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Influence of Cooking Methods on Antioxidant Activity of Vegetables

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ABSTRACT: The influence of home cooking methods (boiling, microwaving, pressure-cooking, griddling, frying, and baking) on the antioxidant activity of vegetables has been evaluated in 20 vegetables, using different antioxidant activity assays (lipoperoxyl and hydroxyl radicals scavenging and TEAC). Artichoke was the only vegetable that kept its very high scavenging-lipoperoxyl radical capacity in all the cooking methods. The highest losses of LOO· scavenging capacity were observed in cauliflower after boiling and microwaving, pea after boiling, and zucchini after boiling and frying. Beetroot, green bean, and garlic kept their antioxidant activity after most cooking treatments. Swiss chard and pepper lost OH· scavenging capacity in all the processes. Celery increased its antioxidant capacity in all the cooking methods, except boiling when it lost 14%. Analysis of the ABTS radical scavenging capacity of the different vegetables showed that the highest losses occurred in garlic with all the methods, except microwaving. Among the vegetables that increased their TEAC values were green bean, celery, and carrot after all cooking methods (except green bean after boiling). These 3 types of vegetables showed a low ABTS radical scavenging capacity. ccording to the method of analysis chosen, griddling, microwave cooking, and baking alternately produce the lowest losses, while pressure-cooking and boiling lead to the greatest losses; frying occupies an intermediate position. In short, water is not the cook's best friend when it comes to preparing vegetables.

Keywords: antioxidants, cooking methods, vegetables

Introduction

Oxidative-free radicals are byproducts of the normal reactions within our bodies. These reactions include the generation of calories, the degradation of lipids, the catecholamine response under stress, and inflammatory processes (Wang and others 2008).

Fruits and vegetables are considered to be the major contributors of Reactive Oxygen Spices (ROS)-scavenging antioxidants. The cancer and other disease-preventing action supposedly resides in the fact that vegetables contain not only abundant nutritional antioxidants, but also a great quantity of nonnutritional antioxidants, such as flavonoids (quercetin, one of the most abundant flavonoids present in vegetables [Wach and others 2007]), betalains, S-allyl cysteine, and S-methyl cysteine (Murcia and others 2006). Flavonoids have numerous beneficial effects on human health, acting as antioxidants because of their ability to act against a wide range of cations in multiple hydroxyl groups and in the carbonyl group on ring C (Shaghaghi and others 2008). However, sometimes, phenolic structures may exhibit prooxidant activity rather than antioxidant action and may play an important role in their anticancer and apoptosis-inducing properties, as dietary phenolic compounds, which could mobilize endogenous copper in human peripheral lymphocytes, leading to oxidative DNA damage (Zheng and others 2008).

Lifestyles that involve diets high in vegetables and fruits have been associated with a reduced risk of cancer and this association has motivated the "5-a-day" program. Furthermore, vegetables are very low in calories and are usually consumed in their fresh state, and also after processing and cooking. A calorie-restricted diet decreases chemically induced tumor incidence and increases life expectancy, reducing oxidative damage, and altering rates of cell division and/or apoptosis. This fact not only attenuates the generation of ROS by liver mitochondria, but also alters the activities of the electron transport chain (Hwang and Bowen 2007). However, cooking, such as boiling, which causes overall flavonol losses (Makris and Rossiter 2001), microwaving (Zhan and Hamauzu 2004), pressure-cooking, griddling, baking, and frying (Young and Jolly 1990), can profoundly affect both the texture and the nutritional value of vegetables. Cooking softens the cell walls and facilities the extraction of carotenoids (Rodríguez-Amaya 1999). Some studies have shown that a loss of vitamins in vegetables during cooking varies with the cooking treatment (Lin and Chang 2005). During deep-fat frying, oils undergo physicochemical changes. Moreover, the food dehydrates and fat penetrates the food. Thus, food fried in used oil contains significantly high levels of thermoxidized and polymerized products that could be undesiderable from a nutritional point of view (Sánchez-Muniz and Bastida 2003).

Vegetables contain several hydrophilic and lipophilic antioxidant compounds and it is important to estimate the antioxidant activity using different methods. They may act together more effectively than singly because they function synergistically and are capable of quenching free radicals in both aqueous and lipid phases (Ohr 2004; Trombino and others 2004). Antioxidant compounds may also act as metal chelators and interfere with the pathways that regulate cell division and proliferation and detoxification; they also may regulate inflammatory and immune responses, and may have antiulcerative properties (Hamauzu and others 2008). They may inhibit or activate a large variety of mammalian enzyme systems, exhibiting biphasic dose responses in cells at low doses. Phytochemicals activate signaling pathways that result in the

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increased expression of genes-encoding cytoprotective proteins, including antioxidant enzymes (Dragsted and others 2006), protein growth factors, and mitochondrial proteins. Examples of the phytochemicals: sulphorane or isothiocyanates (present in broccoli) and allicine (present in garlic) (Mattson 2008).

Most of common vegetables are consumed after being cooked. Thus, it is important to know what happens to their antioxidant activity or free radical capacity during common domestic processes (boiling, frying, microwave cooking).

Objective

Because modern day consumers seek to avoid aggressive cooking methods which may affect the functionality of foods, there is growing interest in the phytochemical profiles and antioxidant activities of cooked (boiled, microwaved, steamed, griddled, fried, and baked) vegetables. This article attempts to determine the optimum cooking method that results in the highest retention of the antioxidant capacity and radical scavenging activity of vegetables, to improve their functional activity.

Materials and Methods

Raw vegetables

Artichoke (*Cynara scolymus*, L.), asparagus (*Asparragus officinalis*, L.), beetroot (*Raphanus sativus*, L. cv. *cruenta*, L.), broad bean (*Vicia faba*, L. cv. *mayor*, L.), broccoli (*Brassica oleracea*, L. *botrytis* cv. *cymosa* Duch), Brussels sprout (*Brassica oleracea*, L. cv. *gemmifera*, Zenker), carrot (*Daucus carota*, L. cv. *sativa*, D.C.), cauliflower (*Brassica oleracea* L. cv. *botrytis* f. *cauliflora*, Duch), celery (*Apium graveolens*, L. cv. Dulce), eggplant (*Solanum melongena*, L.), garlic (*Allium sativum*, L.), green bean (*Phaseolus vulgaris*, Savi.), leek (*Allium porrum*, L.), maize (*Zea mays*, cv. saccharatum), onion (*Allium cepa*, L.), pea (*Pisum sativum*, L. cv. *vulgare*, L.), pepper (*Capsium annuum*, L. cv. grosum, Bailey), spinach (*Spinacia oleracea, L.*), Swiss chard (*Beta vulgaris*, L. cv. *cycla*, L.), and zucchini (*Cucurbita pepo*, L. cv. *medullusa*, Alef). The vegetables were purchased on 5 different occasions in a local supermarket and were from different lots.

Chemicals

All the chemicals used were of chromatographic grade and were purchased from Sigma Chemical Co. (Poole, Dorset, UK). The food additives widely used by food industry and compiled in Alimentarius Codex, Buthylated hydroxyanesole (BHA, E-320), Buthylated hydroxytoluene (BHT, E-321), and propyl gallate (E-310) at the permitted concentration of 100 μg/g were analyzed as antioxidant standards. From a human health perspective, the Codex Alimentarius and the FDA allow the use of phenolic antioxidants in foods and they are regarded as safe (GRAS) chemicals. The maximum usage level of single or multiple antioxidants approved in the legislation mentioned above is 200 ppm (Codex Alimentarius 2006).

Sample preparation

The vegetables were cooked in our laboratory, after cleaning and washing with water (in consumer conditions) and after separating the nonedible portion and cutting into small pieces. The vegetable samples (3.5 kg) were divided into 7 parts (500 g each), keeping 1 portion as control (uncooked, stored at 4 °C in the refrigerator in home consumer conditions), and the rest subjected to different cooking treatments (Table 1). These were:

(1) Boiling in a stainless steel vessel: each vegetable (500 g) was added to the boiling water (1000 mL) and cooked until tender.

(2) Microwaving: the vegetable (500 g) was placed in a glass dish without additional water, and cooked in a domestic microwave oven (Moulinex® model; type 45; 062001-E; 1500 W; Moulinex, AFM 441, Moulinex Intl., Barcelona, Spain).

(3) Pressure-cooking: vegetables (500 g) were placed in a pressure cooker (stainless steel, 22 cm diameter, Magefesa®, Zaragoza, Spain), containing water (300 mL) and a pressure valve for high pressure-cooking.

(4) Frying: the vegetable sample (500 g) was placed in a frying pan with hot refined olive oil (169 °C) (100 mL), and stirred until the sample became crisp-tender.

(5) Griddling: vegetable (500 g) cooked in a thick frying pan with no oil.

(6) Baking in a domestic oven (Tefal® model; 18 L; 5265.46; 2200 W; Tefal, Toastngrill, Groupe SEB IBERICA SA, Barcelona, Spain):

Table 1 — Cooking treatment and times used to cook the vegetables.

	Cooking methods					
	Boiling	Pressure-cooking	Microwave cooking (medium power)	Baking (200 °C)	Griddling	Frying
Vegetables	Times (min)					
Artichoke	10	4	3	20	13	10
Asparagus	17	5	4	39	18	12
Beetroot	24	5	2.5	30	15	8
Broad bean	20	5	4	30	14	10
Broccoli	16	5	2	49	20	15
Brussels sprout	22	5	2	35	18	14
Carrot	23	5	3	35	17	10
Cauliflower	17	5	2.5	34	20	13
Celery	20	4	3	20	20	10
Eggplant	16	4	3.5	38	10	10
Garlic	20	4	2.5	30	12	8
Green bean	21	4.5	3	35	15	15
Leek	15	3	2	30	12	8
Maize	47	10	2	40	10	7
Onion	20	5	3.5	35	17	12
Pea	12	4	4	25	10	6
Green pepper	20	5	4.5	47		17
Spinach	13	3	2	14	18	12
Swiss chard	20	3	3.5	30	15	10
Zucchini	20	4.5	4	24	15	10

vegetables (500 g) were placed in a ceramic container and were submitted to dry heat.

For all these cooking treatments, the best cooking times were previously established for each vegetable by an informal testing panel consisting of 3 trained panelists, so that vegetables had the color and texture of home-cooked products (Table 1).

After cooking, the vegetables were cooled for a few minutes at room temperature before being ground, homogenized, and stored at −20 °C. Raw and cooked vegetables were assayed by lipoperoxyl and hydroxyl radicals and Trolox equivalent antioxidant capacity (TEAC) methods to evaluate their free radical scavenging and antioxidant capacity. Several *in vitro* analytical methods are available to characterize the antioxidant propensity of bioactive compounds in plant foods, although the use of more than 1 method is recommended to give a comprehensive assessment of antioxidant efficacy. Oxidants and antioxidants have different chemical and physical characteristics and so there is no simple universal method by which antioxidant capacity can be measured accurately and quantitatively (Dini and others 2008).

Peroxidation of phospholipid liposomes. The fluidity and permeability of the cell membrane is maintained by its composition, including phospholipid, glycerides, and fatty acids. An excess of free radical helps induce attack on these lipids and the chain reaction of lipid peroxidation (Yeng and others 2008).

The experimental conditions were the same as those described in our previous report Martínez-Tomé and others (2004). The experiments were conducted in a physiological saline buffer (PBS) (3.4 mM Na_2HPO_4–NaH_2PO_4 0.15 M NaCl), pH 7.4. In a final volume of 1 mL, the assay mixtures were made up with PBS, 0.5 mg/mL phospholipid liposomes, 100 μM $FeCl_3$, 100 mg of samples, and 100 μM ascorbate (added last to start the reaction). Incubations were at 37 °C for 60 min, at the end of which, 1 mL each of 1% (wt/vol) thiobarbituric acid (TBA) and 2.8% (wt/vol) trichloroacetic acid were added to each mixture. The solutions were heated in a water bath at 80 °C for 20 min to develop the malondialdehyde thiobarbituric adduct ($[TBA]_2$–MDA). The $(TBA)_2$–MDA chromogen was extracted into 2 mL of butan-1-ol and the extent of peroxidation was measured in the organic layer as absorbance at 532 nm.

Hydroxyl radical scavenging. ROS have been suggested to cause structural alterations in DNA, for example, damaged bases and damage to deoxyribose and protein DNA cross-links. ROS are able to directly modify DNA bases, including hydroxyl radical and peroxyl and alkoxyl radicals. Whereas superoxide and hydrogen peroxide do not react with DNA bases directly, hydroxyl radicals generate a multiplicity of products from all 4 DNA bases, especially in the presence of Ca and Fe through Fenton-type reactions. The deoxyribose fragments produced in DNA by free radical attack are potent mutagens and block the action of the DNA polymerase and DNA ligase *in vitro* (Hwang and Bowen 2007).

In a final volume of 1.2 mL, the reaction mixtures contained the following reagents: 10 mM KH_2PO_4–KOH buffer (pH 7.4), 2.8 mM H_2O_2, 2.8 mM deoxyribose (where used), 50 μM $FeCl_3$ premixed with 100 μM EDTA before addition to the reaction mixture, and 100 mg of the tested samples. Ascorbate (100 μM), where used, was added to start the reaction. The tubes were incubated at 37 °C for 1 h. The products of the OH· radical attack on deoxyribose were measured as described in Murcia and others (2002).

ABTS radical cation scavenging activity

This method is based on the inhibition, by antioxidants, of the absorbance of the radical cation ABTS, which has a characteristic long-wavelength absorption spectrum showing a maximum at 734 nm. Antioxidant compounds suppress the absorbance of the ABTS radical cation to an extent and on a time scale dependent on the antioxidant capacity of the substance under investigation. A TEAC value can be assigned to all compounds that scavenge the $ABTS^{\cdot+}$ by comparing with the value Trolox, according to the experimental conditions described in Jiménez and others (2008).

Statistical analysis. Foods were prepared in 5 replicates and were analyzed in triplicate. The results were analyzed statistically using the Statgraphics Plus 5.1 (Open Land Communications, Madrid, Spain), submitting the data to a simple 1 factor analysis of variance (ANOVA) after verifying the existence of a highly significant interaction between the type of cooking and type of vegetable in a prior 2-factor variance analysis. The parameters studied were the antioxidant retention coefficients of all the vegetable in each of the cooking processes, obtained from all the raw and processed vegetable values, using the expression: (content after processing/content in raw vegetable) × 100, so that 100% indicates the absence of any loss and 0% the total loss of antioxidant activity, while a value above 100% would refer to gains over on the initial, raw value.

Results

Free radical scavenging assays

Antioxidant activity expressed as scavenging of lipoperoxyl radical. Table 2 shows the inhibition of lipid peroxidation in the presence of vegetables submission to different cooking treatments, such as boiling, microwaving, pressure-cooking, griddling, frying, and baking.

Boiling. This cooking method produced the greatest decrease in LOO· scavenging capacity in the most of the vegetables analyzed with respect to their fresh counterparts. The highest losses were obtained for pea, cauliflower, and zucchini, with percentages above 50%. Losses of around 30% to 50% were obtained for spinach, garlic, broccoli, Brussels sprout, carrot, leek, and green bean. The rest of the vegetables lost between 5% and 30% of scavenging capacity, except artichoke, eggplant, and onion, which maintained very good antioxidant activity, and asparagus that increased its LOO· scavenging capacity.

Pressure-cooking. After submitting vegetables to pressure-cooking, most of the vegetables showed significant losses ($P < 0.01$) of between 25% and 50%, except broad bean and spinach with losses of 5% and 25%. The vegetables that kept their antioxidant activity were Swiss chard, beetroot, onion, artichoke, and asparagus. Eggplant increased its antioxidant capacity significantly ($P < 0.01$).

Baking. The highest losses of antioxidant capacity were produced in carrot, Brussels sprout, leek, cauliflower, pea, and zucchini, with percentages of between 30% and 50%. Other vegetables (onion, broad bean, celery, beetroot, and garlic) lost between 5% and 30% of their scavenging capacity. The vegetables that kept their LOO· radical scavenging capacity after baking were artichoke, asparagus, broccoli, and pepper, while green bean, eggplant, maize, Swiss chard, and spinach increased their antioxidant activity significantly ($P < 0.05$).

Microwaving. When vegetables were submitted to microwaves, the highest losses were obtained for cauliflower (above 50%). Most of the vegetables decreased their scavenging capacity between 30% and 50%, while broad bean and beetroot lost 5% and 30% scavenging capacity, respectively. Vegetables that kept their antioxidant capacity were artichoke, asparagus, garlic, onion, and spinach. The vegetables that significantly ($P < 0.01$) increased their scavenging capacity were eggplant, maize, pepper, and Swiss chard.

Griddling. After griddling Brussels sprout and pea lost about 30% and 50% of their antioxidant capacity, respectively. The rest of the vegetables lost between 5% and 30%, except artichoke, beetroot, celery, eggplant, garlic, and maize, which maintained their antioxidant activity. Green bean, Swiss chard, spinach, asparagus, broccoli, and onion significantly ($P < 0.01$) increased their scavenging capacity after griddling.

Frying. The greatest losses were obtained for zucchini (above 50%). Other vegetables such as leek, onion, pea, Brussels sprout, and pepper lost between 30% and 50%, while the rest of the vegetables only showed losses of between 5% and 30%, except Swiss chard, artichoke, and green bean, which kept their antioxidant activity. Only eggplant increased the antioxidant activity with respect to the fresh sample.

In summary, artichoke was the only vegetable of those analyzed that kept its very high scavenging lipoperoxyl radical capacity in all the cooking methods applied. The highest losses of LOO· scavenging capacity were observed in cauliflower after boiling and microwaving, pea after boiling, and zucchini after boiling and frying.

Antioxidant capacity expressed as hydroxyl radical scavenging ability. The OH· radical scavenging capacity of vegetables submitted to boiling, pressure-cooking, baking, microwaving, griddling, and frying with respect to raw samples are shown in Table 3.

Boiling. The highest losses were for pepper (above 50% with respect to fresh). Spinach, cauliflower, and Swiss chard lost 25% to 50% of their scavenging capacities. The rest of the vegetables showed significant differences ($P < 0.01$) in the extent to which their OH· scavenging capacity was lost (5% to 25%), although beetroot, garlic, and green bean kept their antioxidant activity after boiling.

Pressure-cooking. The greatest loss in antioxidant activity after pressure-cooking was in pepper (more than 50%). Swiss chard lost between 30% and 50%. The rest of the vegetables lost between 5% and 30%, with significant differences ($P < 0.01$), except broccoli, garlic, green bean, and spinach, which maintained their antioxidant activity with respect to fresh samples. Celery showed a higher scavenging capacity after pressure-cooking than the fresh sample.

Baking. When vegetables were baked on the oven, pepper showed the highest losses with significant differences ($P < 0.01$). The rest of the vegetables also showed significant differences ($P < 0.01$) with losses of between 5% and 30%, except asparagus, broccoli, eggplant, green bean, leek, onion, and pea, which kept their antioxidant capacity. After baking, celery significantly ($P < 0.01$) increased its scavenging capacity with respect to the fresh product.

Microwaving. Again, pepper showed the highest losses after this cooking treatment (above 50%). Swiss chard showed losses of around 30% to 50%. The rest of the vegetables lost between 5% and 25%, except beetroot, broccoli, carrot, eggplant, garlic, green bean, leek, maize, and pea, which showed little variation in their antioxidant activity. This cooking process significantly ($P < 0.01$) increased the antioxidant activity of celery.

Griddling. The results show that beetroot, cauliflower, eggplant, and onion kept their antioxidant activity. In this case, the vegetables with the highest losses (with a significant difference of $P < 0.01$), was pepper. The rest of the vegetables lost 5% to 30% antioxidant activity. Again, celery significantly ($P < 0.01$) increased its OH· scavenging capacity after griddling.

Frying. Swiss chard significantly ($P < 0.01$) decreased its antioxidant activity with losses of between 30% and 50%. The rest of vegetables also decreased their antioxidant activity by between 5% and 30%, except beetroot, garlic, maize, and onion, whose antioxidant activity did not vary after frying. Celery produced higher levels of antioxidant activity than fresh celery, with significant differences ($P < 0.01$).

In summary, beetroot, green bean, and garlic kept their antioxidant activity after most cooking treatments. Swiss chard and pepper lost OH· scavenging capacity in all the processes. Celery increased its antioxidant capacity in all the cooking methods except boiling when it lost 14%.

Table 2—Percentage of losses of LOO radical scavenging in the lipid system using ox-brain phospholipids in vegetables submitted to different cooking methods.

	% Losses					
	Cooking methods					
Vegetables	Boiling	Pressure-cooking	Baking	Microwaving	Griddling	Frying
Artichoke	—[A]	—	—	—	—	—
Asparagus	$-6.7 \pm 1.56_{z}$	—	—	—	$-7.0 \pm 2.18_{yz}$	$6.5 \pm 1.08_{bc}$
Beetroot	$5.0 \pm 0.85_{bc}$	—	$19.6 \pm 1.16_{e}$	$22.0 \pm 1.55_{d}$	—	$21.4 \pm 0.88_{ef}$
Broad bean	$9.7 \pm 0.69_{bc}$	$6.8 \pm 0.60_{c}$	$10.5 \pm 1.09_{d}$	$10.4 \pm 0.72_{c}$	$8.0 \pm 1.42_{def}$	$11.8 \pm 1.15_{cd}$
Broccoli	$32.7 \pm 2.65_{fg}$	$37.4 \pm 1.66_{de}$	—	$34.2 \pm 0.60_{e}$	$-6.5 \pm 1.66_{yz}$	$15.6 \pm 1.03_{de}$
Brussels sprout	$33.0 \pm 1.67_{fg}$	$38.9 \pm 2.19_{def}$	$31.8 \pm 0.84_{f}$	$40.3 \pm 1.50_{fg}$	$35.1 \pm 1.32_{g}$	$42.4 \pm 0.83_{i}$
Cauliflower	$55.0 \pm 1.08_{ij}$	$36.7 \pm 0.61_{d}$	$36.4 \pm 1.06_{fg}$	$56.7 \pm 1.55_{h}$	$11.8 \pm 0.78_{ef}$	$23.9 \pm 0.47_{f}$
Carrot	$33.9 \pm 1.45_{fg}$	$43.7 \pm 1.37_{fg}$	$31.7 \pm 1.92_{f}$	$42.2 \pm 0.96_{g}$	$13.7 \pm 2.04_{f}$	$11.1 \pm 1.47_{cd}$
Celery	$21.2 \pm 1.17_{d}$	$34.3 \pm 1.57_{d}$	$11.2 \pm 1.09_{d}$	$30.7 \pm 2.10_{e}$	—	$18.9 \pm 3.28_{ef}$
Eggplant	—	$-6.4 \pm 2.24_{z}$	$-8.7 \pm 2.11_{xy}$	$-6.7 \pm 2.34_{z}$	—	$-8.4 \pm 3.15_{z}$
Garlic	$32.5 \pm 1.38_{fg}$	$38.9 \pm 1.15_{def}$	$19.9 \pm 1.41_{e}$	—	—	$15.5 \pm 1.79_{de}$
Green bean	$40.0 \pm 2.54_{h}$	$25.9 \pm 2.36_{d}$	$-12.4 \pm 2.00_{x}$	$33.0 \pm 0.44_{e}$	$-14.6 \pm 3.06_{x}$	—
Leek	$36.1 \pm 2.06_{gh}$	$43.3 \pm 1.78_{ef}$	$34.6 \pm 1.77_{fg}$	$36.3 \pm 1.68_{ef}$	$9.1 \pm 2.56_{def}$	$30.7 \pm 2.44_{g}$
Maize	$23.1 \pm 1.95_{de}$	$26.4 \pm 2.00_{d}$	$-8.6 \pm 1.06_{xy}$	$-8.5 \pm 1.38_{z}$	—	$9.5 \pm 1.74_{cd}$
Onion	—	—	$10.1 \pm 0.44_{cd}$	—	$-5.6 \pm 1.25_{yz}$	$34.6 \pm 1.20_{gh}$
Pea	$60.5 \pm 1.42_{j}$	$34.5 \pm 1.04_{d}$	$38.7 \pm 0.86_{g}$	$34.7 \pm 1.05_{ef}$	$34.7 \pm 1.16_{g}$	$38.2 \pm 1.40_{hi}$
Pepper	$27.9 \pm 0.69_{ef}$	$24.5 \pm 1.23_{d}$	—	$-9.8 \pm 3.15_{z}$	$6.6 \pm 2.29_{de}$	$42.9 \pm 1.40_{i}$
Spinach	$31.6 \pm 1.81_{fg}$	$18.3 \pm 1.39_{d}$	$-6.0 \pm 1.71_{yz}$	—	$-10.0 \pm 1.50_{xy}$	$7.8 \pm 1.54_{bc}$
Swiss chard	$10.8 \pm 1.09_{c}$	—	$-8.0 \pm 1.93_{xy}$	$-6.4 \pm 1.81_{z}$	$-10.2 \pm 1.34_{xy}$	—
Zucchini	$51.2 \pm 1.35_{i}$	$49.5 \pm 0.51_{g}$	$47.5 \pm 1.72_{h}$	$36.2 \pm 1.11_{ef}$	$13.1 \pm 1.59_{ef}$	$60.9 \pm 1.71_{j}$

Statistical differences were analyzed by ANOVA ($P < 0.01$). Different letters indicates significant differences among groups.
[A](—) indicates no losses detected.
Negative values indicate increases of antioxidant activity.

Measurement of antioxidant activity by ABTS method. Table 4 shows ABTS radical scavenging capacity for cooked vegetables expressed by TEAC value.

Boiling. This cooking method produced the highest losses in garlic (above 50%) and a significant decrease ($P < 0.01$) in the scavenging capacity in spinach and zucchini of between 10% and 30%. The rest of the vegetables maintained very good ABTS radical scavenging capacity. Carrot and leek increased their antioxidant activity with respect to fresh samples.

Pressure-cooking. After submitting vegetables to pressure-cooking, garlic lost around 50% of its antioxidant activity, while leek, maize, Swiss chard, and zucchini lost 10% to 50%. The rest of the vegetables kept their TEAC value, except carrot, celery, and green bean, which increased their TEAC value with respect to the fresh sample.

Baking. When the vegetables were cooked in an oven, garlic lost more than 50% of its ABTS radical scavenging capacity. The rest of the vegetables did not differ significantly from the fresh samples, except carrot, celery, and green bean, which increased their TEAC value after processing.

Microwaving. In this case, it must be remembered that heat is generated inside the vegetable and moves outward. All the vegetables kept their antioxidant capacity except maize (with losses of around

Table 3—Percentage of losses of OH radical scavenging capacity in vegetables submitted to different cooking methods.

	% Losses					
	Cooking methods					
Vegetables	Boiling	Pressure-cooking	Baking	Microwaving	Griddling	Frying
Artichoke	$9.3 \pm 2.02_{bcd}$	$10.0 \pm 2.51_{de}$	$8.8 \pm 1.13_{def}$	$8.3 \pm 1.89_{efg}$	$8.1 \pm 1.53_{cde}$	$5.4 \pm 2.16_{b}$
Asparagus	$9.1 \pm 1.46_{bc}$	$9.6 \pm 0.38_{de}$	—[A]	$7.4 \pm 1.83_{def}$	$9.2 \pm 0.85_{def}$	$8.0 \pm 1.37_{bc}$
Beetroot	—	$8.2 \pm 1.72_{cde}$	$8.0 \pm 1.00_{de}$	—	—	—
Broad bean	$14.5 \pm 1.11_{de}$	$17.5 \pm 1.25_{fg}$	$13.9 \pm 1.21_{fg}$	$14.9 \pm 1.54_{hi}$	$14.7 \pm 1.22_{fgh}$	$15.6 \pm 1.46_{def}$
Broccoli	$15.2 \pm 1.91_{ef}$	—	—	—	$15.9 \pm 1.33_{gh}$	$12.2 \pm 1.76_{cd}$
Brussels sprout	$22.7 \pm 1.90_{h}$	$20.6 \pm 0.66_{gh}$	$16.7 \pm 1.31_{gh}$	$18.3 \pm 2.07_{i}$	$28.1 \pm 1.63_{j}$	$20.7 \pm 1.14_{fg}$
Cauliflower	$32.2 \pm 1.53_{i}$	$28.4 \pm 1.33_{i}$	$27.4 \pm 1.45_{j}$	$10.4 \pm 1.95_{fgh}$	—	$17.0 \pm 1.67_{def}$
Carrot	$16.2 \pm 1.56_{efg}$	$19.0 \pm 1.71_{gh}$	$22.5 \pm 1.66_{ij}$	—	$12.9 \pm 1.60_{efgh}$	$20.1 \pm 0.93_{fg}$
Celery	$13.8 \pm 1.03_{cde}$	$-12.8 \pm 2.40_{z}$	$-42.7 \pm 2.80_{z}$	$-39.3 \pm 1.53_{z}$	$-42.3 \pm 3.45_{z}$	$-27.4 \pm 2.03_{z}$
Eggplant	$20.4 \pm 0.89_{fgh}$	$7.5 \pm 1.56_{cde}$	—	—	—	$14.3 \pm 1.19_{de}$
Garlic	—	—	$5.5 \pm 1.31b_{cde}$	—	$6.3 \pm 1.61_{cd}$	—
Green bean	—	—	—	—	$11.5 \pm 1.49_{defg}$	$7.1 \pm 0.90_{cd}$
Leek	$8.7 \pm 0.63_{bc}$	$7.6 \pm 1.24_{cde}$	—	—	$12.5 \pm 1.13_{efgh}$	$17.3 \pm 0.92_{def}$
Maize	$8.3 \pm 1.43_{b}$	$8.6 \pm 1.40_{cde}$	$7.2 \pm 1.07_{cde}$	—	$7.7 \pm 1.10_{cde}$	—
Onion	$9.1 \pm 1.32_{bc}$	$5.3 \pm 1.89_{bcd}$	—	$7.0 \pm 1.73_{cdef}$	—	—
Pea	$14.0 \pm 1.93_{cde}$	$12.2 \pm 1.97_{ef}$	—	—	$12.0 \pm 1.32_{efg}$	$17.4 \pm 1.53_{def}$
Pepper	$74.8 \pm 0.88_{k}$	$72.4 \pm 0.84_{k}$	$71.3 \pm 1.32_{k}$	$70.4 \pm 0.71_{k}$	$62.2 \pm 0.69_{k}$	$24.4 \pm 1.11_{g}$
Spinach	$30.6 \pm 1.05_{i}$	—	$9.7 \pm 1.31_{ef}$	$13.5 \pm 1.19_{ghi}$	$17.8 \pm 1.38_{hi}$	$19.2 \pm 1.20_{efg}$
Swiss chard	$43.0 \pm 0.89_{j}$	$48.8 \pm 1.06_{j}$	$21.7 \pm 1.75_{hi}$	$31.3 \pm 1.37_{j}$	$22.6 \pm 0.99_{ij}$	$48.1 \pm 1.46_{h}$
Zucchini	$21.3 \pm 0.64_{gh}$	$23.3 \pm 1.17_{hi}$	$16.9 \pm 1.31_{ghi}$	$6.2 \pm 0.77_{bcdef}$	$15.6 \pm 1.15_{gh}$	$20.7 \pm 1.19_{fg}$

Statistical differences were analyzed by ANOVA ($P < 0.01$). Different letters indicates significant differences among groups.
[A](—) indicates no losses detected.
Negative values indicate increases of antioxidant activity.

Table 4—Percentage of losses of ABTS radical anions scavenging by vegetables submitted to different cooking methods.

	% Losses					
	Cooking methods					
Vegetables	Boiling	Pressure-cooking	Baking	Microwaving	Griddling	Frying
Artichoke	—[A]	—	—	—	—	—
Asparagus	—	—	—	—	—	$37.9 \pm 2.84_{bc}$
Beetroot	—	—	—	—	—	—
Broad bean	—	—	—	—	—	—
Broccoli	—	—	—	—	—	—
Brussels sprout	—	—	—	—	—	—
Cauliflower	—	—	—	—	—	$17.5 \pm 2.75_{ab}$
Carrot	$-66.7 \pm 15.61_{y}$	$-79.8 \pm 16.35_{x}$	$-111.1 \pm 23.68_{y}$	$-142.2 \pm 26.84_{y}$	$-191.1 \pm 25.06_{y}$	$-191.2 \pm 25.41_{x}$
Celery	$-37.3 \pm 13.81_{z}$	$-43.1 \pm 9.26_{y}$	$-149.6 \pm 20.69_{x}$	$-167.0 \pm 18.43_{y}$	$-167.2 \pm 18.79_{y}$	$-154.7 \pm 18.10_{y}$
Eggplant	—	—	—	—	—	—
Garlic	$59.1 \pm 3.83_{c}$	$59.3 \pm 3.08_{e}$	$59.4 \pm 2.55_{b}$	—	$35.5 \pm 3.96_{b}$	$58.2 \pm 2.02_{c}$
Green bean	—	$-10.6 \pm 4.71_{z}$	$-57.8 \pm 6.99_{z}$	$-57.2 \pm 7.42_{z}$	$-53.4 \pm 6.70_{z}$	$-53.6 \pm 8.33_{z}$
Leek	—	$35.9 \pm 4.40_{cd}$	—	—	—	—
Maize	—	$23.5 \pm 3.15_{cd}$	—	$35.0 \pm 3.20_{b}$	—	—
Onion	—	—	—	—	—	—
Pea	—	—	—	$6.1 \pm 1.43_{ab}$	—	—
Pepper	—	—	—	—	—	$20.5 \pm 3.16_{ab}$
Spinach	$11.1 \pm 1.77_{ab}$	—	—	—	—	—
Swiss chard	—	$18.6 \pm 2.94_{bc}$	—	—	—	$14.0 \pm 3.85_{ab}$
Zucchini	$28.5 \pm 3.11_{b}$	$39.3 \pm 1.68_{d}$	—	—	—	—

Statistical differences were analyzed by ANOVA ($P < 0.01$). Different letters indicates significant differences among groups.
[A](—) indicates no losses detected.
Negative values indicate increases of antioxidant activity.

35%) and pea (with losses of around 6%) while carrot, celery, and green bean showed a significant increase ($P < 0.01$) of antioxidant activity.

Griddling. This type of cooking produced significant losses ($P < 0.01$) in garlic. The rest of the vegetables kept their ABTS radical scavenging capacity, except carrot, celery, and green bean, which increased their TEAC value with significant differences ($P < 0.01$).

Frying. After frying, garlic showed losses higher than 50%, asparagus between 30% and 40%, Swiss chard, cauliflower, and pepper between 5% and 30%. The rest of the vegetables did not show variations in their TEAC value, except carrot, celery, and green bean, which increased their antioxidant activity.

In summary, analysis of the ABTS radical scavenging capacity of the different vegetables showed that the highest losses were obtained in garlic using all the methods, except microwaving, which produced a very pronounced loss in maize. After boiling, zucchini decreased its TEAC value and after pressure-cooking a substantial decrease was obtained for Swiss chard, maize, leek, and zucchini.

Among the vegetables that increased their TEAC values were green bean, celery, and carrot after all the processing methods (except green bean after boiling). These 3 types of vegetables showed a low ABTS radical scavenging capacity.

Discussion

When vegetables are submitted to cooking processes, such as pressure-cooking, microwaving, baking, griddling, deep-frying, variations appear in their antioxidant activity or scavenger capacity. These variations depend on the vegetable themselves (bioactive structures), the cooking method, the bioavailability of phenolics (Sultana and others 2007), temperature, the localization of the structures in the vegetables, cutting, chopping (Makris and Rossiter 2001), stability of the structure to heat (Prasad and others 1996; Pedraza-Chaverrí and others 2006), the synergic activity of the structures, and on the reaction systems assayed (for example, β-carotene is an efficient singlet oxygen quencher but is not a hydrogen (donor; Yamaguchi and others 2001).

Four possibilities are suggested for the increase in antioxidant activity of some vegetables after cooking: (1) the liberation of high amounts of antioxidant components due to the thermal destruction of cell walls and sub cellular compartments; (2) the production of stronger radical-scavenging antioxidants by thermal chemical reaction; and/or (3) suppression of the oxidation capacity of antioxidants by thermal inactivation of oxidative enzymes; (4) production of new nonnutrient antioxidants or the formation of novel compounds such as Maillard reaction products with antioxidant activity (Morales and Babel 2002). However, it is not clear to what extent each possible factor contributes to the increase in activity (Yamaguchi and others 2001). It has recently been shown that the thermal processing of sweet corn, tomato, and other vegetables increases antioxidant activity, perhaps as a result of Maillard reaction products (Dewanto and others 2002; Nindo and others 2003). Sultana and others (2007), found an increase in the reducing power of carrot due in part, to the exceptional increase in carotenoids, which could have compensated for the general loss in vitamin C and other nonphenolic antioxidants present in the vegetables.

In the case of boiling or pressure-cooking occurs lixiviation phenomenon that leads to a 64% loss of total carotenoids and a 49% loss of total phenolics (Bunea and others 2008). The phenols enter the cooking water and complex phenol proteins are found, reducing drastically by 90% or more according to Barroga and others (1985), and Rocha-Guzmán and others (2007). The concentration of phenolic acids is highest in the outer layers of some vegetables (Turkmen and others 2005) and these are extremely exposed to the water (Andlauer and others 2003) reducing antioxidant power of some vegetables such as pea, spinach, cauliflower, and cabbage (Sultana and others 2007). However, although total phenolics are usually stored in vegetables in pectin or cellulose networks and can be released during thermal processing, individual phenolics may sometimes increase because heat can break supramolecular structures, releasing the phenolic sugar glicosidic bounds, which react better with the Folin–Ciocalteau reagent (Bunea and others 2008).

On the other hand, boiling may decrease the activity by decreasing ascorbic acid (Yamaguchi and others 2001), while higher activity may occur as consequence of the inactivation of oxidative enzymes such as ascorbate oxidase (Yamaguchi and others 2001). This fact reduces the browning potential and, although chlorogenic acid decreases, the ascorbic acid allows, its concentration to be retained for a longer time (Viña and Chaves 2007).

On the other hand, microwave heating retains the active components in the cooked tissue (Yamaguchi and others 2001). Our results for most of the vegetables analyzed agree with this. The activity of vegetables cooked in the microwave oven was generally higher than that of those cooked in boiling water, because microwave heating, griddling and baking does not stimulate the release of ascorbic acid or other antioxidants from cooked tissue. Allicin preserved its OH radical scavenging properties after microwaving and boiling (Prasad and others 1996; Pedraza-Chaverrí and others 2006).

In the frying process using olive oil, the food looses water but increases its content of α-tocopherol, polyphenols, and even terpenic acids from olive oil (Kalogeropoulos and others 2007). Tocopherol is lost at frying temperatures but its stability increases in low oxygen atmospheres or in the presence of more active primary antioxidants such as ascorbic acid (Boskou 2003). In the aqueous phase, oxygen, prooxidant metals, and water-soluble antioxidants, must diffuse through this phase. For this reason, differences in solubility and mobility and mass transfer rates of transition metals and antioxidant through different phases may significantly affect oxidation. One important property of oxygen is that it is more soluble in oil than water and this may also influence oxidation rates (Jacobsen and others 2008). Oxidative reactions are enhanced after cooking due to the interaction of unsaturated fatty acids with prooxidant substance such as, nonheme iron, oxygen, or due to frying time/temperature (Kalogeropoulos and others 2007). When the frying time exceeds 3 min, proteins can react with TBA solution, reducing TBA values (Du and Li 2008).

Conclusions

The results showed that artichoke was the only vegetable that maintained its very high scavenging lipoperoxyl radical capacity in all the cooking methods. Beetroot, green bean, and garlic kept their OH· antioxidant activity after most cooking treatments. Among the vegetables that increased their TEAC values were green bean, celery, and carrot after all cooking methods (except green bean after boiling). These 3 types of vegetables showed a low ABTS radical scavenging capacity.

Our findings have identified the best methods for cooking vegetables while retaining their radical-scavenging activity and antioxidant activity with its health-related properties. Depending on the vegetable in question, griddling and microwave cooking produced the lowest losses, while pressure-cooking and boiling lead to the greatest losses; in general, frying occupies an intermediate position. In short, water is not the cook's best friend when it comes to preparing vegetables.

The results of the study serve as a database providing information on the effects of different cooking methods on the antioxidant potential of vegetables, and might encourage the food industry to recommended particular cooking methods to help maintain the antioxidant properties of vegetables that we eat. However, further research about cooking's impact on chemical efficacy *in vivo* should be carried about in the future.

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