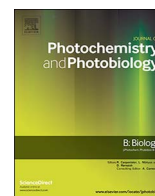




Contents lists available at ScienceDirect

Journal of Photochemistry & Photobiology, B: Biology

journal homepage: www.elsevier.com/locate/jphotobiol

Malvidin and cyanidin derivatives from açai fruit (*Euterpe oleracea* Mart.) counteract UV-A-induced oxidative stress in immortalized fibroblasts



Ganna Petruk^a, Anna Illiano^a, Rita Del Giudice^a, Assunta Raiola^b, Angela Amoresano^a, Maria Manuela Rigano^b, Renata Piccoli^{a,c}, Daria Maria Monti^{a,c,*}

^a Department of Chemical Sciences, University of Naples Federico II, Complesso Universitario Monte Sant'Angelo, via Cinthia 4, 80126 Naples, Italy

^b Department of Agricultural Sciences, University of Naples Federico II, Via Università 100, 80055 Portici, Naples, Italy

^c Istituto Nazionale di Biostrutture e Biosistemi (INBB), Rome, Italy

ARTICLE INFO

Keywords:

Euterpe oleracea
Antioxidants
Phenolics
Malvidin
Oxidative stress
UV-A radiations
Eukaryotic cells

ABSTRACT

UV-A radiations are known to induce cellular oxidative stress, leading to premature skin aging. Consumption of açai fruit (*Euterpe oleracea* Martius) is known to have many health benefits due to its high level of antioxidants. Herein, we analyzed the ability of phenolic compounds extracted from this fruit to attenuate UV-A-induced oxidative stress in immortalized fibroblast.

A methanol/water açai extract was fractionated by HPLC and each fraction tested for anti-oxidant stress activity. Immortalized fibroblasts were pre-incubated with açai fractions and then exposed to UV-A radiations. Açai extract was found to be able to strongly protect cells from oxidative stress. In particular, reactive oxygen species (ROS) production, GSH depletion, lipid peroxidation and no increase in the phosphorylation levels of proteins involved in the oxidative stress pathway was observed in cells pre-incubated with the extract and then irradiated by UV-A. Mass spectrometry analyses of HPLC fractionated extract led us to the identification of malvidin and cyanidin derivatives as the most active molecules able to counteract the negative effects induced by UV-A irradiation.

Our results indicate, for the first time, that açai fruit is a valuable natural source for malvidin and cyanidin to be used as anti-stress molecules and represent good candidates for dietary intervention in the prevention of age related skin damage.

1. Background

In the last few years, an increasing attention has focused on age-related diseases, including skin aging. Oxidative stress caused by aging is considered a general initiating factor of neurodegeneration and carcinogenesis [1]. Indeed, it is known that the skin is constantly exposed to oxidative stress induced by reactive oxygen species (ROS), generated by endogenous (i.e. enzyme activities) or exogenous sources [2,3].

UV radiation from sunlight is one of the most important health-related environmental factors because of its hazardous effects, which include generation of skin cancer, suppression of the immune system, and premature skin aging [2]. In particular, UV-A (400–315 nm)

radiations are weakly absorbed by DNA, but rather excite endogenous chromophores, leading to DNA damage. This occurs through the production of reactive singlet oxygen that specifically reacts with guanine within the DNA molecule [4,5]. UV-A radiations may also promote the formation of hydroxyl radicals via the photosensitized production of superoxide anions. Because of their high reactivity and low specificity, hydroxyl radicals likely induce a wide range of DNA damage [6]. Fibroblasts, cells of the dermis, are continuously exposed to UV-A radiations, which are able to penetrate deeply in the skin. The cellular antioxidant defense system is composed of endogenously produced antioxidant molecules, but the intrinsic mechanism for antioxidant defense is gradually impaired with aging, resulting in an inability to deal with ROS generation [7]. A continuous and regular

Abbreviations: ABTS, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid); DCF, 2',7'-dichlorofluorescein; DTNB, 5,5'-dithiobis-2-nitrobenzoic acid; EDTA, ethylene diamine tetra acetic acid; HAA, hydrophilic antioxidant activity; H₂-DCFDA, 2',7'-dichlorodihydrofluorescein diacetate; MALDI, matrix-assisted laser desorption/ionization; MS, mass spectrometry; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; P-HSP-27, phosphorylated heat shock protein; P-p38, phosphorylated p38 MAP kinase; P-MAPKAPK-2, phosphorylated MAP kinase-activated protein kinase; ROS, reactive oxygen species; r.t., room temperature; TBA, thiobarbituric acid; TBARS, TBA reactive substances; TFA, trifluoroacetic acid; TNB, 5-thio-2-nitrobenzoic acid

* Corresponding author at: Department of Chemical Sciences, University of Naples Federico II, Complesso Universitario Monte Sant'Angelo, via Cinthia 4, 80126 Naples, Italy.

E-mail address: mdmonti@unina.it (D.M. Monti).

<http://dx.doi.org/10.1016/j.jphotobiol.2017.05.013>

Received 27 February 2017; Received in revised form 5 May 2017; Accepted 9 May 2017

Available online 12 May 2017

1011-1344/ © 2017 Elsevier B.V. All rights reserved.

Table 1
Total phenolic acids, total flavonoids and hydrophilic antioxidant activity in açai extract.

Açai antioxidant determination	Value
Total phenols (mg/100 g DW)	192.41 ± 10.78
Total flavonoid (mg/100 g DW)	159.67 ± 7.65
HAA (mmol TE/100 g DW)	2.1 ± 0.17

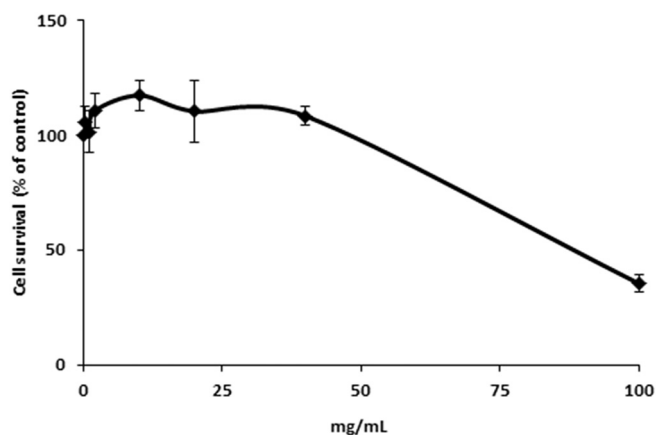


Fig. 1. Effect of açai extracts on the viability of BALB/3T3 fibroblasts. Dose-response curve of BALB/3T3 cells after 48 h incubation with increasing concentrations of açai extracts. Cell viability was assessed by the MTT assay and expressed as described in Materials and Methods section. Values are given as means ± S.D. (n ≥ 3).

intake of vitamins, trace metals, polyunsaturated fatty acids, and polyphenols from food sources contributes to counteract oxidative stress [8,9] and in preventing or retarding age-related diseases [10–12]. As an example, we recently report the beneficial effects of tomato extracts in counteracting oxidative stress in different cell lines [13,14]. Therefore, a continuous search for natural extracts highly rich in antioxidant molecules is needed to identify and provide novel natural drugs against aging-associated diseases.

Açai (*Euterpe oleracea Martius*) tree is a large palm found in the Amazon flood plain. The fruit of this palm is a small purple-black berry which reaches about 10 mm in diameter and is usually consumed in all states of Brazil since it is rich in α -tocopherol, fibers, lipids, polyphenols (including anthocyanins), and mineral ions [15,16]. The high polyphenol content, mostly composed of anthocyanins and flavones, is

thought to confer to açai fruit several health-promoting effects, including anti-inflammatory, immunomodulatory, antinociceptive, and antioxidant properties [17–25]. In particular, açai extracts were shown to increase plasma antioxidant capacity [21], to decrease oxidative stress in endothelial cells [26], to attenuate tumor growth in mice affected by esophageal cancer [27] and to lower the level of blood cholesterol in animal models for hypercholesterolemia [28].

A positive role of açai in modulating ROS production and activating antioxidant genes expression in rat liver was also reported [29] and, more recently, Peixoto and colleagues demonstrated the beneficial effect of açai extract in counteracting oxidative stress and aging in *C. elegans* [18].

Although several reports on the spectrum of health benefits of açai have been reported, only few studies are available so far on the identification of the antioxidant molecules responsible for the reported beneficial effects on human health.

Here, a methanol/water extract from *Euterpe oleracea* fruits was analyzed for its antioxidant activity on fibroblasts exposed to UV-A-induced insults. A combined approach of bioassays and mass spectrometry analyses led to the identification of açai bioactive compounds.

2. Methods

2.1. Açai Extracts

Methanolic extracts from açai fruit were obtained as reported by Rigano et al. [30], starting from commercially available dried powder (2 g, Tuialimentos, Brasil). The mixture was dried in a rotovapor (R-210, Buchi), and dissolved in 5% dimethyl sulfoxide (DMSO) in PBS (1 mL).

2.2. Antioxidant Compounds Determination and Antioxidant Activity Analysis

Total phenols content was determined in the whole açai extract according to the method of Singleton et al. [31] modified as reported by Rigano et al. [30]. Briefly, an equal volume of Folin-Ciocalteu's phenol reagent and two volumes of ddH₂O were added to the hydrophilic extract. After 6 min, Na₂CO₃ was added (7% final concentration). After 90 min the absorbance was read at 760 nm. A standard curve was obtained by using gallic acid in the range 0–70 μ g/mL. The total phenolic content was expressed as mg of gallic acid equivalents (GAE)/100 g dry weight (DW) of açai. Three independent analyses

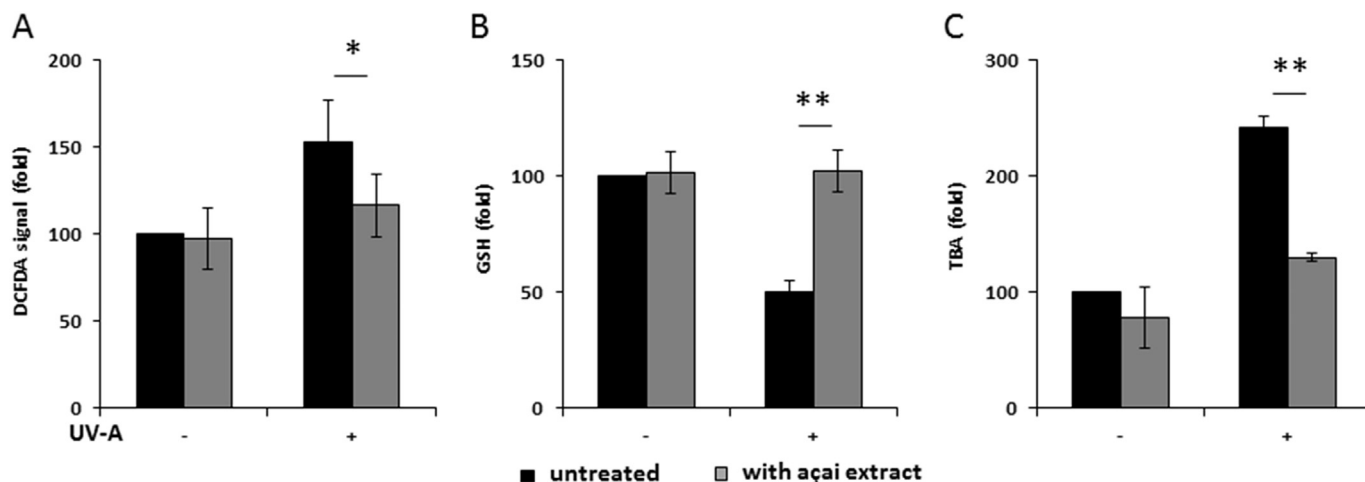


Fig. 2. ROS production, GSH oxidation and lipid peroxidation in BALB/3T3 cells irradiated by UV-A in the presence of açai extracts. Cells were pre-incubated in the presence of 10 mg/mL açai extract (grey bars) for 2 h and then irradiated by UV-A (100 J/cm²). A, intracellular ROS levels were determined by DCFDA assay; B, intracellular GSH levels determined by DTNB assay; C, lipid peroxidation levels determined by TBARS assay. Values are expressed as fold increase with respect to control (i.e. untreated) cells. Data shown are the means ± S.D. of three independent experiments. * indicates p < 0.01; ** indicates p < 0.001.

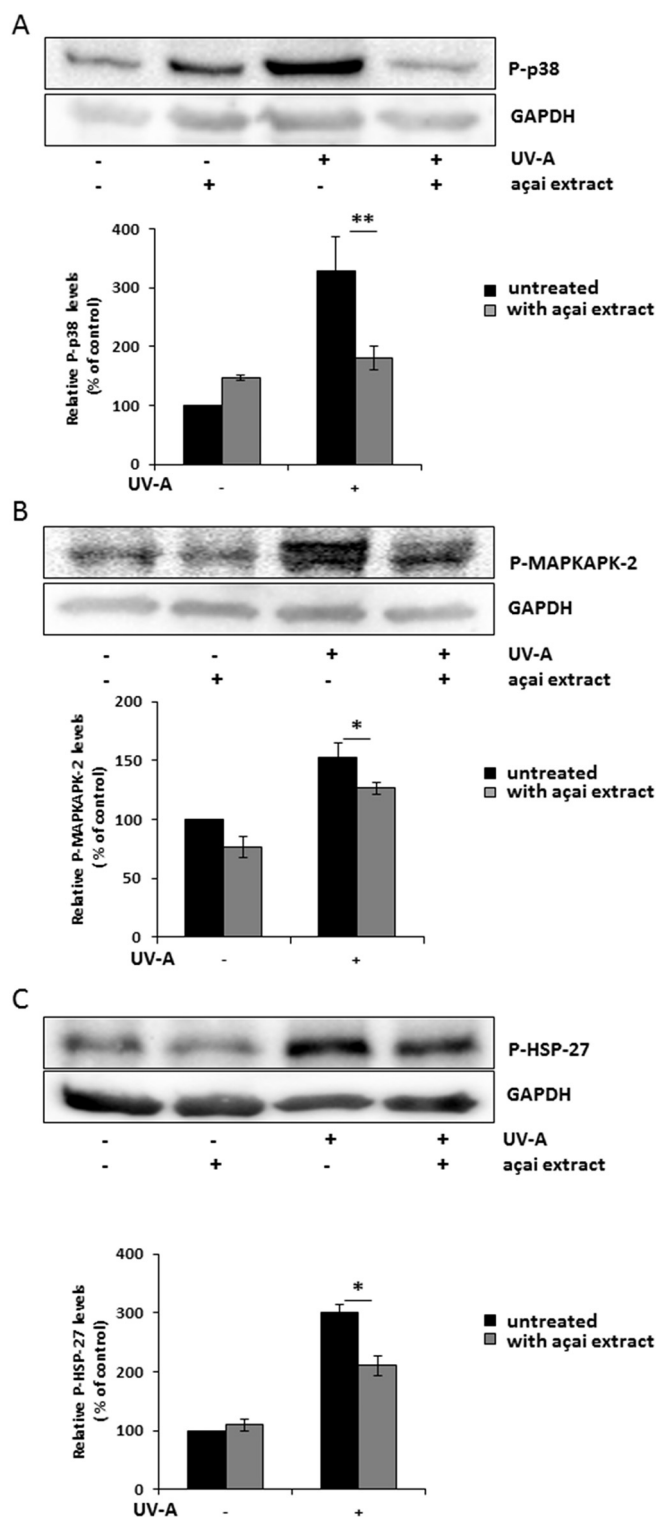


Fig. 3. Effect of açai extracts on UV-A-induced oxidative stress markers in BALB/3T3 fibroblasts. Cells were incubated with 10 mg/mL açai extract 2h prior to UV-A irradiation (100 J/cm²) and then cells were incubated for 90 min. Western blots show the phosphorylation levels of p38 (A), MAPKAPK-2 (B) and HSP-27 (C), with the relative densitometric analysis in the absence (black bars) or in the presence (grey bars) of açai extract. GAPDH was used as internal standard. Data shown are the means \pm S.D. of three independent experiments. * indicates $p < 0.01$; ** indicates $p < 0.001$.

were carried out.

Total flavonoids were estimated by the aluminum chloride colorimetric assay reported by Marinova et al. [32] with modifications reported by Raiola et al. [33]. Briefly, NaNO₂ (5%) was added to

methanolic extract and, after 5 min incubation, AlCl₃ (10% w/v) was added. After 6 min, NaOH (1 M) was added and the absorbance measured at 510 nm. A standard curve was obtained by using quercetin in the range 0–100 μ g/mL. Total flavonoids content was expressed as mg quercetin equivalents (QE)/100 g DW. Three independent analyses were carried out.

HAA was evaluated in the water-soluble fraction using the protocol described by Miller et al. [34] with modifications reported by Del Giudice et al. [35]. Briefly, methanolic extracts were allowed to react with an ABTS⁺ solution for 2.5 min, and then the absorbance was measured at 734 nm using a spectrophotometer. A standard curve, obtained by using Trolox, was linear between 0 and 20 μ M Trolox. Values were expressed as mmol TE/kg DW of açai. Three separate analyses were carried out with each sample.

2.3. Cell Culture and MTT Assay

BALB/3T3 fibroblasts (clone A31, from ATCC) were cultured in Dulbecco's Modified Eagle's Medium (Sigma-Aldrich, St Louis, Mo, USA), supplemented with 10% foetal bovine serum (HyClone), 2 mM L-glutamine and antibiotics, all from Sigma-Aldrich, in a 5% CO₂ humidified atmosphere at 37 °C.

Cells were seeded in 96-well plates (100 μ L/well) at a density of 5×10^3 /well. For dose-dependent cytotoxicity assays, 24 h after seeding, increasing volumes of açai methanolic extracts were added to the cells, to reach a final concentration ranging from 0.2 to 100 mg/mL. After 48 h incubation, cell viability was assessed by the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay, as described by Monti et al. [36]. Cell survival was expressed as the percentage of viable cells in the presence of extract compared to that of control samples. Two groups of cells were used as control, i.e. cells untreated with the extract and cells supplemented with identical volumes of DMSO. Each sample was tested in three independent analyses, each carried out in triplicates.

2.4. Oxidative Stress

To analyze oxidative stress, cells were plated at a density of 4×10^4 cells/cm². 24 h after seeding, cells were incubated for 2 h in the presence or absence of 10 mg/mL of açai extract, or equivalent amount of each isolated fraction, and then irradiated for 10 min with UV-A light (100 J/cm²) (treatment before injury). In a second group of experiments, cells were irradiated for 10 min with UV-A (100 J/cm²) and then incubated for 2 h in the presence or absence of açai extract or equivalent amount of each isolated fraction (treatment after injury).

2.5. DCFDA Assay

To estimate ROS production, the protocol described in [13] was followed. Briefly, at the end of incubation, cells were incubated with 2',7'-dichlorodihydrofluorescein diacetate (H₂-DCFDA, Sigma-Aldrich). Fluorescence intensity was measured by a Perkin-Elmer LS50 spectrofluorimeter (525 nm emission wavelength, 488 nm excitation wavelength, 300 nm/min scanning speed, 5 slit width for both excitation and emission). ROS production was expressed as percentage of DCF fluorescence intensity of the sample under test, with respect to the untreated sample. Each value was assessed by three independent experiments, each with three determinations.

2.6. DTNB Assay

To estimate intracellular glutathione levels, a procedure previously described was followed [14]. Briefly, at the end of incubation, cells were detached by trypsin, lysed and protein concentration was determined by the Bradford assay. Then, 50 μ g of proteins were incubated with 3 mM EDTA, 144 μ M 5,5'-dithiobis-2-nitrobenzoic acid (DTNB) in

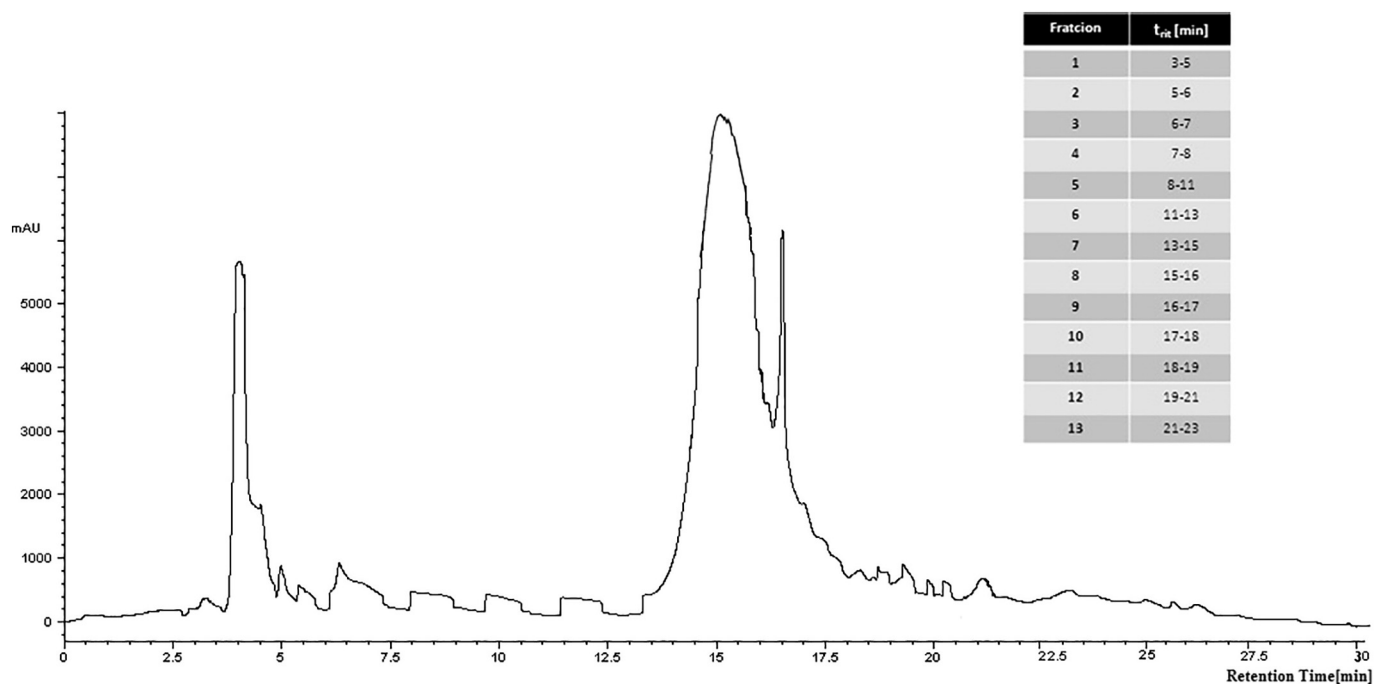


Fig. 4. Fractional analysis of açai extract by reverse-phase HPLC. Reverse phase C18 column was used with a linear gradient from 5% to 95% in 0.1% trifluoroacetic acid; elution was monitored at 278 nm. Thirteen fractions were manually collected.

30 mM TrisHCl pH 8.2, centrifuged at 14,000g for 5 min at 4 °C and the absorbance of the supernatant was measured at 412 nm by using a multiplate reader (Biorad). GSH levels were expressed as the percentage of TNB absorbance in the sample under test with respect to the untreated sample. Values are the mean of three independent experiments, each with triplicate determinations.

2.7. Measurement of Lipid Peroxidation

The thiobarbituric acid reactive substances (TBARS) assay was performed as described by Del Giudice et al. [13]. Briefly, after irradiation with UV-A, cells were kept for 90 min at 37 °C, then detached and suspended (5×10^4 cells) in 0.67% thiobarbituric acid (TBA) and 20% trichloroacetic acid (1:1 v/v). After heating and centrifugation at 3000g for 5 min at 4 °C, samples were read at 532 nm. Lipid peroxidation levels were expressed as the percentage of the absorbance at 532 nm of the sample under test, with respect to untreated cells (100%). Three independent experiments were carried out, each one with three determinations.

2.8. Western Blot Analyses

Cells were plated at a density of 2×10^4 cells/cm² in complete medium for 24 h and then treated as described above (paragraph 2.4). When açai extract was used before UV-A exposure, cells were incubated for further 90 min at 37 °C. Alternatively, following the induction of UV-A stress, cells were treated with the extract for 120 min at 37 °C. Cell lysates were then analyzed by Western blotting performed as reported by Galano et al. [37]. Phosphorylation levels of p38, MAPKAPK-2 or HSP-27 were detected by using specific antibodies purchased from Cell Signal Technology (Danvers, MA, USA). To normalize protein intensity levels, specific antibodies against internal standards were used, i.e. anti- β -actin (Proteintech, Manchester, UK) or anti-GAPDH (ThermoFisher, Rockford, IL, USA). The chemiluminescence detection system (SuperSignal® West Pico) was from Thermo Fisher.

2.9. Reverse-phase HPLC Analysis

Methanol/water açai extract (2 g/mL, 1 mL) was fractionated by reverse-phase HPLC on a Phenomenex Jupiter C18 column (250 \times 2.00 mm 5 μ m, 300 Å pore size) (Phenomenex, Torrance, California, USA) with a linear gradient from 5% to 95% acetonitrile (Sigma Aldrich, Saint Louis, USA) in 0.1% trifluoroacetic acid (TFA) (Carlo Erba Reagents S.r.l, Milan, Italy) over 40 min, at a flow rate of 200 μ L/min; elution was monitored at 278 nm. The eluate was collected manually in thirteen fractions (1 mL). Fractions were lyophilized and dissolved in 5% DMSO in PBS, and their content identified by MALDI-TOF (AB SCIEX, Milan, Italy) analysis.

2.10. Mass Spectrometry Analyses

Matrix-assisted laser desorption/ionization (MALDI) mass spectrometry (MS) experiments were performed on a 5800 MALDI-TOF-TOF AB SCIEX equipped with a nitrogen laser (337 nm) (AB SCIEX, Milan, Italy). Aliquots of açai HPLC fractions (0.5 μ L) were mixed (1:1, v/v) with 2.5 dihydroxybenzoic acid (10 mg/mL) (Sigma-Aldrich, Saint Louis, USA) in acetonitrile:water (90:10) solution. Calibration was performed by using AB SCIEX calibration mixture (Monoisotopic ($M + nH$)ⁿ⁺: 904.46 Da des-Arg-Bradykinin, 1296.68 Da Angiotensin I, 1570.67 Da Glu-Fibrinopeptide B, 2093.08 Da ACTH (clip 1–17), 2465.19 Da ACTH (clip 18–39), 3657.92 Da ACTH (clip 7–38)). For polyphenols identification, MS and tandem mass (MS/MS) spectra were acquired using a mass (m/z) range of 100–4000 Da.

2.11. Statistical Analyses

In all the experiments samples were analyzed in triplicate. Quantitative parameters were expressed as the mean value \pm SD. Significance was determined by Student's *t*-test at a significance level of 0.01. Differences among fractions were determined by using SPSS (Statistical Package for Social Sciences) Package 6, version 15.0 (SSPS Inc., Chicago, IL, USA). Significance was determined by Duncan's-test at a significance level of 0.05.

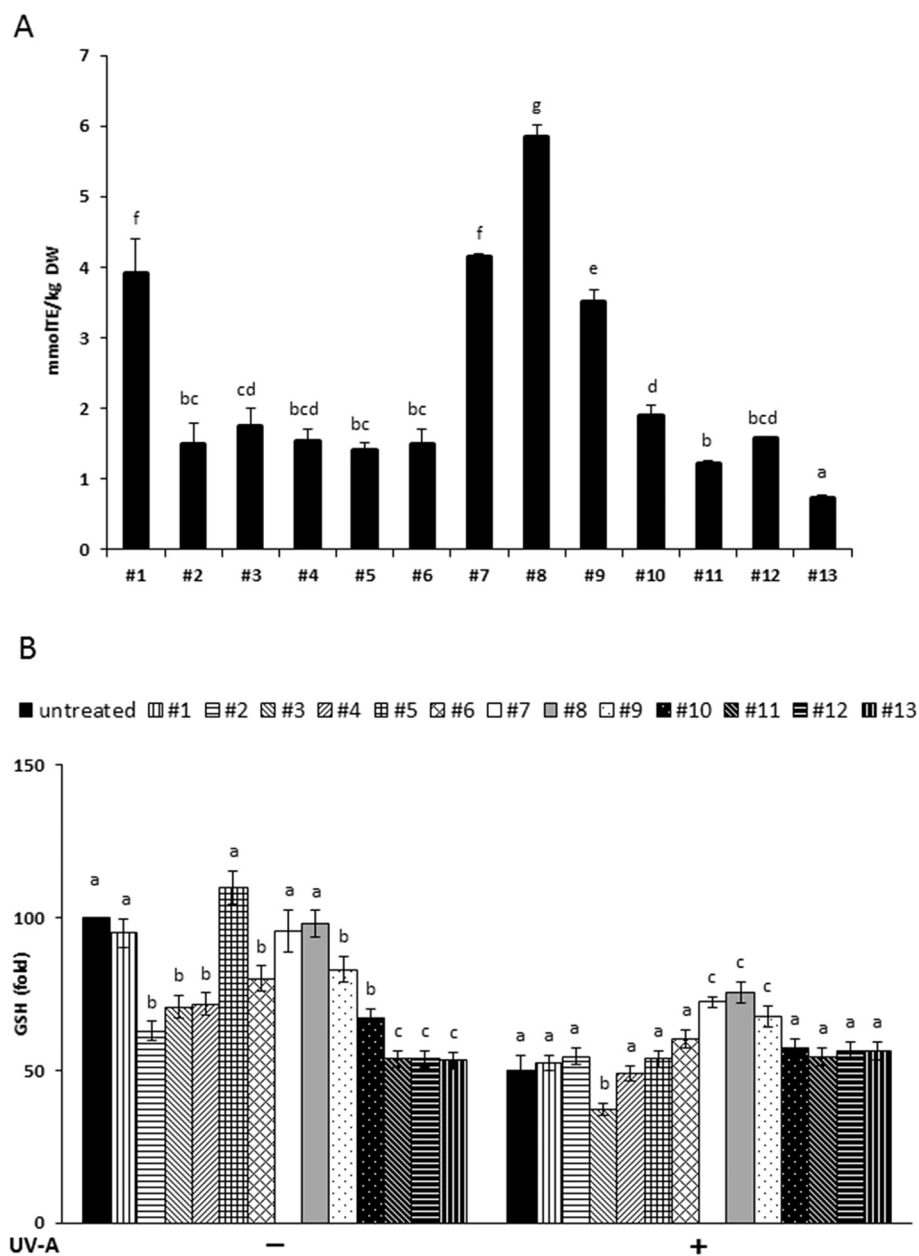


Fig. 5. Analysis of the antioxidant activity of açai fractions. A, Hydrophilic antioxidant activity (mmol TE/kg DW) in fractions evaluated by ABTS test. Data shown are the means \pm S.D. of three independent experiments. Significance was determined by Duncan's-test at a significance level of 0.05. Values with different letters are significantly different. B, Changes in intracellular GSH levels of cells before (–) and after (+) UV-A treatment. Cells were incubated with açai extract, or equivalent amount of fractions obtained by HPLC, for 2 h prior to UV-A irradiation (100 J/cm²). Data shown are the means \pm S.D. of three independent experiments. Values with different letters are significantly different compared to untreated cells, indicated by black bars ($p < 0.05$).

3. Results

3.1. Antioxidant Compounds Determination

We first characterized the methanol/water açai extract by measuring its antioxidant compounds and determining the antioxidant activity. By colorimetric assays, the total phenol content was estimated to be 192.41 ± 10.78 mg GAE/100 g DW, whereas that of total flavonoids was found to be 159.67 ± 7.65 mg QE/100g DW. The mean antioxidant activity was 2.1 ± 0.17 mmol TE/100 g DW. Data are reported in Table 1.

3.2. Biocompatibility of Açai Extracts on Fibroblasts

The biocompatibility of the açai extract on BALB/3T3 fibroblasts

was tested by a dose-response test, in a range from 0.2 to 100 mg/mL of extract. As shown in Fig. 1, cell viability was not affected up to 40 mg/mL, whereas at the highest concentration tested (100 mg/mL) a 50% reduction of cell viability was observed. On the basis of these results, subsequent experiments were carried out at 10 mg/mL of açai extract, corresponding to a flavonoid content of 0.16 mg QE/mL and to a phenolic content of 0.19 mg GAE/mL.

3.3. Pretreatment With Açai Extracts Inhibits UV-A-Induced Damage in BALB/3T3 Fibroblasts

In all the experiments reported below, BALB/3T3 fibroblasts were pretreated with açai extract for 2 h before the induction of oxidative stress by UV-A irradiation (100 J/cm²). Immediately after UV-A irradiation, ROS production was determined by using H₂DCF-DA (2,7-

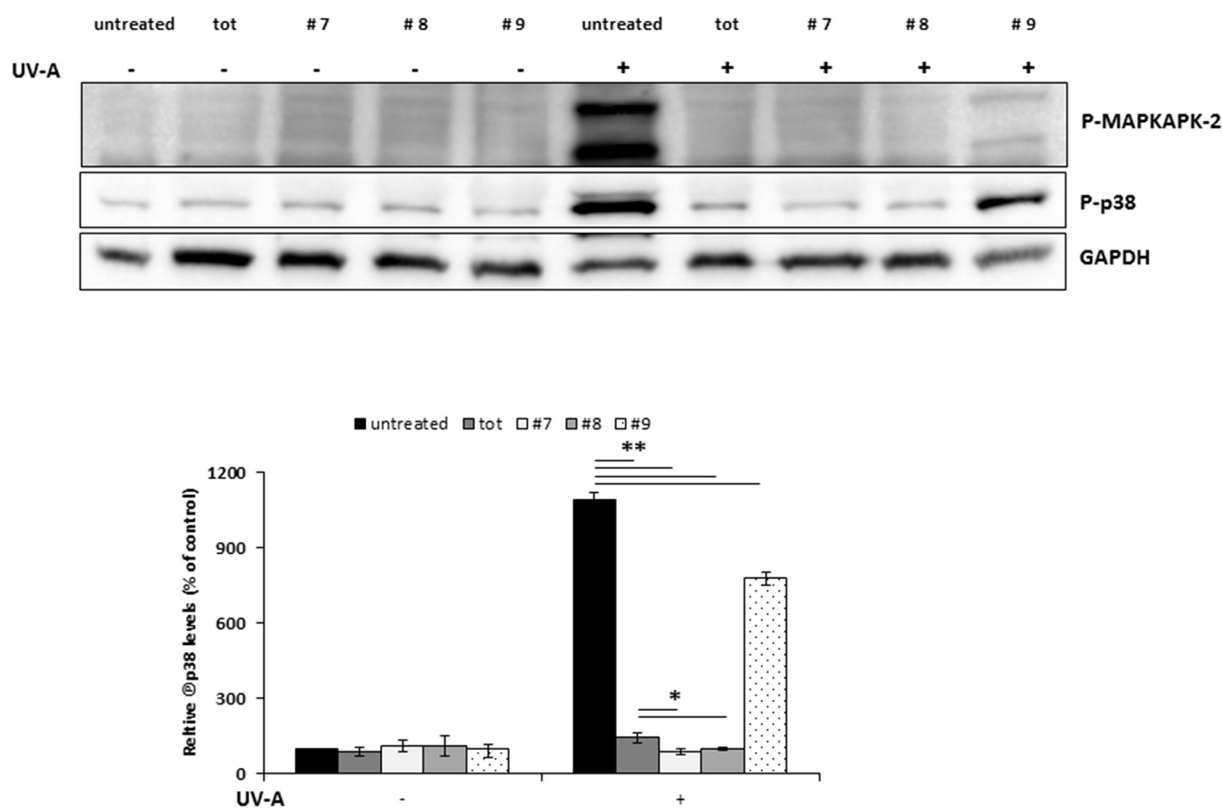


Fig. 6. Western blot analysis of UV-A-treated BALB/3T3 cells in the presence of HPLC fractions. Cells were incubated with different fractions for 2h prior to UV-A (100 J/cm²) irradiation and then cells were incubated for further 90min at 37 °C. Representative western blots show the phosphorylation levels of p38 (A) and MAPKAPK-2 (B), with the relative densitometric analysis in the absence (black bars) or in the presence (white bars) of extract. GAPDH was used as internal standard. Data shown are the means \pm S.D. of three independent experiments. * indicates $p < 0.01$, ** indicates $p < 0.001$.

dichlorofluorescein diacetate) (Fig. 2A). As expected, UV-A treatment significantly increased DCF fluorescence intensity, compared to the non-irradiated samples, whereas açai extract had no effect on ROS levels. Interestingly, pretreatment of cells with açai extract prior to UV-A exposure, resulted in the inhibition of ROS production. Moreover, following UV-A-oxidative stress induction, we found that intracellular GSH was significantly decreased with respect to untreated cells, whereas no alteration in intracellular GSH levels was observed when cells were pretreated with açai extract before being irradiated by UV-A (Fig. 2B). The protective effect of açai extract was confirmed by analyzing the lipid peroxidation levels after 90 min from irradiation. Lipid peroxidation level was measured by thiobarbituric acid reactive substances (TBARS). A significant increase in lipid peroxidation levels after UV-A treatment was observed but, noteworthy, this effect was abolished when cells were pretreated with açai extract. Again, pretreatment of cells with açai extract significantly counteracted the increase of lipid peroxidation (Fig. 2C).

The protective effect of açai extract was further confirmed by Western blot experiments, in which the phosphorylation levels of p38 and its direct target, MAPKAPK-2, were analyzed (Fig. 3). These proteins are directly involved in signaling stress pathways induced by UV-A [38]. When cells were UV-A irradiated, we observed a significant increase in the phosphorylation levels of p38 and MAPKAPK-2, as expected (Fig. 3A and B, third lane). Instead, although the treatment of non-irradiated cells with açai extract slightly altered the phosphorylation level of p38 (Fig. 3A, second lane), when cells were exposed to açai extract prior to UV-A treatment, the phosphorylation levels of p38 and MAPKAPK-2 were similar to those observed in non-irradiated cells (Fig. 3A and B, fourth lane). A similar trend was observed for HSP-27, a heat shock protein whose phosphorylation is directly related to oxidative stress [39,40] (Fig. 3C).

3.4. Antioxidant Activity of Açai Fractions

In order to identify which açai compounds were responsible for the antioxidant activity observed in irradiated fibroblasts, the extract was fractionated by HPLC on a reverse phase C18 column, from which the profile shown in Fig. 4 was obtained. 13 HPLC fractions were collected, as reported in the Figure, and their antioxidant activity was tested in vitro. The HAA analysis (Fig. 5A) revealed that the highest antioxidant activity was associated to fractions 7 and 8, for which the HAA mean values of 4.13 mmol TE/kg DW and 5.85 mmol TE/kg DW, respectively, were calculated. High antioxidant power was also identified in fractions 1 and 9, with HAA mean values of 3.92 and 3.49 mmol TE/kg DW, respectively. Lower values were detected in the other fractions, ranging from 0.72 mmol TE/kg DW (fraction 13) to 1.89 mmol TE/kg DW (fraction 10).

Then, we analyzed the intracellular GSH levels in BALB/3T3 cells pretreated with each HPLC fraction, before and after UV-A treatment. As shown in Fig. 5B, fractions 7 and 8 were able to contrast the detrimental effects of UV-A irradiation on GSH depletion, whereas fraction 9 was less efficient, in agreement with the HAA analysis. By contrast, fraction 1 did not show any protective effect from oxidative stress. Western blot experiments confirmed the strong antioxidant activity of fraction 7 and 8 on UV-A-stressed fibroblasts. In particular, the increase in p38 and MAPKAPK phosphorylation levels induced by UV-A irradiation was attenuated when cells were pretreated with açai fractions 7 and 8, whose effect was found to be slightly, but significantly, more pronounced than that of the whole extract (Fig. 6). Noticeable, a lower decrement in p38 phosphorylation level was observed when cells were pre-treated with fraction 9.

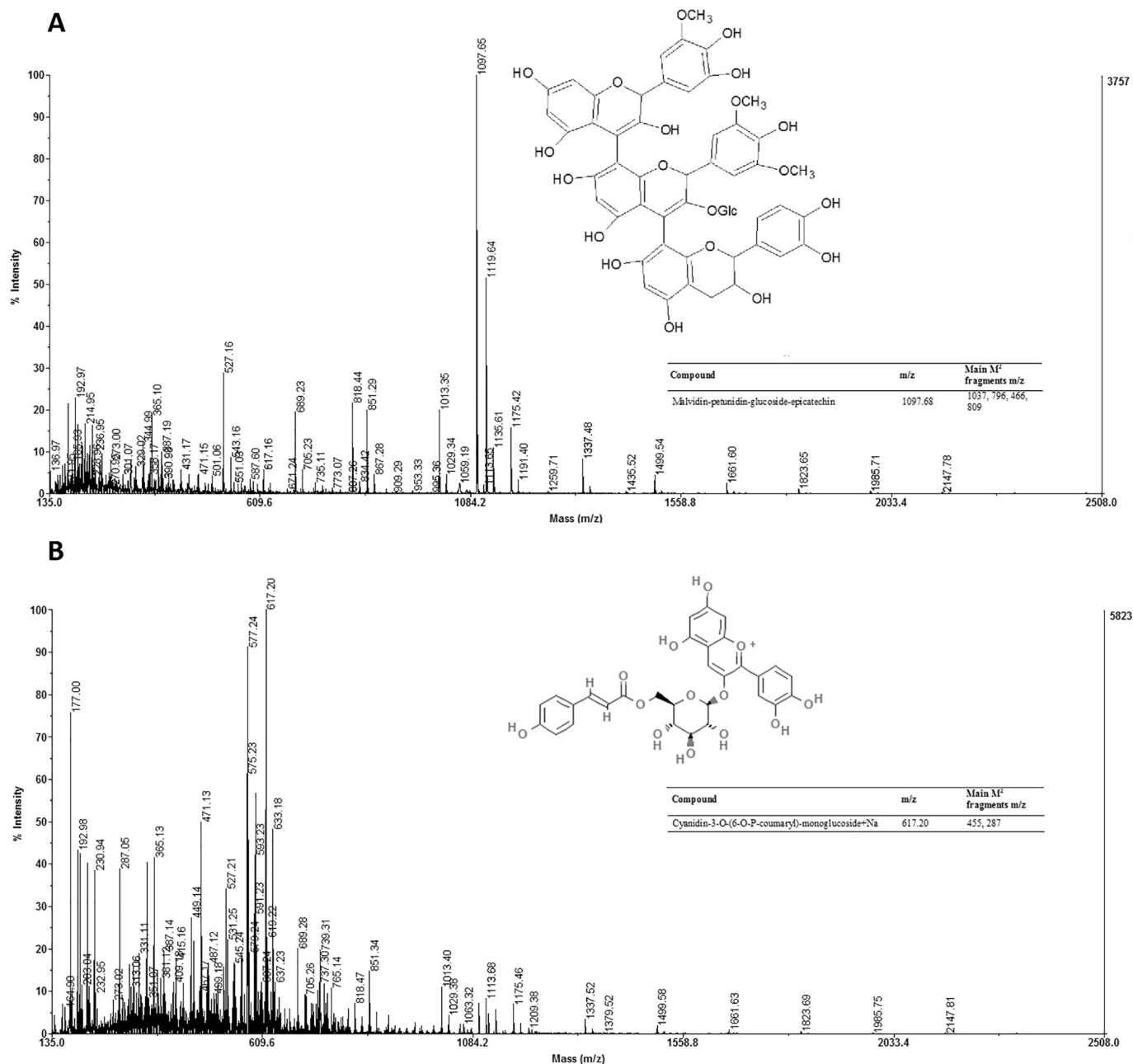


Fig. 7. MALDI-MS analysis of selected HPLC fractions from açai extract. The MALDIMS spectra of fractions 7 and 8 are reported in panels A and B, respectively. The attributions are reported.

3.5. Malvidin and Cyanidin are Responsible for the Açai Antioxidant Activity on Immortalized Fibroblasts

HPLC fractions 7 and 8 were submitted to MALDI-MS analysis, as described in the Material and Method section. The different species detected in the MALDI spectra (Fig. 7A-B) were identified on the basis of MSMS MALDI TOF analysis and compared with literature data [41]. Mass spectral identification is reported in Table 2. Qualitative and semi-quantitative differences can be appreciated in the MALDI spectra showing the presence of different flavonoids in açai fractions. Among them, malvidin-petunidin-glucoside-epicatechin was found to be the predominant compound in fraction 7, whereas cyanidin-3-O-(6-O-p-coumaryl)-monoglucoside was found to be the most abundant species in fraction 8. DCFDA assay indicated that malvidin and cyanidin enriched fractions were able to mitigate ROS production as well as the whole extract (Fig. 8A). However, the TBARS assay (Fig. 8B) revealed that the

activity of malvidin enriched fraction was comparable to that of the whole extract, whereas cyanidin enriched fraction was lower.

Finally, to evaluate if malvidin and cyanidin derivatives protect cells after UV-A injury and to avoid any sunscreen effect, fibroblasts were first irradiated by UV-A (100 J/cm²), and afterwards incubated for 2h with either açai extract or its fractions and then ROS, GSH levels and the phosphorylation levels of p38 were evaluated (Fig. 9). Interestingly, UV-A induced ROS production was mitigated by the presence of either açai extract or its active fractions. Furthermore, no alteration in intracellular GSH levels was measured and a significant decrease in the phosphorylation levels of p38 was also observed.

4. Discussion

Among the most severe environmental injuries to which humans are exposed, UV-A radiations are known to be the main cause of different

Table 2

MALDI mass spectral analysis of açai fractions after reverse-phase HPLC. The identified species and the relative percentages are reported (G = glucose).

Compound	<i>m/z</i>	Main M ² fragments <i>m/z</i>	Fraction 7	Fraction 8
Petunidin-3-acetyl	365.10	305, 203	20%	43%
Peonidin-3-O-acetyl monoglucoside	527.19	467, 365, 203	32%	38%
Cyanidin-3-O-(6-O-P-coumaryl)-monoglucoside + Na	617.20	455, 287	10%	100%
Petunidin-3-acetyl diglucoside	689.23	527, 629	22%	20%
Malvidin-3-acetylglucose-ethyl-catechin/Petunidin-3-acetyl triglucoside	851.31	791, 689	20%	17%
Petunidin-3-acetyl tetraglucoside	1013.35	952, 851	20%	14%
Malvidin-petunidin-glucoside-epicatechin	1097.68	1037, 796, 466, 809	100%	5%
Petunidin-3-acetyl + 5G	1175.42	1115, 1013	16%	8%
Petunidin-3-acetyl + 6G	1337.48	1277, 1175	12%	5%
Petunidin-3-acetyl + 7G	1499.54	1439, 1337	8%	3%
Petunidin-3-acetyl + 8G	1661.6	1601, 1499	6%	3%
Petunidin-3-acetyl + 9G	1823.65	1763, 1661	6%	2%
Petunidin-3-acetyl + 10G	1985.71	1925, 1823	4%	1%
Petunidin-3-acetyl + 11G	2147.78	2087, 1985	2%	1%

detrimental effects on the skin, such as inflammation, premature skin aging and development of skin cancer [42,43]. In recent years, an increasing body of research has focused on antioxidants since they are able to counteract oxidative stress-induced pathological conditions. However, studies are usually conducted on commercial molecules, supposed to have antioxidant activity [43–45].

Here, we analyzed hydrophilic extracts from açai berries to evaluate their dermato-protective properties against UV-A irradiation. Açai is the name commonly used for the tree *Euterpe oleracea Martius*, a South American palm [25,46], whose berry is largely consumed since it shows several beneficial activities, including anti-inflammatory and antioxidant properties. These activities are mainly due to açai high content in polyphenol content, especially anthocyanins and flavones [20–25].

Immortalized fibroblasts, exposed to UV-A radiations, showed increased levels of intracellular ROS and lipid peroxidation, and decreased levels of intracellular GSH. Noteworthy, we found that a pre-treatment of cells with açai extracts clearly inhibited the detrimental effect of oxidative stress, since ROS generation, lipid peroxidation, intracellular GSH levels and p38 phosphorylation levels were all found to be almost unaltered with respect to UV-A unexposed cells. It is

interesting to notice that the açai extract is able to efficiently protect immortalized, non-cancer cells from the deleterious effects of UV-A irradiation when tested at concentrations compatible with cell viability, i.e. non associated to any cytotoxicity. Moreover, we demonstrated that the protective effect of açai extract is not due to a sunscreen effect, as the extract was able to protect cells also after UV-A induced damage.

Mass spectrometry analyses of HPLC fractionated extract led us to the identification of the main agents responsible for the potent antioxidant activity of açai extracts on fibroblasts. These are two flavonoids, cyanidin and malvidin, both endowed with strong antioxidant activity, with malvidin the most active.

Cyanidin is considered the widest spread anthocyanin in the plant kingdom and it is known to be a strong natural antioxidant. Amorini and colleagues [47] demonstrated that cyanidin-3-glucoside, also known as kuromanin, has a remarkable antioxidant capacity in the model of Cu²⁺-mediated human low density lipoprotein oxidation, even higher than resveratrol and ascorbic acid and independent from pH variations (from 4 to 7.4). In addition, it is reported that cyanidin-3-glucoside may attenuate obesity-associated insulin resistance and hepatic steatosis in high-fat diet-fed mice via the transcription factor FoxO1 [48].

Malvidin, normally present at high concentration in red wine and black rice, and responsible for their color, plays an important role in protecting plants from UV irradiation [49,50]. Accordingly, it has been reported that malvidin has antioxidant activity in vitro [51], as well as in neuronal and in endothelial cells [52,53]. Recently, it has been demonstrated that malvidin is also able to inhibit aging by attenuating oxidative stress in human diploid fibroblasts [54]. Moreover, several authors have shown that malvidin inhibits the growth of different tumor cell lines in vitro or in vivo [55–57].

Polyphenols are known to inhibit free radicals' production by donating hydrogen atoms, which break oxidation chains and chelate transition metal ions. Açai polyphenols may upregulate antioxidant enzymes by activating the Nrf2/Keap1 pathway [58], downregulate the expression of the pro-inflammatory transcription factor NF-κB, which in turn targets pro-inflammatory cytokines, and reduces the accumulation of intracellular lipids in differentiated adipocytes by downregulating the expression of adipogenic genes transcription factors [59]. Additionally, different studies performed on healthy women, which consumed 200 g/day of açai pulp for 4 weeks, revealed that some antioxidant enzymes, i.e. superoxide dismutase, catalase and glutathione, were increased [59–61].

Our findings now reveal that açai extract protects BALB/3T3 cells against UV-A irradiation by neutralizing the negative effects of stress induced by UV-A, as it interferes with ROS generation and keeps intracellular GSH and lipid peroxidation close to the normal cellular levels. This work reveals, for the first time, that malvidin present in açai

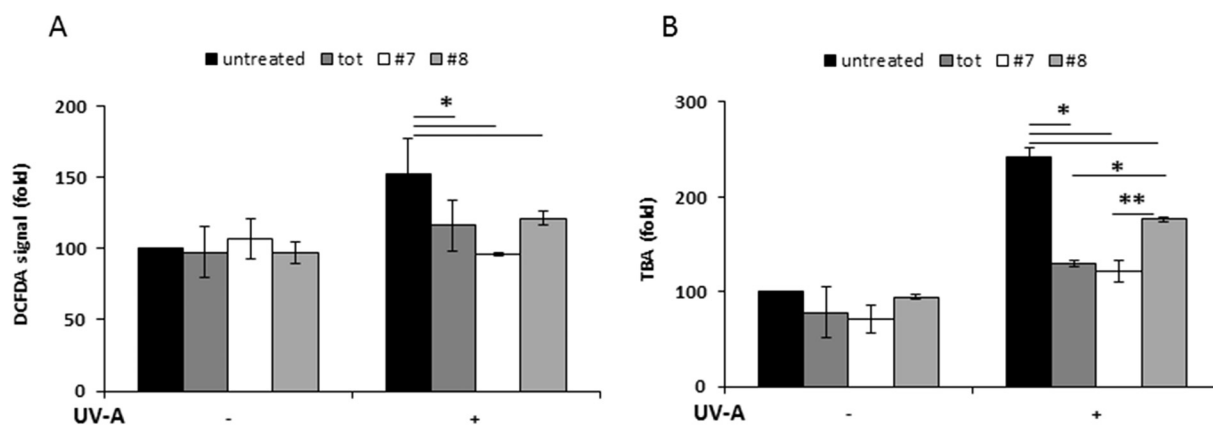


Fig. 8. ROS production and lipid peroxidation in UV-A-treated BALB/3T3 fibroblasts in the presence of açai fractions. Cells were pre-incubated with açai extract (dark grey bars), fraction 7 (white bars) and fraction 8 (light grey bars) for 2h and then irradiated by UV-A (100 J/cm²). A, intracellular ROS levels; B, lipid peroxidation levels. Values are expressed as fold increase with respect to control (i.e. untreated) cells. Data shown are the means ± S.D. of three independent experiments. * indicates p < 0.01; ** indicates p < 0.05.

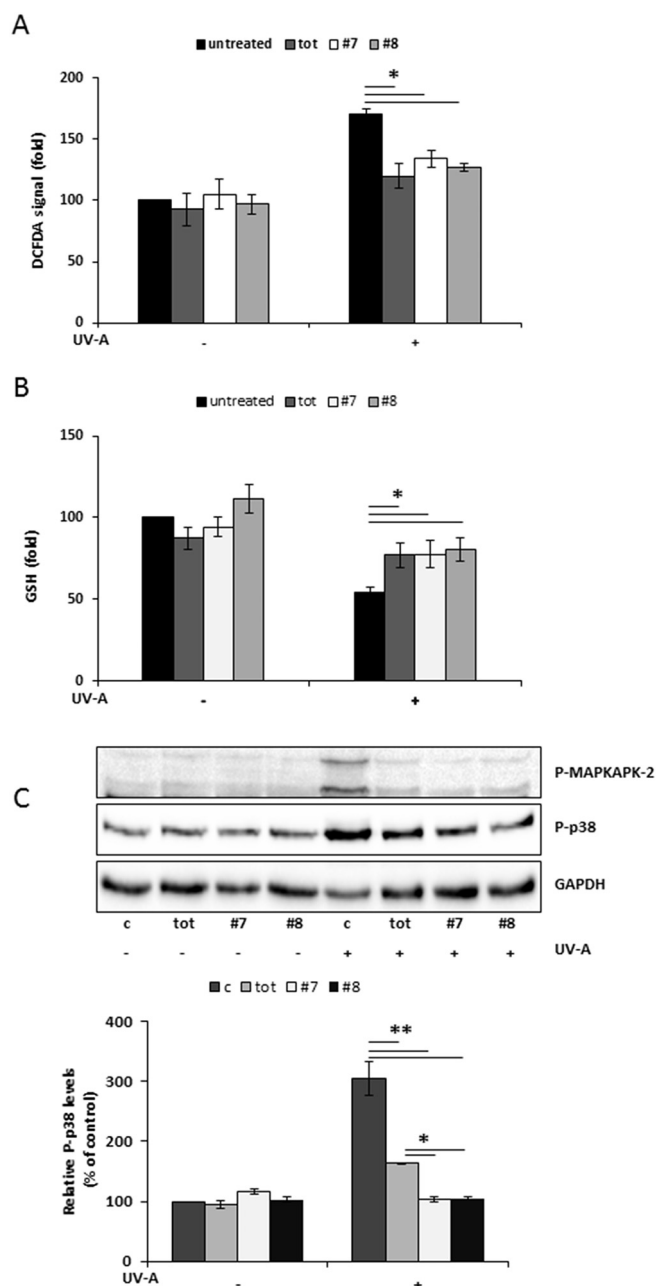


Fig. 9. Analysis of oxidative stress in BALB/3T3 fibroblasts incubated in the presence of açai fractions after UV-A treatment. Cells were irradiated by UV-A (100 J/cm²) and then incubated with açai extract, fraction 7 or fraction 8 for 2h. A, intracellular ROS levels; B, intracellular GSH levels; C, Western blotting of the phosphorylation levels of p38 and MAPKAPK-2, with the relative densitometric analysis. GAPDH was used as internal standard. In all panels, untreated cells (named c in western blotting analysis) are represented by black bars, cells incubated with açai extract by dark grey bars, with fraction 7 by white bars and with fraction 8 by light grey bars. Values are expressed as fold increase with respect to control (i.e. untreated) cells. Data shown are the means \pm S.D. of three independent experiments. * indicates $p < 0.05$; ** indicates $p < 0.01$.

extract can be considered as a good and safe candidate for dietary intervention in the prevention of age related diseases, since the use of anthocyanins as food additives (indicated as “E163”) has been already approved in the European Union, Australia, and New Zealand [62].

Author contribution

G.P. and D.M.M. conceived, performed and analyzed cell biology experiments; A.R. and M.M.R. performed in vitro experiments; R.D.G.

performed spectrofluorimetric experiments; A.A. and A.I. conceived, performed and analyzed the HPLC and MS experiments; R.P. analyzed and discussed the data; D.M.M. wrote the paper with the contribution of all the authors.

Conflict of interest

The authors declare no competing financial interest.

Acknowledgement

The authors thank Dr. Carla Damiano for the work done during her master thesis.

References

- [1] R. Thanan, S. Oikawa, Y. Hiraku, S. Ohnishi, N. Ma, S. Pinlaor, et al., Oxidative stress and its significant roles in neurodegenerative diseases and cancer, *Int. J. Mol. Sci.* 16 (2014) 193–217.
- [2] D.R. Bickers, M. Athar, Oxidative stress in the pathogenesis of skin disease, *J. Invest. Dermatol.* 126 (2006) 2565–2575.
- [3] S. Briganti, M. Picardo, Antioxidant activity, lipid peroxidation and skin diseases. What's new, *J. Eur. Acad. Dermatol. Venereol.* 17 (2003) 663–669.
- [4] J. Cadet, R. Teoule, Comparative study of oxidation of nucleic acid components by hydroxyl radicals, singlet oxygen and superoxide anion radicals, *Photochem. Photobiol.* 28 (1978) 661–665.
- [5] J.L. Ravanat, C. Saint-Pierre, P. Di Mascio, G.R. Martinez, M.H.G. Medeiros, J. Cadet, Damage to isolated DNA mediated by singlet oxygen, *Helv. Chim. Acta* 84 (2001) 3702–3709.
- [6] T. Douki, A. Reynaud-Angelin, J. Cadet, E. Sage, Bipyrimidine photoproducts rather than oxidative lesions are the main type of DNA damage, *Biochemistry* 42 (2003) 9221–9226.
- [7] S. Raha, B.H. Robinson, Mitochondria, oxygen free radicals, disease and ageing, *Trends Biochem. Sci.* 25 (2000) 502–508.
- [8] L.A. Pham-Huy, H. He, C. Pham-Huy, Free radicals, antioxidants in disease and health, *Int. J. Biomed. Sci.* 4 (2008) 89.
- [9] L. Gil del Valle, Oxidative stress in aging: theoretical outcomes and clinical evidences in humans, *Biomed. Aging Pathol.* 1 (2011) 1–7.
- [10] A. Scalbert, I.T. Johnson, M. Saltmarsh, Polyphenols: antioxidants and beyond, *Am. J. Clin. Nutr.* 81 (2005) 215S–217S.
- [11] B.E. van Wyk, M. Wink, *Phytomedicines, Herbal Drugs, and Poisons*, first ed., (2015) (Chicago, IL).
- [12] M. Wink, Modes of action of herbal medicines and plant secondary metabolites, *Fortschr. Med.* 2 (2015) 251–286.
- [13] R. Del Giudice, G. Petruk, A. Raiola, A. Barone, D.M. Monti, M.M. Rigano, Carotenoids in fresh and processed tomato (*Solanum lycopersicum*) fruits protect cells from oxidative stress injury, *J. Sci. Food Agric.* 97 (2017) 1616–1623, <http://dx.doi.org/10.1002/jsfa.7910>.
- [14] G. Petruk, A. Raiola, R. Del Giudice, A. Barone, L. Frusciante, M.M. Rigano, D.M. Monti, An ascorbic acid-enriched tomato genotype to fight UV-A-induced oxidative stress in normal human keratinocytes, *J. Photochem. Photobiol. B* 163 (2016) 284–289.
- [15] H. Rogez, Açai: Preparo, Composicao e Melhoramento da Conservacao, first ed., EDUFPA, Brazil, 2000.
- [16] M.S.M. Rufino, J. Perez-Jimenez, S. Arranz, R.E. Alves, E.S. de Brito, M.S. Oliveira, F. Saura-Calixto, Açai (*Euterpe oleracea*) BRS Para: a tropical fruit source of antioxidant dietary fiber and high antioxidant capacity oil, *Food Res. Int.* 44 (2011) 2100–2106.
- [17] J. Holderness, I.A. Schepetkin, B. Freedman, L.N. Kirpotina, M.T. Quinn, J.F. Hedges, M.A. Jutila, Polysaccharides isolated from Açai fruit induce innate immune responses, *PLoS One* 6 (2011) e17301.
- [18] H. Peixoto, M. Roxo, S. Krstin, T. Röhrlig, E. Richling, M. Wink, An Anthocyanin-rich extract of açai (*Euterpe precatoria Mart.*) increases stress resistance and retards aging-related markers in *Caenorhabditis elegans*, *J. Agric. Food Chem.* 64 (2016) 1283–1290.
- [19] M.M. dos Santos Dias, H.S.D. Martino, G. Noratto, A. Roque-Andrade, P.C. Stringheta, S. Talcott, A.M. Ramos, S.U. Mertens-Talcott, Anti-inflammatory activity of polyphenolics from açai (*Euterpe oleracea Martius*) in intestinal myofibroblasts CCD-18Co cells, *Food Funct.* 6 (2015) 3249–3256.
- [20] R. Lichtenthaler, R.B. Rodrigues, J.G.S. Maia, M. Papagiannopoulos, H. Fabricius, F. Marx, Total oxidant scavenging capacities of *Euterpe oleracea Mart.* (Açai) fruits, *Int. J. Food Sci. Nutr.* 56 (2005) 53–64.
- [21] S.U. Mertens-Talcott, J. Rios, P. Jilma-Stohlawetz, L.A. Pacheco-Palencia, B. Meibohm, S.T. Talcott, H. Derendorf, Pharmacokinetics of anthocyanins and antioxidant effects after the consumption of anthocyanin-rich açai juice and pulp (*Euterpe oleracea Mart.*) in human healthy volunteers, *J. Agric. Food Chem.* 56 (2008) 7796–7802.
- [22] J. Kang, C. Xie, Z. Li, S. Nagarajan, A.G. Schauss, T. Wu, X. Wu, Flavonoids from açai (*Euterpe oleracea Mart.*) pulp and their antioxidant and anti-inflammatory activities, *Food Chem.* 128 (2011) 152–157.
- [23] J. Kang, K.M. Thakali, C. Xie, M. Kondo, Y. Tong, B. Ou, G. Jensen, M.B. Medina,

- A.G. Schauss, X. Wu, Bioactivities of açai (*Euterpe precatoria* Mart.) fruit pulp, superior antioxidant and anti-inflammatory properties to *Euterpe oleracea* Mart., *Food Chem.* 133 (2012) 671–677.
- [24] H.A. Favacho, B.R. Oliveira, K.C. Santos, B.J. Medeiros, P.J. Sousa, F.F. Perazzo, J.C.T. Carvalho, Anti-inflammatory and antinociceptive activities of *Euterpe oleracea* Mart., *Areaceae*, oil, *Rev. Bras* 21 (2011) 105–114.
- [25] A.G. Schauss, X. Wu, R.L. Prior, B. Ou, D. Huang, J. Owens, A. Agarwal, G.S. Jensen, A.N. Hart, E. Shanbrom, Antioxidant capacity and other bioactivities of the freeze-dried Amazonian palm berry, *Euterpe oleracea* Mart. (açai), *J. Agric. Food Chem.* 54 (2006) 8604–8610.
- [26] G.D. Noratto, G. Angel-Morales, S.T. Talcott, S.U. Mertens-Talcott, Polyphenolics from açai (*Euterpe oleracea* Mart.) and red muscadine grape (*Vitis rotundifolia*) protect human umbilical vascular Endothelial cells (HUVEC) from glucose and lipopolysaccharide (LPS)-induced inflammation and target micro RN A-126, *J. Agric. Food Chem.* 59 (2011) 7999–8012.
- [27] G.D. Stoner, L.S. Wang, C. Seguin, C. Rocha, K. Stoner, S. Chiu, A.D. Kinghorn, Multiple berry types prevent N-nitrosomethylbenzylamine-induced esophageal cancer in rats, *Pharm. Res.* 27 (2010) 1138–1145.
- [28] M. Oliveira de Souza, M. Silva, M.E. Silva, R. de Paula Oliveira, M.L. Pedrosa, Diet supplementation with açai (*Euterpe oleracea* Mart.) pulp improves biomarkers of oxidative stress and the serum lipid profile in rats, *Nutrition* 26 (2010) 804–810.
- [29] J.F. Ferreira da Costa Guerra, C.L. Lopes de Brito Magalhaes, D.C. Costa, M.E. Silva, M.L. Pedrosa, Dietary açai modulates ROS production by neutrophils and gene expression of liver antioxidant enzymes in rats, *J. Clin. Biochem. Nutr.* 49 (2011) 188–194.
- [30] M.M. Rigano, A. Raiola, G.C. Tenore, D.M. Monti, R. Del Giudice, L. Frusciantè, A. Barone, Quantitative trait loci pyramiding can improve the nutritional potential of tomato (*Solanum lycopersicum*) fruits, *J. Agric. Food Chem.* 62 (2014) 11519–11527.
- [31] V.L. Singleton, J.A. Rossi, Colorimetry of total phenolics with phosphomolybdc-phosphotungstic acid reagents, *Am. J. Enol. Vitic.* 16 (1965) 144–158.
- [32] D. Marinova, F. Ribarova, M. Atanassova, Total phenolics and total flavonoids in bulgarian fruits and vegetables, *J. Univ. Chem. Technol. Metall.* 40 (2005) 255–260.
- [33] A. Raiola, R. Del Giudice, D.M. Monti, G.C. Tenore, A. Barone, M.M. Rigano, Bioactive compound content and cytotoxic effect on human cancer cells of fresh and processed yellow tomatoes, *Molecules* 21 (2016) 33.
- [34] J.N. Miller, C.A. Rice-Evans, Factors influencing the antioxidant activity determined by the ABTS^{•+} radical cation assay, *Free Radic. Res.* 26 (1997) 195–199.
- [35] R. Del Giudice, A. Raiola, G.C. Tenore, L. Frusciantè, A. Barone, D.M. Monti, M.M. Rigano, Antioxidant bioactive compounds in tomato fruits at different ripening stages and their effects on normal and cancer cells, *J. Funct. Foods* 18 (2015) 83–94.
- [36] D.M. Monti, D. Guarnieri, G. Napolitano, R. Piccoli, P. Netti, S. Fusco, A. Arciello, Biocompatibility, uptake and endocytosis pathways of polystyrene nanoparticles in primary human renal epithelial cells, *J. Biotechnol.* 10 (2015) 3–10.
- [37] E. Galano, A. Arciello, R. Piccoli, D.M. Monti, A. Amoresano, A proteomic approach to investigate the effects of cadmium and lead on human primary renal cells, *Metalomics* 6 (2014) 587–597.
- [38] L.O. Klotz, C. Pellieux, K. Briviba, C. Pierlot, J.M. Aubry, H. Sies, Mitogen-activated protein kinase (p38-, JNK-, ERK-) activation pattern induced by extracellular and intracellular singlet oxygen and UV-A, *Eur. J. Biochem.* 260 (1999) 917–922.
- [39] W.R. Swindell, Heat shock proteins in long-lived worms and mice with insulin/insulin-like signaling mutations, *Aging* 1 (2009) 573.
- [40] A. Strayer, Z. Wu, Y. Christen, C.D. Link, Y. Luo, Expression of the small heat-shock protein Hsp16 – 2 in *Caenorhabditis elegans* is suppressed by *Ginkgo biloba* extract Egb 761, *FASEB J.* 17 (2003) 2305–2307.
- [41] R. Flamini, Mass spectrometry in grape and wine chemistry. Part I: polyphenols, *Mass Spectrom. Rev.* 22 (2003) 218–250.
- [42] D. Kulms, T. Schwarz, Molecular mechanisms of UV-induced apoptosis, *Photodermatol. Photoimmunol. Photomed.* 16 (2000) 195–201.
- [43] Y.-C. Hseu, C.W. Chou, K.J. Senthil Kumar, K.T. Fu, H.M. Wang, L.S. Hsu, Y.H. Kuo, C.R. Wu, S.C. Chen, H.L. Yang, Ellagic acid protects human keratinocyte (HaCat) cells against UV-A-induced oxidative stress and apoptosis through the upregulation of the HO-1 and Nrf-2 antioxidant genes, *Food Chem. Toxicol.* 50 (2012) 1245–1255.
- [44] Y.-C. Hseu, H.W. Lo, M. Korivi, Y.C. Tsai, M.J. Tang, H.L. Yang, Dermato-protective properties of ergothioneine through induction of Nrf2/ARE-mediated antioxidant genes in UV-A-irradiated human keratinocytes, *Free Radic. Biol. Med.* 86 (2015) 102–117.
- [45] I.F. Almeida, A.S. Pinto, C. Monteiro, H. Monteiro, L. Belo, J. Fernandes, A.R. Bento, T.L. Duarte, J. Garrido, M.F. Bahia, J.M. Sousa Lobo, P.C. Costa, Protective effect of *C. sativa* leaf extract against UV mediated-DNA damage in a human keratinocyte cell line, *J. Photochem. Photobiol. B* 144 (2015) 28–34.
- [46] A.G.V. Costa, D.F. Garcia-Diaz, P. Jimenez, P.I. Silva, Bioactive compounds and health benefits of exotic tropical red–black berries, *J. Funct. Foods* 5 (2013) 539–549.
- [47] A.M. Amorini, G. Fazzina, G. Lazzarino, B. Tavazzi, D. Di Piero, R. Santucci, F. Sinibaldi, F. Galvano, G. Galvano, Activity and mechanism of the antioxidant properties of cyanidin-3-O-βglucopyranoside, *Free Radic. Res.* 35 (2001) 953–966.
- [48] H. Guo, M. Xia, T. Zou, W. Ling, R. Zhong, W. Zhang, Cyanidin 3-glucoside attenuates obesity-associated insulin resistance and hepatic steatosis in high-fat diet-fed and db/db mice via the transcription factor FoxO1, *J. Nutr. Biochem.* 23 (2012) 349–360.
- [49] U. Matern, B. Grimmig, Polyphenols in plant pathology, *Polyphenolic Phenomena*. Paris: INRA Editions, 1993, pp. 143–147.
- [50] E. Bognar, Z. Sarszegi, A. Szabo, B. Debrececi, N. Kalman, Z. Tucek, B. Sumegi, F. Gallyas Jr, Antioxidant and anti-inflammatory effects in RAW264.7 macrophages of malvidin, a major red wine polyphenol, *PLoS One* 8 (2013) e65355.
- [51] R. Pop, M.N. Stefanut, A. Cata, C. Tanasie, M. Medeleanu, Ab initio study regarding the evaluation of the antioxidant character of cyanidin, delphinidin and malvidin, *Cent. Eur. J. Chem.* 10 (2012) 180–186.
- [52] N. Matsunaga, S. Imai, Y. Inokuchi, M. Shimazawa, S. Yokota, Y. Araki, H. Hara, Bilberry and its main constituents have neuroprotective effects against retinal neuronal damage *in vitro* and *in vivo*, *Mol. Nutr. Food Res.* 53 (2009) 869–877.
- [53] W. Huang, Y. Zhu, C. Li, Z. Sui, W. Min, Effect of blueberry *Anthocyanins Malvidin* and glycosides on the antioxidant properties in endothelial cells, *Oxidative Med. Cell. Longev.* (2016), <http://dx.doi.org/10.1155/2016/1591803>.
- [54] H.R. Seo, M.J. Choi, J.M. Choi, J.C. Ko, J.Y. Ko, E.J. Cho, Malvidin protects WI-38 human fibroblast cells against stress-induced premature senescence, *Eur. J. Cancer Prev.* 21 (2016) 32–40.
- [55] J.W. Hyun, H.S. Chung, Cyanidin and malvidin from *Oryza sativa* cv. Heugjinjubyeo mediate cytotoxicity against human monocytic leukemia cells by arrest of G2/M phase and induction of apoptosis, *J. Agric. Food Chem.* 52 (2004) 2213–2217.
- [56] P.H. Shih, C.T. Yeh, G.C. Yen, Effects of anthocyanidin on the inhibition of proliferation and induction of apoptosis in human gastric adenocarcinoma cells, *Food Chem. Toxicol.* 43 (2005) 1557–1566.
- [57] S.J. Patterson, J.G. Fischer, R.V. Dulebohn, DNA damage in HT-29 colon cancer cells is enhanced by high concentrations of the anthocyanin malvidin, *FASEB J.* 22 (2008) 890–900.
- [58] W. Li, Y. Guo, C. Zhang, R. Wu, A.Y. Yang, J. Gaspar, A.N. Kong, Dietary phytochemicals and cancer chemoprevention: a perspective on oxidative stress, inflammation, and epigenetics, *Chem. Res. Toxicol.* 29 (2016) 2071–2095.
- [59] H.S.D. Martino, M.M. dos Santos Dias, G. Noratto, S. Talcott, S.U. Mertens-Talcott, Anti-lipidaemic and anti-inflammatory effect of açai (*Euterpe oleracea* Martius) polyphenols on 3T3-L1 adipocytes, *J. Funct. Foods* 23 (2016) 432–443.
- [60] P.O. Barbosa, D. Pala, C.T. Silva, M.O. de Souza, J.F. do Amaral, R.A.L. Vieira, G.A. Folly, A.C. Volp, R.N. de Freitas, Açai (*Euterpe oleracea* Mart.) pulp dietary intake improves cellular antioxidant enzymes and biomarkers of serum in healthy women, *Nutrition* 32 (2016) 674–680.
- [61] D. Pala, P.O. Barbosa, C.T. Silva, M.O. de Souza, F.R. Freitas, A.C. Volp, R.C. Maranhão, R.N. Freitas, Açai (*Euterpe oleracea* Mart.) dietary intake affects plasma lipids, apolipoproteins, cholesteryl ester transfer to high-density lipoprotein and redox metabolism: a prospective study in women, *Clin. Nutr.* (2017), <http://dx.doi.org/10.1016/j.clnu.2017.02.001>.
- [62] C.K. Singh, I.A. Siddiqui, S. El-Abd, H. Mukhtar, N. Ahmad, Combination chemoprevention with grape antioxidants, *Mol. Nutr. Food Res.* 60 (2016) 1406–1415.