parentheses; NS, not significant; ***, $p < 0.001$.

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