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Pistachio (*Pistacia vera* var Kerman) from Argentinean cultivars. A natural product with potential to improve human health

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ABSTRACT

The chemical profile, mineral content as well as antioxidant activities of three cultivars of *Pistacia vera* cv Kerman were investigated. The total phenolic (TP) content flavonoids (FT) and anthocyanins (TA) were measured. Additionally, the profile of polyphenols was analyzed. A slight, not significant, increment was observed in TP content between cultivars with different age (5, 9 and 11 years old). The 9 years old cultivar showed the highest FL value, while the 11 years old cultivar had the higher TA content. Main polyphenols were separated by HPLC and identified by electrospray ionization (ESI) coupled to quadrupole-time of flight mass spectrometry (LC–ESI–QTOF–MS). Gallic acid and (+)-catechin were present in higher amounts. The presence of myricetin, isoquercitrin and a dimer of procyanidin are reported for the first time in pistachio. Additionally, K, Ca and Mg were found in high proportion. The highest antioxidant capacity was measured in the 11 years old pistachio cultivar. This work presents the first evidence that *Pistacia vera* cv Kerman from Argentinean cultivars could be considered as a functional food or ingredient in a diet, with potential to improve human health.

1. Introduction

A diet rich in fruits, vegetables, nuts and minimally refined cereals is associated with a lower risk of chronic degenerative diseases. Since the oxidative stress is commonly associated with these diseases, dietary antioxidants, particularly those from plants, may provide a beneficial effect on human health (John & Shahidi, 2010).

Extensively studied sources of natural antioxidants are fruits and vegetables, seeds, cereals, berries, wine, tea, onion

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Abbreviations: DPPH, 1,1-diphenyl-2-picrylhydrazyl; FRAP, ferric-reducing antioxidant power; PE, petroleum ether; DCM, dichloromethane; MeOH-H$^+$, acidified methanol; MeOH-H$^+$ E acidified methanol extract, MeOH-H$^+$ E S acidified methanol skin extract; TP, total phenolics; FT, flavonoids; TA, total anthocyanin; GAE, gallic acid equivalents; QE, quercetin equivalents.

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bulbs, olive oil and aromatic plants. Attempts have also been made to identify and evaluate antioxidants in agricultural by products, ethnic and traditional products, herbal teas, cold pressed seed oils, exudate resins, hydrolysis products, and other raw materials rich in antioxidant phenols that have nutritional importance and/or potential for applications in the promotion of health and prevention against damages caused by free radicals (Dimitrios, 2006).

The last decades were characterized by a growing interest of consumers, food industry and researchers, in evaluating different ways to improve human health by consumption of either natural or man-made foods. So far, the development of functional foods, nutraceuticals, designer foods; therapeutic foods, superfoods, or medicinal foods appear to play an important role in current dietary habits (Nagai & Inoue, 2004). Nuts, including pistachio, are known as nutritious food with a high content of healthful lipids (Shahidi, Alasalvar, & Liyana-Pathirana, 2007) in addition to a high content of polyphenols (Alturfan, Emekli-Alturfan, & Uslu, 2009; Arcan & Yemenicioglu, 2009; Gentile et al., 2007; Kornsteiner, Wagner, & Elmadfa, 2006; Tomaino et al., 2010). Mandalari et al. (2013) demonstrated that bioactive compounds from pistachios become rapidly accessible in the stomach, maximizing the possibility of absorption in the upper small intestine, which would contribute to the beneficial relation between pistachio consumption and health-related outcomes.

Recently, an important increase in the cultivation of non-traditional crops is observed in northwest areas of Argentina. New cultivars include cherries, capers, cranberries, hazelnuts, walnuts and pistachios.

Pistachio (*Pistacia vera* L.) is a member of the *Anacardiaceae* family. This is a native species of arid zones from Central and Western Asia. They were brought to the Mediterranean basin about 2000 years ago. The USDA Plant Introduction Department introduced pistachio in California around 1904, but it was not promoted as a commercial crop in California until 1929 (Anderson & Smith, 2005; Gentile et al., 2007). Though the pistachio tree grows virtually in all soil types; high temperatures in addition to deep, sandy loam soils favours its healthy development (Shokraii, 1977).

In the 1980s, the first seeds of pistachio were introduced to Argentina (Andean provinces of Mendoza, La Rioja, Catamarca and San Juan) from California (USA). The first commercial pistachio cultivars were grafted with the Kerman variety from Iran. Currently, there are over 1000 hectares with an annual pistachio production of 400 tonnes, the Province of San Juan being the main producing area (500 ha). Cultivation areas within this province are well suited for growing pistachio, with sandy loam soils and summer temperatures above 37 °C, which are described as ideal for this plant.

Pistachio is mostly used as a snack or as ingredient in the food industry. The consumption of pistachio has been shown to significantly decrease the oxidative stress, improving both total cholesterol and LDL levels. It is also well known for its antioxidant capacity, which could be associated to its high total phenolic content (Arcan & Yemenicioglu, 2009; Ballistreri, Arena, & Fallico, 2009; Gentile et al., 2007; Halvorsen et al., 2006; Mandalari et al., 2013).

Among the common foodstuffs, nuts have a mineral profile that is beneficial for human health (Segura, Javierre, Lizarraga, & Ros, 2006). Pistachios are an excellent source of potassium, phosphorus, magnesium and calcium (U.S.D.A, 2010).

To the best of our knowledge, there are no reports on chemical and nutritional characteristics of *Pistacia vera* cv. Kerman produced in Argentinean cultivars. Thus, the main goal of this study was to characterize Argentinean pistachios, considering the antioxidant capacity and the mineral content from three cultivars, with ages between 5 and 11 years. Additionally we were interested in evaluating the profile of polyphenols, looking to match beneficial antioxidant capacity with this profile, enabling a better understanding of claimed health benefits.

2. Materials and methods

2.1. Chemicals

Ultra-pure water (<5 μg/L TOC) was obtained from a water purification system Arium 126 61316-RO, plus an Arium 611 UV unit (Sartorius, Germany). Methanol (HPLC grade) and formic acid (puriss. p.a. for mass spectroscopy) were obtained from J. T. Baker (State of México, México) and Fluka (Steinheim, Germany), respectively. Commercial Folin–Ciocalteu (FC) reagent, HNO_3 (63%) and HCl (37%) were purchased from Merck Química Argentina (Buenos Aires, Argentina). 1,1-Diphenyl-2-picrylhydrazyl (DPPH), gallic acid, quercetin, myricetin, malvidin-O-glucoside and trichloroacetic acid (TCA) were from Sigma to Aldrich (Buenos Aires, Argentina). Commercial standards of (+)-catechin, (−)-epicatechin, quercetin, isoquercitrin, apigenin and naringenin were obtained from Extrasynthese (Genay, France). Inductively coupled plasma multielement standard solution, Merck VI CertiPUR®, was obtained from Merck Química Argentina (Buenos Aires, Argentina). The composition and concentration of the Merck VI standard was as described in the accompanying certificate of analysis. HNO_3 (63.7%) sub-boiling grade was prepared from analytical grade acid using a distiller (Figmay Sub-boiling distiller, Córdoba, Argentina). The purity of the nitric acid was verified by ICP-MS before use. NIST 1548 Typical Diet was used as certified reference material (CRM) (NIST, Montgomery County, MD, USA). All other chemicals were of analytical grade.

2.2. Samples

The sampling areas were located at both riverbanks of the San Juan River (lat. 31°S; long. 69°W). The altitude that varies from 650 to 750 m.a.s.l. Pistachio grows in large clusters, similar to grapes, having a fleshy coating. They mature in late summer or early autumn, when their covers turn pink and shells are naturally divided along their sutures. After harvesting, pistachio covers are mechanically removed from pistachio hard shells and dried in ovens until reaching a moisture content of 5–6%.

2.3. Sample preparation

Dry pistachio samples (unroasted) (2 kg each) were provided by Pisté S.R.L. (a local grower from Carpintería, Pocito district, province of San Juan, Argentine). Pistachio cultivars analyzed during this study were five (Cultivar1), nine (Cultivar2) and eleven (Cultivar3) years old. To determine the effect of cultivar

age on the pistachio composition, samples were collected as follows: Cultivar1 ($n = 5$), Cultivar2 ($n = 5$) and Cultivar3 ($n = 6$). All samples were stored at 4–8 °C in the darkness and analyzed within two months.

Pistachio were shelled to release kernels with their skin (seed coat), and ground in a coffee grinder for 5 min. Ground pistachio (10 g; 40 mesh) was defatted in a Soxhlet extractor during 60 min using 200 mL petroleum ether (PE). The residue was further extracted with dichloromethane (DCM) using the same procedure. Soluble phenolic compounds present in the defatted samples were extracted using acidified methanol (0.1% HCl, v/v) (MeOH-H^+) under reflux. Solvents from different extracts were evaporated under reduced pressure (40 °C), yielding dry extracts designated as PEE, DCME and MeOH-H^+E. Dry extracts were stored in the dark at −20 °C until analysed within three months. Dry extracts yields (w/w) were calculated in terms of dry starting material (Table 1). The acidified methanolic extracts were used for total phenolics, flavonoids, total anthocyanins and antioxidants assays.

A second set of pistachio samples (100 g kernels, randomly taken from each sample) were completely dehulled by hand, to afford 88.85 g of seeds and 11.15 g of skins. Aliquots (200 mg) of crushed skins were mixed with 2 mL acidified methanol, placed in falcon tubes and sonicated (40 kHz) during 30 min at 25 °C (ultrasound bath model TB02TACA, TESTLAB S.R.L, Buenos Aires, Argentina). The homogenate was then centrifuged at 10,000*g* during 10 min using a Biofuge® 28RS Heraeus Sepatech Centrifuge (Heraeus Instruments, Hanau, Hesse, Germany). The supernatant, acidified methanol skin extract (MeOH-H^+SE), was separated (Goli, Barzegar, & Sahari, 2005; Seeram et al., 2006; Tomaino et al., 2010), filtered (0.45 μm) and injected into an LC–ESI–QTOF–MS system for polyphenols analyses (see 2.7). Taking into account that the skin represents 11.15% of the whole pistachio, we extrapolated the average concentration of individual polyphenols to the weight of the whole pistachio kernel, in agreement with Tomaino et al. (2010).

2.4. Determination of total phenolics content

The total phenolic (TP) content of acidified methanolic extract (MeOH-H^+E) was determined using the method described by Heldrich (1990). An extract dilution (1 g/L) was oxidized using Folin–Ciocalteu reagent (125 μL) and neutralized with sodium carbonate (20% w/v). After 30 min, the absorbance of the resulting blue solution was measured at 765 nm using a Shimadzu UV-160A spectrophotometer (Shimadzu Corporation, Kyoto, Japan MultiSpec-1501, equipped with a holder for multiple cells and temperature control). TP were determined by linear regression from a calibration plot constructed using gallic acid (0, 25, 50, 100, 150 and 250 μg/mL), and expressed as mg of gallic acid equivalents per 100 g of pistachio on a dry weight (dw) (mg GAE/100 g dw). Data from triplicates are reported as mean ± SD.

2.5. Determination of flavonoids content

The total flavonoids (FT) content in the MeOH-H^+E was determined following the procedure described by (Chang, Yang, Wen, & Chern, 2002), using a colorimetric method with $AlCl_3$ hexahydrate as a complex-forming reagent and known quercetin concentrations as a standard to construct the calibration plot. One milligram of quercetin was dissolved in 95% ethanol and then diluted to 5, 10, 25, 50 and 100 mg/L. The diluted standard solutions were separately mixed with 750 μL of 95% ethanol, 50 μL of 10% aluminium chloride, 50 μL of 1 M potassium acetate and 1400 μL of ultrapure water. After incubation at room temperature for 30 min, the absorbance of the reaction mixture was measured at 415 nm. The amount of 10% aluminium chloride was substituted by the same amount of ultrapure water in the reference. Similarly, 250 μL of extracts were reacted with aluminium chloride to determine the flavonoid content. The absorbance of the reaction mixture was read at 415 nm using a spectrophotometer (Shimadzu Corporation, Kyoto, Japan MultiSpec-1501, equipped with a holder for multiple cells and temperature control). Results are expressed as mg of quercetin equivalents per 100 g of pistachio on a dry weight (dw) basis (mg QE/100 g dw). Data from triplicates are reported as mean ± SD.

2.6. Determination of total anthocyanins content

Total anthocyanins (TA) content in the MeOH-H^+E was determined using a modified pH differential method, previously described by Meyers et al. (2003). A spectrophotometer (Shimadzu Corporation, Tokyo, Japan, MultiSpec-1501) was used to measure the absorbance at 510 and 700 nm in buffers at pH 1.0 and 4.5. Absorbance readings were converted to total μg cyanidin 3-glucoside/100 g of pistachio on a dry weight (dw) using a molar extinction coefficient of 26,900, calculating the absorbance as follows:

$$A = [(A_{510} - A_{700})_{pH1.0} - (A_{510} - A_{700})_{pH4.5}]$$

Data from triplicates are reported as mean ± SD.

Table 1 – Yield extracts from pistachio cultivars of different age. Values are reported as percentage (% w/w) in terms of dry starting material (dw). Results are presented as mean ± standard deviation.

Samples	Yield extracts (% w/w)		
	PEE	DCME	MeOH-H^+E
Cultivar1 ($n = 5$)	45 ± 3^a	3 ± 1^a	11 ± 2^a
Cultivar2 ($n = 5$)	47 ± 2^a	2 ± 1^a	11 ± 1^a
Cultivar3 ($n = 6$)	45 ± 3^a	2 ± 1^a	12 ± 2^a

ANOVA. Different letters indicate significant difference among cultivars, Duncan ($p < 0.05$).

2.7. Antioxidant activity

2.7.1. Free radical scavenging activity on DPPH

Free radical scavenging effects of the MeOH-H^+E were assessed by the fade of a methanolic solution of 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) as previously reported by Tapia et al. (2004). Extracts were assayed at concentrations 50, 100, 250, 500, 1000 and 2000 μg/mL. Scavenging activities were evaluated at 517 nm in a UV–Vis spectrophotometer (Shimadzu Corporation, Kyoto, Japan, MultiSpec-1501). Quercetin was used as a reference compound. The loss of colour (fade percentage) indicated the free radical scavenging efficiency of the substances. DPPH antioxidant capacity was expressed as % of DPPH decolouration using the equation:

$$\% \quad \text{Scavenging effect} = [1 - (A_{\text{sample}} - A_{\text{blanck}})/A_{\text{DPPH}}] \times 100$$

The extract concentration providing 50% of radicals scavenging activity (EC_{50}) was calculated by plotting the inhibition percentage at A_{517} against the extract concentration. Results were extrapolated from the plot by linear regression. Analyses were performed in triplicate; values are reported as mean ± SD.

2.7.2. Ferric-reducing antioxidant power assay (FRAP)

FRAP assay, measures the reducing capability of the samples, evaluating the conversion of a Fe^{3+}/ferricyanide complex to Fe^{2+}. The iron-reducing power of the samples was tested using the assay reported by Oyaizu (1986). Briefly, 200 μL extract (0.1, 0.2, 0.5 and 1.0 mg/mL) were added to 500 μL of phosphate buffer (0.1 M, pH 6.6) and 500 μL of potassium ferricyanide (1%, w/v). Afterwards, the mixture was incubated at 50 °C for 20 min, with further addition of 500 μL trichloroacetic acid (TCA) (10%, w/v) and it was vortex for shaken for 20 s. Then, 1000 μL of this solution were mixed with 1000 μL of ultrapure water and 200 μL of $FeCl_3$ (0.1%, w/v). After 30 min incubation, the absorbance was read at 700 nm using a UV–Vis spectrophotometer (Shimadzu Corporation, Kyoto, Japan, MultiSpec-1501). Increased absorbance of the reaction means increased reducing power. Analyses were performed in triplicate; values are reported as mean ± SD.

2.8. Identification and quantification of skin phenolics by HPLC–ESI–QTOF MS

Evaluation of the phenolic profile was performed on an Agilent Series 1200 LC System (Agilent, Santa Clara, CA, USA) coupled in tandem to a PDA detector (Agilent Series 1200) and a MicrOTOF Q II (Bruker Daltonics, Billerica, MA, USA) high resolution mass spectrometer (MS and MS/MS) equipped with an ESI source. The HPLC system was equipped with a binary gradient pump, solvent degasser, and autosampler (Agilent Series 1200 L).

HPLC analyses were performed on a thermostated (40 °C) Luna C18 250 × 4.6 mm (5 μm) column (Phenomenex, Torrance, CA, USA), at 0.4 mL/min flow rate, using 0.5% (v/v) formic acid–water (solvent A) and 0.5% (v/v) formic acid–methanol (solvent B). HPLC runs were performed using the following gradient: starting with 20% B, changing to 50% B along 3 min, kept for 5 min, followed by a second ramp to 80% B during 5 min, maintained for 17 min, returning to 20% B in 1 min, remaining at this last condition for 10 min before the next run. The injection volume was 40 μL.

ESI–MS and MS/MS detection was performed in successive runs using both negative and positive ionization modes, with mass acquisition between 100 and 1500 Da. Nitrogen was used as drying and nebulizer gas (7 L/min and 3.5 bar, respectively), and 180 °C for drying temperature. For MS/MS experiments fragmentation was achieved by using the auto MS^2 option of the equipment. UV–Vis analyses were carried out in the range between 200 and 700 nm (PDA).

The identification of pistachio constituents was achieved by comparison of the spectral properties (UV, ESI–MS and MS/MS) of eluted compounds with those of reference samples when available, or by comparison with literature data. The standards gallic acid, naringenin, apigenin, quercetin, isoquercitrin, (+)-catechin, (−)-epicatechin and myricetin, were prepared at a stock concentration of 1000 mg/L. Calibration standard samples were prepared by appropriate dilutions with methanol from the stock solutions and filtered on Millipore filters (0.45 μm) before use. MS analysis was used for quantification of the compounds with specific calibration plot. When reference compounds were not available, the calibration plots from structurally related compounds were used. Compounds concentrations were measured in triplicate and the mean value and the standard deviation in each case was reported.

2.9. Elemental analysis

The pistachio fraction (Cultivars 1–3) was prepared for elemental analysis as follows: pistachios were shelled, and kernels with their skin (seed coat) were milled using a coffee grinder. Ground samples (particle size 0.5 mm) were accurately weighted and mineralized by acid digestion using a microwave oven (Anton Paar Multiwave 3000; Graz, Styria, Austria). Samples (0.2 g) were introduced in quartz vessels, followed by the addition of 8 mL concentrated nitric acid, keeping vessels open until no fumes were observed (2–3 h). Afterwards, vessels were cap closed and heated using the following power sequence: starting with a 15 min ramp until reaching 600 W, holding for 45 min (maximal T = 169 °C; max pressure = 75 bar) and a final 15 min step disabling power to reach pressure equilibration. Mineralized samples were quantitatively transferred to 25 mL volumetric flasks, adjusting the volume with ultrapure water, followed by filtration using 0.45 μm filters. Spiked samples were also prepared by adding varying amounts of individual standard solutions (1000 mg/L in 1% nitric acid), doubling the starting concentration for each element. The rest of the procedure was the same as that used for non-spiked samples. All recoveries were between 84% and 116%. All samples were prepared in duplicate. A certified reference material (CRM: NIST 2548 a- typical diet) was analyzed for quality control using the same procedure. Recovery of elements measured in this work from CRM was between 80% and 110% of certified values.

2.9.1. Quantification of elements by Q–ICP–MS

Twenty-nine elements were quantified in pistachio samples: Li, Be, B, Na, Mg, Al, K, Ca, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Ga, As, Se, Rb, Sr, Mo, Ag, Cd, Te, Ba, Tl, Pb and Bi. Elemental analyses were carried out on a Quadrupole Inductively Coupled Plasma Mass Spectrometer (Q-ICP-MS) (Agilent Technology

7500 cx Series, Santa Clara, CA, USA), equipped with an ASX-500 series autosampler model (Agilent Technology, Santa Clara, CA, USA). The sample introduction system consisted of a microflow concentric nebulizer, Peltier cooled spray chamber and 2.5 mm ID fixed injector torch. The RF power was 1500 W for all the experiments and the interface was fitted with Ni sampling and skimmer cones designed for low polyatomic formation. Two operation modes were used: with and without collision cell technology (CCT). CCT mode measurements were performed for Mg, Al, K, Ca, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Ga, As, Se, Rb, Sr and Mo. For the CCT mode the collision cell was flushed with a collision gas (He). The elements Li, Be, B, Ag, Cd, Te, Ba, Tl, Pb and Bi were measured without operating the collision cell with gas, reaching full sensitivity. The oxide ratio and double charged species was maintained below 1% for both modes of operation. All measurements were performed using Sc, In and Re as internal standards. Instrumental and procedural blanks were determined together with samples. Three replicates were obtained for each sample. Full quantitative analysis was performed from calibration plots, constructed using linear regression from standards for each element ($R^2 \geqslant 0.99$). Sodium measurements were carried out by Flame Atomic Absorption Spectrometry (FAAS) using a Perkin Elmer 3030 (Waltham, MA, USA) in an air-acetylene flame. All pistachio samples (digested) were diluted tenfold using HNO_3 (2% sub-boiling grade in ultrapure water) before Q-ICPMS measurements. Standards and blanks were prepared using the same mixture (2% HNO_3). All samples were analyzed in duplicate (two independent samples measured in triplicate each).

2.10. Statistical analysis

Results were analyzed by one-way ANOVA and significant differences between mean values were determined by Duncan's test ($p < 0.05$) using the software InfoStat (2002). Pearson's correlation analysis was used to determine correlation coefficients and their statistically significance.

3. Results and discussion

3.1. Pistachios yield extracts

The yields of PEE constituted 45–47% of the whole pistachio, DCME were between 2% and 3%, while MeOH-H$^+$E represented 11–12% (Table 1). Significant differences ($p < 0.05$) between cultivars of different age were not observed.

3.2. Total phenolics, flavonoids, and total anthocyanins content

The TP content in MeOH-H$^+$E of pistachio cultivars (1–3) varied from 360 to 463 mg GAE/100 g dw (Fig. 1). A slight but not significant increment was observed in TP content between cultivars of different age. The concentration of TP found during this work was similar than those reported in pistachio from Turkey (461 mg GAE/100 g dw) (Arcan & Yemenicioglu, 2009), and from California (USA) (572 ± 7 mg GAE/100 g dw) (Yang, Liu, & Halim, 2009). A higher concentration of TP was reported by Wu et al. (2004) and Kornsteiner et al. (2006) in pistachio from USA, Austria and Greece. However, (Ballistreri et al., 2009; Gentile et al., 2007) studied *P. vera* L. from Italy and reported values lower than those found in this work.

Regarding flavonoid contents, cultivar 2 (9 years old) showed 20.6 ± 2.5 mg QE/100 g dw, with significant differences from cultivars1 (Fig. 1). (Ballistreri et al., 2009) reported similar values for Italian pistachios.

The AT content from cultivars 1–3 was 0.7 ± 0.1, 0.9 ± 0.1 and 1.2 ± 0.3 μg cyanidin 3-glucoside/100 g dw, respectively. These results are lower than those reported in pistachio skin from Italy and USA (Ballistreri et al., 2009; Bellomo & Fallico, 2007; Wu et al., 2006). Furthermore, the Cultivar3 presented the higher anthocyanins content, which was significantly different in relation to the concentration observed in cultivars 1 and 2.

3.3. Antioxidant activity

The MeOH-H$^+$E from pistachio cultivars (1–3) were evaluated for antioxidant capacity by the DPPH radical scavenging and the ferric-reducing antioxidant power (FRAP) assays.

DPPH is widely used for assessing the ability of polyphenols to transfer labile H atoms to radicals, a likely antioxidant mechanism. The free DPPH radical scavenging capacities of MeOH-H$^+$E are summarized in Fig. 2A. The highest antioxidant capacity was detected for Cultivar3 (EC_{50} = 280 μg/mL), in agreement with the highest content of TP and TA observed for this cultivar. Moreover, a positive significant Pearson's correlations ($r^2 = 0.79$ at $p < 0.01$) was found between the TP content and the DPPH activity. This positive correlation suggests that the antioxidant activity is primarily related to phenolics compounds present in the MeOH-H$^+$E. These results are coincident with those reported by Arcan and Yemenicioglu (2009), matching antioxidant activity (ABTS) and phenolic content ($r^2 = 0.70$).

Regarding the FRAP assay, Fig. 2B shows that the reducing antioxidant power increased when the concentration of MeOH-H$^+$E was increased. At the same dose, the reducing power of MeOH-H$^+$E from Cultivar2 was higher than values for Cultivar3 and Cultivar1, respectively. The level of flavonoids in MeOH-H$^+$E in Cultivar2 correlated well (Pearson, $r^2 = 0.67$) with values from the corresponding FRAP assay ($p < 0.01$). This suggests that FL also contributed to the reduction power, confirming that pistachios have antioxidant capacity.

3.4. Identification and quantification of skin phenolics by HPLC–ESI–QTOF

The composition and concentrations of major phenolic were determined by HPLC–ESI–MS and MS/MS analysis (Table 2). The MeOH-H$^+$ SE were used to analyze the phenolic profile, considering that phenolics are found at higher concentrations in the pistachio skin (Tomaino et al., 2010). Thus, we analyzed phenolics from the skin, extrapolating values, considering the skin weight relative to the whole pistachio (see Section 2.3). Major flavonoids found were: (+)-catechin (16 μg/g dw seed), procyanidin dimer (6 μg/g dw seed), isoquercitrin (6 μg/g dw seed), luteolin (3 μg/g dw seed) and (−)-epicatechin (3 μg/g dw seed). Eriodictyol, eriodictyol-O-hexoside, quercetin, quercetin-O-hexoside, myricetin and naringenin were detected in

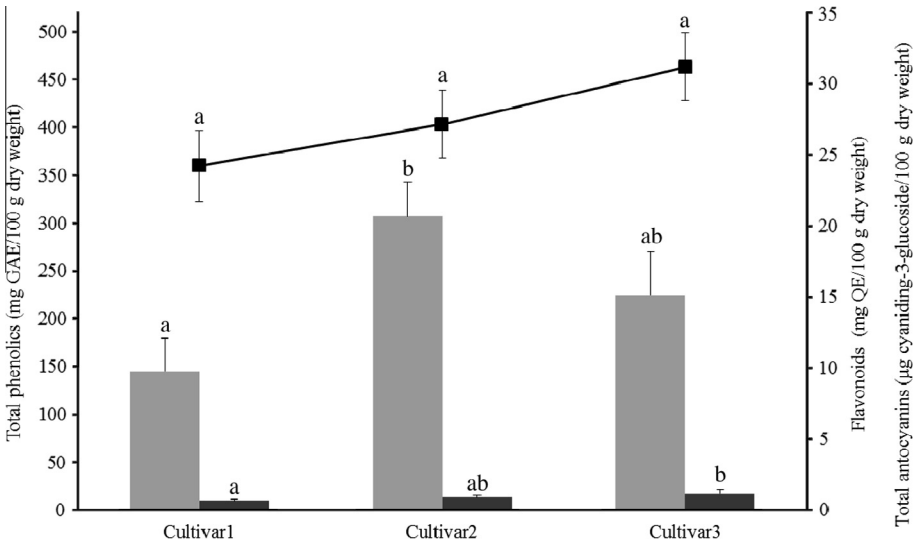

Fig. 1 – Total Phenolic (dot), Flavonoids (grey bars) and total anthocyanin (black bars) contents of acidified methanolic extracts between pistachios cultivars with different age. Results are expressed as mean ± SD (standard deviation). Different letters indicate significant difference among cultivars, Duncan ($p < 0.05$).

minor concentrations. Also, gallic acid was detected (8 μg/g dw seed). Moreover, myricetin (0.2 μg/g dw seed), isoquercitrin (6 μg/g dw seed) and procyanidin dimer (6 μg/g dw seed) are reported for the first time in pistachios.

The anthocyanins identified were cyanidin-3-O-galactoside (0.2 μg/g dw seed) and cyanidin-3-O-glucoside (0.01 μg/g dw seed). Similar values have been reported by (Ballistreri et al., 2009; Bellomo & Fallico, 2007; Seeram et al., 2006).

Main phenolic compounds identified in Argentinean pistachio are known for their antioxidant activity in different trials, mainly gallic acid, catechin and epicatechin. According to Frankel (1999), the relative antioxidant activity of pure phenolic compounds tested at two concentrations decreased in the following order: catechin > myricetin = epicatechin–rutin > gallic acid > quercetin > cyanidin.

The antioxidant effect of (+)-catechin on lipid peroxidation and as an inhibitor of COX-1 and COX-2 enzymes has been reported (Gorelik & Kanner, 2001; Noreen, Ringbom, Perera, & Bohlin, 1997; Schmeda-Hirschmann et al., 2003). On the other hand, procyanidins are also reported to be potent antioxidants. Studies on humans show that a diet rich in procyanidins decreases/inhibits the lipid peroxidation of LDL cholesterol, increasing the free radical scavenging capacity (Fuhrman, Lavy, & Aviram, 1995; Natella, Belelli, & Gentili, 2002). Luteolin is a compound with anti-inflammatory, antiallergenic, antiviral, anticarcinogenic actions (Van Zanden et al., 2004), in addition to attenuation of multiple sclerosis (Verbeek, van Tol, & van Noort, 2005) and rheumatoid arthritis (Hou, Wu, Huang, & Guo, 2009).

3.5. *Mineral content*

Twenty-nine elements (Li, Be, B, Na, Mg, Al, K, Ca, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Ga, As, Se, Rb, Sr, Mo, Ag, Cd, Te, Ba, Tl, Pb and Bi) from three studied cultivars were quantified (Table 3). The elemental analysis shows that K was the most abundant nutrient, followed by Ca and Mg, The average content of minor and trace elements decrease as follow Na > Fe > Zn > Cu > Mn. Concentrations from these elements were similar to the pattern generally observed in nuts (Yang et al., 2009). B, Ba, Be, Cr, Li, Mo, and Se were not detected. Our current results are in agreement with values reported by (U.S.D.A. United State Department of Agriculture, 2010) for pistachio. Furthermore, concentrations of Ca, K, Mg, Fe, Zn, Cu and Mn were similar to those reported for Californian pistachios (variety Kerman) (Anderson & Smith, 2005). Conversely, Iran and Turkish pistachios showed higher values than those found in this work (Anderson & Smith, 2005).

The median concentration of Mg was significantly different within the three cultivars. These could be attributed to the selectivity process of mineral bioaccumulation within the vegetable/fruit varies with different trace elements (Anderson & Smith, 2005).

A slight increment was observed in K, Ca, Fe, Cu, Mn, Zn and Rb content among cultivars of different age. Cultivar3 showed the highest mineral content, which was significantly different in relation to the concentration observed in Cultivar1 and in Cultivar2 (Duncan, $p > 0.05$). The content of Na and Sr not presented significant differences between cultivars (Table 3). Additionally, Argentinean pistachios showed minor Sr concentrations respect to other geographic regions (Turkey, California and Iran) reported by Anderson and Smith (2005).

Since metal contamination could take place during handling and processing of pistachio, the presence of twelve heavy metals (Ag, Al, As, Bi, Cd, Co Ga, Ni, Pb, Te, Tl and V) was analyzed. It is important to note that levels of these twelve metals were below LOD in Argentinean pistachio (Table 3); only Al (1.2 ± 0.3 mg/100 g dw) was above the LOQ but below the mean dietary intake suggested (2.5–6.3 mg/day) (WHO, 1997).

3.6. *Nutritional value*

Pistachios are very rich in phytosterols, potassium, vitamin B6, carotenoids, and tocopherols and have been ranked

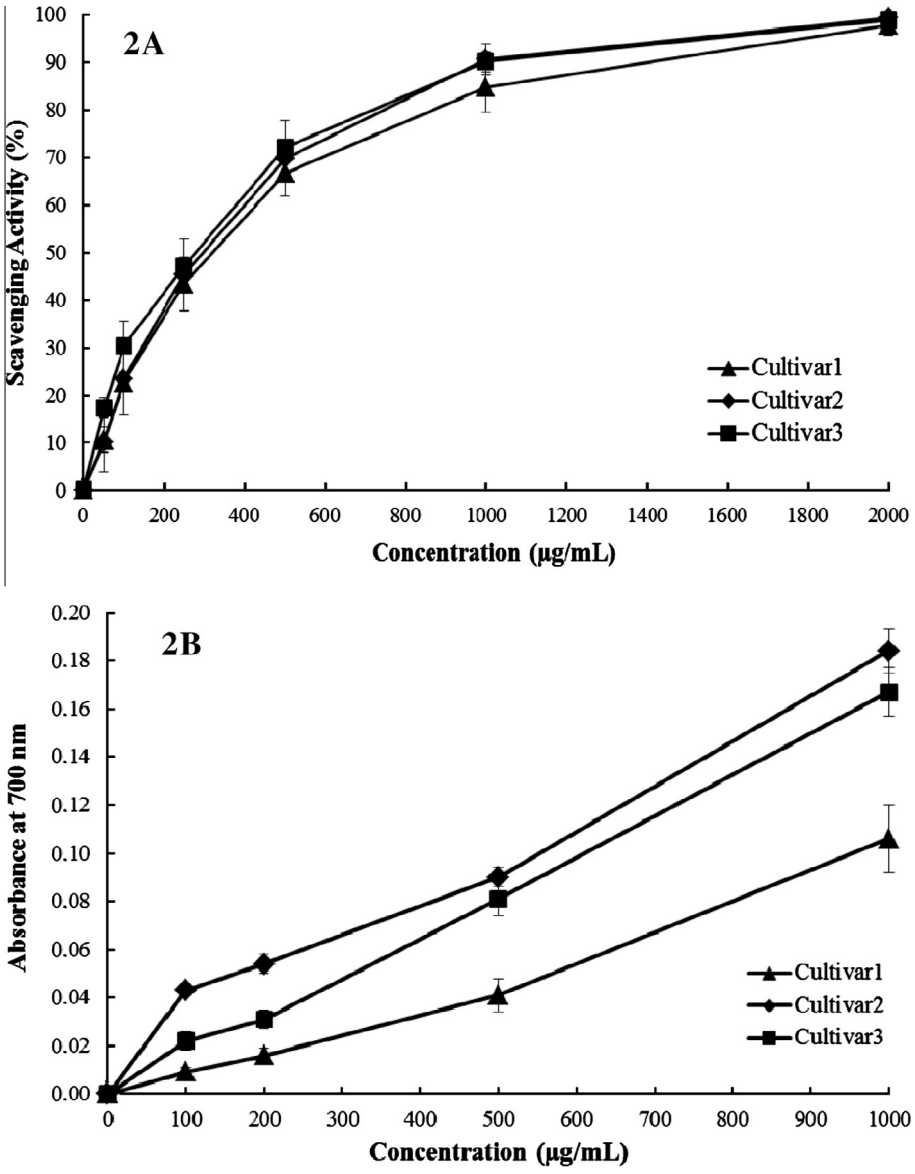

Fig. 2 – Scavenging activity of acidified methanolic extracts on 1,1-diphenyl-2-picrylhydrazyl (DPPH) (2-A) and ferric reducing power (FRAP) (2-B), between pistachios cultivars with different age.

among the 50 foods highest in antioxidants (Mandalari et al., 2013). In addition, pistachio nuts contain some important vitamins and minerals (Khatib et al., 2010).

Phenolic compounds have different structures and specificities (Fu et al., 2011). They can participate in the antioxidant defence system by preventing the formation of pro-oxidants, scavenging activated oxidants, reducing reactive intermediates and inducing repair systems. Natural antioxidants in vegetables and fruits, such as vitamins and polyphenols, are considered to make a major contribution in the prevention and treatment of some chronic and degenerative diseases, including cancer, heart disease, cataracts, and cognitive dysfunction.

The evaluation of minerals and trace elements in foods is an important part of nutritional and toxicological analyses. Main nutritional elements found in studied cultivars include K, Ca and Mg. Potassium, the major intracellular cation in the body, is required for a normal cellular function, while Mg is the most abundant intracellular divalent cation, being an essential cofactor for more than 300 enzymatic reactions. Ca is an essential nutrient, quantitatively the most abundant of the body's minerals as well as a vital electrolyte. The intake of potassium is beneficial for the cardiac conduction, bone mineralization and insulin function; besides, K has a calcium-sparing effect in the kidney (Segura et al., 2006).

A low concentration of sodium, with average values of the 9.2 mg/100 g dw, was found in studied cultivars. Na is required together with chloride to maintain the extracellular volume and plasma osmolality (Food & Nutrition Board, 2001). Thus, pistachio has a beneficial contribution to the diet, with low-sodium and high-potassium amounts. In 2003, the Food and Drug Administration (FDA, 2003) approved the first qualified health claim specific to nuts, decreasing the risk of heart disease: 'Scientific evidence suggests, but does not fully

Table 2 – Compounds identified and quantified from Argentinean pistachio extracts.

Phenolics compounds	$[M–H]^-(-MS^2[M–H])^-$	Qtof–MS $[M–H]^-$ m/z	Accuracy (ppm)	Identification quantification procedure	Concentration (μg/g dw)	
					Skin	Pistachio seeds[a]
Gallic Acid	169 (125)	169.0128	8.89	*a*	75 ± 5	8
Procyanidin dimer	577 (289, 407, 425)	577.1340	1.56	*b*, *c*	55 ± 3	6
(+)-catechin	289 (245)	289.0713	1.38	*a*	140 ± 10	16
(−)-epicatechin	289 (245)	289.0712	1.73	*a*	27.53 ± 0.03	3
Eriodictyol-O-hexoside	449 (287)	449.1096	−1.55	*b*, *d*	3.35 ± 0.03	0.4
Eriodictyol-O-hexoside	449 (287)	449.1101	−2.67	*b*, *d*	0.21 ± 0.01	0.02
Quercetin-O-hexoside	463 (301)	463.0886	−0.86	*b*, *e*	2.68 ± 0.03	0.3
Isoquercitrin	463 (301)	463.0882	0	*a*	49.3 ± 0.6	6
Myricetin	317 (178)	317.0311	−2.52	*a*	1.6 ± 0.1	0.2
Eriodictyol	287	287.0563	−0.69	*b*, *d*	13.7 ± 0.9	2
Quercetin	301 (179)	301.0359	−1.66	*a*	13.7 ± 1.2	2
Naringenin	271 (177)	271.0619	−2.58	*a*	1.9 ± 0.2	0.2
Luteolin	285 (175, 199, 217, 241)	285.0414	−3.51	*b*, *f*	30.4 ± 1.6	3
	$[M–H]^+(-MS^2[M–H])^+$	Qtof–MS $[M–H]^-$ m/z				
Cyanidin-O-galactoside	449 (287)	449.1153	0.67	*b*, *g*	21.14 ± 0.05	0.2
Cyanidin-O-glucoside	449 (287)	449.1122	7.57	*b*, *g*	0.55 ± 0.01	0.01

Procedures used for either full or tentative identification: *a*, co-analysis relative to a pure compound showing identical retention and mass data; *b*, comparison of MS, MS/MS and UV data with the literature. Quantification was made using a calibration plot by linear regression of the corresponding standard, except when indicated: *c*, quantified as catechin; *d*, quantified as naringenin; *e*, quantified as isoquercitrin; *f*, quantified as apigenin; *g*, quantified as malvidin-O-glucoside. Results are expressed as mean ± SD (standard deviations) from three independent measurements.

a Values extrapolated from skin-content, considering that the skin represents 11.15% of the total pistachio weight.

Table 3 – Multielement composition of studied pistachios. Results are reported as mean ± SD (mg/100 g dw).

Element[a]	Cultivar1 ($n = 5$)	Cultivar2 ($n = 5$)	Cultivar3 ($n = 6$)
Ca[b]	93 ± 11^{A}	104 ± 8^{A}	118 ± 7^{B}
Cu[b]	1.33 ± 0.09^{A}	1.6 ± 0.1^{B}	1.7 ± 0.1^{B}
Fe[b]	3.6 ± 0.8^{A}	3.5 ± 0.5^{A}	4.5 ± 0.5^{B}
K[b]	923 ± 33^{A}	940 ± 26^{AB}	976 ± 32^{B}
Mg[b]	93 ± 7^{A}	101 ± 6^{B}	112 ± 5^{C}
Mn[b]	0.7 ± 0.1^{A}	0.9 ± 0.1^{B}	1.0 ± 0.1^{B}
Na[b]	9.4 ± 0.7^{A}	9.2 ± 1.6^{A}	9.1 ± 0.8^{A}
Zn[b]	1.7 ± 0.3^{A}	2.2 ± 0.5^{AB}	2.3 ± 0.2^{B}
Al	0.9 ± 0.2^{A}	1.2 ± 0.3^{B}	1.1 ± 0.1^{AB}
Rb	0.44 ± 0.06^{A}	0.42 ± 0.06^{A}	0.8 ± 0.1^{B}
Sr	0.17 ± 0.03^{A}	0.21 ± 0.03^{A}	0.18 ± 0.02^{A}

a LOD (μg/g): Cr (0.56), Mo (0.065), Se (0.03), Li (0.01), Be (0.01), B (0.82), Ba (0.04), Ag (0.002), As (0.03), Bi (0.01), Cd (0.01), Co (0.01), Ga (0.006), Ni (0.40), Pb (0.01), Te (0.07), Tl (0.44) and V (0.005).

b Mineral nutrients in daily diet. Different letters in the same line indicate significant difference between cultivars, Duncan ($p < 0.05$).

prove, that eating 1.5 oz (42.5 g) per day of most nuts (including pistachios) as part of a diet, helps lowering saturated fat and cholesterol, which may reduce the risk of heart disease'. Therefore, incorporating this quantity of pistachios in the daily diet should provide ca. 402 mg K, 43 mg Mg and 45 mg Ca.

In according to the Nutrient Composition Data, published by the US Department of Agriculture (U.S.D.A.) in 2010, a portion (28.35 g) of shelled pistachios contains around 116 mg of TP, representing 10% of the suggested daily intake. When comparing with a single serving of apple (150 g), which can provide 210 mg of TP; 150 g of pistachio can afford 50% of the daily intake (613 mg), which is three fold the amount provided by an apple serving.

4. Conclusions

The results of this work show that *Pistacia vera* var Kerman from Argentinean cultivars are rich in phenolic including flavonoids, which are useful for blocking the action of reactive oxygen species (ROS), involved in cardiovascular disease and cancer and, thus, may provide significant protection against the oxidation of essential biological macromolecules. The macro- and micro-mineral nutrients observed in pistachio makes it an ideal component for a healthy diet.

The phenolic profile revealed fifteen constituents, gallic acid and (+) catechin being the predominant phenolic compounds identified in studied pistachios cultivars. Thus,

Argentinean pistachios may be considered as a functional food or ingredient in the diet, with good potential for improving human health.

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