Effect of steaming and *sous vide* processing on the total phenolic content, vitamin C and antioxidant potential of the genus *Brassica*

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Abbreviations

ANOVA: Analysis of variance; DPPH: 2,2-Diphenyl-1-picrylhydrazyl; FRAP: Ferric reducing antioxidant power; h^0: Hue angle; HPLC: High-performance liquid chromatography; S.D: Standard deviation; TPTZ: 2,4,6-Tris(2-pyridyl)-s-triazine; UV: Ultraviolet; TCEP: tris(2-carboxyethyl)phosphine hydrochloride

Abstract

This study evaluated the effect of thermal processing on the antioxidant potential, vitamin C, and total phenolic content of various parts, including edible co-products, of several *Brassica* vegetables. Overall, no significant differences were observed in the lightness of the samples after thermal processing, although the greenness and h^0 values of the samples were affected (p<0.05). Similar profiles were observed for the leaves, inflorescences, and stalks of the studied crucifers. The stalks of some varieties, including broccoli cv. Parthenon and kale cv. Crispa, showed higher vitamin C contents compared to that of their inflorescences (p<0.05). Both steaming and *sous-vide* processing significantly reduced the vitamin C and total phenolic content of the crucifers studied (p<0.05). The results demonstrated that *Brassica* co-products contain valuable and health-promoting substances that can be lost during thermal processing; this must be considered when calculating the dietary intake of these compounds from cooked vegetables.

Industrial relevance

The results obtained herein suggest that *Brassica* stalks are as nutritious and healthy as the florets or leaves, which are more commonly consumed as part of our diet. In addition, this study demonstrates the potential of *Brassica* co-products as a resource for the extraction of antioxidant compounds and opens new commercial opportunities for their use beyond their current applications in the food industry. The results also highlight that these compounds are mostly lost during processing and that the processing conditions should be carefully optimized to minimize their degradation.

Keywords: thermal processing, *sous vide*, antioxidant activity, vitamin C, ascorbic acid, phenolic content, food co-products, cruciferous vegetables, waste reduction

1. **Introduction**

To aid processing, extend shelf-life and to obtain low-cost and good-tasting foods, the food industry has relied on the use of fats, sugars, chemical processing aids, and plastics (Lamppa, Horn, & Edwards, 2014). However, consumers are now becoming more aware of the relationship among food, diet, and health, and this has led to increased interest in natural ingredients and the consumption of food products that are tasty, nutritious and healthy (Grasso, Brunton, Lyng, Lalor, & Monahan, 2014). The results obtained from several epidemiological studies have encouraged a high intake of plant products, which are associated with a reduced risk for several chronic diseases, such as atherosclerosis and cancer (Podsędek, 2007). Furthermore, the number of consumers following a vegan diet and the demand for vegan food have notably increased in many countries, and it is likely that this trend will continue to grow (Janssen, Busch, Rödiger, & Hamm, 2016).

The family *Brassicaceae* (or *Cruciferae*) consists of 350 genera and over 3500 species, which include the genera *Camelina*, *Crambe*, *Sinapis*, and *Brassica* (Cartea, Francisco, Soengas, & Velasco, 2010). *Brassica* plants are informally known as cruciferous vegetables, crucifers, cabbages, or mustard plants and they are economically the most important genus in the *Brassiceae* family (Rakow, 2004). *Brassica* species that are commonly used for food include broccoli, cauliflower, cabbage (*B. oleracea*), and turnip (*B. rapa*). These vegetables are known for their anti-carcinogenic properties (Podsędek, 2007; Singh, Upadhyay, Prasad, Bahadur, & Rai, 2007), and several studies recently reported the occurrence of antioxidant compounds in several crucifers, such as Chinese cabbage (Seong, Hwang, & Chung, 2016) and red and green cabbages (Upadhyay, Sehwag, & Singh, 2016).

Research on cruciferous vegetables has focused mainly on the plant parts that are most commonly consumed. The non-consumed parts, which are the crucifer co-products, are

usually discarded as waste or used for low-value purposes (Ngu & Ledin, 2005). It is not practical to discard co-products and wastes, especially when these contain a significant amount of bioactive compounds that can promote human health and a nutritious diet. Waste revalorization and the reutilization of co-products are important issues in the food industry (Lafarga, Gallagher, Walsh, Valverde, & Hayes, 2013). Furthermore, although some crucifers can be eaten fresh, these vegetables are most commonly consumed after cooking. The temperature may affect the antioxidant content of foods due to antioxidant release, destruction, or even the creation of new metabolites (Wachtel-Galor, Wong, & Benzie, 2008). There is a need for quantitative data on the nutritional and bioactive properties of cooked *Brassica* vegetables. The effects of cooking and cooking methods on the bioactive properties of vegetables have not been well studied. Such information would not only help to better understand the function of antioxidant phytochemicals but also promote their consumption and promote health.

In this work, the authors report the effect of two cooking methods (a conventional thermal treatment and *sous-vide*) on the colour, antioxidant activity, total phenolic content, and total vitamin C content of different *Brassica* vegetables, including broccoli, cauliflower, and cabbage. The analysed parts of the plants included those that are regularly consumed and those that are not currently used as food sources, opening new commercial opportunities for their use in the food industry.

2. Materials and methods

2.1 Chemicals and reagents

Methanol, sodium acetate, acetic acid, hydrochloric acid, and ferric chloride hexahydrate were obtained from Panreac (Barcelona, Spain). Gallic acid, ascorbic acid, metaphosphoric acid, 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ), 2,2-diphenyl-1-picrylhydrazyl (DPPH), tris(2-carboxyethyl)phosphine hydrochloride (TCEP), and sodium carbonate were purchased from Sigma-Aldrich (Steinheim, Germany). Folin-Ciocalteu's reagent was purchased from VWR (Llinars del Vallès, Spain). All reagents used were of analytical grade.

2.2 Plant material: Collection and processing

Brassica vegetables, including broccoli cv. Marathon (*Brassica oleracea* var. *italica)*, broccoli cv. Parthenon (*Brassica oleracea* var. *italica)*, broccoli cv. Graffiti (*Brassica oleracea* var. *botrytis)*, broccoli cv. Pastoret (*Brassica oleracea* var. *botrytis)*, Espigall del Garraf (*Brassica oleracea* var. *acephala)*, and kale cv. Crispa (*Brassica oleracea* var. *acephala)*, were provided by the Fundació Miquel Agustí, Barcelona, Spain. The plants were grown at Agròpolis, Baix Llobregat, Barcelona, Spain (41°17'18.6"N 2°02'39.7"E) and collected during November 2015.

The sample processing was performed at the pilot plant of the IRTA Fruitcentre in Lleida, Spain. Upon collection, the samples were frozen using liquid nitrogen and stored at -80 °C until further use. Sample processing consisted of dividing the samples into six replicates of approximately 100 g each of either the leaves/stems or inflorescences/stems. Three replicates were used for the steaming and *sous-vide* treatments.

Before the *sous-vide* treatment, the samples were rinsed using tap water for 10 seconds and vacuum-sealed in a polyethylene vacuum-sealable bags designed for this treatment. The samples were vacuum-sealed using a "soft vacuum" programme. Both treatments, the conventional processing and *sous-vide*, were performed in a Rational SCC WE-101 convection oven (Rational AG, Landsberg am Lech, Germany). The treatment conditions for steaming were: 100 °C and 15 min for inflorescences or leaves and 100 °C and 65 min for stems. The treatment conditions for the *sous vide*-treated samples were: 80 °C and 15 min for inflorescences or leaves and 80 °C and 90 min for stems. After treatment, the samples were quickly chilled to approximately 3-4 °C before freezing using liquid nitrogen and storage at -80 °C.

2.3 Colour measurement

Eight colour recordings were taken per part of the plant (stem, leaf, or inflorescence) and per treatment (fresh and after the conventional treatment or *sous-vide*) for each sample using a Minolta CR-200 colorimeter (Minolta INC, Tokyo, Japan). The CIE values were recorded in terms of L^* (lightness), a^* (redness, greenness), and b^* (yellowness/blueness). A calibration was performed using a standard white tile (Y:92.5, x:0.3161, y:0.3321) provided by the manufacturer and the D65 illuminant, which approximates daylight.

The hue angle (h^0) was calculated with the obtained L^*, a^*, and b^* values using the equation:

$$h^0 = \tan^{-1}\frac{b^*}{a^*}$$

2.4 Determination of the content of vitamin C

The total vitamin C content (ascorbic acid and dehydroascorbic acid) was determined in triplicate with high-performance liquid chromatography (HPLC) using a Waters 717 plus Autosampler HPLC system (Waters Corp., NJ, USA) coupled to an ultraviolet (UV) detector following the method previously described by Plaza et al. (2011). Briefly, six grams of a frozen sample were homogenized with 20 mL of an extraction solution that contained 30 g/L meta-phosphoric acid and 80 mL/L acetic acid in HPLC-grade water. The resulting mixture was centrifuged using a Sigma 3-18KS centrifuge (Osterode am Harz, Germany) at 12,000 rpm for 20 min at 4 °C and filtered, and the total volume was adjusted to 25 mL with the extraction solution. The samples were further filtered through 0.45-µm filters, and an aliquot (1.9 mL) of the mixture was obtained to react with 0.1 mL of 0.04 M TCEP for 3 h at room temperature in the dark.

Vitamin C was separated on a reversed-phase Supelcosil™ LC18 (5 µm) stainless-steel column (250 × 4.6 mm i.d., Supelco, USA). An isocratic solvent system was used (0.1 mL/L of sulphuric acid, pH 2.5-2.6). The flow rate was fixed at 1 mL/min, and the UV-vis photodiode array detector was set at 254 nm. The identification of vitamin C was performed by comparing the retention time and the obtained spectra to those previously obtained with a standard.

2.5 Determination of the total phenolic content

The total phenolic content was determined using Folin-Ciocalteu's method following the modifications described by Altisent, Plaza, Alegre, Viñas, and Abadias (2014). Briefly, for the extraction, six grams of a frozen, fresh sample were homogenized with 20 mL of methanol 70% (v/v), centrifuged using a Sigma 3-18KS centrifuge (Osterode am Harz, Germany) at 12,000 rpm for 20 min at 4 °C, and filtered. The extraction solution was added to the extract to obtain a final volume of 25.0 mL. The assay was performed in triplicate by adding 4.3 mL of deionized water and 0.5 mL of Folin-Ciocalteu's reagent

to 0.7 mL of each extract. After 5 min of incubation, 2.0 mL of a saturated sodium carbonate solution was added. The mixture was shaken and further incubated for 1 h in the dark, and the absorbance was read at 760 nm using a GENESYS^{TM} 10S UV-Vis spectrophotometer (Thermo Fisher Scientific, MA, USA). The results are expressed as mg of gallic acid per kilogram of fresh weight.

2.6 Antioxidant activity

The antioxidant activity was measured using two different methods: the ferric reducing antioxidant power (FRAP) and DPPH· scavenging activity assay. The extraction methodology was the same for both methods. A sample extract was obtained by mixing 20 mL of methanol 70% (v/v) with six grams of sample and followed by homogenization for 5 min and centrifugation using a Sigma 3-18KS centrifuge (Osterode am Harz, Germany) at 12,000 rpm for 20 min at 4 °C. The extracts were diluted to 25 mL with the extraction solution.

2.6.1 Ferric reducing antioxidant powder (FRAP)

The total antioxidant potential of the samples was determined for each sample using the FRAP assay previously described by Benzie and Strain (1996). Briefly, the FRAP reagent was freshly prepared by mixing 0.3 M acetate buffer (pH 3.6), 10 mM TPTZ in 40 mM HCl and 20 mM $FeCl_3$ in the proportion 10:1:1 (v/v/v), respectively. The assay was performed by adding 1.4 mL of the FRAP reagent to 0.1 mL of the generated extract. After 20 min of incubation in the dark at 37 °C with shaking, the absorbance was read at 593 nm using a GENESYS^{TM} 10S UV-Vis spectrophotometer (Thermo Fisher Scientific, MA, USA). The results were run in triplicate and compared to a standard curve prepared daily with different concentrations of ascorbic acid.

2.6.2 The 2,2-diphenyl-1-picrylhydrazyl radical (DPPH·) scavenging activity

The antioxidant activity was also measured following the method described by Hidalgo, Sánchez-Moreno, and de Pascual-Teresa (2010). Briefly, the assay was performed by adding 1.4 mL of a 0.1 mM DPPH solution to 0.1 mL of the generated extract. After 60 min of incubation at room temperature in the dark, the absorbance was read at 515 nm using a GENESYS^{TM} 10S-UV Vis spectrophotometer (Thermo Fisher Scientific, MA, USA). The results were run in triplicate and compared to a standard curve prepared daily with different concentrations of ascorbic acid.

2.7 Statistical analysis

All tests were replicated three times, except for the colour readings, which were recorded eight times per sample. The results are expressed as the mean ± standard deviation (S.D.). Samples were analysed using analysis of variance (ANOVA). Statistical analysis was done using JMP 8 (SAS Institute Inc., Cary, USA). Tukey's pairwise comparison of the means was conducted to identify differences between treatments, and Student's t-test was used to identify differences between different parts of the same crucifer. The criterion for statistical significance was $p<0.05$.

3. Results and discussion

3.1 Effect of thermal processing on the colour

The present study evaluated the effect of thermal processing on the colour of selected *Brassica* vegetables. Colour is a key parameter in food products because food colour is important for humans' innate perceptions of the value of food items and is the first parameter of quality evaluated by consumers (Markovic, Ilic, Markovic, Simonovic, & Kosanic, 2013). Colour is a reliable indicator of the healthful quality of foods. In this study, eight recordings of the L^*, a^*, and b^* values were taken per part per plant. L^* is defined as the lightness (L^*=0 yields black, and L^*=100 indicates diffuse white).

Table 1 shows the L^* and h^0 values, which were calculated from the a^* and b^* values. The colours of the florets and stems of the raw and thermally processed crucifers were comparable to those obtained in previous studies (Brewer, Begum, & Bozeman, 1995; Miglio, Chiavaro, Visconti, Fogliano, & Pellegrini, 2007). In the current study, no significant differences were observed between the lightness of the inflorescences and the stalks of the fresh broccoli species. Only broccoli cv. Pastoret presented a higher L^* value in the stalk compared to the inflorescence (p<0.05). Thermal processing did not affect the lightness of the inflorescences of the different broccoli varieties and the leaves of Espigall del Garraf. However, steaming affected the lightness of the leaves of kale cv. Crispa (p<0.05). In addition, thermal processing resulted in a significant increase in the L^* value of the stalks of all the studied varieties (p<0.05). The results contrast with previous studies, which reported a decrease in the lightness of broccoli after thermal processing (Miglio, et al., 2007). Pellegrini et al. (2010) also reported differences in the lightness of *Brassica* vegetables after thermal processing.

No differences were observed between the h^0 value of the fresh broccoli inflorescences or leaves of Espigall del Garraf and their stalks, except for kale cv. Crispa, which showed a higher h^0 value for the leaves (p<0.05). Overall, thermal processing significantly reduced the h^0 value in both the inflorescences and stalks, except for the inflorescences of broccoli cv. Graffiti and the stalks of broccoli cv. Parthenon and broccoli cv. Pastoret. A decrease in the h^0 value is associated with a loss of greenness. Similar results were obtained by Miglio et al. (2007), who observed a loss of greenness and a reduction in the h^0 value of *Brassica* samples after both steaming and frying. During processing, chlorophyll is converted into pheophytin and pyropheophytin, turning vegetables from a bright green to an olive green colour (Bongoni, Verkerk, Steenbekkers, Dekker, & Stieger, 2014). The observed reduction in greenness in the h^0 value of the crucifers after thermal processing could be caused not only by the degradation of chlorophyll but also by changes in the surface reflectance and depth of light penetration in thermally processed vegetables, which are caused by a loss of air and other dissolved gases (Tijskens, Schijvens, & Biekman, 2001). Although processing resulted in a reduction of the h^0 value in most samples, *sous-vide* processing of the stalks of broccoli cv. Marathon and broccoli cv. Graffiti resulted in higher h^0 values (p<0.05). This trend was also previously observed after steaming crucifer stems (Miglio, et al., 2007).

3.2 Effect of thermal processing on the vitamin C content

Vitamin C includes ascorbic acid, and its oxidation product, dehydroascorbic acid, has several biological activities in the human body and is thought to have cancer-protective capacities (Bakker, et al., 2016). Over 85% of vitamin C in human diets is supplied by fruits and vegetables (Podsędek, 2007). Indeed, the concentration of vitamin C in blood is an excellent biomarker of vegetable and fruit consumption and provides better

approximations of the concentration available to cells than dietary questionnaires (Block, Norkus, Hudes, Mandel, & Helzlsouer, 2001). However, the content of vitamin C in fruits and vegetables can be significantly reduced during processing and storage due to its solubility in water and its sensitivity to high temperatures and oxidation conditions (Gamboa-Santos, Cristina Soria, Pérez-Mateos, Carrasco, Montilla, & Villamiel, 2013). For example, conventional cooking of broccoli for 0.5, 1.5, and 5.0 min results in vitamin C losses of 19.2, 47.5, and 65.9%, respectively (Zhang & Hamauzu, 2004). Vitamin C loss caused by food processing can be reduced. For example, Lin and Brewer (2005) observed that steam blanching resulted in better vitamin C retention in peas compared to treatments with boiling water for equal blanching times.

The vitamin C content of *Brassica* vegetables varies significantly between subspecies. For example, Pfendt, Vukašinović, Blagojević, and Radojević (2003) reported the ascorbic acid content of kale as 92.6 mg/100 g of edible portion. This value is much higher than the ascorbic acid content of white cabbage reported by Bahorun, Luximon-Ramma, Crozier, and Aruoma (2004), 8 mg/100 mg edible portion. In the current study, broccoli cv. Graffiti florets showed the highest vitamin C content with a concentration of 220.3 ± 17.1 mg/100 g of fresh sample ($p<0.05$). The vitamin C contents of the studied fresh and processed vegetables are shown in Figure 1. Overall, the inflorescences/leaves of fresh vegetables presented higher vitamin C contents than the stem. However, for some varieties, including broccoli cv. Parthenon and kale cv. Crispa, the vitamin C contents of the fresh stalks were higher ($p<0.05$). The results compared favourably with those obtained previously by Zhang et al. (2004) who reported a higher vitamin C content in the stem than the floret of broccoli. Thermal processing significantly reduced the vitamin C content of all analysed samples ($p<0.05$). The results are in line with previous studies that reported a reduction in the vitamin C content of vegetables after thermal processing

(Wachtel-Galor, et al., 2008). The observed reduction was especially high in the inflorescences of broccoli cv. Marathon, broccoli cv. Graffiti, and broccoli cv. Pastoret and in the leaves of Espigall del Garraf compared to the reduction in the vitamin C contents of their stems ($p<0.05$). The vitamin C contents of the stems of broccoli cv. Marathon, broccoli cv. Graffiti, broccoli cv. Pastoret, Espigall del Garraf, and kale cv. Crispa were higher than those observed in their inflorescences/leaves after both steaming and *sous-vide* processing ($p<0.05$). However, this trend was not observed in broccoli cv. Parthenon, and no differences were observed between the two plant parts after processing. Steaming resulted in higher vitamin C losses in both the stems and leaves of kale cv. Crispa compared to *sous-vide* processing ($p<0.05$). This could be caused by the reduced amount of oxygen present when cooking by *sous-vide*, and previous studies have suggested that oxygen is probably the most important factor in vitamin C degradation (Verbeyst, Bogaerts, Van der Plancken, Hendrickx, & Van Loey, 2013).

3.3 Effect of processing on the total phenolic content

Natural antioxidants can be divided into three main groups: vitamins, carotenoids, and phenolic compounds (Thaipong, Boonprakob, Crosby, Cisneros-Zevallos, & Hawkins Byrne, 2006). Polyphenols possess ideal structural chemistry for free radical-scavenging activities. Over 8,000 phenolic compounds, including phenolic acids and flavonoids, have been identified in various plant species and have been linked to anti-diabetic, anti-ageing, anti-cancer, neuro-protective, and cardio-protective effects (Pandey & Rizvi, 2009). Although several authors published extensive reviews on the phenolic profiles in different *Brassica* species (Cartea, et al., 2010; Podsędek, 2007), data on the phenolic compounds in plant parts that are not commonly consumed are scarce. For this reason, this study aimed to quantify the phenolic compounds in the various parts of these vegetables. Figure 2 shows the total phenolic content of the studied *Brassica* vegetables. The highest

phenolic content was found in the fresh leaves of kale cv. Crispa, which had a total phenolic content of 158.8 ± 3.5 mg/100 g sample. Results obtained herein were higher when compared to those reported by Leja, Mareczek, Starzyńska, and Rożek (2001) who observed that broccoli florets contained 56.2 mg TPC/100 g of fresh weight. Similar results were also reported by Zhang et al. (2004) who published a concentration of 34.5 mg TPC/100 g of fresh broccoli. No differences were observed between the total phenolic content of the stems and the inflorescences of fresh broccoli cv. Parthenon and broccoli cv. Pastoret. The inflorescences and leaves of broccoli cv. Graffiti, Espigall del Garraf, and kale cv. Crispa showed a higher total phenolic content than their stems (p<0.05). This trend was not observed in broccoli cv. Marathon, which showed a higher phenolic content in its stem than the inflorescence (p<0.05). Thermal processing significantly reduced the phenolic content of the studied vegetables. Similar results were obtained previously (dos Reis, de Oliveira, Hagen, Jablonski, Flôres, & de Oliveira Rios, 2015; Pellegrini, et al., 2010). Other previously studied treatments, such as microwaving, also resulted in high losses of flavonoids (97%), sinapic acid derivatives (74%), and caffeoyl-quinic acid derivatives (87%) in *Brassica* species (Vallejo, Tomás-Barberán, & García-Viguera, 2003). Overall, no differences were observed between the samples treated by steaming or *sous-vide* processing for most of the varieties. However, the phenolic contents of the stems of broccoli cv. Parthenon and broccoli cv. Pastoret treated using *sous-vide* were significantly higher than those obtained after steaming (p<0.05). However, the opposite trend was observed after processing the inflorescences of broccoli cv. Parthenon, and *sous-vide* processing resulted in a higher phenolic content loss (p<0.05).

3.4 Measurement of the antioxidant activity

Natural antioxidants in fruits and vegetables have gained increasing interest over the last decade (Thaipong, et al., 2006). As mentioned previously, several studies have

317 highlighted the antioxidant potential of *Brassica* species (Bekhit, Lingming, Mason,
318 Zhou, & Sedcole, 2013; Podsędek, 2007; Seong, et al., 2016; Upadhyay, et al., 2016;
319 Wachtel-Galor, et al., 2008). Although some studies suggested that plant parts that are
320 not commonly consumed have similar antioxidant potentials to those that are eaten
321 (Balasundram, Sundram, & Samman, 2006; Wijngaard, Rößle, & Brunton, 2009), most
322 studies have focused on the antioxidant potential of commonly consumed parts. The
323 current study evaluated the antioxidant potential of several *Brassica* species using two
324 independent methods: the FRAP assay and DPPH radical assay.
325 The FRAP assay uses antioxidants as reductants in a redox-linked colorimetric method
326 employing an easily reduced oxidant. The ferric to ferrous ion reduction at low pH values
327 causes the formation of a coloured ferrous-tripyridyltriazine complex (Benzie, et al.,
328 1996). Figure 3 shows the antioxidant activity of the fresh and thermally treated samples
329 measured using the FRAP method. The inflorescences of fresh broccoli cv. Marathon and
330 broccoli cv. Graffiti and the leaves of Espigall del Garraf and kale cv. Crispa showed
331 higher antioxidant activities, as measured using the FRAP assay, compared to their stems
332 (p<0.05). However, the opposite trend was observed in the samples of broccoli cv.
333 Pastoret, which presented a higher antioxidant activity in the stems (p<0.05); no
334 differences were observed between the stems and inflorescences of broccoli cv.
335 Parthenon. *Sous-vide* processing resulted in a significant reduction in the antioxidant
336 potential of the inflorescences and leaves of all the studied vegetables (p<0.05). However,
337 no differences were observed between the antioxidant activities of fresh and *sous-vide*-
338 treated stems of broccoli cv. Parthenon, Espigall del Garraf, and kale cv. Crispa, and an
339 increase was observed in the antioxidant potential of the stems of broccoli cv. Marathon
340 after both steaming and *sous-vide* processing compared to that of the fresh stems
341 (p<0.05). For some parts of some varieties, including the inflorescences of broccoli cv.

342 Marathon and broccoli cv. Parthenon, *sous-vide* processing resulted in a higher loss of
343 antioxidant potential compared to steaming. However, for the stems of broccoli cv.
344 Parthenon and the leaves of Espigall del Garraf and kale cv. Crispa, a significantly higher
345 loss of antioxidant potential was observed after steaming ($p<0.05$).
346 The results obtained using the DPPH· assay, as shown in Figure 4, did not correlate well
347 to those obtained using the FRAP method in terms of the antioxidant potential. The
348 antioxidant potential was higher in the inflorescences of fresh broccoli cv. Graffiti and
349 kale cv. Crispa compared to their fresh stems ($p<0.05$), and no differences were observed
350 between the inflorescences or leaves and stems of fresh broccoli cv. Parthenon, broccoli
351 cv. Marathon, and Espigall del Garraf. Overall, the antioxidant activity of the analysed
352 samples measured using the DPPH· assay increased after thermal processing. This
353 increase in antioxidant activity was higher after the *sous-vide* processing of the
354 inflorescences of broccoli cv. Marathon and the stems and inflorescences of broccoli cv.
355 Pastoret and after steaming the inflorescences of broccoli cv. Pastoret and the stems of
356 broccoli cv. Parthenon and Espigall del Garraf. These results are in line with those
357 obtained previously by Juániz et al. (2016) and by Wachtel-Galor et al. (2008) who
358 reported an increase in the antioxidant activity after cooking several *Brassica* vegetables.
359 Similar results were also obtained by Turkmen, Sari, and Velioglu (2005) who observed
360 an increase in the antioxidant activity of broccoli after boiling, microwaving, and
361 steaming. The observed increase in the antioxidant activity could be caused by the
362 liberation of antioxidants from insoluble portions or the formation of novel antioxidants
363 caused by temperature-dependent reactions (Hwang, Shin, Lee, Lee, & Yoo, 2012;
364 Manzocco, Calligaris, Mastrocola, Nicoli, & Lerici, 2000; Martins, Jongen, & Van
365 Boekel, 2000). The water lost during processing may result in a concentration of the
366 antioxidant compounds, which could be another reason for the observed increase in the

antioxidant activity. The observed differences between the antioxidant activities obtained using both methods might be due to the different principles on which these methods are based. Although both methods are redox-linked colorimetric methods, the DPPH radical assay and FRAP assay are based on the acceptance of either hydrogen atoms or electrons from antioxidants, respectively.

4. Conclusions

In this study, the antioxidant potential and antioxidant contents, such as vitamin C or phenolic compounds, in *Brassica* vegetables showed significant losses during thermal processing. The results were in line with previous studies where thermal processing resulted in decreased antioxidant activity. This must be considered when calculating the dietary intake of these compounds from cooked vegetables. The cooking conditions evaluated in this study were strong, and smaller losses would be expected under milder cooking conditions. However, this must be confirmed *in vitro*. This study also demonstrated that non-commercial parts of crucifers can be as rich in nutrients as the currently commercially used parts of these plants. For example, the uncooked stems of broccoli could be used as resources for the generation of extracts rich in antioxidants and other value-added ingredients. Future studies would include a sensorial analysis of these underused plant parts and an investigation of their acceptance by consumers. The results obtained herein open new commercial opportunities for *Brassica* producers for their use as novel ingredients in healthy foods. This would not only promote health but also reduce the amount of co-products discarded as waste or used for low-value purposes.

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Figure captions

Figure 1. Effect of treatment on the vitamin C content of A) Broccoli cv. Marathon; B) Broccoli cv. Parthenon; C) Broccoli cv. Graffiti; D) Broccoli cv. Pastoret; E) Espigall del Garraf; F) Kale cv. Crispa

Values represent the mean of three independent experiments ± S.D. Capital letters indicate significant differences between different parts of the same sample. Lower case letters indicate significant differences between treatments. The criterion for statistical significance was $p<0.05$.

Figure 2. Effect of steaming and *sous-vide* processing on the total phenolic content of A) Broccoli cv. Marathon; B) Broccoli cv. Parthenon; C) Broccoli cv. Graffiti; D) Broccoli cv. Pastoret; E) Espigall del Garraf; F) Kale cv. Crispa

Values represent the mean of three independent experiments ± S.D. Capital letters indicate significant differences between different parts of the same sample. Lower case letters indicate significant differences between treatments. The criterion for statistical significance was $p<0.05$.

Figure 3. Antioxidant activity measured using the FRAP assay of A) Broccoli cv. Marathon; B) Broccoli cv. Parthenon; C) Broccoli cv. Graffiti; D) Broccoli cv. Pastoret; E) Espigall del Garraf; F) Kale cv. Crispa

Values represent the mean of three independent experiments ± S.D. Capital letters indicate significant differences between different parts of the same sample. Lower case letters indicate significant differences between treatments. The criterion for statistical significance was $p<0.05$.

Figure 4. Antioxidant activity measured using DPPH· assay of A) Broccoli cv. Marathon; B) Broccoli cv. Parthenon; C) Broccoli cv. Graffiti; D) Broccoli cv. Pastoret; E) Espigall del Garraf; F) Kale cv. Crispa

Values represent the mean of three independent experiments ± S.D. Capital letters indicate significant differences between different parts of the same sample per treatment. Lower case letters indicate significant differences between treatments. The criterion for statistical significance was $p<0.05$.

Table 1. Colour recordings for the fresh and treated samples. Values represent mean of three independent experiments ± S.D. The criterion for statistical significance was $p<0.05$.

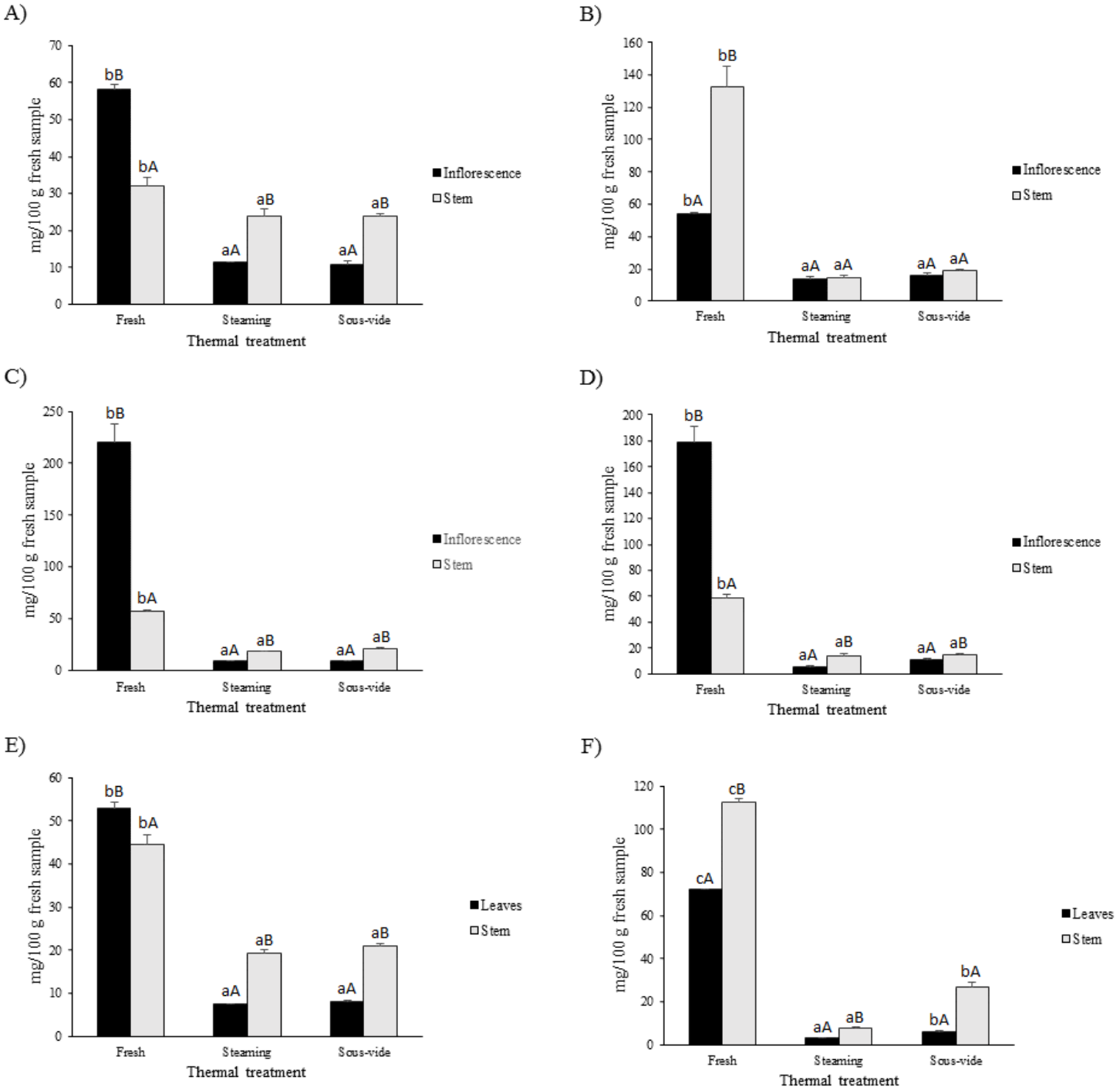
Sample	Part	Treatment	L^*	h^0
Broccoli cv. Marathon	Inflorescence	Fresh	44.14 ± 4.79 ^{aA}	122.73 ± 4.29 ^{cA}
		Steaming	40.21 ± 11.42 ^{aA}	96.92 ± 2.02 ^{aA}
		Sous-vide	44.88 ± 16.61 ^{aA}	106.09 ± 2.22 ^{bA}
	Stalk	Fresh	47.02 ± 2.18 ^{aA}	122.29 ± 1.43 ^{aA}
		Steaming	62.23 ± 3.11 ^{bB}	123.12 ± 2.07 ^{aB}
		Sous-vide	59.81 ± 2.48 ^{bA}	127.28 ± 1.70 ^{bB}
Broccoli cv. Parthenon	Inflorescence	Fresh	49.30 ± 6.49 ^{aA}	121.47 ± 2.52 ^{bA}
		Steaming	46.33 ± 17.37 ^{aA}	101.22 ± 6.79 ^{aA}
		Sous-vide	47.85 ± 12.63 ^{aA}	110.86 ± 4.78 ^{aA}
	Stalk	Fresh	50.24 ± 3.62 ^{aA}	125.69 ± 2.01 ^{aA}
		Steaming	60.07 ± 3.91 ^{bA}	124.28 ± 5.61 ^{aB}
		Sous-vide	59.78 ± 2.33 ^{bA}	124.39 ± 1.23 ^{aB}
Broccoli cv. Graffiti	Inflorescence	Fresh	40.75 ± 9.64 ^{aA}	111.43 ± 15.01 ^{aA}
		Steaming	42.95 ± 3.80 ^{aA}	105.71 ± 7.21 ^{aA}
		Sous-vide	48.18 ± 9.08 ^{aA}	106.25 ± 9.23 ^{aA}
	Stalk	Fresh	45.04 ± 2.97 ^{aA}	122.87 ± 1.94 ^{aA}
		Steaming	63.08 ± 2.20 ^{cB}	122.82 ± 2.69 ^{aA}
		Sous-vide	56.78 ± 2.34 ^{bA}	130.55 ± 4.87 ^{bB}
Broccoli cv. Pastoret	Inflorescence	Fresh	42.64 ± 7.16 ^{aA}	124.22 ± 8.90 ^{bA}
		Steaming	42.13 ± 19.74 ^{aA}	100.35 ± 5.35 ^{aA}
		Sous-vide	41.31 ± 13.96 ^{aA}	109.80 ± 5.76 ^{aA}
	Stalk	Fresh	57.28 ± 2.53 ^{aB}	115.77 ± 2.35 ^{aA}
		Steaming	63.99 ± 4.31 ^{bA}	116.65 ± 4.38 ^{aB}
		Sous-vide	65.08 ± 5.00 ^{bB}	120.89 ± 8.09 ^{aA}
Espigall del Garraf	Leaves	Fresh	44.91 ± 6.52 ^{aA}	133.42 ± 3.17 ^{cA}
		Steaming	44.88 ± 13.20 ^{aA}	105.74 ± 4.56 ^{aA}
		Sous-vide	36.80 ± 7.17 ^{aA}	114.40 ± 3.00 ^{bA}
	Stalk	Fresh	44.79 ± 1.57 ^{aA}	129.51 ± 3.96 ^{bA}
		Steaming	61.74 ± 2.87 ^{bB}	120.69 ± 2.95 ^{aB}
		Sous-vide	59.52 ± 2.82 ^{bB}	130.92 ± 20.28 ^{abA}
Kale cv. Crispa	Leaves	Fresh	37.58 ± 3.36 ^{cA}	136.12 ± 2.68 ^{bB}
		Steaming	34.76 ± 1.51 ^{bA}	110.17 ± 3.87 ^{aA}
		Sous-vide	30.24 ± 1.96 ^{aA}	114.52 ± 3.93 ^{aB}
	Stalk	Fresh	54.38 ± 1.07 ^{aB}	113.76 ± 2.80 ^{bA}
		Steaming	59.94 ± 3.89 ^{bB}	95.13 ± 1.98 ^{aA}
		Sous-vide	58.70 ± 1.48 ^{bB}	98.02 ± 2.27 ^{aA}

Capital letters indicate significant differences between different parts of the same plant for fresh, steamed, or sous-vide processed samples. Lower case letters indicate significant differences between treatments for the same part of the plant.

427

428

429 **Figure 1**


A)
mg/100 g fresh sample
70
60
50
40
30
20
10
0
bB
bA
aA
aB
aA
aB
Fresh
Steaming
Sous-vide
Thermal treatment
Inflorescence
Stem
B)
mg/100 g fresh sample
160
140
120
100
80
60
40
20
0
bB
bA
aA
aA
aA
aA
Fresh
Steaming
Sous-vide
Thermal treatment
Inflorescence
Stem
C)
mg/100 g fresh sample
250
200
150
100
50
0
bB
bA
aA
aB
aA
aB
Fresh
Steaming
Sous-vide
Thermal treatment
Inflorescence
Stem
D)
mg/100 g fresh sample
200
180
160
140
120
100
80
60
40
20
0
bB
bA
aA
aB
aA
aB
Fresh
Steaming
Sous-vide
Thermal treatment
Inflorescence
Stem
E)
mg/100 g fresh sample
60
50
40
30
20
10
0
bB
bA
aA
aB
aA
aB
Fresh
Steaming
Sous-vide
Thermal treatment
Leaves
Stem
F)
mg/100 g fresh sample
120
100
80
60
40
20
0
cB
cA
aA
aB
bA
bA
Fresh
Steaming
Sous-vide
Thermal treatment
Leaves
Stem

431 **Figure 2**

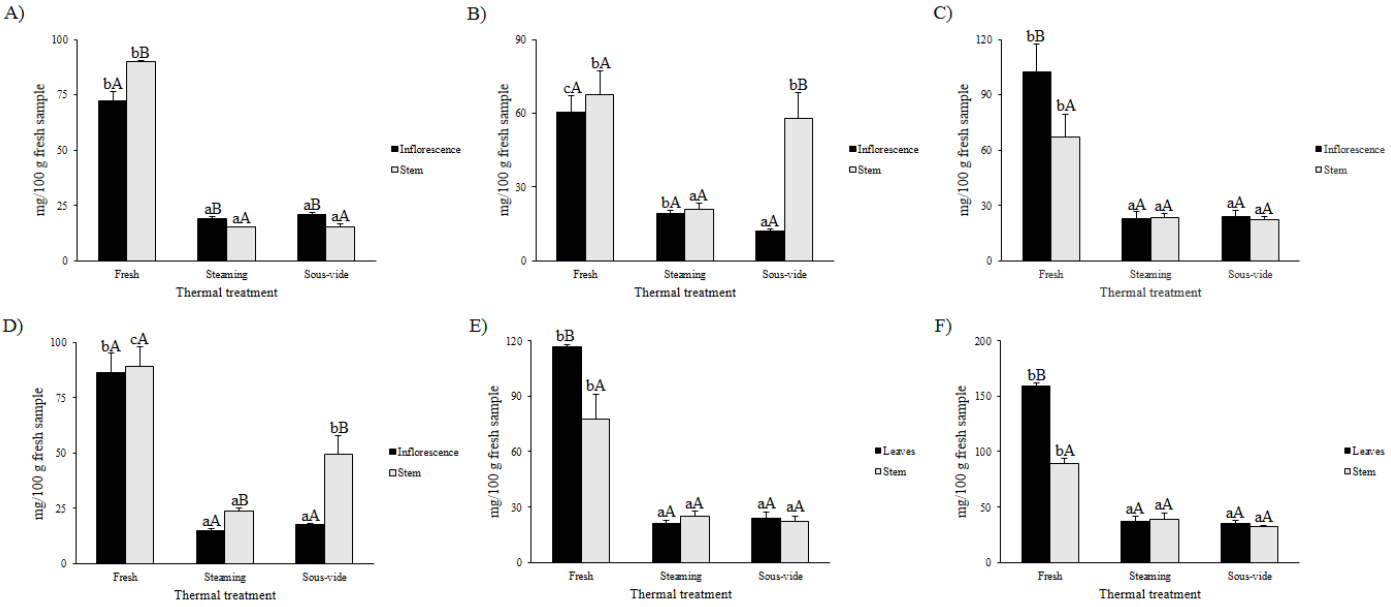
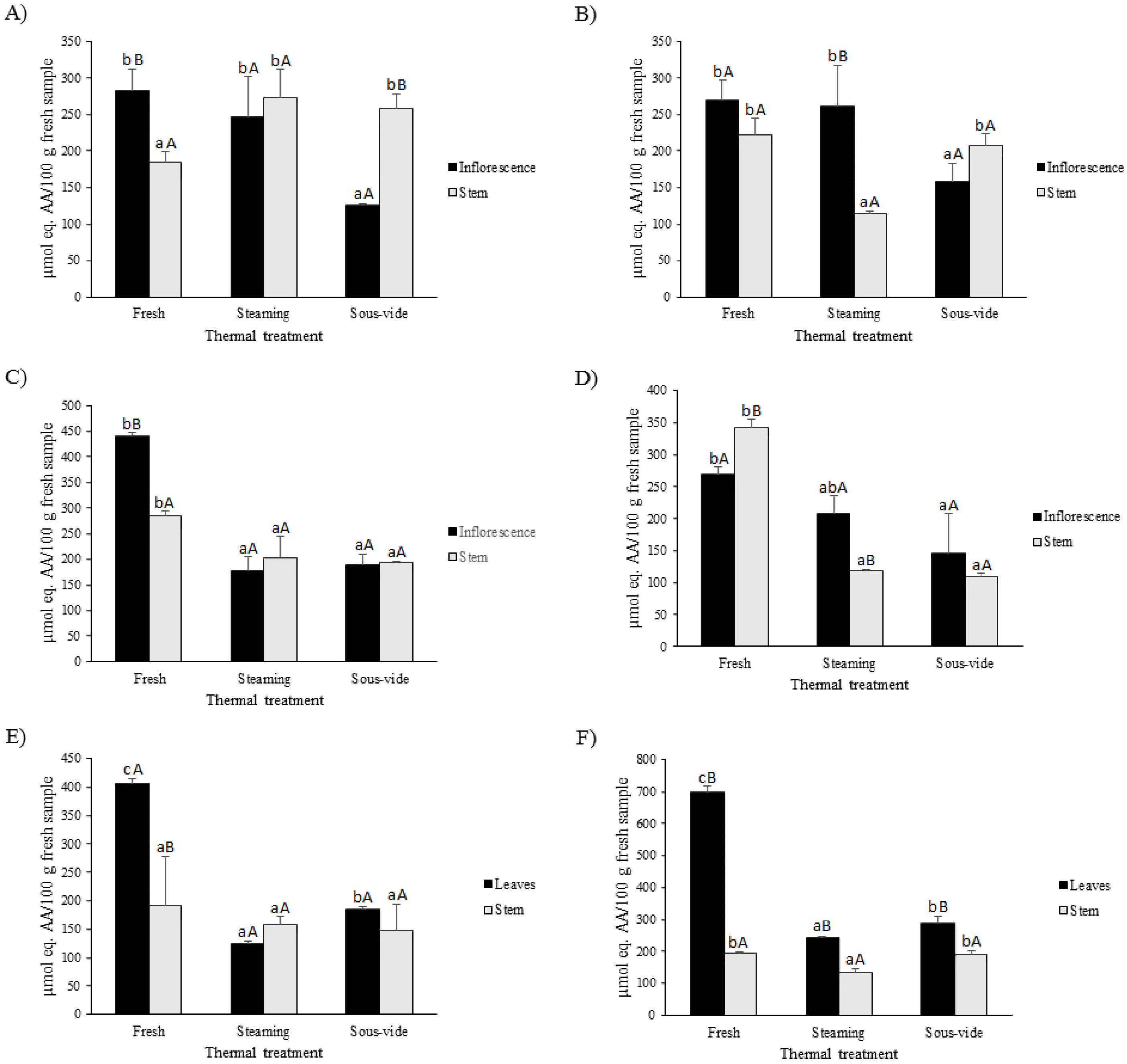
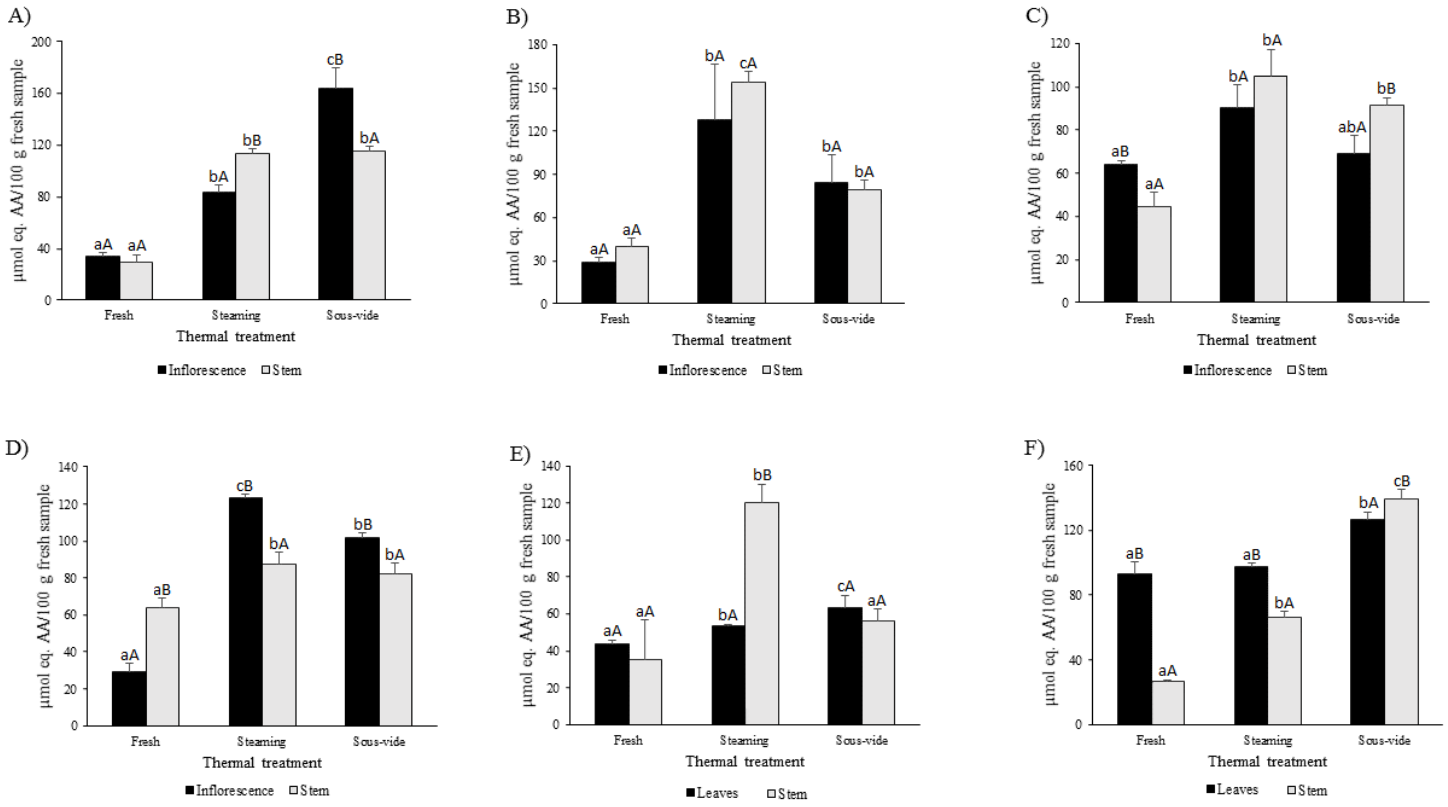

A)
B)
C)
D)
E)
F)
mg/100 g fresh sample
Thermal treatment
Fresh
Steaming
Sous-vide
Inflorescence
Stem
Leaves
bA
bB
cA
aA
aB

Figure 3


A)
B)
C)
D)
E)
F)
µmol eq. AA/100 g fresh sample
Thermal treatment
Fresh
Steaming
Sous-vide
Inflorescence
Stem
Leaves
bB
aA
bA
bA
aA
bB
bA
bA
bB
aA
aA
bA
bB
bA
aA
aA
aA
aA
bA
bB
abA
aB
aA
aA
cA
aB
aA
aA
bA
aA
cB
bA
aB
aA
bB
bA

434 **Figure 4**


A)
B)
C)
D)
E)
F)
µmol eq. AA/100 g fresh sample
Fresh
Steaming
Sous-vide
Thermal treatment
Inflorescence
Stem
Leaves

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