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Comparison of Cardioprotective Abilities between the Flesh and Skin of Grapes

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Recent studies have documented that grapes and grape juices are equally cardioprotective as red wine. The existing reports implicate that the skin and seeds of the grapes containing polyphenolic antioxidants are instrumental for the cardioprotective properties of grapes. The present study examines if the flesh of grapes also possesses any cardioprotective abilities. Three groups of randomly selected rats were fed, water only (control), flesh of the grapes (2.5 mg/kg b. wt.) or the skins (2.5 mg/kg b. wt.) for 30 days. At the end of the 30 days, isolated perfused hearts were made ischemic for 30 min followed by 2 h of reperfusion in the working mode. The results demonstrated that both flesh and skin of the grapes could protect the hearts from ischemic reperfusion injury as evidenced by improved postischemic ventricular recovery and reduced myocardial infarct size. High performance liquid chromatography (HPLC) revealed that skin and flesh contained comparative amounts of glucose, fructose, tartaric acid, malic acid, shikimic acid, and *trans*-caftaric acid. In addition, the flesh contained reduced amounts (compared to skin) of *cis*-coutaric, *trans*-coutaric, caffeic, *p*-coumaric, cinnamics, and catechin/epicatechin. Total polyphenolic index was also lower in flesh compared to skin. The anthocyanins were present exclusively in the skin. Electron paramagnetic resonance (EPR) spectrometry of hydroxy radicals indicated that both flesh and skins possessed equal amount of ROS scavenging activities. Total malonaldehyde content in the heart was reduced comparatively with either flesh or skin. The results indicate for the first time that the flesh of grapes are equally cardioprotective as skin, and antioxidant potential of skin and flesh of grapes are comparable with each other despite of the fact that flesh does not possess any anthocyanin activities.

KEYWORDS: Grapes; flesh; skin; cardioprotective; antioxidant; ROS; EPR spectroscopy

INTRODUCTION

Epidemiological as well as experimental studies have demonstrated that daily consumption of moderate quantity of wine, especially red wine, reduces the risk of cardiovascular diseases (*1*−*3*). The original observation by Renaud et al. that the morbidity and mortality due to coronary heart diseases in France has been significantly lower than that in other developed countries, despite the high consumption of fat and saturated fatty acids by the French (*4*), was confirmed by subsequent studies (*5*, *6*). Subsequent studies indicated that some of the white wines could also reduce myocardial ischemic injury (*5*), and the grapes from which wines are manufactured also possess cardioprotective abilities (*6*).

The cardioprotective properties of grapes have been shown to be exclusively located in the seeds and skin of grapes, which contain large varieties of polyphenolic antioxidants. For example, grape seeds contain proanthocyanidins, which possess cardioprotective properties (*7*, *8*). Grape skins are rich in resveratrol, a phytoalexin, which possesses diverse health benefits including cardioprotective abilities (*9*−*12*). Red wines made of the grapes are believed to inherit the health benefits from grape skins, which are rich in resveratrol and proanthocyanidins.

There is an increasing amount of evidence that patients who drink grape juice are protected against LDL oxidation beyond that seen with antioxidant supplementation alone (*13*−*15*).

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Short-term ingestion of purple grape juice improved flow-mediated vasodilation and LDL susceptibility to oxidation in the patients with coronary artery disease (*14*), suggesting that nonalcoholic contents of red wine containing polyphenolic antioxidants were mainly responsible for improved endothelial function. Despite the fact that the majority of the grape juice comes from the flesh, this compoent of grapes was never studied.

In the present study, we compared the cardioprotective abilities of skin and flesh of grapes. The rats were given either extracts of skin or flesh for a period of 30 days. After 30 days, the hearts were subjected to ischemia reperfusion injury. Surprisingly, grape flesh was found to be equally cardioprotective as grape skin. Many of the antioxidants and some sugars and acids possessing antioxidant properties are present in the flesh of the grapes. Electron paramagnetic resonance spectroscopy of hydroxyl radicals demonstrated that the ROS scavenging activity was similar between flesh and skin of grapes.

MATERIALS AND METHODS

Animal Preparation. Sprague Dawley male rats of about 300 gm body weight were used for our study. All animals used in this study received humane care in compliance with the principles of laboratory animal care formulated by the National Society for Medical Research and Guide for the Care and Use of Laboratory Animals prepared by the National Academy of Sciences and published by the National Institutes of Health (Publication Number NIH 85-23, revised 1985). The rats were fed ad libitum regular rat chow with free access to water. A group of rats were given (orally) for 30 days one of the three preparations: (i) water only (control); (ii) extracts of grape skin; or (iii) extracts of grape flesh. After 30 days, rats were sacrificed, and hearts were excised and perfused via working-mode.

Isolated Working Rat Heart Preparation. The rats were properly anesthetized with pentobarbital (65 mg/kg). After intravenous administration of heparin (500 IU/kg), the chests were opened, and the hearts were rapidly excised and mounted on a non-recirculating Langendorff perfusion apparatus (*9*). Retrograde perfusion was established at a pressure of 100 cm H_2O with an oxygenated normothermic Krebs−Henseleit bicarbonate (KHB) buffer with the following ion concentrations (in mM): 118.0 NaCl, 24.0 $NaHCO_3$, 4.7 KCL, 1.2 KH_2PO_4, 1.2 $MgSO_4$, 1.7 $CaCl_2$, and 10.0 glucose. The KHB buffer has been previously equilibrated with 95% O_2/5% CO_2, pH 7.4 at 37 °C. After perfusing the heart via the Langendorff mode for 10 min, the pulmonary vein was cannulated and the Langendorff perfusion discontinued for subsequent working heart perfusion as described previously (*9*). It is essentially a left-heart preparation in which oxygenated KHB at 37 °C enters the cannulated pulmonary vein and left atrium at a filling pressure of 17 cm H_2O. The perfusion fluid then passes to the left ventricle from which it was spontaneously ejected through the aortic cannula against a pressure of 100 cm H_2O. Aortic flow can be measured by a calibrated rotameter while coronary flow is measured by the timed collection of the coronary perfusate dripping from the heart. The aortic flow was recirculated while coronary effluent can be collected or reccirculated. Heart rate, left ventricular developed pressure, and its first derivative (dp/dt_{max}) were acquired and recorded. Coronary flow was terminated for 15 min to induce global ischemia which is followed by 2 h of reperfusion. Aortic pressure was measured using a Gould P23XL pressure transducer connected to a sidearm of the aortic cannula, and the signal was amplified using a Gould 6600 series signal conditioner and monitored on a real-time data acquisition and analysis system (CORDAT II). For the measurement of left ventricular developed pressure (LVDP), a Millar catheter was inserted in the left ventricle via the tube and cannula of left atrium and the mitral valve as described previously (*10*, *11*), and the exact LVDP was measured and recorded.

At the end of 10 min, after the attainment of steady-state cardiac function, baseline functional parameters were recorded and coronary effluent collected for biochemical assays. The circuit was then switched back to the retrograde mode and heart perfused for 15 min with KHB buffer. Hearts was then subjected to global ischemia for 30 min followed by 2 h reperfusion.

Measurement of Myocardial Infarct Size. At the end of reperfusion, a 10% (w/v) solution of triphenyltetrazolium in phosphate buffer was infused into the aortic cannula (*12*). The hearts were excised and stored at −70 °C. Sections (0.8 mm) of frozen heart were fixed in 2% paraformaldehyde, placed between two cover slips, and digitally imaged using a Microtek ScanMaker 600z. To quantitate the areas of interest in pixels, a NIH Image 5.1 (a public-domain software package) was used. The Infarct size (transmural) was quantified in pixels.

Estimation of malonaldehyde (MDA). MDA was assayed in the heart as described previously (*19*) to monitor the development of oxidative stress. The MDA was derivatized using 2,4-dinitrophenylhydrazine (DNPH). Aliquots of 25 μL of derivatized MDA in acetonitrile were injected onto a Beckman Ultrasphere C_{18} (3 mm) column in a Waters HPLC (Waters Corp., Milford, MA). The products were eluted isocratically and detected at 307, 325, and 356 nm. The amount of MDA was quantitated using Maxima software program (Waters).

Preparation of Flesh and Skin of Grapes for ROS Scavenging Activities and HPLC Analysis. Four different red grape varieties of *Vitis vinifera* from central Italy were used for chemical analysis. The varieties were:

"Cesanese d'Affile"	(variety 1)
"Montepulciano"	(variety 2)
"Nero buono di Cori"	(variety 3)
"Sangiovese"	(variety 4)

The grapes were pressed by a squeezer, the resulting must was cleaned by centrifugation and analyzed for its sugar content (g/L) by refractive index, and pH and total acidity were expressed as tartaric acid content (g/L) according Official Standard Methods (*22*). Selected grape samples were carefully treated with a lancet for the separation of flesh from the skin, excluding the grape seeds. After separation, the sample of flesh and skin was dipped in a EtOH/CH_3COOH 9:1 solution at 0 °C, and the final volume was adjusted to a 1:2 ratio vegetal matter vs extraction solvent. The resulting mixture was homogenized for 30 s at 10000 rpm with Ultra-Turrax and centrifuged at 0 °C for 30 min at 25000*g*. After separation of the supernatant, each sample was divided into little aliquots that were stored at −80 °C until use.

Chemical Analyses. All chemicals used were from Sigma-Aldrich (USA). For each dissolution or dilution, buffers and all other solutions used for antioxidant and antiradical assays were previously deareated by bubbling with pure N_2. The results were expressed as mg/100 g fresh weight of grape materials. Each analysis was repeated five times.

Analysis of Sugar and Organic Acids. Simple sugars and organic acid contents of grape extracts were analyzed by HPLC. As for sugars, they were separated with a CarboSep Coregel 87C carbohydrate column with a 0.78 × 30 cm bed packed with a cation-exchange resin in the Ca^{2+} ionic form. The mobile phase was water at 0.5 mL/min, and the elution was performed at 85 °C, while the sugars were revealed by a refractive index detector. Glucose and fructose solutions were used as external standards (retention times 11.5 and 14.7 min, respectively). Each analysis was repeated three times.

As for organic acids, the separation was carried out on an Inertsil ODS-3 column 5 mm of particle diameter and 0.46 × 25 cm dimension. The elution was carried out at 20 °C with H_3PO_4 0.02 mol/L in water as mobile phase at 0.6 mL/min. The revelation was spectrophotometrically made at 210 nm. Tartaric, malic, and shikimic acid solutions were used as external standards (retention times 7.8, 9.8, and 10.3 min, respectively). The analyses were three times repeated.

Total Polyphenol and Anthocyanin Index. The extracts of the flesh and skin from the grapes were assayed for polyphenols by the Folin−Ciocalteau reaction as follows: 1 mL of extract was diluted with 20 mL water in a 50 mL flask and then was treated with 3 mL of Folin−Ciocalteu reagent and well mixed. The mixture was treated with 6 mL of Na_2CO_3 20% solution in water, kept at a final volume of 50 mL, and stored 1 h in the dark. After the complete reaction time, the absorbance of the solutions were read at 730 nm for a 1 cm cuvette

against blank obtained by treating as previously 1 mL of H_2O/EtOH/CH_3COOH 33:60:7, the same composition as the grape extraction solution. Results were expressed as mg/L of gallic acid, after calibration with solutions of gallic acid treated in the same way as the samples.

The method for the total anthocyanins assay was based on the different color of anthocyanins at different pH values. At pH 1.0 anthocyanins gave a stabilized red-purple color, while at pH 4.5 anthocyanins are not colored, owing to colorless carbinol formation; total anthocyanin content of the grape extract is related to the difference in absorbance at 510 nm between the samples buffered at pH 1.0 and pH 4.5. The values were expressed as mg/L of cyanidin chloride. Assays of both analyses were done in triplicate.

Separation of the Polyphenols. Grape polyphenols were separated by HPLC by a method that avoided the interference of anthocyanins in the skin. Two types of chromatogram were obtained according the detector wavelength: 320 nm for the cinnamates and 280 for other phenolic compounds. The interference of anthocyanins was evaluated by plotting the chromatograms at 525 nm.

Two columns in series were used: an Inertsil ODS-3 column 5 mm of particle diameter and 0.46 × 25 cm dimension and an Inertsil C8 column 5 mm of particle diameter and 0.46 × 25 cm dimension. The elution was performed at 40 °C at a flow rate of 0.8 mL/min by a linear gradient of two mobile phases: solvent A CH_3COOH 2% in H_2O v/v, solvent B CH_3CN. The elution program is shown below:

time (min)	solvent A (%)	solvent B (%)
0	90	10
15	90	10
25	70	30
60	40	60
80	95	5

The identified and quantified compounds at 320 nm were *trans*-caffeoyl-tartaric acid (caftaric acid) (retention time14.7 min), *cis*-cumaroyl-tartaric acid (*cis*-coutaric acid) (16.5 min), *trans*-coumaryl-tartaric acid (*trans*-coutaric acid) (19.5 min), *trans*-caffeic acid (22.1 min), *trans*-*p*-coumaric acid (24.1 min), and *trans*-resveratrol (33.5 min). The compounds evaluated at 280 nm were gallic acid (13.3 min), (+) catechin (16.3 min), and (−) epicatechin (20.1 min) (see HPLC diagrams).

The peaks from the retention time of about 25 min and about 30 min, that are very visible in the skin extract, are probably related to anthocyanins, whose amount was spectrophotometrically evaluated as total anthocyanin index.

The quantification of the compounds was made by external standard technique, and the values were expressed as mg/kg fresh weight of grape material. The dosages of *trans*-caffeic acid, *trans*-*p*-coumaric acid, *trans*-resveratrol, gallic acid, (+)catechin, and (-)epicatechin were made by direct comparison with the calibration curve of each standard compound, while for *trans*-caffeoyl-tartaric acid, *cis*-cumaroyl-tartaric acid, and *trans*-coumaryl-tartaric acid were respectively used the calibration parameters of *trans*-caffeic acid, *cis*-*p*-coumaric acid, and *trans*-*p*-coumaric acid owing to the lack of commercial standards. The analysis was repeated three times.

Electron Paramagnetic Resonance Spectrometry (EPR). The ROS scavenging activity of grape extracts was evaluated by their abilities to scavenge the most potent active oxygen species, •OH. The standard for •OH reaction were prepared in the following concentrations: a stock solution of $FeSO_4 \cdot 7H_2O$ 10 mmol/L in 2Na EDTA 12 mmol/L, and a stock solution of H_2O_2 10 mmol/L were freshly prepared in a phosphate buffer solution 0.1 mol/L pH 7.4 (PBS). These solutions were diluted 6.5-fold with PBS, to obtain the Fenton reaction mixtures at the following final concentrations: $FeSO_4 \cdot 7H_2O$ 1.54 mmol/L, 2Na EDTA 1.85 mmol/L, and H_2O_2 1.54 mmol/l. The reaction was initiated by mixing the Fe-EDTA solution with the grape skin or flesh extracts or PBS (control) and then the reaction started by adding the H_2O_2 solution.

The hydroxyl radical produced in the Fenton reaction mixture was trapped with 5,5-dimethylpyrrolidine-*N*-oxide (DMPO), according to methods already described (*24*, *25*) with some modifications. The resultant adduct DMPO-•OH, consisting of a quartet resonance composed of resonances with 1:2:2:1 relative intensities composed of a doublet of triplet resonances well described in a previous paper (*20*), was detected by an X-Band EPR spectrometer Varian E-line Century series. The triplet resonance, with relative peak intensities of 1:1:1, arises from free radical coupling with the $I = 1$ ^{14}N, the more abundant isotope. The doublet structure superimposed on the triplet structure arises from free radical coupling with the β vinylic proton ($I = 1/2$). For •OH measurement, the mixture without the scavenging compounds (blank) contained: 0.7 mL of PBS; 0.2 mL of Fe-EDTA; 0.2 mL of 50 mmol/L DMPO in PBS; 0.2 mL of H_2O_2. The sample preparation for the EPR test was made by solvent evaporation from 1 mL of each extract in a centrifugal evaporator at 0 °C. The dry extract was twice dissolved in 2 mL of bi-distilled water and again evaporated in order to eliminate each solvent trace, which could interfere with the EPR analysis. Finally, samples were dissolved in 2 mL of PBS ready for the analysis.

A blank confirmation was made by evaporating in vacuum the same solvent amount used for samples, subsequently treated as the samples. The mixture with the scavenging solution contained 0.5 mL of PBS, 0.2 mL of scavenger (grape extract in PBS), 0.2 mL of Fe-EDTA, 0.2 mL of 50 mmol/L DMPO in PBS, and 0.2 mL of H_2O_2. These solutions were accurately mixed in a glass tube assay, and successively placed in the EPR probe, a capillary tube of 100 mm length and 0.7 mm internal diameter. EPR spectra were recorded after exactly 2 min. The instrumental parameters were as follows: frequency, 9.26 GHz; power, 5 mW; field set, 3390 G; scan time, 64 s; time constant, 0.5 s; gain, 16000; modulation, 1 G. The scavenger activity percent of the assayed solution for •OH was expressed as the following formula:

$$I = 100(1 - h_x/h_o)$$

where I was scavenger activity, h_o and h_x were the relative heights of the highest resonance signal (mm) of the DMPO-•OH adduct spectra in a reaction mixture without and with the scavenger solution, respectively. Each assay was repeated four times.

Scavenging Activity of DPPH Radical. We also used the ability of grape extracts to quench DPPH•, as a measure of their ROS scavenging activities. DPPH (1,1-diphenyl-2-picrylhydrazyl) is a synthetic and stable free radical product, whose quenching by a scavenger substrate could be spectrophotometrically followed at 517 nm. One mL of grape extract was diluted with 1 mL of ethanol 96°. An aliquot of 0.5 mL of the resulting solution was added to 1.5 mL of ethanol 96° and 0.5 mL ethanolic solution containing DPPH• 0.5mmol/L. The blank sample was prepared using 2.0 mL ethanol and 0.5 mL of the same ethanolic solution containing DPPH• in order to check the radical stability in the used conditions. The ethanolic solution containing DPPH• was kept at room temperature in the dark. The absorbance of the remaining DPPH• in the shaken reaction mixture was measured in a 1 cm cuvette with a Unicam UV/vis spectrophotometer at 517 nm and at 20 °C and followed each 30 s for 10 min, until reaching a plateau.

Trolox (6-hydroxy-2,5,7,8-tetramethilchroman-2-carboxylic acid), an α-tocopherol (vitamin E) derivative, is known as one of the most powerful antioxidants, with relatively high selectivity for scavenging peroxynitrite and hydroxyl radical and is currently used as standard reference method (Trolox Equivalent Antioxidant Capacity, TEAC). Trolox, 0.1 mmol/L (0.5 mL of the same solvent as used for grape extracts), was used as reference. The radical scavenging activity (S) of each extract after 10 min was expressed in percent as the following formula:

$$S = 100 - [(A_x/A_o)] \times 100$$

where A_x was the optical density of DPPH• solution in presence of grape extract and A_o the optical density of DPPH• solution in the absence of the sample. Each assay was performed in quadruplicate.

Statistical Analysis. For statistical analysis, a two-way analysis of variance (ANOVA) followed by Scheffe's test was first carried out to test for any differences between groups. If differences were established, the values were compared using Student's t-test for paired data. The vaues were expressed as mean ± SEM. The results were considered significant for $p < 0.05$.

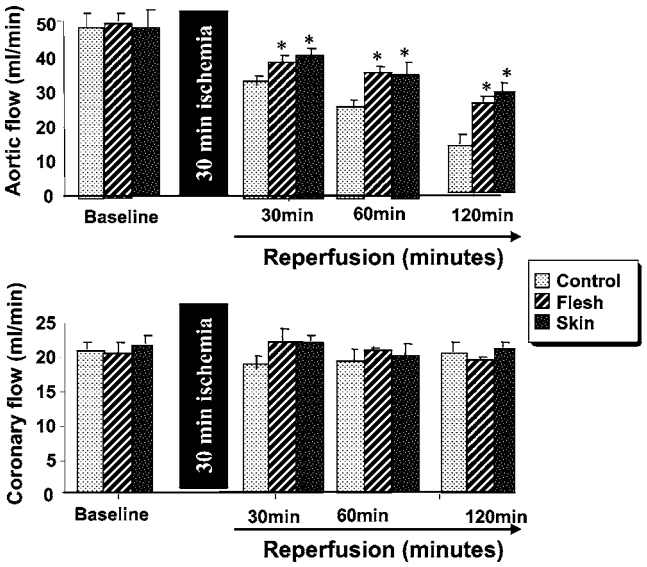

Figure 1. Effects of flesh and skin of grapes on the aortic flow (top) and coronary Flow (bottom). Rats were given either skin or flesh of grapes for 30 days while control experiments were performed by giving the rats a mixture of 45 μg/100 g glucose and 45 μg/100 g fructose. At the end of 30 days, isolated rat hearts were subjected to 30 min ischemia followed by 2 h of reperfusion. Aortic flow and coronary flow were determined at baseline and during the postischemic reperfusion. Results are expressed as mean ± SEM of six hearts per group.

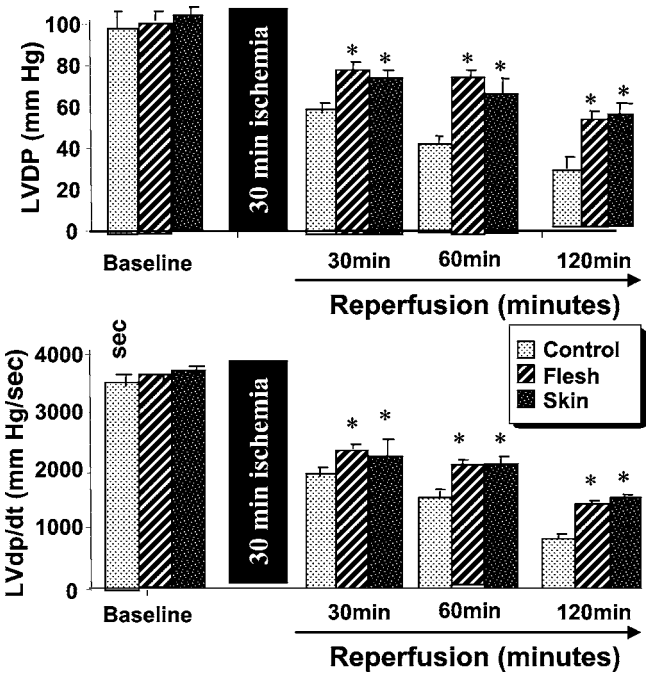

Figure 2. Effects of flesh and skin of grapes on LVDP and $LV_{max}dp/dt$. Rats were given either skin or flesh of grapes for 30 days while control experiments were performed by giving the rats a mixture of 45 μg/100 g glucose and 45 μg/100 g fructose. At the end of 30 days, isolated rat hearts were subjected to 30 min ischemia followed by 2 h of reperfusion. LVDP and $LV_{max}dp/dt$ were determined at baseline and during the post-ischemic reperfusion. Results are expressed as mean ± SEM of six hearts per group

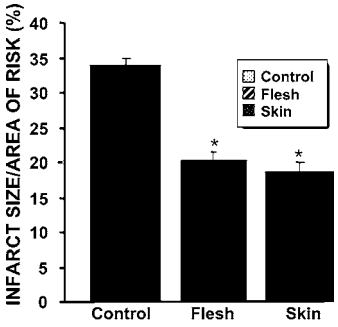

Figure 3. Effects of flesh and skin of grapes on myocardial infarct size. Rats were given either skin or flesh of grapes for 30 days while control experiments were performed by giving the rats a mixture of 45 μg/100 g glucose and 45 μg/100 g fructose. At the end of 30 days, isolated rat hearts were subjected to 30 min ischemia followed by 2 h of reperfusion. Myocardial infarct size was determined at the end of each experiment as described in the Methods Section. Results are expressed as mean ± SEM of six hearts per group.

RESULTS

Effects of Flesh and Skin of Grapes on Myocardial Function. The hearts of the rats given grape products orally for 30 days significantly improved postischemic contractile function as compared to those given control diets. Surprisingly, both flesh and skin improved postischemic ventricular function in an identical manner. As shown in **Figure 1** (top), aortic flow was reduced in all groups during the postischemic reperfusion. Both skins and flesh of grapes significantly improved the aortic flow during the reperfusion as compared to control group. There was no difference in the pattern of coronary flows between any of the groups (**Figure 1**, bottom). Postischemic LVDP (**Figure 2**, top) and $LV_{max}dp/dt$ (**Figure 2**, bottom) also showed significant improvement for the hearts of the rats given either of the grape products.

Effects of Skin and Flesh of Grapes on Myocardial Infarct Size. Myocardial infarct size expressed as the percent infarct of the entire risk area was only 34.5% for the control hearts subjected to 30 min ischemia followed by 2 h of reperfusion (**Figure 3**). There was a significant reduction in the infarct size for the heart of the animals given either skin or flesh of the grapes. However, there was no difference in infarct size between the two experimental groups.

Effects of Skin and Flesh of Grapes on MDA Formation in the Heart. The MDA is the presumptive marker for the development of the oxidative stress and ROS activity in the heart. As shown in **Figure 4**, the MDA content of the heart was significantly less in the hearts of the animals fed either skin or flesh of grapes. Interestingly, there were no differences between the skin and flesh fed groups.

Sugar and Acid Content. As mentioned in the Methods Section, we analyzed four different varieties of grapes separately. Sugar content (refractometry) was about 200 g/L in all samples, with the highest content in variety 1 (**Table 1**). The ethanol/acetic acid extraction of skin and flesh revealed the presence of simple sugars; in fact, the quantity of sugar present in grape extracts for each grape variety resembles the normal sugar content of grapes at full maturity. Total sugar content detected by HPLC (**Figure 5**) in skin and flesh is shown in **Table 2**. In all samples there was slightly higher content of fructose than glucose.

The organic acid profile of grape skin and flesh (**Table 3**) was expressed as the amounts of tartaric, malic, and shikimic acids.

Phenolics Content. Total phenolics were quantified by the Folin−Ciocalteau method. As it was expected (**Table 4**), the phenolic contents were much higher in the skins compared to those present in the flesh. The highest content was found in the variety 4, followed by variety 3. The anthocyanins were found

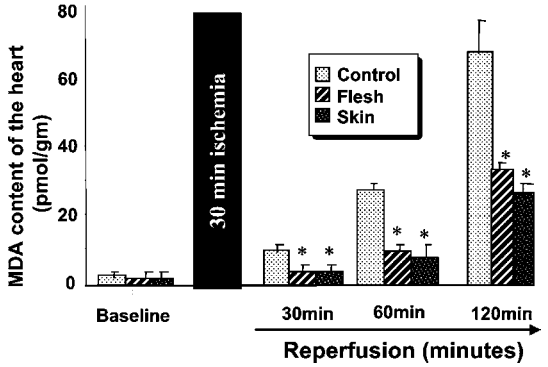

Figure 4. Effects of flesh and skin of grapes on the MDA content of the heart. Rats were given either skin or flesh of grapes for 30 days while control experiments were performed by giving the rats a mixture of 45 μg/100 g glucose and 45 μg/100 g fructose. At the end of 30 days, isolated rat hearts were subjected to 30 min ischemia followed by 2 h of reperfusion. MDA content was determined at the end of each experiment as described in Materials and Methods. Results are expressed as mean ± SEM of six hearts per group.

Table 1. The Sugar Content (refractometry), pH, and Acidity of Grape Samples from Different Varieties

	grape variety[a]			
	1	2	3	4
total sugars, g/L	202.2	214.3	208.0	201.0
pH	3.10	3.08	3.09	3.07
total acidity, g/L of tartaric acid	11.3	8.6	8.6	7.2

[a] 1, 2, 3, and 4 are the four different varieties of grape used. For each variety $n = 5$.

Table 2. Sugar Content (g/100 g f. w.) of Skin and Flesh from Different Varieties of Grapes.

	grape variety[a]			
	1	2	3	4
	Skin			
glucose	8.2	9.1	6.0	8.7
fructose	7.1	7.3	5.4	7.4
sum	15.3	16.4	11.4	16.1
	Flesh			
glucose	10.8	11.5	9.3	8.8
fructose	8.0	9.4	8.5	8.0
sum	18.8	20.9	17.8	16.8

[a] 1, 2, 3 and 4 are the four different varieties of grape used. For each variety $n = 3$.

only in skin extract of grape. The highest value was found in the third variety. The most important phenolics of grape, apart from anthocyanins in red grape cultivars, are represented by products related to the cinnamic acid structure, both free and esterified by tartaric acid as shown in **Table 5**.

Coumaryl-tartaric acid esters were found in skin, while flesh had a prevalence of *trans*-caffeoyl-tartaric ester. Free cinnamates (caffeic and *p*-coumaric acids) were generally low respect to esterified forms, except for the variety 2, which showed its presence as free cinnamates with respect to esterified ones. Total cinnamics yielded higher amounts in skin than in flesh.

Table 5 also shows calculated amounts of *trans*-resveratrol. *Trans*-resveratrol was present in the highest amount in the skin of variety 3, followed by variety 1, while traces of this compound were also found in flesh probably due to interference during the difficult separation procedure of skins and flesh. As

Table 3. Organic Acids Content (mg/100 g f. w.), by HPLC, of Skin and Flesh from Different Varieties of Grapes.

	grape variety[a]			
	1	2	3	4
	Skin			
tartaric acid	35.9	32.4	24.7	34.6
malic acid	1081.6	428.2	836.7	418.4
shikimic acid	14.1	6.2	3.8	6.6
sum	1131.6	466.8	865.2	459.6
	Flesh			
tartaric acid	26.9	73.5	30.2	39.0
malic acid	759.8	372.5	422.0	240.9
shikimic acid	3.3	5.3	1.5	0.6
sum	790.0	451.3	453.7	280.5

[a] 1, 2, 3, and 4 are the four different varieties of grape used . For each variety $n = 3$.

Table 4. Total Polyphenols Index (Folin-Ciocalteau) and Total Anthocyanins of Skin and Flesh from Differrent Grape Varieties.

grape variety[a]	total polyphenols index (mg/100 g f. w.)	total anthocyanins (mg/100 g f. w.)
	Skin	
1	182.7	81.3
2	196.3	125.6
3	287.6	164.3
4	327.9	144.6
	Flesh	
1	43.7	-
2	31.8	-
3	28.6	-
4	30.6	-

[a] 1, 2, 3, and 4 are the four different varieties of grape used. For each variety $n = 3$.

for flavan-3-ols, they were detected at 280 nm, and the sum (+) catechin and (−) epicatechin is reported in **Table 5**. Their content was generally higher in skin than in flesh.

Antioxidant Properties: EPR Spectroscopy. The results of $^{\bullet}$OH scavenging activities of the skin and flesh of grapes are shown in **Figure 6**. Surprisingly, grape skin extracts did not show higher $^{\bullet}$OH scavenging activity with respect to flesh.

In fact, flesh extracts showed slight but significant higher scavenging activity than skin (about 90% $^{\bullet}$OH inhibition in all samples vs 85% in skin extracts). The values between each variety did not show any changes, except for the skin of the variety 3, that resulted lower than other varieties of $^{\bullet}$OH inhibition (82%).

In contrast to $^{\bullet}$OH scavenging activities of the grape products, the scavenging action against DPPH$^{\bullet}$ was significantly ($p < 0.05$) higher for the skin extracts compared to that of flesh (**Figure 7**, left). There were some differences between the grape varieties.

DISCUSSION

There are several salient features in this study: (i) the most important finding is that the flesh and skin of grapes are equally cardioprotective; (ii) EPR spectroscopy demonstrated that flesh and skin possess the same degree of OH scavenging abilities despite the fact that skins exclusively contain the anthocyanins and higher amounts of polyphenols; (iii) flesh and skin of grapes contain comparable amounts of sugars and organic acids including tartaric, malic, and shikimic acids; the flesh and skin reduced the oxidative stress in the heart to the same extent.

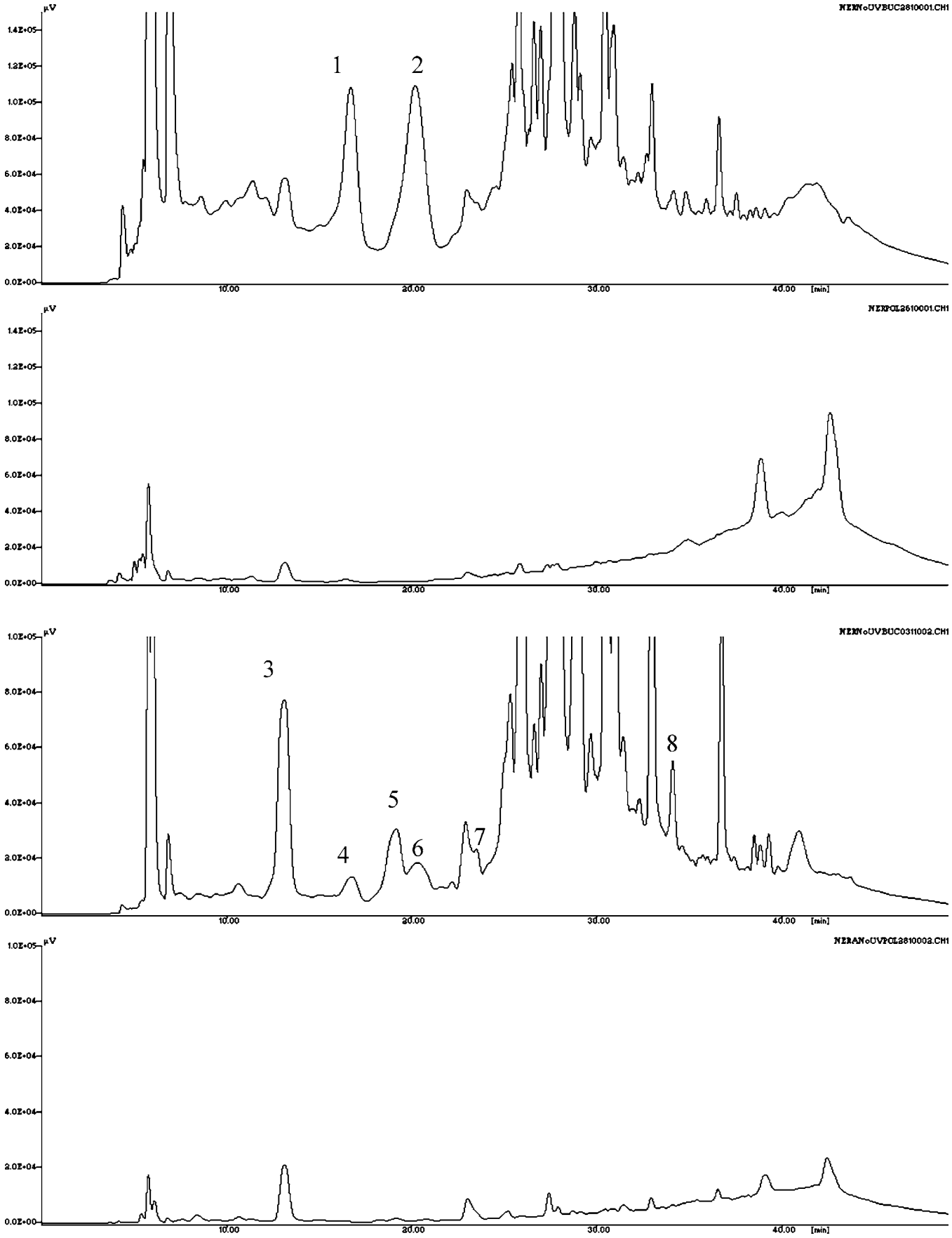

Figure 5. HPLC analysis of a sample of skin and flesh from the grapes: 1 = (+) catechin; 2 = (–) epicatechin; 3 = *trans*-caftaric acid; 4 = *cis*-coutaric acid; 5 = *trans*-coutaric acid; 6 = *trans*-caffeic acid; 7 = *trans-p*-coumaric acid; 8 = *trans*-resveratrol.

Oxygen-derived free radicals and/or oxidative stress play a significant role in a large variety of cardiovascular diseases including congestive heart failure, valvular heart disease, cardiomyopathy, hypertrophy, atherosclerosis, and ischemic

Table 5. Cinnamic Acid Derivatives, *trans*-Resveratrol, and Flavan-3-ols (mg/kg f.w.) in Skin and Flesh of Different Grape Varieties.

grape variety	*trans*-caftaric	*cis*-coutaric	*trans*-coutaric	caffeic acid	*p*-coumaric acid	*trans*-resveratrol	total cinnamics	(+)catechin + (–)epicatechin
				Skin				
1	2.87	3.22	14.65	0.07	1.68	3.48	22.49	1.90
2	2.63	4.93	0.79	5.13	15.86	2.99	29.34	0.90
3	1.31	7.54	19.07	0.75	6.69	4.78	35.36	1.39
4	2.43	4.88	11.20	0.32	0.36	0.78	19.18	2.58
				Flesh				
1	5.93	1.82	3.80	0.03	0.03	0.38	11.61	0.79
2	7.09	0.71	1.22	0.05	0.82	0.13	9.89	0.90
3	1.72	0.28	0.53	0.02	0.04	0.18	2.59	0.87
4	3.44	0.47	1.29	0.06	0.01	0.05	5.26	0.27

[a] 1, 2, 3, and 4 are the four different varieties of grape used . For each variety $n = 3$.

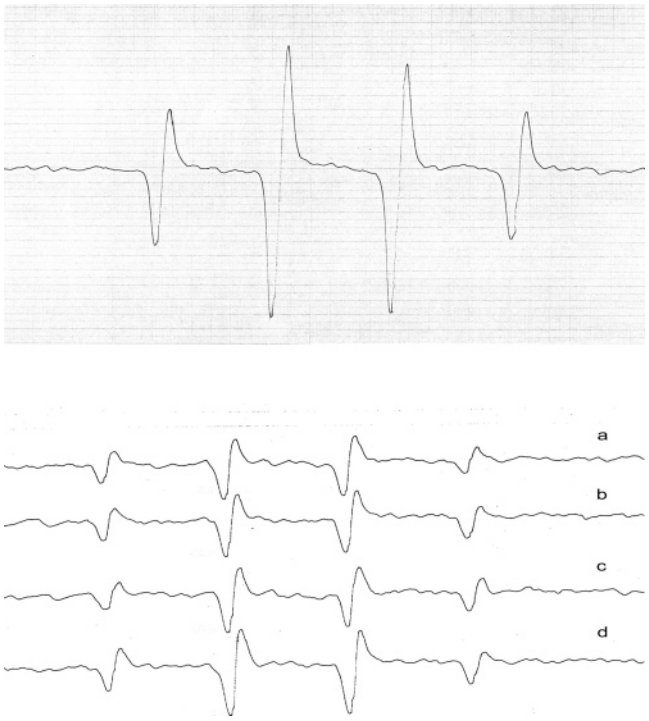

Figure 6. EPR spectrum of the DMPO-•OH adduct in the absence of antioxidant activity from grape extracts is shown on the top. Ordinate: EPR signal amplitude (arbitrary units). Abscissa: magnetic field intensity (in the range between 3340 and 3440 G). At the bottom are shown EPR spectra of the DMPO-•OH adduct in the presence of different grape extracts: (a) from entire grape; (b) from skin; (c) from flesh; (d) from seeds.

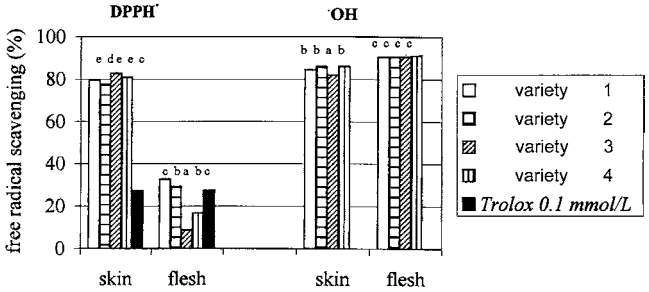

Figure 7. Scavenging activities of grape skin and flesh as percent lowering of the basal radical activity of •OH from the Fenton reaction (EPR method) and of DPPH• (DPPH• quenching method). The scavenging ativity of 0.1 mmol/L Trolox is also shown as a reference for DPPH• test. Each sample was analyzed in quadruplicate. $p < 0.05$ for flesh scavenging activity by EPR method vs flesh scavenging activity by DPPH quenching method. Small letters above the columns indicate differences among the grape varieties: equal letters = no significative difference; different letters = significative difference with variable degree of $p < 0.05$.

heart disease (*13*, *14*). Since the implication of oxygen free radicals in the pathogenesis of myocardial ischemia/reperfusion injury more than two decades ago (*15*), the role of these reactive oxygen species in many other cardiovascular diseases is becoming increasingly apparent. Under normal conditions there is a balance between the formation of pro-oxidants (oxygen free radicals) and the amount of antioxidants present. This steady-state condition is interrupted in pathophysiological conditions because of the excessive production of free radicals, or decrease in antioxidants or both.

Substantial evidence exists to support the notion that ischemia and reperfusion generate superoxide and hydroxyl radicals among other cytotoxic free radicals (*15*). The presence of reactive oxygen species were confirmed directly by estimating free radical formation and indirectly by assessing lipid peroxidation and DNA breakdown products (*11, 17*). Among the oxygen free radicals, superoxide anion (O_2^-) is the most innocuous free radical while the hydroxyl radical (•OH) proves the most detrimental to cells. Virtually all the biomolecules including simple carbohydrates (*20*), lipids, proteins, and DNAs are potential targets for •OH radical attack. The results of our study demonstrated that standardized grape extract (SGE) used in our study can directly scavenge both superoxide and hydroxyl radicals. These results were further supported by a significant reduction of MDA content in the SGE-treated hearts.

Antioxidants have long been known to protect against the damaging effects of free radical-mediated tissue injury especially ischemia reperfusion injury of the heart and other organs. Amidst the intense interest generated in light of the various findings which support red wine as being a plausible preventive intervention against coronary heart disease, we were interested if grapes, from which wines are manufactured, also possess the same cardioprotective properties. Similar to wine, grapes contain polyphenolic antioxidants including resveratrol, anthocyanins, catechin, quercetin, flavans, and several phenolics. All of these polyphenolic compounds have been shown to possess potent antioxidant properties (*5*).

In the case of grape, the skins are noted for their high phenolic content with respect to flesh. Especially in red grape varieties, high amount of anthocyanins are present, which contributes to the red color for the grapes. The wine making process for the red wines makes provision for skin maceration into the fermenting must in order to extract anthocyanins. Not only do the anthocyanins render the red color to the wine, but also they are involved in the wine aging processes by their ability to copolymerize with other flavonoids, such as catechins or flavan-

3-ols. The present data (**Table 4**) of total polyphenol index from red grape varieties confirmed this fact. Generally, polyphenols are 8-fold higher in skin than in flesh and the anthocyanins are present only in the skin extracts in relevant amounts (about 50% of total phenolic content). Grapes do not possess significant amounts of ascorbic acid and carotenoids, which could also be responsible for the reduction of ROS. The ROS scavenging properties against •OH of the grapes are mainly due to the presence of the polyphenols (anthocyanins and cinnamates) as shown in the present study.

The most important finding of the present study was that the antioxidant properties of grape skin and flesh were comparable despite of the fact that the skin exclusively contain anthocyanins and higher amounts of polyphenols. Interestingly, both skins and flesh contain almost identical amounts of sugars (**Table 2**) such as glucose and fructose, and organic acids such as tartaric, malic, and shikimic acid (**Table 3**). Many sugars contain antioxidant activities (*20*). Sugars are also involved in stress response and may act as signaling molecules facilitating cross-talk between two or more transduction pathways resulting in gene expression and modification of proteins. Signal transduction mediated by sugars and sugar-mediated gene expression can regulate the oxidative stress. While sugars certainly possess antioxidant properties, skin and flesh of grapes contain comparable amounts of glucose, fructose, and total sugar. Thus, it is unlikely that differences in antioxidant potency between skin and flesh are due to their sugar content. *Solidago* species have been used in phytotherapy for centuries to cure urological and antiphlogistical problems (*30*). *Solidago canadensis* (Asteraceae) contain, among many ingredients, cinnamates and shikimic acid connected to sugar components, which were also found in the flesh of grapes. Antioxidant activity of shikimic acid was recently identified in liposomes, emulsions, and bulk oil (*31*, *32*). Recently, antioxidant activity of white wine has been attributed to tartaric acid (*33*, *34*), which was found in the grape flesh. Alpha hydroxy acids including tartaric acid prevented skin irritation by virtue of their antioxidant function (*35*). In a recent study, cinnamic acid was found to possess potent antioxidant activity (*36*). Hypocholesterolemic and antiatherosclerotic activities of flaxseed were attributed to its cinnamic acid component (*37*). Antioxidant activity of cinnamic acids was comparable with the activities of ascorbic acid or α-tocopherol (*38*). The radical scavenging activities of *Echinacea* root extracts were attributed to the polyphenols including caftaric acid and caffeic acid (*39*), found in the flesh of grapes. A recent study attributed the antioxidant effects of chardonnay white wine in diabetic rats to several polyphenols including caffeic acid and caftaric acid (*40*). In a related study, antioxidant capacity of some varieties of French white wine was also attributed to caffeic and caftaric acids among other polyphenols (*41*). In fact, large varieties of white wines, champagnes, and chardonnay have been found to contain caftaric acid, coutaric acid, cinnamics acid, coumaric acid, and caffeic acid, all of which were detected in the flesh of grapes (*42*, *43*). In the present study, the amount of caftaric acid, an *o*-diphenol compound, was higher in grape flesh than in the skin of red grapes. Conversely, coutaric acid, the monophenol derivative of caftaric acid, was found higher in skin than in flesh. It is well-known that *o*-diphenols are more effective than the corresponding monophenols in the antioxidant action against free radicals, so the presence of a significant amount of *o*-diphenol compounds in the flesh could have contributed to the scavenging activity of OH, despite the lower absolute amounts of total polyphenols with respect to skins.

In a previous study, the free radical scavenging activity of grapes was shown to be different in skin with respect to flesh extracts (*32*, *33*). However, the study was performed using DPPH• method. As shown in the present study, DPPH method cannot detect the ROS scavenging activities of every constituent of grapes. Indeed, DPPH reacts preferentially with hydrogen donors like polyphenols (*23*), and it resulted in a higher activity of skin extract compared to that of flesh. The ROS scavenging activities were not only comparable between the two groups, but flesh contained higher scavenging activities compared to skins when EPR spectroscopy of •OH was used. Some carbohydrates such as the disaccharides maltose and sucrose at a concentration 8 mmol/L showed values very close to a powerful antiradical product such as chlorogenic acid at the same molar concentration (71% of •OH quenching at the same experimental conditions used in the present research). On the other hand, DPPH• showed a very low reactivity, practically absent, toward simple carbohydrates (*20*).

Hence, it seems likely high amount of •OH quenching was derived also from the activity of the carbohydrates and or organic acids, which were present in both in skin and in flesh. Especially, tartaric acid and malic acid show a significant ROS scavenging activity against •OH.

Grapes as opposed to other fruits and vegetables as sources of polyphenols and antioxidants are unique in a number of ways. First of all, resveratrol and a few other polyphenols are present only in grapes and are virtually absent from commonly consumed fruits and vegetables, and as such the consumption of grapes would constitute their only source in the diet. Resveratrol, a stilbene polyphenol, has recently been found to protect the hearts from ischemic reperfusion injury by its ability to reduce both necrosis and apoptosis (*45*). Resveratrol also possesses cancer chemopreventive activity (*46*) and is able to inhibit platelet aggregation (*47*) and to reduce oxidative stress in PC 12 cells (*48*). In addition, proanthocyanidins have also been found to be cardioprotective by its ability to reduce ventricular arrhythmias and cardiomyocyte apoptosis (*8*). Grape seed proanthocyanidins were also found to potentiate a signal transduction cascade by inhibiting proapoptotic genes Jnk and Jun (*7*). On the basis of the findings that resveratrol and proanthocyanidins are present in the skins and seeds of the grapes, much attention has been paid on these parts and not the flesh. The present study indicates that several organic acids and polyphenols possessing potent antioxidant activities present in the flesh of grapes are also found in white wines. The experiment was performed under identical conditions, and the animals were fed the same diet except for supplementation with either grape skin or grape flesh. Thus, the results truly reflect the effects of skin or flesh of the grapes and are not due to any changes in general nutritional condition or calorific intake. Although further study is needed to identify the principle ingredients responsible for the cardioprotective abilities of the grape flesh, to the best of our knowledge, our study provides evidence for the first time that the flesh of grapes is equally cardioprotective with respect to skins.

LITERATURE CITED

(1) Sato, M.; Ray, P. S.; Maulik, G.; Maulik, N.; Engelman, R. M.; Bertelli, A. A.; Bertelli, A.; Das, D. K. Myocardial protection with red wine extract. *J. Cardiovasc. Pharmacol.* **2000**, *35*, 263−268.

(2) Rimm, E. B.; Giovannucci, E. L.; Willett, W. C.; Colditz, G. A.; Ascherio, A.; Rosner, B.; Stampfer, M. J. Prospective study of alcohol consumption and risk of coronary disease in men. *Lancet* **1991**, *338*, 464−486.

(3) Gaziano, J. M.; Buring, J. E.; Breslow, J. L.; Goldhaber, S. Z.; Rosner, B.; VanDenburgh, M.; Willett, W.; Hennekens, C. H. Moderate alcohol intake, increased levels of high-density lipoprotein and its sub-fractions and decreased risk of myocardial infarction. *New England J. Med.* **1993**, *329*, 1829−1834.

(4) Renaud, S.; De Lorgeril, M. Wine, alcohol, platelets and the French Paradox for coronary heart disease. *Lancet* **1992**, *339*, 1523−1526.

(5) Cui, J.; Tosaki, A.; Cordis, G. A.; Bertelli, A. A.; Bertelli, A.; Maulik, N.; Das, D. K. Cardioprotective abilities of white wine. *Ann. N.Y. Acad. Sci.* **2002**, *957*, 308−316.

(6) Cui, J.; Juhasz, B.; Tosaki, A.; Maulik, N.; Das, D. K. Cardioprotection with grapes. *J. Cardiovasc. Pharmacol.* **2002**, *40*, 762−769.

(7) Sato, M.; Bagchi, D.; Tosaki, A.; Das, D. K. Grape seed proanthocyanidin reduces cardiomyocyte apoptosis by inhibiting ischemia/reperfusion-induced activation of JNK-1 and c-Jun. *Free Radical. Biol. Med.* **2001**, *31*, 729−737.

(8) Sato, M.; Maulik, G.; Ray, P. S.; Bagchi, D.; Das, D. K. Cardioprotective effects of grape seed proanthocyanidin against ischemic reperfusion injury. *J. Mol. Cell. Cardiol.* **1999**, *31*, 1289−1297.

(9) Das, S.; Alagappan, V. K.; Bagchi, D.; Sharma, H. S.; Maulik, N.; Das, D. K. Induction of iNOS-VEGF-KDR-eNOS after resveratrol consumption: a potential mechanism for resveratrol preconditioning of the heart. *Vasc. Pharmacol.* **2005**, *42*, 281−289.

(10) Das, S.; Tosaki, A.; Bagchi, D.; Maulik, N.; Das, D. K. Resveratrol-mediated activation of cAMP response element-binding protein through adenosine A3 receptor by Akt-dependent and -independent pathways. *J. Pharmacol. Exp. Ther.* **2005**, *314*, 762−769.

(11) Das, S.; Cordis, G. A.; Maulik, N.; Das, D. K. Pharmacological preconditioning with resveratrol: role of CREB-dependent Bcl-2 signaling via adenosine A3 receptor activation. *Am. J. Physiol. Heart Circ. Physiol.* **2005**, *288*, H328−H335.

(12) Hattori, R.; Otani, H.; Maulik, N.; Das, D. K. Pharmacological preconditioning with resveratrol: role of nitric oxide. *Am. J. Physiol. Heart Circ. Physiol.* **2002**, *282*, H1988−H1995.

(13) Demrow, H. S.; Slane, P. R.; Folts, J. D. Administration of wine and grape juice inhibits in vivo platelet activity and thrombosis in stenosed canine coronary arteries. *Circulation* **1995**, *91*, 1182−1188.

(14) Stein, J. H.; Keevil, J. G.; Wiebe, D. A.; Aeschlimann, S.; Folts, J. D. Purple grape juice improves endothelial function and reduces the susceptibility of LDL cholesterol to oxidation in patients with coronary artery disease *Circulation* **1999**, *100*, 1050−1055.

(15) Mark, D.; Maki, K. C. Concord grape juice reduces blood pressure in men with high systolic blood pressure. *FASEB J.* **2003**, *17*, 693.

(16) Chou, E. J.; Keevil, J. G.; Aeschlimann, S.; Wiebe, D. A.; Folts, J. D.; Stein, J. H. Effect of ingestion of purple grape juice on endothelial function in patients with coronary heart disease. *Am. J. Cardiol.* **2001**, *88*, 553−555.

(17) Engelman, D. T.; Watanabe, M.; Engelman, R. M.; Rousou, J. A.; Kisin, E.; Kagan, V. E.; Maulik, N.; Das, D. K. Hypoxic preconditioning preserves antioxidant reserve in the working rat heart. *Cardiovasc. Res.* **1995**, *29*, 133−140.

(18) Hattori, R.; Otani, H.; Maulik, N.; Das, D. K. Pharmacological preconditioning with resveratrol: role of nitric oxide. *Am. J. Physiol. Heart Circ. Physiol.* **2002**, *282*, H1988−H1995.

(19) Cordis, G. A.; Maulik, N.; Das, D. K. Detection of oxidative stress in heart by estimating the dinitrophenylhydrazine derivative of malonaldehyde. *J. Mol. Cell. Cardiol.* **1995**, *27*, 1645−1653.

(20) Morelli, R.; Russo-Volpe, S.; Bruno, N.; Lo Scalzo, R. Fenton-dependent damage to carbohydrates: free radicals scavenging activity of some simple sugars. *J. Agric. Food Chem.* **2003**, *51*, 7418−7425.

(21) Morelli, R.; Lo Scalzo, R.; Stradi, R.; Bertelli, A.; Falchi, M. Evaluation of the antioxidant activity of new carotenoid-like compounds by electron paramagnetic resonance. *Drugs Exp. Clin. Res.* **2003**, *29*, 95−100.

(22) Bertelli, A.; Falchi, M.; Lo Scalzo, R.; Morelli, R. EPR evaluation of the antiradical activity of wines containing high concentrations of resveratrol. *Drugs Exp. Clin. Res.* **2004**, *30*, 111−115.

(23) Brand-Williams, W.; Cuvelier, M. E.; Berset, C. Use of a free radical method to evaluate antioxidant activity. *Lebensm.-Wiss.u. Technol.* **1995**, *28*, 25−30.

(24) Das, D. K.; Maulik, N. Evaluation of antioxidant effectiveness in ischemia reperfusion tissue injury. *Methods Enzymol.* **1994**, *233*, 601−610.

(25) Das, D. K.; Maulik, N. Protection against free radical injury in the heart and cardiac performance. In *Exercise and Oxygen Toxicity*; Sen, C. K.; Packer, L.; Hanninen, O., Eds.; Elsevier Science: Amsterdam, 1995; pp 359−388.

(26) Arroyo, C. M.; Kramer, J. H.; Dickens, B. F.; Weglicki, W. B. Identification of free radicals in myocardial ischemia/reperfusion by spin trapping with nitrone DMPO. *FEBS Lett.* **1987**, *221*, 101−104.

(27) Tosaki, A.; Bagchi, D.; Pali, T.; Cordis, G. A.; Das, D. K. Comparisons of ESR and HPLC methods for the detection of hydroxyl radicals in ischemic/reperfused hearts. A relationship between the genesis of oxygen-free radicals and reperfusion-induced arrhythmias. *Biochem. Pharmacol.* **1993**, *45*, 961−969.

(28) Cordis, G. A.; Maulik, G.; Bagchi, D.; Riedela, W.; Das, D. K. Detection of oxidative DNA damage to ischemic reperfused rat hearts by 8-hydroxydeoxyguanosine formation. *J. Mol. Cell. Cardiol.* **1998**, *30*, 1939−1944.

(29) Saija, A.; Scalese, M.; Lanza, M.; Marzullo, D.; Bonina, F.; Castelli, F. Flavonoids as antioxidant agents: importance of their interaction with biomembranes. *Free. Rad. Biol. Med.* **1995**, *19*, 481−486.

(30) Apati, P.; Houghton, P. J.; Kery, A. HPLC. investigation of antioxidant components in solidaginins herba. *Acta Pharm. Hung.* **2004**, *74*, 223−231.

(31) Chang, Y. C.; Almy, E. A.; Blamer, G. A.; Gray, J. I.; Frost, J. W.; Strasburg, G. M. Antioxidant activity of 3-hydroshikimic acid in liposomes, emulsions, and bulk oil. *J. Agric. Food Chem.* **2003**, *51*, 2753−2757.

(32) Li, M K.; Mikola, R.; Draths, K. M.; Worden, R. M.; Frost, J. W. Fed-batch fermenter synthesis of 3-dehydroshikimic acid using recombinant Escherichia coli. *Biotechnol. Bioeng.* **1999**, *64*, 61−73.

(33) De Beer, D.; Joubert, E.; Gelderblom, W. C.; Manley, M. Antioxidant activity of South African red and white cultivar wines: free radical scavenging. *J. Agric. Food Chem.* **2003**, *51*, 902−909.

(34) Kilmartin, P. A.; Zou, H.; Waterhouse, A. L. A cyclic voltammetry method suitable for characterizing antioxidant properties of wine and wine phenolics. *J. Agric. Food Chem.* **2001**, *49*, 1957−1965.

(35) Berardesca, E.; Distante, F.; Vignoli, G. P.; Oresajo, C.; Green, B. Alpha hydroxyacids modulate stratum corneum barrier function. *Br. J. Dermatol.* **1997**, *137*, 934−938.

(36) Cos, P.; Hermans, N.; Calomme, M.; Maes, L.; De Bruyne, T.; Pieter, L.; Vli etinck, A. J.; Vanden Berghe, D. Comparative study of eight well-known polyphenolic antioxidants. *J. Pharmacy Pharmacol.* **2003**, *55*, 1291−1297.

(37) Prasad, K. Hypocholesterolemic and antiatherosclerotic effect of flax lignan complex isolated from flaxseed. *Atherosclerosis* **2005**, *179*, 269−275.

(38) Kim, D. O.; Lee, C. Y. Comprehensive study on vitamin C equivalent antioxidant capacity (VCEAC) of various polyphenolics in scavenging a free radical and its structural relationship. *Crit. Rev. Food Sci. Nutr.* **2004**, *44*, 253−273.

(39) Pellati, F.; Benvenuti, S.; Magro, L.; Melegari, M.; Soragni, F. Analysis of polyphenolic compounds and radical scavenging activity of Echinacea spp. *J. Pharm. Biomed. Anal.* **2004**, *35*, 289−301.

(40) Landrault, N.; Poucheret, P.; Azay, J.; Krosniak, M.; Gasc, F.; Jenin, C.; Cros G.; Teissedre, P. L. Effect of a polyphenols-enriched chardonnay white wine in diabetic rats. *J. Agric. Food Chem.* **2003**, *51*, 311−318.

(41) Landrault, N.; Poucheret, P.; Ravel, P.; Gasc, F.; Cros, G.; Teissedre, P. L.; Antioxidant capacities and phenolics levels of French wines from different varieties and vintages. *J. Agric. Food Chem.* **2001**, *49*, 3341−3348.

(42) Chamkha, M.; Cathala, B.; Cheynier, V.; Douillard, R. Phenolic composition of champagnes from chardonnay and pinol noir vintages. *J. Agric. Food Chem.* **2003**, *51*, 3179−3184.

(43) Pozo-Bayon, M. A.; Hernandez, M. T.; Martin-Alvarez, P. J.; Polo, M. C. Study of Low Molecular Weight Phenolic Compounds during the Aging of Sparkling Wines Manufactured with Red and White Grape Varieties. *J. Agric. Food Chem.* **2003**, *51*, 2089−2095.

(44) Sánchez-Moreno, C.; Larrauri, J. A.; Saura-Calixto, F. Free radical scavenging activity and inhibition of lipid oxidation of wines, grape juices and related poliphenolic constituents. *Food Res. Int.* **1999**, *32*, 407−412.

(45) Ray, P. S.; Maulik, G.; Cordis, G. A.; Bertelli, A. A.; Bertelli, A.; Das, D. K. The red wine antioxidant resveratrol protects isolated rat hearts from ischemia reperfusion injury. *Free Radic. Biol. Med.* **1999**, *27*, 160−169.

(46) Jang, M.; Cai, L.; Udeani, G. O.; Slowing, K. V.; Thomas, C. F.; Beecher, C. W.; Fong, H. H.; Farnsworth, N. R.; Kinghorn, A. D.; Mehta, R. G.; Moon, R. C.; Pezzuto, J. M. Cancer chemopreventive activity of resveratrol, a natural product derived from grapes. *Science* **1997**, *275*, 218−220.

(47) Bertelli, A. A.; Giovannini, L.; Giannessi, D.; Migliori, M.; Bernini, W.; Fregoni, M.; Bertelli, A. Antiplatelet activity of synthetic and natural resveratrol in red wine. *Int. J. Tissue React.* **1995**, *17*, 1−3.

(48) Chanvitayapongs, S.; Draczynska-Lusiak, B.; Sun, A. Y. Amelioration of oxidative stress by antioxidants and resveratrol in PC12 cells. *Neuroreport* **1997**, *6*, 1499−1502.

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