Antioxidant Capacities and Daily Antioxidant Intake from Vegetables Consumed in Taiwan

Ray-Yu Yang[1]*, Samson C. S. Tsou[1], Tung-Ching Lee[2], Peter M. Hanson[1] and Po-Yung Lai[3]

[1] AVRDC–The World Vegetable Center, PO Box 42, Tainan, Taiwan ROC

[2] Department of Food Science and the Center for Advanced Food Technology, Rutgers, the State University of New Jersey, 65 Dudley Road, New Brunswick, NJ 08901, U.S.A.

[3] Institute of Tropical Agriculture and International Cooperation, National Pingtung University of Science and Technology, Pingtung, Taiwan ROC

*Corresponding author (E-mail: ryy074@netra.avrdc.org.tw)

ABSTRACT

Epidemiological studies have demonstrated protective effects on human health from consumption of vegetables and fruits. Information on plant-sourced antioxidant capacity and intake is crucial to better understand the health implications of various dietary patterns. The objective of this study was to determine daily intake of antioxidant activity (AOA) from vegetables for the Chinese population in Taiwan. AOA intakes from vegetables were calculated to be 2014 μmol TE/day for methanol-soluble antioxidants and 750 μmol TE/day for water-soluble antioxidants based on a vegetable consumption survey in Taiwan in 2000. Sweet potato leaves, leafy Chinese cabbage, bamboo shoot, and spinach contributed 46% of the daily AOA intake for methanol-extracted antioxidants; whereas, sweet potato leaves, spinach, white cabbage and radish contributed 57% of the water-soluble AOA intake. Highly consumed vegetables, including white cabbage, bamboo shoot and loofah, were low in AOA. Increased consumption of green leafy vegetables could greatly improve overall AOA intake.

Keywords: Antioxidant activity, Antioxidant intake, Vegetables.

INTRODUCTION

Increased vegetable consumption has been widely promoted in recent years because of their value to human diets by providing basic nutrients as well as non-nutrient phytochemicals associated with health maintenance and prevention of chronic diseases and cancers (Steinmetz & Potter, 1996). Dietary antioxidants are critical, especially when the human body is under oxidative stress due to imbalances between generation of reactive oxygen species and endogenous antioxidants (Launer & Kalmijn, 1998; Aruoma & Halliwell, 1997).

Numerous groups of phytochemicals in vegetables, such as carotenoids, ascorbic acid, α-tocopherol and polyphenols are recognized for their antioxidative ability (Salah *et al.*, 1995; Edge, McGarvey & Truscott, 1997; Papas, 2002). However, vegetables differ in the types and levels of antioxidants they contain. The synergies and antagonisms of antioxidants in crude mixtures add complexity in attempts to explain their antioxidant capacities.

Instead of measuring antioxidant content, potential antioxidants can be evaluated for properties that inhibit generation of free radicals, directly scavenge free radicals, and delay or inhibit lipid peroxidation in vivo or in food. Several methods have been reported to characterize dietary antioxidants and determine their activities (Aruoma *et al.,* 1997). So far, no single method has been accepted to evaluate overall antioxidant capacity of food. A commonly used method, Trolox equivalent antioxidant capacity (TEAC, or ABTS method named in this study) reflects the ability of hydrogen- or electron-donating antioxidants to scavenge the ABTS radical cation ($ABTS^{\bullet +}$) compared with that of Trolox. This method is correlated with total phenolic content and antioxidant activity (AOA) measured by ORAC (oxygen radical absorbance capacity) and FRAP (ferric reducing antioxidant power) methods for common vegetables and fruits (Proteggente *et al.*, 2002; Pellegrini *et al.*, 2003). Other reported methods such as DPPH decomposition (Goupy *et al.*, 1999) and superoxide scavenging (SOS) (Murakami *et al.*, 1996) differ in the free radicals generated and their antioxidative mechanism. They have not yet been systematically compared.

The kinds and quantities of vegetables consumed by people vary greatly, depending upon culture, preference and availability. Per capita vegetable consumption in Taiwan is among the highest in the world. The antioxidant composition and capacity of vegetables relative to vegetables intake data are important to understand the health implications of various dietary patterns. The objective of this study was to assess the antioxidant capacities of common vegetables consumed by the Chinese population in Taiwan using three different assays (TEAC, DPPH and SOS) and to investigate the dietary antioxidant intake from vegetables based on collected data from a food consumption survey (Ali *et al.*, 2000).

MATERIALS AND METHODS

Chemicals

XOD (xanthine oxidase, grade III from butter milk, 1.0 unit/mg protein), NBT (nitro blue tetrazolium), ABTS (2,2-azino-bis (3-ethylbenz-thiazoline-6-sulfonic acid)), HRP (horseradish peroxidase, type VI-A, 1000 unit/mg solid), DPPH (1,1-diphenyl-2-picryl-hydrazyl) and linoleic acid were purchased from Sigma Chemical Co., St. Louis, MO. Trolox (6-hydroxy-2,5,7,8-tetramethyl-chroman-2- carboxylic acid) was purchased from Aldrich, St. Louis, MO. Other reagents used in this study were all analytical reagent grade.

Plant materials and preparations

The selection of the vegetables (Table 1) was based on household consumption survey conducted by Asian Vegetable Research and Development Center throughout Taiwan including 1457 households during 1998−1999 (Ali *et al.,* 2000). It was developed on the basis of 24 hrs recall.

Fresh materials were purchased in 2001 – 2003 from local markets in southern Taiwan. About 2 kg of edible portion of each fresh sample were collected and washed with tap water followed by distilled water, cut into 1 x 1 cm pieces and well mixed. Exactly 20 g of cut samples were homogenized with 80 mL of deionized water and 20 g for 80 mL of methanol, respectively, in a homogenizer. Juices of water extracts were squeezed from samples with a steel press. Both water and methanol extracts were centrifuged at 15,000 rpm for 10 min at 10℃, divided into several 1.5 mL volume in microtubes, and frozen at –70 ℃ immediately for subsequent AOA analysis. Each AOA value was an average of two replications per extract. About 200 g of chopped vegetables were hot-air dried (50℃ for 2 days), ground into fine powder for dry matter determination (135 °C/ 4 hr).

Antioxidant activity assay (TEAC method)

The ABTS/H_2O_2/HRP decoloration method was carried out as described in Arnao *et al.* (2001) with some modifications indicated in Hanson et al. (2004). The capacity of different components to scavenge the ABTS radical cation was compared to Trolox (0, 1, 2, 3 and 4 mM) in a dose response curve. The reaction mixture was prepared freshly containing 20 mM ABTS, 0.41 mM H_2O_2 and 50 units of HRP in 50 mM Na-phosphate buffer (pH7.5) in a total volume of 10 mL. The reaction mixture was further diluted to 100 mL with 50 mM phosphate buffer for water-extracted samples or diluted to 100 mL with ethanol for methanol-extracted samples. Stable absorbance at 730 nm was obtained in 2 min and reached OD_{730nm} = 1.0. Then 20 µL of the plant extract with appropriate dilution in water or methanol was added to the 1 mL of the reaction medium. The decrease in absorbance, which is proportional to the ABTS radical quenched, was determined after 5 min.

DPPH method

The DPPH assay was performed as described in Goupy *et al.* (1999) with some modifications. The radical form of DPPH has an absorbance at 517 nm, which disappears upon reduction by H· pulled from antioxidant compounds. Each plant extract was diluted with water (or methanol) to an appropriate concentration in a total volume of 2 mL; then 2 mL of 60 µM DPPH in water (or methanol) was added. The absorbance decrease was determined after 30 min in the dark. Trolox (0, 4, 8, 12, 16, 20 µM) was used as standard.

SOS method

The SOS was measured by the xanthine/XOD system as described by Murakami et al. (1996) with some modifications. The superoxide radical is generated from the oxidation of xanthine to uric acid by XOD. The radical reacts with NBT to cause a color change from light yellow to dark purple, which can be measured at $OD_{560\ nm}$. The ability of sample antioxidants to scavenge superoxide radicals can be determined by inhibition of NBT reductions. Methanol extracts of samples were N_2 air dried and dissolved in water with a few drops of Tween 20 before the assay. In a 20 mL tube, we added 20 µL of sample, 500 µL of PBS (pH7.2) containing 0.24 mM NBT and 0.4 mM xanthine, followed by 500 µL of a XOD solution (0.049 unit/mL) in PBS. After incubation at 37 °C for exactly 20 min, the reaction was stopped by adding 1 mL of a sodium dodecyl sulfate solution (69 mM). Visible absorption of the reaction mixture at 560 nm was measured. The absorbance difference of the reaction without XOD and with XOD was represented as 100% of NBT reduction inhibition. A sample blank was included without NBT to determine the sample's color interference to readings. A sequence of sample dilutions (at least 4 dilutions) with water was carried out until the NBT inhibition fell below 10%. Ascorbate (0, 0.5, 1, 2, 5, 10 mM) was used as standard.

RESULTS AND DISCUSSION

The 27 most consumed vegetables in Taiwan were identified by a food consumption survey and their AOA of water and methanol extracts were evaluated using three AOA assays. So far there is no optimal solvent to extract all antioxidants from plants. Vegetables are commonly prepared and consumed in a water-rich food matrix; methanol is a widely used solvent to extract phytochemicals in plants, especially for Chinese herbs, and ethanol with similar polarity to methanol is commonly added in Chinese household preparation by using rice wine containing about 18% ethanol. Thus we used water and methanol as the solvents in this study to reflect the AOA in food matrix instead of using combinations of solvents with various polarities in acidic or alkaline condition. The hydropholic AOAs were not included in this study due to their very low values compared to water and methanol extracts in our preliminary test. The low contribution of hydrophobic antioxidants to overall AOA was also reported by Wu et al. (2004).

AOA of the most consumed vegetables

The AOA values of vegetables for the six assays and overall AOA ranking are listed in Table 1. he overall rank was average rank over all assays. The ranking of vegetables for each assay, though different, showed similar trends. Vegetables ranked in the top five in one or more AOA assays included sweet potato leaf, old ginger, amaranth, spinach, eggplant, pak choi, leafy Chinese cabbage, tomato, welsh onion and kang-kong. Among these, sweet potato leaf and ginger were in the top five vegetable lists in every assay. Sweet potato leaf showed the greatest AOA by ABTS and DPPH methods, whereas ginger was the vegetable with highest SOS activity. Most green leafy vegetables exhibited high AOA by ABTS and DPPH methods, where 5 of the top 7 by overall ranking were green leafy types. It is noteworthy that eggplant, tomato and welsh onion had high SOS activity but relatively low AOAs by ABTS and DPPH methods. The lowest-ranking vegetables for AOA were wax gourd, loofah, cucumber and bottle gourd.

Regarding the extraction solvents, methanol-soluble antioxidants exhibited greater AOA by ABTS method for the vegetables tested; incontrast, the water extracts of 18 vegetables out of 27 showed higher AOA values by DPPH method. For, SOS, 17 vegetables showed greater activity with water extracts versus methanol extracts. However, vegetables with DPPHw > DPPHm are not necessary the same as those with SOSw > SOSm. The greatest AOA values by each method were all found from methanol extracts of vegetables.

Correlation coefficient between AOA values determined from six assays were all significant. The highest correlation coefficient was obtained with AOA values by ABTSm and DPPHm ($r=0.9548$, $p<0.0001$), whereas AOA values by SOSm and DPPHw ($r=0.3955$, $p=0.0412$) and AOA values by SOSm and DPPHw ($r=0.4436$, $p=0.0205$) had smaller but statistically significant correlation coefficient.

Table 1. Antioxidant activity[1] (AOA) of common vegetables[2] in Taiwan and overall AOA rank[3].

Scientific name	Common name	AOA Rank	ABTSm TE	ABTSw TE	DPPHw TE	DPPHm TE	SOSw AE	SOSm AE
Brassica oleracea	Cabbage, white	20	1.60	0.80	1.45	0.70	0.3	ND
Bambusoideae spp	Bamboo shoot	17	3.28	0.52	0.39	0.20	8.5	5.9
Luffa cylindrica	Loofah	26	0.86	0.25	0.12	0.29	ND	ND
Raphanus sativus	Radish	15	3.21	3.50	2.42	0.38	6.6	ND
Lactuca sativa	Heading lettuce	23	1.75	0.50	0.90	0.20	0.0	ND
Ipomoea aquatica	Kang kong	14	4.75	0.35	0.94	0.53	18.2	11.3
Ipomoea batatas	Sweet potato, green leaf	1	28.33	15.49	12.12	19.40	24.7	118.9
Brassica campestris	Leafy Chinese cabbage	7	10.71	3.94	3.03	3.56	6.8	9.2
Spinacia oleracea	Spinach	4	10.30	5.10	7.90	5.25	31.9	ND
Brassica campestris	Chinese cabbage	19	2.40	1.05	1.25	0.65	0.0	ND
Daucus carota	Carrot	18	2.85	0.31	0.51	0.41	9.2	5.8
Cucumis sativus	Cucumber	25	1.40	0.24	0.21	0.14	1.7	ND
Allium fistulosum	Welsh onion	13	6.15	1.64	1.47	0.43	6.8	13.2
Lagenaria siceraria	Bottle gourd	24	1.62	0.51	0.20	0.10	3.7	ND
Brassica oleracea	Cauliflower	8	7.57	2.11	2.59	2.52	17.7	4.6
Benincasa hispida	Wax gourd	27	0.21	0.18	0.58	0.03	ND	ND
Lycopersicon esculentum	Tomato	10	3.41	3.20	0.98	0.81	13.0	28.0
Apium graveolens	Celery	22	2.05	0.45	0.65	0.35	4.0	0.8
Momordica charantia	Bitter gourd	9	7.80	1.92	1.62	3.65	9.7	4.0
Amaranthus spp	Amaranth, green	3	14.07	3.58	4.02	10.35	16.6	9.8
Vigna unguiculata	Yard long bean	11	9.10	1.66	1.57	3.81	8.0	3.7
Phaseolus vulgaris	Kidney bean	12	10.30	1.50	1.60	4.40	5.7	ND
Zingiber officinale	Ginger, old	2	15.31	11.04	6.40	6.16	32.4	131.0
Solanum melongena	Eggplant	5	7.12	3.77	1.29	4.15	18.9	62.5
Zizania latifolia	Water bamboo	21	2.22	0.82	0.76	0.41	4.9	ND
Vigna radiata	Mungbean sprouts	16	8.41	1.12	1.27	0.54	5.8	0.8
Brassica campestris	Pak Choi	6	10.84	3.17	3.52	4.62	12.9	0.8
	Mean		6.58	2.55	2.21	2.74	9.9	15.2
	SD		6.08	3.45	2.70	4.18	9.2	34.2
	Min		0.21	0.18	0.12	0.03	ND	ND
	Max		28.33	15.49	12.12	19.40	32.4	131.0

[1] Values are means, n=2, in µmol TE/g or µmol AE/g on fresh weight basis. TE, Trolox equivalent; AE, ascorbate equivalent. ND= not detected.

[2] The selection of vegetables was based on the food consumption survey in Taiwan (Ali et al. 2000). Vegetables are in the sequence of the daily consumption from high to low.

[3] The overall ranking was based on average rank over each AOA assay.

AOA Intake from Vegetables

In Table 2, the average antioxidant per capita intake from vegetables on the basis of AOA values by ABTS method were calculated to be 2014 μmol TE/day for methanolic antioxidants and 750 μmol TE/day for aqueous antioxidants. Sweet potato leaves, leafy Chinese cabbage, bamboo shoot, and spinach together contributed the 46% of daily intake of methanol-soluble antioxidants; whereas, sweet potato leaves, spinach, white cabbage and radish contributed 57% of AOA from water-soluble antioxidants. In spite of high AOA of amaranth and ginger, their contribution to daily AOA intake was only about 4 – 5 % of the total AOA due to their low consumption. White cabbage, bamboo shoot and loofah were the most consumed vegeables, however, their overall AOA rankings were 20, 17 and 26, respectively, and their AOA contribution to daily antioxidant intake was only 1 – 6%.

Table 2. Daily AOA intakes[1] from vegetables in Taiwan.

	Daily intake (g/person)	Daily antioxidant intake[2] Methanol extractable AOA		Water extractable AOA	
		μmol TE	%	μmol TE	%
Cabbage, white	45.5	73	4%	66	9%
Bamboo shoot	34.8	114	6%	14	2%
Loofah	25.0	22	1%	3	0%
Radish	22.2	71	4%	54	7%
Heading lettuce	21.3	37	2%	19	3%
Kangkong	20.3	96	5%	19	3%
Sweet potato, green leaf	18.6	526	26%	225	30%
Leafy Chinese cabbage	15.4	165	8%	47	6%
Spinach	10.9	112	6%	86	11%
Chinese cabbage	10.8	26	1%	14	2%
Carrot	9.9	28	1%	5	1%
Cucumber	9.4	13	1%	2	0%
Welsh onion	9.0	55	3%	13	2%
Bottle gourd	8.9	14	1%	2	0%
Cauliflower	8.6	65	3%	22	3%
Wax gourd	8.4	2	0%	5	1%
Tomato	7.9	27	1%	8	1%
Celery	7.8	16	1%	5	1%
Bitter gourd	7.7	60	3%	12	2%
Amaranth, green	7.0	99	5%	28	4%
Yardlong bean	6.9	63	3%	11	1%
Kidney bean	6.4	66	3%	10	1%
Ginger, old	6.4	97	5%	41	5%
Eggplant	6.3	45	2%	8	1%
Water bamboo	5.9	13	1%	4	1%
Mungbean sprouts	5.7	48	2%	7	1%
Pak Choi	5.4	59	3%	19	3%
Total	352	2014	100%	750	100%

[1] The data source of the consumption amounts was referred to Ali *et al.* (2000).

[2] The AOA intakes were calculated based on AOA values by ABTS method.

The types of vegetables eaten daily were very different in the US versus Taiwan. Antioxidants from dry beans and peas contributed 64% of the daily AOA in the US, followed by lettuce (8%) and tomato (8%). The major antioxidants were quercetin and kaempferol flavonoids in beans and peas (Plumb *et al.*, 1999), quercetin in lettuce and carotenoids in tomato (Bravo, 1998). In Taiwan, antioxidants from leafy vegetables contributed the most to total AOA, especially sweet potato leaves, leafy Chinese cabbage, spinach, kangkong and amaranth. Their major antioxidants were beta-carotene, lutein, vitamins C, E, phenolic acids (such as gallic acid, chlorogenic acid, ferulic acid) and flavonols (such as quercetin and kaempferol) (Bravo, 1998) It seems that Taiwan people consume both more amount and types of antioxidants. The health benefits of antioxidants derived from different types of vegetable might be different and worth further investigation.

It is worth noting that sweet potato leaf contributed almost one-third of overall AOA intakes from vegetables. Sweet potato leaves are eaten as a cooked leafy vegetable in many countries (Villareal *et al.*, 1982) and the leaves contain anthocyanins and phenolic acids (Islam *et al.*, 2002a, 2002b), and showed anti-mutagenic properties (Yoshimoto *et al.*, 2002).

Vegetable consumption in Taiwan is one of the highest in the world with a daily per capita intake of about 350–420 g including a diversity of species (Ali et al. 2000; Wu et al. 1999). Several species including sweet potato leaves, amaranth and ginger have higher AOA; however, the most consumed vegetables tended to have lower AOA. To increase antioxidant consumption in Taiwan, people should eat more green leafy vegetables or additional vegetables with high AOA. In our recent survey of more than 120 vegetables species for AOA We found several very high AOA plant species, such as Chinese cedar and Moringa. Given the wide range of AOA among edible plants, there is great potential to increase antioxidant consumption. Because AOA of certain species such as Chinese cedar is 100 −1000 times greater than common vegetables, even consumption of a small quantity could greatly increase AOA consumption.

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