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Total Phenolics and Antioxidant Capacity of Vegetables Grown in the Southwestern Andes Region of South America

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Abstract The antioxidant richness of 69 species and/or varieties of vegetables grown in the southwestern Andes region of South America, including some endemic varieties, was evaluated in terms of their total phenolics (TP) and oxygen radical absorbance capacity (ORAC). Parsley, basil and coriander rank notably higher among those species that are commonly consumed in their raw state, exhibiting TP and ORAC values that are comparable to those reported earlier by us for various antioxidant-rich indigenous berries. For a given species, major differences were also seen among its varieties; in the case of tomatoes and lettuce, the maximal-to-minimal differences in ORAC values reached 16- and 28-fold, respectively. For those vegetables that are not consumed raw, the boil-cooking process affected differentially the antioxidant richness; for instance, while in the case of asparagus, chard, and lima beans boil-cooking induced major drops in ORAC (40-60%), in the case of artichoke bottoms a marked increase (35%) in ORAC was seen. Within the frame of building rankings of antioxidant richness, in the present work substantial data are also presented to highlight the concept that when assessing the antioxidant supply potential of any vegetable, besides quantifying its antioxidant richness per 100 g, considering its actual portion of consumption is also fundamental. On such basis, among the 29 vegetables analyzed in their cooked state, the endemic and regionally-grown Michuñe, Cabrita, and purple varieties of potatoes emerge as the top 3 antioxidant-rich plant foods.

Keywords: *antioxidants, vegetables, total phenolics, ORAC*

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1. Introduction

The continuing interest in characterizing the antioxidant richness of fruits and vegetables (F&V) emerged from the early recognition that these foods can be an excellent source of antioxidants, and that by elevating their consumption the relative risk of developing certain cardiovascular and tumoral diseases can be significantly lowered [1,2,3,4,5,6]. Although several etiologic factors are likely to be involved in the initiation and/or progression of such diseases, most facts support the hypothesis that preventing the occurrence of oxidative stress and/or ameliorating its effects be fundamental towards achieving several of the health-beneficial effects associated with a higher consumption of antioxidant-rich F&V [6,7]. However, the protective cardiovascular (CV) effects of incrementing F&V consumption seems to arise not only from the ability of some of its bioactive compounds to act as antioxidants, but also from their ability to exert anti-inflammatory, anti-thrombotic, endothelial dysfunction-lessening, blood pressure-lowering, and/or lipid profile-normalizing actions [8]. In the case of tumoral diseases, although the overall cancer-preventive effects of enhancing the consumption of antioxidant-rich F&V in humans are still inconclusive [9], a broadly accepted scientific notion exists that some of the compounds present in F&V, besides having antioxidant properties, have the potential to exert chemotherapeutic actions [10]. From a mechanistic point of view, some of the antioxidant activity-carrying molecules could also exert anti-tumoral effects by promoting anti-mutagenic, anti-proliferative, pro-apoptotic, and/or anti-angiogenic actions [11,12].

With very few exceptions, phenolic compounds account for most of the antioxidant activity found in F&V, and although some of these foods can also be an excellent source of certain non-phenolic antioxidants, vitamins, trace minerals and dietary fiber, the high presence of polyphenols is still regarded as a major contributor to the CV benefits associated with an elevated consumption of F&V [13]. In view of the latter, the total content of these compounds (i.e., total phenolics or TP) has been long assayed as an indirect but suitable form of estimating the polyphenol-related antioxidant richness of plant-derived foods. At the epidemiological level, inverse correlations between the dietary intake of flavonoids and the incidence of coronary heart disease and mortality have been established [13,14].

Evidence emerged during the last decade indicates that while every plant-derived polyphenol has the ability to exert a direct antioxidant action by scavenging free radicals and/or by reducing other pro-oxidant species, some of polyphenols are also able to exert antioxidant actions via indirect mechanisms [12]. The latter comprise, mostly, regulating in a favorable manner the expression of genes coding for the synthesis of antioxidant and pro-oxidant enzymes, and/or modulating the activity of such enzymes. Since the concentrations of polyphenols required to promote such effects are known to be much lower than those needed to scavenge free radicals and/or pro-oxidant species (ROS), the indirect mechanisms are likely to significantly contribute to the overall antioxidant effects exerted by some of these compounds *in vivo* [12]. The latter has prompted many researchers to assess the concentration of individual phenolic compounds in foods [15,16]. However, when it comes to directly evaluate the capacity of a given (whole) food to scavenge ROS, the antioxidant activity is likely to result not only from the simple sum of the individual contribution of each phenolic, but also from a synergistic effect that could emerge from the interaction between these compounds [17], and/or from their interaction with other non-phenolic ROS-scavenging carrying molecules (like carotenoids, and vitamins C and E). Among the most common and widespread used analytical approaches to assess *in vitro* the ROS scavenging properties of foods is the ORAC (Oxygen Radical Absorbance Capacity) assay [18]. When ORAC is assumed to be a method to assess the so-called total antioxidant capacity of a food, it should be beard in mind that it only serves as an approach to quantitatively evaluate the *in vitro* potential that, in a given food, its extractable components have to scavenge certain peroxyl and alkoxyl radicals [19]. Like with any other *in vitro* run antioxidant assay, the ROS-scavenging capacity of food components assessed through ORAC shall not be considered to imply that such components will necessarily be bioavailable to the organism, or that they will overall exert such ROS-scavenging action *in vivo* at the target sites. Nonetheless, establishing the ORAC value of a given food has long represented and is still considered a practical index to define the potential that -throughout its extractable components- different foods would have to contribute with ROS-scavenging antioxidants to the organism.

Our laboratory launched in 2012 the first web-based database on total phenolics and ORAC values of over 120 species and/or varieties of fruits produced and consumed within the South Andes region of South America [20]. Interestingly, recent meta-analysis of cohort studies indicate that for certain pathologies (i.e. gastric cancer) only the consumption of fruits, but not of vegetables, is able to reduce the relative risk of disease development [21]. In turn, in other pathologies, only an increased intake of vegetables, but not of fruits, has been associated with a health protecting benefit (i.e. hepatocellular carcinoma, a leading cause of cancer mortality worldwide) [22]. In view of the increasingly recognized importance that vegetables have as health-protective foods, in the present study we have characterized the antioxidant richness of a total of 69 species and/or varieties of vegetables grown in the west-south Andean region of South America. Part of the results reported here were recently digitally tabulated and incorporated into a database of the web site www.portalantioxidantes.com. In the present work, detail rankings of the antioxidant richness of such vegetables and correlations between their ORAC and TP values are presented and discussed. In addition, the effects of cooking on the antioxidant richness of those vegetables that are often eaten in their cocked form, and the influence that the variety or the cultivar has on the TP and ORAC is reported, highlighting the particularly greater antioxidant richness displayed by certain native vegetable varieties.

2. Materials and Methods

2.1. Chemicals

2,2′-Azobis(2-amidinopropane) dihydrochloride (AAPH) was purchased from Wako Chemicals (Richmond, VA, USA). 6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox®), fluorescein (sodium salt), Folin-Ciocalteu's phenol reagent, sodium carbonate, and gallic acid were all obtained from Sigma-Aldrich (St. Louis, MO, USA). All other reagents, including organic solvents, were of analytical grade.

2.2. Vegetable Sampling

Vegetables were sampled from supermarkets belonging to the two major food retail chains in Chile, located in 10 different important cities, from Antofagasta to Puerto Montt, located at latitudes of 23° and 41°, respectively. Vegetables distributed by these retailers are supplied by nearby growers as well as growers located at some distance from such cities along the indicated latitudes. In addition, some vegetables available at the above-mentioned sampling points occasionally originated from growers located in the Chiloé Island (off coast Chile, 42° 40′36″S 73° 59′36″W), Peru, Bolivia, and the Argentinian Patagonia region. In each sampling, approximately 2.0 Kg of each vegetable (amount that secured a representative number of individual items) was randomly selected from bins located at the selling rooms of the retail outlet. Samples in their ready-to-be eaten fresh form were taken on, at least, three different occasions during two consecutive sampling years (from March 2013 to April 2015). Immediately after sampling, the vegetables were stored in iced coolers and transported to reach within 12−18 h the laboratory where the analyses were finally conducted. Upon arrival, samples were duly labeled and kept at 4 °C until analysis; the latter took place the same day or, at the latest, 24 h after arrival. All analyses were conducted by the Laboratory of Analysis of Antioxidants in Foods, located at the Nutrition and Food Technology Institute, University of Chile, Santiago, Chile.

Whereas most vegetables were analyzed in their fresh condition (i.e., as they are regularly consumed), the drained pulps of some vegetables were analyzed following their cooking in boiling in water (mass of vegetables, water volumes, and cooking times are detailed in complementary data). In the case of tomatoes and cucumbers, which are not necessarily amenable to consumption with their peels, samples were also analyzed in their peeled form. Whenever possible, each of the varieties of vegetables consumed by the population, as these were available to be sampled, was subjected to separate analysis. The latter applied to lettuces, tomatoes, onions, potatoes and beans.

2.3. Sample preparation

From a pool consisting of not less than 1500 g of each of the vegetables to be analyzed, three portions were independently weighted (10−30 g each of the edible parts), and then 150 mL of a solution consisting of acetone/water/acetic acid (70:29.5:0.5; AWA) was added. The mixtures were homogenized, at a controlled medium speed during 3-5 min, by means of an Ultraturrax® homogenizer (IKA, Wilmington, NC, USA). The resulting homogenates were incubated at 23°C during 40 min in a shaking bath. The tubes were then centrifuged (2500g for 15 min at 4°C) and the supernatants separated and kept at 4°C. The pellets resulting from such centrifugation were subjected to a second extraction by adding 150 mL of AWA, homogenized, incubated, and centrifuged as described above. Each supernatant resulting from the second extraction was pooled with its corresponding first extraction supernatant. The three resulting supernatant pools were independently subjected to the total phenolic and ORAC determinations.

2.4. Measurement of Total Phenolic Content and ORAC Activity

Total phenolic contents were assayed in the above-referred supernatant pools, using the Folin−Ciocalteu's method (F-C) as described by Wu *et al*. [23]. Briefly, 15 µL of the samples (diluted in AWA) or standards was mixed with 200 µL of a solution containing the F-C reagent (previously diluted 1:10 v/v in distilled water), 40 µL of sodium carbonate (20% w/v), and 45 µL of distilled water. Following incubation of the resulting solution at 37 °C for 30 min, the OD at 765 nm was measured in a 96-well plate using a Multi-Mode Microplate Reader (Synergy HT, Winooski, VT, USA). The analysis of each supernatant pool was done in triplicate. The results of TP were estimated on the basis of a standard curve of gallic acid and were expressed for fresh raw vegetables, as milligrams of gallic acid equivalents (GAE) *per* 100 g of sample weight (fw). When the F-C analyses were performed in cooked vegetables, the TP content was expressed as milligrams of GAE *per* 100 g of drained-cooked samples weight (cw). For the TP assay, the within-day repeatability ranged from 0.2 to 2%, the between-day repeatability was <2.25%, and variation between replicates was typically between 2 and 7.5 RSD%.

The ORAC activity was assayed in the above-referred supernatant pools, using AAPH as a source of peroxyl radicals and fluorescein as oxidizable probe [23]. In brief, 45 µL of the above-mentioned supernatant pools (diluted in AWA) was transferred to 96-well microplates containing each 50 µL of AAPH (18 mM) and 175 µL of fluorescein (108 nM). The plates were placed in a Multi-Mode Microplate Reader (Synergy HT) and incubated for 60min at 37°C with shaking of the plates every 3 min. During the incubation, the fluorescence (485 nm Ex/520 nm Em) was monitored continuously every 3 min. The analysis of each pooled supernatant was done in triplicate. The results of ORAC activity were estimated on the basis of a standard curve of Trolox®, using a quadratic regression equation obtained between the Trolox® concentration and net area under the fluorescence decay curve. ORAC activity was expressed as micromoles of Trolox® equivalents (TE) *per* 100 g of fresh raw vegetable weight (fw) or as micromoles of TE *per* 100 g of drained-cooked vegetable weight (cw). The within-day repeatability of the ORAC assay, measured as relative standard deviation (RSD) in standard solutions, ranged from 0.4 to 2.5%. The between-day repeatability was <3%. The variation in the values for replicate food items obtained from the same source was typically between 2.5 and 8 RSD%. In assessing the antioxidant activity of foods, some investigators distinguish between the contribution of the hydrophilic and that of the lipophilic extractable compounds to the ORAC activity [23]. The ORAC values informed in the here-reported database correspond to those arising from the hydrophilic acetone/water/acetic acid extractable compounds.

2.5. Cocking Conditions

Out of the 69 species/varieties of vegetables under study, 29 were analyzed in their cooked state. Excepting for broccoli and cauliflower, which were vapor-cooked (with no direct water contact), all other vegetables were boiled in water. The cooking time (min), and the ratio of the whole vegetable mass to boiling water volume (g/mL), shown under brackets, were as follows: artichoke bottom (60 and 0.11), asparagus (green type; 15 and 0.16), beetroot (35 and 0.23), Black beans (26 and 0.2), broccoli (15 and 0.49), Bruja potato (30 and 0.2), Butter potato (30 and 0.2), Cabrita potato (30 and 0.2), cauliflower (15 and 1.17), chard (15 and 0.15), Desirée potato (30 and 0.2), eggplant (20 and 0.16), green beans (25 and 0.21), Green Pallar beans (Chiloé-grown; 26 and 0.2), Haricot beans (26 and 0.2), Lengua de vaca potato (30 and 0.2), lima beans (30 and 0.2), Michuñe potato (30 and 0.2), New potato (30 and 0.2), peas (20 and 0.33), Pinto beans (26 and 0.2), Purple Pallar beans (Chiloé-grown; 26 and 0.2), Purple potato (30 and 0.2), Shelled beans (26 and 0.2), squash (Camote variety; 20 and 0.3), White beans (26 and 0.2), Winter squash (20 and 0.27), Yellow corn (24 and 0.35), zucchini (20 and 0.41).

2.6. Statistics

Descriptive statistical analysis was performed using Microsoft Excel and/or GraphPad Prism version 5.01 for Windows, GraphPad software (San Diego, CA, USA). Because the intent of the present study was to provide data on those vegetables that are most commonly consumed by populations living around the south Andes region of South America, and not to evaluate the influence of factors that could affect the antioxidant capacity of vegetables (such as environmental or post harvesting factors), results shown in the present study represent, for a given vegetable, a composite (mean) of the ORAC and TP values obtained upon the analyses of such vegetable, independent of its geographic site of sampling or origin. Thus, the ORAC and TP values of a vegetable correspond to those of a particular species and, in some cases, a particular variety of vegetable.

3. Results

For a total of 69 species and/or varieties of vegetables that were analyzed in their fresh and raw condition, a ranking of the 36 species which show the higher ORAC activity is depicted in Figure 1. For each of such species, the variety or cultivar (indicated under bracket after the specie) that exhibited the highest ORAC value was selected. Among the lowest ORAC exhibiting vegetables (exhibiting less than 2000 µmol TE/100 g fw) were: carrot (1), celery (2), peas (3), Yellow corn (4), White radish (5), zucchini (6), cauliflower (7), Green or snap beans (8), cucumber (9), leek (10), squash (Camote) (11), bean sprout (12), eggplant (13), broccoli (14), chili pepper (Red) (15), Green asparagus (16), beetroot (17) and watercress (18). Medium ORAC value vegetables (defined from 2000−5000 µmol TE/100 g fw) included: mushroom (White common) (19), tomato (Alicante) (20), cabbage (Purple) (21), ciboulette (22), chard (23), garlic (24), onion (Shallot Purple) (25), artichoke -bottom part (26), capsicum (Red) (27) and potatoes (Cabrita) (28). Vegetables with the highest ORAC (from 5000 to near 30000 µmol TE/100 g fw) were: spinach (29), arugula (30), lettuce (Sangria) (31), lima beans (32), coriander (33), bean (Pinto) (34), basil (35) and parsley (36). Insert to Figure 1 depicts the ranking curve of the ORAC values for the 69 species and/or cultivars of vegetables included in the study. Besides the formerly referred vegetables (numbered from 1 to 36), the insert to Figure 1 includes the following 33 others varieties or cultivars: tomato (Beefsteak) (37), cucumber (Kirby) (38), lettuce (Iceberg) (39), tomato (Salad) (40), squash (Winter) (41), hydroponic tomato (42), New onion (43) and New potato (their "so called" new varieties) (44), tomato (Roma) (45), cabbage (Green) (46), scallions (47), mushroom (Boletus luteus) (48), mushroom (Shiitake) (49), lettuce (Romaine) (50), tomato (Cherry) (51), beans (Pallar green) (52), beans (Shelled) (53), potato (Butter) (54), capsicum (Green) (55), chili pepper (Green) (56), potato (Desirée) (57), potato (Bruja) (58), potato (Lengua de vaca) (59), beans (White) (60), lettuce (Butter) (61), beans (Haricot) (62), onion (Shallot White) (63), garlic (Chilota) (64), lettuce (Big Boston) (65), potato (Purple) (66), potato (Michuñe) (67), beans (Pallar purple) (68) and beans (Black) (69).

ORAC data from the 36 vegetables presented in Figure 1 were correlated with their corresponding total phenolic contents (Figure 2). According to the curve obtained (y= 29.8x-1155), the ORAC and TP parameters correlate with an r^2 value of 0.86. When each of the 69 fresh and raw vegetables were considered, the ORAC versus TP parameters were estimated to correlate with an r^2 value of 0.84 (y=26.2x-602; not shown). While the ranking of ORAC values shown in Figure 1 was constructed on the basis of comparing only the 36 higher ORAC-presenting vegetables, the differences in antioxidant richness that arise from comparing, within a given species, different varieties or cultivars of some of such vegetables are shown in Figure 3 (A and B). Towards that end tomatoes and lettuces were taken as examples of vegetables belonging to the medium and higher ORAC ranges, respectively. Among the six studied varieties of tomatoes, the Beefsteak exhibits the lowest ORAC and TP, while the Alicante and Cherry ones exhibit the highest ORAC and TP, respectively (Figure 3 A). The maximal difference in TP, which amounted to almost 3-fold, was seen between the Cherry and the Beefsteak varieties. Amongst some west-south Andes communities, many people consume tomatoes without peel; such practice has been presumed to be an effective manner of avoiding exposure to surface-contained agrochemicals. Taking into consideration the latter, and the fact that the peel of many fruits and vegetables can be an important source of antioxidants [24,25], the ORAC and TP values corresponding to each of the tomato varieties analyzed in their peeled forms were included in Figure 3 A (as numbers under brackets). When the highest and the lowest ORAC values of the peeled varieties were compared (Alicante versus Beefsteak, respectively), a difference of near 12-fold was found. In turn, the difference in TP amounted to only 1.2 fold when the same varieties were compared. Figure 3 B compares the ORAC and TP values of five different varieties of lettuces. While the Iceberg variety exhibits the lowest ORAC and TP contents, the Sangria variety ranks highest for such parameters.

Table 1. Influence of The Cooking Process on The Antioxidant Richness of 13 Vegetables

VEGETABLES	mg GAE / 100 g fw or cw		µmol TE / 100 g fw or cw	
	RAW	COOKED	RAW	COOKED
Artichoke (bottom part)	96	130	3641	3680
Asparagus (green type)	94	56	1555	847
Beetroot	126	95	1781	1357
Broccoli	111	91	1338	1095
Cauliflower	61	46	516	447
Chard	121	50	2612	1148
Eggplant	86	79	1229	1078
Lima beans	413	174	7473	3505
Peas	41	34	357	263
Squash (camote variety)	49	41	859	670
Winter squash	26	23	295	427
Yellow corn	57	40	360	500
Zucchini	45	42	508	567

Results are presented as mg GAE or as µmol TE per 100 g of either fresh (fw) or cooked (cw) weight of total phenolics and ORAC respectively.

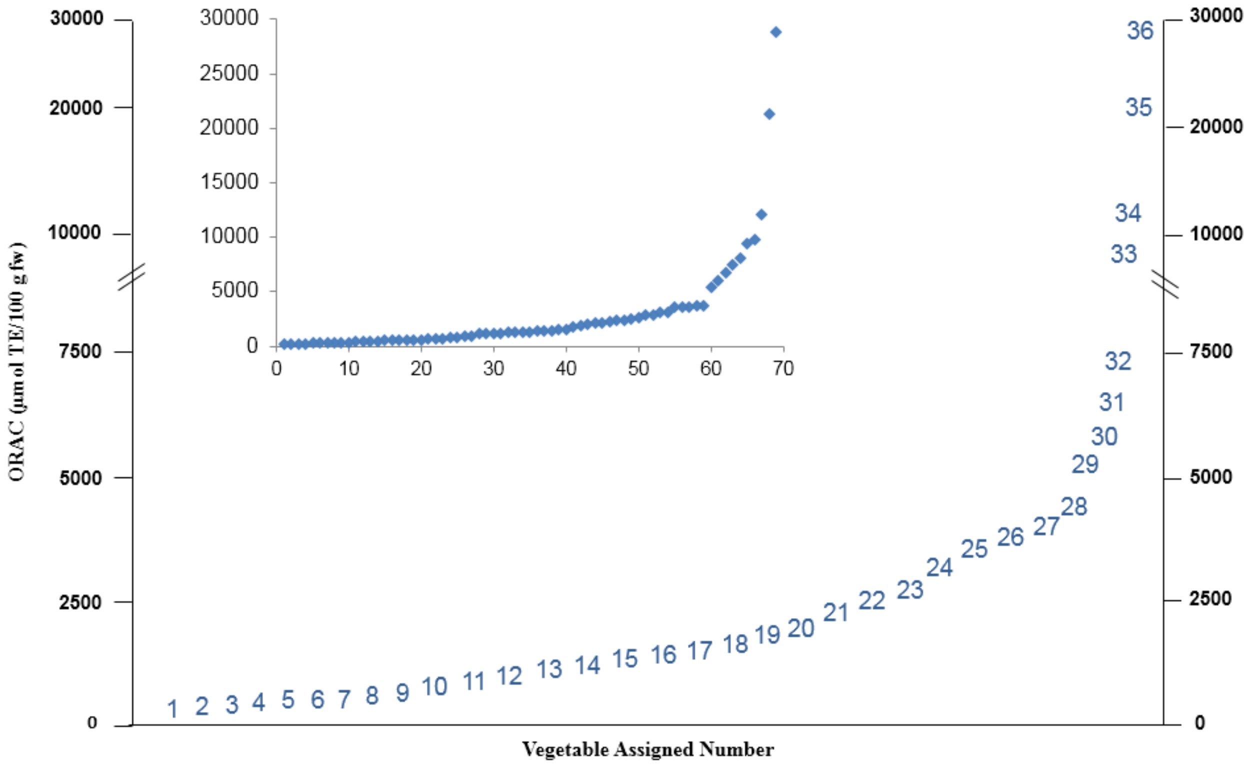

Figure 1. Ranking of commonly consumed vegetables based on their ORAC values. A ranking of the ORAC values of 36 species of vegetables that are frequently consumed by populations from the south Andes region of South America in their fresh and raw states is shown. The species corresponding to each assigned number are described in the text. In the case of those vegetables that present more than one variety, the results shown correspond to the highest ORAC-exhibiting variety. The insight figure depicts the ORAC values obtained for the 69 species/varieties of vegetables under study, each analyzed in its fresh and raw state. ORAC values are expressed as μmol TE/100 g fw

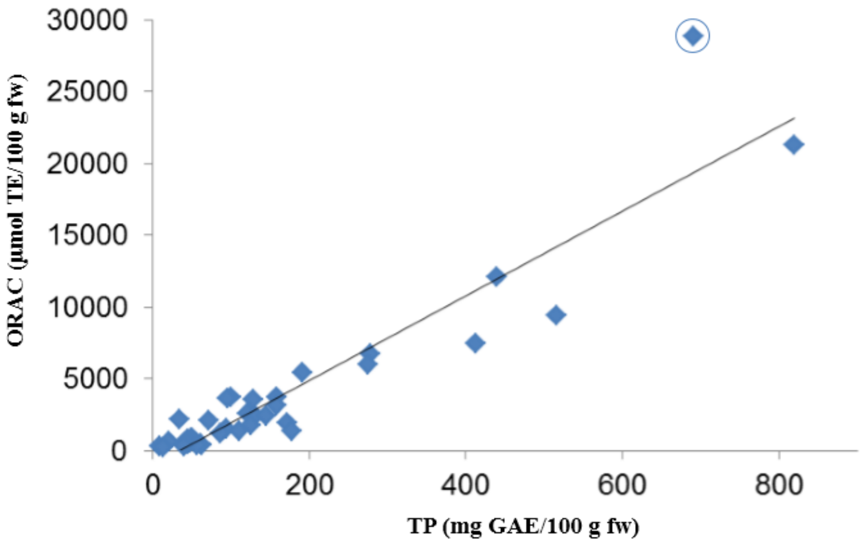

Figure 2. Correlation between total phenolics and ORAC values of 36 species of vegetables analyzed in their fresh and raw state. While ORAC values are expressed as μmol TE/100 g fw (as in Figure 1), TP are expressed as mg GAE/100 g fw. The circled diamond shown at the top part of this figure corresponds to parsley. As explained in the text, it is highlighted to point out its correlation-distancing effect this vegetable

Out of the 69 species and/or cultivars of vegetables under study, 29 are regularly consumed as cooked foods. Since exposure of plant foods to high temperatures can potentially alter their antioxidant contents [26,27], the effect cooking on the antioxidant richness (ORAC and TP) was evaluated, initially in the following 13 vegetables: artichoke (bottom), asparagus (Green), beetroot, broccoli, cauliflower, chard, eggplant, lima beans, peas, squash (Camote), squash (Winter), corn (Yellow) and zucchini. Beetroot, chard and zucchini were included in this list since these can be ingested both, in their raw as well as in their cooked states. Excepting for broccoli and cauliflower, which were steam-cooked, all other vegetables were boil-cooked. Cooking conditions (namely, cooking mode, temperature and time, and the vegetable mass to water volume ratio) are described in the material and methods section. Table 1 shows the TP content and ORAC activity of those vegetables that were analyzed in their raw and cooked states. As shown, the cooking process significantly ($p < 0.05$) affected the antioxidant richness, decreasing TP and ORAC in 8 of the 13 studied vegetables, while increasing TP in one (artichoke) and ORAC in two other vegetables (Winter squash and Yellow corn). In the two remaining vegetables, no significant changes were detected (eggplant and zucchini) in such parameters. In addition to the already referred 13 vegetables (shown in Table 1), the effect of boil-cooking on TP and ORAC was studied in eight varieties of beans (Figure 4A) and eight varieties of potatoes (Figure 4B). The following varieties of beans were included: Green, Green Pallar, Shelled, White, Purple Pallar, Black, Haricot and Pinto. In the case of potatoes, the varieties under study were: Bruja, Cabrita, Desirée, Butter, Lengua de vaca, Michuñe, New and Purple potato.

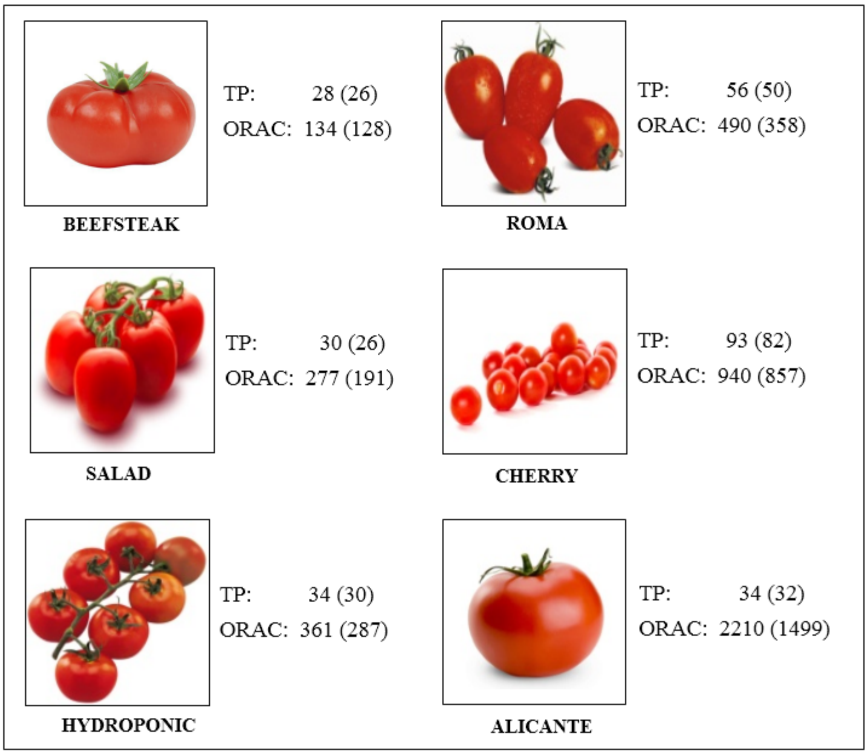

Figure 3A. Total phenolics and ORAC values of six different varieties of tomatoes. Tomatoes were analyzed in their fresh and raw state. For each variety, the TP and ORAC values of unpeeled tomatoes are side-accompanied by results obtained from analyzing the same but in their peeled forms. The latter results are presented as bracketed numbers. ORAC and TP values are expressed as µmol TE/100 g fw and as mg GAE/100 g fw, respectively

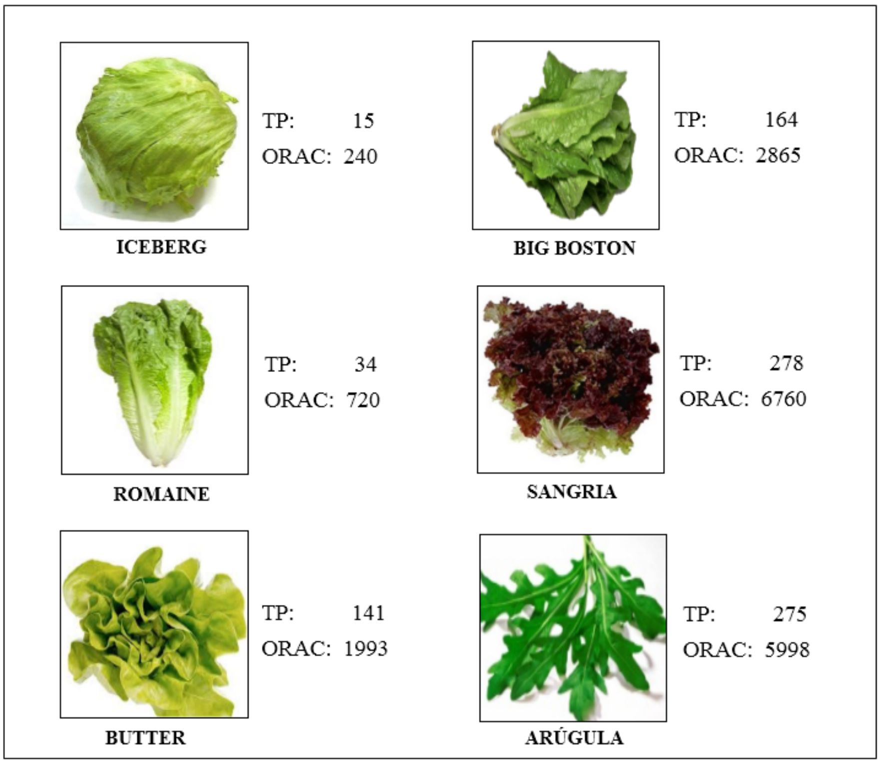

Figure 3B. Total phenolics and ORAC values of five different varieties of lettuces and arugula. These vegetables were analyzed in their fresh and raw state. ORAC and TP values are expressed as µmol TE/100 g fw and as mg GAE/100 g fw, respectively

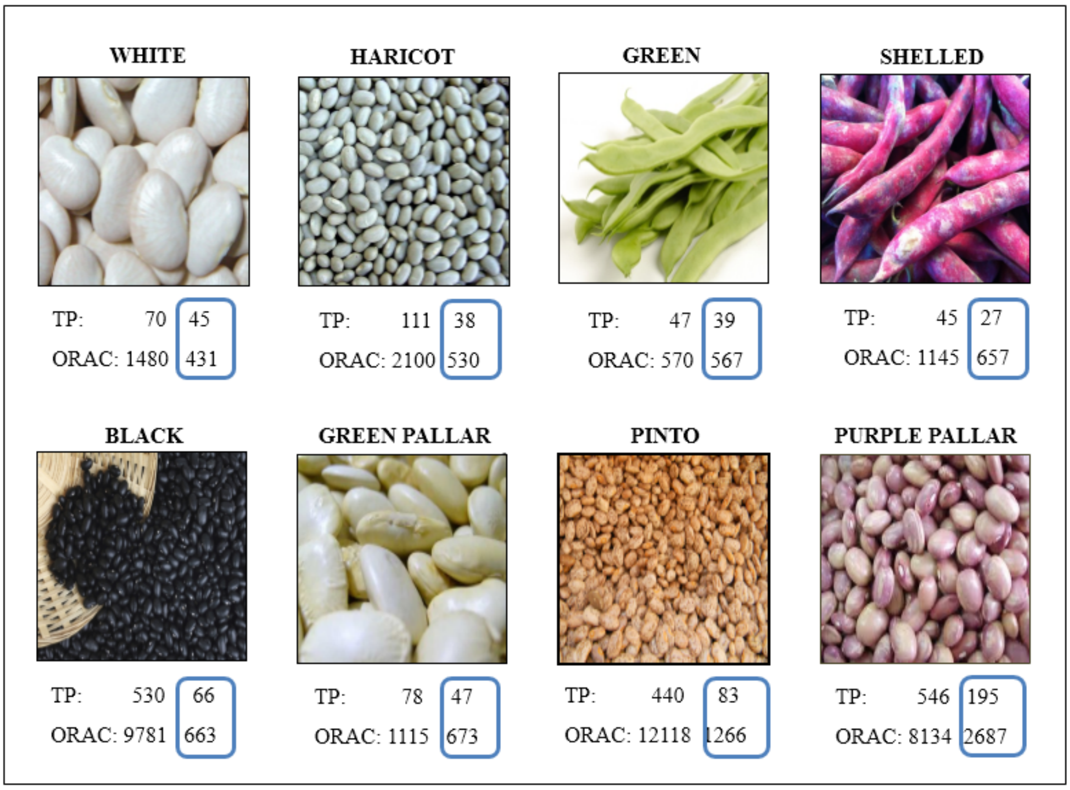

Figure 4A. Total phenolics and ORAC values of eight different varieties of beans. The eight varieties of beans were analyzed in their raw and cooked states. The cooking conditions are detailed in Material and Methods. TP and ORAC values of cooked beans are presented as numbers within a rectangle. ORAC values are expressed as µmol TE/100 g fw (raw) or /100 g cw (cooked beans). TP are expressed as mg GAE/100 g of fw and cw

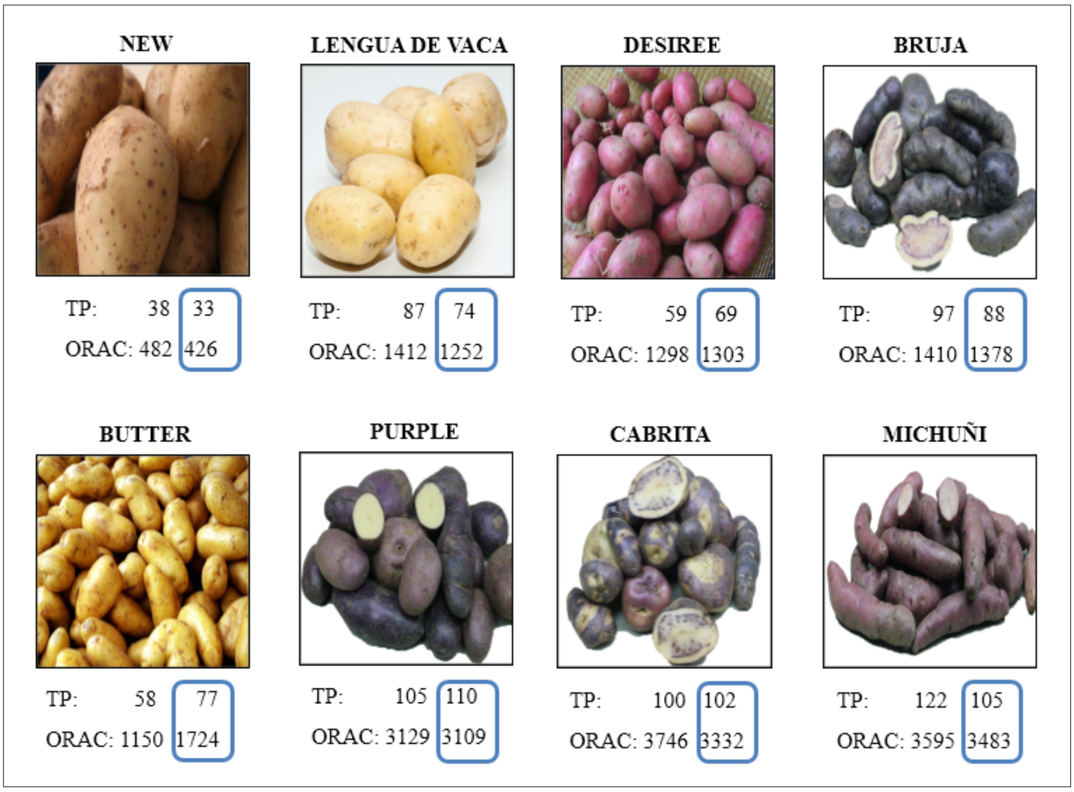

Figure 4B. Total phenolics and ORAC values of eight different varieties of potatoes. The following varieties were studied: Bruja potato (Chiloé-grown long and round shaped, skin is dark-purple and flesh is spotted with purple and light-yellow streaks), Cabrita potato (Chiloé-grown long and round shaped, skin and flesh are heterogeneously spotted with purple and light-yellow bands), Desirée potato (pink-to-red skin and light-yellow flesh), Butter potato (yellow skin and flesh), Lengua de vaca potato (Chiloé-grown round shaped, yellow skin and white flesh), Michuñe potato (Chiloé-grown long shaped, brown-pink skin and light-yellow flesh), New potato (early harvested, pink skin and white flesh) and Purple potato (Chiloé-grown purple skin and white flesh). Analyses were applied to potatoes in their raw and cooked states, using whole unpeeled potatoes as samples. The cooking conditions are detailed in Material and Methods. TP and ORAC values of cooked potatoes are presented as numbers within a rectangle. ORAC values are expressed as µmol TE/100 g fw (raw) or /100 g cw (cooked potatoes). TP are expressed as mg GAE/ 100 g of fw and cw

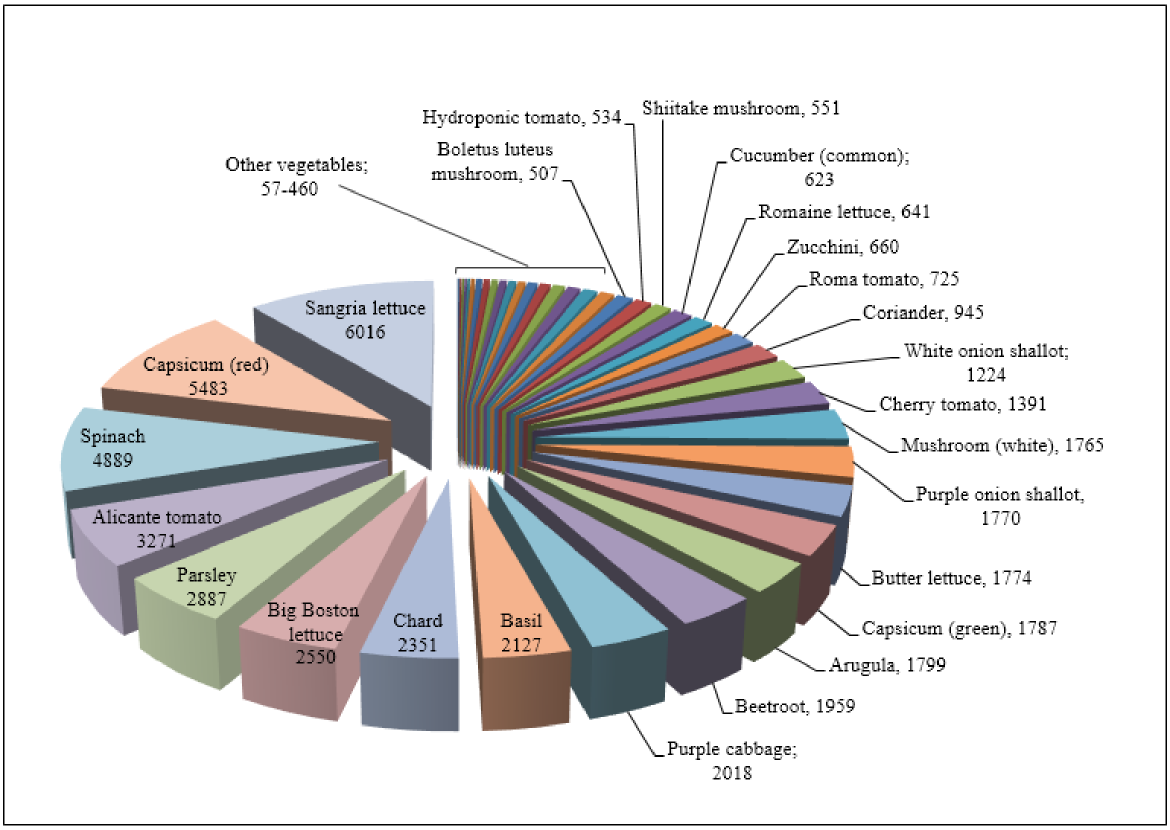

Figure 5A. Ranking of the *per* portion ORAC values of vegetables that are consumed in their raw state. Out of the 69 vegetables under study, 43 were selected and shown in the figure as these are either regularly or occasionally consumed in their raw state. Values were calculated by multiplying the ORAC of each vegetable (expressed µmol TE/100 g of weight of the edible raw part) by the portion at which such vegetable is regularly consumed (expressed in g; complementary table)

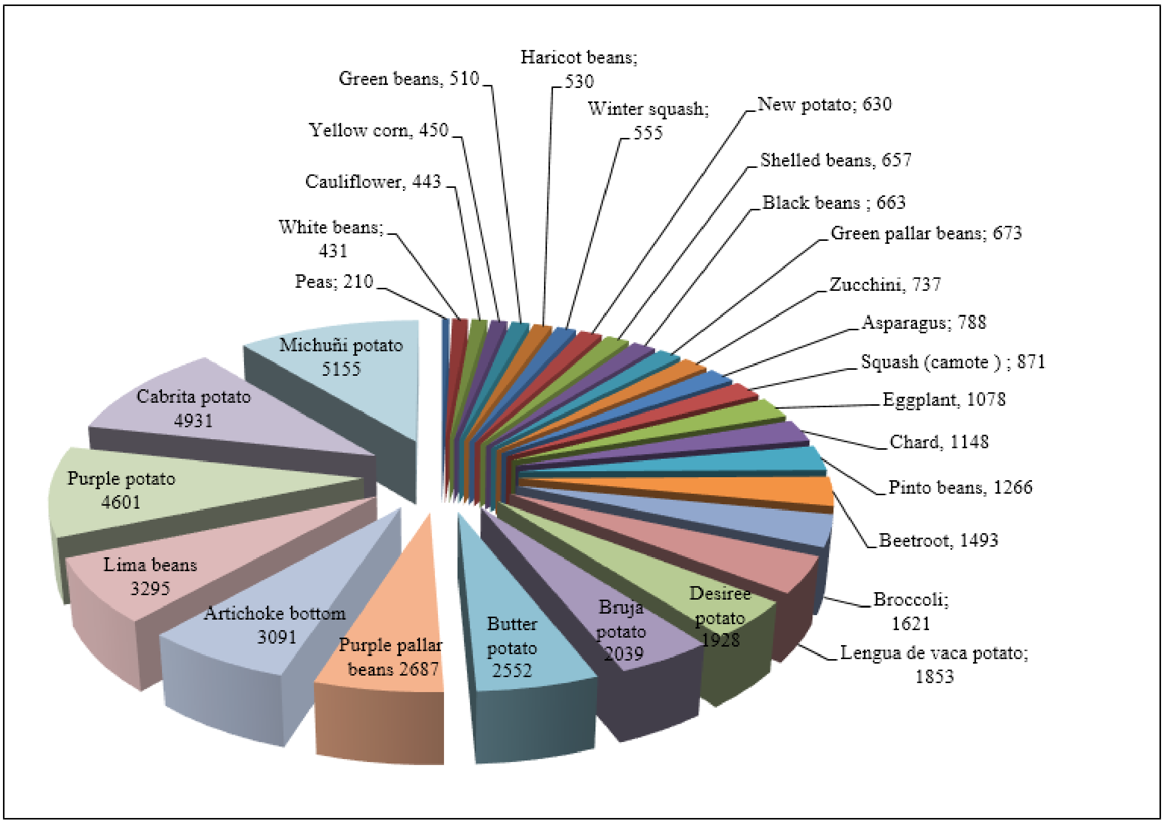

Figure 5B. Ranking of the *per* portion ORAC values of vegetables that are consumed in their cooked state. Out of the 69 vegetables under study, 29 were selected and shown in the figure as these are regularly consumed in their cooked state. Values were calculated by multiplying the ORAC of each vegetable (expressed µmol TE/100 g of weight of the edible cooked part) by the portion at which such vegetable is regularly consumed (expressed in g; complementary table)

As seen in Figure 4A, boil-cooking led to had significant decrements in the TP of each of the eight studied varieties of beans, ranging these from 17% in Green beans to 88% in Black beans. Green beans were the only varieties exhibiting no significant descent in ORAC. The range of decrements in ORAC among the seven remaining varieties varied from 40% in Green Pallar beans, to 93% in Black beans. On the other hand, in the case of the potatoes (Figure 4B), out of the eight studied varieties, only two exhibited significant decrements in their TP, 13% in the New potato and 15% in the Lengua de vaca variety, and ORAC, 12% in the New potato and 11% in the Lengua de vaca. Five potato varieties showed no significant descents in ORAC (of which four showed no significant drops in TP). The only variety of potato which exhibited an increment in TP (of 33%) and ORAC (of 50%) was the Butter potato.

Expressing the antioxidant richness of different vegetables as ORAC values *per* 100 g of raw or cooked weight allows their comparison and ranking in terms of their *in vitro* potential to provide antioxidants. However, in order to estimate the actual potential of such foods to supply the organism with their *in vitro* assayed antioxidants, a theoretical supply value was calculated by multiplying the ORAC value of each vegetable (expressed *per* 100 g of weight) by the portion at which such vegetable is regularly consumed (expressed in g). In the complementary data section, a table with the size portions used to estimate each of the theoretical supply values is included. Figure 5A depicts a ranking of the *per* portion ORAC values for a total of 43 vegetables that are either regularly (40 out of 43) or occasionally (only 3 out of 43) consumed in their raw state. While 9 out of the 43 vegetables exhibit ORAC values (µmol TE/portion) higher than 2000, 8 are between 1959 and 1224, and 26 are equal to or lower than 945. Of the latter ones, 18 would supply even less than 500 µmol TE/portion.

In terms of per cooked portion, the theoretical ORAC supply values of the formerly referred 29 vegetables that are consumed in their cooked state, of which (excepting for beetroot, chard and zucchini) 26 are consumed solely in their cooked state, are depicted in Figure 5B. As shown, out of the 29 vegetables, 8 exhibit ORAC values (µmol TE/portion) higher than 2000, 7 in the range 1928 to 1078, and 14 equal to or lower than 871. Of the latter ones, 4 would supply less than 500 µmol TE/portion.

4. Discussion

The recognition that populations showing a higher consumption of F&V have a lower relative risk of developing oxidative stress-related diseases has prompted the assessment of the antioxidant richness of plant-based foods and led to the construction of diverse on-line available databases. Examples of the latter include the databases launched by the USDA on flavonoids in 2003 and updated in 2014 [28] for 506 food items, on isoflavones in 1999 and updated in 2008 of 560 food items [29], and on proanthocyanidins, for 284 food items, launched in 2004 [30]. A totally separate but highly comprehensive database on 500 specific polyphenols in over 400 foodstuffs is the Phenol-Explorer database launched in 2009 and continually updated by Scalbert and co-workers [15,31]. Other databases have addressed the antioxidant richness of plant foods by assessing the total phenolic contents and ORAC activity. Pioneer work in that direction was the launching by the USDA in 2010 of a database with such antioxidant parameters for several hundred of foodstuffs commonly consumed by the American population (USDA database for the oxygen radical absorbance capacity (ORAC) of selected foods, release 2. Removed by the USDA's Nutrient Data Laboratory (NDL) in 2012 (http://www.ars.usda.gov/Services/docs.htm?docid=15866)). Subsequent to such endeavor our laboratory launched in 2012 a database with ORAC and TP for a large number of fruits produced and consumed within the south Andes region of South America [20]. Results from the present study extend the latter initiative to define ORAC and TP of 69 species and/or cultivars of vegetables grown in the same geographical region, including some varieties of potatoes, beans, onions and garlic that are believed to be native to the Chiloé Island. Among the 69 studied vegetables (of which 36 are species) the antioxidant richness was found to spread along a broad range of values, with differences of over 90-fold for TP (e.g. basil *versus* celery with 819 and 9 mg of GAE/100 g fw, respectively) and of near 215-fold for ORAC (e.g. parsley *versus* Beefsteak tomato, with 28865 and 134 micromol TE/100 g fw, respectively). Such differences are c.a. 3-fold greater than those estimated previously by us for a large group of fruits grown in the same areas, as the latter showed a near 30-fold difference in TP and an 80-fold difference in ORAC values [20] (www.portalantioxidantes.com). Nonetheless, when instead of comparing vegetables and fruits in terms of their highest and lowest TP and ORAC values, these are compared in terms of the difference that exists between the highest TP-containing foods, fresh basil (819 mg GAE/100 g fw) is estimated to be almost half as rich in TP as the maqui fruit (1664 mg GAE/100 g fw), according to data reported previously by us [20]. When such comparison is made on the basis of the highest ORAC-containing plant foods, parsley and basil (28865 and 21269 micromol TE/100 g fw, respectively) are found to exhibit values that are even slightly higher than those formerly seen by us [20] for calafate and maqui berries (25662 and 19850 micromol TE/100 g fw, respectively). The two latter fruits are recognized amongst the antioxidant-richest known berries [32,20]. Thus, it would be too adventurous to asseverate or generalize that, as a group of plant foods, either vegetables or fruits are a better potential source of antioxidants.

In terms of their relative TP and ORAC ranking positions, the here-presented results show that for those vegetables that are consumed in their raw and fresh state, parsley, basil and coriander rank as the top-three antioxidant-richest plant foods. Previous studies addressing the antioxidant richness of these three last vegetables, may have failed to remark their particularly high antioxidant properties, possibly, because their TP and/or ORAC were primarily examined solely in their dried states or in relation to a large group of other culinary herbs or spices [23,33]. In line with the abundance of phenolics in many vegetables and their recognized contribution to the whole antioxidant activity, the present study demonstrates the existence of high correlations between TP and ORAC in both, the 36 species and the 69 species and/or varieties of vegetables under study (r^2= 0.86 and r^2= 0.84, respectively). Previously, a good correlation between these two antioxidant parameters has been seen in fruits [20] as well as in several other plant foods [23,34]. Relative to most vegetables included in the here-shown correlation (Figure 2), parsley showed a greater separation from the curve, being its ORAC value considerably higher than that expected from its TP content. When parsley was not included among the 36 vegetables, the r^2 value increased from 0.86 to 0.92 (y = 24.12x - 452; data not shown). While the reasons for the latter are not clear, it would seem reasonable to assume that relative to most studied vegetables, parsley could be either particularly richer in non-phenolic molecules that actively contribute to its overall ROS-scavenging capacity, or rich in phenolic compounds whose structure endow them with a significantly higher efficiency to scavenge ROS.

The present study also remarks the great importance that the variety can have on the antioxidant richness of certain vegetables. For instance, in the case of tomatoes (analyzed unpeeled), a difference of over 16-fold higher ORAC can be calculated when comparing the Alicante and Beefsteak varieties (shown in Figure 3A). Yet, such difference amounted to only 1.2-fold when these varieties are compared in terms of their TP. Interestingly, when analyzed without peel, the ORAC value of the Alicante variety (but not that of Beefsteak) decreased by near one third, suggesting that the peel of the Alicante tomatoes might contain a substantially greater concentration of ORAC active non-phenolic compounds, of which one could be lycopene, known to be near 3-fold more concentrated in tomato skin than in flesh [35]. For lettuces, the importance of the variety in the antioxidant richness was evidenced as a 28-fold difference for ORAC and a near 18-fold difference for TP when the Sangria and the Iceberg varieties are compared. Although arugula is not strictly a variety of lettuce, this world-wide increasingly consumed green salad, was found to exhibit, after the Sangria lettuce, the highest ORAC and TP values.

Since many vegetables are often consumed in their cooked state, the effects of cooking on the antioxidant richness were investigated in 29 out of the 69 species and/or varieties of vegetables under study. Results indicate that while in species like asparagus, chard and lima beans, the boil-cooking process can have a severe antioxidant-lowering effects (with drops from 40-60%), in other species, like squash-Camote, peas, broccoli, beetroot and cauliflower, the effect of such process was either intermediate (17-25%) or only marginal (9% in eggplant). In previous works, an antioxidant-lowering effect of boil-cooking has also been observed in vegetables such as broccoli and carrots [36], peas [37], kale, spinach, cabbage, swamp cabbage, and shallots [38]. It should be noted, however, that the boiling process produced, in the case of artichokes an increment (of near 35%) in TP but not in ORAC, and that in the cases of yellow corn and Winter squash, significant increments that manifested only in their ORAC values (39% and 45% respectively). Regarding artichoke, Ferracane *et al.* [39] had already reported that the antioxidant capacity of this vegetable is found to be increased after boiling by near 66% in total caffeoylquinic acids, and up to 8-fold when assessed by TEAC, FRAP or TRAP assays. Presumably, the cooking process could have had a matrix-softening effect which makes more available and/or easier to extract from Winter squash and Yellow corn the ORAC-active assessable molecules, and from artichokes some phenolic compounds. Noteworthy, it has been reported that due to certain heat-induced chemical reactions, the cooking process could itself involve the formation of other Folin Ciocalteu-reducing and/or ORAC active assessable compounds [36]. Thus, it should point out that an increment in the analytically assessable antioxidant compounds should not necessarily be equated to an actual increment in the concentration or in the bio-accessibility of its health-promoting constituents.

On the other hand, the present study reveals that the magnitude of the antioxidant-lowering effects of boil-cooking would depend not only on the species of vegetable under study, but also on its tested varieties. For instance, among the eight varieties of beans examined, Black beans show the greatest susceptibility, as after cooking these underwent an over 8-fold greater drop in ORAC and 5-fold greater drop in TP compared to Green Pallar beans. It should be noted however that, since in raw black beans are near 8-fold richer in ORAC and TP than Green Pallar beans, it is not possible to assume that the antioxidant richness, and/or the relative ranking position of a given variety of bean will be the same after being cooked. Thus, Pinto beans, which rank first in ORAC in their raw state (12118 µmol TE/100 g) becomes second in their cooked state (1266 µmol TE/100 g), and raw Purple Pallar beans which rank third (8134 µmol TE/100 g) become first after cooking (2687 µmol TE/100 g). In contrast to what is seen with beans, the susceptibility of potatoes to boil-cooking was, in general, minimal and found not to differ essentially among the eight studied varieties. Out of the three purple varieties of potatoes included in the present study, two underwent either no decrement in TP (Cabrita and Purple potatoes) or just a 9% drop in the case of the third (Bruja potatoes). Recently, Lemos *et al*. [40] reported a near 30% drop in TP for baked Purple Majesty potato (purple skin and flesh), a highly pigmented anthocyanin-rich cultivar registered on the British Potato Variety Database. However, there are contradictory results in the literature regarding the effect of boil-cooking on the levels of total phenolics, with some authors reporting gains [41] and others losses [42,43] in TP after boil-cooking potatoes.

When it comes to make some possible nutritional interpretations, besides attending to the antioxidant richness that every plant food has in the state at which the plant foods is consumed (namely, raw or cooked), it should be also considered the amount or portion at which such food is on average regularly consumed. The latter contention allowed us to generate a theoretical antioxidant supply value, which in the case of ORAC, was calculated multiplying the per 100 g ORAC value of each vegetable by the portion at which such vegetable is consumed. Upon ranking the 43 vegetables that are consumed as raw foods a near 100-fold difference (from 57 to 6016 µmol TE/portion) could be estimated between the lowest and the highest theoretical ORAC-supplying vegetables. In the case of the 29 vegetables that are consumed as cooked-foods, such differences amount to 24-fold (from 210 to 5155 µmol TE/portion). Thus, it becomes evident that certain plant foods that rank highly on their per 100 g basis, rank only marginally when the comparison is made in terms of their contribution of ORAC per portion of consumption. Examples of the latter are parsley, basil and coriander, which move from being the top three among the raw vegetables, to the 5th, 8th and 18th position in the per portion ranking, respectively. Conversely, in contrast to their 6th, 10th, 8th and 17th position in the per 100 g basis, Sangria lettuce, capsicum Red, spinach and Alicante tomato emerge now as the four top ORAC-supplying raw vegetables when considered on a per portion basis, respectively. In the case of cooked eaten vegetables, the Michuñe potato, Cabrita potato, Purple potato and lima beans are the top four *per* portion ORAC-supplying foods, followed by artichoke bottom, Purple Pallar beans, Butter potato, Bruja potato, Desirée potato and Lengua de vaca potato. Noteworthy, out of these ten plant foods, seven are potatoes. Thus, when it comes to compare the 29 species and/or varieties of ORAC-supplying vegetables that are eaten as cooked foods, half of the top-ten ones are comprised by varieties of potatoes which are grown and in the present study were collected from the Chiloé Island. The latter is particularly significant since potatoes are regarded as a staple food amongst several populations of the west-south Andes region of South America. Interestingly, in the case of the North American diet, potatoes would account for up to 25% of the vegetable phenolics. On the basis of these results and upon considering the high frequency and consumption of potatoes among the latter populations, it becomes warranted the need to further characterized in terms of their antioxidant composition, not only the five formerly mentioned varieties of potatoes, but also a significant number of other so far no studied varieties of colored potatoes believed to be endemic or indigenous from this geographical region.

5. Conclusions

As a plant food group, some vegetables can be an excellent source of antioxidants. Among those consumed in their fresh and raw forms, parsley, and basil and coriander remark for their high ORAC and TP contents, approaching values found in some of the antioxidant richest berries. The variety can be determinant; in the case of tomatoes and lettuces, differences in ORAC of 16- and 28-fold, respectively, can be found. On the other hand, boil-cooking can affect the antioxidant richness of some but no other vegetable species. While major antioxidant-lowering effects are evident in asparagus, chard and lima beans (with drops in ORAC from 40-60%), a marginal effect is seen in eggplants. In contrast, a significant increase in TP (of near 35%, but not in ORAC) is seen when artichokes bottoms are subjected to cooking. Results of this study reveal that to assess the antioxidant-supplying potential of any vegetable, besides considering its antioxidant richness per 100 g of weight, the portion of consumption of any vegetable must be considered. A significant case of lessening the relative antioxidant-supplying potential is that of parsley, basil and coriander. An opposite case is that of Sangria lettuce, which on a per consumption portion becomes an excellent potential source of ORAC-related compounds. In the case of cooked foods, potatoes, whose antioxidant richness on a per 100 g basis does no remark as such, acquire a particularly important position as a source of antioxidants when its antioxidant-supplying potential is estimated on a per portion consumption basis. In fact, on such basis, different varieties of potatoes pass to represent seven out the ten most ORAC-contributing cooked foods. Remarkably, out of such seven varieties five correspond to potatoes regularly grown in Chiloé Island.

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Vegetables	Portion of Consumption (g)
Artichoke (bottom)	84
Arugula	30
Asparagus	93
Basil	10
Lima bean	94
Bean	100
Beetroot	110
Broccoli	148
Cabbage	84
Capsicum	148
Carrot	78
Cauliflower	99
Chard	100
Celery	110
Ciboulette	10
Coriander	10
Corn	90
Cucumber	99
Bean sprout	50
Eggplant	100
Garlic	2
Green bean	90
Leek	30
Lettuce	89
Mushroom	84
Onion	50
Parsley	10
Peas	80
Pepper	5
Potato	148
Radish	85
Scallions	15
Spinach	90
Squash	130
Tomato	148
Watercress	20

Appendix A. Supplementary Data

List of the portions of consumption of the vegetables analyzed in the present study. The values represent the amount (expressed in g) of the edible portion of the vegetable customarily consumed per eating occasion. In the present study, the listed values were employed to estimate the per portion ORAC values shown in Figure 5A and Figure 5B. Most portion values were derived from the U.S. Food and Drug Administration, Food Guidance Regulation (http://www.fda.gov/downloads/Food/GuidanceRegulation/ucm063477.pdf), January 2008, and whenever necessary, from data contained in the "Tabla de Composición de los Alimentos Chilenos", 8th edition, 1990 (http://www.libros.uchile.cl/files/presses/1/monographs/426/submission/proof/files/assets/common/downloads/publication.pdf).

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