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Phytochemical profile and functionality of *Brassicaceae* species

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author statement

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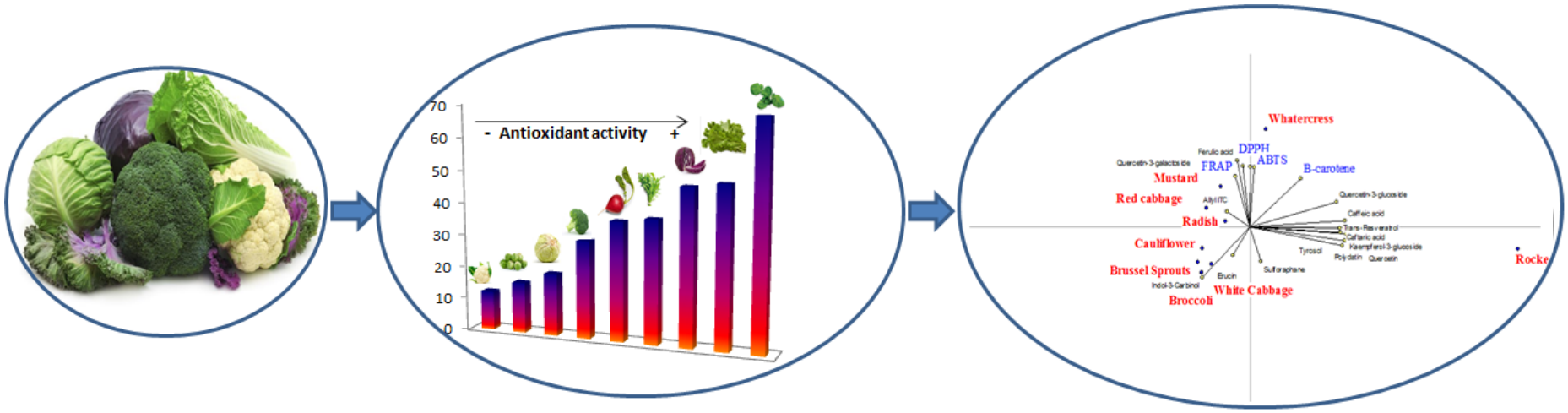

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Phytochemical profile and functionality of *Brassicaceae* species

Running title: Phytochemical and functional profiling in cruciferous

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ABSTRACT

The most widely consumed Brassicaceae species were characterized and compared in the present study. The isothiocyanates and phenolic profiles were measured. The *in vitro* antioxidant and antiradical activities were determined using 2,2′-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid diammonium salt, 1,1-diphenyl-2-picrylhydrazyl radical, ferric reducing antioxidant potential and β-carotene methods. The results showed that all Brassicaceae species evaluated had antioxidant properties, with watercress and green mustard being the most active antioxidant species. Every vegetable studied had a unique sulfur and phenolic profile. Twenty-five phytochemicals were found in Brassicaceae species and their antioxidant activity measured using pure compounds. The results showed that the strongest antioxidant compounds in decreasing order were myricetin, quercetin-3-galactoside, quercetin-3-glucoside, pterostilbene, ferulic acid, kaempferol, allyl isothiocyanate, and (-)-epicatechin. Besides, the phenolic compound *trans*-resveratrol was found in these species. The highest concentration of *trans*-resveratrol was observed in rocket leaves at up to 84 μg/g dry weight.

KEYWORDS

Brassica sp., Isothiocyanates, Phenolic compounds, *trans*-Resveratrol, Cruciferous.

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1. INTRODUCTION

The Brassicaceae (= Cruciferae) family contains more than 350 genera and 3,000 species worldwide (Fahey et al., 2001; Marzouk et al., 2010). Despite the great diversity among the Brassicaceae family members, few species are eaten, mainly from the *Brassica* genus. Other important species are *Eruca sativa* (rocket), *Nasturtium officinale* (watercress) and *Raphanus sativus* (radish) (Thomson et al., 2007).

Brassicaceae vegetable consumption is recommended due to its nutritional composition and phytochemical richness. They are low in fat and high in vitamins, minerals and fiber (Dias, J., 2012). They are also good sources of different phytochemicals such as isothiocyanates and phenolic compounds that have an important role in chronic diseases prevention. Brassicaceae vegetables provide two sources of organosulphur compounds; those derived from the glucosinolate-myrosinase system and S-methyl cysteine sulphoxide (Stoewsand, 1995), which lead to several sulfur-containing volatile metabolites. The first system, results in isothiocyanate (ITC) formation, which is responsible for the pungent taste associated with these plant species. Depending on the glucosinolate chemical structure, the ITC can have either indolic, aliphatic or aromatic side-chains. Some authors have reported that ITC can lower the incidences of different cancers (Dinkova-Kostova & Kostov, 2012). The chemopreventive properties of ITC are shown by their participation in multiple anticancer mechanisms such as modifications of the chemical carcinogenesis process due to changes in the activities of drug-metabolizing enzymes, induction of cell cycle arrest and apoptosis, inhibition of angiogenesis and metastasis, changes in histone acetylation status, as well as antioxidant, anti-inflammatory, and immunomodulatory activities (Camargo & Manucha, 2016; Dinkova-Kostova & Kostov, 2012). The characterization of the ITC's antioxidant properties is significant because they may protect the human body against the oxidative

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damage mediated by reactive oxygen species (ROS). They can effectively depurate hydrogen peroxide and organic hydroperoxides, and they can induce phase II enzymes (Burčul et al., 2018).

Other important bioactive compounds found in Brassicaceae species are the phenolic compounds, which have been reported as major antioxidants of *Brassica* plants (Soengas et al., 2011).

The evaluation of the antioxidant capacity of vegetables is a complex issue due to the diversity of oxidants and the different possible mechanisms needed to depurate or scavenge them. There is not a single test which comprehensively reflects the antioxidant capacity of the samples. Consequently, an evaluation of the antioxidant capacity must use different tests that involve multiple factors and mechanisms to inhibit the oxidative process (Frankel & Meyer, 2000).

Previous studies only measured the radical scavening activity of different *Brassica sp*. using the 1,1-diphenyl-2-28 picrylhydrazyl radical (DPPH) and the ferric reducing antioxidant potential (FRAP) of these plant extracts (Cartea & Velasco, 2008; Kaulmann et al., 2014). Moreover, there is no data concerning the protective ability of the Brassicaceae plant extracts against oxidative processes or the correlation between ITC and phenolic compounds or their antioxidant activities measured using different methods.

The main aims of the present study were to analyze the phenolic and sulfur profiles of 9 of the main edible Brassicaceae species, to measure the antioxidant capacity using 4 different methodologies and to discuss the associations among phytochemical contents and the primary antioxidant mechanisms for each species. The principal component analysis (PCA) was carried out to determine the main mechanism(s) of action.

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2. MATERIALS AND METHODS

2.1. Chemicals

Linoleic acid (99% v/v), potassium persulphate (99% w/v), trichloroacetic acid (99% w/v), Tween 20 (97% v/v), 2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), sulforaphane (SF) (90% v/v), allyl ITC (AITC) (95% v/v), indole-3-carbinol (I3C) (>96% v/v), soybean lipoxidase (LOX) type 1-S (46,000 units/mg solid), *trans*-β-carotene (95%), 1,1-diphenyl-2-picrylhydrazyl radical (DPPH), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) (98% v/v), iron (III) chloride (99% w/v) gallic acid (99% w/v), caffeic acid (99% w/v), caftaric acid (≥97% w/v), (-)-gallocatechin gallate (≥99% w/v), (+)-catechin (≥99% w/v), (-)-epicatechin (≥95% w/v), syringic acid (≥95% w/v), p-coumaric acid (98% w/v), ferulic acid (≥99% w/v), *trans-r*esveratrol (≥99% w/v), polydatin (≥95% w/v), quercetin 3-β-D-glucoside (≥90% w/v), quercetin 3-β-D-galactoside (≥97% w/v), myricetin (≥96% w/v) and quercetin hydrate (95% w/v) were obtained from Sigma Aldrich Co. (St. Louis, MO, USA). The standard of 2-(4-hydroxyphenyl) ethanol (tyrosol) (≥99.5% w/v) was obtained from Fluka (Buchs, Switzerland) and kaempferol (≥98% w/v) was purchased from Alfa Aesar (Tewksbury, MA, USA). Formic acid (>88% w/v) was obtained from the Cicarelli Co. (San Lorenzo, Santa Fe, Argentina). Methanol (MeOH), acetonitrile (ACN) and chloroform were HPLC grade and were purchased from Sintorgan (Villa Martelli, Buenos Aires, Argentina). Sodium borate anhydrous and ferrous sulfate were obtained from Biopack (Buenos Aires, Argentina). Ultrapure water (18 MΩ·cm) was obtained from a Milli-Q water purification system (Millipore, Paris, France). Stock solutions of compounds were prepared in MeOH at 1000 mg/mL. Calibration standards were dissolved in MeOH (50% v/v).

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Erucin was extracted from rocket (*Eruca sativa)* seeds, according to the method of Vaughn et al. (2005). Briefly, defatted seeds (10 g), were mixed with 25 mL of 0.005 M potassium phosphate buffer, pH 7.0 and 50 mL of CH_2CL_2, the mixture was kept in an incubator shaker set at 25°C and 200 rpm for 8 h. Following hydrolysis, 10 g of sodium chloride and 10 g of anhydrous sodium sulfate were added and mixed thoroughly. The CH_2Cl_2 was decanted and filtered through Whatman No. 1 filter paper (Sigma Aldrich) and the residual seeds were extracted an additional three times. The extracts were combined, analyzed and its purity was determined.

2.2. Plant material and sample conditioning

Samples of 9 commonly consumed Brassicaceae vegetables, including broccoli (*Brassica oleracea* var. italica), cabbage (*Brassica oleracea* var. capitata), Brussels sprouts (*Brassica oleracea* var. gemmifera), radish (*Raphanus sativus*), green mustard (*Brassica juncea*), cauliflower (*Brassica oleracea* var. botrytis), rocket (*Eruca sativa*), and watercress (*Nasturtium officinale*), were purchased from local grocery stores located in the Cooperative Market of Mendoza, which represent a convergence point of the predominant vegetable producers, exporters and traders of the midwestern region of Argentina. One kg of each species was purchased in 5 different stores and immediately sent to the laboratory, in autumn 2017. A single batch of one kg of each species was randomly extracted for the analysis, which was formed mixing all the vegetables of the same species.

A subsample of each batch was measured in triplicate. The edible part was washed with tap water. ITC extraction and moisture content determination were done on the day of purchase. For dry matter determinations, samples were processed, weighed (3 g of each vegetable) and dried in a convection oven (Dalvo, Santa Fe, Argentina) at 70 ± 10°C until constant weigh. Results were expressed as g dw (dry weight)/100 g fw (fresh weight).

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2.3.Phytochemical extraction

An ultrasound-assisted extraction was carried out using an optimized technique (Fusari et al., 2015). Ten g of fresh vegetable was placed in a blender with 50 mL of ultrapure water and homogenized for 9 min (Blender, 600 W, 60 Hz, model HR2030/10, Phillips, Buenos Aires, Argentina); then, the homogenate was sonicated in an ultrasound bath for 5 min (40 kHz and 600 W, model TB 04, Testlab, Buenos Aires, Argentina). ITC formation was carried out by stirring an aliquot of 5 mL homogenate at 37°C for two h (Ares et al., 2014).

2.4.Phytochemical analysis

2.4.1. ITC determination using a HPLC-DAD (diode array detector)

ITC analysis was done using a miniaturized technique (Fusari et al., 2018) called dispersive liquid-liquid microextraction (DLLME). Briefly, one mL ACN was mixed with 700 µL chloroform and rapidly injected into 3 mL of sample solution using a syringe. The mixture was centrifuged at 2000 x g for 2 min at 25ºC (Gelec, G142, Buenos Aires, Argentina). The organic solvent phase was dried under a nitrogen stream and dissolved in 500 µL MeOH. Finally, it was filtered using a 0.45 µm polytetrafluoroethylene (PTFE) membrane (Sigma Aldrich) before injection into the HPLC (Shimadzu LC 20A, Shimadzu Corp., Columbia, MD, USA), a DAD (Dionex Softron GmbH, Thermo Fisher Scientific Inc., Germering, Germany) with a wavelength set to 241 nm for analysis (Wilson et al., 2012).

The chromatographic analysis was done using an ODS Waters RP-C18 column (150 x 4.6 mm x 5 µm) (Phenomenex, Torrance, CA, USA) and a guard-column with the same characteristics (10 x 4.6 mm x 5 µm) (Phenomenex). The elution of the analytes was done with a mobile phase using different ratios of MeOH (A) and water (B) at a flow rate of 0.6 mL/min for 30 min. Both solvents had 0.1% v/v formic acid. The system was equilibrated using the starting conditions for 10 min before the injection of the next sample. Before use,

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mobile phases were filtered using a 0.45 µm polytetrafluoroethylene (PTFE) membrane (Sigma Aldrich). The linear gradient program used was: 0 min 50% A, 0–20 min 80% A, 20–30 min 80% A. The injection volume was 10 µL, and the oven temperature was 25°C. Peak identification and quantification were carried out by comparing retention times and response signals with reference standards. Sample's analytes were quantified using external calibration with pure standards to determine each compound-specific response signal. Calibration curves were found to be linear in a concentration range of 5–100 mg/mL, with correlation coefficients R>0.91 for all analytes.

2.4.2. Phenolic compounds determination using HPLC-DAD

For phenolic compounds profiling, an aliquot of the extract obtained in Section 2.3 was centrifuged at 12.000 x g for 10 min at 25ºC. The supernatant was filtered using a 0.2 µm polytetrafluoroethylene (PTFE) membrane (SKC Ltd., Blandford Forum, Dorset, UK) and diluted with 0.1 mL ACN. Mobile phases were ultrapure water with 0.1% formic acid (A) and ACN (B). Analytes were separated using a previously reported method (Fontana et al., 2016) with the following gradient: 0–2.7 min, 5% B; 2.7–11 min, 30% B; 11–14 min, 95% B; 14–15.5 min, 95% B; 15.5–17 min, 5% B; 17–20, 5% B. The mobile phase flow was 0.8 mL/min. The column temperature was 35°C, and the injection volume was 10 µL. The quantification was made with a multi-wavelength's detector (254, 280, 320, and 370 nm) for different analytes (Fontana et al., 2016). Samples were quantified using an external calibration with authentic standards to determine each compound specific response signal. Linear ranges between 0.1 and 20 mg/L with a coefficient of determination (R^2) > 0.9 were obtained. The software used to control all parameters of the HPLC-DAD system and to process the data was the Chromeleon™ Chromatography Data System Software v. 7.1 (Thermo Fisher Scientific Inc., Buenos Aires, Argentina)

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2.5. Antiradical and antioxidant capacity *in vitro* assays

2.5.1. DPPH$^{\bullet}$ scavenging assay

Free-radical scavenging activity was measured using the DPPH$^{\bullet}$ bleaching method (Brand-William et al., 1995). An aliquot of the aqueous extract was added to 3 mL DPPH$^{\bullet}$ methanolic solution and measured at 515 nm using a DU-530 UV-Visible spectrophotometer (Beckman Coulter, Buenos Aires, Argentina). The decrease in absorbance was determined by monitoring the absorbance changes every 30 s for 10 min. Antiradical activity (ARA) was calculated according to Burda & Oleszek (2001) as shown in Equation 1, where A_{SS} is the absorbance of the solution at the steady state and A_0 is the absorbance of DPPH$^{\bullet}$ solution before the antioxidant addition. A_{SS} was estimated by the mathematical fitting of kinetic curves obtained using Origin Pro v. 8.0 software (OriginLab Corp., Northampton, MA, USA).

$$ARA\ \% = (Ass/A_0)x\ 100 \qquad (1)$$

ARA was expressed as antiradical activity/100 mg of dw. All determinations were done in triplicate for each extract.

2.5.2. ABTS$^{+\bullet}$ scavenging assay

ABTS was dissolved in distilled water to give a 7 mM solution, according to Locatelli (2017). The radical solution was prepared by incubating the ABTS solution with the same proportion of 2.45 mM potassium persulphate solution for 16 h in the dark at room temperature (20 to 25°C), and this was subsequently diluted with distilled water to a final absorbance of 1.00 at 734 nm. For ARA determinations, an aliquot of aqueous extracts was added to 3 mL ABTS$^{+\bullet}$. The decrease in absorbance was determined by monitoring the absorbance changes every 30 s for 10 min. All determinations were done in triplicate. The

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212 percentage inhibition of $ABTS^{+\bullet}$ by the samples was calculated using Equation 1. ARA was
213 expressed as antiradical activity/100 mg of dw.

214 *2.5.3. Ferric reducing capacity assay (FRAP)*

215 The ability to reduce ferric ions was measured using the procedure described by Marazza
216 (2012). An aliquot of 1 mL of sample was mixed with 1 mL 0.2 M sodium phosphate
217 buffer (pH 6.6) and 1 mL 1% (w/v) potassium ferricyanide. The mixture was incubated at
218 50°C for 20 min. Then, 1 mL of 10% (v/v) trichloroacetic acid was added. The mixture was
219 centrifuged at 15,900 x g for 10 min at 4 °C. The supernatant (1.5 mL) was mixed with 0.3
220 mL of 0.1% (w/v), ferric chloride and 1.5 mL of ultrapure water. After 10 min, the
221 absorbance at 700 nm was measured. The ferric cation reducing power was expressed in
222 Trolox equivalent antioxidant capacity (TEAC) in µmol TEAC/g dw. The percentage of
223 ferric reduction was calculated using Equation 2 (Canabady-Rochelle et al., 2015), where
224 C_0 is the concentration of $FeSO_4$ (µM) with absorbance equal to 1.00 and C_s is the
225 equivalent concentration of $FeSO_4$ (µM) observed with each vegetable extract.

226 $$Reducing\ capacity\ (\%) = 100 - ((Co - Cs)/Co)x\ 100) \qquad (2)$$

227

228 *2.5.4. β-carotene bleaching assay*

229 The antioxidant capacity (AOA) of the extracts and fractions was determined using the
230 enzymatically induced *β*-carotene bleaching method, according to Chaillou and Nazareno
231 (2006). An aliquot of 500 µL of a saturated stock solution of β-carotene in chloroform was
232 mixed with 500 µL of Tween 20. The mixture was evaporated using a nitrogen stream for
233 15 min to remove chloroform. The final solution was obtained by adding 0.01 M borate
234 buffer (pH 9) to an absorbance of 1.3 at 460 nm. The linoleic acid solution was prepared by
235 mixing 50 µL with 200 µL of Tween 20 and diluted with 0.01 M borate buffer (pH 9). LOX

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236 solution was obtained by dissolving 10 mg of the enzyme in 0.01 M borate buffer (pH 9)
237 brought to 10 mL. Assays were done by mixing 2 mL β-carotene solution with 300 μL
238 linoleic acid with 300 μL 0.01 M borate buffer (pH 9), 100 μL sample solution (or distilled
239 water in control assay) and 400 μL of LOX were used to initiate the reaction.
240 Spectrophotometric measurements were carried out at 460 nm. All assays were carried out
241 in triplicate at room temperature. AOA was calculated following Burda and Oleszek
242 (2001), as the percentage of inhibition of the β-carotene bleaching of the samples compared
243 to that of the control as described below in Equation 3. A_s^0 and A_c^0 are the absorbance values
244 measured at the initial incubation time for the samples and control, respectively. Parameters
245 A_s^∞ and A_c^∞ , are the absorbance values at the steady-state measured for the samples and
246 control, respectively, which were estimated by the mathematical fitting of kinetic curves
247 (linear) obtained using the Origin Pro software. Values were expressed as AOA/100 mg
248 dw.

249 $$AAO\ (\%) = 100 \times [1- (A_s{}^0 - A_s{}^{00})/ (A_c{}^0 - A_c{}^{00})] \quad (3)$$

250 **2.6. Statistical analysis**

251 Data were expressed as the mean ± standard deviation (SD). Pearson's correlation analysis
252 and principal component analysis (PCA) were done using C.W. InfoStat version 2013
253 (Grupo Infostat, FCA, Universidad Nacional de Cordoba, Argentina. URL
254 http://www.infostat.com.ar).

255 For supervised PCA only variables with loadings values higher than zero were considered
256 (InfoStat). Mean value comparisons were calculated using the least significant difference
257 (Tukey's LSD) test, and $p<0.05$ was considered significant. For ABTS, DPPH and β-

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carotene bleaching assays, Origin Pro software were used for mathematical fitting of kinetic curves.

3. RESULTS AND DISCUSSION

3.1.Phytochemical profile of Brassicaceae species

Bioactive compound contents in the 9 analyzed species are shown in Tables I and II. Total ITC contents varied from 30.6 to 427 µg/g dw. The species-specific ITC profiles mainly were: sulforaphane in broccoli, indol-3-carbinol in Brussels sprouts, broccoli, and watercress and allyl-ITC in green mustard and watercress.

The most abundant ITC compound was allyl-ITC and was found in all species. Erucin was found only in broccoli and sulforaphane was absent in cauliflower, watercress, and green mustard.

On the other hand, TPC varied from 42.7 (red cabbage) to 2.3 x 10^3 (radish) µg/g dw. The most prevalent phenolic compound was (-)-epicatechin in broccoli, cauliflower and green mustard, (+)-catechin in Brussel sprout, procyanidin B1 in radish, ferulic acid in red cabbage, kaempferol-3-glucoside in rocket, quercetin-3-glucoside in watercress and *p*-coumaric acid in white cabbage. These results indicated that flavonoid compounds, mainly flavonols, and flavan-3-ols, are the most abundant phenolic compounds in these species. In addition, tannins and phenolic acids were found in white cabbage and radish. The latter fraction represented the dominant group of phenolic compounds. Recently Li et al. (2018) measured the phenolic compounds in 12 Brassicaceae species including pakchoi, choysum, Chinese cabbage, kailan, Brussels sprout, cabbage, cauliflower, broccoli, rocket salad, red cherry radish, daikon radish, and watercress and reported that the main phenolic compounds were hydroxycinnamic acids and derivatives, and flavonoids and derivatives,

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but no (-)-epicatechin, proanthocyanidins and stilbenes were reported. This could suggest that the present study found a wider set of phenolic compounds.

Noteworthy, *trans*-resveratrol was measured and quantified in broccoli, Brussels sprouts, green mustard, radish, rocket, watercress, and white cabbage. Previously, *trans*-resveratrol was reported within the Brassicaceae family only in *Brassica napus L*. and in *Arabidopsis thaliana L.* (Řezanka et al., 2018). Moreover, *trans-r*esveratrol levels in green mustard and rocket were similar, and in some cases higher, than those previously reported in foods and beverages thought of as good sources of this compound, such as blueberries and grapes, peanuts, peanut butters and red wines (King et al., 2006). Rocket and green mustard leaves showed from 33 to 84 µg/g dw of *trans*-resveratrol, which was up to 4 times higher than the levels observed in some cultivars of berries which ranged between 18 to 50 µg/g dw according to previous studies (Sebastià et al., 2017; Shrikanta et al., 2015). Furthermore in grapes often considered the most abundant source of *trans*-resveratrol, mean levels ranged from 65 to 328 µg/g dw (Fontana et al., 2017; Shi et al., 2003; Vicenzi et al., 2013). Other good sources of *trans*-resveratrol are peanuts (*Arachis hypogaea* L.), whose leaves ranged from 0.02 to 1.79 µg/g dw (Meredith & Alfred, 2003; Sales & Resurreccion, 2009). However, the levels of resveratrol in processed products derived from peanuts can reach up to 5 µg/g dw (Sobolev & Cole, 1999). These data suggested that Brassicaceae species could be considered good food sources of *trans*-resveratrol compared with blueberries and grapes. Due to the absence of any report of *trans*-resveratrol in these species, a confirmation was done for its presence using gas chromatography-mass spectrometry (GC-MS) using a previously reported method, with some modifications according to the detector used (Montes et al., 2010). It can be observed in Figure 1, the mass spectra obtained after the analysis of a commercial standard sample of *trans*-resveratrol and the sample of rocket

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(given as an example) are similar in terms of the observed fragments and the distribution of their intensities.

These results indicated that rocket and green mustard are sources of *trans*-resveratrol. Future studies involving *trans*-resveratrol bioavailability as well as studies related to absorption and bioavailability will be needed to understand the physiological processes after consumption. These processes depend mainly on the food matrix and would be important to elucidate which is the best *trans*-resveratrol dietary source.

3.2.Variation in antioxidant capacity among Brassicaceae species

Antioxidant effects measured as an antioxidant (β-carotene bleaching method), antiradical (DPPH or ABTS bleaching methods) and reducing (FRAP) activities were detected in all aqueous vegetable extracts analyzed (Figure 2). Watercress and green mustard were the strongest antioxidant vegetables analyzed; cauliflower and Brussels sprouts were the weakest. These results are consistent with Soengas et al. (2011), who determined the antioxidant strength of 6 *Brassica* vegetables using FRAP and DPPH and the relative order for broccoli, cabbage, and cauliflower was the same as reported here. These three species also resulted in the weakest antioxidants among the 6 *Brassica* species in that study. Upadhyay et al. (2016) measured AOA in *Brassica oleracea* species and observed an antioxidant strength in decreasing order as red cabbage > green cabbage > broccoli > cauliflower, which is similar to these results despite working with other AOA methodology. Mean antiradical activities, measured by DPPH, ABTS, and FRAP methods, varied more than 12-fold and ranged from 7.1 to 89.2 AOA/100 mg dw for radish and watercress, respectively. In addition, these results are consistent with Sikora et al. (2008) who found an antioxidant ranking headed by Brussel sprouts, broccoli, and cauliflower using the DPPH assay.

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Mean antioxidant activities values, measured using the β-carotene bleaching assay, varied more than 9-fold and ranged from 8.6 to 78.4 AOA/100 mg dw in Brussel sprouts and rocket, respectively. β-carotene bleaching assay results are consistent with CORFO-Chile (2015), which measured the AOA using the oxygen radical absorbance capacity (ORAC) assay and the total phenolic content in these vegetables. This database includes a study of 50 vegetables, among them, rocket showed the highest activity.

Several authors have compared the antioxidant activities in *Brassica* species (Li et al., 2018; Mizgier et al., 2016; Murador et al., 2016; Podsędek et al., 2006; Sikora et al., 2008; Zieliński et al., 2007), but to date, the 9 species have not been studied using the 4 tests used in this study. The assay of β-carotene bleaching in the coupled oxidation with linoleic acid is a good indicator of the protective ability of the active compounds against the oxidative process induced by LOX (Chaillou & Nazareno, 2006). In this method, the lipid fraction is emulsified in micelles in an aqueous environment where the phenolic compounds are partitioned, while the oxidative enzyme is located in the interface. This system could constitute an acceptable model for most foods and even some biological systems (Prieto et al., 2012).

3.3.Relationships between antioxidant activities and their bioactive compounds content

Correlation analysis was done to explore the relationships between the content of bioactive compounds and the antioxidant activities of plant extracts (Table III and Supplementary data Table S1). Significant positive strong correlations were observed between allyl ITC, (+)-catechin, ferulic acid, quercetin-3-galactoside, (-)-epicatechin and kaempferol with FRAP, between caffeic acid, ferulic acid, quercetin-3-glucoside and myricetin with the β-carotene method, between allyl ITC, ferulic acid, pterostilbene and myricetin with ABTS.

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353 Medium correlations were observed between FRAP and gallic acid, syringic acid and
354 myricetin, between the β-carotene method with pterostilbene and kaempferol-3-glucoside,
355 between ABTS and (+)-catechin, ferulic acid and quercetin-3-glucoside and between DPPH
356 and (+)-catechin. Among ITC, only allyl ITC showed significant correlation with FRAP,
357 which also suggested that an electron transfer mechanism is involved instead of a hydrogen
358 transfer mechanism. Phenolic compounds apparently exert their antioxidant action in these
359 species by both mechanisms as was already proposed by Cartea et al. (2008) who reported
360 that antioxidant capacity of phenolic compounds is related to its chemical structure, and
361 they had an important role in neutralizing reactive oxygen species, quenching singlet and
362 triplet oxygen, or decomposing peroxides. Total phenolic compounds were only correlated
363 significantly with ABTS suggesting that antiradical mechanisms of quenching of ROS are
364 more effective than reducing mechanism in these species. Total ITC content was negatively
365 correlated with DPPH and β-carotene assays.

366 **3.4. PCA**

367 Supervised PCA was applied to the whole data set of 9 Brassicaceae species. The
368 dimensionality of the data was reduced to 2 uncorrelated principal components (PC), PC1
369 and PC2, accounting for 69.9% of the observed variation. The loading, eigenvalues, and
370 percentage of cumulative variance are shown in Table IV. PC1 was positively correlated
371 with *trans*-resveratrol, quercetin-3-glucoside, caffeic acid, kaempferol-3-glucoside, and
372 caftaric acid and negatively with indol-3-carbinol, pterostilbene, and allyl ITC. PC2 was
373 mainly correlated with the 4 methodologies of antioxidant and antiradical activities and
374 with quercetin-3-galactoside, erucin, and sulforaphane. The variation of the data is
375 explained mainly by phenolic compounds such as *trans*-resveratrol, quercetin-3-glucoside,
376 caffeic acid, kaempferol-3-glucoside and caftaric content; and by antioxidant activities

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measured using ABTS and FRAP. The graphic representation of the scores and loadings in Figure 3, show a separation of the species. Rocket is located in the medium right side of the plot, which is characterized by high phenolic content (mainly phenolic acids and flavonoids). White cabbage, broccoli, cauliflower, and Brussel sprouts are located in the bottom left side of the plot characterized by high contents of some isothiocyanates such as of indol-3-carbinol and erucin. Red cabbage, green mustard, and radish are located in the upper right side of the plot, characterized by high allyl ITC, ferulic acid and quercetin-3-galactoside content. Finally, watercress is located in the upper right side of the plot, which is characterized by high antioxidant and antiradical activity and phenolic compounds content (mainly quercetin-3-glucoside and caffeic acid). PCA showed the strong correlations observed between ABTS, DPPH, and FRAP with some phenolic compounds and to a lesser extent with ITC. Moreover, the strongest antioxidant species are located in opposite quadrants of the plots (CP1) suggesting that both, phenolic and sulfur compounds, found in these species contribute to these properties.

Based on the PCA and the correlation analysis, caffeic acid, ferulic acid, (-)-epicatechin, quercetin-3-glucoside, myricetin, and kaempferol are the main antioxidants found in these species. Watercress had the strongest antioxidant activity, of all the species. Green mustard was positioned second in this ranking for antioxidant strength, and its phenolic profile was headed by (-)-epicatechin. When the phenolic profile of the species that evidenced lesser antioxidant activity including cauliflower and broccoli were considered, (-)-epicatechin was also the main compound found, although in lower concentrations compared with green mustard. This suggested that the antioxidant potential of each species was not determined by only one compound, but rather by the interaction among different compounds. Allyl ITC was the most closely related to the antioxidant activities studied here. The iron-reducing

401 capacity suggested that the ITC antiradical mechanism could be an electron transfer in
402 neutral pH and aqueous media. The high ITC content found in rocket and watercress could
403 explain the high activity observed in these species. Some authors have proposed that the
404 sulfur atom in the methylation group present in the side chain of some ITC can act as an
405 electron donor, switching from a reduced form (the sulfide group CH_3-S), to an oxidized
406 form (the sulphinyl group CH_3–S=O); thus, this generates redox couples (e.g.,
407 erucin/sulforaphane in rocket) (Barillari et al., 2005; Papi et al., 2008).
408 PCA and correlation analysis suggested that both groups of compounds, phenolic and sulfur
409 ones, are associated with ARA and AOA. It is possible that the Brassicaceae antioxidant
410 capacity could be explained by synergistic effects among different compounds.
411 All the samples assayed showed a strong antiradical behavior, mainly using the electron
412 donor capacity to reduce species, rather than the mechanism of hydrogen atom transfer.
413 This can be explained considering that some ITC may act as electron donors (Barillari et
414 al., 2005).
415

416 **4. CONCLUSIONS**

417 Detailed aspects of antioxidant capacity have been shown, and it was possible to find a high
418 correlation between allyl ITC and ABTS and FRAP protection, as well as between
419 individual phenolic compound contents and DPPH and ABTS for Brassicaceae species.
420 These results allowed proposing that a hydrogen transfer mechanism was the main
421 antioxidant mechanism involved for cruciferous phenolic compounds and electron transfer
422 mechanism for cruciferous sulfur compounds.

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Moreover, several phenolic compounds and the main isothiocyanates for each species were described, including the presence of *trans*-resveratrol in all species. The levels observed in some samples are promising from a nutritional point of view.

Watercress and green mustard were the strongest antioxidant species, being the most promising vegetable of this family for their potential functional activities.

Correlation analysis suggested that both sulfur and phenolic compounds contribute to Brassicaceae antioxidant effects to different extents. Future studies that address the behaviour of each compound individually and the combinations would be interesting to elucidate the possible interactions between compounds and the possible effect of the matrix of each food.

5. CONFLICT OF INTERESTS

The authors confirm that they have no conflicts of interest with respect to the study described in this manuscript.

6. FUNDING

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Table I. Isothiocyanate concentration determined in the Brassicaceae species.

Vegetable	Sulforaphane	Indol-3-Carbinol	Allyl ITC	Erucin	Total ITC	Dry weight
Broccoli	260 ± 10	55 ± 2	98± 7	12 ± 1	430 ± 10	14.0 ± 0.1
White cabbage	10.1 ± 0.3	26 ± 2	8.9 ± 0.5	ND	45 ± 1	16.0 ± 0.1
Red cabbage	4.2 ± 0.5	18 ± 1	77 ± 1	ND	99.0 ± 0.3	8.0 ± 0.1
Brussels sprouts	2.6 ± 0.3	70 ± 2	12.0 ± 0.2	ND	85 ± 1	11.0± 0.1
Radishes	16 ± 1	18.5 ± 0.5	70 ± 3	ND	110 ± 20	5.0 ± 0.3
Watercress	ND	41 ± 2	88.0 ± 0.5	ND	130 ± 10	8.0 ± 0.1
Rocket	110 ± 20	ND	59 ±1	ND	170± 10	8.20 ± 0.02
Cauliflower	ND	24 ± 1	6.3 ± 0.4	ND	31.0 ± 0.5	9.80 ± 0.04
Green mustard	ND	11 ± 1	90 ± 2	ND	100 ± 10	7.09 ± 0.02

Results are expressed as mean (µg/g dw) ± SD for total and individual ITC and for dry weight as g dw/100 g fw. ND: non-detected means the level of the compound is under the limit of detection of the technique (Limit of quantification of the methodology used: SF=0.3; I3C=1.6; AITC=2.7; ER=7.4 µg/g dw).

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601 **Table II.** Phenolic compounds content determined in the Brassicaceae species.

Vegetable	Gallic acid	Procyanidin B1	(+)-Catechin	Caffeic acid	p-Coumaric acid	Ferulic acid	*Trans*-resveratrol	Pteroestilbene	Quercetin-3-galactoside	Quercetin-3-glucoside
Broccoli	4.5 ± 1.1	ND	ND	2.6 ± 0.5	ND	1.72 ± 0.01	0.88 ± 0.01	0.7 ± 0.1	ND	ND
Brussels sprouts	3.2 ± 2.2	34 ± 2	35 ± 1	12 ± 1	34 ± 3	2.1 ± 0.6	3.0 ± 0.5	0.7 ± 0.1	ND	ND
Cauliflower	16 ± 1	ND	47 ± 6	ND	33 ± 1	14 ± 2	ND	1.1 ± 0.1	26 ± 10	ND
Green mustard	2.9 ± 0.4	ND	ND	26 ± 3	3.2 ± 1.4	20 ± 3	33 ± 4	ND	76 ± 2	8.2 ± 1.5
Radish	2.9 ± 0.3	2.2 ± 0.3 ($\times 10^3$)	ND	ND	15 ± 1	ND	9.0 ± 0.5	ND	ND	ND
Red cabbage	16 ± 2	ND	ND	ND	ND	21 ± 3	ND	ND	ND	ND
Rocket	9.8 ± 0.6	ND	110 ± 20	170 ± 30	ND	ND	84 ± 1	ND	0.7 ± 0.4	250 ± 50
Watercress	4.4 ± 1.3	ND	ND	55 ± 18	44 ± 27	54 ± 11	6.5 ± 0.3	ND	73 ± 3	170 ± 70
White cabbage	7.2 ± 0.7	ND	33 ± 8	12 ± 3	110 ± 10	11 ± 2	4.5 ± 0.5	ND	ND	ND
Vegetable	**Syringic acid**	**(-)-Epicatechin**	**Caftaric acid**	**Tyrosol**	**Polydatin**	**Myricetin**	**Quercetin**	**Kaempferol**	**(-)-Gallocatechin gallate**	**Total phenolics**

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Broccoli	ND	100 ± 10	ND	ND	ND	2.8 ± 0.1	ND	9.2 ± 0.2	43 ± 5	170 ± 20
Brussels sprouts	ND	ND	ND	ND	ND	ND	ND	ND	ND	120 ± 10
Cauliflower	7.7 ± 0.6	310 ± 40	ND	ND	ND	ND	ND	ND	12 ± 1	470 ± 70
Green mustard	31 ± 1	870 ± 20	4.32 ± 0.04	ND	ND	ND	ND	37 ± 1	ND	1.1 ± 0.2 (x 10^3)
Radish	ND	ND	ND	ND	ND	ND	ND	ND	40 ± 3	2.3 ± 0.5 (x 10^3)
Red cabbage	ND	ND	ND	ND	ND	5.9 ± 0.4	ND	ND	ND	40 ± 5
Rocket	8.5 ± 0.6	ND	13 ± 3	70 ± 5	14.3 ± 0.1	15 ± 2	16 ± 2	9.8 ± 8.9	ND	1.5 ± 0.2 (x 10^3)
Watercress	ND	ND	ND	ND	ND	53 ± 3	ND	ND	ND	550 ± 40
White cabbage	ND	25 ± 4	ND	15 ± 3	ND	4.7 ± 0.1	ND	ND	ND	220 ± 30

Values are expressed as mean µg/g dw ± SD. Limit of quantification of quantified compounds: gallic acid=0.1; procyanidin B1=0.5; (+)-catechin=0.25; caffeic acid=0.05; p-coumaric acid=0.05; ferulic acid=0.05; *trans*-resveratrol=0.1; quercetin-3-galactoside=0.1; quercetin-3-glucoside=0.25; syringic acid=0.1; (-)-epicatechin=0.25; caftaric acid=2.5; tyrosol=0.5; polydatin=0.1; myricetin=0.5; quercetin=0.5; kaempferol=0.25; (-)-gallocatechin gallate=0.5 µg/mL *Brassicaceae* extract.

606 **Table III.** Significative ($p<0.05$) pairwise correlation values (R) among antioxidant
607 activities, isothiocyanates, and phenolic compound contents.

Variable 1	Variable 2	R	p value
ABTS	DPPH	0.86	0.010
β-carotene	DPPH	0.51	0.006
FRAP	ABTS	0.41	0.034
Total phenolic compounds	ABTS	0.39	0.044

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627 **Table IV.** Loadings, eigenvalues and percentage of cumulative variance for the first two
628 principal components of the whole data set (above) for 9 Brassicaceae species and groups
629 of compounds (below).

Variables	CP 1	CP 2
trans-Resveratrol	0.33	-0.05
Quercetin-3-glucoside	0.32	0.11
Caffeic acid	0.34	-0.01
Kaempferol-3-glucoside	0.35	-0.08
Caftaric acid	0.33	-0.08
Tyrosol	0.33	-0.14
Polydatin	0.34	-0.12
Quercetin	0.34	-0.12
DPPH	0.02	0.43
ABTS	0.04	0.41
β-Carotene	0.20	0.29
FRAP	-0.04	0.26
Pteroestilbene	-0.14	-0.28
Ferulic acid	-0.03	0.40
Sulforaphane	0.03	-0.22
Indol-3-Carbinol	-0.19	-0.31
Allyl ITC	-0.08	0.05
Cumulative variance (%)	44.4	69.9

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638 **Figure legends:**

639 **Figure 1. Chromatograms and mass spectrum corresponding to *trans*-resveratrol**
640 **standard (left) and a Brassicaceae sample (rocket) containing quantifiable levels of**
641 ***trans*-resveratrol (right).**

642 **Figure 2. Antioxidant capacity of 9 Brassicaceae species determined using 4**
643 **methodologies.** Brassicaceae species are located according to their overall antioxidant
644 strength, but each analytical method can be visualized in a different color.

645 **Figure 3. Principal components analysis of Brassicaceae antioxidant properties and**
646 **phytochemical contents.** PCA was done with the whole data of phytochemical content and
647 antioxidant capacity for all species.

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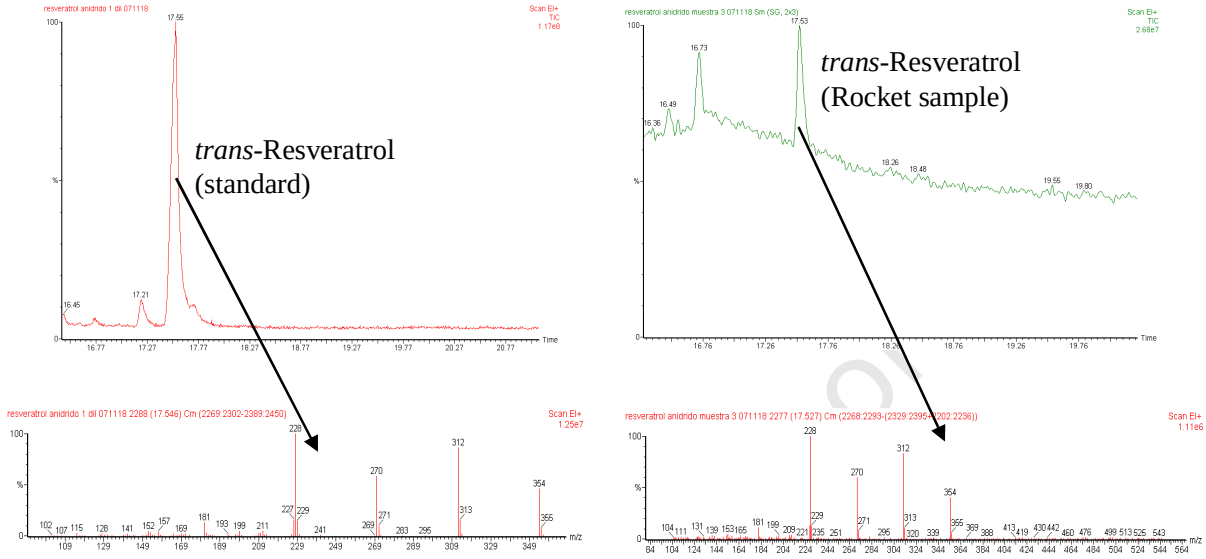
Figure 1



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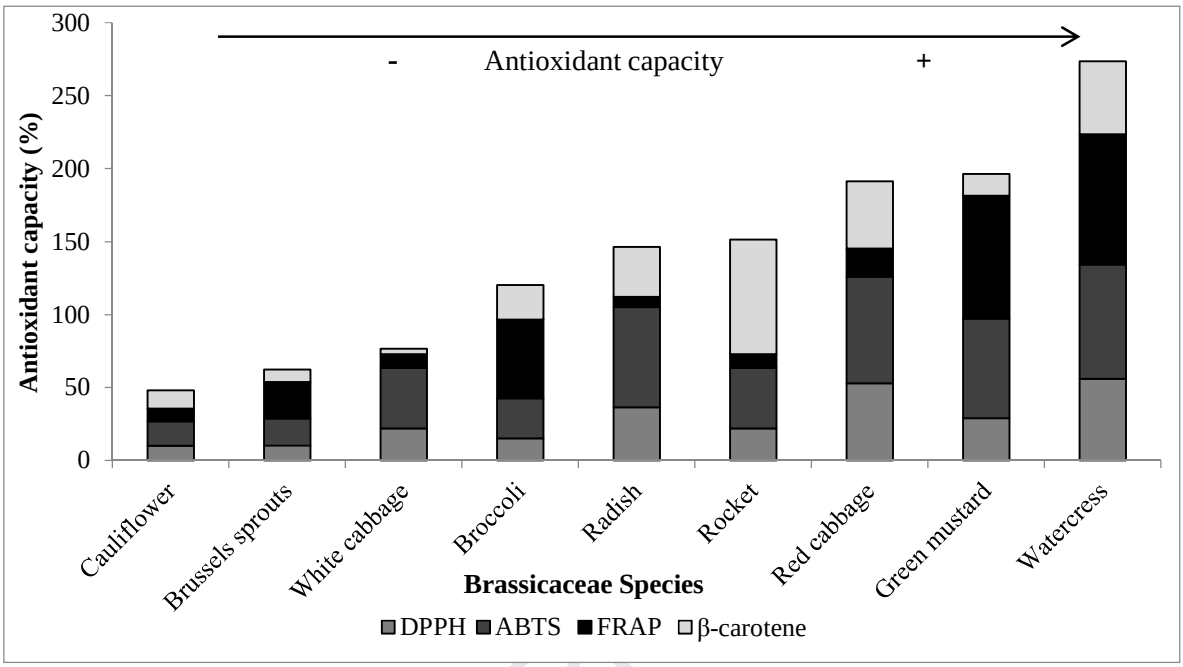
672 **Figure 2**



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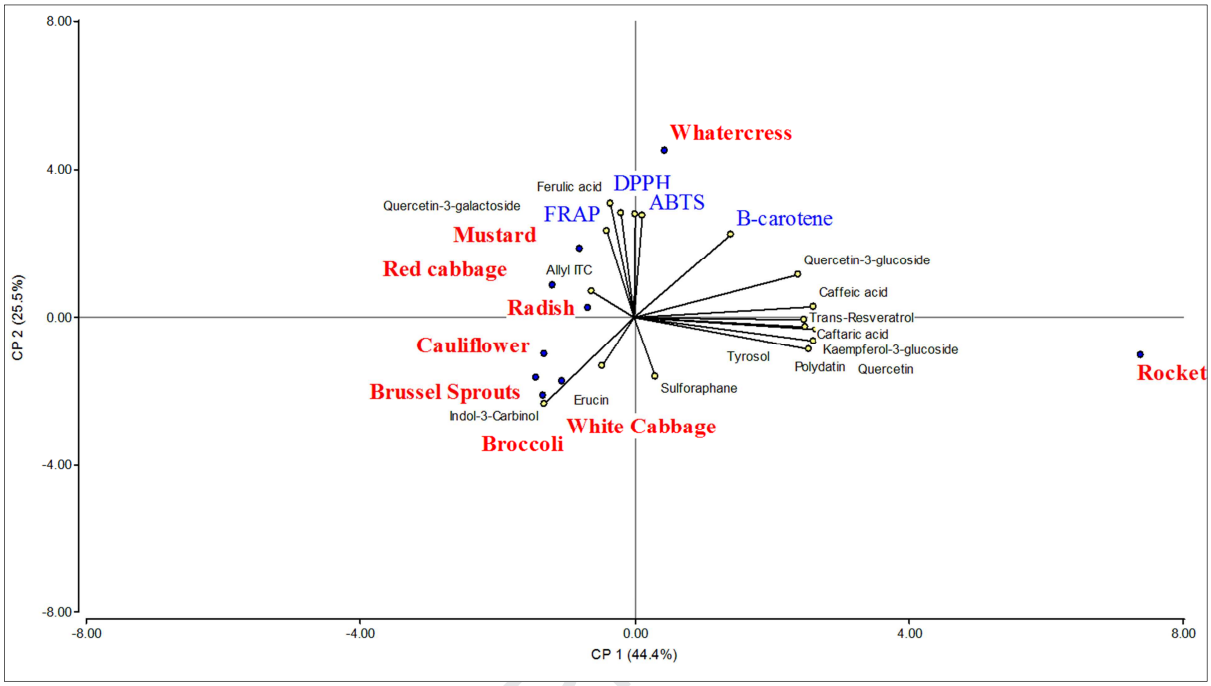
685 **Figure 3**



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Supplementary material:

Table S1. Significative ($p<0.05$) pairwise correlation values (R) among antioxidant activities, individual isothiocyanates and individual phenolic compounds.

Variable 1	Variable 2	R	P value
ABTS	DPPH	0.86	0.010
β-Carotene	DPPH	0.51	0.007
FRAP	ABTS	0.41	0.035
Dry matter	total ITC	0.45	0.018
Dry matter	DPPH	-0.39	0.045
Dry matter	ABTS	-0.39	0.047
Dry matter	FRAP	0.46	0.017
Sulforaphane	total ITC	0.97	0.000
Indol-3-Carbinol	total ITC	0.43	0.034
Indol-3-Carbinol	DPPH	-0.41	0.047
Indol-3-Carbinol	ABTS	-0.58	0.003
Indol-3-Carbinol	Dry matter	0.81	0.000
Indol-3-Carbinol	Sulforaphane	0.45	0.048
Allyl ITC	total ITC	0.61	0.001
Allyl ITC	DPPH	0.59	0.001
Allyl ITC	ABTS	0.7	0.000
Allyl ITC	β-Carotene	0.46	0.016
Allyl ITC	FRAP	0.65	0.000
Allyl ITC	Sulforaphane	0.55	0.007
Erucin	total ITC	0.93	0.000
Erucin	Dry matter	0.47	0.013
Erucin	Sulforaphane	0.92	0.000
Erucin	Indol-3-Carbinol	0.43	0.035
Erucin	Allyl ITC	0.41	0.032
Gallic acid	FRAP	-0.38	0.048
Procyanidin B1	Dry matter	-0.5	0.007
(+)-Catechin	DPPH	-0.46	0.015
(+)-Catechin	ABTS	-0.47	0.013
(+)-Catechin	FRAP	-0.51	0.007
(+)-Catechin	Allyl ITC	-0.48	0.010
Caffeic acid	β-Carotene	0.55	0.003
Caffeic acid	(+)-Catechin	0.76	0.000
p-Coumaric acid	Allyl ITC	-0.48	0.011
Ferulic acid	DPPH	0.61	0.001
Ferulic acid	ABTS	0.49	0.010
Ferulic acid	β-Carotene	0.54	0.004
Ferulic acid	FRAP	0.61	0.001

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Ferulic acid	Sulforaphane	-0.48	0.020
Ferulic acid	(+)-Catechin	-0.38	0.049
trans-Resveratrol	Indol-3-Carbinol	-0.44	0.032
trans-Resveratrol	(+)-Catechin	0.72	0.000
trans-Resveratrol	Caffeic acid	0.92	0.000
Pteroestilbene	DPPH	-0.67	0.001
Pteroestilbene	ABTS	-0.67	0.000
Pteroestilbene	β-Carotene	-0.41	0.031
Pteroestilbene	Dry matter	0.44	0.022
Pteroestilbene	Indol-3-Carbinol	0.47	0.022
Pteroestilbene	Allyl ITC	-0.44	0.020
Quercetin-3-galactoside	ABTS	0.46	0.017
Quercetin-3-galactoside	FRAP	0.83	0.000
Quercetin-3-galactoside	Ferulic acid	0.75	0.000
Quercetin-3-glucoside	β-Carotene	0.77	0.000
Quercetin-3-glucoside	(+)-Catechin	0.57	0.002
Quercetin-3-glucoside	Caffeic acid	0.92	0.000
Quercetin-3-glucoside	*trans*-Resveratrol	0.72	0.000
Kaempferol-3-glucoside	β-Carotene	0.45	0.017
Kaempferol-3-glucoside	(+)-Catechin	0.82	0.000
Kaempferol-3-glucoside	Caffeic acid	0.96	0.000
Kaempferol-3-glucoside	*trans*-Resveratrol	0.91	0.000
Kaempferol-3-glucoside	Quercetin-3-glucoside	0.85	0.000
Syringic acid	FRAP	0.48	0.012
Syringic acid	Indol-3-Carbinol	-0.47	0.021
Syringic acid	*trans*-Resveratrol	0.44	0.022
Syringic acid	Quercetin-3-galactoside	0.61	0.001
(-)-epicatechin	FRAP	0.54	0.004
(-)-epicatechin	Indol-3-Carbinol	-0.44	0.031
(-)-epicatechin	Quercetin-3-galactoside	0.65	0.000
(-)-epicatechin	Syringic acid	0.94	0.000
Caftaric acid	Indol-3-Carbinol	-0.42	0.039
Caftaric acid	(+)-Catechin	0.75	0.000
Caftaric acid	Caffeic acid	0.91	0.000
Caftaric acid	*trans*-Resveratrol	0.98	0.000
Caftaric acid	Quercetin-3-glucoside	0.73	0.000
Caftaric acid	Kaempferol-3-glucoside	0.94	0.000
Caftaric acid	Syringic acid	0.41	0.034
Tyrosol	(+)-Catechin	0.87	0.000
Tyrosol	Caffeic acid	0.92	0.000
Tyrosol	*trans*-Resveratrol	0.9	0.000
Tyrosol	Quercetin-3-glucoside	0.73	0.000
Tyrosol	Kaempferol-3-glucoside	0.94	0.000

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Tyrosol	Caftaric acid	0.89	0.000
Polydatin	(+)-Catechin	0.85	0.000
Polydatin	Caffeic acid	0.94	0.000
Polydatin	*trans*-Resveratrol	0.93	0.000
Polydatin	Quercetin-3-glucoside	0.77	0.000
Polydatin	Kaempferol-3-glucoside	0.98	0.000
Polydatin	Caftaric acid	0.93	0.000
Polydatin	Tyrosol	0.98	0.000
Myricetin	DPPH	0.55	0.003
Myricetin	β-Carotene	0.9	0.000
Myricetin	FRAP	0.46	0.015
Myricetin	Caffeic acid	0.39	0.042
Myricetin	Ferulic acid	0.78	0.000
Myricetin	Quercetin-3-galactoside	0.51	0.006
Myricetin	Quercetin-3-glucoside	0.66	0.000
Quercetin	(+)-Catechin	0.84	0.000
Quercetin	Caffeic acid	0.93	0.000
Quercetin	*trans*-Resveratrol	0.92	0.000
Quercetin	Quercetin-3-glucoside	0.79	0.000
Quercetin	Kaempferol-3-glucoside	0.99	0.000
Quercetin	Caftaric acid	0.95	0.000
Quercetin	Tyrosol	0.95	0.000
Quercetin	Polydatin	0.99	0.000
Kaempferol	FRAP	0.6	0.001
Kaempferol	Allyl ITC	0.44	0.023
Kaempferol	*trans*-Resveratrol	0.43	0.025
Kaempferol	Quercetin-3-galactoside	0.53	0.005
Kaempferol	Syringic acid	0.91	0.000
Kaempferol	(-)-Epicatechin	0.86	0.000
Kaempferol	Caftaric acid	0.43	0.024
(-)-Gallocatechin gallate	total ITC	0.6	0.001
(-)-Gallocatechin gallate	Sulforaphane	0.56	0.005
(-)-Gallocatechin gallate	Erucin	0.66	0.000
(-)-Gallocatechin gallate	Procyanidin B1	0.59	0.001
(-)-Gallocatechin gallate	Ferulic acid	-0.4	0.037
Total phenolic compounds	ABTS	0.39	0.044
Total phenolic compounds	Dry matter	-0.53	0.005
Total phenolic compounds	Indol-3-Carbinol	-0.46	0.025
Total phenolic compounds	Procyanidin B1	0.77	0.000
Total phenolic compounds	*trans*-Resveratrol	0.5	0.008
Total phenolic compounds	Pteroestilbene	-0.39	0.044
Total phenolic compounds	Caftaric acid	0.43	0.026

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HIGHLIGHTS

- Bioactive phytochemicals in 9 cruciferous species were investigated.
- Twenty five phytochemicals were quantified.
- All species analyzed show antioxidant activity.
- Each cruciferous vegetable had its own phenolic and sulphur compound profile.
- Watercress and green mustard were the strongest antioxidant cruciferous.
- Cruciferous vegetables are a sources of trans-resveratrol.

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Dear Editor-in-Chief
Food Bioscience

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome. We confirm that the manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed. We further confirm that the order of authors listed in the manuscript has been approved by all of us.

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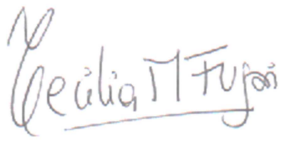

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