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Analysis of Antioxidant Activities of Common Vegetables Employing Oxygen Radical Absorbance Capacity (ORAC) and Ferric Reducing Antioxidant Power (FRAP) Assays: A Comparative Study

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A total of 927 freeze-dried vegetable samples, including 111 white cabbages, 59 carrots, 51 snap beans, 57 cauliflower, 33 white onions, 48 purple onions, 130 broccoli, 169 tomatoes, 25 beets, 88 peas, 88 spinach, 18 red peppers, and 50 green peppers, were analyzed using the oxygen radical absorption capacity (ORAC) and ferric reducing antioxidant capacity (FRAP) methods. The data show that the ORAC and FRAP values of vegetable are not only dependent on species, but also highly dependent on geographical origin and harvest time. The two antioxidant assay methods, ORAC and FRAP, also give different antioxidant activity trends. The discrepancy is extensively discussed based on the chemistry principles upon which these methods are built, and it is concluded that the ORAC method is chemically more relevant to chain-breaking antioxidants activity, while the FRAP has some drawbacks such as interference, reaction kinetics, and quantitation methods. On the basis of the ORAC results, green pepper, spinach, purple onion, broccoli, beet, and cauliflower are the leading sources of antioxidant activities against the peroxyl radicals.

KEYWORDS: ORAC; FRAP; antioxidant activity; chain-breaking antioxidant; free radical; hydrogen atom transfer; single electron transfer

INTRODUCTION

There is now increasing interest in antioxidant activity of phytochemicals present in the diet. Antioxidants are believed to play a very important role in the body defense system against reactive oxygen species (ROS), which are the harmful byproducts generated during normal cell aerobic respiration (*1*). Increasing intake of dietary antioxidants may help to maintain an adequate antioxidant status and, therefore, the normal physiological function of a living system (*2*−*3*). Some functional foods and vegetables are the important sources of exogenous antioxidants. Their nutritional values are normally quantified by the total amount of certain components such as "total fat", "total calories", and "total carbohydrate" which are labeled in the nutrition facts sheet appearing on food packages. These indexes are intended to provide very useful nutritional information to consumers. Although antioxidants are recognized as important phytonutrients (*4*), currently there is no "total antioxidant" as a nutritional index available for food labeling because of the lack of standard quantitation methods. Unlike other nutrients, antioxidants are chemically diverse. The most common antioxidants present in vegetables are vitamins C and E, carotenoids, flavonoids, and thiol (SH) compounds, etc. The chemical diversity of antioxidants makes it difficult to separate and quantify individual antioxidants from the vegetable matrix. Therefore, it is desirable to establish a method that can measure the total antioxidant activity level directly from vegetable extracts. Recently, several methods have been developed to measure "total antioxidant activity" (*5*), "total antioxidant capacity" (*6*−*7*), or "total antioxidant potentials" (*8*−*9*). Among them, Trolox equivalent antioxidant capacity (TEAC) (*10*), total radical absorption potentials (TRAP) (*11*), ferric reducing/antioxidant power (FRAP) (*12*), and oxygen radical absorption capacity (ORAC) assays (*13*) are the representative ones. Mechanistically, these methods are based on either single electron transfer (SET) reaction or a hydrogen atom transfer (HAT) reaction between an oxidant and a free radical. For the SET-based methods (eq 1, M = metal ion), such as FRAP and TEAC, antioxidants are oxidized by oxidants, such as Fe (III) or $ABTS^{+\bullet}$ (eq 1). As a result, a single electron is transferred from the antioxidant molecule to the oxidant. The change of absorbance of either antioxidant or oxidant is measured by an ultraviolet−visible spectrometer and the absorbance value is used as the quantitation for the reducing capability of the antioxidant.

$$M(n) + A{-}H \rightarrow M(n-1) + A{-}H^{+} \quad (1)$$

$$ROO^{\bullet} + A{-}H \rightarrow ROOH + A^{\bullet} \quad (2)$$

$$ROO^{\bullet} + FL{-}H \rightarrow ROOH + FL^{\bullet} \quad (3)$$

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The HAT-based method (eqs 2 and 3, FL = fluorescent probe), such as ORAC and TRAP, utilizes a radical initiator to generate peroxyl radical ROO•. The ROO• abstracts a hydrogen atom from antioxidant preferably. As a result, the reaction between ROO• and the target molecule probe is retarded or inhibited. Because these chemically distinct methods are based on different reaction mechanisms, it is necessary to evaluate whether different methods can provide comparable antioxidant values for the same sample. In this paper, for the first time, the antioxidant activities of common vegetables, in a large sample size (total 927), from the U.S. market were analyzed using ORAC and FRAP procedures. Our results indicated that the two sets of results did not correlate well. This discrepancy has been extensively discussed from the mechanistic point of view.

MATERIALS AND METHODS

Chemicals. 2,4,6-tripyridyl-s-triazine (TPTZ) and $FeCl_3 \cdot 6H_2O$ were purchased from Sigma (St. Louis, MO). Fluorescein disodium (FL) and Trolox were obtained from Aldrich (Milwaukee, WI). 2,2′-azobis (2-amidino-propane) dihydrochloride (AAPH) was purchased from Wako Chemicals USA (Richmond, VA).

Vegetable Samples. A total of 927 fresh vegetables were collected from various U.S. marketplaces at different harvesting seasons. They included 111 white cabbages, 59 carrots, 51 snap beans, 57 cauliflower, 33 white onions, 48 purple onions, 130 broccoli, 169 tomatoes, 25 beets, 88 peas, 88 spinach, 18 red peppers, and 50 green peppers. Freeze-drying was carried out for all the vegetables to remove the moisture. The freeze-dried vegetable samples were then packed in N_2-vacuumed amber bottles and stored in −80 °C before analysis.

Sample Preparation. Freeze-dried samples were accurately weighed into 0.5-g aliquots and 20 mL of acetone/water (50:50, v/v) extraction solvent was added. The mixture was shaken at 400 rpm at room temperature on an orbital shaker for an hour. The extracts were centrifuged at 14000 rpm for 15 min, and the supernatant was ready for analysis after appropriate dilution with 75 mM potassium phosphate buffer solution (pH 7.4).

Experimental Conditions. *ORAC Assay.* The automated ORAC assay was carried out on a COBAS FARA II spectrofluorometric centrifugal analyzer (Roche Diagnostic System Inc., Branchburg, NJ). The procedure was based on a previous report by Ou and co-workers (*14*). Trolox, a water-soluble analogue of vitamin E, was used as a control standard. The experiment was conducted at 37 °C under pH 7.4 condition with a blank sample in parallel. The analyzer was programmed to record the fluorescence of FL every minute after addition of AAPH. All fluorescent measurements are expressed relative to the initial reading. The final results were calculated using the differences of areas under the FL decay curves between the blank and a sample and were expressed as micromole Trolox equivalents (TE) per gram (μmol TE/g).

FRAP Assay. The FRAP assay was performed as previously described by Benzie and Strain (*12*), and was also carried out on a COBAS FARA II spectrofluorometric centrifugal analyzer (Roche). The experiment was conducted at 37 °C under pH 3.6 condition with a blank sample in parallel. In the FRAP assay, reductants ("antioxidants") in the sample reduce Fe (III)/tripyridyltriazine complex, present in stoichiometric excess, to the blue ferrous form, with an increase in absorbance at 593 nm. ΔA is proportional to the combined (total) ferric reducing/antioxidant power (FRAP value) of the antioxidants in the sample. The final results were expressed as micromole Trolox equivalents (TE) per gram on dried basis (μmol TE/g, db).

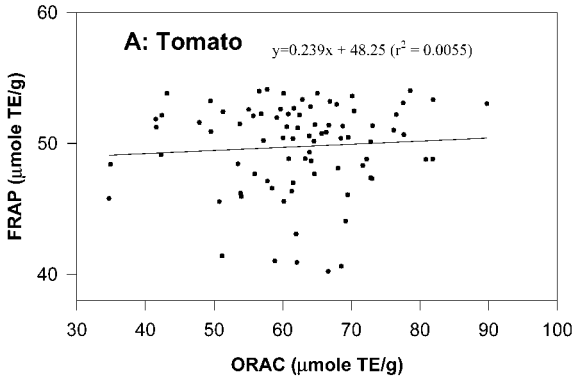
RESULTS

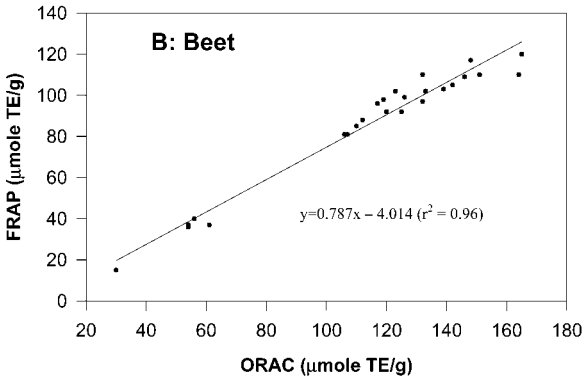
The vegetable extracts were analyzed by the standard FRAP and ORAC procedures, and the values obtained from the two methods were normalized to Trolox equivalents per gram on a freeze-dried basis. The distributions of the ORAC and FRAP values are illustrated in **Figure 1A−M**. The figures not only show cultivation dependency of the ORAC and FRAP values, but they also reveal the irregular relationship between ORAC and FRAP values. **Table 1** summarizes the maximum, minimum, and median values of the antioxidant activities of the vegetable samples. **Figures 2** and **3** show the antioxidant activity rank order of vegetables based on the data in **Table 1**.

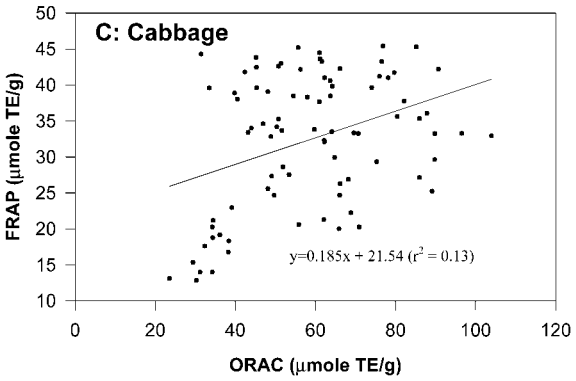
DISCUSSION

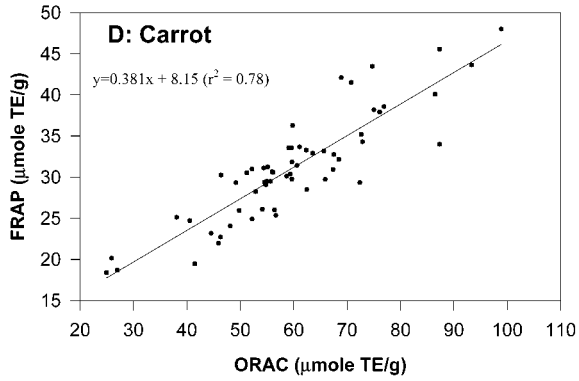
Cultivation Dependency of Antioxidant Capacity of Vegetables. Although we sampled the vegetables from various U.S. marketplaces without knowing the specific cultivation information for each vegetable species, from our results, one conspicuous conclusion is that the antioxidant activity of vegetables is highly dependent on cultivation conditions, as is reflected in **Figure 1a−m**. The antioxidant activity varies considerably from variety to variety. For instance, broccoli shows nearly 10-fold differences between highest and lowest values, green pepper exhibits almost 6-fold differences, and spinach reveals 2-fold differences. This large variability among the same vegetable can be apparently explained by the influences of different variety, location, and harvest season, etc., which would affect the level of antioxidants present in these vegetables. As reported by Prior et al., the major phytochemicals responsible for the antioxidant capacity most likely can be accounted for by the flavonoid compounds, which are known as secondary natural product metabolites (*15*). Apparently, the biosynthesis of these natural products is profoundly influenced by a number of factors, such as locations, weather conditions, and harvest periods, etc. Therefore, it is expected that the ORAC values vary accordingly. This similar phenomenon was observed for the FRAP group. Not only do vegetables show ORAC dependency on cultivar, but fruits also exhibit this same dependency. Prior and co-workers reported the ORAC values of fruit and leaf tissues of 87 highbush blueberries and the results show a wide range of ORAC values for different blueberry species (*16*). Another recent study has also shown that the antioxidant capacities of apples are dependent on cultivar (*17*). Currently, we are conducting further studies to characterize the major antioxidants present in these vegetables, and the outcomes will ultimately reveal the correlation between cultivation conditions and antioxidant activity from the chemistry point of view.

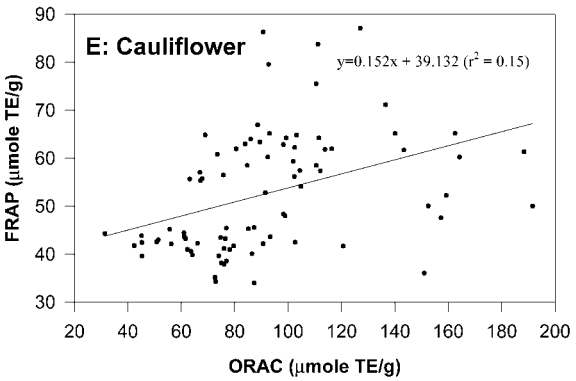
Antioxidant Activity Rank Order of the Vegetables. The comparative study of antioxidant activity is desirable not only from an academic point of view but also in the interest of vegetable producers and consumers. Consequently, there are plenty of papers attempting to rank antioxidant capacities of different plant extracts, including fruits and vegetables. For example, antioxidant activity of some vegetables based on ORAC results have been previously reported by Cao et al. (*18*). However, because of their limited sample size and drawbacks of the original ORAC method, previous ORAC values may not be representative in terms of antioxidant activity rank order (*14*). In the present study, for the first time, a large number of vegetables from various locations at different harvest seasons were evaluated using the ORAC and FRAP assays. To compare antioxidant activity on an equal basis, the moisture contained in the samples was removed by freeze-drying; thus, the results from this study provide us a fully comprehensive antioxidant activity profile of each examined vegetable. **Figures 2** and **3** are the rank orders based on ORAC and FRAP values, respectively. Apparently, one cannot draw a clear conclusion on rank because the ORAC/FRAP data of different vegetables cover a broad range and overlap significantly among different vegetables, albeit the median values have some trend. For

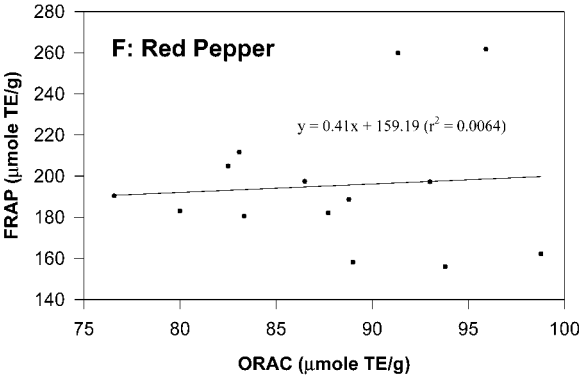

A: Tomato
y=0.239x + 48.25 (r^2 = 0.0055)
FRAP (μmole TE/g)
ORAC (μmole TE/g)

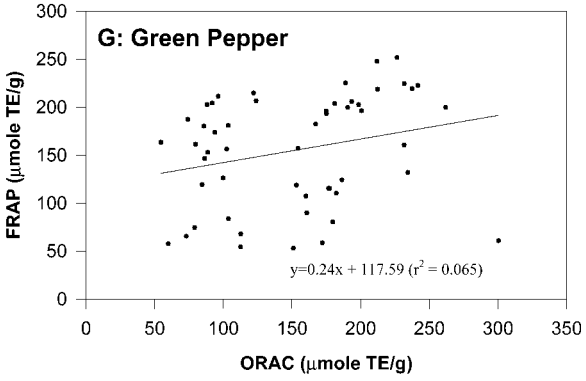

B: Beet
y=0.787x – 4.014 (r^2 = 0.96)
FRAP (μmole TE/g)
ORAC (μmole TE/g)

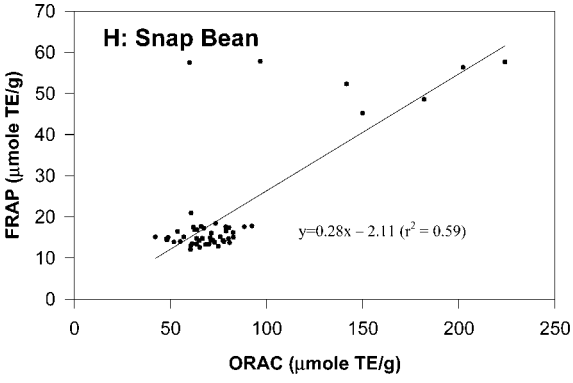

C: Cabbage
y=0.185x + 21.54 (r^2 = 0.13)
FRAP (μmole TE/g)
ORAC (μmole TE/g)


D: Carrot
y=0.381x + 8.15 (r^2 = 0.78)
FRAP (μmole TE/g)
ORAC (μmole TE/g)


E: Cauliflower
y=0.152x + 39.132 (r^2 = 0.15)
FRAP (μmole TE/g)
ORAC (μmole TE/g)


F: Red Pepper
y = 0.41x + 159.19 (r^2 = 0.0064)
FRAP (μmole TE/g)
ORAC (μmole TE/g)


G: Green Pepper
y=0.24x + 117.59 (r^2 = 0.065)
FRAP (μmole TE/g)
ORAC (μmole TE/g)


H: Snap Bean
y=0.28x – 2.11 (r^2 = 0.59)
FRAP (μmole TE/g)
ORAC (μmole TE/g)

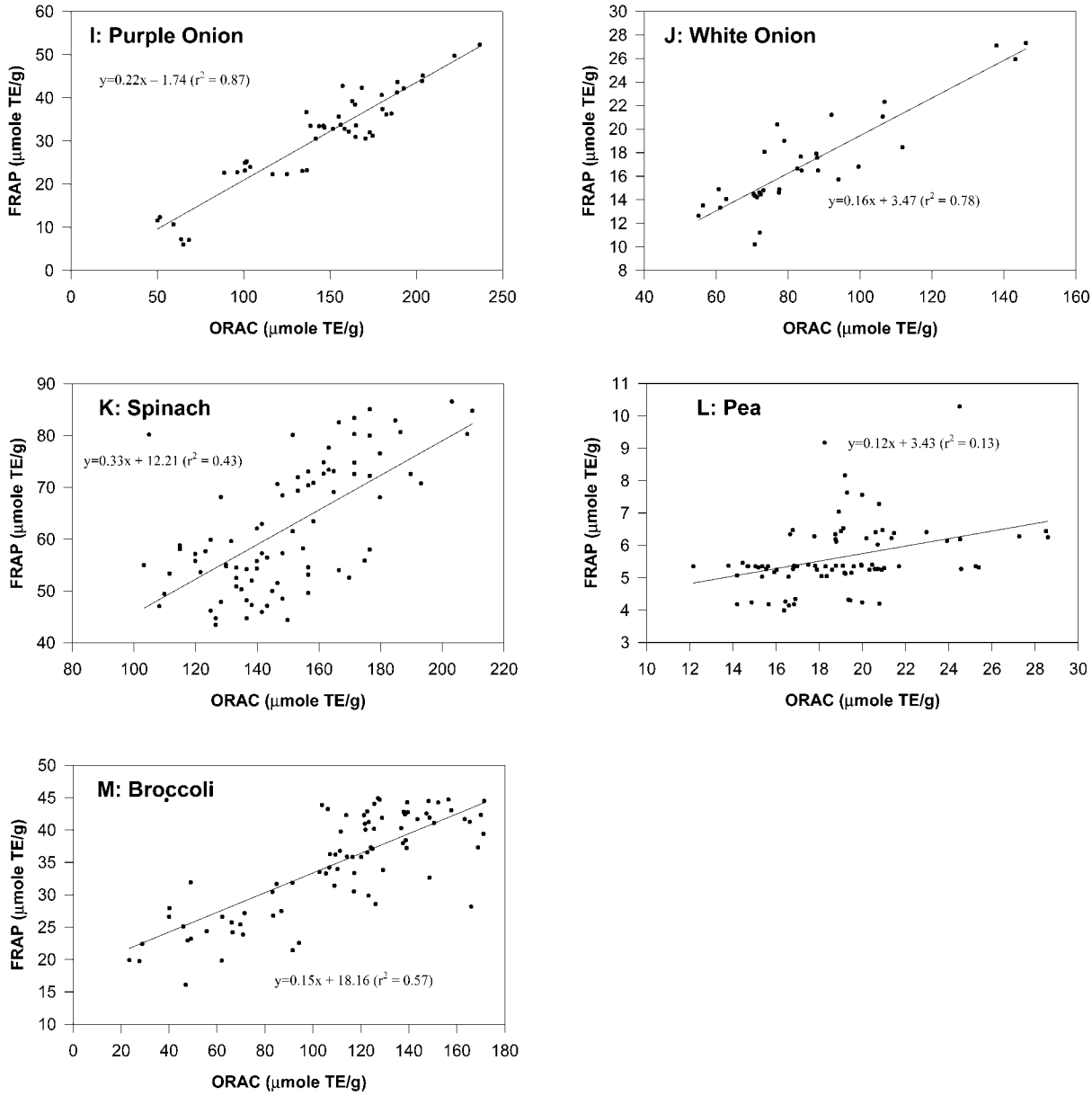


Figure 1. A–M. Correlation between the ORAC value and the FRAP value. The results are expressed as micromole Trolox equivalents per gram based on the freeze-dried weight (μmol TE/g, d.w.). With the exception of beet, carrot, purple onion, and white onion, there is no linear correlation between the ORAC value and the FRAP value.

Table 1. ORAC and FRAP Values of Vegetables (μmol TE/g) (n > 4)

		ORAC					FRAP				
species	sample size	max	min	median	mean	SD	max	min	median	mean	SD
pea	88	29	12	18	19	3	10	4	5	6	1
carrot	59	99	25	57	60	15	48	18	25	31	7
white cabbage	111	146	23	60	61	21	125	13	38	39	17
tomato	169	112	33	66	67	13	83	40	54	56	8
snap bean	51	223	42	70	79	37	58	12	15	20	13
white onion	33	146	55	78	85	23	27	10	16	17	4
red pepper	18	161	73	86	97	43	261	123	183	185	49
cauliflower	57	152	62	91	102	28	83	36	59	61	12
beet	25	165	30	120	115	36	120	12	96	86	29
broccoli	130	208	23	132	126	42	71	16	42	41	11
purple onion	48	237	50	153	143	46	52	6	33	31	11
spinach	88	234	103	148	152	26	94	43	58	64	13
green pepper	50	300	54	160	154	60	251	161	53	157	58

example, from a statistical point of view, the ORAC values among green pepper, spinach, and purple onion are the same. Therefore, for the rank to make sense, one has to specify the vegetable origin, harvest time, and data acquisition procedures.

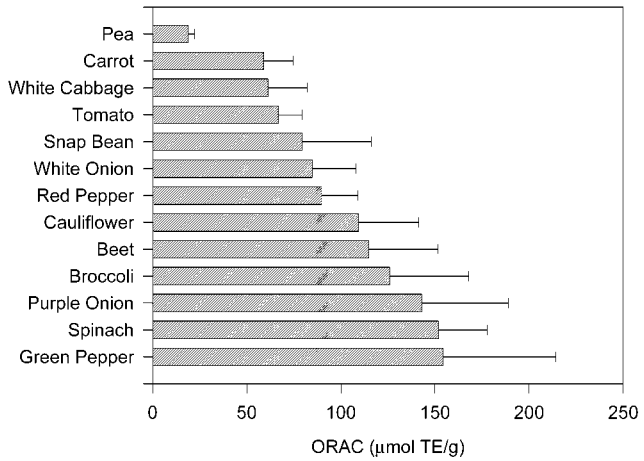


Figure 2. Rank order for antioxidant activities of common vegetables based on the ORAC values.

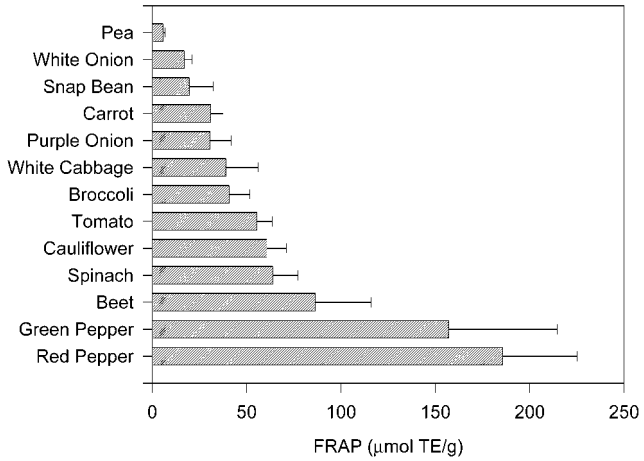


Figure 3. Rank order for ferric reducing capabilities of common vegetables based on the FRAP values.

Rank order based on the limited sample size and varieties is not representative and can result in misleading conclusions.

Antioxidant Reaction Mechanism. Both ORAC and FRAP values have been claimed to reflect total antioxidant activity (*19*−*20*). If this claim is valid, regardless of the different reaction mechanisms, their values should be comparable, and one should expect that the antioxidant active rank based on either ORAC or FRAP should have a similar trend. On the contrary, our data reveal that the FRAP and ORAC values do not correlate well. For example, the ratio of mean ORAC over FRAP value ranges from 0.52 to 5.00 for the twelve vegetables. The rank order based on the absolutely ORAC mean values is green pepper > spinach > purple onion > broccoli > beet > cauliflower > red pepper > white onion > snap bean > tomato > white cabbage > carrot > pea, whereas the rank based on the FRAP results is red pepper > green pepper > beet > spinach > cauliflower > tomato > broccoli > white cabbage > purple onion > carrot > snap bean > white onion > pea. Within vegetables, the plots of ORAC against FRAP (**Figure 1a**−**m**) also exhibit no trend for most vegetables, except for those of beet, onions, and carrots. The different results from the two assays warrant some discussions.

Herein, we attempted to analyze the two assays from the chemistry principles upon which they are based. In general, the antioxidants can be classified into two mechanistic categories: preventive antioxidants and chain-breaking antioxidants. Preventive antioxidants, such as superoxide dismutase, catalase, peroxidase, and transferrin, inhibit formation of reactive oxygen species. Chain-breaking antioxidants are compounds that scavenge oxygen radicals and thereby break radical chain sequences. They include vitamin C, vitamin E, uric acid, bilirubin, and polyphenols, et al. For chain-breaking antioxidants, there are two possible pathways in which antioxidants can play a role. The first pathway involves a hydrogen atom transfer (HAT), where the oxygen radical abstracts a hydrogen from the antioxidant, resulting in formation of a stable antioxidant radical. The following equations illustrate the stepwise process of HAT. We use an azo compound as a representative radical generator and LP−H as the lipid substrate:

$$R{-}N{=}N{-}R \rightarrow 2\,R^{\bullet} + N_2 \quad (4)$$

$$R^{\bullet} + O_2 \rightarrow ROO^{\bullet} \quad (5)$$

$$ROO^{\bullet} + LP{-}H \rightarrow ROOH + LP^{\bullet} \quad (6)$$

$$LP^{\bullet} + O_2 \rightarrow LPOO^{\bullet} \quad (7)$$

$$LPOO^{\bullet} + LP{-}H \rightarrow LPOOH + LP^{\bullet} \quad (8)$$

As illustrated, once the peroxyl radical (ROO•) is generated, the chain reactions of eqs 5−7 start, and, as a consequence, lipid molecules (LP−H) would be oxidized to lipid peroxides (LP−OOH). In the presence of antioxidants (ArOH), the lipid peroxidation chain reaction can be interrupted as follows:

$$ROO^{\bullet} + ArOH \rightarrow ArO^{\bullet} + ROOH \quad (9)$$

For phenolic antioxidants, the formed phenoxyl radical ArO• is relatively stable, and it only reacts slowly with substrate LPH but rapidly with peroxyl radical ROO•. For example, α-tocopherol (α-TOH), known as the most effective lipid-soluble chain-breaking antioxidants in vivo, reacts with peroxyl radical with a rate constant of about 10^6 $M^{-1}s^{-1}$, which is much faster than the reaction of peroxyl radicals with lipid substrate, typically 10^1 $M^{-1}s^{-1}$. The second possible pathway in which antioxidant de-activative free radicals is single electron transfer (SET) as illustrated below:

$$ROO^{\bullet} + ArOH \rightarrow ROO^{-} + ArOH^{+\bullet} \quad (10)$$

$$ROO^{-} + ArOH^{+\bullet} \rightarrow ArO^{\bullet} + ROOH \quad (11)$$

The net result from above is the same as from the HAT mechanism. However, when compared to HAT, the SET mechanism is strong-solvent-dependent due to solvent stabilization of the charged species. The question raised here is which mechanism physiologically reflects the antioxidant preventive action. More recently, Wright and co-workers used a procedure based on density functional theory to calculate the gas-phase bond dissociation enthalpy (BDE) and ionization potential for molecules belonging to the class of phenolic antioxidants, including tocopherols, catechins, aminophenols, and stilbenes related to resveratrol. Their results demonstrated that in most cases HAT will be dominant (*21*). It is logical with the biological oxidation processes, in which an oxygen molecule is reduced to the final product H_2O with some degree of SET involvement (oxygen oxidation state changed from 0 to −2). More importantly, it is also a hydrogen atom transfer process because oxygen is hydrogenated and the reductants are dehydrogenated. For example, dihydroflavin oxidizing to flavins, hydroquinones oxidizing to quinones, and thiols oxidizing to disulfides. It is

clear that hydrogen atom transfer (HAT) reaction concurs with electron transfer reaction and plays a dominant role in biological redox reactions. Therefore, the ORAC principle is closely related to biological functions of chain-breaking antioxidants.

Principle of FRAP. The relevant chemical reaction of the FRAP method involves a single electron reaction between Fe $(TPTZ)_2$ (III) and a single electron donor ArOH.

$$Fe(TPTZ)_2(III) + ArOH \rightarrow Fe\ (TPTZ)_2(II) + ArOH^{+\bullet} \quad (12)$$

Benzie and co-workers (*12*) considered the antioxidant as any species that reduces the oxidizing species that would otherwise damage the substrates. And the authors further treat the "total antioxidant power" as the "total reducing power". The antioxidant activity is then interpreted as the reducing capability.

To accurately measure the total reducing power, the following conditions must be met. (1) All, and only, antioxidants can reduce Fe $(TPTZ)_2$(III) under the reaction conditions (thermodynamics). (2) The reaction rate must be sufficiently fast enough that the reaction can be completed in a short assay time (e.g., 4 min in the actual FRAP assay) (kinetics). (3) The oxidized antioxidant, $ArOH^{+\bullet}$, and its secondary reaction products should have no absorption at 593 nm, the maximum absorption of Fe $(TPTZ)_2$ (II).

In fact, these conditions are very difficult to meet. First, the standard redox potential of Fe(III)/Fe(II) is 0.77 V; any compound with lower redox potential can theoretically reduce Fe (III) to Fe (II) and contributes to the FRAP values resulting in falsely high FRAP values. Therefore, the reason for choosing Fe (III) as an oxidant seems to be too arbitrary. Second, not all antioxidants reduce Fe (III) at a fast rate as anticipated. For example, Pulido and co-workers (*22*) recently examined the FRAP assay of dietary polyphenols in water and methanol. The absorption (A_{593}) was slowly increasing even after several hours of reaction time. The polyphenols with such behavior include caffeic acid, tannic acid, ferulic acid, ascorbic acid, and quercetin, etc. Besides polyphenols, thiol compounds also react with Fe $(TPTZ)_2$(III) slowly. Our own experiment shows that the reaction of glutathione with Fe $(TPTZ)_2$(III) is a very slow reaction and the reducing power cannot be correctly measured (unpublished results). At this point, the FRAP reaction is too slow to be of any practical use. Third, another limitation of the FRAP assay is the possible interference due to the UV−Vis absorption at 593 nm by compounds other than Fe $(TPTZ)_2$ (II). For example, Benzie and co-workers (*12*) reported an unusually high FRAP value for bilirubin (twice that of Trolox and ascorbic acid). In fact, it is known that when bilirubin is oxidized, it is transformed to beliverdin which has a strong absorption at 593 nm ($\epsilon_{593} = 1 \times 10^4$). Therefore, the FRAP assay cannot be used in biological samples. Many vegetable extracts are colored and may have similar interference. Finally, the FRAP assay depends on the reduction of a ferric tripyridyltriazine Fe $(TPTZ)_2$(III) complex to the ferrous tripyridyltriazine Fe$(TPTZ)_2$(II) by an antioxidant at a low pH of 3.6. However, the low pH can significantly inhibit one electron transfer from the antioxidant to the ferric ion. FRAP results reflect only the antioxidant reducing potential based on ferric ion instead of the antioxidant preventive effect. Clearly, the FRAP assay actually measures the reducing capability based upon ferric ion, which is not relevant to antioxidant activity mechanistically and physiologically, let alone the total antioxidant capacity. On the basis of these facts, we feel that it is not appropriate to use the FRAP value as an indicator for "total antioxidant power".

Principle of the ORAC Assay. The ORAC assay was initially developed by Cao et al. and was significantly improved by Ou and co-workers (*14*). In the improved ORAC assay, fluorescein was the chosen fluorescent probe. Ou and co-workers have also identified the oxidized fluorescein products and the reaction mechanism was determined to follow the HAT mechanism. Under this reaction condition, one mole of AAPH loses a dinitrogen to generate two moles of AAPH radical at a constant rate (eq 4). In air-saturated solution, the generated AAPH radical reacts with O_2 rapidly (eq 5; $k_5 \sim 10^9$ $mol^{-1}\cdot s^{-1}$) to give a more stable peroxyl radical $ROO^{\bullet}$. The loss of fluorescence of fluorescein is an indication of the extent of damage from its reaction with the peroxyl radical. In the presence of antioxidant, $ROO^{\bullet}$ abstracts a hydrogen atom from the antioxidant to form hydroperoxide (ROOH) and a stable antioxidant radical ($ArO^{\bullet}$); as a result, the damage to fluorescein induced by peroxyl radical is inhibited. The protective effect of an antioxidant is measured by assessing the area under the fluorescence decay curve (AUC) of the sample compared to that of the blank in which no antioxidant is present. Ou and co-workers have shown that under the ORAC experimental conditions fluorescence decrease is independent of concentrations of FL but first order with AAPH concentration. Thus, the reaction rate is limited by eq 4 ($k_4 = 3.19 \times 10^{-7}$ $mol^{-1}\cdot s^{-1}$). Most of the samples do not affect the thermo-decomposition rate of AAPH, and AAPH itself does not react directly with the sample. As a result, the ORAC assay directly measures the antioxidant activities of chain-breaking antioxidants against peroxyl radicals. Therefore, we suggest that ORAC values be used as a guideline for "peroxyl radical absorption capacity" of vegetables.

In summary, the antioxidant activities of 927 vegetables have been measured by the ORAC and FRAP assays. On the basis of our knowledge of antioxidant chemistry, it is concluded that the ORAC values reflect the peroxyl radical scavenging activity of vegetables. In contrast, the FRAP assay estimates only the Fe (III) reducing activity, which is not necessarily relevant to antioxidant activity physiologically and mechanistically. Thus, we suggest that the antioxidant rank order should be based on ORAC results. To our best knowledge, the study reported here is the most comprehensive antioxidant study on the common vegetables so far, in which nearly 1,000 vegetable extracts were analyzed. Hence, our results are representative and provide some valuable data for establishment of recommended antioxidant daily allowance in the future. However, the ORAC assay is not a "total antioxidant activity assay", because it only measures antioxidant activity against peroxyl radicals. Biologically relevant reactive oxygen species (ROS) also include $O_2^{-\bullet}$, $HO^{\bullet}$, ONOO−, and singlet oxygen. As different ROS have different reaction mechanisms, to completely evaluate antioxidant activity is a rather difficult task without a short-cut, and using one assay result to claim "total antioxidant activity" is oversimplified and thus inappropriate. To elucidate a full profile of antioxidant activity against various ROS, comprehensive assays are needed.

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ABBREVIATIONS USED

Trolox, 6-hydroxy-2,5,7,8-tetramethyl-2-carboxylic acid; AAPH, 2,2′-azobis (2-amidino-propane) dihydrochloride; ABTS, 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonate); ORAC, oxygen radical absorbance capacity; HAT, hydrogen atom transfer;

TEAC, Trolox equivalent antioxidant capacity; FRAP, ferric reducing antioxidant power.

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