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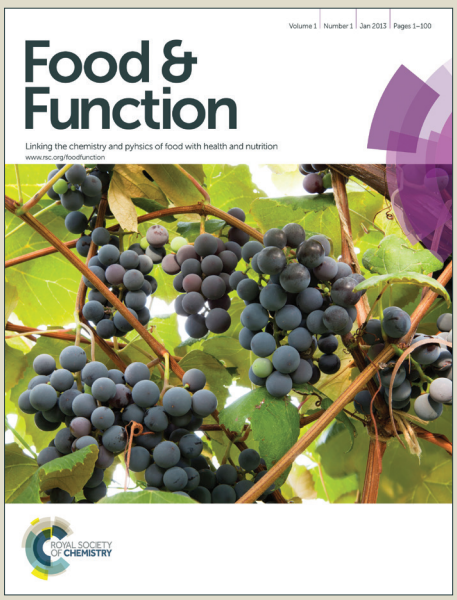


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# Impact of boiling on phytochemicals and antioxidant activity of green vegetables consumed in the Mediterranean diet

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## Abstract

The effect of boiling (10 minutes) on eleven green vegetables frequently consumed in the Mediterranean diet was evaluated. For that, some physicochemical parameters and the contents of vitamin C, phenolics and carotenoids, as well as the antioxidant activity, were determined in raw and boiled samples.

The raw vegetables analysed in this study were good sources of vitamin C, carotenoids and phenolic compounds, with contents ranging from 10.6 to 255.1 mg/100 g, 0.03 to 3.29 mg/100 g and 202.9 to 1010.7 mg/100 g, respectively. Boiling promoted losses in different extensions considering both the different bioactive compounds and the distinct vegetables analysed. Contrary to phenolics (more resistant), vitamin C was the most affected compound. Boiling also originated significant losses in the antioxidant activity of the vegetables. Considering all the parameters analysed, the vegetables most affected by boiling were broccoli and lettuce. The least affected ones were collard and *tronchuda* cabbage.



**Keywords:**

Green vegetables, boiling, carotenoids, phenolics, ascorbic acid, antioxidant activity.


## Introduction



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A characteristic of the so-called Mediterranean diet, recently recognized by UNESCO as Intangible Cultural Heritage, is the inclusion of a large variety of vegetables in the dishes. This behavior has, undoubtedly, a positive impact on the population's health. It has already been scientifically established that the ingestion of large amounts of vegetables reduces the risk of chronic injuries, particularly, cardiovascular diseases, cancers, cataract and macular degeneration, obesity and type 2 diabetes, and several degenerative disorders.[1-5] Inevitably, all these effects are related to the chemical composition of the consumed vegetables. Besides being rich in water and fiber and poor in fat and carbohydrates, many raw vegetables are also naturally rich in minerals, vitamins, and phytochemicals (carotenoids and phenolic compounds). Another feature of the Mediterranean diet is the minimal processing of foods. Vegetables are mostly ingested *in natura*, especially in salads or after being boiled in water. The main purpose of cooking is to make vegetables more edible, palatable and digestible. Additionally, by eliminating potential pathogens and reducing the intake of some anti-nutrients, boiling contributes for the safety of these foods.[6] The downside is that cooking may adversely affect the levels of nutrients and bioactive compounds, especially the heat-sensitive and water soluble ones. Although some studies reported the improvement of *in vivo* bioavailability of some phytochemicals when vegetables are cooked[7,8] many others pointed out significant losses of minerals,[9] vitamins,[10] total soluble proteins and soluble sugars,[11] carotenoids[12,13] and phenolic compounds.[14,15]

Nevertheless, the available data about boiling effects on the contents of vitamin C, carotenoids and phenolic compounds, as well as on the antioxidant activity, of green vegetables traditionally consumed in the Mediterranean diet are still scarce. The aim of this research was, therefore, to develop a comprehensive study about this topic, enabling a comparative analysis of 11 selected vegetables, encompassing 4 different genera.



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## Results and Discussion

*Raw vegetables*

Chemical characterization of the eleven green vegetables analyzed in this study, is presented in **Table 1**. Our results reveal that all the vegetables are quite similar in terms of moisture content (ranging from 96.2 to 98.0 %) and pH (values varied between 6.1 and 7.1). According to Morales et al.,[16] higher moisture contents provide higher tender and succulent properties in leafy vegetables. The presented results are in agreement with those authors, since lettuce stands out with the highest moisture value (98.0 g/ 100 g) and cabbage showed the lowest one (96.2 g/ 100 g). In what concerns to total soluble solids (TSS), the values are more dissimilar, ranging from 1.16 to 6.89 ºBrix. It is important to note that the highest and lowest values were determined in broccoli samples (rabe buds and rabe leaves, respectively). A similar behavior can be described for ash content, except that lettuce presented the lowest ash value (0.66 %), reflecting a comparatively lower mineral content. In turn, spinach presented the highest ash content (1.31 %).

The similarity in terms of physicochemical parameters contrasts with the diversity observed relatively to the contents of the analyzed bioactive compounds. Vitamin C levels of the analysed vegetables were quite variable. Lettuce presented the lowest content (10.6 mg/ 100 g), while collard displayed the highest one (255 mg/ 100 g).

The variability in terms of carotenoids was even larger: cauliflower had only 0.03 mg/ 100 g while spinach presented a 100-fold higher value (**Table 1**). Phenolic compounds were the most representative phytochemicals (from 203 mg/ 100 g for cauliflower and 1011 mg/ 100 g for spinach). Notably, all vegetables presented significant differences in these bioactive compounds. **Figure 1** depicts the clusters classification of the selected



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vegetables, considering the physicochemical parameters and contents of bioactive compounds, which reflects the described diversity.

The most similar samples, that constitute a well-defined cluster, are broccoli, broccoli rabe leaves and broccoli rabe buds, all presenting analogous amounts of carotenoids and phenolic compounds. The collard is closer to watercress than to the other Brassicaceae because both have higher contents of vitamin C, carotenoids and phenolic compounds. The cauliflower distinguishes itself from the other vegetables due to the lowest values of natural pigments (carotenoids and phenolic compounds). Spinach is also a vegetable apart because it has an exceptionally high carotenoid content.

***Boiled vegetables***

Consumers are aware to ingest a variety of vegetables and maximize the intake of beneficial antioxidants. However, in Mediterranean gastronomy, vegetables are often boiled to become more edible. It is known that antioxidant composition and bioavailability of vegetables are greatly affected by cooking methods.[14,15] According to **Table 1**, some of the raw vegetables are excellent sources of bioactive compounds. However, cooking can drastically influence the content and bioavailability of phytochemical compounds. In this study, 10 minutes of boiling had a minor influence in their pH and ash contents. The first parameter remained statistically unaltered ($p > 0.05$) for all. Only the broccoli rabe leaves and watercress experienced a significant decrease ($p < 0.05$) in their ash contents (-10.6 % and -17.8 %, respectively). TSS values were more affected by boiling, indicating the leaching of soluble sugars and organic acids into the water. Just five of the vegetables experienced significant reductions of TSS (broccoli rabe buds -20.6 %; cabbage -22.1 %; cauliflower -30.5 %; spinach -23.3 %; and watercress -16.9 %).



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In the other hand, the boiling effect on the contents of bioactive compounds seemed to be somewhat detrimental. **Figure 2** shows significant losses of these compounds for all the studied vegetables after boiling. Another feature that becomes apparent is a different extension loss for the various bioactive compounds and, for a given compounds class, it depended upon the vegetable. Thermal degradation and leaching into the boiling water are expected to be the primarily responsible for the verified decreases, explaining the high losses observed in this and in similar studies for other foods.[17]

Overall the major loss occurs in the vitamin C content with 77.7 % decrease in spinach. Somsub and colleagues[18] reported losses in ascorbic acid content between 24 % and 95 % in 13 selected Thai vegetables subjected to boiling for just 4 minutes.

The carotenoid degradation may reach 40 %, in the case of lettuce, savoy cabbage, and broccoli. Carotenoids are a class of lipophilic compounds, less susceptible to leaching and also less heat sensitive than vitamin C. There are even studies referring the bioavailability enhancement of some carotenoids with cooking.[7,8] This can be attributed to cell walls disruption with food processing procedures, facilitating their liberation from proteins. But this behavior can vary with the food matrix, being reported increases and decreases of these bioactive compounds.[19] For instance, Chang, Prasad and Amin[12] studied the effect of different domestic cooking methods on carotenoids retention in 7 commonly consumed leafy Malaysian vegetables. The authors concluded that 8 minutes of boiling imply large variations of lutein retention (from 0 to 418 %) and β-carotene (from 18 to 380 %). In a study with five tropical leafy vegetables from Africa, Djuikwo et al.[20] also recorded losses of total carotenoids from 5 to 20 % after 10 minutes boiling. Kao and colleagues[13] evaluated the effect of boiling in various carotenoid-rich green leafy vegetables, including Thai basil leaves and cilantro, and noted that total carotenoids content reached the maximum after boiling those vegetables for 5 minutes



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and 10 minutes, respectively. A negative effect on the total carotenoids contents of the vegetables was noticed with more boiling time.

Comparatively, total phenolic losses were smaller; nonetheless, they can account to nearly 30% in the case of lettuce and 20 % in broccoli (**Figure 2**). Watercress, spinach, savoy cabbage and the broccoli rabe leaves did not undergo substantial losses of phenolic compounds ($p > 0.05$).

Turkmen et al.[21] studied the effect of 5 minutes boiling on the contents of total phenolics of spinach and boccoli from Turkey and measured losses of about 6 % for the latter vegetable. Mazzeo and collaborators[22] also detected losses of about 4 % when the spinach was boiled for 10 minutes. Although they measured a reduction of approximately 31 % in the phenolics content of cauliflower, a value quite superior to that recorded here (~ 14 %) it that may be correlated with differences on the chemical composition of the vegetable (edaphoclimatic, cultivation and post-harvest conditions).

As noted above, the effect of cooking depends on the type of vegetable.[23] This has been mainly related with the fact that the morphology of the cells and organelles containing the various phytochemicals differs among vegetables.[24] However, the remaining chemical makeup and structure of the vegetables should also play a determinant role. For example it is known that the leaves of collard and *tronchuda* cabbage are covered by a relatively thick epicuticular waxy layer which may provide an additional barrier reducing the wettability and the mass and heat transfer, thus hampering the leaching of the compounds during boiling. Probably this is one of the reasons why such vegetables present lower losses than, for instance, lettuce. Anyway, a cluster analysis taking into account the losses of bioactive compounds reported above, suggests that the studied vegetables can be divided into two groups (**Figure 3**): a first one, comprising collard, *tronchuda* cabbage, cauliflower, savoy cabbage and watercress, i.e., vegetables that lost



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nearly half of the vitamin C and did not undergo significant losses in phenolic compounds; and another group of vegetables that suffered substantial losses of all bioactive compounds (cabbage, broccoli rabe leaves, broccoli rabe buds, broccoli and lettuce). Spinach was not grouped, probably due to the higher decrease in vitamin C contents.

***Effect of boiling on the antioxidant potential***

The antioxidant activity (AA) is a parameter that measures the combined effect of all antioxidants in preventing the harmful action of free radicals. Owing to the very different chemical nature of the antioxidants, the activity should be assessed by complementary methods. In this study, the DPPH$^{\bullet}$ radical scavenging activity and the β-carotene linoleate model assays were chosen for that purpose. Among all the tested vegetables, savoy cabbage, spinach and collard greens presented an exceptionally high percentage of antioxidant potential (> 80 %). A moderate antioxidant potential (50-70 %) was shown by watercress and *tronchuda* cabbage, while cauliflower and lettuce presented the lowest free radical scavenging activities (< 30 %) (**Figure 4**).

According to **Figure 4**, boiling can be associated with losses in the antioxidant activity of the vegetables commonly used in the Mediterranean diet, reaching nearly 58 % in the antioxidant capacity measured by the β-carotene bleaching (broccoli rabe buds) and 36 % against the DPPH$^{\bullet}$ radical (cauliflower). These values are similar to those already reported by other authors. For example, Faller and Fialho[25] in a study with vegetables registered radical scavenging capacity variations between -93 % and +16 %. It should be noted that the antioxidant activity may not be directly correlated with the total concentration of antioxidant compounds, due to synergistic or antagonistic effects.[26]

The correlation results (**Figure 5**) have revealed that AA is directly related to the



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phenolic compounds content (Pearson's $r_{DPPH}$ = 0.941, Pearson's $r_{\beta CL}$ = 0.484) and moderately correlated with carotenoids content (Pearson's $r_{DPPH}$ = 0.399, Pearson's $r_{\beta CL}$ = 0.580) and vitamin C (Pearson's $r_{DPPH}$ = 0.394; Pearson's $r_{\beta CL}$ = 0.224). These findings suggest that the reduction in antioxidant potential might be primarily related with the depletion of phenolic compounds and secondly of carotenoids compounds. Accordingly, vegetables that lost considerable amounts of phenolic and carotenoids compounds (cauliflower, lettuce and broccoli rabe buds) also exhibit substantial reduction in their antioxidant activity.

## Experimental

### *Standards and reagents*

2,6-Dichlorophenol-indophenol (Tillman's reagent), ascorbic acid, metaphosphoric acid, acetone, petroleum ether, sodium carbonate, ethanol, 2,2-diphenyl-1-picrylhydrazyl radical ($DPPH^{\bullet}$), β-carotene, chloroform, and Tween 40 emulsifier were all obtained from Sigma-Aldrich (St. Louis, MO, USA). The Folin-Ciocalteu reagent, gallic acid, and linoleic acid were purchased from Panreac Química S.L.U. (Barcelona, Spain). Ultrapure water from a Simplicity 185 system (resistivity 18.2 MΩ.cm; Millipore, Belford, USA) was used for all aqueous solutions preparation.

### *Samples and sample preparation*

According to the high cultural relevance in the Mediterranean diet, eleven green vegetables (**Table 2**) where chosen to perform this study. They were all obtained from local markets, in the district of Porto, Portugal.

About 2 kg of each vegetable were sampled. In the same day, each vegetable was prepared, cooked and submitted to the extraction process. All the material was washed



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220 in running water and dried with absorbing paper before random sampling in batches of
221 ~500 g. The edible parts of the vegetables (leaves and blooms) were cut into small
222 pieces.
223 All batches were divided into two equal portions, one maintained raw and the other
224 submitted to boiling. The boiling procedure was performed by adding each vegetable
225 sample to boiling tap water (~ 100 ºC) in a covered stainless-steel pot (1:5 vegetable/
226 water) and letting it there for exactly 10 minutes. This period was selected as the
227 minimum cooking time needed for adequate sample palatability and taste. Afterwards
228 the boiled samples were drained and dried carefully with absorbing paper prior to
229 further analysis.
230 Before physicochemical and phytochemicals evaluation, samples (raw and cooked)
231 were homogenized in a grinder (Grindomix GM 200, Retsch, Haan, Germany).
232 Aqueous extracts were obtained and used to determine some parameters, like phenolics
233 content and antioxidant capacity. In these cases, samples (~ 5 g) were stirred with 100
234 mL of water, at 25 ºC, for 1 h, protected from light, and the solids separated from the
235 extract by vacuum filtration. The extracts were stored at -20 ºC prior analysis.
236
237 ***Physicochemical characterization***
238 Moisture and ash contents, pH and total soluble solids (TSS) were determined in all
239 samples. A gravimetric assay was performed to evaluate the moisture content. Samples
240 (~5 g) were dried in a stove (WTC binder Klasse 2.0, Tuttlingen, Germany) at 105 ºC ±
241 1 ºC, followed by regular weighing up to a constant weight. Results were expressed as
242 water percentage (%). The mineral content was evaluated by incineration at 450 ºC and
243 results expressed in percentage (%). The pH value was measured in triplicate with a pH-
244 meter (Microprocessor pH Bench-top HI 8417, Hanna Instruments). TSS were



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quantified with an Atago, NAR-3T refractometer, properly adjusted and calibrated at 20 ºC with distilled water and the results expressed as ºBrix (an approximate value of total sugar content).

***Determination of antioxidant compounds***

***Determination of vitamin C***

Ascorbic acid content was determined according to a previously described method.[27] Briefly, samples were mixed with metaphosphoric acid (0.1 g/ L) for 45 min at room temperature, and filtered through Whatman nº 4 filter paper. Then, 1 mL of filtrate was mixed with 2,6-dichlorophenol-indophenol and absorbance was measured at 515 nm. A calibration curve of ascorbic acid (linearity range: 0.5-100 μg/ mL, $r$ = 0.9994) was prepared and the results were expressed in mg of ascorbic acid per 100 g of fresh sample.

***Total carotenoids content***

Total carotenoids content was determined according to Wang and Liu[28] with some modifications. Each vegetable (raw and boiled) was submitted to a previous extraction before quantification. Briefly, 5 g of homogenized sample was added to 50 mL of petroleum ether/acetone mixture (1:1, v/v), wrapped with aluminum foil and subjected to constant shaking during 30 min, at room temperature (25 ºC). After filtration, 3 mL of supernatant were collected and absorbance was measured at 445 nm. The total carotenoids content (mg/ 100 g of fresh sample) was determined according to the following equation:



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Total carotenoids (mg/ 100g) = ($A$ x $y$ x $10^6$)/ ( $A^{\%}_{1cm}$ x 1000 x $w$), where $A$ represents the absorbance of the extract at 445 nm; $y$ is the volume of extract (mL); $A^{\%}_{1cm}$ represents the extinction coefficient of carotenoids ( $A^{\%}_{1cm}$ = 2592), and w is the sample weight (g).

***Determination of total phenolic content***

Total phenolic contents were determined according to Costa and colleagues.[29] Aliquots of 0.5 mL of extract were added to Folin-Ciocalteu reagent (2.5 mL, previously diluted with water 1:10 v/v) and 2 mL of a sodium carbonate solution (7.5 % m/v). After 30 min incubation at room temperature, absorbance readings were performed at 765 nm. Total phenolics were quantified by means of a calibration curve of gallic acid (linearity range = 2-200 μg/ mL, $r$ = 0.9989) and results expressed in mg per 100 g of fresh weight.

***Antioxidant activity***

***DPPH• radical-scavenging activity***

This assay was evaluated according to Vinha et al.[30] in a microplate reader (BioTek Synergy HT, GENS5). The aqueous extracts (300 μL) were added to 2.7 mL of an ethanolic DPPH• solution (6 x $10^{-5}$ mol/ L). The mixture was vigorously stirred and absorbance determined at 515 nm, until a stable plateau was reached. DPPH• scavenging activity (RSA) was determined as the percentage of DPPH• discoloration using the following equation: % RSA = [($A_{DPPH•}$ – $A_S$)/ $A_{DPPH•}$] x 100, where $A_S$ represents the absorbance of the sample with DPPH• and $A_{DPPH}$ is the absorbance of the DPPH• solution.



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292 ***Inhibition of β-carotene bleaching***

293 β-carotene bleaching inhibition by neutralization of linoleate free radicals was also
294 evaluated according to Vinha et al.[27] A solution of β-carotene (2 mg in 10 mL of
295 chloroform) was prepared and then, 2 mL pipetted into a 100 mL round-bottom flask.
296 After the chloroform removal under vacuum (40 ºC), ~40 mg of linoleic acid, 400 mg of
297 Tween 40 emulsifier, and 100 mL of distilled water were added and vigorously shaken.
298 5 mL of this emulsion were transferred into different test tubes containing 1 mL of
299 sample extracts. Immediately after adding the emulsion to the test tubes, the zero time
300 absorbance was read at 470 nm. The tubes were incubated in a water bath at 50 ºC.
301 Measurement of absorbance continued until complete β-carotene bleaching and the
302 antioxidant activity was obtained by the following equation: (β-carotene content after 2
303 h of assay/initial β-carotene content) x 100.

304

305 ***Statistical analysis***

306 Statistical analysis was performed using SPSS v. 21 (IBM Corp., Armonk, NY, USA).
307 Data of all analysis, in triplicate, are expressed as mean ± standard deviation. After
308 validating the assumptions of multivariate normality and homogeneity of variance-
309 covariance, a MANOVA analysis was used to compare different vegetables. Whenever
310 the MANOVA analysis detected significant effects, an ANOVA, for each of the
311 parameters experimentally determined, was performed followed by Tukey's HSD post-
312 hoc test. The vegetables were grouped into homogeneous groups through a hierarchical
313 Cluster analysis by the method of least distance (Nearest Neighbor) using the squared
314 Euclidean distance as the measure of dissimilarity. A 0.05 significance level was



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315 considered for all tests. *p*-values inferior to 0.05 were considered to be statistically
316 significant.
317 Mean comparison between raw and boiled vegetables was made through independent
318 samples t-test. Pearson correlation tests were used to ascertain the existence of linear
319 relationships between the contents of bioactive compounds and antioxidant activity.
320

321 **CONCLUSIONS**

322 Upon boiling (10 min), the vegetables analysed in this study suffered considerable
323 losses of vitamin C and carotenoids. In what concerns to phenolic compounds, the
324 decrease content was not so substantial and generalized. In addition to the intrinsic
325 properties of the compounds, boiling effect seemed to be influenced by the nature of the
326 vegetable matrix, probably due to differences in chemical composition and
327 cellular/organelle structure. Globally, the vegetables most affected were broccoli rabe
328 leaves and buds, broccoli and lettuce. The least affected were collard and *tronchuda*
329 cabbage. A cluster analysis taking into account the losses of bioactive compounds
330 evidences two groups of vegetables. Collard, *tronchuda* cabbage, cauliflower, savoy
331 cabbage and watercress formed a group that lost nearly half of the vitamin C and did not
332 undergo significant losses of phenolic compounds. The other group included vegetables
333 that lost substantial amounts of all bioactive compounds (cabbage, broccoli rabe leaves,
334 broccoli rabe buds, broccoli and lettuce). Since the antioxidant potential was directly
335 correlated with the contents of bioactive compounds, particularly with total phenolics,
336 this parameter was also significantly reduced upon boiling, especially with cauliflower,
337 lettuce and broccoli rabe buds. Our results may be useful to consumers on the choice of
338 each vegetable, considering their losses after boiling. Moreover, this study improves the
339 consumption of all vegetables, which may provide benefits to health.



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433 **Figure Captions**

434

435 **Figure 1.** Dendrogram resulting from a cluster analysis of the studied vegetables,
436 considering the physicochemical parameters and contents of bioactive compounds.

437

438 **Figure 2.** Contents of bioactive compounds of the different vegetables before and after
439 boiling. * indicates significant differences ($p < 0.05$).

440

441 **Figure 3.** Overall similarity of the vegetables in terms of bioactive compounds loss.

442
443
444 **Figure 4.** Antioxidant activity (A.A.) of vegetables, before and after boiling, by the β-
445 carotene linoleate model system (βCL) and on DPPH• radical scavenging activity. *
446 indicates significant differences ($p < 0.05$) caused by boiling.

447

448 **Figure 5.** Pearson correlation analysis between the antioxidant activity (βCL and
449 DPPH) and the bioactive contents (vitamin C, total carotenoids and phenolics). *
450 indicates that the correlation is significant at the 0.05 level.

451

452

453

454

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456 **Table 1.** Physicochemical parameters and bioactive compounds of 11 types of raw vegetables.

| | Physicochemical Parameters | | | | Bioactive Compounds | | |
|---|---|---|---|---|---|---|---|
| **Fresh Vegetable** | **Moisture (%)** | **pH** | **TSS (ºBrix)** | **Ash (%)** | **Vitamin C (mg/100g)** | **Carotenoids (mg/100g)** | **Phenolics (mg/100g)** |
| **Broccoli** | 97.3±0.1$^{a,b,c,d}$ | 6.37±0.06$^{d,e,f}$ | 3.09±0.06$^{d}$ | 0.97±0.02$^{c}$ | 49.6±1.0$^{h}$ | 1.11±0.01$^{f}$ | 455.9±1.6$^{h}$ |
| **Broccoli rabe buds** | 96.7±0.2$^{c,d,e}$ | 6.36±0.10$^{d,e,f}$ | 6.89±0.05$^{a}$ | 1.04±0.03$^{b,c}$ | 100.8±1.6$^{d,e}$ | 1.09±0.01$^{f}$ | 554.3±0.8$^{f}$ |
| **Broccoli rabe leaves** | 97.7±0.4$^{a,b,c}$ | 6.52±0.11$^{c,d,e,f}$ | 1.16±0.07$^{g}$ | 0.80±0.04$^{d,e}$ | 115.0±7.9$^{b,c}$ | 1.21±0.01$^{e}$ | 403.6±1.6$^{i}$ |
| **Cabbage** | 96.2±0.3$^{e}$ | 6.50±0.08$^{c,d,e,f}$ | 2.04±0.06$^{f,e}$ | 0.95±0.07$^{c,d}$ | 90.8±3.4$^{e,f}$ | 0.41±0.01$^{i}$ | 561.8±0.9$^{e}$ |
| **Cauliflower** | 96.9±0.4$^{b,c,d,e}$ | 6.82±0.03$^{a,b,c}$ | 3.97±0.08$^{c}$ | 0.95±0.02$^{c,d}$ | 69.1±1.7$^{g}$ | 0.03±0.01$^{j}$ | 202.9±0.6$^{k}$ |
| **Collard** | 96.5±0.3$^{d,e}$ | 6.13±0.02$^{f}$ | 2.98±0.03$^{d}$ | 0.96±0.03$^{c,d}$ | 255.1±9.7$^{a}$ | 2.50±0.07$^{b}$ | 747.8±0.6$^{c}$ |
| ***Tronchuda* cabbage** | 96.5±0.6$^{d,e}$ | 6.93±0.10$^{a,b}$ | 2.20±0.13$^{e}$ | 1.04±0.08$^{b,c}$ | 105.8±8.0$^{d,c}$ | 1.39±0.01$^{d}$ | 608.9±0.9$^{d}$ |
| **Lettuce** | 98.0±0.4$^{a}$ | 6.43±0.42$^{d,e,f}$ | 1.83±0.05$^{f}$ | 0.66±0.03$^{e}$ | 10.6±0.9$^{i}$ | 0.80±0.01$^{h}$ | 393.0±1.5$^{j}$ |
| **Savoy cabbage** | 96.4±0.2$^{d,e}$ | 6.37±0.03$^{d,e,f}$ | 3.95±0.10$^{c}$ | 1.05±0.05$^{b,c}$ | 70.7±1.4$^{g}$ | 0.97±0.01$^{g}$ | 816.8±0.8$^{b}$ |
| **Spinach** | 97.8±0.3$^{a,b}$ | 7.10±0.04$^{a}$ | 2.02±0.07$^{f,e}$ | 1.31±0.04$^{a}$ | 40.8±1.6$^{h}$ | 3.29±0.05$^{a}$ | 1010.7±1.1$^{a}$ |
| **Watercress** | 96.4±0.6$^{d,e}$ | 6.63±0.02$^{b,c,d,e}$ | 1.89±0.04$^{f}$ | 1.18±0.08$^{a,b}$ | 122.3±1.6$^{b}$ | 1.98±0.02$^{c}$ | 502.7±1.4$^{g}$ |

457 *Values expressed as mean±standard deviation obtained from 3 measurements per replicate. Within each column, different superscript letters represent significant differences
458 between samples ($p < 0.05$).

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460 **Table 2.** List of the 11 samples selected for this study, identified by their common and scientific names, respectively.

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| ***Vegetables*** | |
|---|---|
| **Common name** | **Scientific name** |
| Broccoli | *Brassica oleracea* var. *italica* |
| Broccoli rabe buds | *Brassica rapa* var. *rapa* |
| Broccoli rabe leaves | *Brassica rapa* var. *rapa* |
| Cabbage | *Brassica oleracea* var. *capitata* |
| Cauliflower heads | *Brassica oleracea* var. *botrytis* |
| Collard | *Brassica oleracea* var. *acephala* |
| *Tronchuda* cabbage | *Brassica oleracea* L. var. *costata* DC |
| Lettuce | *Lactuca sativa* var. *latina* |
| Savoy cabbage | *Brassica oleracea* var. *sabauda* L. |
| Spinach | *Spinacia oleracea* |
| Watercress | *Nasturtium officinale* |

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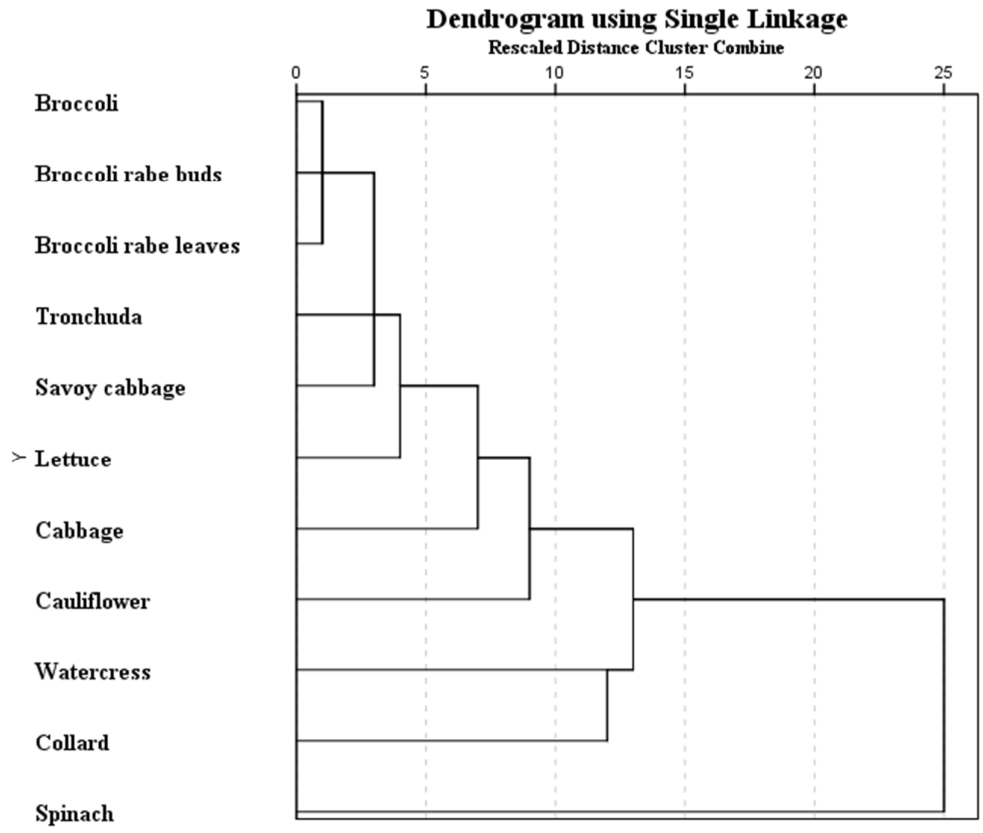


**Figure 1.**

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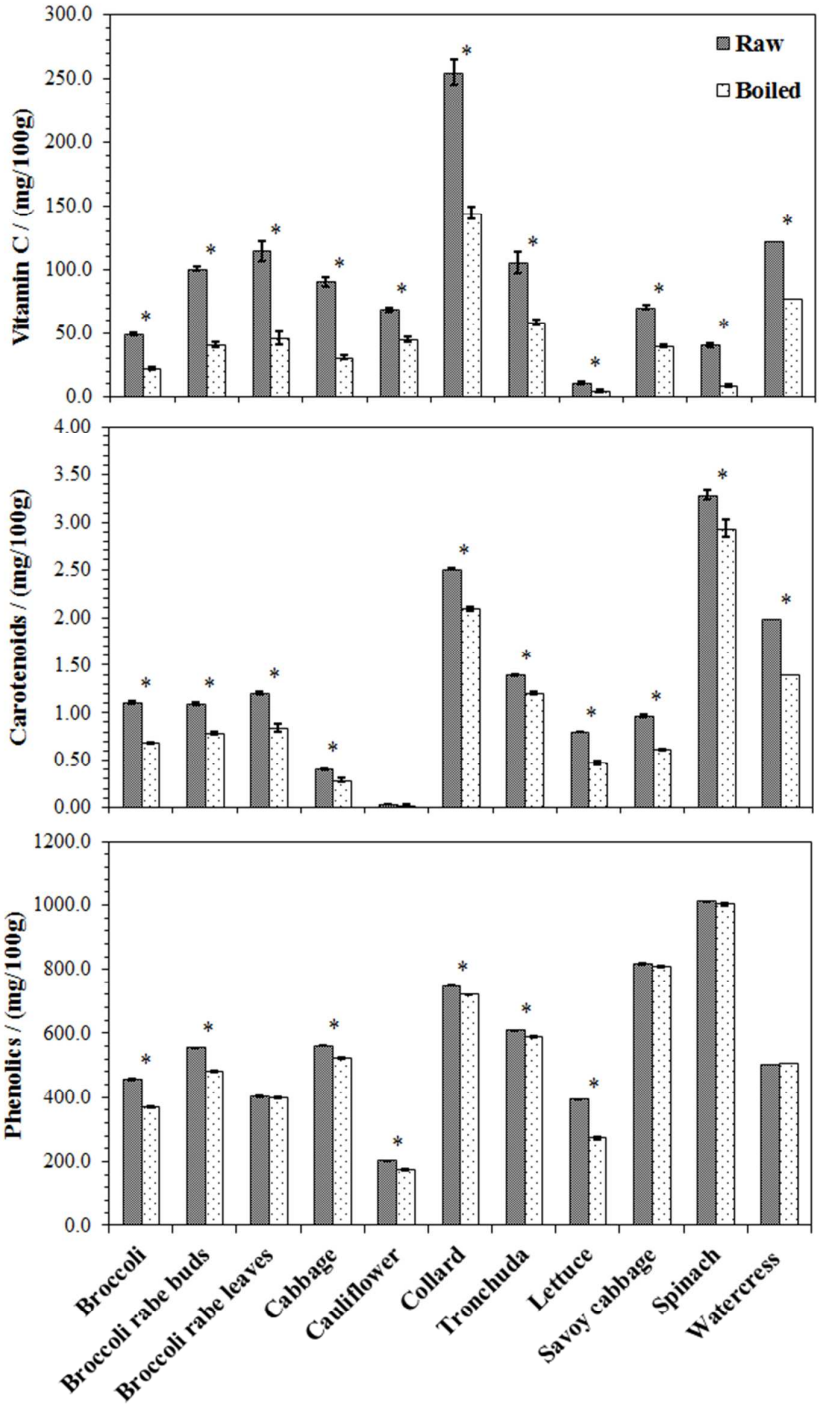


**Figure 2.**

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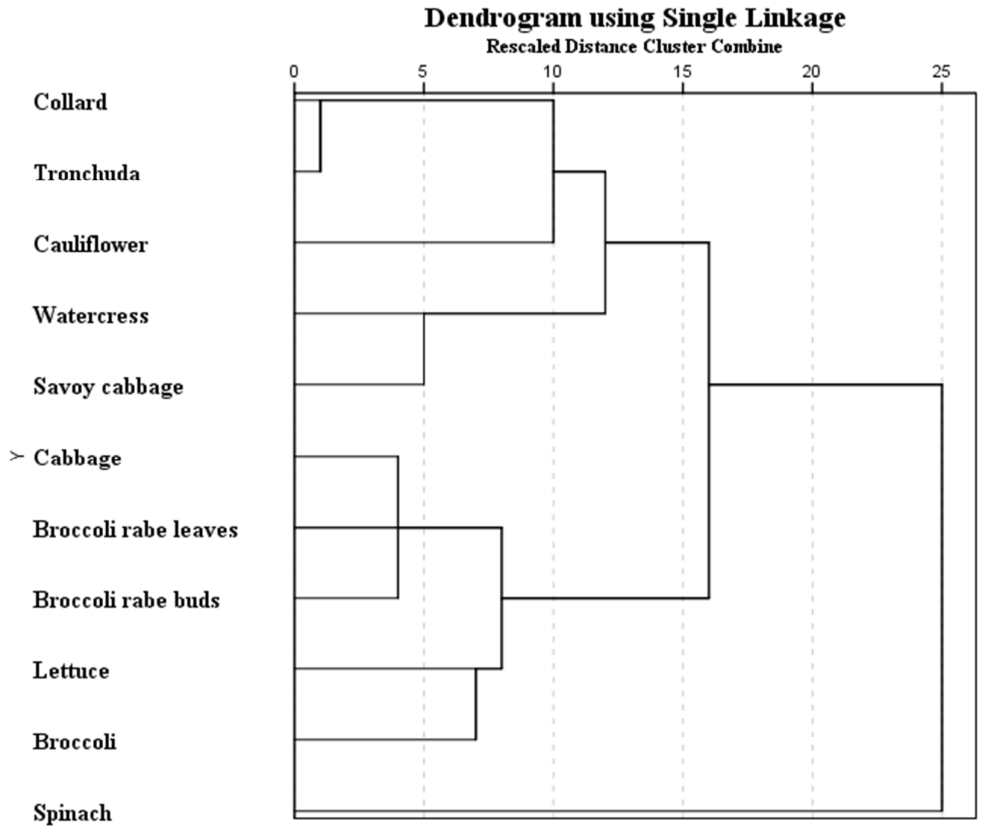


**Figure 3.**

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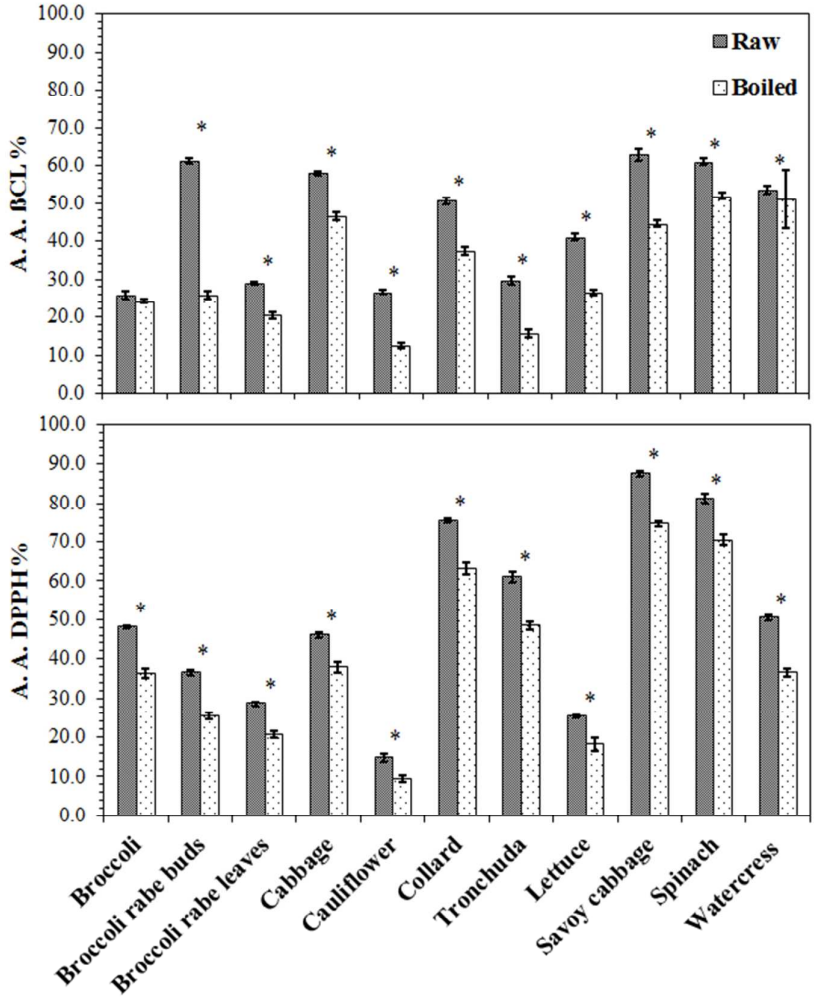


**Figure 4.**

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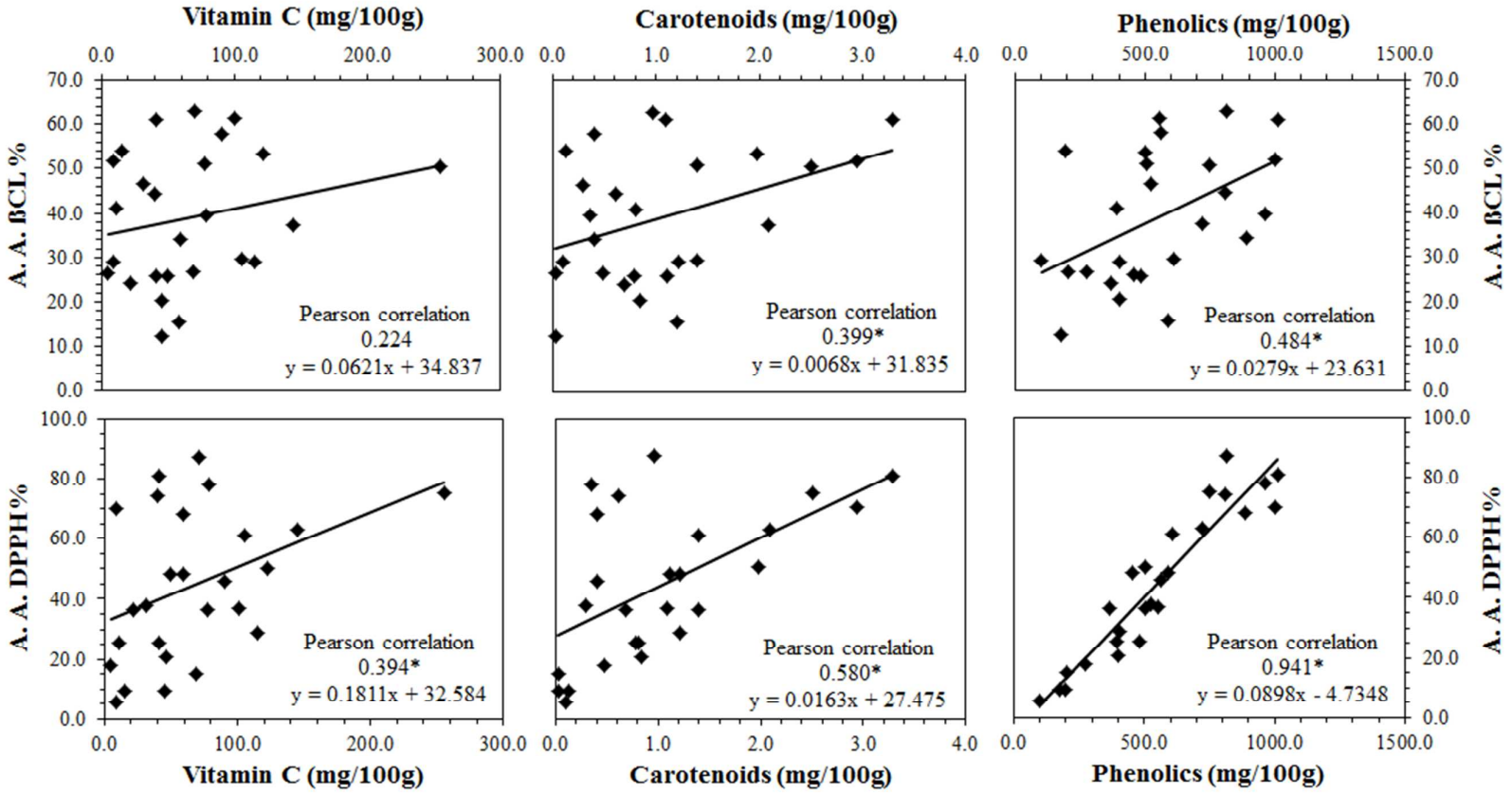


**Figure 5.**

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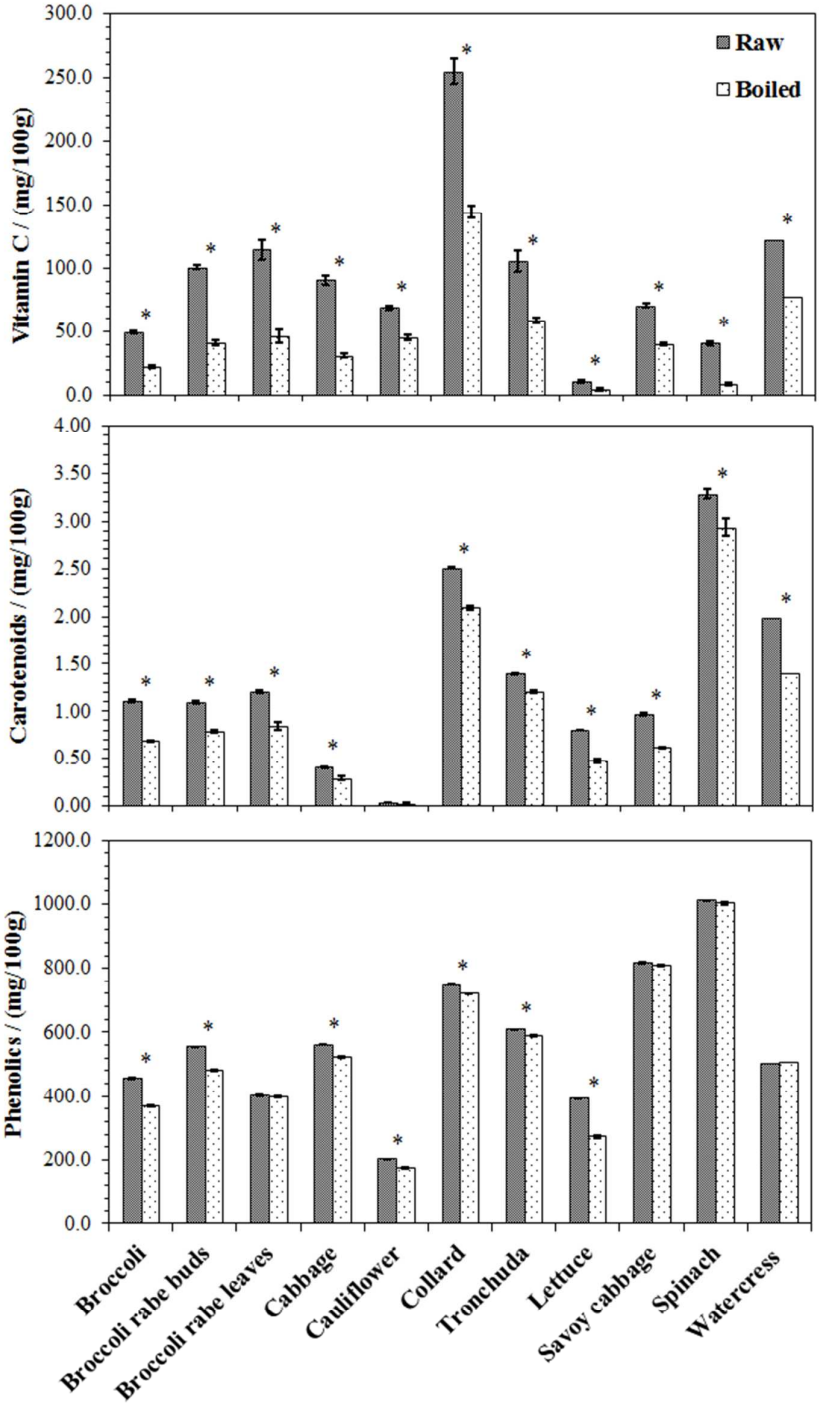
TOC graphic/Graphical abstract

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