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# Prune melanoidins protect against oxidative stress and endothelial cell death

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## 1. ABSTRACT

The health-promoting effects of fruit and vegetable consumption are thought to be due to phytochemicals contained in fresh plant material. Whether processed plant foods provide the same benefits as unprocessed ones is an open question. Melanoidins from heat-processed plums (prunes) were isolated and their presence confirmed by hydroxymethylfurfural content and browning index. Oxidative-induced endothelial cell (EC) damage is the trigger for the development of cardiovascular diseases (CVD); therefore the potential protective effect of prune melanoidins on hydrogen peroxide-induced oxidative cell damage was investigated on human endothelial ECV304 cells. Cytoplasmic and mitochondrial redox status was assessed by using the novel, redox-sensitive**,** ratiometric fluorescent protein sensor (roGFP), while mitochondrial membrane potential (MMP) was investigated with the fluorescent dye, JC-1. Treatment of ECV304 cells with hydrogen peroxide dose-dependently induced both mitochondrial and cytoplasmic oxidation, in addition to MMP dissipation, with ensuing cell death. Pretreatment of ECV304 with prune melanoidins, significantly counteracted and ultimately abolished hydrogen peroxide elicited phenomena, clearly indicating that these polymers protect human EC against oxidative stress.

## 2. INTRODUCTION

Inverse associations between diets rich in plant foods and pathological conditions such as cancer and cardiovascular diseases (CVD) have been reported in several epidemiological studies (1). The health-promoting effect of fruit and vegetable consumption is thought to be due to fresh plant-contained phytochemicals, including antioxidant compounds such as phenolic compounds, carotenoids and vitamins (1). However, a remarkable amount of the food intake in the human diet comes from processed foodstuffs, and whether processed foods provide less benefit than unprocessed ones is uncertain.

Food processing operations are mostly based on heating. Thermal treatments often result in non-enzymatic browning (NEB), which occurs through sugar thermal degradation (caramelisation), or, when more acidic conditions are present, by the Maillard reaction (MR) between sugar and organic acids (2). The high molecular-weight heterogeneous polymers formed in the last stage of the NEB reaction are called melanoidins. Melanoidins are widely distributed in home- and industrial-processed foodstuffs and may have various *in vitro* functions, for instance as an antioxidant (3), an antihypertensive (4) and a metal-binder (5). Of particular interest is the antioxidant

activity of melanoidins; since these products are naturally formed during food processing and storage, they can influence the oxidative and shelf life of several type of foods including cereals (6), coffee and tomatoes (7). In line with their antioxidant activity, some physiological effects, including the protection of cells from oxidative-induced damage, have been reported (8-10).

Prunes are obtained by drying the fruit of certain cultivars of *Prunus domestica* L. (*Rosaceae*), and possess the highest antioxidant activity among the most commonly consumed fruits and vegetables (11). The biological effect of prunes on human health has been attributed, in part, to their high polyphenol content and antioxidant capacity (11, 12), which is due to their large amounts of caffeoylquinic acid isomers (12, 13) and flavonoids (14). *In vivo* and *in vitro* experiments indicate that prunes have high antioxidant capacity (15) along with the ability to inhibit LDL oxidation (12) and to reduce atherosclerosis lesions (16). We have previous reported that drying two common plum varieties to produce prunes resulted in a two to three-fold increase in antioxidant activity, even though it considerably reduced the phenol content (17, 18). We hypothesized that this increase might have been due to the formation of non-enzymatic browning products (NEBPs) (e.g. Melanoidins) after drying. Thus, although the effect of polyphenolic compounds cannot be ruled out, melanoidins appear to be the prevailing contributors to the reported antioxidant activity of prunes *in vitro*. In this regard, although the antioxidant properties of melanoidins have been studied *in vitro* for several years their potential antioxidant effects on *in vivo* biological systems such as human cells has been little investigated and is largely unknown.

Association of increased reactive oxygen species (ROS) with many pathological conditions (19, 20) suggests that counteracting oxidative stress with antioxidants could prevent disease occurrence or ameliorate its effects. For this reason a great deal of attention is now focusing on naturally occurring antioxidants as potential candidates for disease prevention and/or treatment. The endothelial cell (EC) plays a crucial role in the integration and modulation of signals within the vascular wall (21) and perturbation of such homeostasis by oxidative damage is the trigger for the development of CVD (22). The present work was undertaken with the intent to investigate whether food melanoidins isolated from prunes might protect human EC against $H_2O_2$-induced oxidative stress and cell damage.

## 3. MATERIALS AND METHODS

### 3.1. Chemicals

Unless stated in the text all the reagents used were from Sigma (Sigma, St Louis, MO).

### 3.2. Sampling and dehydration

Prunes were prepared using fruit of the President cultivar. These were bought in a local market at an optimum stage of ripening, pre-treated and dried at 85°C as previously described (17). Before analysis, the dried fruit was packed in co-extruded plastic bags and kept in a freezer at -20 °C.

### 3.3. Determination of HMF, phenolic content and total antioxidant activity

The polyphenol fraction, which was used to assess both phenols and hydroxymethylfurfural (HMF), was extracted and analyzed by HPLC as previously reported (17, 18) using well-established methods (12, 23). A Hewlett Packard Series 1090 liquid chromatograph coupled with a diode array detector was used and operating conditions were as previously reported (17, 18). A Gemini 5 µm C18, 250x4.6 mm column was fitted with a Gemini pre-column C18, 4.0x3.0 mm (Phenomenex, Torrance, CA) and used. The injection sample was 10 µL. Spectra acquisitions and quantifications for HMF and phenols were performed as detailed by us elsewhere (17). All values were expressed as milligrams per kilogram of dry matter (dm) and were calculated as the average of four measurements. The total phenol content was obtained by adding the values of the single phenols detected. Antioxidant activity was evaluated using the radical DPPH method as previously described in detail (17) and expressed as $Abs^{-3}$ $min^{-1}$ $g^{-1}$ of dm.

### 3.4. Melanoidins extraction

The extraction of melanoidins was carried out following a previously published method (24). In detail, 100 g of pitted and ground prunes were defatted with $CHCl_3$ while stirring. After solvent evaporation, the operation was repeated twice more. Solvent traces were eliminated by rotary evaporation. 200 ml of bi-distilled water were added to this residual solid, and the resulting slurry was sonicated for 30 minutes at 40°C. The water fraction was collected and the operation repeated on the solid phase. The two water fractions were combined and centrifuged at 8400 g for 15 minutes at 15°C, and the supernatant was then evaporated under vacuum at the maximum temperature of 50°C (fraction I). The residual solid was added to that of fraction I and dissolved in 200 ml of ethanol/water (60:40 V/V), and the resulting slurry was then sonicated for 30 minutes at room temperature. This operation was repeated. The two ethanol:water fractions were combined and centrifuged at 8400 g for 15 minutes at 15°C, and the supernatant was then evaporated under vacuum at the maximum T of 50°C (fraction II). The residual solid was added to that of fraction II and dissolved in 200 mL of 2-propanol/water (50:50 V/V), and the resulting slurry was then sonicated for 60 minutes at room temperature. This operation was repeated. The two propanol/water fractions were combined and centrifuged at 8400 g C for 15 minutes at 15°, and the supernatant was then evaporated under vacuum at the maximum T of 50°C (fraction III). The remaining solid fraction, which consisted of pieces of fruit, was fraction IV. The yield of each fraction (as g per 100 g of dried fruit) was recorded. Fraction IV had no *in vitro* antioxidant activity, and thus it was not taken into consideration.

### 3.5. Measurements of browning index

The formation of brown pigment due to the NEB reaction can be estimated as a brown index from spectrophotometric readings at 420 nm. For this reason the polyphenolic fractions from fresh and dried samples, as well as the melanoidin fractions extracted from dried

prunes were subjected to a spectrophotometric reading in absorbance mode at 420 nm in a 1 cm glass cuvette. The samples were appropriately diluted in water to give absorbance values of <1. These values were used to give an absorbance value per g dm of each diluted fraction. Five measurements were made for each sample.

### 3.6. Cells culture, treatments, and viability assay

ECV304 is an EC line established from the vein of an apparently normal human umbilical cord. This cell line has been proposed as a suitable model for providing novel insights into the mechanisms governing EC biology under both physiological and pathological conditions (25-29). ECV304 were provided by the European Collection of Animal Cell Cultures (ECACC Salisbury, UK). Cells were grown in medium M199 supplemented with 10% fetal bovine serum (Invitrogen, Carlsbad, CA), 100 µg/m1 penicillin, and 100 µg/m1 streptomycin (Invitrogen,). Cells were maintained in a standard culture incubator with humidified air containing 5% $CO_2$ at 37° C. The day before each experiment, cells were plated in 24-well plates (Corning, Lowell, MA) at a concentration of 100,000 cells per well and pretreated with melanoidins for 6 hrs before oxidative stress was induced in the last 2 hrs, by treatment with the indicated concentration of hydrogen peroxide ($H_2O_2$). In accord with a previously study using coffee melanoidins on human hepatoma cells (9), the doses of 2, 6 and 12 µg/ml were tested in our human vascular model. Cell viability for treated and untreated cell was assessed after 24 hrs by automatic cell counting (Countess® Invitrogen) and expressed as number of cells per ml.

### 3.7. Determination of cellular redox status

Cellular redox status was investigated by using the redox-sensing green fluorescent protein (roGFP), which reports the redox status of the GSH/GSSG pool *in vivo* in both plant and mammalian cells (30, 31). Plasmids coding for roGFP2 expression were obtained starting from p*CVU55762-roGFP2* (kindly provided by Dr. Andreas J. Meyer, University of Heidelberg, Germany). Cyt-roGFP2 was obtained by restriction cloning using *Bam*HI and *Not*I restriction enzymes into pcCDNA3 vector (Invitrogen); mit-roGFP2 was obtained by cloning a PCR amplification product into pCMV/myc/mito (Invitrogen) using *Pst*I and *Xho*I sites. Plasmids containing cytoplasmic roGFP2 (cyt-RoGFP) and a mitochondrial targeted roGFP2 (mit-RoGFP) were transfected in HCV304 by using the lipofectamine 2000 reagent following the provider protocol (Invitrogen). Transfected cells were selected using 0.8 mg/mL of G418 in the media for 3 to 4 weeks. Positive stably transfectants were selected by serial dilution of G418-resistant clones which constitutively expressed both cyt- and mit-RoGFP2 under a fluorescence microscope (Olympus XI70). RoGFP2 has two fluorescence excitation maxima at 400 (oxidized form) and 485 nm (reduced form) and display rapid and reversible ratiometric changes in fluorescence in response to changes in ambient redox potential. The ratios of fluorescence from excitation at 400 and 485 nm indicate the extent of oxidation and thus the redox potential while canceling out the amount of indicator and the absolute optical sensitivity (31). In place of confocal imaging analysis we used a recently developed fluorometer-based method for monitoring roGFP oxidation (32). Fluorescence measurements were performed in clear 24-well plates (Corning, Lowell, MA) on a fluorescence plate reader GENios plus (Tecan, Männedorf, CH) from the upper side using multiple reads per well (the read pattern was square, and the number of reads was 2 x 2). Cells were excited by using 400 and 485 nm filters and fluorescence values were measured using 535 nm emission filter. For background correction emission intensities were determined for non-transformed cells (4 discs each experiment) exposed to same excitation wavelengths under the same conditions. These values were averaged and subtracted from the fluorescence values of roGFP2. The degree of oxidation of the roGFP2 was estimated from the ratios of light intensities obtained during 1-min intervals under 400- and 485-nm excitation. Treatment-induced variations of roGFP2 oxidation were estimated by comparison with roGFP oxidation in control untreated cells.

### 3.8. Measurement of mitochondrial membrane potential

Measurement of mitochondrial membrane potential (MMP) was performed with the JC-1 stain (Invitrogen), a lipophilic cation fluorescent dye that accumulates in mitochondria in a MMP-dependent manner, showing red fluorescent JC-1 aggregates (590 nm emissions) at higher MMP. When MMP decreases, JC-1 aggregates depart from mitochondria and change to green fluorescent JC-1 monomers (535 nm emissions). Therefore, the ratio of the red signal to the green can been used to detect the occurrence of MMP depolarization in the early stages of cell death due to mitochondrial damage (33, 34). After treatments cells were incubated at room temperature in the dark with 5µg/ml JC-1 in HBSS for 30 minutes. The cells were then washed twice with HBSS and fluorescence levels were immediately acquired with excitation and emission wavelengths set at 535 and 590 nm, respectively, for red fluorescence, and 485 and 535 nm, respectively, for green fluorescence. Measurements were performed in clear 24-well plates (Corning, Lowell, MA) on a fluorescence plate reader GENios plus (Tecan, Männedorf, CH) from the upper side using multiple reads per well (the read pattern was square, and the number of reads was 2 x 2). For each sample, the results were calculated as the ratio (red/green) of fluorescence of sample, averaged after the fluorescence values had been corrected for the background and protein content.

### 3.9. Statistical analysis

Data were expressed as means ± S.D. of three or four different experiments. One-way analysis of variance (ANOVA) followed by a post-hoc Newman-Keuls Multiple Comparison Test were used to detect differences of means among treatments with significance defined as $P < 0.05$. Statistical analysis was performed using GraphPad Prism version 5.00 for Windows, GraphPad Software, San Diego California USA.

## 4. RESULTS AND DISCUSSION

During their lifetime cells are subjected to oxidative damage, which is reported to be associated with several pathological conditions including cancer and CVD

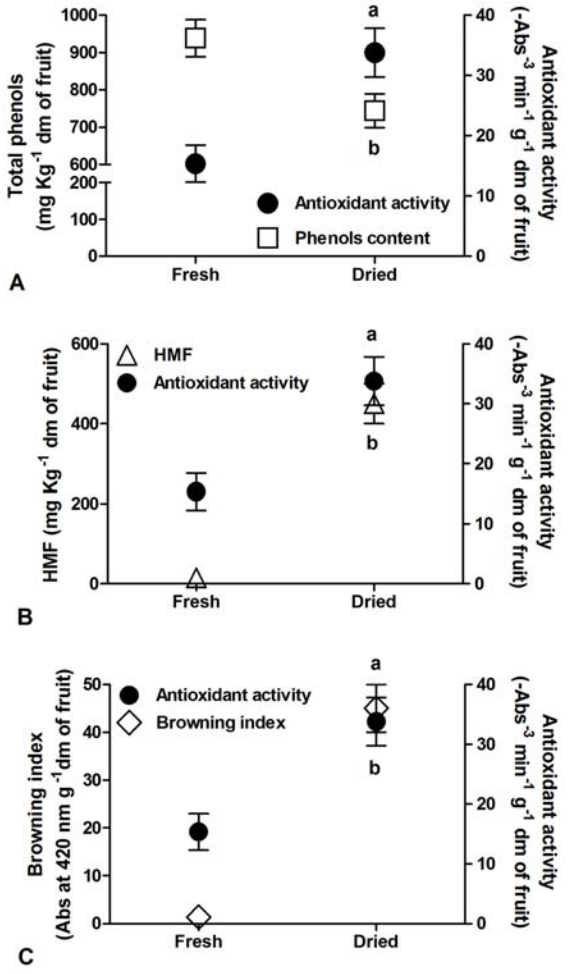


**Figure 1.** Changes in chemical parameters elicited by fruit processing. Changes in antioxidant activity (A-C), Phenols content (A), hydroxymethylfurfural (HMF) (B) and Browning index (C) during transformation of fresh fruit (plums) to dried (prunes). Data are the mean ± standard deviation (SD) from four measurements. (A-B) a; b, significantly different from the fresh sample.

(19, 20). Although endogenous antioxidants play an important role in protecting cells against oxidative insults, additional antioxidants (e.g. dietary antioxidants) appear to be required to prevent or to protect living cells from oxidation (1). In this context, health benefits exerted by plant-derived compounds and extracts have been mainly ascribed to their antioxidant potential and the resulting capability to counteract oxidative-induced damage (1). However, during food processing and storage, chemical reactions among food components lead to both destruction and formation of phytonutrients (7), therefore whether processed plant foods provide the same benefits as those ascribed to unprocessed ones is uncertain. For instance, melanoidins are heterogeneous polymeric structures formed during food processing in the last stage of the MR whose effects on human health are largely unknown. Indeed, although the antioxidant properties of melanoidins have been studied *in vitro* for several years their potential antioxidant effects on *in vivo* biological systems such as human cells has been little investigated and is barely known. In this light, we explored the effect of melanoidins formed during the transformation of plums to prunes against $H_2O_2$-induced oxidative stress and cell damage. Using a previously published procedure (17), fresh fruit was processed by standardized drying and heating conditions, then both fresh fruit (plums) and processed (prunes) were chemically characterized on the basis of commonly recognized parameters (17, 18), namely the presence of intermediate (hydroxymethylfurfural) and final (NEBPs) products of the MR. The compound hydroxymethylfurfural (HMF) is practically absent in fresh food, but it is naturally created in sugar-containing food during heat-treatments like drying or cooking. HMF is formed in the MR and is one of the intermediate products of the NEB (12, 23). Despite the decreased total phenol content (Figure 1A) an increase in antioxidant capacity was elicited by the food transformation process, which strongly correlated with the paralleled increase of HMF content (Figure 1B). The processing-induced increase of HMF was also accompanied by a significant rising in the browning index (Figure 1C), suggesting NEBPs may be responsible for the increase in chain-breaking activity observed after fruit transformation. Indeed, the absorbance at 420 nm, which represents the browning index, is an important parameter for the presence of NEBPs and is related to the brown pigment formation caused by the NEB reaction during plum processing (Figure 1C) (35). The measurements of the browning index indicate that NEBPs were presents in all melanoidin fractions extracted from dried samples, while (as with whole fresh fruit samples) they were completely absent on those melanoidin fractions extracted from fresh fruit (Table 1). Noteworthily, the amount of NEBPs in the whole dried fruit samples was basically the sum of the NEBPs detected in the three melanoidins fractions, indicating they were present in the same amount in the prunes and in the melanoidin fractions extracted from prunes (Table 1). Fraction I showed the highest amount of NEBPs among all the melanoidin fractions, and was therefore chosen to be tested for its antioxidant activity on cells exposed to oxidative stress.

A variety of pathogenic stimuli can increase ROS production within the EC, and oxidative-induced EC dysfunction is emerging as the probable initial step in the progression toward pathological conditions such as atherosclerosis and hypertension (22). Given the pivotal role played by the endothelium in cardiovascular homeostasis and the involvement of EC dysfunction in CVD pathogenesis (22), it was reasonable for us to use a human EC line to investigate the effect of melanoidins on $H_2O_2$-induced oxidative damage. In order to mimic oxidative damage and set standard conditions for the following experiments, we first investigated the effect of different doses of $H_2O_2$ on ECV304 cell death and mitochondrial damage. As expected, 2hr-treatment of ECV304 cells with $H_2O_2$ resulted in a dose-dependent decrease of cell survival as evidenced by the significant decrease in the number of viable cells in comparison with the untreated control group (Figure 2A). Some pathological

**Table 1.** browning index of whole fruit sample and of melanoidins extracted from fresh plums and dried prunes

| Samples | Fraction I[1] | Fraction II[1] | Fraction III[1] | Total[2] |
|---|---|---|---|---|
| Fresh plums | 0 | 0 | 0 | |
| Dried prunes | 23.02 ±2.00[3] | 11.49±1.45[3] | 12.43±2.20[3] | 46.95±5.24[3] |

[1] melanoidins fractions, [2] whole fruit, [3] non enzymatic browning products (NEBPs), Abs at 420nm $g^{-1}$ dm of fruit. Data are the mean ± standard deviation (SD) from four measurements

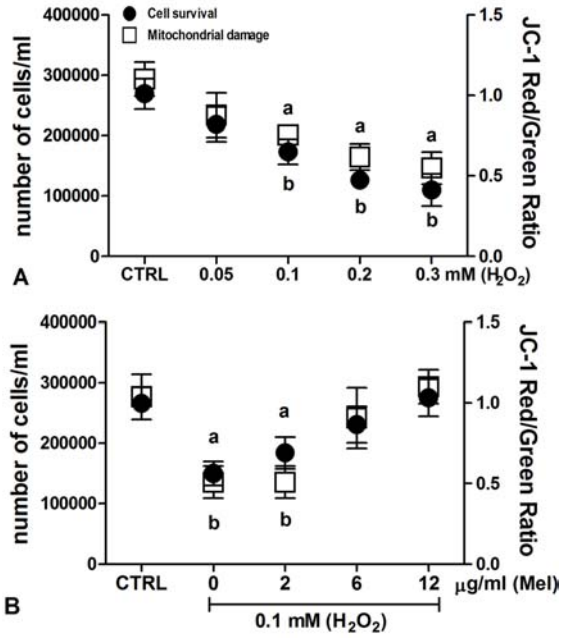


**Figure 2.** Melanoidins protect human endothelial cells from hydrogen peroxide-induced cell death. Dose-dependent effect of hydrogen peroxide ($H_2O_2$) on cell survival and mitochondrial damage (A). Dose-dependent effect of melanoidins on $H_2O_2$-induced mitochondrial damage and cell death. Data are the mean ± standard deviation (SD) of four experiments. (A-B) a; b, significantly different from the control.

stimuli, including ROS, can trigger an increase in mitochondrial membrane permeability promoting the release of toxic factors and the dissipation of MMP. The consequence of such mitochondrial dysfunction is a bioenergetic catastrophe culminating in the disruption of plasma membrane integrity with ensuing cell death (36). Thus regulation of cell death has emerged as a second major function of mitochondria, in addition to their established role in energy metabolism (36). Our experimental results indicated that $H_2O_2$-induced cell death was indeed associated with a superimposable loss of MMP, which strongly implicated mitochondria in the cell death elicited by $H_2O_2$ (Figure 2A). Based on these experiments, around 50% of $H_2O_2$-induced mitochondrial impairment and cell death was observed at 0.1 mM of $H_2O_2$., We therefore used this concentration as the standard condition to mimic oxidative-induced cell damage in the following experiments. Previous studies indicate that pretreatment of human hepatoma cells with coffee (9) and biscuit (10) melanoidins exerts remarkable protection against oxidative-induced cell death; therefore we investigated whether prune melanoidins would exert any protective effect on the observed $H_2O_2$-induced cell damage. To this end, cells were treated with melanoidins for 6 hrs and $H_2O_2$ was added during the last 2 hrs of incubation to induce oxidative stress. As reported in Figure 2B melanoidin pretreatment was able to dose-dependently counteract both cell death and MMP impairment as induced by 0.1 mM $H_2O_2$, strongly indicating a protective effect of these polymeric compounds against oxidative stress and mitochondrial-mediated cell death.

With the intention of elucidating the molecular mechanisms underpinning prune melanoidin protection, we used a redox-sensitive green fluorescent protein (roGFP) to investigate the effect of these polymers on $H_2O_2$-induced intracellular oxidative change. Fluorescence imaging of ROS in live cells has been widely used to assess intracellular oxidation in different cellular compartments and under various experimental conditions (37). However, many of the methods so far employed to determine the levels of intracellular ROS suffer from various pitfalls (37). Because electrons for ROS detoxification derive at least in part from the GSH pool, variations of ROS levels are manifested in concomitant changes in the thiol redox potential, which is reflected in the reduced to oxidized glutathione ratio (GSH/GSSG) (38). RoGFP2 can be targeted to various cellular compartments and due to two engineered cysteine thiols is sensitive to environment redox change resulting in a thiol-thiol bond (30, 31). Dithiol formation causes reciprocal changes in roGFP emission intensity when excited at two different wavelengths. Thus the analysis of roGFP fluorescence measures the redox status of the intracellular GSH/GSSG pool (30, 31). To follow intracellular redox changes during our experimentation we established two ECV304 lines constitutionally expressing mit- and cyt-roGFP2 (Figure 3). As reported in Figure 4A, the treatment of roGFP expressing cells with different $H_2O_2$ concentrations, dose-dependently shifted the ECV304 intracellular redox status toward a more oxidative condition in both mitochondrial and cytosolic compartments, indicating that under our experimental conditions roGFP2 has a significant dynamic range and responds linearly to increasing doses of a well-known oxidant. Note that the observed increase of intracellular oxidative conditions elicited by the applied doses of oxidant was paralleled by a corresponding dose-dependent increase in mitochondrial damage and cell death (Figure 2A), confirming the relationship between these $H_2O_2$-elicited phenomena.

We next wanted to determine whether the cellular protection elicited by melanoidins was due to counteraction of $H_2O_2$-induced intracellular oxidation. To this end, roGFP expressing cells were treated with melanoidins for 6 hrs and 1 mM of $H_2O_2$ was added during the last 2 hrs of incubation. At the end of the experiment both mit- and

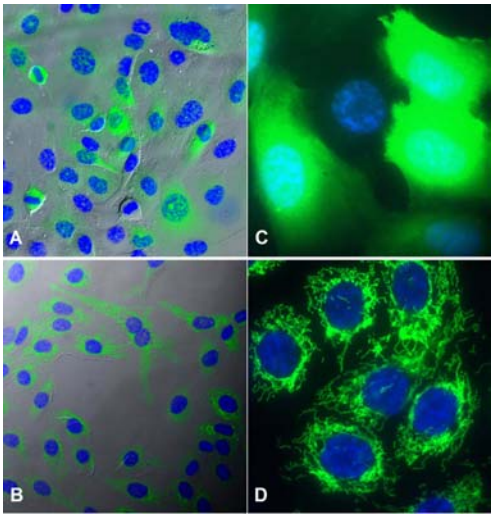


**Figure 3.** ECV304 cells lines constitutively expressing cytoplasmic and mitochondrial roGFP. Cells were grown in glass chamber slides at concentrations to allow 70-80% confluence in 24 hrs. On the day of experiments, cells were washed with PBS three times, fixed with 4% paraformaldehyde, counterstained with Hoechst, mounted and visualized by fluorescence microscopy. Right images depict cytoplasmic (A) and mitochondrial (B) merged images of *roGFP2* (green), nuclear hoechst staining (blue) and bright-field of ECV304 cells at 40X magnification. Left images depict cytoplasmic (C) and mitochondrial (D) merged images of *roGFP2* (green) and nuclear Hoechst staining (blue) of ECV304 cells at 100X magnification.

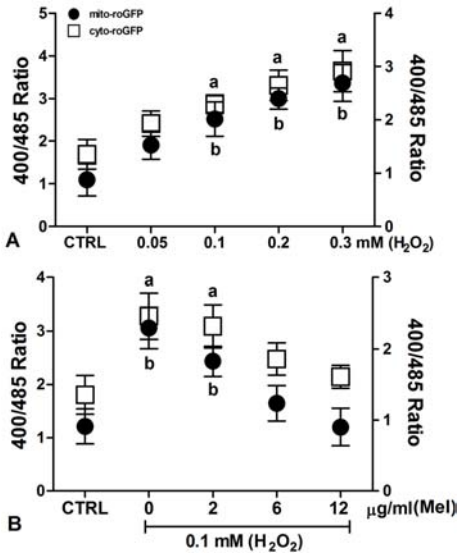


**Figure 4.** Melanoidins protect human endothelial cells from hydrogen peroxide-induced oxidative stress. Dose-dependent effect of hydrogen peroxide (H2O2) on cytoplasmic (cyt-roGFP) and mitochondrial (mit-roGFP) ro GFP2 oxidation (A). Dose-dependent effect of melanoidins on H2O2-induced cytoplasmic (cyt-roGFP) and mitochondrial (mit-roGFP) ro GFP2 oxidation. Data are the mean ± standard deviation (SD) of four experiments. (A-B) a; b, significantly different from the control.

cty-roGFP fluorescence were recorded. Data reported in Figure 4B indicate that melanoidins were able to dose-dependently inhibit intracellular oxidation elicited by 0.1 mM $H_2O_2$ and reestablish an intracellular redox state similar to that of control cells. Consistent with this antioxidant effect is the observed dose-associated protection exerted by prune melanoidins against $H_2O_2$-induced MMP depolarization and cell death (Figure 2B), indicating a tight link between their antioxidant activity and cellular protection.

The direct link between oxidative stress, MMP depolarization and cell death is now clearly demonstrated and widely accepted (36). In our vascular cell model $H_2O_2$ was able to dose-dependently elicit all three of the above-mentioned phenomena, clearly indicating that cell death occurred because of the intracellular oxidative conditions and the mitochondrial damage. Pretreatment of cultured cells with prune melanoidins, significantly counteracted and ultimately abolished $H_2O_2$-induced intracellular oxidation, MMP depolarization and cell death. To our knowledge, this is the first work reporting the protective effect of prune melanoidins against oxidative-induced cell death. Moreover, using a novel genetically engineered fluorescence protein to assess the redox status of intracellular compartments, our data confirm and reinforce previously published works using coffee (9), biscuit (10) and synthesized melanoidins (8). Melanoidins indeed, appear to work as antioxidants by positively modulating the GSSG/GSH ratio in favor of the reduced form, and thus favorably preparing the cell to face oxidative insult. The present results extend previously published observations by studying in detail the mechanism of cellular protection afforded by melanoidins. This mechanism clearly involves protection against intra-mitochondrial oxidation and oxidative-induced MMP depolarization. We believe this work adds new insight concerning the effect of processed plant foods on cellular physiology. Indeed, melanoidins from different sources could have different effects on the same (or on different) cellular models, and because of the lack of knowledge in this field, it is imperative that various melanoidins be evaluated under different experimental conditions to determine their effects. Our results demonstrated a protective effect of prune melanoidins in an oxidatively stressed human vascular model, suggesting that these polymeric compounds are the principal candidates for the cardiovascular benefit exerted by prunes *in vivo* (11, 15, 16).

## 5. ACKNOWLEDGEMENT

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**Abbreviations:** CVD, cardiovascular diseases; EC, endothelial cell; MMP, mitochondrial membrane potential; NEB, non-enzymatic browning; MR, maillard reaction; NEBPs, non-enzymatic browning products; ROS, reactive oxygen species; HMF, hydroxymethylfurfural; MMP, mitochondrial membrane potential.

**Key Words:** Maillard Reaction Products, Prunes, Melanoidins, Reactive Oxygen Species, Antioxidant, Endothelial Cell Protection

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