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Phenolic contents and bioactive potential of peach fruit extracts

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ABSTRACT

Several cultivars of peach fruit (*Prunus persica* L.) were investigated. Their phenolic composition and concentration were assessed by LC–MS. Concentrations were calculated in mg per g of dry weight extract. Their antioxidant capacity (Folin–Ciocalteu, ORAC, DPPH, ABTS, PFRAP and ICA), inhibitory property against β-amyloid and α-synuclein fibril formation and protective capacity against Aβ-induced toxicity on PC12 cell lines (viability assessed by MTT assay and intracellular ROS production by DCFH-DA assay) were evaluated. Fifteen different phenolic compounds were identified and quantified. In particular, new isorhamnetin derivatives were identified. Phenolic contents were ranged between 19 and 82 mg/g. Spring Belle extract had the highest content and Romea the lowest. Except for the ICA assay, a good correlation between phenolic content and the antioxidant capacities of peach fruit extracts was found, indicating that phenolic compounds are major contributors to their antioxidant capacity. Results indicate that the phenolic extract of peach cultivars inhibits Aβ and αS fibril formation and protects PC12 cell lines against Aβ-induced toxicity.

1. Introduction

The peach (*Prunus persica* L.) is a deciduous tree that is native to South Asia and cultivated worldwide. The world production is about ten million tons per year. Peach is one of the most widely consumed fruits in several European countries, especially those with the Mediterranean diet (Konopacka et al., 2010; Saura-Calixto & Goñi, 2006). Epidemiological studies suggest that the Mediterranean diet, which is based partly on fruit consumption, may play a role in preventing several diseases (Sofi, Cesari, Abbate, Gensini, & Casini, 2008). The benefits of fruit consumption have been linked with phenolic compounds (Arts & Hollman, 2005). Peaches are known to contain phenolics (Aleixandre, Aleixandre-Tudó, Bolaños-Pizzaro, & Aleixandre-Benavent, 2013; Tomás-Barberán et al., 2001; Vizzotto, Cisneros-Zevallos, Byrne, Ramming, & Okie, 2007) that have been shown to display a high antioxidant and chemopreventive potential (Chang, Tan, Frankel, & Barrett, 2000; Gasparotto et al., 2014; Noratto, Porter, Byrne, & Cisneros-Zevallos, 2009; Noratto, Porter, Byrne, Cisneros-Zevallos, 2014). Nevertheless, as mentioned by Tomás-Barberán et al. (2001), very few studies have investigated both the phenolic profiles and their content in several fruit peach cultivars (Di Vaio, Marallo, Graziani, Ritieni, & Di Matteo, 2014). Furthermore, phenolic compounds have a crucial impact on the visual aspect and taste of fruit (Lee, Kagan, Jaworski, & Brown, 1990) and could therefore be used to assess fruit quality.

The aim of the present study was to compare the phenolic content of seven fruit peach cultivars grown in Algeria: four freestone cultivars: Cardinal (medium sized fruit, bright red over yellow skin, yellow flesh, early midseason harvest), Flavorcrest (large round fruit, bright red over yellow skin; yellow flesh, midseason harvest), Red Top (large fruit, almost fully blushed over yellow skin, yellow flesh, midseason harvest), Spring Belle (medium round fruit, orangey skin, yellow flesh, midseason harvest); a semi-freestone cultivars: Dixired (medium sized fruit, red skin, yellow flesh, early midseason harvest) and two clingstone cultivars: Romea (medium sized fruit, yellow skin, yellow-orange flesh, midseason harvest) and Tebana (medium sized fruit, orangey skin, yellow flesh, midseason harvest). Moreover, as the antioxidant potential of these fruit peach varieties has never been compared, this potential was assessed by several tests involving different mechanisms

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(Dudonné et al., 2011; Prior, Wu, & Schaich, 2005) in order to cover all the aspects of antioxidant efficacy. The following assays were performed: Folin–Ciocalteu (F–C), Oxygen Radical Absorbance Capacity (ORAC), 2,2-DiPhenyl-PicrylHydrazyl radical (DPPH), 2,2′-Azinobis(3-ethylBenzoThiazoline-6-Sulfonic acid) (ABTS), Potassium Ferricyanide Reducing Antioxidant Power (PFRAP) and Iron Chelating Activity (ICA). Phenolic compounds have recently received particular attention owing to their possible preventive role in neurological disorders (Basli et al., 2012; Esposito et al., 2002). β-amyloid (Aβ) and α-synuclein (αS) aggregation are considered to be a critical step during neurodegenerative processes associated with Alzheimer's disease (AD) and Parkinson's disease (PD) (Butterfield, Reed, Newman, & Sultana, 2007; Irwin, Lee, & Trojanowski, 2013; Rochet & Lansbury, 2000). In this study, the inhibitory capacity of phenolic extracts of different fruit peach cultivars on Aβ and αS fibril formation was evaluated. Moreover, the protective effects of peach extracts on Aβ-induced toxicity in PC12 cell lines were measured.

2. Materials and methods

2.1. Reagents and standards

Chlorogenic acid, quercetin 3-*O*-glucoside, quercetin 3-*O*-galactoside, kaempferol 3-*O*-glucoside, isorhamnetin 3-*O*-glucoside and cyanidin 3-*O*-glucoside were obtained from Extrasynthese (France). Folin–Ciocalteu reagent, sodium carbonate, catechin, rutin, aluminum chloride, 2,2′-azobis(2-amidinopropane) dihydrochloride (AAPH), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), potassium ferricyanide, trichloroacetic acid, ferric chloride, 3-(2-pyridyl)-5,6-diphenyl-1,2,4-triazine-4′,4″-disulfonic acid sodium salt (ferrozine), 1,1-diphenyl-2-picrylhydrazyl radical (DPPH), 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), 3′,6′-dihydroxy spiro[2-benzofuran-3,9′-xanthene]-1-one (fluorescein), phosphate buffer (0.1 M, pH 7.4), Phosphate Buffered Saline (PBS), 2′,7′-dichlorofluorescein diacetate (DCFH-DA), dimethyl sulfoxide (DMSO), L-ascorbic acid, L-glutamine, fetal horse serum, fetal bovine serum, penicillin, streptomycin, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) and Thioflavin T (THT) were obtained from Sigma (France). Chlorhydric acid, formic acid and acetonitrile were supplied by Fisher Scientific. Ethylenediaminetetraacetic acid (EDTA) and sodium hydroxide were obtained from Fluka. Acetone, methanol, ferrous sulfate, sodium nitrate and potassium persulfate were supplied by Prolabo (France). Curcumin was purchased from Bachem (Germany). Amyloid-β peptide 25–35 ($A\beta_{25-35}$) was provided by Synvec (France). Purified recombinant human α-synuclein was purchased from Alexotech AB (Umeå, Sweden). Water was purified using an Elga water purification system (ElgaLabWater, USA) with a resistivity of no less than 18 MΩ/cm.

2.2. Plant material

The fruit peach samples used in this study were obtained from the Technical Institute of Fruit Tree Cultivation and Vine (Institut Technique de l'Arboriculture Fruitière et de la Vigne, ITAFV, Tessala El Merdja, Birtouta, Algiers, Algeria). Seven varieties of peach (*p. persica* L.) were randomly harvested between May and June 2011: Cardinal, Dixired, Flavorcrest, Red Top, Romea, Spring Belle and Tebana. To check that fruits had reached maturity, Brix was measured with a refractometer before harvest. Maturity was deemed to be reached when the sugar content plateaued, as measured by total soluble solids assessed by refractometry. After the harvest, whole fruits were washed, dried, pitted, cutted into slices and then homogenized in a blender before being freeze-dried in a lyophilizer. The freeze-dried peach samples obtained were grounded into fine powder to ensure uniformity and kept at 4 °C until extraction.

2.3. Sample preparation

The freeze-dried fruits (20 g) were twice extracted with 200 mL of acetone/water (60/40, v/v). Extraction was performed at room temperature for two hours and under agitation. After centrifugation (20 min, 4000 rpm), the supernatants were combined and concentrated with a vacuum rotary evaporator at 40 °C before being freeze-dried. The dried supernatants were dissolved in 20 mL methanol/water (30/70, v/v). Each extract was purified on a Supelclean™ LC-18 solid phase extraction (SPE) column (Supelco, USA). After elution with methanol/water 90/10 (v/v), resulting purified extracts were evaporated at 40 °C and then freeze-dried. Samples were kept at 4 °C until analysis.

2.4. HPLC and HPLC-mass spectrometry (MS/MS) analysis

The chromatography apparatus, an Agilent 1200 (Agilent Technologies, Santa Clara, CA, USA), consisted in an autosampler module, a degasser, a binary pump, a column heater/selector and a UV–visible-DAD detector from the same provider. The column was a Prontosil C_{18} (5 μm, 250 mm × 4.6 mm), Bischoff (Leonberg, Germany). Lyophilized peach extract was diluted in 50% methanol containing 1% formic acid at the concentration of 50 mg/mL and filtered through a 0.45 μm PTFE filter. The flow rate was typically 0.8 mL/min and the sample injection volume was 20 μL. Fractions were eluted with a water gradient containing 5% formic acid (solvent A) and acetonitrile–5% formic acid (solvent B) according to the following gradient program (v/v): 0 min 90% A 10% B, 85 min 50% A 50% B, 95 min 100% B linear for 10 min, followed by 10 min for re-equilibration. Detection wavelengths were set at 280, 360 and 520 nm with a diode array detector (DAD).

This HPLC was coupled to an Esquire 3000+ ion trap mass spectrometer using an ESI source from Bruker Daltonics (Billerica, MA, USA). The HPLC output flow was split with a passive splitter at an average 1:100 ratio depending on the flow solvent, viscosity and rate. Drying gas was set at 9.0 L/min and 350 °C, nebulizer pressure was set to 27 psi. ESI-MS parameters: HV capillary – 4100 V, end plate offset – 500 V, capillary exit 134.3 V, skimmer 40 V, trap drive 59.3, scan 25,000 μs, rolling average 2 and trap averages 5.

Individual polyphenols were quantified by means of a calibration curve using external standards. Flavan-3-ols were quantified as catechin at 280 nm; hydroxycinnamic acids as chlorogenic acid at 280 nm; flavonols as quercetin 3-*O*-glucoside at 360 nm and anthocyanins as malvidin 3-*O*-glucoside at 520 nm. Concentrations were calculated in mg per g of dry weight extract (mg/g). Mean values of each peach cultivar were calculated from three technical replicates.

2.5. Antioxidant properties

2.5.1. Total phenolic content

The total phenolic content of peach extracts was determined by the Folin–Ciocalteu colorimetric method adapted to 96-well plates (Singleton & Rossi, 1965). Dried extracts were resuspended in methanol before dilution. To 20 μL of peach extract or methanol (blank), 100 μL of Folin–Ciocalteu reagent (diluted previously 10 times with water) were added. After 2–3 min, 80 μL of sodium carbonate (75 g/L) solution were added. The mixture was then kept in the dark at room temperature for 1 h. The absorbance was measured at 765 nm using a plate reader (Fluostar Optima; BMG Labtech). The total phenolic content was expressed as mg

chlorogenic acid equivalent per g of dry weight extract (mg CAE/g). Samples were analyzed at least three times and with triplicate samples.

2.5.2. DPPH assay

Antioxidant activity of the extracts was measured in terms of radical scavenging ability by using the stable radical DPPH, as described by Blois (1958). Various concentrations of the samples (50 μL) were mixed with 150 μL of 200 μM methanolic solution of DPPH in a 96-well plate. The plate was allowed to stand at room temperature in the dark for 20 min. Absorbance was measured at 520 nm against methanol as a blank. A standard Trolox curve was plotted and results were expressed as mg of Trolox equivalent per g of dry extract (mg TE/g DE). All samples were analyzed in triplicate in at least three different experiments.

2.5.3. ORAC assay

The oxygen radical absorbance capacity (ORAC) assay allows the determination of free radical scavenging capacity. It was performed according to the method of Ou, Hampsch-Woodill, and Prior (2001) modified by Dávalos, Gómez-Cordovés, and Bartolomé (2004) using the automated plate reader (Fluostar Optima; BMG Labtech). The antioxidant capacities of the peach extracts were expressed as mg of Trolox equivalent per g of dry weight extract (mg TE/g). All samples were analyzed in quadruplicate and in at least three different experiments.

2.5.4. ABTS assay

The scavenging activity of peach extract on ABTS radical cation was estimated according to the method of Re et al. (1999). The ABTS radical cation ($ABTS^{+}$) solution at an absorbance of 0.700 ± 0.020 at 734 nm was generated as indicated by Re et al. (1999). The initial protocol was slightly modified for analysis in microplate: to 10 μL of peach extract (or Trolox), 250 μL of the diluted $ABTS^{+}$ solution were added. Results were expressed as mg of Trolox equivalent per g of dry weight extract (mg TE/g). All samples were analyzed in triplicate in at least three different experiments.

2.5.5. PFRAP assay

The capacity of peach extracts to reduce Fe^{3+} was assessed by the method of Oyaizu adapted for a 96-well microplate (Oyaizu, 1986). To 10 μL of methanolic dilution of extract (or ascorbic acid as standard), 30 μL of phosphate buffer (0.2 M, pH 6.6) and 30 μL of 1% potassium ferricyanide were added. The plate was incubated at 50 °C for 20 min and 30 μL of 10% trichloroacetic acid were added to each well. Absorbance was measured at 700 nm after addition of 100 μL of distilled water and 20 μL of 0.1% ferric chloride. Results were expressed as mg ascorbic acid equivalent per g of dry weight extract (mg AA/g). All samples were analyzed in triplicate in at least three different experiments.

2.5.6. ICA assay

The chelating activity of peach extracts for ferrous ions Fe^{2+} was measured according to the method of Dinis, Madeira, and Almeida (1994). 40 μL of methanolic dilution of peach extract, 80 μL of deionized water and 40 μL of $FeSO_4$ (0.2 mM) were mixed in a 96-well microplate. The reaction was initiated by addition of 40 μL of ferrozine (2 mM). Ferrozine reacted with the divalent iron to form stable magenta complex species that were very soluble in water. After 10 min at room temperature, the absorbance of the Fe^{2+}–ferrozine complex was measured at 562 nm. Methanol instead of peach extract was used as positive control and distilled water as blank instead of ferrozine. EDTA was used as standard and results were expressed as mg EDTA per g of dry weight extract (mg EDTA/g). All samples were analyzed in triplicate in at least three different experiments.

2.6. Fibril aggregation inhibitory activity

2.6.1. Amyloid-β fibril inhibition

Amyloid-β (Aβ) fibril aggregation inhibition was measured according to the method of Rivière et al. (2007). UV–visible measurements were used to search for inhibitors of Aβ fibril formation. A stock solution of $Aβ_{25–35}$ peptide at 1 mM was prepared in sterile water at 4 °C and aliquots were stored at −20 °C. All steps were carried out at 4 °C to prevent any Aβ peptide polymerization. Lyophilized peach extract containing polyphenols was solubilized in methanol to a concentration of 2 mg/mL. Stock solutions were aliquoted and stored at -20 °C. To study Aβ fibril inhibition, experiments were carried out by using a reaction mixture containing 80 μL phosphate buffer (10 mM final concentration, pH 7.2), 10 μL Aβ peptide (0.1 mM final concentration) and 10 μL tested extracts (200 μg/mL final concentration) or 10 μL of methanol as negative control. Sonication was performed for 5 min to avoid peptide aggregation as much as possible at time $t = 0$ h. UV–visible spectroscopic analysis was performed on a Cary 300 bio UV–visible spectrophotometer (Varian, USA) and polymerization kinetics were monitored for 5 h at 200 nm corresponding to the peptide bond absorbance wavelength. Typical spectroscopic experiments were performed with six samples containing respectively Aβ peptide alone, peach extract alone, a curcumin/Aβ peptide mixture, and three peach extract/Aβ peptide mixtures. The sample with the extract alone was used to check that it had no influence on the absorbance, while the curcumin/Aβ peptide mixture was used as positive control. At least three independent measurements were made for each experiment.

2.6.2. α-synuclein fibril inhibition

A solution of 140 μM α-synuclein (αS) was prepared in a 25 mM Na_2HPO_4, 140 mM NaCl buffer at pH 7.4 and sonicated 2 min prior to each experiment. Peach extract was diluted in DMSO at the concentration of 40 mg/mL stock solution and then diluted in the abovementioned buffer to a concentration of 200 μg/mL in 2% DMSO solution. To 30 μL of diluted peach extract, an equal volume of αS at 140 μM was added in a 96-well plate. Thioflavin T (ThT) was added to a final concentration of 20 μM. Each condition was performed in triplicate ($n = 3$). The plate was incubated at 37 °C, 300 rpm. Fluorescence was measured every 2 h for 4 days with a Fluostar Optima plate reader (BMG Labtech, Germany) set at 450 nm for excitation and 485 nm for emission. Blanks of each compound were subtracted from the measured fluorescence.

2.7. Protective effects against Aβ-induced cytotoxicity on PC12 cells

PC12 cells obtained from a rat pheochromocytoma were obtained from the American Type Culture Collection (ATCC, Manassas, USA). They were maintained in DMEM-Glutamax supplemented with 100 IU/mL penicillin, 100 μg/mL streptomycin, 15% of fetal horse serum and 2.5% of fetal bovine serum at 37 °C in a humidified atmosphere of 5% CO_2. The cells were subcultured in 96-well culture plates (30×10^3 cells/well) for 24 h and then treated with $Aβ_{25–35}$ peptide in the absence or presence of extracts in serum-free culture medium. Extracts were dissolved in DMSO at a final concentration of 0.1%, which is a subtoxic concentration. All experiments were repeated at least in triplicate and performed in quadruplicate.

2.7.1. MTT assay

Cell viability was determined by using the MTT reduction assay. After treatment with 10 μM of $Aβ_{25–35}$ peptide in the absence or

Table 1
Chromatographic data (peak number, retention time and UV_{max}), and MS^2 *m/z* values (molecular and fragment ions) of peach phenolic compounds.

Compound	No.	t_R (min)	UV_{max} (nm)	MH[a] (Frag. MS^2 *m/z*)
Hydroxycinnamates				
Neochlorogenic acid	**1**	3.3	325, 290sh	353 (191, 179)
Chlorogenic acid	**4**	6.4	325, 290sh	353 (191)
Flavan-3-ols				
Procyanidin dimer	**2**	4.1	280	577 (451, 425, 407, 289, 287)
Catechin	**3**	5.2	280	289
Procyanidin dimer	**5**	11.9	280	577 (451, 425, 407, 289)
Anthocyanins				
Cyanidin-3-glucoside	**6**	13.5	515, 280	449 (287)[a]
Cyanidin-3-rutinoside	**7**	15.4	515, 280	595 (449, 287)[a]
Flavonols				
Quercetin-3-galactoside	**8**	22.0	350	463 (301)
Quercetin-3-rutinoside	**9**	22.5	350	609 (301)
Quercetin-3-glucoside	**10**	23.0	350	463 (301)
Kaempferol-3-galactoside	**11**	26.2	345	447 (285)
Kaempferol-3-rutinoside	**12**	27.9	345	593 (285)
Kaempferol-3-glucoside	**13**	28.2	345	447 (285)
Isorhamnetin-3-rutinoside	**14**	28.8	345	623 (315)
Isorhamnetin-3-glucoside	**15**	29.5	345	477 (315)

[a] MS data in negative mode except for anthocyanins in positive mode.

presence of different concentrations of peach extracts for 24 h (50, 100 and 200 μg/mL), PC12 cells were incubated in 0.5 mg/mL MTT at 37 °C for 3 h. Then, the MTT solution was removed and the resulting formazan crystals were dissolved with DMSO. Absorbance values were read at 540 nm on a microplate reader (MRII, Dynex).

2.7.2. ROS measurement

The concentration of intracellular reactive oxygen species (ROS) was determined by using an intracellular peroxide-sensitive fluorescent DCFH-DA probe. PC12 cells were seeded in 96-well black culture plates (30×10^3 cells/well). After treatment with $A\beta_{25-35}$ (20 μM) and peach extracts for 6 h (50, 100 and 200 μg/mL), cells were washed with PBS and then incubated with 100 μl of DCFH-DA (5 μM). The fluorescence intensity was immediately measured for 30 min at 37 °C at an excitation wavelength of 485 nm and an emission wavelength of 520 nm using a spectrofluorometer (FLUOstar Optima, BMG Labtech).

2.8. Statistical analysis

All samples were analyzed at least in triplicate. Data are expressed as means ± standard error mean (SEM). Concerning the antioxidant measurements, parametric tests were used to assess variance and correlation after a Kolmogorov–Smirnov test to confirm the normality of the data. Differences were evaluated by one-way analysis of variance (ANOVA) and Tukey's test. The Pearson correlation test was used to compare the different values of antioxidant activities obtained in our extracts after all types of antioxidant measurement. Differences were considered statistically significant at $p < 0.05$. The statistical tests for PC12 cell experiments were performed with one-way ANOVA followed by Dunnett's multiple comparison post-hoc test. Significance was set at $p < 0.05$. GraphPad Prism 5.03 for Windows (GraphPad Software, San Diego, CA, USA) was used for these analyses.

3. Results and discussion

3.1. Identification of phenolic compounds

In this study, phenolic compounds were identified by HPLC-MS/MS analysis. The combination of HPLC–DAD analysis and electrospray ionization mass spectrometry allows the efficient identification and quantification of polyphenols in extracts (Tomás-Barberán et al., 2001). Phenolic compounds were tentatively identified by examining the mass spectra in positive and/or in negative mode (MS and MS/MS). To confirm the identification of the phenolic compounds, several markers were available: chlorogenic acid (hydroxycinnamic acid), catechin (flavan-3-ol), cyanidin-3-*O*-glucoside (anthocyanin), quercetin-3-*O*-glucoside, rutin, quercetin-3-*O*-galactoside, kaempferol-3-*O*-glucoside, isorhamnetin-3-*O*-glucoside (flavonol). Fifteen phenolic compounds were identified in fruit peach cultivars with this procedure (Table 1). These compounds originate from four phenolic groups: phenolic acids, flavan-3-ols, anthocyanins and flavonols. The UV–visible detector system was set at 280 nm for phenolic acids and flavan-3-ols, 360 nm for flavonols and 520 nm for anthocyanins (Fig. 1).

For mass analysis of phenolic acids the negative mode was selected. Two isomers of chlorogenic acid were observed. The assignment of chlorogenic acids was based on the observation of the ion fragments *m/z* 353 $[M - H]^-$, 191 and 179 corresponding to the deprotonated molecule, the deprotonated quinic acid and the deprotonated caffeic acid, respectively. Chlorogenic acid (**4**) was identified by chromatographic comparison with the authentic marker. Neochlorogenic acid (**1**) was tentatively identified by comparison with literature data (Tomás-Barberán et al., 2001).

Concerning flavan-3-ol derivatives, catechin (**3**) was identified by comparison with the standard. In the negative mode, catechin mass spectra showed characteristic fragments at *m/z* 289 $[M - H]^-$ and 179 (Sun, Liang, Bin, Li, & Duan, 2007). In addition to catechin, two procyanidin dimers were observed at peaks **2** and **5**. MS/MS spectra in the negative mode showed a molecular ion at *m/z* 577 $[M - H]^-$ and characteristic fragmentation signals at *m/z* 451, 425, 407 and 289 (Sun et al., 2007).

Two anthocyanins were identified in the extract. They present a characteristic UV spectrum with maximum absorption near 520 nm. The mass spectra were acquired in the positive mode for anthocyanins. Cyanidin-3-*O*-glucoside (**6**) yielded fragment ions at *m/z* 449 and 287 (Acevedo de la Cruz et al., 2012). The fragment at *m/z* 287 observed in the MS/MS spectrum was used to identify the aglycone. The identification was confirmed by comparison with the pure standard. The second anthocyanin, peak **7**, was identified as cyanidin-3-*O*-rutinoside. Peak **7** had an *m/z* 595 $[M - H]^-$, dissociating to yield fragment ions *m/z* 449 $[M - H - 146]^-$ and 287

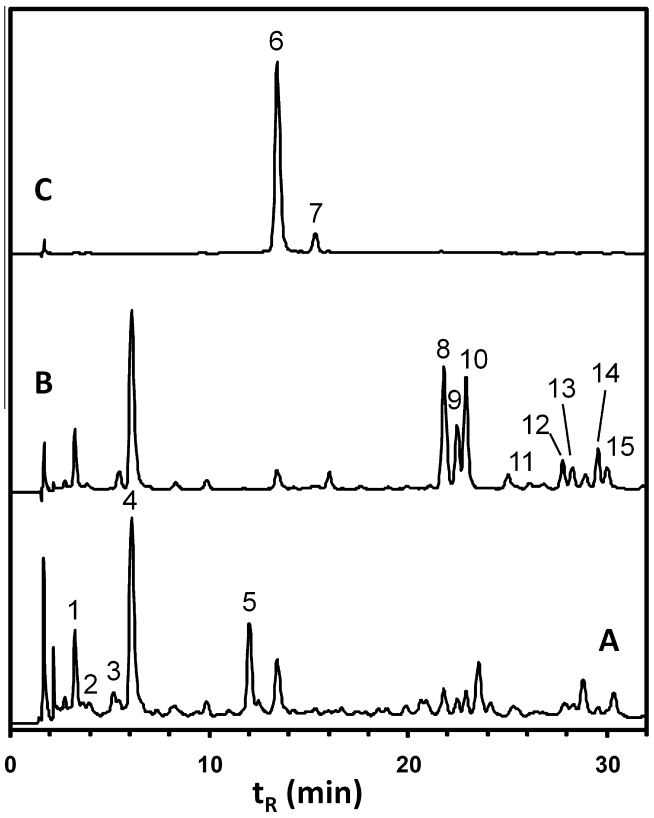

Fig. 1. HPLC chromatograms of Spring Belle extract recorded at 280 nm (A), 360 nm (B) and 520 nm (C).

$[M - H - 146 - 162]^-$, and indicating the loss of a rhamnosyl unit and a rhamnosyl hexosyl group, respectively. The fragment ion at *m*/*z* 287 corresponds to cyanidin aglycone, so this compound was identified as cyanidin-3-*O*-rutinoside (Tomás-Barberán et al., 2001).

All the flavonols identified were conjugated with a glycosidic unit. Mass spectra in the negative mode (MS and MS/MS) allowed the identification of each flavonol aglycone by the observation of characteristic *m*/*z* fragmentation values (301 quercetin; 285 kaempferol; 315 isorhamnetin) owing to the loss of the sugar moiety. The assignment of the sugar was based on the characteristic loss of a fragment of *m*/*z* 162 (glucosides and galactosides), as well as the characteristic loss of a fragment of *m*/*z* 308 (rhamnoglucosides). The identification of flavonol derivatives was confirmed by comparison with pure standards. To our knowledge, this is the first report of isorhamnetin derivatives in fruit peach extracts.

3.2. Phenolic contents

The seven peach fruit extracts can be classified into three groups based on their phenolic contents (Table 2). Spring Belle extract has the highest concentration (81.5 mg/g). Cardinal, Dixired and Red Top constitute the second group with a concentration range between 34.3 and 37.9 mg/g. Finally, Flavorcrest and Romea have the lowest phenolic contents (23.1 and 19.8 mg/g, respectively). The different cultivar fruit extracts were found to have highly variable concentrations in phenolic compounds, in agreement with the literature data (Andreotti, Ravaglia, Ragaini, & Costa, 2008; Tomás-Barberán et al., 2001). This variability depends on both the cultivar and the environmental conditions (Andreotti et al., 2008).

Cinnamic acids were represented by two compounds: neochlorogenic and chlorogenic acids. The maximum level was found in Spring Belle (16.0 mg/g) followed by Cardinal and Dixired (9.5 and 6.5 mg/g, respectively). Flavocrest, Romea and Tebana had the lowest level (2.0, 2.0 and 1.9 mg/g). Chlorogenic acid was the main compound of this class of phenolics in all cultivars. This result is in agreement with literature data and confirms that chlorogenic is the main hydroxycinnamate in peaches (Andreotti et al., 2008; Tomás-Barberán et al., 2001).

Three flavan-3-ols were identified and quantified: catechin and two procyanidin dimers. Epicatechin was also observed but in very low concentrations. Except for Spring Belle, procyanidins were the main class of phenolic compounds accounting for an average percentage higher than 53% of all phenolics. Spring Belle was the richest cultivar, containing 26.6 mg/g followed by Red Top and Dixired, containing 23.1 and 20.8 mg/g, respectively. Romea and Flavorcrest were the cultivars containing the lowest level of flavan-3-ols (12.4 and 14.0 mg/g, respectively). Previous studies showed that catechin is the main monomeric flavan-3-ol, with minor amounts of epicatechin (Tomás-Barberán et al., 2001). Our results confirm that catechin is the main monomeric flavan-3-ol in peaches. In addition to catechin, flavan-3-ol dimers were also detected. The occurrence of flavan-3-ol dimers (B1 to B4) has already been reported in peaches (Tomás-Barberán et al., 2001).

Two anthocyanidins were identified and quantified: cyanidin 3-glucoside and 3-rutinoside. The maximum level was found in Spring Belle (7.7 mg/g), followed by Red Top (1.9 mg/g). No anthocyanidins were identified in Romea and only a small amount of cyanidin 3-glucoside was observed in Tebana. Cyanidin 3-glucoside was the main anthocyanidin identified. Only small amounts of cyanidin 3-rutinoside were detected. This result is in agreement with literature data confirming that only cyanidin derivatives are present in peaches (Tomás-Barberán et al., 2001).

Eight flavonols were identified: quercetin 3-galactoside, 3-rutinoside and 3-glucoside; kaempferol 3-galactoside, 3-rutinoside and 3-glucoside; isorhamnetin 3-rutinoside and 3-glucoside. Flavonols were the main class of phenolic compounds in Spring Belle, accounting for an average percentage of 38% of all phenolics. Spring Belle was the richest cultivar containing 31.2 mg/g, followed by Red Top and Tebana containing 9.6 and 9.2 mg/g, respectively. Romea and Cardinal were the cultivars containing the lowest level of flavonols (5.4 and 6.1 mg/g, respectively). All flavonols detected were substituted by different sugars. The main flavonols identified were rutinoside derivatives (range between 2.6 and 13.9 mg/g) followed by glucoside derivatives. The quercetin derivatives were the main flavonols detected except for Romea and Tebana. In these two cultivars, the majority of flavonols were isorhamnetin derivatives.

3.3. Antioxidant capacity

The following assays were performed: Folin–Ciocalteu (F–C), Oxygen Radical Absorbance Capacity (ORAC), 2,2-DiPhenyl-PicrylHydrazyl radical (DPPH), 2,2′-Azinobis(3-ethylBenzoThiazoline-6-Sulfonic acid) (ABTS), Potassium Ferricyanide Reducing Antioxidant Power (PFRAP) and Iron Chelating Activity (ICA). These different assays allowed us to evaluate the electron transfer and the hydrogen atom transfer capacity, as well as the ability to inhibit interactions between metals and lipids of the seven studied peach extracts (Table 3). As the fruit peach extracts displayed different antioxidant capacities in relation to the method applied, the rank of the antioxidant capacity value obtained is specified for each assay.

The total phenolic content (TPC) based on the Folin–Ciocalteu assay (F–C) varied significantly between 80.8 and 34.5 mg CAE/g of peach extract (dry weight) according to the peach cultivar. In

Table 2
Content of phenolic compounds (mg/g of extract) in peaches.

Compound	Cardinal	%	Dixired	%	Flavorcrest	%	Red Top	%	Romea	%	Spring Belle	%	Tebana	%
Cinnamates														
Neochlorogenic acid	2.4 ± 0.1	7	1.7 ± 0.2	5	0.6 ± 0.1	3	1.0 ± 0.1	3	0.6 ± 0.1	3	2.9 ± 0.2	3	0.5 ± 0.1	2
Chlorogenic acid	7.1 ± 0.9	20	4.8 ± 0.1	14	1.4 ± 0.2	6	2.3 ± 0.1	6	1.4 ± 0.1	7	13.1 ± 0.5	16	1.4 ± 0.1	5
Total	9.5	27	6.5	19	2.0	9	3.3	9	2.0	10	16.0	19	1.9	7
Flavan-3-ols														
Procyanidin dimer	0.3 ± 0.1	1	0.7 ± 0.1	2	0.6 ± 0.1	3	0.4 ± 0.1	1	0.4 ± 0.1	2	3.0 ± 0.2	3	NQ	<1
Catechin	1.1 ± 0.2	3	0.9 ± 0.2	3	1.1 ± 0.2	5	1.5 ± 0.3	4	0.3 ± 0.1	1	1.0 ± 0.1	1	0.6 ± 0.1	2
Procyanidin dimer	16.8 ± 1.2	49	19.2 ± 1.4	56	12.3 ± 0.9	53	21.2 ± 0.5	56	11.7 ± 0.1	59	22.6 ± 0.1	28	17.3 ± 0.1	59
Total	18.2	53	20.8	61	14.0	61	23.1	61	12.4	62	26.6	32	17.9	61
Anthocyanins														
Cyanidin glucoside	0.5 ± 0.1	1	0.4 ± 0.1	1	0.7 ± 0.03	2	1.9 ± 0.2	5	-	-	7.0 ± 0.1	9	NQ	<1
Cyanidin rutinoside	NQ	<1	NQ	<1	NQ	<1	NQ	<1	-	-	0.7 ± 0.1	1	-	
Total	0.5	2	0.4	1	0.7	2	1.9	5	-	-	7.7	10	NQ	<1
Flavonols														
Quercetin galactoside	0.8 ± 0.2	2	1.0 ± 0.2	3	0.4 ± 0.1	2	1.0 ± 0.1	3	0.6 ± 0.1	3	4.5 ± 0.1	5	0.8 ± 0.1	3
Quercetin rutinoside	1.1 ± 0.1	3	1.2 ± 0.1	3	1.5 ± 0.2	7	2.5 ± 0.1	7	0.5 ± 0.1	3	9.5 ± 0.1	12	1.3 ± 0.1	4
Quercetin glucoside	1.1 ± 0.1	3	1.2 ± 0.1	3	1.9 ± 0.3	8	2.7 ± 0.2	7	0.4 ± 0.1	2	9.0 ± 0.1	11	1.1 ± 0.1	4
Kaempferol galactoside	NQ	<1	NQ	<1	NQ	<1	NQ	<1	NQ	<1	NQ	<1	NQ	<1
Kaempferol rutinoside	0.6 ± 0.1	2	0.3 ± 0.1	1	NQ	<1	NQ	<1	NQ	<1	1.0 ± 0.1	1	NQ	<1
Kaempferol glucoside	0.7 ± 0.1	2	0.8 ± 0.2	2	0.4 ± 0.1	2	0.8 ± 0.1	2	1.2 ± 0.1	6	2.0 ± 0.1	3	1.6 ± 0.1	5
Isorhamnetin rutinoside	1.5 ± 0.3	4	1.8 ± 0.1	5	1.4 ± 0.1	6	1.8 ± 0.3	5	2.1 ± 0.1	10	3.4 ± 0.3	4	3.7 ± 0.1	13
Isorhamnetin glucoside	0.3 ± 0.1	1	0.4 ± 0.1	1	0.8 ± 0.1	3	0.6 ± 0.1	1	0.5 ± 0.1	3	1.8 ± 0.1	2	0.7 ± 0.1	3
Total	6.1	18	6.7	19	6.4	28	9.6	25	5.4	27	31.2	38	9.2	32
Total phenolic	34.3		34.4		23.1		37.9		19.8		81.5		29.0	

Table 3
Antioxidant activities and inhibitory properties against of $A\beta_{25-35}$ and αS fibril formation of the seven studied peach cultivars.

	Cardinal	Dixired	Flavorcrest	Red Top	Romea	Spring Belle	Tebana
Antioxidant assays							
F–C (mg of CA /g)	44.1 ± 2.6^{c,d}	53.2 ± 4.2^{b}	34.5 ± 3.4^{e}	49.2 ± 5.5^{b,c}	40.2 ± 3.6^{d,e}	80.8 ± 7.7^{a}	38.2 ± 3.3^{d,e}
Rank	4	2	7	3	5	1	6
PFRAP (mg of AA/g)	31.8 ± 4.1^{c}	36.2 ± 3.5^{b}	22.1 ± 2.8^{e}	33.4 ± 5.1^{c,d}	23.7 ± 0.8^{c}	56.2 ± 1.0^{a}	27.6 ± 1.9^{d,e}
Rank	4	2	7	3	6	1	5
DPPH (mg of TE/g)	26.9 ± 3.4^{c}	35.9 ± 2.8 ^{b}	16.0 ± 0.9^{d}	29.8 ± 1.2^{c}	20.6 ± 1.7^{d}	48.9 ± 1.8^{a}	20.1 ± 2.9^{d}
Rank	4	2	7	3	5	1	6
ABTS (mg of TE/g)	57.1 ± 8.5^{b}	47.9 ± 7.8^{b,c}	33.3 ± 3.3^{c,d}	46.1 ± 1. 4^{b}	28.9 ± 5.9^{d}	90.1 ± 3.1^{a}	35.5 ± 3.5^{c,d}
Rank	2	3	6	4	7	1	5
ORAC (mg of TE/g)	118.7 ± 17.6^{b}	141.7 ± 5.7^{b}	91.6 ± 8.6^{c}	128.6 ± 7.0^{b}	98.9 ± 13.1^{c}	229.6 ± 3.6^{a}	93.3 ± 11.8^{c}
Rank	4	2	7	3	5	1	6
ICA (mg of EDTA/g)	24.8 ± 0.2^{b}	24.5 ± 0.5^{b}	17.3 ± 0.5^{c}	21.8 ± 1.4^{b,c}	38.9 ± 1.8^{a}	13.3 ± 1.8^{d}	37.3 ± 1.2^{a}
Rank	3	4	6	5	1	7	2
Inhibitory properties							
$A\beta_{25-35}$ (%)	66 ± 5^{a}	36 ± 10^{b}	44 ± 6^{b}	72 ± 2^{a}	37 ± 6^{b}	39 ± 6^{b}	63 ± 8^{a}
Rank	2	7	4	1	6	5	3
αS (%)	15 ± 8^{d}	68 ± 11^{a,b}	30 ± 6^{c}	72 ± 5^{a}	52 ± 13^{b}	67 ± 17^{a,b}	88 ± 15^{a}
Rank	7	3	6	2	5	4	1

$^{a–e}$ Significant difference at $p < 0.05$ by Tukey's test within the same line.

increasing order, the following for the TPC values was noted: Spring Belle > Dixired > Red Top > Cardinal > Romea > Tebana > Flavorcrest. In the literature, TPC values are often specified for 100 g of fresh weight. In this manner, the values of the TPC in the different *p. persica* L. varieties are considered to range from 345 to 808 mg of CAE/100 g fresh weight (FW), given the fact that peaches contain 90% of water. The values were of the same order as those previously reported (Cevallos-Casals, Byrne, Okie, & Cisneros-Zevallos, 2006; Los, Wilska, & Pawlak, 2000).

The antioxidant activity of the fruit extracts evaluated with the PFRAP assay was the highest in Spring Belle with a value of 56.2 mg AA/g. Dixired displayed intermediate activity (36.2 mg AA/g). Red Top, Cardinal, Tebana, Romea and Flavorcrest had the lowest values (33.4, 31.8, 27.6, 23.7 and 22.1 mg AA/g, respectively). These results are in same order of magnitude as those reported elsewhere (Liu, Zhao et al., 2014).

With the DPPH assay, Spring Belle extract exhibited the highest scavenging activity, followed by Dixired, Red Top, Cardinal, Romea and Tebana. Flavorcrest exhibited the lowest DPPH antioxidant capacity. In the literature, fruit peach extracts have been reported to display equivalent values (Cevallos-Casals et al., 2006).

According to the ABTS assay in which values are expressed in mg TE/g, Spring Belle was the peach variety with the greatest antioxidant capacity (90.1 mg TE/g), followed by Cardinal (57.1 mg TE/g). Dixired and Red Top had intermediate values (47.9 and 46.1 mg TE/g, respectively) while Tebana, Flavorcrest and Romea (35.5, 33.3and 28.9 mg TE/g, respectively) had lower values (mg TE/g).

When antioxidant activity was measured with the ORAC method, the highest activity was that of Spring Belle (229.6 mg TE/g). Lower values were found for Dixired, Red Top and Cardinal (141.7, 128.6 and 118.7 mg TE/g, respectively) and still lower ones for Romea, Tebana and Flavorcrest (98.9, 93.3 and 91.6 mg TE/g, respectively). Relative data were obtained by Campbell, Merwin, and Padilla-Zakour (2011) in New York peach varieties with values ranging from 37.50 to 75 mg TE/g DW. The data obtained with F–C, PFRAP, DPPH, ABTS and ORAC assays showed that polyphenols present in the all fruit peach extracts were potent anti-oxidative agents. Moreover, the results corroborated across all the experimental methods and with the same ranking: Spring Belle > Dixired > Red Top > Cardinal > Romea > Tebana > Flavorcrest. This suggested that variety might have a significant influence on the antioxidant activity of peach. It also means that phenolic compounds present in these cultivars are major contributors to reducing power and scavenging radical capacities (DPPH, ABTS and ORAC).

With regard to iron chelating activity, all extracts displayed such ability. Tebana and Romea extracts had the highest ICA values and Spring Belle displayed the lowest. Cardinal and Dixired had intermediate values, as Red Top and Flavorcrest. With the exception of Flavorcrest, an inverse relationship tends to appear between total polyphenol content of peach extracts and ICA. Based on the content of each phenolic in the peach extracts obtained by HPLC-MS/MS, it was not able to find a potential positive correlation between specific polyphenols and ICA. These results suggest that iron chelating activity of peach extracts cannot be mainly attributed to the presence/content of phenolic compounds but rather to other compounds as carotenoids, polysaccharides, peptides, etc. These last compounds were probably present in different amount in the extracts, as the ICA values differed considering the peach variety. Absence of correlation between ICA and total polyphenol content has already been mentioned (Kalogeropoulos, Yanni, Koutrotsios, & Aloupi, 2013; Saiga, Tanabe, & Nishimura, 2003; Sánchez-Vioque et al., 2013; Toth & Pavia, 2000).

To investigate relationships between all the antioxidant assays, a regression analysis was used. Table 4 shows the Pearson correlation coefficients between the antioxidant capacities obtained with the different methods for quantifying antioxidant activity. Correlations between F–C, PFRAP, DPPH, ABTS and/or ORAC were positively high ($0.91 < r < 0.99$ with a p value of at least < 0.001). We hypothesize that the hydrogen and electron donating abilities of fruit peach extracts are directly proportional to their concentration in total phenolics. However, ICA values correlated neither with F–C, PFRAP, DPPH, ABTS and/or ORAC (r ranging from −0.65 to −0.56). Others also reported that ferrous ion-chelating ability displayed poor correlations with both F–C values and other antioxidant activities (DPPH and/or ABTS and/or ORAC) (Wang, Jónsdóttir, & Ólafsdóttir, 2009; Zhao et al., 2008). The following hypothesis was proposed for this lack of correlation. Non-phenolic compounds such as polysaccharides, peptides and proteins might be involved in chelating potency (Wang et al., 2009). Briefly, these results indicate that the fruit peach polyphenols contributed similarly to global radical scavenging capacities, but not to ferrous ion-chelating activity. It can be speculated that the major role of fruit peach polyphenols is that they act as potent radical scavengers and primary chain-breaking antioxidants.

Table 4
Correlations between the antioxidant values of the 7 studied peach cultivars obtained by different assays (F–C, PFRAP, DPPH, ABTS, ORAC, ICA).[a]

	PFRAP	DPPH	ABTS	ORAC	ICA
F–C	0.96***	0.98***	0.93**	0.99***	−0.58ns
PFRAP		0.96***	0.92**	0.97***	−0.58ns
DPPH			0.91**	0.97***	−0.56ns
ABTS				0.95**	−0.65ns
ORAC					−0.63ns

[a] Data represents Pearson correlation coefficient r.
ns Indicates non significant.
** Indicates significant at $p < 0.01$.
*** Indicates significant at $p < 0.001$.

3.4. Protective activities against Aβ cytotoxicity and αS aggregation

3.4.1. Inhibition of Aβ and αS aggregation

Because Aβ and αS are the primary components of amyloid plaques and Lewy bodies, respectively, the aggregation of these proteins is considered to be a critical step during neurodegenerative processes associated with AD and PD, respectively (Rochet & Lansbury, 2000). In this study the inhibitory properties of fruit peach extracts against Aβ and αS aggregation were examined. The effect of each peach extract on $A\beta_{25-35}$ aggregation was monitored by using a routine *in vitro* assay (Rivière et al., 2007) with curcumin serving as positive control (Ono, Hasegawa, Naiki, & Yamada, 2004). The inhibitory property of peach extract on αS aggregation was studied by using the thioflavin T binding assay (Ono & Yamada, 2007). The inhibitory activities of the seven cultivars against Aβ and αS aggregation are presented in Table 3.

Concerning Aβ fibril formation, Red Top, Cardinal and Tebana extracts had the strongest inhibition percentage (72%, 66% and 63%, respectively). The other cultivars were less active with comparable percentages around 40%. Four extracts had a strong inhibitory activity against αS fibril formation: Spring Belle, Dixired, Red Top and Tebana (67%, 68%, 72% and 88%, respectively). Cardinal displayed the weakest activity (15%) with Romea and Flavorcrest showing moderate values (52% and 30% respectively). These data suggest that the constituents of each cultivar might influence inhibition. Nevertheless, at present it is difficult to draw conclusions about the phenolic composition–activity relationship because of the highly variable concentration of each phenolic class.

3.4.2. Cell viability and Aβ cytotoxicity

The MTT assay was used to determine the protective effect of the peach fruit extracts against Aβ-induced toxicity in the PC12 cell line. MTT is a tetrazolium salt reduced to formazan by mitochondrial dehydrogenase only in living cells. First, the cytotoxic potential of each phenolic extract on PC12 cells was measured with the MTT assay. The phenolic extracts of Dixired, Red Top, Romea, Spring Belle and Tebana were not cytotoxic to concentrations below 200 μg/mL, while Cardinal and Flavorcrest reduced cell viability at 100 and 200 μg/mL (data not shown).

As shown in Fig. 2A, treatment of PC12 cells with 10 μM $A\beta_{25-35}$ reduced cell viability, ranging from 42% to 51% of control. Induction of cytotoxicity by $A\beta_{25-35}$ at 10 μM was then used for all subsequent experiments to evaluate the protective effect of the cultivars. Incubation of PC12 cells with 100 and 200 μg/mL of extracts of all cultivars significantly prevented the cytotoxic effect of $A\beta_{25-35}$. The extracts increased cell viability in a dose-dependent manner. Aβ-induced cytotoxicity was prevented at levels higher than 90% at 200 μg/mL except for Romea (85%). Dixired extracts at the highest concentration tested (200 μg/mL) completely reversed the toxic effect of $A\beta_{25-35}$.

ROS play a critical role in neurodegenerative diseases. Oxidative stress appears at the early stage of AD and Aβ present in AD brain is

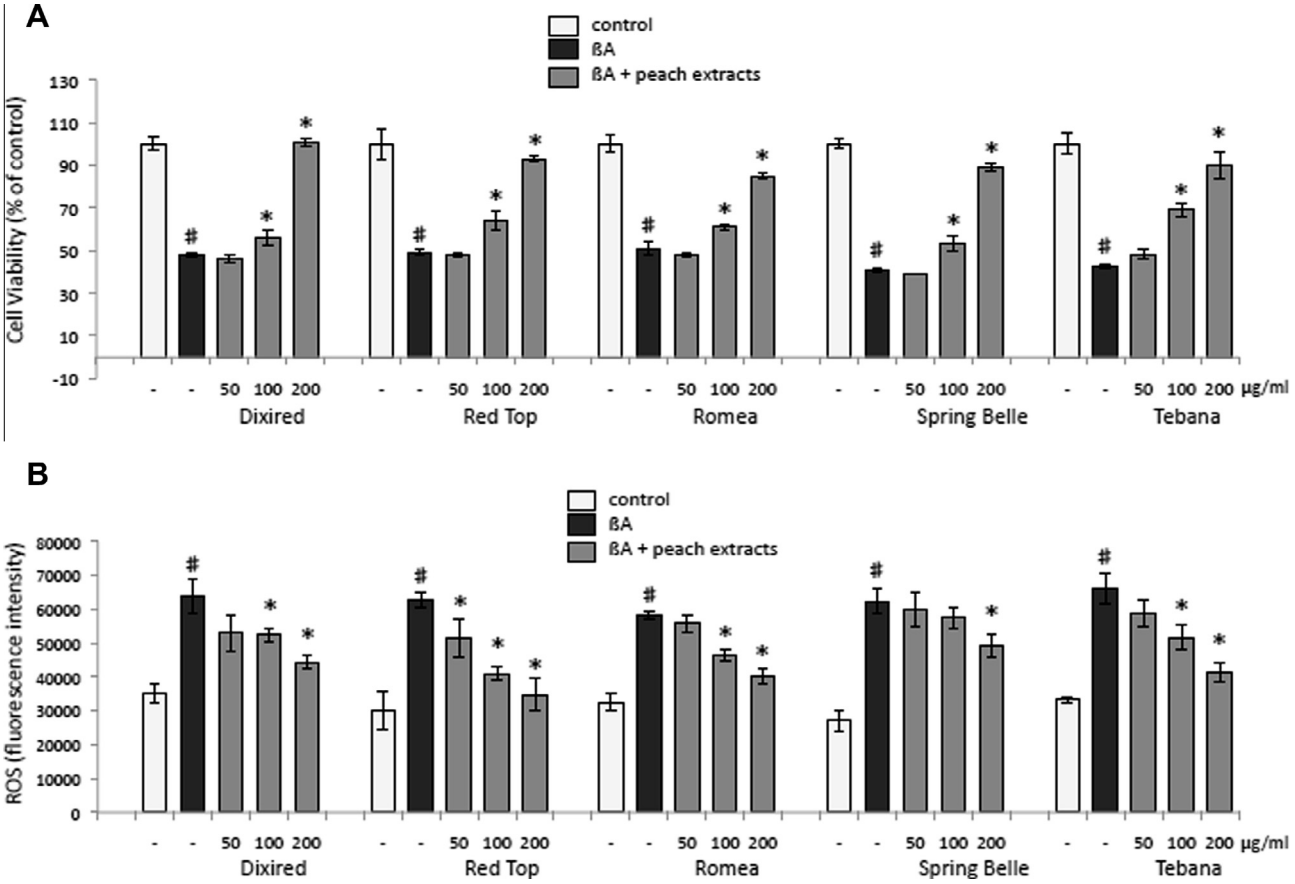

Fig. 2. Cytotoxicity and ROS production. PC12 cell viability was determined by the MTT assay. Cells were treated for 24 h by peach extracts in presence or absence of 10 µM of βA_{25-35} (A). Production of intracellular ROS was measured by the fluorescent probe DCFH-DA. Cells were treated for 6 h by peach extracts (50, 100 and 200 µg/mL) in presence or absence of 20 µM of $A\beta_{(25-35)}$ (B). Results are expressed as mean SEM of four replicates ($n = 4$). $^{\#}p < 0.05$ $A\beta_{(25-35)}$ versus control, $^{*}p < 0.05$ extract versus $A\beta_{(25-35)}$.

known to induce oxidative stress. ROS are able to damage macromolecules including lipids, proteins and nucleic acids and thereby induce neuronal death. ROS production was evaluated by measuring intracellular ROS levels with the DCFH-DA assay. Exposure of PC12 cells to $A\beta_{25-35}$ for 6 h induced an increase in ROS production as compared to control cells (1.8- to 2.3-fold). Treatment with the different extracts reduced ROS generation induced by Aβ (Fig. 2B). Red Top extract inhibited ROS production at all the concentrations tested, whereas Dixired, Romea and Tebana extracts exerted significant protective effects at 100 and 200 µg/mL. Spring Belle extract displayed the lowest effect on ROS production.

Several studies have shown that extracts from medicinal plants exhibit beneficial effects against H_2O_2 and Aβ-induced oxidative injury in PC12 cells (Liu, Xu et al., 2014; Ng et al., 2013). The extracts were shown to protect cells via several mechanisms related to oxidative stress, inflammation and apoptosis. In our study, no correlation was found between the antioxidant capacity of the different varieties and their inhibition of ROS production (especially for Spring Belle extract). Thus the protective effects of fruit peach extracts in PC12 cells could depend on the direct radical scavenging due to the phenolic content itself and on other more specific biological activities. For example, procyanidins are known to present anti-oxidant and anti-apoptotic activities in PC12 cells. They protect cells against 4-hydroxynonenal-induced apoptosis by blocking mitogen-activated protein kinase kinase 4 (MKK4) activity as well as ROS accumulation (Cho et al., 2009). In our study, procyanidins, which are major phenolic compounds in peach extracts, may have largely contributed to the protective effect on Aβ. In conclusion, Dixired, Red Top, Romea, Spring Belle and Tebana extracts could prevent the neurotoxicity induced by Aβ in part by decreasing ROS production.

4. Conclusion

Peach cultivars were found to be rich in phenolic compounds, the content depending on the cultivar. Concerning their antioxidant properties, the F–C, PFRAP, DPPH, ABTS and ORAC data showed that fruit peach extracts are potent antioxidants. Good correlations found between antioxidant activities and total phenolic content indicated that the phenolic compounds present in these fruit peach cultivars are major contributors to their total antioxidant potential. Protective activity assays indicated that the extracts inhibited αS and Aβ fibril formation, the degree of inhibition depending on the cultivar. Moreover, the extracts protected PC12 cells against $A\beta_{25-35}$-induced cytotoxicity and reduced ROS production.

To conclude, these results suggest that consumption of various peach cultivars could serve as a good source of natural antioxidant compounds and that peach phenolic extracts could potentially be exploited for nutritional pharmaceutical purpose. However, further studies were recommended to identify which phenolic compounds contribute to the biological activities of peaches as well as their mode of action.

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