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FULL ARTICLE

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Chemical characterization and functional properties of selected leafy vegetables for innovative mixed salads

Cintia A. Mazzucotelli[1,2] | Gustavo A. González-Aguilar[3] | Mónica A. Villegas-Ochoa[3] | Abraham J. Domínguez-Avila[3] | María R. Ansorena[1,2] | Karina C. Di Scala[1,2]

[1]Grupo de Investigación en Ingeniería en Alimentos, Facultad de Ingeniería, Universidad Nacional de Mar del Plata, Mar del Plata, Argentina

[2]Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Buenos Aires, Argentina

[3]Coordinación de Tecnología en Alimentos de Origen Vegetal, Centro de Investigación en Alimentación y Desarrollo, A. C., Carretera a La Victoria km 0.6, Col. Ejido La Victoria, Hermosillo, C.P. 83304, Sonora, México

Correspondence
Cintia A. Mazzucotelli, Grupo de Investigación en Ingeniería en Alimentos, Facultad de Ingeniería, Universidad Nacional de Mar del Plata, Juan B. Justo 4302, CP B7608FDQ, Mar del Plata, Provincia de Buenos Aires, Argentina.
Email: cmazzuco@fi.mdp.edu.ar

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Abstract

The content of bioactive compounds and antioxidant capacity of nine vegetables of conventional and unconventional utilization in salad mixtures were studied. The total phenolic and flavonoid contents ranged between 39.6–148.5 mg GAE/100g FW and 76.3–217.4 mg QE/100g FW, respectively. Ascorbic acid content ranged between 16.4 and 198.8 mg AAE/100g FW. Antioxidant capacity was assessed using DPPH, FRAP, and ORAC methods; values were in the range of 48.9–245.8 mg TE/100g FW, 67.7–335.8 mg TE/100g FW, and 104.86–833.9 mg TE/100g FW, respectively. Red cabbage, beet greens, parsley, and rocket exhibited the highest antioxidant capacities. Catechin was the most abundant phenolic compound identified in the free fraction, and *p*-coumaric acid, quercetin, and caffeic acid in the hydrolyzed fraction. Results suggested that the presence of these phenolics could be of great importance in preventing some chronic and degenerative diseases when regularly consumed. Nonconventional vegetables showed high antioxidant properties, therefore, it is important to promote their consumption.

Practical applications

Not all vegetables have the same phenolic composition, and not all phenolics have the same antioxidant capacity. Knowledge of the bioactive content and antioxidant capacity profile in each vegetable could be of interest to consumers and the food industry for selecting the more suitable leaves to make salad mixtures with high nutritional and functional values. These compounds can prevent some chronic-degenerative diseases related to oxidative stress, so it is important introduce them regularly into the diet. Moreover, the evaluation of nontraditional vegetables is intended to bring consumers toward a new source of bioactive compounds, prompting their consumption, and providing added value to certain plant parts that are sometimes considered as waste products.

KEYWORDS
antioxidant capacity, flavonoids, functional food, phytochemicals, polyphenols, vegetables

1 | INTRODUCTION

Epidemiological studies have shown an inverse correlation between a diet rich in fruits and vegetables and the incidence of chronic-degenerative diseases such as certain cancers and cardiovascular diseases (Liu, 2013; Ruiz & Hernández, 2014). The beneficial effects of these crops are partially attributed to the biological activities of their phytochemical constituents, such as phenolic compounds, anthocyanins, vitamins, carotenoids, flavonoids, and among others (Bernal, Mendiola, Ibáñez, & Cifuentes, 2011; Espín, García-Conesa, & Tomás-Barberán, 2007; Moyo et al., 2013). Phytochemicals are secondary metabolites synthesized by plants, and their consumption has been associated with beneficial effects on the health of consumers. Their antioxidant properties are well recognized, and can mitigate oxidative stress induced by free radicals, which is involved in the etiology of a

Abbreviations: AAC, ascorbic acid content; AAE, ascorbic acid equivalents; DW, dry weight; FW, fresh weight; FF, free fraction; GAE, gallic acid equivalents; HF, hydrolyzed fraction; TE, Trolox equivalents; TFC, total flavonoid content; TPC, total phenolic content.

wide range of degenerative diseases such as cardiovascular, neurodegenerative, and certain types of cancer. (Liu, 2013; Moreno et al., 2012; Zhang & Tsao, 2016). The growing evidence of the positive role of bioactive compounds on human health has increased consumer demand for food products rich in these compounds. Moreover, consumers have changed their food concept: food constituents go beyond their role as dietary essentials for sustaining life and growth, to one of preventing, managing, or delaying the premature onset of chronic diseases later in life (Folta, Brown, & Blumberg, 2015; Pushpangadan et al., 2014). It is important to consider that not all vegetables have the same phenolic composition, and that not all phenolics have the same antioxidant capacity. It is therefore important to recognize which vegetables have the highest antioxidant capacity and introduce them regularly into the diet (Liu, 2013). In addition, although the antioxidant activities and phenolic compounds of some vegetables have been reported in the literature, these values are highly influenced by geographical region, cultivar, climate, degree of ripeness, water availability, light exposure, as well as storage conditions (Santos, Oliveira, Ibanez, & Herrero, 2014). Their contribution as sources of food antioxidants can be further substantiated if more studies are done on their health-promoting potential. Conversely, the evaluation of nonconventional vegetables is intended to bring consumers toward a new source of bioactive compounds, prompting their consumption, and providing added value to certain plant parts that are sometimes considered as waste products.

The aim of this study was to characterize nine selected vegetables of conventional and unconventional use, in new salad mixtures, based on their bioactive content and antioxidant capacity. Thus, the vegetables that showed the best bioactive properties were selected as potential constituents of this new mixed salad recipe.

2 | MATERIALS AND METHODS

2.1 | Plant material

Nine leafy vegetables were selected for their characterization. Among them, six are considered as conventional leafy vegetable in salad mixture elaboration (commonly consumed as salad mixtures ingredient): green lettuce (*Lactuca sativa* L. var. *longifolia*), red leaf lettuce (*Lactuca sativa* L. cv Lollo Rosso), white cabbage (*Brassica oleracea* L. var. capitata), red cabbage (*Brassica oleracea* var. *capitata* f. *rubra*), spinach (*Spinacia oleracea* L.), and rocket (*Eruca sativa* Mill.). The other three are considered as unconventional leafy vegetable in salad mixture elaboration: beet greens (*Beta vulgaris* L.), radish leaves (*Raphanus raphanistrum* L. subsp sativus), and parsley (*Petroselinum crispum* Mill.). The first two are commonly considered as a residue, and parsley is usually used as a seasoning in the elaboration of other types of foods. All vegetables were bought from a local distributor in Hermosillo, Mexico (29.07° N, 110.95° W), and maintained at 5 ± 1°C in darkness prior to processing. The samples were washed by hand in running tap water to eliminate any surface contamination. All samples were lyophilized (Labconco Freezone 6, Kansas City, Misuri) and kept in dry and dark conditions until processing. The humidity of each vegetable was determined gravimetrically, by following the recommendations of the Association of Official Analytical Chemists (AOAC, 1990).

2.2 | Extraction of bioactive compounds

The extraction of bioactive compounds was conducted according to the method reported by Viacava, Gonzalez-Aguilar, and Roura (2014) with some modifications. For chemical extraction, 0.3 g of freeze-dried samples were homogenized in 20 mL of methanol/water (80/20 vol/vol). The homogenate was sonicated for 30 min and then centrifuged at 18,400 × g for 15 min at 4°C in 50 mL plastic tubes. The supernatant was collected, and the precipitate was re-extracted twice with 10 mL of 80% methanol, under the previously described conditions. The three supernatants were mixed and filtered using Whatman filter paper No. 1. The final methanolic extract was stored at −20°C to be used in the determination of total phenolic content (TPC), total flavonoid content (TFC), and antioxidant capacity. Extractions were performed in three different samples of each vegetal product.

2.3 | Quantification of bioactive compounds

2.3.1 | Total phenolic content

TPC was determined spectrophotometrically using the Folin–Ciocalteu reagent, according to the methodology described by Singleton, Orthofer, and Lamuela-Raventós (1999) with some modifications. For all determinations, the extracts were diluted with the same solvent used for extraction (80% methanol) to a suitable concentration for analysis. The reaction mixture was prepared into a microplate well by combining 30 μL of diluted methanolic extract with 150 μL of Folin–Ciocalteu solution (previously diluted 1:10 vol/vol, with distilled water). After 3 min of incubation at room temperature, 120 μL of an aqueous Na_2CO_3 solution (7.5% wt/vol) were added, and the reaction mixture was incubated for 60 min under the same conditions. The absorbance was measured at 765 nm in a spectrophotometer (FLUOstar Omega, BMG Labtech Inc., Offenburg, Germany). TPC was calculated with a standard curve prepared with gallic acid (GA) as standard, under the same conditions as the samples. Results were expressed as mg of gallic acid equivalents (GAE)/100 g FW.

2.3.2 | Total flavonoid content

TFC of vegetal extracts was quantified by following the methodology described by Zhishen, Mengcheng, and Jianming (1999) with some modifications. An aliquot of diluted methanolic extract (100 μL) was added to 430 μL of an aqueous $NaNO_2$ solution (0.35% wt/vol), and the mixture was incubated for 5 min at room temperature. 30 μL of an $AlCl_3$ solution (10% wt/vol) were added, the mixture was incubated for 1 min, and 440 μL of NaOH 0.454 M were added. 300 μL were pipetted into a microwell plate, and the absorbance was read at 496 nm (FLUOstar Omega). TFC was calculated from a calibration curve prepared with quercetin as a standard, and the results are expressed as mg of quercetin equivalents (QE)/100 g FW.

2.3.3 | Ascorbic acid content

Ascorbic acid content (AAC) was determined by the titrimetric method described by Moreira, Roura, and del Valle (2003). A fresh sample of each vegetable (20 g) was homogenized in 40 mL of a 0.2% oxalic acid solution. The mixture was vacuum-filtered through fiberglass. A 5 mL aliquot of the filtrate was titrated with 2,6-dichloroindophenol. AAC was calculated and expressed as mg of ascorbic acid equivalents (AAE)/100 g FW.

2.4 | Determination of in vitro antioxidant capacity

The antioxidant capacity was determined by three different methodologies, the scavenging activity of the stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical, the ferric-reducing antioxidant power (FRAP) assay, and the oxygen radical absorbance capacity (ORAC) assay.

2.4.1 | 2,2-Diphenyl-1-picrylhydrazyl

The DPPH assay was conducted according to the method reported by Palafox-Carlos et al. (2012). The DPPH solution was prepared by dissolving the DPPH radical in pure methanol, and adjusting the absorbance of the solution (515 nm) at 0.700 ± 0.02. A 20 μL aliquot of methanol extract of each vegetal sample was pipetted into a microplate well, and 280 μL of the DPPH radical were then added. The mixture was incubated in the dark for 30 min. The absorbance was read at 515 nm in a spectrophotometer (FLUOstar Omega). A blank was prepared by replacing the methanolic extract with 80% methanol. The DPPH solution was incubated with serial dilutions of Trolox as standards (40–400 μM), under the same described conditions. The percentage of DPPH radical inhibition was calculated for the standards and for each sample according to the Equation 1:

$$\%In = [(Abs_0 - Abs_s)/Abs_0] \times 100\% \quad (1)$$

Where "%In" was the percentage of DPPH radical inhibition; "Abs_0" was the absorbance of the blank sample; and "Abs_s" was the absorbance of the sample. The results were expressed as mg of Trolox equivalents (TE)/100g FW.

2.4.2 | Ferric-reducing antioxidant power

The FRAP assay was performed according to the method of Benzie and Strain (1996) with some modifications. The FRAP solution consisted of a mixture of 5 mL of acetate buffer (300 mM, pH 3.6), 500 μL of 2,4,6-Tri (2-pyridyl)-s-triazine (TPTZ, 10 mM in 40 mM HCl), and 500 μL of $FeCl_3.6H_2O$ (20 mM). 20 μL of the methanolic extract were pipetted into a microplate well, and 280 μL of the FRAP reagent were added. The mixture was incubated for 30 min in the dark. The increase in absorbance was monitored at 593 nm using a spectrophotometer (FLUOstar Omega). The final absorbance of each sample was compared with those obtained from a Trolox standard curve, and the results were expressed as mg TE/100 g FW.

2.4.3 | Oxygen radical absorbance capacity

The ORAC assay was done as described by Ou, Hampsch-Woodill, and Prior (2001). The reaction mixture was prepared by mixing 25 μL of the sample with 150 μL of 10 nM fluorescein. The reaction was initiated by adding 25 μL of the AAPH radical [2,2′-azobis(2-amidinopropane) dihydrochloride, 240 mM]. Phosphate buffer (75 mM, pH 7.0) was used as the solvent in each solution. The decrease in fluorescence was measured every 90 s for 30 min at an excitation wavelength of 485 nm and emission wavelength of 520 nm in a microplate reader (FLUOstar Omega). Phosphate buffer (75 mM, pH 7.0) was used as a blank, and serial dilutions of Trolox were used as a standard (6.25–200 μM). The results were calculated from the Trolox standard curve, and are expressed as mg TE/100 g FW.

2.5 | Identification and quantification of phenolic compounds

2.5.1 | Hydrolysis and extraction of phenolic acids

Phenolic compound extraction was performed according to the methodology described by Mattila and Kumpulainen (2002). To obtain free and identifiable phenols, this methodology combines an alkaline and an acid hydrolysis of each vegetal sample in order to release phenolic acids from the food matrix, and to break ester bonds and glycosylations. 0.3 g of lyophilized samples were homogenized in 7 mL of a mixture of methanol (containing 2 g/L of 2,(3)-tert-butyl-4-hydroxyanisole) and 10% acetic acid (85:15 vol/vol). Samples were sonicated for 30 min, made up to 10 mL with distilled water, and 1 mL was filtered through a membrane filter (0.22 μm) for subsequent chromatographic analysis of free phenolic acids (free fraction, FF). Afterward, 12 mL of distilled water and 5 mL of NaOH (10 M) were added into the test tube, and its content was bubbled with nitrogen, sealed, and stirred overnight at room temperature for approximately 16 hr using a magnetic stirrer. After that, the solution was adjusted to pH 2, and liberated phenolic acids were extracted three times with 10 mL of a mixture of cold diethyl ether (DE) and ethyl acetate (EA, 1:1) by manually shaking and centrifuging. DE/EA layers were combined, dried in a rotary evaporator, and dissolved into 1.5 mL of methanol. After alkaline hydrolysis, the samples were filtered through a membrane filter and analyzed by liquid chromatography. An acid hydrolysis was also performed after the alkaline hydrolysis was completed; 2.5 mL of concentrated HCl were added into the test tube and incubated in a water bath (85°C) for 30 min. The sample was then allowed to cool, and the pH was adjusted to 2. The DE/EA extraction performed was similar to that described for alkaline hydrolysis. The extracts were then dissolved into 1.5 mL of methanol, filtered through a membrane filter, and analyzed by liquid chromatography (Mattila & Kumpulainen, 2002).

2.5.2 | Identification and quantification of phenolic compounds by UPLC-DAD

The three fractions obtained from the previous extraction were analyzed individually by liquid chromatography. The results from the analysis of the first fraction represent the FF. The results from the alkaline and acid hydrolyzates were added together to represent the hydrolyzed fraction (HF).

To identify and quantify the phenolic compounds, an ultra-performance liquid chromatography apparatus was used (UPLC Waters Acquity System-Waters Co., Milford, MA). The system was equipped

with a diode array detector (DAD), a BEH C18 precolumn (130Å, 1.7 μm, 2.1 × 5 mm), and a BEH C18 column (3.0 × 100 mm, 1.7 μm). Each phenolic compound was identified by comparing its retention time and absorption spectra with those obtained from HPLC-grade standards under the same operating conditions.

To quantify each individual compound, a calibration curve was prepared. Peak areas were plotted against the known concentrations of stock solutions (2–100 μg/mL). The mobile phases were aqueous formic acid (0.5%) (A) and pure HPLC-grade methanol (B). The gradient program was as follows (A:B): 80:20, 0.20 mL/min flow rate, for 5 min; 55:45, 0.18 mL/min, 7 min; 0:100, 0.10 mL/min, 13 min; 60:40, 0.20 mL/min, 1 min; 80:20, 0.40 mL/min, during 30 min. Results were expressed as μg of standard/g dry weight (DW).

2.6 | Statistical analysis

The quantification of bioactive compounds and antioxidant capacity of all samples were performed in triplicate. The results were reported as mean ± standard deviation (mean ± *SD*). Statistical significance between mean values of each vegetable sample was evaluated by analysis of variance (ANOVA) in the R software (version 2.14.0) using multiple comparisons and the Tukey method. The statistical differences among means were considered significant at $p < .05$. In addition, Pearson's correlation coefficients (r) to determine the relation between two variables were analyzed using InfoStat 2013 statistical software.

3 | RESULTS AND DISCUSSION

3.1 | Total phenolic compounds

Table 1 shows the TPC of vegetable samples, the values were in the range of 39.6–148.5 mg GAE/100 g FW. The vegetable with the highest TPC value was red cabbage (148.5 mg GAE/100 g FW), while parsley, beet greens, spinach, and rocket did not show significant differences between them ($p > .05$), with an average TPC value of 107.6 mg GAE/100 g FW. In addition, the vegetable with the lowest TPC was white cabbage (39.6 mg GAE/100 g FW). Many factors affect polyphenol biosynthesis, such as plant breeding, ontogenetic stage, geographical region, climate, and postharvest handling. (Deng et al., 2013; Goyeneche et al., 2015), it is therefore common to find certain differences between the values reported by different authors for the same products. For example, Sosnowska, Redzynia, and Anders (2006) reported TPC values between 134.8 and 171.4 mg GAE/100 g FW for red cabbage, and between 20.8 and 29.7 mg GAE/100 g FW for white cabbage, similar values to those found in this work. However, Stratil, Klejdus, and Kuban (2006) reported a threefold higher TPC value for the same products. For spinach, TPC values reported by other authors (Karaca & Velioglu, 2014; Ninfali & Bacchiocca, 2003; Stratil et al., 2006; Tiveron et al., 2012), were similar to those obtained in the present work, ranging from 90.0 to 116.2 mg GAE/100 g FW. Regarding parsley, although it is not a traditional vegetable consumed in mixed salads, it is widely studied because it is used as a spice/seasoning when preparing other foods. Reported TPC values for parsley range from 82.5 to 262.9 mg GAE/100 g FW (Karaca & Velioglu, 2014; Stratil et al., 2006; Tiveron et al., 2012), and is comparable to the TPC value obtained for the parsley sample in our study. Rocket has been reported as one of the most consumed leafy vegetables in mixed salads in recent years. The leaves and young stems are specially appreciated due to their unique, slightly spicy flavor (Char et al., 2012). Several authors have studied this vegetable because of its potential as an antioxidant source. Tiveron et al. (2012) reported a TPC of 110 mg GAE/100 g FW for rocket, similar to the value obtained in the present study; while Char et al. (2012) reported a sixfold higher polyphenol content. It should be mentioned that beet greens is not considered a vegetable of traditional consumption, so studies about its phenolic content or antioxidant capacity are relatively scarce. Ninfali and Bacchiocca (2003) studied the polyphenols and antioxidant capacity of beet greens under fresh and frozen conditions, and reported a TPC value of 118.23 mg GAE/100 g FW. The high content of phenolic compounds found in beet greens highlights the importance of promoting their incorporation into the diet of consumers.

TABLE 1 Quantification of bioactive compounds of the selected leafy vegetables

Vegetable	TPC (mg GAE/100 g FW)	TFC (mg QE/100 g FW)	AAC (mg AAE/100 g FW)
Red cabbage	148.5 ± 13.6^{a}	176.4 ± 6.5^{bc}	49.9 ± 6.4^{cd}
Parsley	115.1 ± 8.5^{b}	165.2 ± 7.4^{c}	198.8 ± 10.8^{a}
Beet greens	106.6 ± 5.4^{b}	217.4 ± 17.6^{a}	43.5 ± 8.7^{d}
Spinach	104.7 ± 10.6^{b}	160.6 ± 10.9^{cd}	60.8 ± 4.1^{c}
Rocket	103.9 ± 9.5^{b}	173.7 ± 4.6^{bc}	93.3 ± 8.6^{b}
Red lettuce	80.0 ± 3.0^{c}	193.9 ± 10.8^{ab}	21.4 ± 0.9^{e}
Radish leaves	78.3 ± 6.3^{cd}	203.5 ± 9.9^{a}	54.8 ± 4.7^{cd}
Green lettuce	60.3 ± 7.4^{d}	137.5 ± 11.4^{d}	16.4 ± 2.4^{e}
White cabbage	39.6 ± 1.5^{e}	76.3 ± 8.8^{e}	19.7 ± 2.3^{e}

Different letters in the same column are significantly different ($p > .05$).

3.2 | Total flavonoids

Flavonoids are natural pigments present in most plant tissues, and are one of the major classes of polyphenols. The antioxidant capacity of these compounds is associated with the number of hydroxyl groups in their structure and their great capacity to chelate iron and other transition metals (Selvaraj, Krishnaswamy, Devashya, Sethuraman, & Krishnan, 2014). Numerous studies have related flavonoid consumption with beneficial effects on human health, proving their anti-inflammatory, antithrombotic, and anticarcinogenic capacities (Knab et al., 2013; Selvaraj et al., 2014; Yang, Lin, & Kuo, 2008). TFC of vegetable samples is presented in Table 1. The TFC values were in the range of 76.3 to 217.4 mg QE/100 g FW. Beet greens, radish leaves, and red lettuce showed the highest TFC, without significant differences between them ($p > .05$), and an average value of 204.95 mg QE/100 g FW. Conversely, white cabbage had the lowest TFC (76.3 mg QE/100 g FW). In comparison to the flavonoid content observed in

TABLE 2 Pearson's coefficients of correlation (r) between bioactive compounds (phenolics, flavonoids, and ascorbic acid) and antioxidant capacities (measured by FRAP, DPPH, and ORAC assays)

	Phenolics	Flavonoids	Ascorbic acid	DPPH	FRAP	ORAC
Phenolics		0.567	0.464	0.886**	0.937***	0.892**
Flavonoids			0.146	0.491	0.578	0.721*
Ascorbic acid				0.107	0.197	0.656
DPPH					0.922***	0.677*
FRAP						0.797*
ORAC						

*Correlation is significant at $p < .05$.
**Correlation is significant at $p < .005$.
***Correlation is significant at $p < .0005$.

traditional vegetables such as green lettuce and spinach, samples of beet greens, radish leaves, and red lettuce had a TFC approximately 1.4-fold higher. These vegetables could therefore represent excellent sources of bioactive compounds with high impact on the nutrition and health of consumers. Similar behavior was found by Moreno-Escamilla et al. (2017), reporting a flavonoid content in red lettuce 1.62-fold higher than in green lettuce. In the same way, Ninfali, Mea, Giorgini, Rocchi, and Bacchiocca (2005) reported a flavonoid content in beet greens 1.36-fold higher than in green lettuce and spinach. In radish leaves the found content was superior to that reported by Goyeneche et al. (2015) (203.5 versus 135.6 mg QE/100 g FW). Similarly, in spinach, the found flavonoid content was higher to those reported by Lin and Tang (2007) (160.6 versus 133.1 mg QE/100 g FW). Bahorun, Luximon-Ramma, Crozier, and Aruoma (2004) evaluated bioactive compounds and antioxidant capacity in 10 Mauritian vegetables, and found that those with the lowest flavonoids contents were tomato, white cabbage, green lettuce, and carrot, with values between 45 and 102 mg QE/100 g FW.

The vegetables that showed the highest TPC were not the same as those with the highest TFC. Consequently, the relationship between total flavonoids and total phenolic compounds in vegetables samples was analyzed (Table 2), and the results indicated that this correlation was not significant ($r = 0.57$, $p > .1$). The same behavior was found by Miliauskas, Venskutonis, and Van Beek (2004), who analyzed some medicinal and aromatic plant extracts, and not correlation were found between the amount of flavonoids and phenolics compounds ($r = 0.43$). Conversely, Maisuthisakul, Suttajit, and Pongsawatmanit (2007) examined ethanolic extracts from various parts of 26 indigenous Thai plants, and obtained good *r* values ($r = 0.9$), indicating that there was a significant positive correlation between the total phenolic and flavonoid contents of all plant extracts selected in that study. They explained that the TPC differed among the different types and parts of plants (seeds, skin, pulp, leaves), and in the same way, plant extracts contain different levels of total flavonoids as a proportion of the total phenolic compounds, depending on the type and part of the product under study (Maisuthisakul et al., 2007). Therefore, the correlation between phenolics and flavonoids depend on the plant tissue, and on the part of the plant analyzed.

3.3 | Ascorbic acid content

Ascorbic acid is a water-soluble vitamin that acts as an enzyme cofactor, a radical scavenger with strong antioxidant capacity, and as a donor/acceptor in electron transport on the cell membrane, which makes it a bioactive compound whose consumption is related to beneficial health effects (Kamiloglu et al., 2016; Podsędek, 2007). AAC of vegetal samples is shown in Table 1. AAC ranged from 16.4 to 198.8 mg AAE/100 g FW. Parsley showed the highest AAC (198.8 mg AAE/100 g FW), with significant differences with respect to the other vegetables under study ($p < .05$). Rocket had an AAC of 93.3 mg AAE/100 g FW, with significant differences with the other samples. Regarding the other studied vegetables, red cabbage, spinach, and radish leaves showed an average content of 55.1 mg AAE/100 g FW, without significant differences between them ($p > .05$), while green lettuce had the lowest AAC. The AAC values of parsley, lettuce, and spinach were comparable to the values obtained by Karaca and Velioglu (2014) for the same vegetables (126, 10, and 35 mg AAE/100 g FW, respectively). However, the values obtained for spinach and green lettuce were nine- and eightfold higher than the values reported by Proteggente et al. (2002) for these products. The AAC of rocket was in the range of the values reported by Martínez-Sánchez, Gil-Izquierdo, Gil, and Ferreres (2008) of 80–103 mg AA/100 g FW. Koh, Charoenprasert, and Mitchell (2012) evaluated the AAC of 27 varieties of spinach, and found that it varied from 13.4 to 53.7 mg/100 g FW, depending on cropping system (organic or conventional) and cultivar. Moreover, Podsędek (2007) explained that the cause of variations in AAC reported by different authors for the same type of product might be related to the differences in genotype, climatic conditions, nitrogen concentration in fertilization, and also to the quantification method used.

3.4 | Antioxidant capacity

The antioxidant activity value of a sample will differ according to the method used to quantify it, which makes it necessary to perform more than one type of antioxidant capacity determination to take into account the various mechanisms of antioxidant action (Deng et al., 2013). The antioxidant capacity of the vegetables was measured by

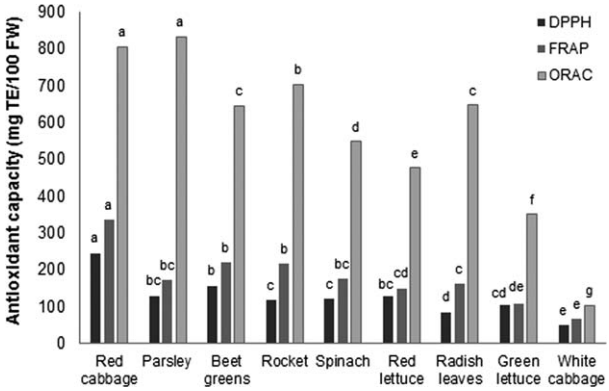

FIGURE 1 Antioxidant capacities of leafy vegetable samples. Different letters in the same assay indicate significant difference between samples ($p \leq .05$)

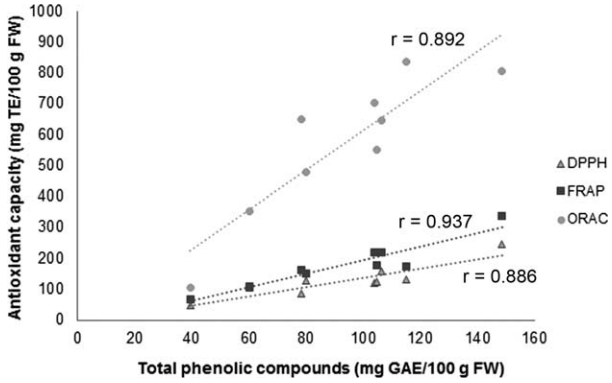

FIGURE 2 Correlations between total phenolic content and antioxidant capacity (DPPH, FRAP, and ORAC)

means of the DPPH, FRAP, and ORAC methods. The results are summarized in Figure 1.

DPPH values were in the range of 48.9–245.8 mg TE/100 g FW. Red cabbage had the highest DPPH value (245.8 mg GAE/100 g FW), followed by beet greens, parsley, and red lettuce, which showed no significant differences between them ($p > .05$), with an average DPPH value of 138.9 mg TE/100 g FW. FRAP values ranged from 67.7 to 335.8 mg TE/100 g FW, with red cabbage showing the highest FRAP value (335.8 mg TE/100 g FW). Beet greens, rocket, spinach, and parsley exhibited an average FRAP value of 196.7 mg TE/100 g FW, without significant differences between them ($p > .05$). ORAC values ranged from 104.8 to 833.9 mg TE/100 g FW. Red cabbage and parsley had the highest ORAC values, with an average of 820.3 mg TE/100 g FW. Rocket showed an ORAC value of 702.6 mg TE/100 g FW, with significant differences with the other vegetables ($p < .05$).

Red cabbage showed the highest antioxidant capacity obtained with the FRAP and DPPH methods, and did not present significant differences with parsley when ORAC was used ($p > .05$). The important antioxidant capacity shown by red cabbage is strongly related to its concentration of bioactive compounds, since it showed the maximum concentration in phenolics. Other authors have also reported high antioxidant activities for red cabbage. For example, Stratil et al. (2006), reported FRAP and DPPH values of 775.9 mg TE/100 g FW and 125.1 mg TE/100 g FW, respectively. This FRAP value is twofold higher than that obtained in our red cabbage sample, and the value obtained with the DPPH method was half than the value reported in our investigation. As mentioned above, the differences between the vegetable samples studied and that reported by other authors are mainly due to preharvest and postharvest factors/processes of the biological material, and also to differences in the extraction and quantification methods (Deng et al., 2013). Based on the analysis of the obtained results, it is possible to observe that parsley showed the second highest overall antioxidant capacity. Parsley did not show significant differences with red cabbage in the ORAC assay, and showed the highest antioxidant capacity in the FRAP and DPPH methods. Antioxidant capacity values obtained for parsley were similar to those reported by Stratil et al. (2006), but other authors have reported lower values (Karaca & Velioglu, 2014; Ogita et al., 2016; Stratil et al., 2006; Tiveron et al., 2012), which suggest that the parsley sample under study presented a greater antioxidant capacity than the average of the same vegetable.

Beet greens and rocket were third in terms of antioxidant capacity values, between all the studied vegetables. It is noteworthy that the data available in the literature for beet greens are scarce, because this vegetable is mostly considered as a food residue. Although rocket is one of the vegetables of recent insertion in the diet of consumers, it was possible to find some reports about this product. Tiveron et al. (2012) reported an antioxidant capacity for rocket (DPPH) almost fourfold lower than the value obtained for the rocket sample in the present study. In the same way, FRAP and DPPH values of the rocket samples used in this work were twice the value reported by Martínez-Sánchez, Marín, Llorach, Ferreres, and Gil (2006).

In the vegetable samples under study, the relationship between antioxidant capacity and TPC was evaluated (Figure 2, Table 2). Significantly higher correlation values were found between TPC and FRAP ($r = 0.937$, $p < .0005$), ORAC ($r = 0.892$, $p < .005$), and DPPH ($r = 0.886$, $p < .005$). The high correlation coefficient explains that variations in phenolic content of a sample, has a significant influence on its antioxidant capacity. We observed that the antioxidant activities analyzed with the ORAC, DPPH, and FRAP assays, showed positive trends in all cases. Antioxidant capacity is increased in parallel to the phenolic content of the vegetable, and tends to zero as the phenolics decrease. This indicates that in these vegetables, phenolic compounds were one of the major contributors to antioxidant capacity.

The correlation between total antioxidant capacities obtained from the DPPH and FRAP methods was significant in a high level ($r = 0.922$, $p < .0005$), suggesting that the antioxidant compounds in the vegetables efficiently reduced the TPTZ-iron complex and scavenged the DPPH radical through an electron transfer mechanism. A possible reason for the lower DPPH values as compared to the FRAP values, could be the presence of compounds that are poor radical scavengers, and are therefore not reactive toward the DPPH radical. Antioxidant compounds such as polyphenols, may be more efficient iron-reducing agents, but some may not scavenge DPPH free radicals as efficiently due to steric hindrance (Wong, Leong, & Koh, 2006). The correlation between total antioxidant capacities obtained by ORAC and by FRAP

and DPPH methods were analyzed, and it was found that the correlation were significant ($p < .05$) in both cases: $r = 0.677$ (ORAC vs. DPPH) and $r = 0.797$ (ORAC vs. FRAP), with lower correlation coefficients than those obtained for the correlation between DPPH and FRAP. The results presented could indicate that not all the antioxidant compounds in the vegetable samples have the same antioxidant capacities or mechanisms of action (as previously mentioned). The DPPH and FRAP assays are based on a single electron transfer mechanism to a stable molecule (DPPH and FRAP), while ORAC uses a radical initiator to generate the peroxyl radical, and its mechanism of action is hydrogen atom transfer (Ou, Huang, Hampsch-Woodill, Flanagan, & Deemer, 2002).

A significant correlation was observed between TFC and ORAC ($r = 0.721$, $p < .05$), but not between TFC and DPPH and FRAP. The comparison of correlation results indicate that the contribution of total flavonoids to the antioxidant capacity is lower than that of total phenolics. The correlation between AAC and antioxidant capacity (ORAC, DPPH, and FRAP) was not significant ($p > .05$). At this respect, is possible to observe in sample results that in many cases, AAC was low and antioxidant capacity was high, suggesting ascorbic acid is a minor contributor to antioxidant capacity. A similar observation was made by Bahorun et al. (2004), who also suggests that ascorbic acid makes a small contribution to the antioxidant capacity of fruits and vegetables.

3.5 | Identification and quantification of individual phenolic compounds

In plants, phenolic compounds occur in soluble forms as well as in combination with cell wall components (bound phenolics). Besides, phenolics in vegetables exist in both free and conjugated forms (glycosylates). Only conjugated compounds are generally present in fresh vegetables, but aglycones may be produced as a result of food processing (Podsędek, 2007). Most studies on vegetable phenolics rely on obtaining free aglycones (by heat, acid or alkaline hydrolysis of vegetal extracts), because determination of individual phenolic glycosides is difficult due to a lack of reference compounds (Podsędek, 2007).

The phenolic profile of each vegetable and the quantitation of the identified compounds is presented on Table 3. The FF refers to the phenolic compounds that can be directly extracted from the food matrix (not bound), and are not glycosylated. The compounds released during alkaline and acid hydrolysis are those that were trapped in the matrix (bound) or were glycosylated. This fraction was identified as the HF, and consist of the sum of the compounds liberated after alkaline and acid hydrolyses.

We determined that, except for parsley, most of the phenolic compounds in the studied vegetables were in the HF, indicating that they were bound to the cell wall or were present as glycosylated compounds, so it would not have been possible to identify/quantify them without an initial hydrolysis. While some phenolic compounds could be identified in both fractions, some of them can be found only in a specific one. In this regard, catechin was only identified in the FF (with the exception of white cabbage), while the hydroxybenzoic acid, *p*-coumaric acid, ferulic acid, sinapic acid, quercetin, and kaempferol, were only detected in HF. Regarding the FF, catechin was the main compound, having been identified in six of the nine vegetables. While in the HF, the most common compounds were *p*-coumaric acid (identified in all nine vegetables), quercetin (in seven vegetables), and caffeic acid (in six vegetables).

Figure 3 shows a representative chromatogram of all fractions obtained from green lettuce. Peaks were identified using pure standards and by analyzing their spectrum. Catechin and chlorogenic acid were only detected in the FF of green lettuce, while GA, *p*-coumaric acid, and quercetin were only detected in the HF, both in alkaline- and in acid-hydrolyzed samples. Caffeic acid was detected in the three fractions, and it was the main one from the HF, corresponding to 76% of phenols quantified in this fraction. While in the FF, chlorogenic acid and rutin were the main compounds (38.5% and 36.5%, respectively).

We determined that in spinach, the main compound identified in the FF was catechin (78%), while the main compound in the HF was quercetin (46%). Beet greens, parsley, and radish leaves also presented catechin as the main identified compound in the FF, with percentages of 100%, 98.6%, and 98.9%, respectively. While the preponderant compounds identified in the HF were ferulic acid in beet greens (76%), *p*-coumaric acid in parsley (60.7%), and kaempferol in radish leaves (53.2%). No compounds were identified in the FF of white and red cabbage, while in the HF, the sinapic acid was the main identified molecule, with 60.7 and 68.5%, respectively. Caffeic acid was the main compound in the HF (79%) of red lettuce, and rutin in the FF (79%).

Red cabbage, beet greens, parsley, and rocket exhibited the highest antioxidant capacities, based on the results obtained from the DPPH, FRAP, and ORAC methods. Because phenolic content showed a good correlation with antioxidant capacity, the main phenolic compounds of these four vegetables were subsequently analyzed to understand their potential benefits on human health; their chemical structures are shown in Figure 4. The reducing properties of these chemicals (as hydrogen or electron-donating agents) predicts their potential for action as free-radical scavengers (antioxidants) (Prakash & Gupta, 2009). Catechins have been widely studied, since they are present in green tea and the health effects of tea have been attributed to them. In standard green tea (2 g of tea leaves in 100 mL boiling water), catechin is found at a concentration of 10 mg/100 mL (Henning et al., 2003); an equivalent dose can be obtained by consuming 92.6 g of fresh beet greens or 30.6 g of fresh parsley. Numerous studies have demonstrated that catechins exerted vascular protective effects through multiple mechanisms, including antioxidant, anti-hypertensive (regulate vascular tone by activating endothelial nitric oxide), anti-inflammatory (suppression of leukocyte adhesion to endothelium and subsequent transmigration through inhibition of NF-B-mediated cytokine production), anti-proliferative (inhibit proliferation of vascular smooth muscle cells by interfering with vascular-cell growth factors involved in atherogenesis), antithrombotic (suppress platelet adhesion), and lipid lowering effects (Babu, Pon, & Liu, 2008; Obrenovich, Nair, Beyaz, Aliev, & Reddy, 2010).

Another of the main phenolic compounds identified in our vegetable samples was quercetin. Onions are considered a major source of dietary quercetin (Lee et al., 2011), with an average content of

TABLE 3 Identification and quantification of free and bound individual phenolic compounds in leafy vegetable samples[a]

Vegetable	Fraction	1[b]	2	3	4	5	6	7	8	9	10	11	12
Spinach	FF[c]	–	–	2,198.7 ± 14.2	–	–	–	–	–	–	621.5 ± 12.0	–	–
	HF[d]	11.9 ± 1.4	–	–	–	20.8 ± 1.72	–	1,274.8 ± 40.4	1,474.9 ± 51.3	–	13.8 ± 2.3	2,365.5 ± 49.9	–
Green lettuce	FF	–	–	1,902.1 ± 62.4	3,182.1 ± 196.0	–	160.2 ± 23.1	–	–	–	3,012.3 ± 210.9	–	–
	HF	125.2 ± 15.7	–	–	–	–	12,636.8 ± 388.4	190.8 ± 9.8	–	–	2,423.9 ± 30.9	839.3 ± 34.3	–
Beet greens	FF	–	–	1,219.8 ± 28.6	–	–	–	–	–	–	–	–	–
	HF	304.8 ± 14.5	–	–	–	–	–	32.5 ± 2.3	2,634.4 ± 32.6	–	–	502.0 ± 6.3	–
White cabbage	FF	–	–	–	–	–	–	–	–	–	–	–	–
	HF	159.9 ± 1.4	–	179.6 ± 18.1	–	–	39.5 ± 3.0	43.8 ± 3.8	35.0 ± 1.2	707.0 ± 25.7	–	–	–
Parsley	FF	42.3 ± 1.7	–	2,969.7 ± 82.2	–	–	–	–	–	–	–	–	–
	HF	–	–	–	–	55.5 ± 5.9	–	756.9 ± 22.7	–	–	427.7 ± 2.2	–	–
Red lettuce	FF	–	–	1,402.7 ± 83.6	–	–	175.8 ± 5.1	–	–	–	11,454.0 ± 15.7	–	–
	HF	55.87 ± 0.5	173.3 ± 15.8	–	–	–	22,757.6 ± 220.3	299.0 ± 10.2	–	–	6,198.3 ± 40.3	2,361.1 ± 17.4	–
Rocket	FF	–	–	–	–	–	–	–	–	–	1,408.2 ± 45.2	–	–
	HF	–	–	–	–	58.8 ± 8.9	379.0 ± 4.3	47.3 ± 3.6	272.3 ± 12.3	902.3 ± 8.8	641.8 ± 22.0	1,303.6 ± 14.6	26.0 ± 4.3
Red cabbage	FF	–		–	–	–	–	–	–	–	–	–	–
	HF	188.0 ± 4.1	–	–	–	18.4 ± 1.2	754.0 ± 16.2	824.6 ± 9.6	962.7 ± 7.4	6,119.9 ± 54.2	–	61.6 ± 1.0	10.7 ± 0.3
Radish leaves	FF	–	–	2,814.8 ± 114.5	–	–	34.4 ± 7.6	–	–	–	–	–	–
	HF	–	–	–	–	270.0 ± 3.7	674.4 ± 2.7	1,632.7 ± 3.5	2,206.9 ± 62.8	–	–	17.6 ± 1.1	5,468.6 ± 28.4

[a]Data are expressed as mean ± standard deviation ($n = 3$), in dry weight basis of the original vegetal sample (μg/g DW).
[b]1: Gallic acid; 2: protocatechuic acid; 3: catechin; 4: chlorogenic acid; 5: hydroxybenzoic acid; 6: caffeic acid; 7: *p*-coumaric acid; 8: ferulic acid; 9: sinapic acid; 10: rutin; 11: quercetin; 12: kaempferol.
[c]FF = Free fraction.
[d]HF = Hydrolyzed fraction.

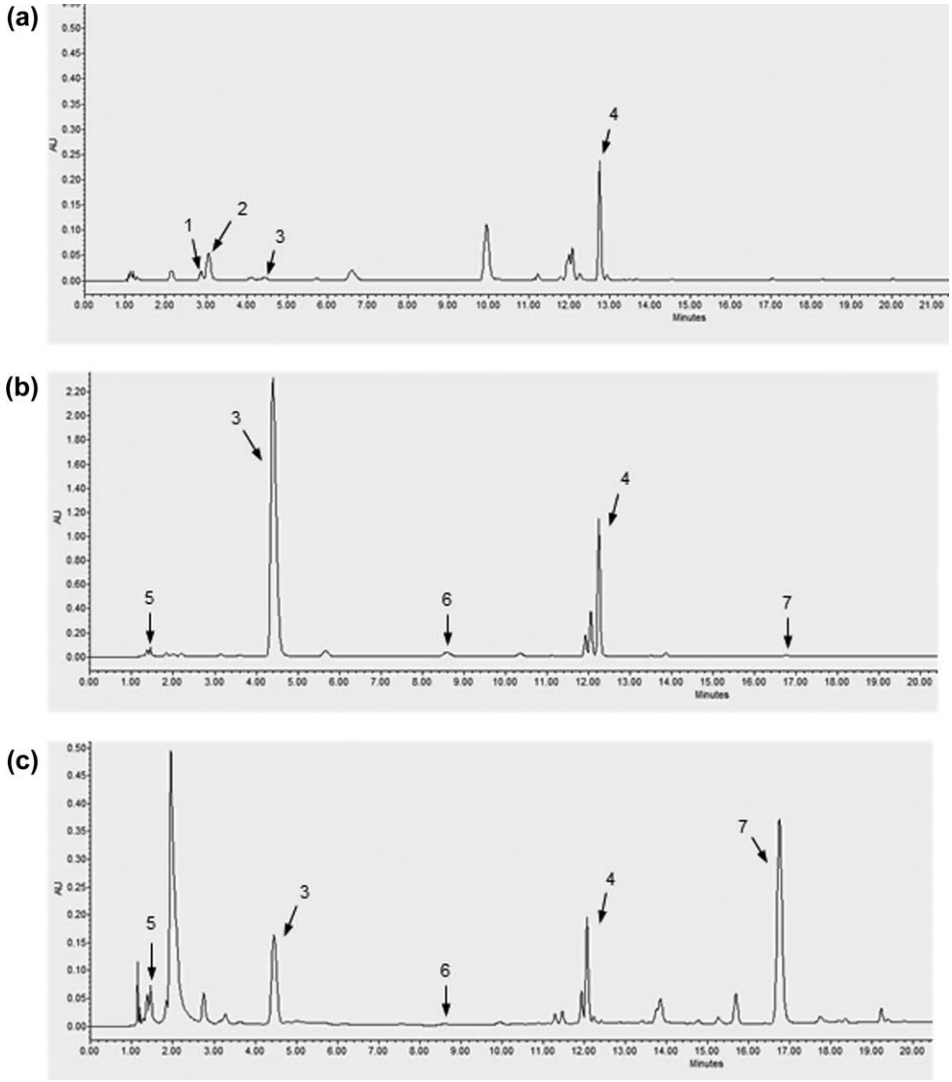

FIGURE 3 A representative UPLC chromatogram of green lettuce samples: (a) free fraction; (b) fraction after alkaline hydrolysis; (c) fraction after acid hydrolysis. The resulting peaks were identified by comparing their retention times and spectra, with those obtained from pure HPLC-grade standards. Peaks were identified as follows: catechin (1), chlorogenic acid (2), caffeic acid (3), rutin (4), gallic acid (5), *p*-coumaric acid (6), and quercetin (7)

15.5 mg/g FW (Yoo, Lee, & Patil, 2010). In contrast to fresh onion, rocket presented a slightly lower quercetin content (14.3 mg/g FW), but in beet greens it was threefold lower (5.02 mg/g FW). Quercetin has anti-inflammatory effects (Hämäläinen, Nieminen, Vuorela, Heinonen, & Moilanen, 2007; Kleemann et al., 2011) and can act as a cardioprotective compound, by mitigating atherosclerosis (Kleemann et al., 2011; Prakash & Gupta, 2009). Anticarcinogenic effects have also been associated with quercetin consumption, since it has apoptosis-inducing abilities in human tumor cells (Chen et al., 2005; Xavier et al., 2009).

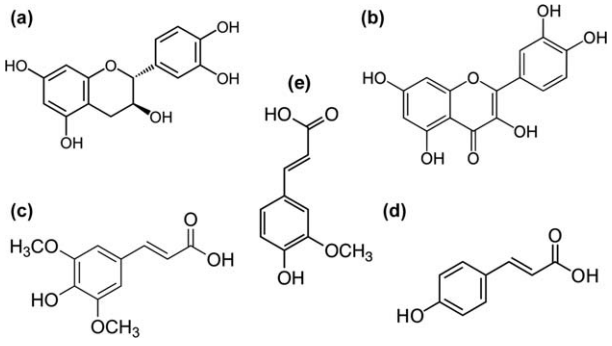

FIGURE 4 Chemical structures of the predominant phenolic compounds identified in our samples: (a) catechin, (b) quercetin, (c) sinapic acid, (d) *p*-coumaric acid, and (e) ferulic acid

One of the hydroxycinnamic acid derivatives found was *p*-coumaric acid. Radish leaves had the most *p*-coumaric acid content (21.2 mg/100 g FW), followed by spinach (9.37 mg/100 g FW), parsley (8.16 mg/100 g FW), and red cabbage (7.35 mg/100 g FW). *p*-coumaric acid exerts benefits effects on human health that are related to its antioxidant properties (Liu, 2013; Yoon et al., 2013). This compound was reported by Yoon et al. (2013) to treat metabolic disorders, preventing or improving insulin resistance and type 2 diabetes by modulating glucose and lipid metabolism. *p*-coumaric acid has also been reported by Vauzour, Corona, and Spencer (2010) to exert neuroprotective effects that could be utilized to treat Parkinson's disease. Roy and Prince

(2013) reported that *p*-coumaric acid exhibited preventive effects on dyslipidemia, and prevention of cardiac hypertrophy.

Ferulic acid is another hydroxycinnamic acid derivative identified in our samples. This compound is one of the major phenolics present in cereal grains (24–54 mg/100g FW), various citrus fruits (1.5–11.6 mg/100g FW), tomatoes (0.29–6 mg/100g FW), and a wide range of other vegetables (1.2–25 mg 100g FW) (Kumar & Pruthi, 2014; Staniforth, Huang, Aravindaram, & Yang, 2012). The results obtained in the present work suggest that beet greens are a high source of ferulic acid (26.2 mg/100g FW), followed by red cabbage (8 mg/100g FW). The reported health effects are related to the treatment of Alzheimer's disease, anticancer properties, and cardioprotection (Kumar & Pruthi, 2014; Mancuso & Santangelo 2014; Staniforth et al., 2012). Lin et al. (2010) reported that ferulic acid plays a novel role in angiogenic effects, and is a potential new therapeutic agent for ischemic diseases (Lin et al., 2010).

Sinapic acid was another major compound identified. It is typically found in Brassicaceae species (Yun et al., 2008). This fact was evidenced in the phenolic profile of our samples, since sinapic acid was only detected in the vegetables belonging to the Brassicaceae family: rocket, red cabbage, and white cabbage (861.0, 6,119.9, and 692.2 μg/g DW, respectively). Sinapic acid and some of its derivatives have recently drawn attention because of their various biological activities. For example, Yun et al. (2008) demonstrated that sinapic acid exerts anti-inflammatory and antiedema effects. Lee et al. (2012) suggested that sinapic acid has neuroprotective effects and may be used as a treatment for Alzheimer's disease. Moreover, Silambarasan et al. (2014) demonstrated that sinapic acid may be potentially therapeutic in hypertensive heart disease.

The three hydroxycinnamic acid derivatives previously described (ferulic acid, *p*-coumaric acid, and sinapic acid) were only found in the HFs; other authors have explained that these metabolites are primarily present in the bound form, connected to cell wall structural components such as cellulose, lignin, and proteins through ester bonds. They can only be identified if previously released by hydrolysis (Kumar & Pruthi, 2014; Liu, 2013; Nićiforović & Abramovič, 2014), it would otherwise be difficult or impossible to detect them and may be overlooked.

4 | CONCLUSIONS

The chemical characterization of nine selected vegetables of conventional and unconventional use in salads was carried out in this work. Their content of bioactive compounds and their antioxidant capacities were determined. Diverse antioxidant capacities were detected among the different vegetables. Red cabbage, beet greens, parsley, and rocket exhibited the highest antioxidant capacities (DPPH, FRAP, and ORAC). These results suggest that these four vegetables could be important sources of natural antioxidants that can prevent some chronic-degenerative diseases related to oxidative stress, which are fairly common in most Western countries. TPC and the antioxidant capacities of the studied vegetables exhibited a strong positive correlation, indicating that phenolic compounds could be one of the main contributors to the total antioxidant capacity of these vegetables. In addition, the phenolic profile of each vegetable was analyzed. Several phenolic compounds were identified, such as catechin, which was the main compound found in the FF, and *p*-coumaric acid, quercetin, and caffeic acid, which were the main compounds in the HF.

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ORCID

Cintia A. Mazzucotelli http://orcid.org/0000-0002-3771-255X

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