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Influence of Treatment and Cooking Time on the Antioxidant Capacity of Different Vegetables Used in Atlantic and Mediterranean Diets

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Abstract Evidence from epidemiological studies has strongly suggested that diets rich in fruits and vegetables play a vital role in disease prevention. The aim of this study was to determine total phenolic content (TPC) and reducing power (RP) for nine vegetables that are normally consumed in Atlantic and Mediterranean diets. In this study vegetables were analyzed when fresh, and then again after heat processes (cooking in boiling water and steam) were applied for different lengths of time. The vegetable showing highest total phenolic content was the Brussels sprout; while zucchini had the lowest content. Green beans presented the highest reducing power, while peppers and cauliflower presented the lowest values. Heat treatment significantly reduced the concentration of total phenolic content and reducing power in all of tested vegetables; however, steam cooking resulted in lower losses. The loss of the total phenolic content and reducing power were higher when heat processes were applied for longer amounts of time. From a nutritional standpoint, it is advisable to use the least aggressive method (steaming) for as short a time as possible

Keywords: *total phenolic content, reducing power, Atlantic and Mediterranean diet, vegetables*

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1. Introduction

Vegetables, fruit, and their nutrients as vitamins, fiber and bioacitve compounds are potent effectors of biological systems in humans. The protective effects of these foods indicated in epidemiologic studies, are not only observed when given in pharmacologic doses of plant foods or their constituents, but do occur when intakes are a normal part of a one's diet [1]. Therefore, diet-derived antioxidants are especially important in protecting organisms against chronic diseases [2]. Epidemiologic data support the association between the high intake of vegetables and fruits and a lower risk for cardiovascular disease [3], several common cancers [4], central nervous system disorders, as well as aging [5].

The wide variation in total polyphenol content in vegetables might arise from the variety of the plant, stage of ripening, place of cultivation, climate condition, fertilization, sample collection, transportation, sample preparation, and methods of analysis [6]. The content of polyphenols in foods is also influenced by the cooking method [7].

Reactive oxygen species (ROS) are a group of oxidants formed during oxygen metabolism that appears to be involved in the pathogenesis of many human diseases. Oxidative stress develops when the generation of ROS overwhelms the scavenging capacity of antioxidants [8]. Hence, certain amounts of exogenous antioxidants are constantly required to maintain an adequate level of antioxidants in order to balance the ROS [9]. As exogenous antioxidants, polyphenols take part in antioxidant defense mechanisms, preventing damaging effects of reactive oxygen species (ROS) on DNA, proteins, and lipids [10].

Polyphenols represent the largest group of water-soluble phytochemicals [10,11,12]. The large groups of compounds that are known as polyphenols are products which come from the secondary metabolism of plants; during this process they may perform various functions as protection against pathogens and herbivores, and are also pigments that attract pollinators. They have structures with hydroxil group bound to at least one aromatic ring and conjugated double bonds, which exert their antioxidant activity [13]. Flavonoid and phenolic acids are the polyphenols that are most commonly found in plant foods [14].

Polyphenols are common constituents of the human diet, with fruits and vegetables being the major dietary source of these bioactive compounds [15]. Polyphenols can interact with specific steps and/or proteins regulating the apoptotic process in different ways depending on their concentration, cell system, and on the type or stage of the pathological process. Because of their ability to modulate cell death, polyphenols have been proposed to be chemo preventive and therapeutic agents [5]. Other studies support the beneficial effects of the intake of polyphenols on the cardiovascular system [7, 11].

The aim of the present study was to measure the total phenolic content (TPC) and reducing power (RP) in different vegetables commonly included in the Atlantic and Mediterranean diets.

2. Material and Methods

2.1. Chemical and Reagents

Acetone, Sodium carbonate anhydrous, Folin-Ciocalteu´s phenol reagent 2N, Gallic acid 98%,Trichloroacetic acid, Ferric chloride hexahydrate, Potassium ferricyanide, Methanol HPLC-grade, Sodium phosphate dibasic and Milli-Q water were used. Reagents were analytical grade and were purchased from Acros, Panreac, Scharlau or Sigma-Aldrich

2.2. Samples

In the present study, nine fresh plant products were used: green beans (Phaseolus vulgaris L. var helda), pepper (Capsicum spp.), zucchini (Cucurbita pepo L. var elite), chard (Beta vulgaris L. var. cicla L. subvar. lyon), spinach (Spinacia oleracea L. var. inermis), broccoli (Brassica oleracea L.var. itálica L. subvar. brocoli), cauliflower (Brassica oleracea L. var. botrytis L. subvar. cordel green), cabbage (Brassica oleracea L. var. capitata L subvar. alba), and brussels sprouts (Brassica oleracea L., var. gemmifera subvar bruselas). We selected these plant foods because there were in season and there are typical of Mediterranean and Atlantic diets. Approximately 1.0 kg of each vegetable were taken at random as samples from the market shelves in a local market in Lugo (Galicia, N.W. Spain) and kept at refrigeration temperature until analysis. The vegetables were always analyzed within 48 h of their acquisition.

Before analysis, the vegetables were washed with water. For green beans, pepper and zucchini, the whole vegetable was used that were cut into 3-4 cm pieces were used. In the case of Swiss chard, spinach, cabbage and Brussels sprouts, the outer or damaged leaves were removed. In broccoli and cauliflower, the flowers were used. Then each batch of clean and sliced vegetables, was divided into three parts, one for analyzing them fresh, and the other two to be subjected to heat treatments of cooking (boiling water and steam), for four different lengths of time in each case (3, 5, 10, and 15 minutes.).

After the heat treatments, vegetables were cooled in an ice-and-water bath for 5 min, then drained and dried on filter paper for another 5 min. Finally, vegetables were refrigerated at 4 °C for 5 minutes.

A total of 81 samples were analyzed in triplicate; thus, there were 9 fresh, 36 boiled and 36 steamed samples.

The measurements were taken with fresh vegetables, and then again after heat processes (cooking in boiling water and steam) were applied for different lengths of time, so as to evaluate the antioxidant potential of these vegetables and to establish which cooking method is the most suitable one to use in order to minimize the loss of these compounds.

2.3. Heat Treatments

For boiling water cooking process, vegetables were added to boiling water at 100 °C in a ratio of 2:1 [16].

For the steam cooking process, specific vessels were used. Boiling water at 100 °C was poured into the bottom of the cooking vessel and then the vegetables were placed on top of the steamer basket.

2.4. Determination of Total Phenolic Content (TPC)

Total phenolic content (TPC) was determined according to the method developed by Otto Folin and Ventila Ciocalteu [17]. 0.3 g of previously crushed fresh samples were accurately weighed and 20 mL of 70% preserved acetone at 4 °C were added [18]. Samples were mixed on the Vortex and left to stand at 4 °C overnight. The solution obtained was filtered through paper Filter-Lab 1238. Ten mL of a solution of Folin-Ciocalteu's 1.10 reagent phenol 2 N were adde to 2 mL of the above filtrate and mixed carefully in a Vortex in order to homogenize the two components. After 3 minutes standing, 8 mL of a solution of sodium carbonate (75 g/L) were added and mixed again in the Vortex. The mixture was incubated at 25 °C for 90 minutes. After that time, the samples showed a blue color, and absorbance at 760 nm was measured. Each determination was performed in triplicate.

The TPC for each sample was calculated and expressed in mg of gallic acid equivalents /100 g of fresh weight from the calibration curve obtained.

2.5. Reducing Power (RP)

The reducing power was determined according to the method indicated by Atmani et al. [19] and Stajčić et al. [20]. Approximately 0.5 g of a previously crushed fresh sample was accurately weighed. Then, 20 mL of methanol maintained at 4ºC were added [18]. Samples were mixed in the Vortex and left to stand at 4 °C overnight. 2.5 mL of the above solution were mixed with a phosphate buffer (2.5 mL, 0.2 M/L, pH 6.6) and potassium ferricyanide [K3Fe(CN)6] (2.5 mL, 1%). The mixture was incubated at 50ºC for 20 min. A portion (2.5 mL) of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged at 6500 rpm for 10 min at room temperature. The upper layer of the solution (5 mL) was mixed with Milli-Q water (5 mL) and FeCl3 (1 mL, 0.1%). After 10 minutes, a prussian blue color (reduced Fe+3 a Fe+2) developes in the samples and the absorbance was read at 700 nm. An increased absorbance of the reaction mixture indicated increased reducing power. All analyses were run in triplicate and averaged.

2.6. Statistical Treatment

Data were reported as mean±SD for triplicate determinations. All statistical calculations were performed using SPSS v.21.0 for Windows (SPSS Inc., Chicago, Ill., U.S.A.).

The existence of significant differences between the fresh products was evaluated by a one-way ANOVA (product) followed by Tukey's test for post hoc comparisons. The existence of significant differences for each product after cooking for different lengths of time was evaluated by a two-way ANOVA, (treatment and cooking time) with interaction, followed by Tukey's test for post hoc comparisons and t-Student comparison of means for dependent samples. If the interaction was significant, a separate study of each factors were performed using one-way ANOVA (cooking time) for each treatment and t-Student (treatment) for each cooking time.

3. Results and Discussion

3.1. TPC of Fresh Vegetables

Total phenolic content was calculated based on the standard curve ($R^2 = 0.9961$). Values (mean±SD) of TPC, (mg gallic acid equivalents (GAE) / 100 g of fresh weight), is shown in Table 1. After one-way ANOVA aplication, significant differences ($p<0.05$) among products were found except for green beans and cabbage (Table 1).

As shown above, the TPC of the vegetables studied varies greatly, in a range of 16.4 to 271.07 mg GAE/100 g of F.W. This variability coincides with that found by different authors. Sreeramulu and Raghunath, [21] indicated that the total phenolic content of vegetables commonly consumed in India (roots, tubers and other vegetables) ranges from 27 to 339 mg gallic acid/100 g fresh weight and that red cabbage had the highest, and ridge gourd the lowest TPC. In the study by Tharasena and Lawan [22], the TPC of twenty-one varieties of selected vegetables purchased from the local market and consumed as side dishes were determined, and the result showed that the TPC ranged from 20 (Boub ngu) to 166 mg gallic acid/100 g fresh weight (Pak bung thai maung). Kaulmann et al. [10] indicated that polyphenol content varied considerably between the different Brassica and plum varieties, with the lowest concentrations for white Brassica varieties (5.4– 61.5 mg gallic acid/100 g fresh weight) and highest concentrations for red and green Brassica varieties (12.5–139.0 mg gallic acid/100 g fresh weight). Equally, Korekar, et al. [23], studied the TPC in seventeen natural populations of Seabuckthorn (SBT), and affirmed that high variability within and among populations were observed. The TPC in SBT ranged from 964 to 10.704 mg gallic acid/100 g fresh weight, between individuals in the population studied. Along other lines, Li et al. [24] indicated that significant differences were found in parts of the same Amaranthus plant and among the different species. In Amaranthus plants the total phenolic content (TPC) ranged from 1.04 to 14.94 mg gallic acid/100 g dry weight. Contents much higher than the above were found by Kongkachuichai et al. [6] who compared the TPC of some Thai indigenous vegetables, such as mon-pu (4762.76 mg gallic acid/100 g fresh weight) and young cashew leaves (4075.79 mg gallic acid/100 g fresh weight).

Due to this variability, it is important to know the polyphenol content of the edible portion of those vegetables most consumed in the evaluated diets. Among the vegetables analyzed in the study at hand, Brussels sprouts presented a significantly higher content of TPC (271.07 mg gallic acid equivalents/100 g of fresh weight) (Table 1). Furthermore, the present work also corroborates the work carried out by Ismail, Marjan, and Foong [25], who found that among all the fresh vegetables studied, spinach had the highest phenolic content, even higher than cabbage.

In the present study, the vegetable showing the lowest TPC content was zucchini (16.36 mg gallic acid equivalents/100 g of fresh weight); this data coincides with that indicated by Cieslik, Greda, and Adamus [26] and Hervert-Hernández et al. [27]. In wheat flake samples, the TPC detected according to the Folin–Ciocalteu method ranged between 39.61 and 88.59 mg gallic acid/100 g fresh weight [28].

3.2. Influence of Treatment and the Cooking Time on TPC of Vegetables Studied

Data on phenolic content in cooked green vegetables are scarce, and further, differences have been found in the effect on the TPC content, depending on the applied treatment, its conditions and vegetable food. The results [29] show that antioxidant components and antioxidant activity in broccoli were lost heavily during conventional and microwave cooking. These losses need to be taken into account when calculating the dietary intake of these compounds from the cooked broccoli.

Wachtel-Galor, Wong, and Benzie [30] studied the TPC for four vegetables unprocessed and processed using three different cooking methods (microwaving, boiling and steaming) were studied. When comparing the effects of the three cooking methods, steaming was found to maintain the highest total phenolics value, followed by boiling. Microwaving showed the lowest value. Their results on total phenolics showed that cooking had a negative effect on the TPC after 5 and 10 min when compared with the raw vegetables. Gorinstein et al. [31] studied garlic, white and red onions subjected to bleaching and boiling, were evaluated and the authors concluded that comparative control shows that bleaching for 90 seconds of all the vegetables studied most fully preserves contents of bioactive compounds and the level of antioxidant activity. Faller and Fialho [32] indicated that cooking was found to lead to reductions in the antioxidant capacity for most vegetables, and suggesting amount consumed of these phytochemical can be overestimated when using data from raw vegetables.

For this reason, the TPC of nine vegetables which are characteristic of Atlantic and Mediterranean diets were determined after heat processing them with the two most commonly used methods (boiling water and steam) and four different lengths of processing time in each case. The results were analyze to know the influence of the type of treatment and cooking time through a two-way ANOVA with interaction (Table 2).

Figure 1 shows the profile of the TPC content, depending on the treatment and cooking time (boiling water and steam) of each of the foods studied compared to baseline (fresh).

Table 1. TPC of 9 fresh vegetables and one-way ANOVA differences

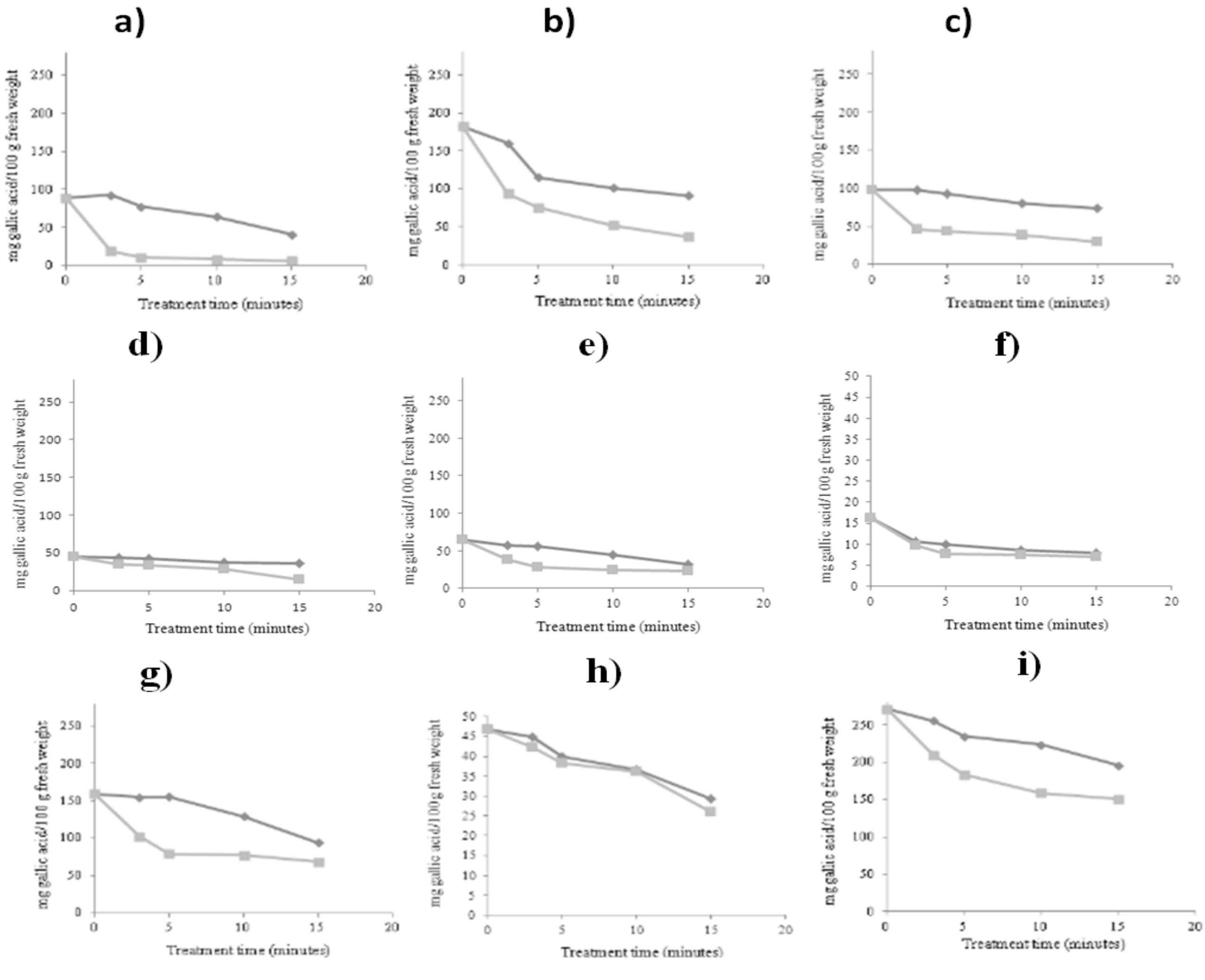
Plant species	TPC (mg GAE/100 g FW)
Broccoli (*Brassica oleracea L.var. itálica L. subvar.brocoli)*	97.61 ±0.40^{d}
Brussels sprouts (*Brassica oleracea L. var. gemmifera subvar bruselas)*	271.07 ±6.10^{f}
Cabbage (*Brassica oleracea L. var. capitata L. subvar. alba)*	46.81 ±1.52^{a}
Cauliflower (*Brassica oleracea L. var. botrytis L. subvar. cordel green)*	64.39 ±1.95^{c}
Chard (*Beta vulgaris L. var. cicla L. subvar. lyon)*	182.52 ±2.04^{g}
Green beans (*Phaseolus vulgaris L. var helda)*	44.95 ± 0.80^{a}
Pepper (*Capsicum spp.)*	159.08 ± 0.16^{b}
Spinach (*Spinacia oleracea L. var. Inermis)*	88.82 ±5.87^{h}
Zucchini (*Cucurbita pepo L. var. elite)*	16.36 ±1.11^{e}

Values are presented as mean values + SD (n=3)
Different letters indicate significant differences (p<0.05).

Table 2. Two-way ANOVA (T, t) with interaction on TPC

Plant species	(T)	(t)	(Txt)
Broccoli (*Brassica oleracea L.var. itálica L. subvar.brocoli*)	***	***	ns
Brussels sprouts (*Brassica oleracea L. var. gemmifera subvar bruselas*)	***	***	*
Cabbage (*Brassica oleracea L. var. capitata L. subvar. alba*)	ns	***	ns
Cauliflower (*Brassica oleracea L. var. botrytis L. subvar. cordel green*)	***	***	***
Chard (*Beta vulgaris L. var. cicla L. subvar. lyon*)	***	***	**
Green beans (*Phaseolus vulgaris L. var helda*)	***	***	**
Pepper (*Capsicum spp.*)	***	***	***
Spinach (*Spinacia oleracea L. var. Inermis*)	***	***	***
Zucchini (*Cucurbita pepo L. var. elite*)	ns	ns	ns

ns = not significant, (*) significant (p<0,05), (**) significant (p<0,01), (***) significant (p<0,001)
(T) Treatment (t) Cooking time (Txt) interaction.



a) Spinacia oleracea L. var. inermis; **b)** Beta vulgaris L. var. cicla L. subvar. Lyon; **c)** Brassica oleracea L. var. itálica L. subvar. Brócoli; **d)** Phaseolus vulgaris L. var helda; **e)** Brassica oleracea L. var. botrytis L. subvar. cordel Green; **f)** Cucurbita pepo L. var. Elite; **g)** Capsicum spp.; **h)** Brassica oleracea L. var. capitata L. subvar. Alba; **i)** Brassica oleracea L. var. gemmifera subvar bruselas, Treatment: ♦ Steaming ■ Boiling

Figure 1. TPC in the fresh product and after boiling or steam for different lengths of time

As can be seen (Figure 1), the TPC decreased with cooking time in both treatments (boiling water and steam). This decrease is significant (Table 2), for all cases except for the treatment in cabbage, and for the treatment and cooking time, in the case of the zucchini.

The reduction in the polyphenol content caused by the heat treatment has been demonstrated by Oboh [18] in other tropical leafy greens. Quiñones et al. [7], indicate that cooking vegetables may decrease the initial content of phenolic compounds by up to 75%.

As per the heat processing in the present study, steaming resulted in a lower loss of TPC (Figure 1) than boiling water did; thus confirming the data found for other vegetables like carrots, broccoli and squash [33] and spinach, carrots and cauliflower [34].

Wachtel-Galor et al. [30] also indicated that steaming was the only method that retained the TPC in cooked vegetables, with an enhancement found in cauliflower. The depletion of the TPC after boiling or microwaving might be due to the breakdown of phenolics during the heating process.

In the present study, all products suffered losses, but the most important ones occurred in spinach (Figure 1.a). This product suffered losses of up to 78% when it is cooked for three minutes in boiling water, and showed losses as high as 93% if treatment was prolonged to 15 minutes, as compared to a 54% loss if the spinach is steamed for the same amount of time. Contrary to what happened when treated with boiling water, the TPC for spinach increased by 4% when it was steamed for three minutes. This behavior was also observed in spinach by Mazzeo et al. [34] and it is attributable to the lack of lignocellulosic plant structure breakdown during the steaming treatment, versus during the dip treatment. Consequently, the phenolic compounds are not released at the plants by the steam treatment. Plants do not release phenolic compounds during steaming. Solubilization does not occur when steaming, which results in a net increase; yet, when boiling the water soluble compounds may be dissolved.

The product that suffered the next-greatest loss after spinach was chard (Figure 1.b), which showed maximum values of 79.6% and 49.8% when boiled and steamed, respectively, for 15 minutes.

In the case of broccoli, losses were 69.2% when it was boiled for 15 minutes and 25.1% when it was steamed for the same amount of time. We also observed (Figure 1.c) a behavior similar to that of spinach, with an increase of about 0.28% in the TPC content (Figure 1.c) after steaming for 3 minutes. For green beans, (Figure 1.d) losses of up to 67.9% were observed when boiling them for 15 min, which can be compared to significantly lower (20.1%) values if they are steamed for the same length of time.

In the case of cauliflower (Figure 1.e), zucchini (Figure 1.f) and capsicum (Figure 1.g), the TPC losses, after 15 minutes of treatment, were 63.2, 56.5 and 56.9% respectively, when boiled and 49.4, 52.3 and 40.7%, respectively, when steamed.

The vegetables that suffered the lowest losses of TPC are: smooth cabbage (Figure 1.h), with reductions of 44.2 and 37.2% when they steamed and boiled for 15 minutes; (no significant differences, as noted above) and Brussels sprouts (Figure 1.i) with reductions of 44.5% when boiled for 15 minutes, and 27.7% when steamed.

A similar variability in the losses for the different vegetables evaluated in this study, was also found by Ismail et al. [26], who indicated that Swamp cabbage lost the highest amount of phenolic compounds (26%), followed by cabbage (20%), spinach (14%), shallots (13%) and kale (12%) after blanching in boiling water for 1 minute. In their study, the ANOVA showed significant differences in the total phenolic content between fresh and treated vegetables. Their findings indicated that phenolic compounds were very sensitive to heat treatment even for short cooking times.

Given the high losses in the TPC when heat processing is involved, from a nutritional standpoint it is advisable to use the least aggressive method (steaming) for as short a time as possible. Steam cooking should be, therefore the method of choice because, as already seen in the results, even when the maximum time of treatment is used (15') TPC losses do not exceed 55% in any case.

3.3. Reducing power (RP) of fresh vegetables

Different authors have suggested that the antioxidant effect increases exponentially as a function of the evolution of the Reducing power (RP), and have indicated that the antioxidant properties are related to the development of the RP [35, 36]. In the present study, the RP was analyzed for nine vegetables that were studied while fresh and after processing. The RP was expressed as absorbance at 700 nm in 0.5 g sample. Table 3 shows the data of nine fresh vegetables based on the spectrophotometric detection of the $Fe3+$ -$Fe2+$ transformation. After applying an ANOVA of 1 factor (product type), on data obtained in RP in the products studied, significant differences ($p <0.05$) among all the products except peppers and cauliflower can be seen (Table 3).

The results showed that green beans had the highest RP, followed by smooth cabbage and chard. This indicates that the methanol extract of green beans has a greater capacity to reduce $Fe3+$ a $Fe2+$, so it may react better with free radicals in order to stabilize and finish radical chain reactions. Of all the products studied, pepper and cauliflower were those with significantly lower values of RP (Table 3).

Shyamala et al. [35] evaluated the reducing power of four leafy vegetables, cabbage (Brassica oleracea var. capitata), coriander leaves (Coriandrum sativum), hongone leaves (Alternanthera sessilis), and spinach (Spinacia oleracea) obtained from local markets, were evaluated and found that RP of the leafy vegetables followed this order—spinach<cabbage<coriander leaves < hongone leaves. The results indicate that the RP of chard was higher than that of broccoli and cauliflower. These data are consistent with those presented in Özen's study [37].

Finally, Kim, Cho, and Han [38] evaluated ten leafy green vegetables commonly consumed in East Asia were evaluated and the reducing power of the leafy green vegetables correlated well with TPC.

3.4. Influence of treatment and cooking time on RP of vegetables studied

Faller and Fialho [32] indicated that, generally, cooking caused reductions in the antioxidant capacity for most vegetables, and that there were small differences in the cooking methods applied. Therefore the reducing power in the study at hand was determined after applying two different cooking methods.

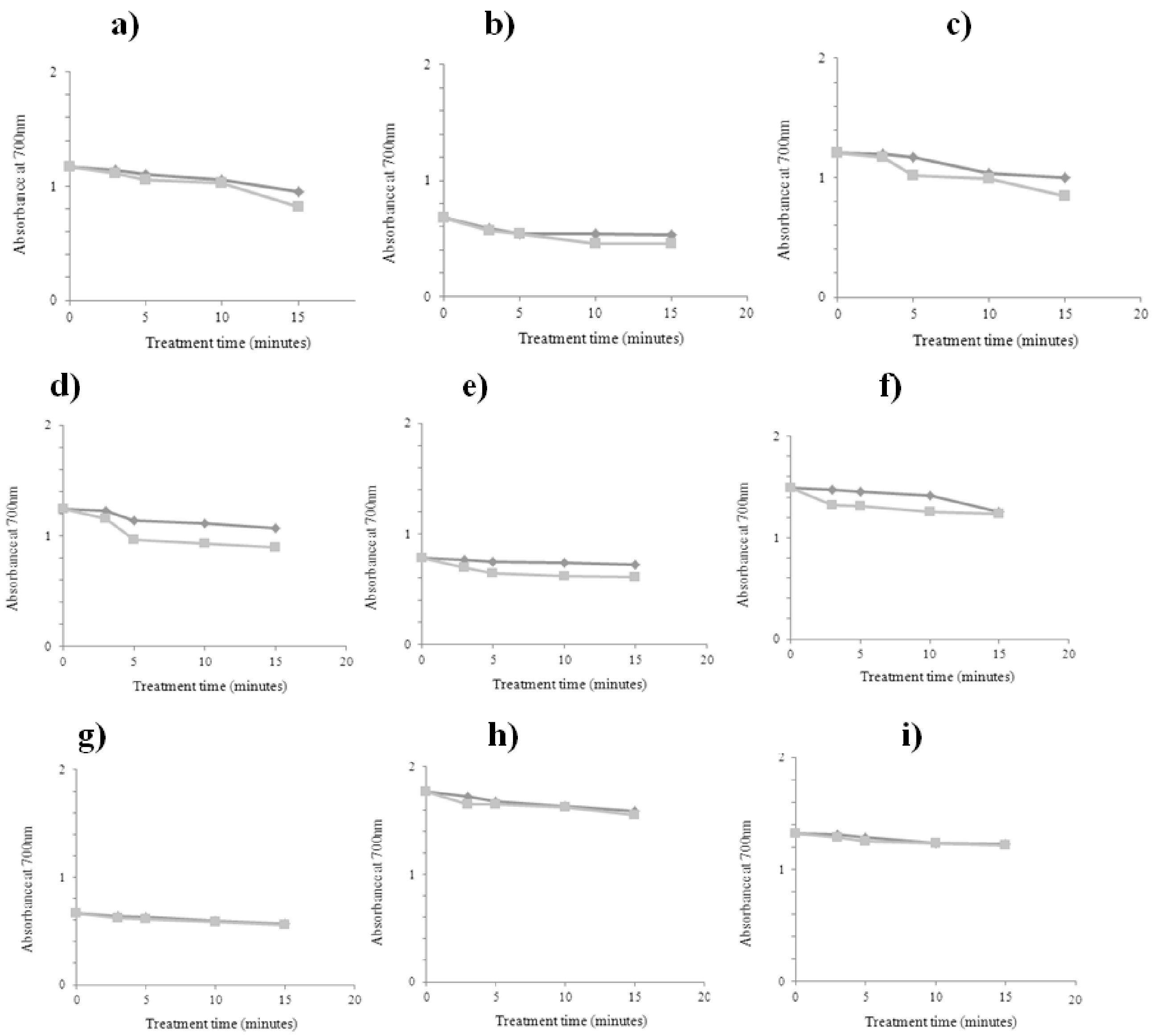
The results obtained after the application of a two-way ANOVA, ("T", treatment and "t", cooking time) with interaction, (T*t), on the RP variable, are summarized in Table 4. The influence of the heat treatment time was significant for the RP in all products (Table 4).

The RP decreased as the intensity of the heat treatment increased for both thermal treatments. The RP was significantly higher in samples subjected to steam cooking (Figure 2).

Table 3. RP of 9 fresh vegetables and one-way ANOVA differences

Plant species	RP
Broccoli (*Brassica oleracea L.var. itálica L. subvar.brocoli*)	$1.25 + 0.01^{d}$
Brussels sprouts (*Brassica oleracea L. var. gemmifera subvar bruselas*)	$1.17 + 0.01^{f}$
Cabbage (*Brassica oleracea L. var. capitata L. subvar. alba*)	$1.49 + 0.01^{c}$
Cauliflower (*Brassica oleracea L. var. botrytis L. subvar. cordel green*)	$0.68 + 0.01^{a}$
Chard (*Beta vulgaris L. var. cicla L. subvar. lyon*)	$1.32 + 0.01^{g}$
Green beans (*Phaseolus vulgaris L. var helda*)	$1.77 + 0.01^{b}$
Pepper (*Capsicum spp.*)	$0.67 + 0.01^{a}$
Spinach (*Spinacia oleracea L. var. Inermis*)	$0.78 + 0.01^{h}$
Zucchini (*Cucurbita pepo L. var. elite*)	$1.21 + 0.01^{e}$

Different letters indicate significant differences (p<0.05).



a) Brassica oleracea L. var. gemmifera subvar bruselas, **b)** Brassica oleracea L. var. botrytis L. subvar. cordel green, **c)** Cucurbita pepo L. var. elite, **d)** Brassica oleracea L. var. itálica L. subvar. Brócoli, **e)** Spinacia oleracea L. var. inermis, **f)** Brassica oleracea L. var. capitata L. subvar. alba, **g)** Capsicum spp, **h)** Phaseolus vulgaris L. var helda, **i)** Beta vulgaris L. var. cicla L. subvar. Lyon. Treatment: ♦ Steaming ■ Boiling

Figure 2. RP in the fresh product and after boiling and steaming for different lengths of time

Table 4. Two-way ANOVA (T, t) with interaction on RP

Plant species	(T)	(t)	(Txt)
Broccoli (*Brassica oleracea L.var. itálica L. subvar.brocoli*)	***	***	***
Brussels sprouts (*Brassica oleracea L. var. gemmifera subvar bruselas*)	***	***	***
Cabbage (*Brassica oleracea L. var. capitata L. subvar. alba)*	***	***	***
Cauliflower (*Brassica oleracea L. var. botrytis L. subvar. cordel green*)	***	***	***
Chard (*Beta vulgaris L. var. cicla L. subvar. lyon*)	***	***	*
Green beans (*Phaseolus vulgaris L. var helda*)	***	***	*
Pepper (*Capsicum spp.)*	***	***	ns
Spinach *(Spinacia oleracea L. var. Inermis*)	***	***	***
Zucchini (*Cucurbita pepo L. var. elite*)	***	***	***

ns = not significant, (*) significant (p<0,05), (**) significant (p<0,01), (***) significant (p<0,001)
(T) Treatment (t) Cooking time (Txt) interaction.

Oboh [18] studied the effect of boiling green leafy vegetables for 5 minutes on the reducing activity of some tropical plant were studied and found a reduction after the heat treatment.

Although the differences were significant (Table 4) they were not as important as in the case of the TPC.

The greatest losses in RP occurred in Brussels sprouts (Figure 2.a) treated by boiling for 15 minutes (34.8%), as compared to a 20.4% loss when samples were steamed for the same amount of time.

The cauliflower (Figure 2.b), zucchini (Figure 2.c) and broccoli (Figure 2.d), suffered similar losses (32.9, 30.2 and 27.8%) when boiled for 15 minutes, yet significantly less when they were steamed (21.0, 17.6 and 14.2% respectively).

When boiled for 15 minutes, the spinach (Figure 2.e), cabbage (Figure 2.f) and pepper (Figure 2.g), suffered losses of 22.0, 17.2 and 17.0%, respectively; and losses of 7.4, 15.8 and 14.8% when steamed for the same length of time.

Finally, minor losses occurred in green beans (Figure 2.h), and chard (Figure 2.i), which showed losses of 7.8 and 12.2% when boiled for 15 minutes as compared to losses of 7.3 and 10.1% when they were steamed for the same amount of time.

Moreover, it is important to note that, in the case of spinach, 3 min of steaming did not change its reducing power when compared to that found in fresh spinach. This lack of decrease in the reducing power might well be attributed to the outstanding TPC increase, as shown above, which could offset the general loss of vitamin C and other non-phenolic antioxidants present in this vegetable [18].

The RP losses indicated here, and shown with TPC, confirm the less aggressive effect of steaming as compared to boiling when applying heat treatment. Pearson correlation analysis between the results of TPC and RP was applied and high correlations with values higher than 0.729 for all the samples were found. The determination of TPC could be used as a measure of the effect of thermal treatments.

4. Conclusions

In conclusion, this research examined the TPC content and RP of nine vegetables bought in a local market in Lugo (Galicia, N.W. Spain), when they were fresh and after heat processing (boiling and steaming) for different amounts of time.

The results from this study showed that the edible part of different vegetables presented differences in their TPC and RP. Among the vegetables tested, Brussels sprouts presented greater TPC than the others, while green beans showed a greater RP.

Both the type of heat processing and the length of cooking time had a significant effect on the reduction of the TPC and the RP of vegetables tested. Steaming the vegetables for shorter amounts of time produced the lowest reductions.

The information obtained in this study reaffirms the importance of applying the least aggressive heat treatment in order to maintain the contribution of antioxidant activity compounds; when consumed regularly, these vegetables may contribute a significant amount of antioxidant compounds to a person's dietary intake.

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