Journal of Functional Foods 16 (2015) 256–264

Available online at www.sciencedirect.com

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Chemical characterization and antioxidant capacity of red radish (*Raphanus sativus* L.) leaves and roots

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ARTICLE INFO

Article history:
Received 13 February 2015
Received in revised form 22 April 2015
Accepted 24 April 2015
Available online

Keywords:
Raphanus sativus
Proximal composition
Polyphenols
Flavonoids
Functional food

ABSTRACT

Red radish roots and leaves were characterized in terms of their physico-chemical, nutritional, antioxidant and microbiological properties. The nutritional value of radish leaves far exceeded the corresponding value for roots. Leaves presented higher percentage of protein, ash and crude fiber than roots. Calcium was found to be the most abundant mineral with a value of 752.64 mg/100 g. Ascorbic acid content in leaves (38.69 mg/100 g) doubled the value found in roots. Total phenolic contents of leaves (695.07 mg GAE/100g d.m.) were almost two times higher than for roots, while total flavonoid levels (1042.73 mg quercetin/100 g d.m.) were four times higher. Leaves' and roots' antioxidant activities were 39.48 mmol and 11.09 mmol TE/100 g d.m., respectively, by means of ORAC analysis. The most abundant free and bound phenolic compounds of roots and leaves were pyrogallol and vanillic acid; and epicatechin and coumaric acid, respectively.

1. Introduction

The adverse effects of oxidative stress on human health have become a serious issue. The World Health Organization (WHO) has estimated that 80% of the earth's inhabitants rely on traditional medicine for their primary health care needs, and most of this therapy involves the use of plant extracts and their active components (Krishnaiah, Sarbatly, & Nithyanandam, 2011). Recent reports indicate that there is an inverse relationship between the dietary intake of antioxidant-rich foods and the incidence of human disease (De la Rosa, Alvarez-Parrilla, & Gonzalez-Aguilar, 2009; García-Andrade et al., 2013). Thus, antioxidant compounds that are contained in natural plant sources can serve as dietary supplement and a type of preventive medicine (García-Andrade et al., 2013).

During the last decades, a number of studies have examined the effect of consumption of cruciferous vegetables on

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http://dx.doi.org/10.1016/j.jff.2015.04.049
1756-4646/

health (Beevi, Mangamoori, & Gowda, 2012). The latest reports suggest that cruciferous vegetables act as a good source of natural antioxidants due to their high levels of phenolic compounds, including tocopherols, as well as carotenoids and ascorbic acid. Foremost are their antioxidative effects, manifested by the ability to scavenge free radicals or to prevent oxidation of low-density lipoproteins. Polyphenolic compounds have become the focus of current nutritional and therapeutic interest largely due to their disease-preventing and health-promoting effects (Beevi et al., 2012). Moreover, they play a significant role as antioxidants in the protective effect of plant-derived foods (Balasundram, Sundram, & Samman, 2006). In particular, Radish (*Raphanus sativus* L.) is a root vegetable of Cruciferaceae family and it is an important vegetable crop worldwide (Tsouvaltzis & Brecht, 2014). Radish composition was found to be of highly medicinal and nutritional value. Thus, it was suggested as an alternative treatment for various ailments including hyperlipidemia, coronary heart diseases and cancer (Curtis, 2003). Previous studies on antioxidant activities of *R. sativus* have been focused mainly on sprouts, which have been reported to contain sinapic acid esters and flavonoids as main phenolic components (Takaya, Kondo, Furukawa, & Niwa, 2003). However, few investigations have been done on the polyphenolics profile and antioxidant activity of red radish (Beevi et al., 2012; Beevi, Narasu, & Gowda, 2010). Apart from these, radish leaves constitute an underutilized leafy vegetable, and practically no information is available on the chemical content of such raw material.

The objective of this work was to characterize the fresh leaves and roots of red radish (*R. sativus* L.) assessing the physico-chemical properties, bioactive compound contents, microbiological analysis as well as their antioxidant capacity in order to determine their potential as functional foods, evaluating them as sources of natural antioxidants for food and medicinal purposes.

2. Materials and methods

2.1. Plant material

Field grown radishes were purchased from a local market in Mar del Plata, Argentina. They were kept at 5 ± 1 °C in darkness prior to processing. Radish roots were separated from leaves, and both were washed in tap water to eliminate any surface contamination. All determinations were made on fresh products, immediately after washing.

2.2. Proximate analysis

Crude protein content was determined using the Kjeldahl method with a conversion factor of 6.25. Lipid content was analyzed gravimetrically following Soxhlet extraction. Crude fiber was estimated by acid/alkaline hydrolysis of insoluble residues. Crude ash content was estimated by incineration in a muffle furnace at 550 °C. The moisture content was determined gravimetrically. All methodologies followed the recommendations of the Association of Official Analytical Chemists (AOAC, 1990). The available carbohydrate was estimated by difference. All measurements were carried out in triplicate.

2.3. Determination of minerals

Minerals (Na, K, Ca, Mg, Cu, Mn, Zn, and Fe) were measured by using an atomic absorption spectrophotometer (AAS, Shimadzu Instruments, Inc., SpectrAA-220, Kyoto, Japan) after the digestion in an H_2SO_4, HNO_3 and $HClO_4$ mixture. All determinations were done in triplicate. The minerals were expressed as g/100 g of Fresh Weight (FW).

2.4. Microbiological determinations

The enumeration and differentiation of microorganisms and particular microbial groups were performed using the following culture media and conditions: mesophilic aerobic bacteria on plate count agar (PCA) incubated at 35 °C for 24 to 48 h (ICMSF, 1983); *Lactobacillus* spp. (n = 5) on MRS agar (*Lactobacillus* agar) incubated in anaerobic jars with Anaerocult C (Merck, Darmstadt, Germany) at 30 °C for 5 days; *Enterobacteriaceae* in Mac Conkey agar incubated at 37 °C for 24 h. Molds and yeast were counted in yeast–glucose–chloramphenicol (YGC) medium incubated at 25 °C for 5 days (ICMSF, 1983). Microbial counts were performed in triplicate. The results were expressed as log CFU/mL. All culture media were from Britania, Buenos Aires, Argentina.

2.5. Nutritional analysis

2.5.1. Determination of ascorbic acid content

Ascorbic acid content was determined by the titrimetric assay described by Moreira, Roura, and del Valle (2003). Twenty grams of radish roots or leaves were homogenized with 40 mL of 0.2% oxalic acid solution. This mixture was vacuum filtered through glass fiber. Five milliliter aliquots of the filtrate were titrated with 2,6-dichloroindophenol. Ascorbic acid contents are calculated as mg of reduced ascorbic acid/100 g FW.

2.5.2. Determination of chlorophyll content

Total chlorophyll (TC) in leaves was determined according to Roura, Moreira, Crapiste, and del Valle (2001). Radish leaves were homogenized with a commercial blender (Multiquick, MR 5550 CA Braun, Espanola S.A., Barcelona, Spain) and two samples (1 g each) were taken from each homogenate. Each sample was then homogenized with 19 mL of a cold solution 18:1 (v/v) acetone:ammonium hydroxide (0.1 mol/L). This homogenate was filtered and water was removed from the filtrate with anhydrous sodium sulfate. Absorbance of the filtrate at 660.0 and 642.5 nm was read with a UV 1601 PC UV–visible spectrophotometer (Shimadzu Corporation). TC was calculated as: $TC\ (mg/L) = 7.12 \times A_{660} + 16.8 \times A_{642.5}$, where TC is the total chlorophyll concentration (mg/L) and A_{660} and $A_{642.5}$ are the absorbances at the corresponding wavelengths. TC was expressed as mg chlorophyll/100 g.

2.6. Color measurements

The color was measured in the inner part of sliced radish roots and on leaves surface with a Colorimeter (Lovibond, RT Series,

London). The colorimeter was standardized against a white tile (L^* = 97.63, a^* = 0.3133, b^* = 0.3192). The measurements were made in triplicate over each surface sample. Color was recorded using a CIE – L* a* b* uniform color space (Lab), where L* indicates lightness (whiteness or brightness/darkness), a* indicates chromaticity on a green (–) to red (+) axis, and b* chromaticity on a blue (–) to yellow (+) axis (CIE, 1978).

2.7. Determination of antioxidant activities

2.7.1. Total phenolic content

Total phenolic content (TPC) was determined colorimetrically by the Folin–Ciocalteu method according to Vega-Gálvez et al. (2014). A 0.5 mL aliquot of the radish extract solution was transferred to a glass tube, 0.5 mL of Folin–Ciocalteu reagent and 2 mL of 20% Na_2CO_3 solution were added and mixed well by using a vortex. After 15 min of incubation at room temperature, 10 mL of ultra pure water were added and the precipitate formed was removed by centrifugation over 5 min at 4000 × *g*. Finally, the absorbance was measured in a spectrophotometer (Spectronic 20® Genesys™, Vernon Hills, IL, USA) at 725 nm and compared with a gallic acid equivalents (GAE) calibration curve. Results were expressed as mg GAE/100 g of dry matter (d.m.). All reagents were purchased from Merck. All measurements were done in triplicate.

2.7.2. Total flavonoids content

Total flavonoid content of the radish extracts is performed following the protocol described by Kim, Chun, Kim, Moon, and Lee (2003) with some modifications. An aliquot of methanolic extract (0.1 mL) was mixed with 2.4 mL deionized water in a 5 mL microcentrifuge tube, added 0.15 mL $NaNO_2$ (50 mg/mL), and allowed to react for 5 min. Following this, 0.15 mL $AlCl_3$ (100 mg/mL) was added and the mixture was allowed to stand for further 6 min. Finally, 1.0 mL 1 mol/L NaOH and 1.2 mL deionized water were added to the reaction mixture and the absorbance at 510 nm was read against a blank, by replacing the extract with deionized water. Total flavonoid content was calculated from a calibration curve using quercetin as standard, and expressed as mg quercetin/100 g d.m. All measurements were done in triplicate.

2.7.3. Antioxidant capacity

The antioxidant capacity was determined by the scavenging activity of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical, the ferric-reducing antioxidant power (FRAP) assay, and oxygen radical absorbance capacity (ORAC) assay. For DPPH, the procedure described by Turkmen, Sari, and Velioglu (2005) was followed with some modifications. An aliquot of 3.9 mL of 0.15 mmol/L DPPH radical in methanol was added to a test tube with 0.1 mL of the sample extract. The reaction mixture was vortex-mixed for 30 s and left to stand at room temperature in the dark for 30 min. The absorbance was measured at 517 nm, using a spectrophotometer (Spectronic® 20 Genesys™). Eighty percent (v/v) methanol was used as blank solution. Calibration curves were made for each assay using Trolox (6-hydroxy-2, 5,7,8-tetramethylchroman-2-carboxylic acid). The results were expressed as mmol TE (Trolox equivalents)/100 g d.m. All measurements were done in triplicate.

The FRAP assay was determined by a modified method according to Benzie and Strain (1999) with some modifications. To prepare the FRAP reagent, a mixture of 0.3 mol/L acetate buffer (pH 3.6), 10 mmol/L TPTZ, and 20 mmol/L ferric chloride (10:1:1 v:v:v) was made. Then, 2.85 mL reagent and 0.15 mL sample were mixed. The reaction mixture was incubated at 37 °C for 30 min. The increase in absorbance was monitored at 593 nm vs. a blank. FRAP reagent was freshly made up each measuring day and the blank consisted in 2.85 mL reagent and 0.15 mL methanol 80% (v/v). The final absorbance of each sample was compared with those obtained from the standard curve made from Trolox and the results were expressed as mmol TE/100 g d.m. All measurements were done in triplicate.

The ORAC assay followed the procedure described by Zhang et al. (2013) with some modifications. 0.5 g of lyophilized tissue was centrifuged 30 min at 5000 × *g* with 25 mL of phosphate buffer (75 mmol/L, pH 7.4). A fluorescein stock solution (100 mol/L) in phosphate buffer was prepared and kept at 4 °C in the dark. Fresh fluorescein solution (100 mmol/L) was prepared daily by diluting the stock solution in phosphate buffer. Next, 200 μL of the working fluorescein solution were added to each 40 μL of mixture sample or Trolox standard prepared in phosphate buffer in a black 96-well plate and incubated for 20 min at 37 °C. The assay was initiated by adding the peroxyl radical generator prepared in phosphate buffer. Specifically, 35 μL of 2,2′-azobis-2-amidinopropane (AAPH, 0.36 mol/L) were added and the fluorescence was measured (ex = 485 nm and em = 535 nm) every minute using a Victor3 multilabel plate reader (Perkin-Elmer, Turku, Finland) maintained at 37 °C until the lecture had declined to less than 5% of the initial reading. Standards and samples were run in triplicate. Results for ORAC were determined using a regression equation relating Trolox concentrations and the net area under the kinetic fluorescein decay curve. The ORAC value of radish extracts was expressed in mmol TE/100 g d.m.

2.8. Extraction of phenolic acids

2.8.1. Free phenolic acids

Extraction was performed by a shaker holding 0.5 g lyophilized tissue in 10 mL 80% methanol for 63 min at 250 rpm, according to the method of Lopez-Martinez et al. (2009) with some modifications. After centrifugation at 5000 × *g* for 3 min, the supernatant was removed and extraction was repeated once more in a similar way for 30 min by a shaker. The combined extracts were evaporated to 37 °C and redissolved in 10 mL MeOH–formic acid 99:1 (v/v). Aliquots of 10 μL were injected into the HPLC column. All measurements were done in triplicate. The same extract was used for the estimation of total phenolics (TPC), FRAP and DPPH assay and flavonoids determination.

2.8.2. Bound phenolic acids

After extraction of free phenolic acids (PAs), 20 mL of 4 N NaOH were added directly to the residue and was put into the shaker for 4 h according to the methodology of Lopez-Martinez et al. (2009) with some modifications. The hydrolyzate was acidified to pH 2 with concentrated HCl. The liberated PAs in the

clear solution were extracted three times with 10 mL ethyl acetate. The pooled ethyl acetate extracts were evaporated under a rotary evaporator in vacuum to 37 °C. The dry residue was dissolved in 10 mL MeOH–formic acid (99:1). Aliquots of 10 μL were injected into the HPLC column. All measurements were done in triplicate.

2.8.3. *Chromatographic conditions: high performance liquid chromatography (HPLC) analysis*

An HPLC system, Agilent 1200 (Santa Clara, CA, United States), equipped with a high pressure pump; automatic injector; a UV–visible diode array detector; controlled by ChemStation software, was used for the analysis. The analytical column was a Kromasil 100-5C18 (250 × 4.6 mm) (Eka Chemical, Stockholm, Sweden). The flow rate was 0.7 mL/min and the eluates were monitored at 280 and 310 nm at 25 °C. The mobile phase was composed of solvent A (formic acid 0.1%, pH 3) and B (100% acetonitrile). The elution was as follows: initial conditions 87% A and 13% B; a linear gradient of solvent B was used from 13 to 55% from 0 to 18 min, from 55 to 60% from 18 to 23 min, from 60 to 13% from 23 to 25 min, and then returned to initial conditions by 2 min. The phenolic extracts and standard compounds were analyzed under the same analysis conditions. Identification of some of the main phenolic acids (pyrogallol, epicatechin, rutin hydrate, tyrosol, gallic, protocatechuic, chlorogenic, caffeic, syringic, vanillic, *p*-coumaric, trans-sinapic, ellagic, salicylic, trans-ferulic and trans-cinnamic acid) in MeOH–formic acid 99:1 (v/v) was performed by comparisons to the retention times and then their spectra, the peak area of maximum absorption wavelength. The results of the main phenolic compounds were expressed as mg/100 g d.m. All measurements were done in triplicate.

3. Results and discussion

3.1. *Proximal composition*

The proximal composition of radish roots and leaves is presented in Table 1. This table shows protein, crude fiber, ash, moisture, lipid and carbohydrate contents. One of the nutritional benefits of radish roots is its high concentration of complex carbohydrates and dietary fiber (Levine et al., 2008). The results for root are comparable with previous works. A report of FAO presented the following values: 94% moisture; 0.54% lipid content; 0.6% protein; 1.6% fiber; 0.54% ash (Sabry & Rizek, 1982). According to Lu et al. (2008), the crude fiber content of Chinese radish lies between 0.450 and 1.855%, and the content of protein varied considerably between 0.115 and 0.034%.

Concerning the proximal composition for radish leaves, comparable results were reported by previous works. Gupta, Jyothi Lakshmi, Manjunath, and Prakash (2005) reported for 13 Indian available underutilized green leafy vegetables values of moisture, ash and lipids in the range of 73–95, 0.77–3.54 and 0.2–0.9 g/100 g, respectively. Protein content was comparable with those reported for pepper tree leaves (Chiffelle, Huerta, Celis, & Araya, 2013) and *Annona senegalensis* leaves (Tijjani et al., 2013). It can be observed that the leaves had higher percent of protein, ashes and crude fiber than those of the roots.

The total carbohydrate content of radish leaves was considerably low compared to other leafy vegetables, like Java, Cassava and Okra leaves (Raimi, Oyekanmi, & Farombi, 2014). This relative low carbohydrate content makes it suitable to be eaten when someone wants to follow a low calorie diet (Adinortey et al., 2012).

3.2. *Mineral content*

Table 2 presents mineral content of radish leaves and roots. As can be observed, leaves have higher amounts of all minerals than the roots. In leaves, calcium was found to be the most abundant mineral with a value of 752.64 ± 1.45 mg/100 g, while copper was the least abundant with a value of 0.11 mg/100 g. Regarding roots, potassium was found to be the most abundant mineral, while copper was the least abundant. Thus, the content of calcium in the leaves was five times than roots. Calcium is required by children, pregnant and lactating women for bones and teeth development (Sodamode, Bolaji, & Adeboye, 2013). The value of calcium in leaves is close to the recommended daily allowance of 800 mg per day for both adults and children (NRC, 1989), and is much higher than other green leafy vegetables (Guil Guerrero, Giménez Martínez, & Torija Isasa, 1998; Gupta et al., 2005). In addition, 100 g of roots will provide almost 20% of recommended dose of calcium.

Radish also contains many other valuable facts being beneficial for human health, such as mineral matter, carotene, among others (Lu et al., 2008). Radish roots and leaves presented high potassium contents. High amount of potassium in the body was reported to increase iron utilization and beneficial to people taking diuretics to control hypertension and suffer from excessive excretion of potassium, through body fluid (Sodamode et al., 2013). The recommended daily allowance of potassium is 2000 mg for adults (NRC, 1989), then 100 g of leaves

Table 1 – Proximal composition of radish roots and leaves.

Parameter	Leaves (g/100 g)	Roots (g/100 g)
Moisture	89.5 ± 0.98	95.24 ± 0.29
Crude protein	3.81 ± 0.32	0.57 ± 0.09
Lipid content	0.37 ± 0.07	0.07 ± 0.01
Ash	1.70 ± 0.06	0.77 ± 0.07
Crude fiber	0.61 ± 0.05	0.32 ± 0.07
Carbohydrates[1]	4.04	3.03

[1] By difference.

Table 2 – Mineral composition of radish leaves and roots.

Mineral (g/100 g)	Leaves	Roots
Cu	0.11 ± 0.00	0.05 ± 0.00
Fe	3.74 ± 0.03	0.15 ± 0.01
Mn	0.50 ± 0.03	0.07 ± 0.01
Zn	0.39 ± 0.01	0.24 ± 0.00
Ca	752.64 ± 1.45	147.87 ± 1.31
Mg	57.04 ± 0.46	14.98 ± 0.02
Na	298.58 ± 2.56	104.95 ± 0.18
K	495.31 ± 0.49	380.11 ± 0.87

will provide 25% of recommended daily dose. Similar values were found by Gupta et al. (2005) for green leafy vegetables. Sodium is an important source of electrolytes within the body (Sodamode et al., 2013). The recommended daily allowance of sodium is 500 mg for adult (NRC, 1989). One hundred grams of leaves will provide almost 50% and 100 g of roots 20%. Magnesium plays an important role in the structure and the function of the human body (Bangash, Arif, Khan, Khan, & Hussain, 2011). The recommended daily allowance for magnesium for adult male is 350 mg (NRC, 1989), so leaves will provide 15%. Iron is required for the formation of hemoglobin and its deficiency leads to anemia (Sodamode et al., 2013). The recommended daily allowance of iron is 10 mg for men and 18 mg for women (NRC, 1989); 100 g of radish leaves will provide 20% for women and 40% for men.

Even though radish leaves are not typically eaten, these results are very interesting because it may be consistent with other leafy vegetable crops and would be important from a food quality standpoint.

3.3. *Microbiological analysis*

Microbial counts for radish roots and leaves are presented in Table 3. Mesophilic microorganisms estimate total viable populations and are indicative of the endogenous microflora and the contamination undergone by the material (Ponce, Roura, Del Valle, & Fritz, 2002). On the other hand, coliforms are indicators of hygiene at the production stage and the maintenance of the cold chain. Fresh vegetables may be contaminated from soil, irrigation water, or improper handling (Ponce et al., 2002). In the present work, mesophilic aerobic bacteria and Enterobacteriaceae load in both roots and leaves were similar, with no significant differences between them, being the value for both populations among 5 log CFU/mL. The role of lactic acid bacteria (LAB) on keeping quality of vegetables is not clear. Breidt and Fleming (1997) have proposed LAB as biocontrol agents in minimally processed refrigerated foods. LAB may exert antimicrobial effects due to one or more of the following mechanisms: lowering the pH, generating hydrogen peroxide, competing for nutrients, and possibly by producing antimicrobial compounds such as bacteriocins (Ponce, Moreira, Del Valle, & Roura, 2008). Lactic acid bacteria in radish leaves were 1 log CFU/mL lower with respect to radish roots.

Yeast and molds sometimes act as strict parasites and sometimes as latent parasites, depending on the plant resistance, the virulence of the strain, the competing microflora, and the ambient conditions. They may present a deep change in the rate of growth after harvest, when the plant resistance is diminished, and lead to rapid spoilage (Ponce et al., 2002). For molds and yeast, the roots showed 1.2 log reduction with respect to radish leaves. For shredded radish, Pushkala, Raghuram, and Srividya (2013) reported total bacterial count of 5.18 log CFU/g and yeast and mold count of 3.54 log CFU/g. Most of the vegetables with higher microbial load grow inside the soil (radish, potato and carrot) or near the soil (cauliflower, cabbage). This may be responsible for their higher count. In addition, other sources of contamination are improper handling, storage and transportation conditions.

Table 3 – Microbiological counts of radish roots and leaves.

Microorganism	Leaves (log CFU/mL)	Roots (log CFU/mL)
Mesophilic aerobic bacteria	5.28 ± 0.13	5.71 ± 0.18
Enterobacteriaceae	5.32 ± 0.10	5.53 ± 0.39
Lactic acid bacteria	2.67 ± 0.00	3.63 ± 0.00
Molds and yeast	6.17 ± 0.13	4.93 ± 0.58

3.4. *Nutritional properties*

Ascorbic acid content of roots was 16.59 ± 0.29 mg/100 g. Comparable values were reported in previous investigations: 14.16 to 33.41 mg/100 g fresh sample for 42 cultivars of radish varied (Lu et al., 2008); 14.75 mg/100 g of shredded radish (Pushkala et al., 2013); 20.0 to 25.0 mg/100 g for sliced and shredded radish contents (del Aguila et al., 2006), and 20.0–22.5 mg/100 g for whole radish (Bangash et al., 2011; Reyes, Villarreal, & Cisneros-Zevallos, 2007).

Regarding leaves, the ascorbic acid content was of 38.69 ± 1.99 mg/100 g. As can be seen, the ascorbic acid content in leaves doubled the content found in roots. Results for ascorbic acid content for radish leaves were not found in literature. Comparison with other green leafy vegetables indicated that radish leaves presented much higher values of this vitamin than fresh Swiss chard (4.68 mg/100 g), butter lettuce (10 mg/100 g) and Java, Cassava and Okra leaves (3.16 mg/100 g) (Agüero, Pereda, Roura, Moreira, & del Valle, 2005; Agüero, Ponce, Bevilacqua, & Roura, 2011; Raimi et al., 2014).

Chlorophyll content of radish leaves was 52.33 ± 0.53 mg/100 g. Urbonaviciute et al. (2006) reported chlorophyll values of 25 mg/100 g for greenhouse radish leaves, while Bacarin, Falqueto, Moraes, Marini, and Löwe (2007) reported values of 16 mg/100 g for radish plants grown in greenhouses. Radish leaves measured in the present work presented much higher values of chlorophyll content than those reported in the literature mentioned above. The fact that radish plants in this study come from field crops could be the cause of the increased value of chlorophyll found in our samples. Comparison of chlorophyll content of radish with other green leafy vegetables indicated that radish leaves presented comparable values: 57.13 mg/100 g for fresh Swiss chard (Agüero et al., 2005) and 10–52 mg/100 g for butter lettuce (Agüero et al., 2011).

3.5. *Color*

The chromatic parameters for internal tissue of radish roots were L = 74.46 ± 0.73, a = –0.38 ± 0.07 and b = 4.82 ± 0.47. The L* value was high, near-white in the scale, which was consistent with the visual appearance of the slices. It was further noted that a* parameter had a value very close to zero, which is the midpoint of the color range between red and green. The b* value was positive, indicating a tendency toward yellow. del Aguila et al. (2008) reported similar chromatic parameters for shredded radish: L = 74.54, a = –0.55 and b = 3.90.

Chromatic parameters for leaves were typical for green leafy vegetables: L = 46.47 ± 3.93, a = –11.45 ± 0.27 and b = 28.64 ± 3.25. Negative a* values together with high positive values for b* are common characteristics of green leafy vegetables.

Porntewabancha and Siriwongwilaichat (2010) reported for lettuce (*Lactuca sativa* L.) values of L* = 67.04, a* = –14.74 and b* = 44.96. Gnanasekharan, Shewfelt, and Chinnan (1992) reported L* = 36.84, a* = –10.86 and b* = 15.04 values for spinach and Gomes et al. (2008) reported L* = 34.47, a* = –8.65 and b* = 19.69 values for baby spinach leaves.

3.6. *Total phenolic and total flavonoids compounds*

Table 4 presents the total phenolic contents (TPC) of radish leaves and roots. The value for radish root is comparable with values reported in a previous work for the same product of 240 mg GAE/100 g d.m. (Tsouvaltzis & Brecht, 2014) but higher compared to the value of 122 mg GAE/100 g d.m. reported by Pushkala et al. (2013). Factors affecting polyphenol biosynthesis includes intraspecific chemodiversity, plant breeding, ontogenetic stage, post-harvest handling, biotic and abiotic factors (Bruni & Sacchetti, 2009).

Concerning leaves, TPC for radish leaves were almost two times higher than roots (Table 4), but lower compared to the value of other leafy vegetables already published (695.07 mg GAE/100g d.m.). For example, TPC for lettuce, spinach and parsley were 870, 1490 and 2390 mg/100 g d.m., respectively (Karaca & Velioglu, 2014).

Regarding flavonoids, total flavonoid levels in leaves were four times higher than roots (Table 4). Flavonoids constitute a special class of phenolic compounds with a structure based on the diphenyl propane carbon skeleton. In general, flavonoids contain multiple hydroxyl groups and exhibit higher antioxidant activities (Kim, Kang, & Gweon, 2013). The potential benefits of flavonoids for human health, as well as other phenolic compounds, are supported by epidemiological and *in vitro* evidence of antioxidant, cardioprotective, and anticarcinogenic activities; they also protect against other non-transmissible chronic diseases (Celli, Pereira-Netto, & Beta, 2011; Grassi, Desideri, & Ferri, 2010). Moreover, the potential toxicity and harmful effects of polyphenols against pathogens result from their prooxidant action. All o-dihydroxylated phenolics, such as quercetin flavonoids, catechin, epicatechin as well as chlorogenic acid and gallic acid derivatives, can produce reactive oxygen species (ROS) during their oxidation (Tuominen, 2013). Flavonoids present in the plant rich diet represent a range of polyphenolic compounds naturally occurring in these foods. In this sense, radish leaves are an excellent source of bioactive compounds with high impact on the nutrition and health of consumers.

Table 4 – Total phenolic compounds and antioxidant capacity of radish roots and leaves.

Phenolic compound and antioxidant capacity	Leaves	Roots
TPC (mg EAG/100g d.m.)	695.07 ± 36.67	341.45 ± 5.70
Flavonoids (mg quercetin/100 g d.m.)	1042.73 ± 49.95	267.47 ± 6.38
DPPH (mmol TE/100 g d.m.)	1.76 ± 0.01	1.36 ± 0.20
FRAP (mmol TE/100 g d.m.)	3.39 ± 0.22	1.96 ± 0.14
ORAC (mmol TE/100 g d.m.)	39.48 ± 1.39	11.09 ± .166

3.7. *Identification of phenolic compounds*

Table 5 shows the free and bound phenolic compounds that have been identified in radish roots and leaves. The detected free phenolic acids for roots were: rutin, vanillic, pyrogallol and gallic acids. The bound phenolic acids found in roots were: vanillic, coumaric, caffeic and trans-ferulic. On the other hand, leaves presented coumaric, caffeic, trans-ferulic, epicatechin, tyrosol and trans-sinapic acids in the free form and vanillic coumaric and trans-ferulic in the bound form. The free form accounted for the 32 and 55% of total phenolic compound for roots and leaves, respectively. It has been reported that free hydroxyl groups in phenolic compounds are mainly responsible for the antioxidant capacity (Zhang et al., 2014).

For roots, the most abundant free and bound phenolic compounds were pyrogallol acid and vanillic acid, respectively. Beevi et al. (2012) reported the sinapic acid as the most abundant phenolic compound in the root of *R. sativus*.

Regarding leaves, the most abundant free and bound phenolic compounds were epicatechin acid and coumaric acid, respectively. Beevi et al. (2010) found vanillic acid as the most abundant phenolic compound in radish leaves, followed by catechin, sinapic acid, o-coumaric and myricetin. Differences in published results could be due to the choice of solvent for the extraction of the polyphenolic compounds from plant materials. Different solvents influence the extraction efficiencies of the different phenolic components in different ways (Su et al., 2014). Trécul et al. (2013) reported tri-vanillate derived with capacity for kinase phosphorylation inhibition in leukemia cells. Sinapic acid is widespread in fruits, vegetables, cereal grains, oilseed crops, and some spices and medicinal plants and as

Table 5 – Phenolic compounds of radish roots and leaves.

Acids (mg/100g d.m.)	F (free) or B (bound)	Leaves	Roots
Rutin hydrate	F	NQ	9.15 ± 1.03
	B	NQ	NQ
Vanillic	F	NQ	23.12 ± 0.76
	B	155.99 ± 61.90	158.63 ± 25.65
p-Coumaric	F	43.48 ± 4.54	NQ
	B	245.94 ± 39.34	52.32 ± 5.39
Caffeic	F	27.28 ± 3.73	NQ
	B	NQ	7.11 ± 0.01
Trans-ferulic	F	7.60 ± 2.72	NQ
	B	98.13 ± 2.70	34.40 ± 0.68
Epicatechin	F	322.10 ± 60.21	NQ
	B	NQ	NQ
Tyrosol	F	180.47 ± 33.27	NQ
	B	NQ	NQ
Trans-sinapic	F	34.04 ± 6.48	NQ
	B	NQ	NQ
Pyrogallol	F	NQ	81.43 ± 5.17
	B	NQ	NQ
Gallic	F	NQ	5.43 ± 0.95
	B	NQ	NQ
TOTAL		1115.01	371.59

NQ: Not quantifiable; Only its retention time matches (not its spectrum).

such is common in the human diet. Sinapic acid shows antioxidant, antimicrobial, anti-inflammatory, anticancer and anti-anxiety activity (Nićiforović & Abramovič, 2014).

3.8. *Antioxidant capacity*

Table 4 showed the antioxidant capacity of roots of leaves of radish by means of DPPH, FRAP and ORAC assays. The antioxidant capacities of leaves to roots ratios assessed by DPPH and FRAP methods were 1.3 and 1.7, respectively. When the antioxidant capacity was determined by ORAC method leaves showed 3.6 times more antioxidant capacity than roots. Effective DPPH· radical scavenging activity exhibited by *R. sativus* extracts could be explained by the presence of polyphenolics, whose radical scavenging properties were reported previously in various model systems (Beevi et al., 2012). FRAP assay measures the capacity of an antioxidant in reduction of an oxidant probe, which changes color when reduced, the degree of color change is proportional to the concentration of antioxidant. FRAP assay measures ferric-to-ferrous reduction capacity of water-soluble antioxidants in acidic pH such as pH 3.6 (Dai & Mumper, 2010). ORAC assay measures the scavenging capacity of antioxidants in samples against the peroxyl radical (ROO·), one of the most common reactive oxygen species (ROS) found in the body. The assay mimics reactions of antioxidants with lipids in a physiological system and has been adopted by the USDA as a standardized test to measure antioxidant potency of foods and nutritional supplements (Levine et al., 2008). According to Levine et al. (2008), root extracts had 22 mmol TE/100 g d.m., which is much higher than that in leaf extract (12 mmol TE/100 g d.m.). The results presented in this work were opposed to the latter authors. The values of the antioxidant capacity for the ORAC assay were greater in leaves than in roots (39.48 and 11.09 mmol TE/100 g d.m., respectively). High antioxidant capacity in leaves corresponded with a high content of polyphenols and flavonoids (Table 4).

4. Conclusions

Food plants consumption has become a health concern due to their vital nutrient content (minerals, fibers, vitamins, phenolic compounds and antioxidants). In particular, the consumption of both radish roots and leaves due to the presence of phytochemicals with diverse beneficial properties confers health promotion with natural medicinal value. In leaves, calcium was found to be the most abundant mineral while in roots was potassium. Total flavonoid levels in leaves were four times higher than roots. The free phenolic acids accounted for the 32 and 55% of total phenolic compound for roots and leaves, respectively. Thus, leaves have shown excellent antioxidant capacity. Our findings suggest the potential of *R. sativus* leaves to be utilized as a source of bioactive compounds. Moreover, the extraction of those compounds from leaves should be optimized to naturally preserve foods, avoiding artificial additives and thus, contributing to the development of new functional products.

Acknowledgments

This work was financially supported by the Consejo Nacional de Investigaciones Científicas y Técnicas (PIP 007 CONICET), the Agencia Nacional de Promoción Científica y Tecnológica (PICT-2008-1724, AGENCIA), the Universidad Nacional de Mar del Plata (UNMDP) and the Research Department of Universidad de La Serena (DIULS), La Serena, Chile.

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