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Comparative study of the phytochemical and mineral composition of fresh and cooked broccolini

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Abstract

Broccolini is originated from crossing the regular broccoli with Chinese kale. Consequently, it has similar properties to these vegetables, but other very particular characteristics. Its consumption has increased in the last few years and, consequently, there have been some studies related to its quality parameters and the influence of different cooking methods. Nevertheless, changes on its phenolic composition and mineral content originated by its cooking have not been investigated in-depth so far. Here we report the phytochemical profile of broccolini before and after boiling, steaming, and griddling cooking treatments. The mineral content and phytochemicals were assessed by inductively coupled plasma-mass spectrometry (ICP-MS) and high performance liquid chromatography-mass spectrometry (HPLC-MS), respectively. The main phenolics (mainly hydroxycinnamic acid derivatives from caffeic, coumaric, ferulic and sinapic acids) were quantified. Three oxylipins, three flavonoid glycosides and the glucosinolate glucobrassicin were also identified. ABTS and DPPH assays were also used as screening methods to assess the antioxidant potential of broccolini. A significant loss of the phenolic compounds and a reduction of the antioxidant activity were observed after the three cooking methods. Clear disadvantages were detected when broccolini was boiled, namely high losses of phenolic acids and derivatives (70%). Steaming and griddling also led to a significant loss of phenolics (50%) from fresh broccolini. The mineral content of this vegetable after domestic cooking procedures is also reported for the first time, calculating the contribution of broccolini consumption to official daily recommendations.

Keywords: broccolini; phenolic profile; mineral content; cooking treatments; total individual phenolic content.

1. Introduction

Vegetables are well-known for being good sources of antioxidants, carotenoids, water-soluble vitamins, and minerals (Kaur & Kapoor, 2008). Among them, *Brassica* is a genus of herbaceous plants in the *Brassicaceae* family that includes a number of the world's most commonly cultivated vegetables. The progenitor species likely originated in the Mediterranean region; nevertheless, *Brassica* species have been cultivated for centuries, adapted for worldwide production and extensively crossed and hybridized. In this genus of plants, *Brassica oleracea* species, that are green fleshy vegetables (e.g., broccoli, cauliflower, and Brussels sprouts), are very common cultivars (Fahey, 2016). *Brassica oleracea* vegetables are known to contain several phytochemicals such as glucosinolates-isothiocyanates (Cartea & Velasco, 2008; Sharma, Sharma, Yadav & Singh, 2016), phenolics (Singh, Upadhyay, Prasad, Bahadur, & Rai, 2007) and carotenoids (Park et al., 2012), which possess both antioxidant and anticarcinogenic properties. They are also an excellent source of essential minerals (Ca, Mg, Na, K, Fe, Zn, etc.) (Moreno, Carvajal, López-Berenguer, & García-Viguera, 2006).

The strong antioxidant properties of flavonoids and other phenolics found in *Brassica oleracea* vegetables are well established and, therefore, their regular consumption could be very useful to promote reduction of oxidative stress (Ahmed & Ali, 2013), and decrease the risk of gastric cardia and esophageal adenocarcinomas (Steevens, Schouten, Goldbohm, & van den Brandt, 2011) and colon and colorectal cancers (Wu et al., 2013) in humans. In addition to free radical scavenging activity (Singh et al., 2007), their phenolic compounds possess different biological activities, being the most important capillary protective effect, antioxidant activity and inhibitory effect in various stages of tumors (Podsędek, 2007). The phenolic content and other

antioxidant phytochemicals of *Brassica* vegetables vary according to factors such as genetic and environmental influences, harvesting stage, post-harvest processing and differences in methods of estimation (Singh et al., 2007; Vallejo, Tomás-Barberán, & García-Viguera, 2003). The significant differences found in the antioxidant phytochemicals within the subspecies indicate that the potential health benefits also depend on the genotype. Nowadays, it is widely known that the most important phenolic compounds in *Brassica* species are flavonoids, and especially flavonols (Cartea, Francisco, Soengas, & Velasco, 2010), which are most commonly found as *O*-glycosides.

In the last years, the phenolic profile of broccoli has been one of the most studied in *Brassica oleracea* species, since it is a very popular vegetable, particularly in the Western Europe countries and in the USA. Broccoli is a source of flavonol derivatives, mainly sophorosides of quercetin and kaempferol, and hydroxycinnamic acid derivatives (Price, Casuscelli, Colquhoun, & Rhodes, 1998).

Broccolini is a new natural hybrid between gai lan (*Brassica oleracea* var. *alboglabra*), also called Chinese broccoli or Chinese kale, and conventional broccoli (*Brassica oleracea*, Italica group). This green vegetable is similar to broccoli, but with completely edible small florets and long and thin stems, and with a milder and sweeter taste (Martínez-Hernández, Gómez, Pradas, Artés, & Artés-Hernández, 2011). Some hydroxycinnamic acids and derivatives (neochlorogenic acid, chlorogenic acid, 1,2-disinapoylgentibiose, 1-sinapoyl-2-feruloylgentibiose, 1,2,2'-trisinapoylgentibiose, 1,2'-disinapoyl-2-feruloylgentibiose, 1-sinapoyl-2,2'-diferuloylgentibiose and 1,2-diferuloylgentibiose) have been identified in methanol extracts of broccolini (Martínez-Hernández et al., 2011). The main hydroxycinnamic acids (caffeic, *p*-coumaric, ferulic and sinapic) were also isolated from broccolini (Lee, Boyce, & Breadmore, 2011).

Nevertheless, to date, a complete study of the phenolic profile of broccolini has not been carried out. Mineral composition of broccolini has also been reported and compared to that of broccoli (Martínez-Hernández, Gómez, Artés, & Artés-Hernández, 2013a).

Broccolini is most frequently consumed slightly cooked, although it can also be consumed raw. Cooking of many vegetables originates physicochemical, sensory and nutritional changes, and the levels of bioactive compounds can be altered (Moreno, Carvajal, López-Berenguer, & García-Viguera, 2006), mainly due to thermal degradation and leakage in the cooking fluids. The direct effect of domestic processing on phenolic content and their potential antioxidant activity can be dramatic (Gawlik-Dziki, 2008; Gil, Ferreres, & Tomás-Barberán, 1999). On the contrary, in some cases cooking treatments increase the release of phenolic compounds from plant tissues (Gil, Tomás-Barberán, Hess-Pierce, Holcroft, & Kader, 2000; Roy, Juneja, Isobe, & Tsushida, 2009). The impact of cooking treatments on the phenolic compounds mainly depends on the type of vegetable and cooking treatment used (Jiménez-Monreal, García-Diz, Martínez-Tomé, Mariscal, & Murcia, 2009; Wachtel-Galor, Wong, & Benzie, 2008). Martínez Hernández *et al*. (Martínez-Hernández et al., 2013b) studied the influence of several cooking treatments in some quality parameters of broccolini, including total phenolic content and antioxidant capacity. Nevertheless, the influence of cooking on the content in phenolic individual compounds has not been evaluated to date.

The main goals of this work were to assess the phenolic and mineral composition of broccolini and study their changes after cooking processes (boiling, steaming and griddling). In addition, we carried out two assays (DPPH and ABTS) to screen the *in vitro* antioxidant capacity of this vegetable (Granato et al., 2018).

2. Experimental

2.1. Chemicals and reagents

All reagents and standards were of analytical reagent grade. We purchased 3-*O*-caffeoylquinic acid (CQA), 4-*O*-CQA, 5-*O*-CQA, ferulic acid, quercetin, vicenin-2, and rutin from Sigma-Aldrich (St. Louis, MO, USA), and prepared individual stock solutions in methanol (MeOH, HPLC grade; Sigma). LC-MS grade acetonitrile (CH_3CN, 99%; LabScan; Dublin, Ireland) and ultrapure water (Milli-Q Waters purification system; Millipore; Milford, MA, USA) were also used. Sodium chloride (NaCl, 99%), sodium hydrogen carbonate ($NaHCO_3$, > 99 %), hydrochloric acid (HCl, 37%), potassium acetate (CH_3COOK, 99 %) and ethanol (EtOH, 96%) were purchased from Panreac (Madrid, Spain). Magnesium chloride ($MgCl_2$, > 99%), 2,2′-azinobis(3-ethylbenzthiazoline-6- sulfonic acid) (ABTS, ≥ 98%), potassium persulfate ($K_2S_2O_8$, > 99%), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox, 97%), and 2,2-diphenyl-1-picrylhydrazyl (DPPH, 95%) were obtained from Sigma-Aldrich.

For ICP-MS analysis, analytical reagent grade nitric acid (HNO_3, 65%; Sigma-Aldrich) was additionally cleaned by sub-boiling distillation with a still built up with components from Savillex (ISC Science; Oviedo, Spain). Hydrogen peroxide solution (H_2O_2, ultratrace analysis) was purchased from Sigma-Aldrich. All calibration standard solutions were prepared from 100 μg mL^{-1} multi-element standard solutions from SCP Science (Paris, France) and ISC Science (Oviedo, Spain) by dilution with 10 % (v/v) sub-boiled HNO_3 in ultrapure deionized water. Certified reference materials "cranberry fruit SRM-3281" and "cabbage powder BCR-679" were obtained from the National Institute of Standards and Technology (NIST) and the Institute for Reference Materials and Measurements (IRMM), respectively.

2.2. Sample preparation

Broccolini samples (1000 g) were purchased in a Spanish local supermarket, stored at 4ºC and used within 24 h. It was harvested in Murcia (Latitude: 37° 59' 13.34" N, Longitude: -1° 07' 48.14" W; Spain) on April 2018. They were mixed into the same pool, washed with Milli-Q water and cut into the edible portions (approximately 16-cm-long pieces). Twenty inflorescences were randomly selected for analytical purposes and divided into five different parts. Two portions of fresh broccolini were used to check its initial mineral and phenolic content, respectively, and the other three portions were submitted to different cooking treatments: (a) steaming: the vegetable was cooked in a traditional stainless steel steamer (three pieces: a pot, a steamer basket, and a lid) for 5 min; (b) boiling: broccolini was boiled for 3 min into a 3 L stainless steel pot containing 100 g of fresh vegetable and 150 mL of tap water; (c) griddling: the vegetable was cooked for 7 min, without oil addition, on an electric griddle at 210 ºC (3,5 min per side). The cooking times were selected according to the instructions of the manufacturer of the broccolini samples in the packaging of the product. They agree with most of the cooking procedures for broccoli and broccolini reported in literature.

The humidity of all samples was measured with a moisture analyzer MA 50.R (RADWAG, Poland). Then, the samples were lyophilized (ModulyoD-23, Thermo Savant; Waltham, MA, USA), except a fresh portion that was subjected to ICP-MS analysis. Samples were stored at -20 ºC until analysis.

2.3 Extraction of phenolic compounds

Powdered samples (2.5 g) were extracted with 50 mL of MeOH in an ultrasonic bath (Bandelin Sonorex Digital 10P; Sigma-Aldrich; Madrid: Spain) at 35 Hz and 280 W for 60 min at room temperature (20-25 ºC). Extractions were performed in triplicate.

The methanolic solutions were filtered through Whatman No.1 filters and the solvent was evaporated under reduced pressure in a Hei-Vap Precision rotary evaporator (Heidolf; Schawabach; Germany) at 40 ºC. Dried extracts (DE) were stored at -20 ºC until analysis.

2.4. HPLC-DAD-MS analysis

DE extracts (5-10 mg) were re-dissolved in 1 mL of MeOH, filtered with 0.45 μm nylon filters, and 10 μL of sample was injected. The HPLC system was an Agilent Series 1100 with a G1315B diode array detector. The separation of the compounds was performed with a reversed phase Luna Omega Polar C_{18} analytical column of 150 x 3.0 mm and 5 μm particle size (Phenomenex). A Polar C_{18} Security Guard cartridge (Phenomenex) of 4 x 3.0 mm was also used. The HPLC instrument was connected to an ion trap mass spectrometer (Esquire 6000, Bruker Daltonics) with an electrospray interface. Chromatographic conditions have been previously detailed (Llorent-Martínez et al., 2018).

2.5. Antiradical activity

DE extracts were analyzed using procedures previously described (Spínola, Llorent-Martínez, Gouveia, & Castilho, 2014) for DPPH and ABTS radical scavenging assays.$DPPH^{\bullet}$ (2,2-diphenyl-1-picrylhydrazyl) is a free radical that is stabilized by virtue of the delocalization of its free electron over the entire molecule in such a manner that it does not dimerize, like most other free radicals. This delocalization determines the occurrence of a deep purple coloration, with absorption in alcoholic solution at 516 nm. On mixing DPPH solution with a hydrogen atom donor, the reduced form is produced with concomitant loss of the purple coloration. The ABTS (2,2'-azino-bis (3-

ethylbenzthiazoline-6-sulphonic acid) method for free radical reduction is based on electron transfer between the antioxidant agent and the bluish-green cation radical $ABTS^{\bullet+}$, which is formed by the loss of an electron by the nitrogen atom of ABTS. This reaction can be monitored at 734 nm as a decrease in the intensity of the absorbance. Radical scavenging results were expressed as g of Trolox equivalent (TE) per 100 g of DE.

2.6. Microwave digestion and ICP-MS analysis

Samples were digested using a MARSXpress (CEM; Gilson; Madrid, Spain) microwave digestion system with 50 mL PFA vessels designed for temperatures up to 260 °C. Digestion was carried out by adding 7 mL sub-boiling HNO_3 and 1 mL H_2O_2 to 0.25 mg fresh sample (powdered). The temperature was increased to 180 ºC in 20 min and kept at 180 ºC for 2 min, with 2 intermediate stages of 100 ºC and 150 ºC. After cooling to room temperature, the digestion solutions were transferred into metal-free plastic containers and diluted to 50 mL with ultrapure water. The accuracy of the method was assessed by analyzing the two certified reference materials above described.

An Agilent 7900 (Agilent Technologies, CA, USA) equipped with a low flow concentric nebulizer (0.2 mL min^{-1}) and high efficiency "MicroMist", a Peltier-cooled quartz spray chamber (Scott type) and a quartz torch (2.5 mm i.d.) were used. The instrument performs AutoTuning and is equipped with a discrete sampling system (ISIS3), a sample introduction system (UHMI), an Autosampler (SPS4), a radio frequency generator (27 MHz), ion lenses off-axis, an octopolar collision-reaction cell

(ORS system), a high-frequency quadrupole (3 MHz) and a secondary electron multiplier detector. Operating conditions are shown in Table S1.

2.7. Statistical analysis

Statistical analysis was performed using SPSS Statistics software v.22 (IBM SPSS Statistics for Windows, IBM Corp., USA). Data of all analyses, in triplicate, are expressed as mean ± standard deviation. A one-way analysis of variance (ANOVA) with Tukey's HSD post-hoc test ($p < 0.05$) was used to look for statistically significant differences among results.

3. Results and discussion

The characterization of phytochemicals was carried out by HPLC-ESI-MS^n using the negative ion mode. The base peak chromatogram is shown in Figure 1. The compounds identified in fresh and cooked broccolini, as well as the concentrations of the main phenolics, are reported in Tables 1 and 2.

FIGURE 1, TABLE 1, TABLE 2

3.1. Phytochemical characterization

Most of the detected compounds corresponded to hydroxycinnamic acids and derivatives, and flavonoid derivatives. This phenolic profile is similar to that described for *Brassica* vegetables (Li et al., 2018). Compound **2** was characterized as a caffeoylquinic acid, based on the deprotonated molecular ion at *m/z* 353 and base peak at *m/z* 191. Compounds **3** and **9** were identified as 5-*O*-, and 3-*O*- caffeoylquinic acids, respectively, by comparison with analytical standards. Compound **4** exhibited the fragmentation 179/135, typical of caffeic acid, so it was tentatively characterized as a derivative. Compounds **5** and **8**, with $[M-H]^-$ at m/z 325, suffered the neutral loss of 162

Da (hexoside), yielding coumaric acid at *m/z* 163 (fragment ion at *m/z* 119), in agreement with coumaric acid-*O*-hexoside. Compound **7**, with $[M-H]^-$ at *m/z* 337, presented base peak at *m/z* 163, indicative of 3-*p*-coumaroylquinic acid (Clifford, Johnston, Knight, & Kuhnert, 2003). Compound **10** presented deprotonated molecular ion at *m/z* 355 and, after the neutral loss of 162 Da, displayed ferulic acid at *m/z* 193 (fragment ions at *m/z* 149 and 134), which corresponded to ferulic acid-*O*-hexoside (Spínola, Llorent-Martínez, Gouveia-Figueira, & Castilho, 2016).

Sinapic acid derivatives were also identified in the broccolini extracts, which is usual in *Brassica* species (Ferreres et al., 2009; Harbaum-Piayda, Palani, & Schwarz, 2016). Compound **11**, with $[M-H]^-$ at *m/z* 385, suffered the neutral loss of 162 Da to yield sinapic acid at *m/z* 223 (fragment ions at *m/z* 208, 179, and 164), corresponding to sinapoylglycoside (sinapic acid-*O*-hexoside). Compounds **15** and **16** were characterized as disinapoyldiglycoside and sinapoylferuloyldiglycoside, respectively, based on the fragmentation patterns previously reported in *Brassica campestris* (Harbaum et al., 2007). Specifically, compounds **15** and **16** may be identified as disinapoylgentiobiose and sinapoylferuloylgentiobiose, respectively, since these hydroxycinnamic derivatives are usually found in *Brassica* vegetables (Neugart et al., 2012; Zietz et al., 2010).

Only three flavonoid glycosides were characterized. Compounds **13** and **14** were kaempferol hexosides, identified by the neutral losses of 162 Da (hexoside) to yield the aglycone at *m/z* 285. On the other hand, compound **17** was tentatively characterized as a quercetin (*m/z* 301) derivative. Compound **6**, with deprotonated molecular ion at *m/z* 447 and base peak at *m/z* 259, was tentatively characterized as the indole glucosinolate glucobrassicin, originally identified in plants of the *Raphanus* and *Brassica* genera (McDanell, McLean, Hanley, Heaney, & Fenwick, 1988). Based on bibliographic information (Jiménez-López, Ruiz-Medina, Ortega-Barrales, & Llorent-Martínez,

2017), compound **12** was characterized as the formate adduct of roseoside (drovomifoliol-*O*-glucoside), which has shown significant insulinomimetic activity along with significant antidiabetic potential (Patel, Prasad, Kumar, & Hemalatha, 2012). Finally, two of the main phenolics found in broccolini were identified as metabolites from unsaturated fatty acids by comparison with previous scientific literature. One of them, compound **18**, showed the deprotonated molecular ion at *m/z* 327, and MS^2 base peak ion at m/z 229. It was identified as an oxo-dihydroxy-octadecenoic acid (oxo-DHODE) (Park et al., 2016; Van Hoyweghen, De Bosscher, Haegeman, Deforce, & Heyerick, 2014). Compound **19** showed a similar fragmentation pattern to that of compound **18**, being also identified as an oxo-DHODE. Another main phenolic, compound **20**, was identified as trihydroxy-octadecenoic acid, taking into account the deprotonated molecular ion at *m/z* value of 329, and its fragmentation pattern (Llorent-Martínez, Spínola, Gouveia, & Castilho, 2015; Park et al., 2016; Van Hoyweghen et al., 2014). These three compounds are called oxylipins, a group of biologically active compounds coming from the oxidative metabolism of polyunsaturated fatty acids. Oxylipins are known as inflammatory modulators, and they have antimicrobial properties (Howe & Schilmiller, 2002) and prevent cardiovascular diseases (Park et al., 2016). Increasing evidence indicates that the collective biological importance of these compounds in plants is comparable to that of the eicosanoid family of lipid mediators in animals (Howe & Schilmiller, 2002).

3.2. Quantitation of individual compounds

The quantification of the main compounds in the broccolini extracts was carried out by UV, using analytical standards of 5-*O*-caffeoylquinic acid (CQA), coumaric acid, ferulic acid, and kaempferol. Calibration curves were prepared in the range 0.2-50 µg

mL^{-1} in MeOH. UV chromatograms were registered at 350 nm for kaempferol and 320 nm for phenolic acids.

The most abundant compounds were individually quantified in fresh and cooked broccolini (Table 2), being mainly hydroxycinnamoyl derivatives. In fresh broccolini two cinnamate esters with quinic acid (5-*O*-caffeoylquinic acid and 3-*p*-coumaroylquinic acid) and two coumaric acid derivatives (coumaric acid hexoside and coumaric acid-*O*-hexoside) accounted for most of the quantified phenolics, 62 and 31 %, respectively. The rest (7%) was made up of sinapic acid derivatives (disinapoyldiglycoside and sinapoylferuloyldiglycoside). Total Individual Phenolic Content (TIPC) was defined as the sum of individually quantified compounds. Only two compounds (**7** and **8**) were quantified in all extracts, observing no significant differences in their concentrations. However, regarding TIPC, statistically significant differences were observed. Fresh samples had the highest TIPC, followed by steamed and griddled (no significant differences between both of them) and boiled, which presented the lowest TIPC. Therefore, as broccoli (Rothwell et al., 2015), broccolini is particularly sensitive to boiling cooking.

The results showed large differences among the three treatments in their influence on hydroxycinnamoyl derivative contents. Clear disadvantages were found when broccolini was boiled, namely high losses of cinnamate esters (78%), sinapic acid derivatives (100%) and coumaric acid derivatives (54%). This can be attributed to the leakage of phenolic compounds in the cooking water. The highest losses were observed for 5-*O*-caffeoylquinic acid and coumaric acid hexoside. Sinapic acid derivatives and 5-*O*-caffeoylquinic acid were completely lost in the steaming treatment. On the other hand, the steaming process had little effects, in terms of loss, on coumaric acid derivatives (16%). Regarding individual hydroxycinnamoyl derivatives,

sinapoylferuloyldiglycoside was not detected after cooking. However, 3-*p*-coumaroylquinic acid and coumaric acid-*O*-hexoside remained in the vegetable tissue. Different authors have studied the effect of cooking in vegetables, including broccolini. In general, authors agree that boiling produces losses in both individual and total phenolic contents (Gawlik-Dziki, 2008; Gliszczyńska-Świgło et al., 2006; Zhang & Hamauzu, 2004), in-line with our results. However, we found losses of phenolics during steaming in broccolini, which is in disagreement with the results observed by other authors in broccoli (Gliszczyńska-Świgło et al., 2006; Martínez-Hernández et al., 2013b). It is necessary to take into account that each food has a characteristic profile of polyphenols and that, in addition, the chemical structure of phenolics has a great influence on the extent of their loss after cooking processes, since it determines their molecular size, polarity and solubility. This may explain, at least partially, the differences found between foods in the same cooking process as well as the differences found between individual polyphenols for the same food and cooking process. In fact, for some phenolics as 5-*O*-caffeoylquinic acid some authors have reported that losses often varied more with food than with the cooking process (Rothwell, 2015). Variability is caused not only by food matrix and phenolic structure, but by slight differences during the cooking procedure such as amounts of sample and water, cooking time, temperature and piece size, as well as the maturity stage and the location of collection of samples.

The flavonoid kaempferol-*O*-hexoside was not detected in fresh broccolini but in the steamed vegetable. Some authors have reported that cooking treatments soften the cell walls and facilitate the extraction of bioactives such as polyphenols, originate modifications in the matrix or the inactivation of the polyphenol oxidases (Ferracane et al., 2008). This could explain the presence of kaempferol-*O*-hexoside in the steamed

broccolini. A similar effect of steaming on the level of flavonoids in broccoli and green beans was observed by other authors (Gliszczyńska-Świgło et al., 2006; Turkmen, Sari, & Velioglu, 2005). It was also reported that heat treatment causes the increase of free flavonols in tomato-based products (Stewart et al., 2000). Similarly, an increase of flavonoids in boiled broccoli and spinach was reported previously by Mazzeo et al. (Mazzeo et al., 2011). Finally, we can conclude that a greater quantity of phenolic compounds will be provided by the consumption of steamed or griddled broccolini as compared with boiled broccolini.

TABLE 2

3.3. Antiradical activity

Antioxidant activity was evaluated by ABTS and DPPH assays as the ability to scavenge free radicals generated in aqueous and lipophilic phases (Figure 2). There were significant differences for ABTS and DPPH results in all the extracts. Antiradical activities of the phenolic extracts, measured by ABTS test, were retained at 35.3%, 28.7%, and 40.4% for steaming, boiling and griddling cooking procedures, respectively. When DPPH test was used, the changes in antiradical activities showed a similar trend, retaining activities of 28.6%, 20.2%, and 35.7% for steaming, boiling and griddling cooking procedures, respectively. Results clearly show that the antiradical activity of cooked broccolini determined by both assays was significantly reduced when compared with the fresh vegetable. Moreover, the highest loss was observed after boiling, in agreement with the results observed for TIPC. These results support the fact that the higher the loss of phenolics, the higher the loss in antioxidant activity.

In general, different studies on broccoli have reported a loss of antioxidant activity after a boiling process (Gawlik-Dziki, 2008; Gliszczyńska-Świgło et al., 2006; Zhang &

Hamauzu, 2004). However, similarly to TIPC, some authors reported increased antioxidant capacity after a steaming process (Gliszczyńska-Świgło et al., 2006; Martínez-Hernández et al., 2013b). These authors attribute these increases to a cellular tissue disruption, releasing phytochemicals from the insoluble part of the vegetable. On the other hand, the observed variability in the antioxidant activity of cooked *Brassica* vegetables could be caused by the broad diversity of chemical compounds present in plant extracts (Lafarga et al., 2018). No matter the conclusions of the study – increased or reduced phenolic content – all reports agree in a close relationship between the amount of phenolics remaining after the cooking process and the observed antioxidant activity. However, the specific assays used for the evaluation of the antiradical/antioxidant activity are critical to perform and accurate comparison of the results obtained by different research groups.

FIGURE 2

3.4. Inorganic content

The mineral content and trace element levels were also determined in fresh broccolini and after the different cooking processes (Table 3). Significant differences between the levels of each element are shown in Table 3. The humidity values were 87.2%, 85.5%, 92.1 and 81.5% for the fresh, steamed, boiled and griddled vegetable, respectively. The following elements were below the method detection limit: Mo, Pb, Se, Sn, Ti, and Tl. Method detection limits are shown in Table S2 (Supplementary Material). It can be observed that the levels of toxic elements are very low: below detection limit or low concentrations that comply with current legislation. In the case of some minerals, the concentration in cooked broccolini is higher than in fresh samples; this can be attributed to the absorption of minerals from water and from the cooking

recipients. Data provided by the European Food Safety Authority indicate increases in the content of some minerals (Ca, Cu, Fe, Se) in broccoli, kale, Brussels sprouts, and cauliflowers (all *Brassica* species) after cooking processes (European Food Safety Authority (EFSA), n.d.).

We used the recommended Dietary Reference Values (DRV) for minerals (adults) published by the European Union (European Food Safety Authority (EFSA), 2017) to check the contribution provided by the consumption of broccolini. As the mineral contents may vary depending on the cooking process, we used contents of fresh broccolini. In addition, a portion of 100 g was supposed for these calculations. Results are summarized in Table 4. It can be observed that Ca, Cu, Fe, Mg, and Zn would contribute less than 10% to the recommended daily ingestion, whereas P (11.6 %), and K (11.5 %) are the minerals with a higher contribution. On the other hand, the mineral concentrations in broccolini are very similar to these ones found in broccoli, and it can be considered to provide a suitable amount of valuable minerals.

TABLE 3, TABLE 4

4. Conclusions

The phenolic and mineral composition of fresh and cooked broccolini (steamed, boiled, griddled) have been reported. Twenty compounds were identified, mainly phenolic acids, and the most abundant compounds were quantified. The main compounds present in broccolini have also been previously reported in *Brassica* vegetables. Three oxylipins with important biological activity were identified in the analyzed extracts. A screening of the potential of broccolini as a source of antioxidant compounds was also assessed by applying *in vitro* assays. All cooking processes produced a decrease in both phenolic content and antioxidant activity. However,

mineral content increased during broccolini cooking. Although the phenolic content was not particularly high, mineral content was very important, representing a high contribution to the recommended daily intake. Hence, this novel vegetable may represent an important source of minerals, as well as some important bioactive compounds, such as oxylipins.

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Conflict of interest

The authors declare no competing financial interest.

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Figure captions.

Fig. 1. Base peak chromatogram of fresh broccolini methanolic extract. Bottom part: enlargement of the chromatogram for minor peaks visualization.

Fig. 2. Influence of cooking treatments on antiradical activity of broccolini extracts. DE: dried extract. TE: Trolox equivalent.

Figure 1

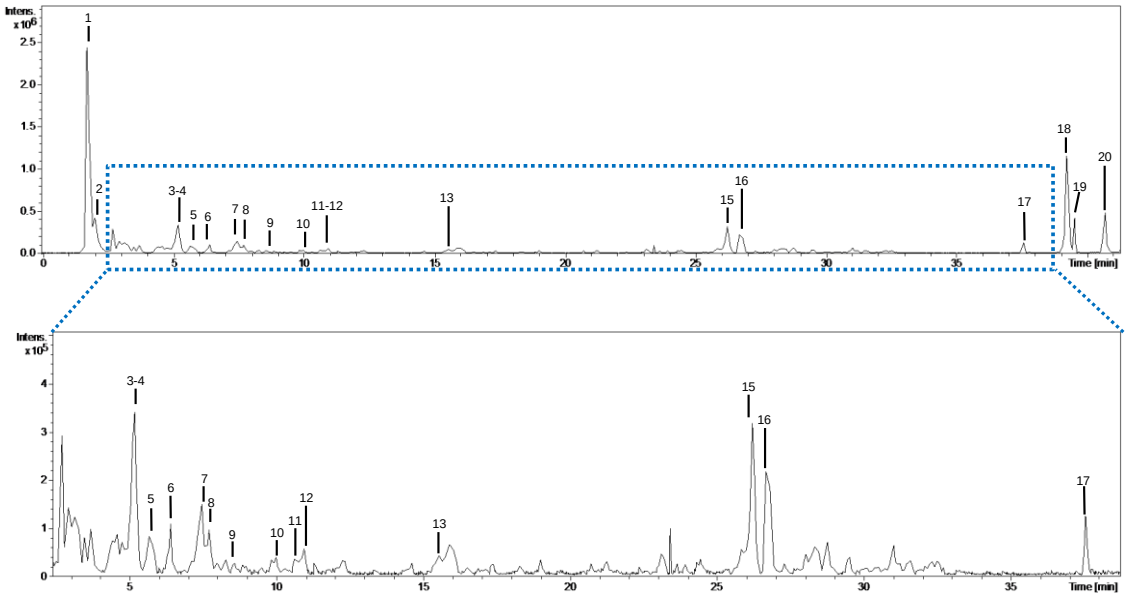

Figure 1

Figure 2

Figure 2

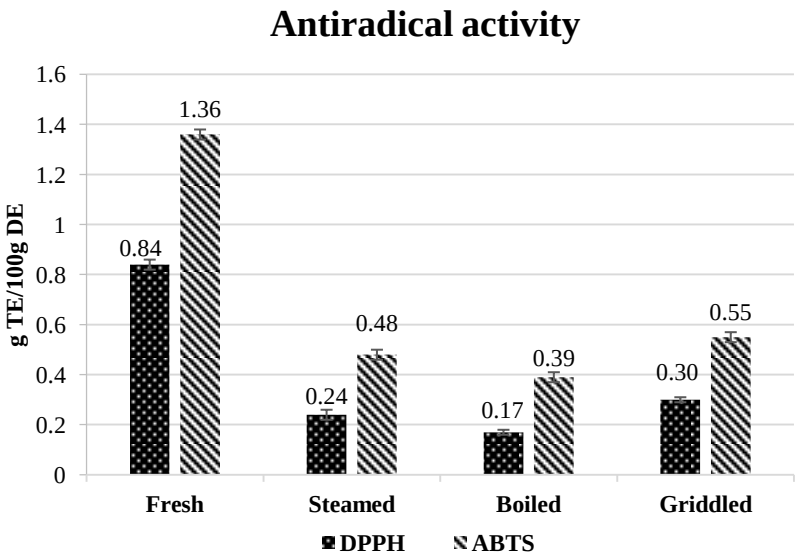

Table 1

Characterization of the compounds found in the analyzed extracts of fresh and cooked broccolini.

N°.	t_R (min)	$[M-H]^-$ *m/z*	m/z (% base peak)	Assigned identification	Fresh	Steamed	Boiled	Griddled
1	1.8	377	MS^2 [377]: 341 (100) MS^3 [377→341]: 179 (100), 161 (48), 143 (22), 131 (5), 119 (48), 113 (13)	Disaccharide (HCl adduct)	✓		✓	
2	2.0	353	MS^2 [353]: 191 (100), 179 (75), 135 (25)	Caffeoylquinic acid	✓			
3	5.2	353	MS^2 [353]: 191 (100), 179 (52), 135 (15)	5-*O*-caffeoylquinic acid*	✓			✓
4	5.2	375	MS^2 [375]: 201 (100), 179 (49) MS^3 [375→179]: 135 (100)	Caffeic acid derivative	✓			✓
5	5.8	325	MS^2 [325]: 163 (100), 119 (11) MS^3 [325→163]: 119 (100)	Coumaric acid-*O*-hexoside	✓	✓		
6	6.4	447	MS^2 [447]: 259 (100)	Glucobrassicin	✓			✓
7	7.5	337	MS^2 [337]: 163 (100) MS^3 [337→163]: 119 (100)	3-*p*-coumaroylquinic acid	✓	✓	✓	✓
8	7.7	325	MS^2 [325]: 163 (100), 119 (12) MS^3 [325→163]: 119 (100)	Coumaric acid-*O*-hexoside	✓	✓	✓	✓
9	8.6	353	MS^2 [353]: 191 (100), 179 (15), 173 (25)	3-*O*-caffeoylquinic acid*	✓			
10	10	355	MS^2 [355]: 193 (100)	Ferulic acid-*O*-hexoside	✓	✓	✓	✓

			MS^3 [355→193]: 149 (32), 134 (100)					
11	10.6	385	MS^2 [385]: 247 (43), 223 (100), 205 (46), 179 (12) MS^3 [385→223]: 208 (89), 179 (30), 164 (100)	Sinapoylglycoside	✓			
12	10.9	431	MS^2 [431]: 385 (100), 223 (12) MS^3 [431→385]: 223 (100), 205 (54), 161 (71), 153 (34) [431→385→223]: 179 (69), 153 (100)	Roseoside (formate adduct)	✓	✓	✓	✓
13	15.4	609	MS^2 [609]: 447 (60), 285 (100) MS^3 [609→447]: 285 (100) MS^4 [609→447→285]: 257 (100), 151 (25)	Kaempferol-*O*-dihexoside	✓	✓	✓	✓
14	24.0	447	MS^2 [447]: 285 (100), 255 (25) MS^3 [447→285]: 255 (100)	Kaempferol-*O*-hexoside		✓	✓	
15	26.2	753	MS^2 [753]: 529 (100), 289 (14) MS^3 [753→529]: 289 (59), 223 (100), 205 (80), 179 (46), 164 (18)	Disinapoyldiglycoside	✓			✓
16	26.6	723	MS^2 [723]: 529 (10), 499 (100), 289 (3), 259	Sinapoylferuloyldiglycoside	✓			✓

			(13) MS^3 [723→499]: 259 (41), 193 (100), 175 (35) MS^4 [723→499→193]: 149 (97), 134 (100)					
17	37.5	421	MS^2 [421]: 375 (100) MS^3 [421→375]: 301 (100), 227 (50)	Quercetin derivative	✓		✓	
18	39.2	327	MS^2 [327]: 309 (21), 291 (30), 229 (100), 211 (54), 171 (32)	Oxo-dihydroxy-octadecenoic acid isomer	✓	✓	✓	✓
19	39.4	327	MS^2 [327]: 291 (42), 229 (100), 211 (57), 171 (58)	Oxo-dihydroxy-octadecenoic acid isomer	✓	✓	✓	✓
20	40.6	329	MS^2 [329]: 311 (28), 229 (100), 211 (60), 171 (10)	Trihydroxy-octadecenoic acid	✓	✓	✓	✓

* Identified with analytical standards

Table 2
Quantification of compounds in extracts of fresh and cooked broccolini.

Nº	Assigned identification	Fresh	Steamed	Boiled	Griddled
Phenolic acids					
3	5-*O*-caffeoylquinic acid	0.77±0.06	-	-	0.29±0.03
5	Coumaric acid hexoside	0.27±0.04	0.25±0.03	-	-
7	3-*p*-coumaroylquinic acid	0.30±0.05^{a}	0.25 ±0.02^{a}	0.24±0.2^{a}	0.27±0.03^{a}
8	Coumaric acid-*O*-hexoside	0.28±0.05^{a}	0.21 ±0.02^{a}	0.25±0.01^{a}	0.23±0.02^{a}
15	Disinapoyldiglycoside	0.06±0.01	-	-	0.02±0.01
16	Sinapoylferuloyldiglycoside	0.05±0.01	-	-	-
Total		1.7±0.2^{c}	0.71±0.07^{b}	0.49±0.03^{a}	0.81±0.08^{b}
Flavonoids					
14	Kaempferol-*O*-hexoside	-	0.10±0.01	-	-
TIPC		1.7±0.2^{c}	0.81±0.08^{b}	0.49±0.03^{a}	0.81±0.09^{b}

TIPC = Total individual phenolic content; defined as the sum of all the quantified compounds. Values (mg g^{-1} DE) are mean ± SD of three measurements. Mean values in the same line not sharing the same letter are significantly different at $p < 0.05$ probability level.

Table 3
ICP-MS results for broccolini, expressed in μg g^{-1} dried vegetable.

	Fresh	Steamed	Boiled	Griddled
Ag	0.98 ± 0.02^{b}	0.890 ± 0.007^{b}	1.6 ± 0.1^{c}	0.69 ± 0.01^{a}
Al	$4.8 \pm 0.5^{a,*}$	$9.6 \pm 0.3^{c,*}$	$9.1 \pm 0.4^{c,*}$	$7.0 \pm 0.5^{b,*}$
As	0.102 ± 0.008^{a}	0.090 ± 0.007^{a}	0.15 ± 0.01^{b}	0.08 ± 0.05^{a}
Ba	2.570 ± 0.008^{a}	3.6 ± 0.2^{b}	4.2 ± 0.2^{c}	2.7 ± 0.5^{a}
Ca	$3.75 \pm 0.04^{a,*}$	$7.2 \pm 0.5^{b,*}$	$8 \pm 1^{b,*}$	$5.9 \pm 0.5^{b,*}$
Cd	1.14 ± 0.04^{b}	1.048 ± 0.007^{b}	1.9 ± 0.1^{c}	0.811 ± 0.005^{a}
Co	0.242 ± 0.008^{a}	1.29 ± 0.06^{b}	N.D	0.9 ± 0.3^{b}
Cr	< LQ	< LQ	< LQ	< LQ
Cu	6.8 ± 0.2^{a}	10.3 ± 0.7^{b}	13 ± 1^{b}	10 ± 2^{ab}
Fe	41 ± 7^{a}	103 ± 7^{b}	127 ± 13^{b}	70 ± 16^{a}
K	$31.6 \pm 0.2^{b,*}$	$33 \pm 1^{b,*}$	$20 \pm 1^{a,*}$	$36 \pm 2^{b,*}$
Mg	$2.02 \pm 0.04^{a,*}$	$2.9 \pm 0.3^{b,*}$	$2.8 \pm 0.4^{ab,*}$	$3.1 \pm 0.4^{b,*}$
Mn	25 ± 2^{a}	53 ± 2^{c}	56 ± 9^{c}	39 ± 1^{b}
Na	$1.445 \pm 0.008^{a,*}$	$1.45 \pm 0.07^{ab,*}$	$1.3 \pm 0.1^{a,*}$	$1.62 \pm 0.05^{b,*}$
Ni	5.3 ± 0.4^{b}	3.6 ± 0.3^{a}	3 ± 1^{a}	3 ± 1^{a}
P	$5.0 \pm 0.2^{a,*}$	$8.0 \pm 0.5^{c,*}$	$6.7 \pm 0.6^{b,*}$	$9 \pm 1^{c,*}$
Sb	0.27 ± 0.02^{a}	0.23 ± 0.01^{a}	0.42 ± 0.01^{b}	N.D
Si	$0.92 \pm 0.02^{b,*}$	$0.79 \pm 0.03^{b,*}$	$1.3 \pm 0.1^{c,*}$	$0.52 \pm 0.02^{a,*}$
V	0.344 ± 0.008^{c}	0.317 ± 0.007^{b}	0.57 ± 0.01^{d}	0.249 ± 0.005^{a}
Zn	34 ± 4^{a}	66.9 ± 0.2^{b}	63 ± 10^{b}	65 ± 11^{b}

N.D.= Not detected. Results are given as mean ± standard deviation. Mean values in the same line not sharing the same letter are significantly different at $p < 0.05$ probability level. * expressed in mg g^{-1}

Table 4
Mean daily intake and percentage of contribution to DRV for minor nutritional elements in 100 g of broccolini.

	Ca	Cu	Fe	K	Mg	Mn	P	Zn
Level in broccolini (mg/100 g)[a]	48	0.087	0.52	404	25.8	0.32	64	0.43
Level in broccoli (mg/100 g)[b]	54	0.06	1.05	309	21	-	77	0.63
DRV (mg/day)								
female	860	1.3	7	3500	300	3	550	6.2-10.2[c]
male	860	1.6	6	3500	350	3	550	7.5-12.7[c]
% DRV[d]								
female	5.6	6.7	7.4	11.5	8.6	10.7	11.6	6.9-4.2
male	5.6	5.4	8.7	11.5	7.4	10.7	11.6	5.7-3.4

[a] Average level of the mineral in fresh broccolini (Table 3). [b] Average level of the mineral in fresh broccoli (European Food Safety Authority (EFSA), n.d.). DRV: Dietary Reference Value for adults (≥ 18 years). [c] DRVs for zinc are provided for four levels of phytate intake: 300-1.200 mg/day. [d] Calculated on the basis of the intake of 100 g of broccolini/day.

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***Graphical Abstract**

*Conflict of Interest form

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Comparative study of the phytochemical and mineral composition of fresh and cooked broccolini

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