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Polyphenols and Antioxidant Capacity of Vegetables under Fresh and Frozen Conditions

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The phenolic and oxygen radical absorbance capacity (ORAC) values have been measured in six fresh and frozen vegetables (beet green, spinach, broccoli, carrot, onion, and celery) from the same cultivar by analyzing the whole juice (WJ) and the acetonic extract of the squeezed pulp. To exploit the effect of the acid environment on the stability and recovery of the phenolics, perchloric acid (PCA) was added directly to WJ and to the pulp before the extraction with acetone. In both fresh and frozen vegetables, PCA markedly increased the recovery of phenolics extracted from the pulp, but PCA had no effect on the WJ. Four of six frozen vegetables showed lower phenolic and ORAC values than the fresh vegetables, whereas in the other two cases, values were significantly higher compared to fresh samples. Among the fresh vegetables, beet green showed the highest ORAC and phenolic values; however, when measured in two different cultivars of beet green, the ORAC value showed as much as 4.5-fold variation, whereas total phenolics and flavonoids showed 1.2- and 3.5-fold variations, respectively. The results show that total phenolics and ORAC, compared in fresh and frozen vegetables, represent an index of the mildness of blanching in the industry of frozen vegetables and provide a measure of the gap in antioxidants in the diet of people who consume frozen instead of fresh vegetables. The plant genotype is an important source of variabilty in the ORAC value, which can be conveniently used to increase the intake of antioxidants from vegetables.

KEYWORDS: Vegetable juices; antioxidants; phenolic compounds; oxygen radical absorbance capacity (ORAC); fresh and frozen vegetables

INTRODUCTION

Fruits and vegetables contain large amounts of natural antioxidant molecules, which can protect against several chronic diseases (*1*−*5*). Among these molecules, we find the liposoluble vitamins A and E, β-carotene, the water soluble vitamin C, and a wide range of amphipathic molecules, broadly termed phenolic compounds. These compounds are divided into several subclasses including phenolic acids, flavonoids, glucosides, and esters (*6*−*8*). Some molecules, representative of each subclass, are specific to only a few plant families or even a single family and may be absent in others (*9*, *10*). Within a vegetable family, the phenolic pool may also change with the cultivar, soil type, growth stage, and fertilization (*11*). After harvest, the relative proportion of the phenolic subclasses can also change with the time of storage and with the technological processes of conservation.

Today, consumers are aware of the need to consume a variety of fresh vegetables to get the most complete antioxidant support (*12*); however, many people do not have the opportunity to eat fresh vegetables every day and frequently use frozen vegetables. Preservation of the nutritive quality of frozen vegetables is closely linked to the lag time between their harvest and their arrival at the processing plant, as well as to the mildness of the blanching in the freezing process. The blanching effect can deplete nutritional values, as vitamins and phenolic compounds are relatively stable when subjected to heat treatments (*12*). Moreover, the freezing procedure varies among different kinds of vegetables. For instance, carrots must be scalded by steam and then peeled before undergoing blanching; other vegetables are subjected to blanching after general cleaning.

The oxygen radical absorbance capacity (ORAC) value is a parameter that shows the efficiency of antioxidant compounds in scavenging free radicals; its value depends on the quality and quantity of the antioxidants contained in the vegetable (*13*−*16*). If the industrial process or storage depletes or modifies the phenolic pool of the vegetable, this could be revealed by a change in the ORAC value.

This kind of study has seldom been performed on frozen vegetables, which are thought to contain lower amounts of phenols with respect to fresh vegetables, but the actual difference between the two categories of vegetables is lacking.

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In this study we measured, in some fresh and frozen vegetables, the total phenolics and ORAC values to detect the extent of their decay from fresh to frozen conditions, to verify if the two parameters may be an index of mildness of the technological processing, and to determine what gap in the antioxidant capacity can potentially adversely affect the diet of people who consume frozen vegetables. The role of the vegetable genotype was considered as well.

MATERIALS AND METHODS

Chemicals. 2,2′-Azobis(2-amidinopropane) dihydrochloride (AAPH) was purchased from Polyscience (Warrington, PA). 6-Hydroxy-2,5,7,8-tetramethyl-2-carboxylic acid (Trolox) was obtained from Aldrich (Milwaukee WI). *Porphiridium cruentum* β-phycoerythrin (B-PE), Folin−Ciocalteu phenol reagents, and all other reagents were purchased from Sigma Aldrich (Milan, Italy).

Vegetables. Broccoli (*Brassica oleracea* Botrytis cv. Cymosa Duch), beet greens (*Beta vulgaris* cv. Bieta Costa bianca and cv. Bieta Verde da taglio), onions (*Allium cepa* cv. Bianca della Regina), celery (*Apium graveolens* cv. Dulce), carrots (*Daucus carota* cv. Nandor), and spinach (*Spinacia oleracea* cv. Kent hybrid) were purchased fresh from farmers who sell them to a local frozen vegetable industry and taken frozen from the bags after processing. Among the frozen vegetables, onion and celery were found in small cubes. The processing conditions used in the production of the frozen vegetables were based on the flo-freezer technology, which allows minimal dehydration of the vegetables and high capacity of production.

Sample Preparation. Each vegetable (100 g) was cut up, ground, and homogenized; the homogenate was collected in vials and centrifuged at 3000*g* for 15 min (4 °C). The supernatant (named WJ) was recovered, and part of it was placed in ice and treated 1:1 v/v with 5% perchloric acid (PCA). This acidified juice, labeled WJ + PCA, was neutralized with 3 M K_2CO_3, centrifuged for 15 min at 3000*g*, and used for the assays. The vegetable pulp was added (1:1 w/v) with 5% PCA, and after 10 min of shaking, we performed an organic extraction with (1:7 w/v) 100% acetone. Typically, the final concentration of the acidified acetone mixture was 80% acetone + 1% PCA. To exploit the contribution of the acid on the organic extraction, a control sample was set up. Briefly, each gram of wet pulp was also extracted (1:1 w/v) with water at room temperature, and after 10 min of shaking, 100% acetone was added, so that the final extractive mixture was 80:20 acetone/water. The acidified and neutral acetonic mixtures were shaken for 30 min at room temperature, and then the supernatants were recovered by 15 min of centrifugation at 3000*g* and 4 °C. The extracts, simply labeled in the following sections as acetone and acetone + PCA, were immediately assayed for the phenolics. Parts of the acetonic extract and WJ were kept frozen at −20 °C until they were used for the ORAC assay, which was performed within 3 days.

Figure 1 shows the scheme for the extraction of phenolic compounds from fresh and frozen vegetables. Trials were also performed by increasing the extraction time to 120 and 240 min or by increasing the PCA in acetone to 5 or 10% final concentration.

ORAC Assay. The method of Cao et al. (*17*) was used with slight modifications (*15*). The final reaction mixture for the assay (2 mL) was prepared as follows: 1600 μL of 0.04 μM B-PE in 0.075 M sodium phosphate buffer, pH 7.0, 200 μL of diluted sample, or 50 μM Trolox. The blank was 0.075 M sodium phosphate buffer, pH 7.0, or acetone. Fluorescence was read every 5 min at 37 °C using a Perkin-Elmer (Norwalk, CT) LS-5 spectrofluorometer at 565 nm emission and 540 nm excitation. When stability was obtained, the reaction was started with 200 μL of 40 mM AAPH and fluorescence measured until zero. The ORAC value refers to the net protection area under the curve (AUC) of B-PE in the presence of samples or Trolox, minus the blank. The formula

$$\text{ORAC } (\mu\text{mol of TE/g of dry tissue}) = [(A_s - A_b)/(A_t - A_b)]kah$$

where A_s is the AUC of the sample, A_b is the AUC of the blank, A_t is the AUC of the Trolox, k is the dilution factor, a is the concentration of the Trolox in μmol/L, and h is the ratio between the liters of juice and the grams of vegetable.

AUC values were calculated with the aid of an integrating program by ORIGIN 2.8 (Microcal Software).

HPLC Analysis. Flavonoid concentration in beet green was determined in the Department of Food Science, University of Perugia. The WJ and the acetone + PCA extract were combined, dessiccated under vacum, resuspended with 80% methanol and then analyzed by HPLC as previously reported (*18*).

Other Assays. Phenolic compounds were assayed according to the Folin−Ciocalteu method (*19*). The total phenolic content was expressed as caffeic acid equivalents in milligrams per gram of juice, milligrams per gram of wet pulp, or milligrams per 100 g of vegetable as consumed.

Statistics. Statistical analysis was carried out using ANOVA with significance level set at $p < 0.05$. Differences among means were evaluated using the ORIGIN 2.8 (Microcal Software) statistic program. All determinations were made at least in duplicate, and a minimum of four samples was employed for each fresh or frozen vegetable.

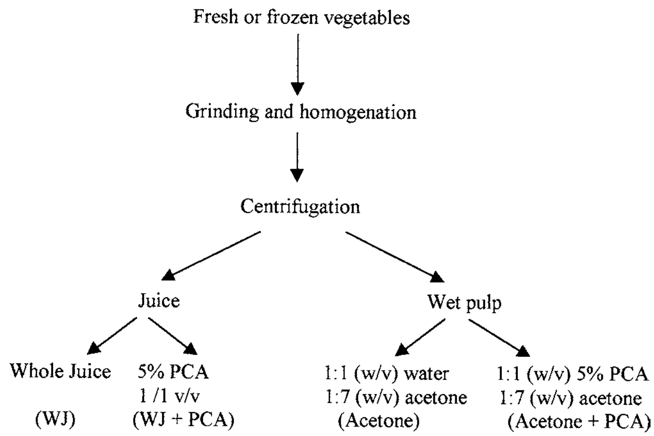

Figure 1. Scheme for the extraction of phenolics from fresh and frozen vegetables. At the end of the flow sheet the effective labels of the extracts used in this study are reported. Concentrations of the final extractive mixtures of the wet pulp were 80% acetone or 80% acetone + 1% perchloric acid (PCA).

Table 1. Phenols in Aqueous Whole Juices of Fresh and Frozen Vegetables[a]

vegetable	fresh		frozen	
	WJ	WJ + PCA	WJ	WJ + PCA
beet green[b]	1.28 ± 0.09	1.19 ± 0.08	0.37 ± 0.04[c]	0.26 ± 0.04[d]
spinach	1.02 ± 0.09	0.54 ± 0.07[e]	0.64 ± 0.05[c]	0.41 ± 0.04[d]
broccoli	0.57 ± 0.06	0.62 ± 0.05	0.55 ± 0.04	0.46 ± 0.04
carrot	0.28 ± 0.08	0.26 ± 0.07	0.20 ± 0.06	0.11 ± 0.03
onion	0.23 ± 0.02	0.25 ± 0.02	0.24 ± 0.04	0.28 ± 0.03
celery	0.09 ± 0.01	0.06 ± 0.01	0.07 ± 0.02	0.07 ± 0.02

[a] Values are expressed as mg/g juice and are the mean ± SD of four independent determinations. [b] Cv. Bieta verde da taglio. [c] Significantly different from WJ of fresh hortages by ANOVA with $p \leq 0.05$. [d] Significantly different from WJ of frozen vegetables. [e] Significantly different from WJ of fresh vegetables.

RESULTS

Table 1 shows the phenolic concentration of the WJ from fresh and frozen vegetables in the presence or absence of PCA. In spinach, the addition of PCA to the WJ decreased the phenolic concentration by about 50 and 30% in fresh and frozen spinach, respectively. In beet green, the addition of PCA depleted the phenolic concentration in frozen samples only. Indeed, in beet green and spinach, the level of total phenols in the WJ was higher in fresh vegetables than in frozen vegetables; in the other

Table 2. Phenols in Acetone Extracts from Pulp of Fresh and Frozen Vegetables[a]

	fresh		frozen	
vegetable	acetone	acetone + PCA	acetone	acetone + PCA
beet green[b]	0.48 ± 0.03	1.38 ± 0.08[c]	0.34 ± 0.03	0.36 ± 0.02
spinach	0.56 ± 0.03	1.13 ± 0.09[c]	0.49 ± 0.03	0.72 ± 0.03[d]
broccoli	0.43 ± 0.02	0.71 ± 0.04[c]	0.45 ± 0.02	0.62 ± 0.03[d]
carrot	0.14 ± 0.02	0.15 ± 0.02	0.10 ± 0.01	0.11 ± 0.02
onion	0.18 ± 0.02	0.25 ± 0.02[c]	0.29 ± 0.03	0.26 ± 0.03
celery	0.10 ± 0.02	0.16 ± 0.02[c]	0.08 ± 0.01	0.09 ± 0.01

[a] Values are expressed as mg/g wet pulp and are the mean ± SD of four independent determinations. [b] Cv. Bieta verde da taglio. [c] Significantly different from acetone of fresh vegetables. [d] Significantly different from acetone of frozen vegetables by ANOVA with $p \leq 0.05$.

Table 3. Total Phenols and ORAC Units in Selected Fresh and Frozen Vegetables[a]

	phenols, mg/100 g of vegetable as consumed		ORAC, μmol of TE/100 g of vegetable as consumed	
vegetable	fresh	frozen	fresh	frozen
beet green[b]	118.23 ± 19	36.5 ± 4[c]	2442.62 ± 200	307.85 ± 30[d]
spinach	107.02 ± 11	71.75 ± 7[c]	734.98 ± 70	419.96 ± 43[d]
broccoli	69.27 ± 7	28.79 ± 3[c]	335.85 ± 30	294.07 ± 30
carrot	20.32 ± 2	8.21 ± 2[c]	212.36 ± 20	100.96 ± 10[d]
onion	24.40 ± 2	52.32 ± 5[c]	540.26 ± 50	707.58 ± 71[d]
celery	12.60 ± 2	15.29 ± 3	180.20 ± 20	100.48 ± 10[d]

[a] Values are mean ± SD of four indipendent determinations. [b] Cv. Bieta verde da taglio. [c] Significantly different from phenols of fresh vegetable. [d] Significantly different from ORAC of fresh vegetables by ANOVA with $p \leq 0.05$.

vegetables, values for fresh samples were almost the same as for frozen samples.

Table 2 shows total phenols of the acetone and acetone + PCA extracts from the pulp. In all fresh vegetables, except carrots, the addition of PCA to acetone increased the extraction of phenols from the pulp. In two of the six frozen vegetables, namely, spinach and broccoli, the effect of PCA added to acetone was significant for the amount of extracted phenols; in the other cases the effect of PCA was irrelevant.

The increase of PCA from 1 to 5 or 10% final concentration provided a negligible increase in the amount of extracted phenols, and prolonging the extraction time of the pulp in acetone + PCA from 30 to 240 min also made no difference (data not shown).

Table 3 shows total phenols and ORAC values in 100 g of fresh or frozen vegetables as consumed. The values were obtained by combining the WJ and the acetone + PCA extract. Except in the cases of onions and celery, total phenols were higher in fresh vegetables than in frozen vegetables. Except in the case of onions, fresh vegetables have higher ORAC values than frozen vegetables. Fresh beet green showed the highest antioxidant capacity with an ORAC value 3 times higher than that of spinach (**Table 3**), although the phenolic concentrations of the two vegetables were not significantly different.

To state the contribution of the genotype, we compared total phenols, flavonoids, and ORAC values of two different cultivars of beet green. Cv. Bieta verde da taglio has ~1.2 times higher phenolic values than cv. Bieta costa bianca (**Figure 2A**), but the former has an ORAC value 4.5 times higher than that of the latter (**Figure 2B**).

Because in the assay of phenolics with the Folin−Ciocalteu reagent other substances can interfere (*8*), we added in this case the HPLC analysis of the phenolic extract. The HPLC profile

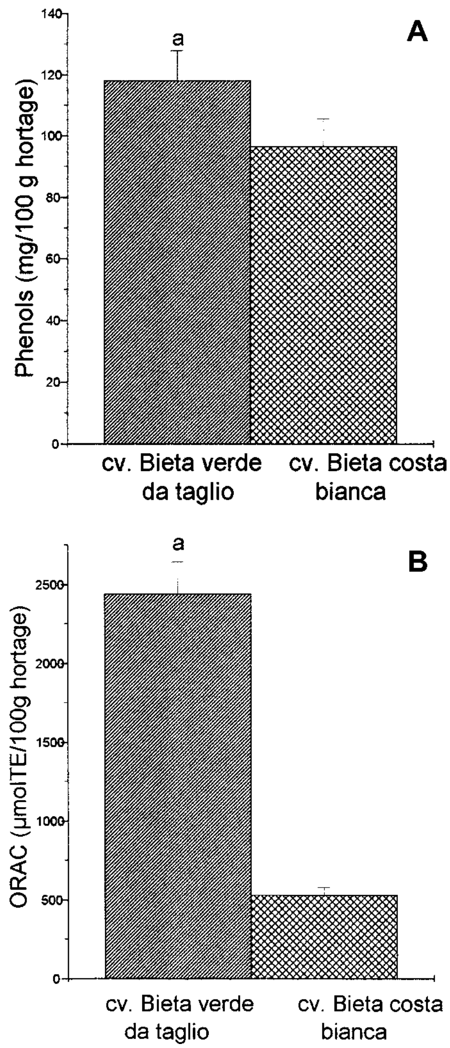

Figure 2. Total phenols and ORAC values of two different cultivars of beet green. Total phenols (A) and ORAC values (B) are means ± SD of six independent determinations. [a], significantly different from cv. Bieta costa bianca by ANOVA with $p \leq 0.05$.

of the beet green phenolic extracts (**Figure 3**) showed that in both cultivars one main peak was present at the retention time of the flavones. This peak accounted for 95% of the total phenols (see **Figure 3**, inset). The concentration of the flavone, which remains unidentified, was obtained using luteolin as standard. The concentrations were 12 mg/100 g of vegetable for cv. Bieta costa bianca and 36 mg/100 g of vegetable for cv. Bieta verde da taglio. This gives a ratio of 3.0, which is higher than the ratio between the total phenolics of the two cultivars but does not account completely for the ORAC discrepancy.

DISCUSSION

Phenolic profiles have been reported mainly for fruits, and their physiological activity has been thoroughly studied (*9*, *20*). Only recently have some reports been published on the phenolic composition of vegetables and aromatic herbs (*7*, *8*, *14*, *21*−*23*).

Methods for isolating phenolic compounds from vegetables vary greatly among laboratories. Ethanol, acetone, and ethyl acetate have been used in combination with inorganic acids to perform acid or alkaline extractions of phenols (*24*). In some cases, few categories of phenolic compounds are extracted to the detriment of others, as it is not easy to extract the integral

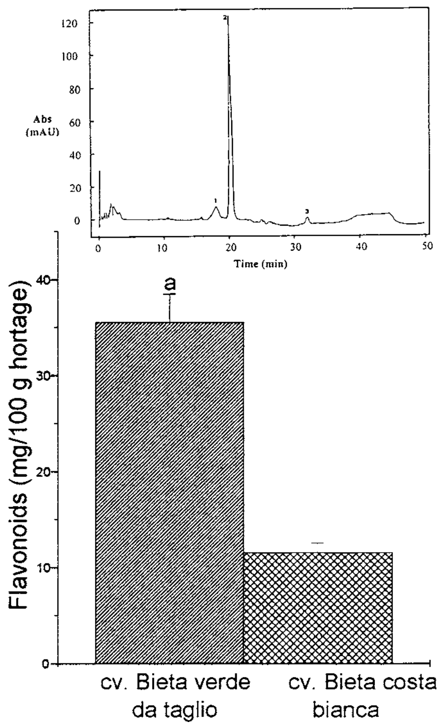

Figure 3. Total flavonoids of two different cultivars of beet green, determined by HPLC analysis. Values are means ± SD of four independent determinations. [a], significantly different from cv. Bieta costa bianca by ANOVA with $p \leq 0.05$. (Inset) HPLC profile of cv. Bieta verde da taglio. Detection was at 280 nm.

phenolic pool. The difficulty of phenol isolation lies in the fact that some phenolic compounds may accumulate in the vacuoles, whereas others are present in the cell wall (*25*).

In this paper, the WJ was obtained first from the vegetables, and then a second phenolic fraction was extracted from the wet pulp with acetone or acetone + PCA. Extraction with acetone + PCA gives a higher quantity of phenols compared to acetone. The effect of PCA in acetone on the yield of phenolics was more relevant in the pulp of fresh vegetables. In this case, all vegetables, except carrots, received a beneficial extractive effect from PCA. The effect of PCA + acetone is remarkable in leafy vegetables, beet green and spinach, which increased phenolic yields 3- and 2-fold, respectively. In frozen vegetables, a positive effect of PCA on the yield of phenolics was found in broccoli and spinach only. Very likely, the PCA softens fibers and completely disrupts vacuoles, thus favoring the mechanical release of phenols and the solubilization of phenolic aggregates (*6*).

WJ was treated with PCA to determine if the block of the enzymes was able to increase or protect the phenolic pool. No additional advantage was observed by the use of PCA added to the WJ in fresh and frozen vegetables. Indeed, in the WJ of spinach, a remarkable detrimental effect of PCA was observed for both fresh and frozen spinach, probably because proteins and chlorophylls precipitate and some phenols were also subjected to coprecipitation. This occurs in particular in spinach because its tender leaves release a high amount of chlorophyll. The fact that frozen beet green showed this PCA effect confirms this interpretation, because following freezing the beet green leaves are very soft.

Our results show the production of WJ and extraction of the wet pulp with acetone + PCA to be an optimal extractive procedure for vegetable phenolics. In our experiments, the acetone + PCA was not neutralized by K_2CO_3, but neutralization is possible after evaporation of the acetone. We do not know if other strong acids, other than PCA, have similar effects in the phenolic extraction from the pulp. We think that the result would be probably the same with HCl, but PCA has the advantage of being easily eliminated by precipitation with K_2CO_3.

The comparative study of the phenols and antioxidant capacity in fresh and frozen vegetables has provided interesting results. Phenols and ORAC values, determined in the WJ and acetone + PCA extracts, were combined and normalized to 100 g of vegetables as consumed. Fresh beet green showed a surprisingly high ORAC value, which means a remarkable concentration of powerful antioxidant molecules. Recent reports have highlighted the powerful antioxidant activity of red and white beet root extracts (*26*, *27*), but to our knowledge no one has described this result in beet greens. In frozen beet green, phenols and ORAC values were about 30 and 12%, respectively, those of fresh vegetable. This loss of phenolics and ORAC probably reflects a drastic processing technology imposed by the texture of the vegetable (*28*). The use of younger beet greens could allow the blanching time to be reduced, thus saving their powerful antioxidant capacity.

Fresh spinach, which has a phenolic value very similar to that of beet green, has an antioxidant capacity 3.5 times lower than that of fresh beet green. This result confirms that the ORAC value is not a direct consequence of the quantity of phenolic compounds but is instead correlated to the quality of the phenols present in the extract and to their interactions, which can amplify or attenuate the total antioxidant capacity (*21*, *29*).

In fresh onions, both the phenolic and ORAC values are lower than those of the frozen sample. Because we used packages of frozen onion cut in small cubes, this result can be explained by the addition of ascorbic acid, which is commonly used for readyy-to-eat packages of frozen vegetables (*30*) to guarantee a better preservation of the product. Ascorbic acid reacts with the Folin reagent and contributes significantly to the ORAC value, so that could account for the difference between fresh and frozen onions.

When compared with the fresh vegetable, the ORAC value of frozen broccoli was conserved. This means that the blanching process of this vegetable was mild and effective (*28*).

In this paper we also showed that the contribution of the cultivar to the nutritive quality of the vegetable is sometimes as important as the effect of the processing. In fact, the ORAC value of cv. Bieta verde da taglio was markedly higher than the ORAC of cv. Bieta costa bianca, although the phenolic concentrations of the two cultivars were only slightly different. The flavonoids of the two cultivars assayed by HPLC showed one main peak of a flavonic compound that remains at present unknown. The difference shown by the concentrations of this flavone does not justify, indeed, the difference in the ORAC values. This result highlights that different cultivars are characterized by different amounts of phenolics (*31*). Tthe ORAC value is necessary and also critical to evaluate the quality of antioxidants and their interactions (*14*); the ORAC value is a parameter of quality that does not always correlate with the total amount of phenolics. The choice of cultivar is important to improve of the mixture of efficient antioxidants in vegetables ingested every day by consumers.

With regard to the amount of ingested vegetables, chemopreventive medicine suggests for Italians an average daily amount of 500 g per person (200 g of vegetables and 300 g of fruits). This amount should give a contribution of phenolics and consequently of ORAC units able to significantly increase the ORAC value in plasma (*32*), with consequent health benefits

(*14*). Our results suggest that people who use frozen vegetables will have a 30−50% drop in antioxidant capacity with respect to the people who eat an identical amount of fresh vegetables.

In Italy, the consumption of frozen vegetables has increased 3-fold from 1982 to 2002. A 2002 statistic survey (www.inrademoskopea.it) has shown that ∼40% of Italians consume daily frozen vegetables. Our results should be taken into consideration by nutrition experts to establish appropriate dietary intakes as well as by the technologists of the frozen vegetable industry to optimize the processing of vegetables.

In conclusion, these results underscore the importance of extracting phenolic compounds from fresh or frozen vegetables by obtaining the WJ first and then by extracting phenols from the pulp by acetone + PCA. The detection of the amount of phenols must be related to their ORAC values, as this assay shows, the potential health benefit of these compounds, which is very often independent of their absolute quantity. The ORAC value is useful in the choice of the plant genotype and also provides information on the optimal time of blanching, which is the most important step to be checked before the freezing of the vegetables to maintain the antioxidant activity of phenolics.

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LITERATURE CITED

(1) Ames, B. N.; Shigenaga, M. K.; Hagen, T. M. Oxidants, antioxidants and the degenerative diseases of aging. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 7915−7922.

(2) Ames, B. N.; Gold, L. S.; Willett, W. C. The causes and prevention of cancer. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 5258−5265.

(3) Giacosa, A.; Filiberti, R.; Hill, M. J.; Faivre, J. Vitamins and cancer chemoprevention. *Eur. J. Cancer Prev.* **1997**, *6* (Suppl. 1), S47−54.

(4) Gariballa, S. E.; Sinclair, A. J. Nutrition, aging and ill health. *Br. J. Nutr.* **1998**, *80*, 7−23.

(5) Muller, H.; Bub, A.; Watzl, B.; Rechkemmer, G. Plasma concentrations of carotenoids in healthy volunteers after intervention with carotenoid-rich foods. *Eur. J. Nutr.* **1999**, *38* (1), 35−44.

(6) Antolovich, M.; Prenzler, P.; Robards, K.; Ryan, D. Sample preparation in the determination of phenolic compounds in fruits. *Analyst* **2000**, *125*, 989−1009.

(7) Vinson, J. A.; Hao, Y.; Su, X.; Zubin, L. Phenol antioxidant quantity and quality in foods: vegetables. *J. Agric. Food Chem.* **1998**, *46*, 3630−3634.

(8) Vinson, J. A.; Proch, J.; Bose, P. Determination of quantity and quality of polyphenol antioxidants in foods and beverages. *Methods Enzymol.* **2001**, *335*, 103−114.

(9) Robards, K.; Antolovich, M. Analytical chemistry of fruit bioflavonoids. A review. *Analyst* **1997**, *122*, 11R−34R.

(10) Servili, M.; Baldioli, M.; Selvaggini, R.; Macchioni, A.; Montedoro, G. F. Phenolic compounds of olive fruit: one-and two-dimensional nuclear magnetic resonance characterization of Nuzhenide and its distribution in the constitutive parts of fruit. *J. Agric. Food Chem.* **1999**, *47*, 12−18.

(11) Waterman, P. G.; Mole, S. In *Analysis of Phenolic Plant Metabolites*; Waterman, P. G., Mole, S., Eds.; Blackwell Scientific Publications: Oxford, U.K., 1994.

(12) Prochaska, L. J.; Nguyen, X. T.; Donat, N.; Piekutowski, W. V. Effects of food processing on the thermodynamic and nutritive value of foods: literature and database survey. *Med. Hypotheses* **2000**, *54* (2), 254−262.

(13) Cao, G.; Sofic, E.; Prior, R. L. Antioxidant capacity of tea and common vegetables. *J. Agric. Food Chem.* **1996**, *44*, 3426−3431.

(14) Prior, R. L.; Cao, G. Analysis of botanicals and dietary supplements for antioxidant capacity: a review. *J. AOAC Int.* **2000**, *83* (4), 950−956.

(15) Ninfali, P.; Aluigi, G.; Bacchiocca, M.; Magnani, M. Antioxidant capacity of vegetable oils. *J. Am. Oil Chem. Soc.* **2001**, *78* (3), 243−247.

(16) Ninfali, P.; Bacchiocca, M.; Biagiotti, E.; Servili, M.; Montedoro, G. F. Validation of the oxygen radical absorbance capacity (ORAC) parameter as a new index of quality and stability of virgin olive oil. *J. Am. Oil Chem. Soc.* **2002**, *79* (10), 977−982.

(17) Cao, G.; Alessio, H. M.; Culter, R. G. Oxygen-radical absorbance capacity assay for antioxidants. *Free Radical Biol. Med.* **1993**, *14*, 303−311.

(18) Montedoro, G. F.; Servili, M.; Baldioli, M.; Miniati, E. Sample and hydrolyzable phenolic compounds in virgin olive oil. 1. Their extraction, separation, and quantitative and semiquantitative evaluation by HPLC. *J. Agric. Food Chem.* **1992**, *40*, 1571−1576.

(19) Singleton, V. L.; Orthofer, R.; Lamuela-Raventos, R. M. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Methods Enzymol.* **1999**, *299*, 152−178.

(20) Wang, H.; Cao, G.; Sofic, E.; Prior, R. L. Total antioxidant capacity of fruits. *J. Agric. Food Chem.* **1996**, *44*, 701−705.

(21) Kähkönen, M. P.; Hopia, A. I.; Vuorela, H. J.; Rauha, J.-P.; Pihlaja, K.; Kujala, T. S.; Heinonen, M. Antioxidant activity of plant extracts containing phenolic compounds. *J Agric. Food Chem*. **1999**, *47*, 3954−3962.

(22) Gu, L.; Weng, X. Antioxidant activity and components of *Salvia plebeia* R.Br.—a Chinese herb. *Food Chem.* **2001**, *73*, 299−305.

(23) Zheng, W.; Wang, S. Y. Antioxidant activity and phenolic compounds in selected herbs. *J. Agric. Food Chem.* **2001**, *49*, 5165−5170.

(24) Pietta, P.; Mauri, P. Analysis of flavonoids in medical plants. *Methods Enzymol.* **2001**, *335*, 26−45.

(25) Waldron, K. W.; Ng, A.; Parker, M. L.; Parr, A. J. Ferulic acid dehydrodimers in the cell walls of *Beta vulgaris* and their possible role in texture. *J. Sci. Food Agric.* **1997**, *74* (2), 221−228.

(26) Kujala, T. S.; Loponen, J. M.; Korel, K. D.; Kalevi, P. Phenolics and betacyanins in red beetroot (*Beta vulgaris*) root: distribution and effect of cold storage and content of total phenolics and three individual compounds. *J. Agric. Food Chem*. **2000**, *48*, 5338−5342.

(27) Ng, A.; Harvey, J.; Parker, M. L.; Smith, A. C.; Waldron, K. W. Effect of oxidative coupling on the thermal stability of texture and cell wall chemistry of beet root (*Beta vulgaris*). *J. Agric. Food Chem*. **1998**, *46*, 3365−3370.

(28) Chu, Y.-H.; Chang, C.-L.; Hsu, H.-F. Flavonoid content of several vegetables and their antioxidant activity. *J. Sci. Food Agric.* **2000**, *80*, 561−566.

(29) Nicoli, M. C.; Anese, M.; Parpinel, M. Influence of processing on the antioxidant properties of fruit and vegetable. *Trends Food Sci. Technol*. **1999**, *10*, 94−100.

(30) Selleck, R. Fruit and vegetable preservative. PCT Int. Appl., Citrus Sensation Pty Ltd., Australia, 2001; 20 pp.

(31) Kurilich, A. C.; Jeffery, E. H.; Juvik, J. A.; Walling, M. A.; Klein, B. P. Antioxidant capacity of different broccoli (*Brassica oleracea*) genotypes using the oxygen radical absorbance capacity (ORAC) assay. *J. Agric. Food Chem.* **2002**, *50*, 5053−5057.

(32) Ninfali, P.; Aluigi, G. Variability of oxygen radical absorbance capacity (ORAC) in different animal species. *Free Radical Res.* **1998**, *29*, 399−408.

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