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Original Article

Development of a database for total antioxidant capacity in foods: a preliminary study[☆]

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Abstract

For the first time, a database of the antioxidant capacities of both the lipophilic and hydrophilic components of foods has been developed using the modified oxygen radical absorbance capacity (ORAC_{FL}) assay and a peroxy radical generator. For lipophilic components, randomly methylated β -cyclodextrin was used as a solubility enhancer. Four representative samples were extracted directly with the hydrophilic solvent (acetone:water:acetic acid, 70:29.5:0.5). Their ORAC_{FL} values were similar to that obtained for hydrophilic ORAC_{FL} (H-ORAC_{FL}) following lipophilic extraction with hexane:dichloromethane (1:1). Lipophilic ORAC values (L-ORAC_{FL}) were relatively low compared to H-ORAC_{FL}, ranging from 0.11 ± 0.06 to 154.70 ± 3.58 $\mu\text{mol TE/g}$ of fresh or dry weight, whereas H-ORAC_{FL} ranged from 1.23 ± 0.17 to 175.24 ± 10.36 $\mu\text{mol TE/g}$ of fresh or dry weight. Total antioxidant capacity (TAC) was calculated as the sum of the lipophilic and hydrophilic ORAC_{FL} values. L-ORAC_{FL} as a percentage of TAC ranged from 0.27% to 63.70%. Sampling time during the year significantly influenced lipophilic and/or hydrophilic ORAC_{FL} values in some food samples. In order to get an accurate total antioxidant capacity of a given food sample, both lipophilic and hydrophilic fractions need to be measured. Food processing, such as cooking or peeling, need to be considered as additional factors which can introduce variation in antioxidant capacity measurements of foods.

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Keywords: Antioxidant capacity; Lipophilic ORAC_{FL}; Hydrophilic ORAC_{FL}

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1. Introduction

Antioxidants have received increased attention by nutritionists and medical researchers for their potential effects in the prevention of chronic and degenerative diseases such as cancer and cardiovascular disease as well as aging (Diaz et al., 1997; Ames et al., 1993, 1995; Young and Woodside, 2001). Epidemiological studies have provided evidence of an inverse association between diets rich in fruits and vegetables and these diseases (Block et al., 1992; World Cancer Research Fund, 1997; Ness and Powles, 1997; Joshipura et al., 1999). These health promotion effects may be related to components in the foods with antioxidant activity (Kaur and Kapoor, 2001). The ability of antioxidants to scavenge free radicals in the human body and thereby decrease the amount of free radical damage to biological molecules like lipids and DNA may be one of their protective mechanisms. However, clinical trials using 'nutritional' antioxidants in food such as vitamins C and E have given equivocal results (Emmert and Kirchner, 1999; Herberg et al., 1999). There are two possible explanations for this phenomenon. One is that other unknown components rather than these well-known 'nutrient' antioxidants also act as antioxidants in food and their activity *in vivo* may be stronger than those mentioned above. In addition, since there are hundreds of antioxidant compounds in most foods, the total antioxidant capacity of a given food may be the integrated action from different compounds instead of that from any single compound. In order to evaluate the total antioxidant capacity of a given food and their health promotion effects accurately, we need to consider the possible interaction of components in their contribution to antioxidant activity.

In general, components in plants can be divided into two fractions, lipophilic and hydrophilic. Although there is not a definite clear demarcation between them, the physio-chemical properties of these two groups of components are quite different. However, most popular *in vitro* antioxidant measurement methods are designed primarily for hydrophilic components, and may not be suitable or adaptable for lipophilic measurements. Some investigators have proposed that, in order to obtain a good measurement of total antioxidant capacity for a given food, lipophilic components need to be separated from that of the hydrophilic components using similar chemical principles (Cano et al., 2000, 2003; Arnao et al., 2001). The original oxygen radical absorbance capacity (ORAC) assay and the modified ORAC_{FL} methods were developed using a hydrophilic environment (Cao et al., 1993, 1995; Ou et al., 2001). However, it has proven to be adaptable for lipophilic antioxidants as well. Recently, Huang et al. (2002) developed a lipophilic ORAC_{FL} measurement method that employed randomly methylated β -cyclodextrin (RMCD) as a solubility enhancer. This allowed for the measurement of the antioxidant capacity of lipophilic and hydrophilic components for a given sample separately, but based on the same peroxy free radical. The ORAC_{FL} method has the advantage that it combines the inhibition degree and time of inhibition into one value.

In this paper, we present for the first time data on the lipophilic, hydrophilic and total antioxidant capacities evaluated by ORAC_{FL} of 28 common foods sampled from four different regions in US markets during two different seasons. Sampling included fruits, vegetables, nuts, dried fruits and rice bran. These foods were selected to represent common foods in the human diet. In addition, they belong to four major food categories of plant origin: fruit, vegetable, nuts and dried fruits. Rice bran was chosen as a comparison for its extremely high lipophilic (L-ORAC_{FL}) to hydrophilic ORAC value (H-ORAC_{FL}). The main purposes of our studies were

to evaluate: (1) the effects of solvent extraction methods and their ability to separate the hydrophilic antioxidants from the lipophilic antioxidants, (2) the effects of sampling season (time of year), and (3) some preliminary comparisons of processing effects on both L-ORAC_{FL} and H-ORAC_{FL}. In addition to the foods mentioned in this paper, other foods will be analyzed and the completed data will be incorporated into the USDA Nutrient Database which will be posted on the USDA Nutrient Data Laboratory web site at: <http://www.nal.usda.gov/fnic/foodcomp>

2. Materials and methods

2.1. Chemicals and apparatus

2,2'-Azobis(2-amidino-propane) dihydrochloride (AAPH) was purchased from Wako Chemicals USA (Richmond, VA). 6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) and Fluorescein (Na salt) (FL) were obtained from Aldrich (Milwaukee, WI). Randomly methylated β -cyclodextrin (Trappsol[®]) (Pharm Grade) was obtained from Cyclodextrin Technologies Development, Inc. (High Springs, FL). All other solvents were purchased from Fisher (Fair Lawn, NJ). Extraction of samples was performed on an ASE[®] 200 accelerated solvent extractor (Dionex Corporation, Sunnyvale, CA). ORAC_{FL} analyses were carried out on a FLUOstar Galaxy plate reader (BMG Labtechnologies, Durham, NC). Fluorescence filters with an excitation wavelength of 485 nm and an emission wavelength of 520 nm were used. The 48 well microplates (Falcon No. 3230) were purchased from VWR (St. Louis, MO).

2.2. Food sampling strategy

Fruits, vegetables, nuts and dried fruits were sampled from retail outlets in 12 cities around the US in two different seasons (Pehrsson et al., 2000; Perry et al., 2001). Approximately 3 pounds of each item were randomly selected from bins in each retail outlet. These samples were composited to form four regional composites: West (Los Angeles, CA, Vancouver, WA, and Longview, WA), Central (Wheaton, IL, Conroe, TX, and Beaumont, TX), South (Mena, AR, Springfield, MO, and Franklin, TN), and Northeast (Springfield, NJ, Canonsburg, PA, and Franklin, PA). However, the timing for all foods was not necessarily at the same time. Fresh fruits and vegetables were collected at a time for peak production within most of the US markets and at a second time when the fruit or vegetable would not likely be produced fresh in most US markets. Rice bran was provided by Dr. N. Fang, Arkansas Children's Nutrition Center, Little Rock, AR.

The fruits and vegetables were freeze-dried and stored in amber bottles under N₂ at -70°C until analysis. Nuts, dried fruits and rice bran were kept at -70°C in their original forms before analysis.

Four vegetables (Russet potato, broccoli, carrot, and tomato) were chosen to make cooked samples. Russet potatoes were baked for 50 min. The others were cooked in boiling water in a stainless-steel sauce pan for 3–4 min. Two varieties of apples (Red Delicious and Golden Delicious) and cucumber were chosen to prepare peeled samples in an effort to compare with samples with peel.

2.3. Sample preparation

Approximately 0.5–1.0 g sample (based on physical properties of samples) were weighed accurately and mixed with 5 g sea sand (Unimin Corporation, Le Sueur, MN). Samples and sand were transferred to a 22 mL extraction cell and were initially extracted with hexane:dichloromethane (1:1, Hex/Dc) followed by acetone:water:acetic acid (70:29.5:0.5, AWA), or directly extracted with AWA. Parameters for ASE[®] 200 Accelerated Solvent Extractor were: static, 5 min, flush, 60%, purge, 60 s, cycle, 3, temperature, 70°C (Hex/Dc extraction), 80°C (AWA extraction), pressure, 1500 psi. Extracts from AWA were transferred to 25 mL volumetric flasks and diluted with AWA to 25 mL total volume. This solution was used to measure H-ORAC_{FL}.

Extracts with Hex/Dc were dried under nitrogen flow in a 30°C water bath. Residues were reconstituted in 10 mL acetone. This sample solution was used to measure L-ORAC_{FL}. The undissolved precipitates of four samples (strawberry, plum, cashew and iceberg lettuce) were dissolved in 2 mL DMSO and L-ORAC_{FL} were measured to compare with that of the acetone solution.

2.4. ORAC_{FL} assay on plate reader

Both hydrophilic and lipophilic ORAC_{FL} assays were carried out on a FLUOstar Galaxy plate reader, which was equipped with an incubator and two injection pumps. The temperature of the incubator was set to 37°C. The procedures were based on our modified ORAC_{FL} method (Prior et al., 2003) and the previous report by Huang et al. (2002). Briefly, AAPH was used as peroxy generator and Trolox as a standard. Forty milliliters of sample, blank and Trolox calibration solutions were transferred to 48-well microplates in duplicate based upon a set layout. The plate reader was programmed to record the fluorescence of FL on every cycle.

Major parameters of assay for the FLUOstar Galaxy were: Cycle number, 35, Cycle time, 210 s with 8 s orbital shaking (4 mm shake width) before each cycle, position delay, 0.3 s, injection speed of 420 μ L/s for both pump 1 and 2.

The final ORAC_{FL} values were calculated using a quadratic regression equation ($y = a + bx + cx^2$) between the Trolox standards or sample concentration and net area under the FL decay curve. The data were analyzed using Microsoft Excel (Microsoft, Roselle, IL). The ORAC_{FL} value was expressed as micromoles of Trolox equivalent (TE) per gram based on fresh weight (for fruits and vegetables) or dry weight (for nuts, dried fruits and rice bran) of sample (μ mol TE/g).

2.5. ORAC_{FL} assay of samples

For the lipophilic ORAC_{FL} assay, the acetone solution was diluted with 7% RMCD in acetone/water (1:1, v/v) to an appropriate concentration to be within the range of the standard curve. The 7% RMCD solution was used as a blank and to dissolve the Trolox standards.

To test for any antioxidants in the particulate matter following dissolving in RMCD, the undissolved material was dissolved in DMSO, and the DMSO solution was diluted with 7% RMCD solution as the final solution for L-ORAC_{FL} measurement. The blank and Trolox standard were used in the same proportion of DMSO in 7% RMCD solution.

For extracts from AWA, sample solutions were diluted with phosphate buffer to the proper concentration range for the standard curve. Trolox standards were prepared with phosphate buffer and the blank for H-ORAC_{FL} assay was phosphate buffer.

2.6. Statistics

Descriptive statistical analyses were performed using Microsoft Excel (Microsoft Corporation, Roselle, IL) and SigmaStat Version 2.03 (Access Softtek Inc.). Graphs were made using SigmaPlot Version 8.0 (SPSS Inc.). The data were expressed as means \pm s.d. for samples with a sample number larger than two. Group differences were evaluated using *t*-tests with $P < 0.05$ considered as indicating a statistically significant difference.

3. Results

3.1. Comparison of L-ORAC_{FL} to H-ORAC_{FL} with/without Hex/Dc extraction

Four foods (strawberry, cashew, lettuce and avocado) were chosen to test the effect of lipophilic extraction on the value for hydrophilic antioxidant capacity. These four samples were directly extracted in the ASE[®] 200 Accelerated Solvent Extractor by AWA using the same conditions described above. Their ORAC_{FL} values as well as the ORAC_{FL} values of the lipophilic and hydrophilic fractions after Hex/Dc extraction are shown in Table 1. From the results, it is clear that there was very little difference between the ORAC_{FL} values for AWA extracts without Hex/Dc and that of AWA extracts with prior Hex/Dc extraction and all were lower than the total antioxidant capacity (TAC = L-ORAC_{FL} + H-ORAC_{FL}).

3.2. Effect of solvent used to dissolve lipophilic components

Four foods (strawberry, plum, cashew and lettuce) which were observed to have precipitates remaining after dissolving the precipitate in acetone were chosen to test if significant antioxidant activity remained that could be dissolved with DMSO. In Table 2, we can see that the ORAC_{FL}

Table 1

Comparison of L-ORAC_{FL} and H-ORAC_{FL} with/without extraction with lipophilic solvents (mean \pm s.d., $n = 3$)^a

Sample name	L-ORAC _{FL} ($\mu\text{mol TE/g}$)	H-ORAC _{FL} (with Hex/Dc) ($\mu\text{mol TE/g}$)	H-ORAC _{FL} (W/O ^b Hex/Dc) ($\mu\text{mol TE/g}$)	TAC ^c ($\mu\text{mol TE/g}$)
Strawberry	3.65 \pm 0.62	441.42 \pm 1.66	440.17 \pm 2.06	445.07
Cashew	5.17 \pm 0.31	11.48 \pm 0.85	12.13 \pm 0.41	16.65
Iceberg lettuce	23.26 \pm 0.13	265.35 \pm 1.21	267.22 \pm 1.01	288.61
Avocado	31.22 \pm 0.22	47.81 \pm 0.07	52.63 \pm 1.57	79.03

^a Data expressed on freeze-dried basis.

^b W/O, without.

^c TAC, total antioxidant capacity.

Table 2

Comparison of ORAC_{FL} values of acetone solution and ORAC_{FL} values of precipitate dissolved in DMSO (mean ± s.d., *n* = 3)^a

Food sample name	ORAC _{FL} of DMSO solution (μmol TE/g)	ORAC _{FL} of acetone solution (μmol TE/g)	ORAC _{FL} -DMSO/ORAC _{FL} -acetone (%)
Strawberry	0.11 ± 0.01	3.65 ± 0.62	3.01
Plum	0.10 ± 0.01	2.83 ± 0.18	3.53
Cashew	0.16 ± 0.05	5.17 ± 0.31	3.09
Iceberg lettuce	0.31 ± 0.07	23.26 ± 0.13	1.33

^aData expressed on freeze-dried basis.

values of these precipitates dissolved in DMSO were very low (<5%) compared to that from the acetone solution.

3.3. L-ORAC_{FL}, H-ORAC_{FL} and TAC of selected food samples and effects of season of sampling

Data on L-ORAC_{FL}, H-ORAC_{FL} and TAC of 28 foods from two different seasons are displayed in Table 3. There is a large range for both L-ORAC_{FL} and H-ORAC_{FL} for the different foods. L-ORAC_{FL} ranged from 0.11 ± 0.05 μmol TE/g (honeydew melon) to 154.70 ± 3.58 μmol TE/g (rice bran). In general, compared to the L-ORAC_{FL}, the H-ORAC_{FL} was much higher. H-ORAC_{FL} ranged from 1.23 ± 0.17 μmol TE/g (watermelon) to 175.24 ± 10.36 μmol TE/g (pecan). Among the food samples tested (Table 3), the percentage of L-ORAC_{FL} to TAC ranged from 0.27% (plum) to 63.7% (rice bran). Forty percent of the foods had L-ORAC_{FL} to TAC proportions above 10%. Fig. 1 shows the ranking of the L-ORAC_{FL} of these foods.

Data in Table 3 also indicate that for some foods, the effect of time of sampling did not affect the measured antioxidant capacity, but for others a difference of up to four-fold was observed.

3.4. Effects of processing on lipophilic and hydrophilic ORAC_{FL}

Figs. 2 and 3 show the comparison of both L-ORAC_{FL} and H-ORAC_{FL} on four cooked and raw food samples. Cooking had variable effects on L-ORAC_{FL} and H-ORAC_{FL}. Figs. 4 and 5 show the comparison of both L-ORAC_{FL} and H-ORAC_{FL} on three peeled and unpeeled samples. L-ORAC_{FL} was markedly decreased when the peel was removed.

4. Discussion

Several in vitro antioxidant capacity assay methods have been used or developed in recent years to evaluate antioxidant capacity of lipophilic components (Naguib, 1998; Pellegrini et al., 1999; Buratti et al., 2001; Cano et al., 2000; Arnao, 2000; Aldini et al., 2001; Kurilich et al., 2002; Huang et al., 2002). Among them, one method was developed and used to measure hydrophilic and lipophilic antioxidant activities separately in foodstuffs (Cano et al., 2000, 2002; Alcolea et al., 2002, 2003). Two methods attempted to measure the ORAC values of lipophilic components in

Table 3
Lipophilic (L-ORAC_{FL}), hydrophilic (H-ORAC_{FL}), and TAC of 28 foods from two different seasons^a

Sample name	Moisture (%)	L-ORAC _{FL} (μmol TE/g)		Mean (μmol TE/g)	H-ORAC _{FL} (μmol TE/g)		Mean (μmol TE/g)	TAC (μmol TE/g)	L-ORAC _{FL} /TAC (%)
		Pass 1 ^b	Pass 2 ^b		Pass 1	Pass 2			
<i>Fruits</i>									
Honeydew	90.0	0.09±0.03	0.12±0.07	0.11±0.05	3.09±0.48	1.51±0.32*	2.30±0.92	2.4	4.5
Cantaloupe	89.7	0.06±0.01	0.21±0.02*	0.15±0.08	3.59±0.30	2.51±0.20*	2.97±0.62	3.1	4.8
Plum	86.7	0.24±0.08	0.10±0.03	0.17±0.10	58.96±18.17	65.47±24.02	62.22±20.22	62.4	0.3
Watermelon	91.7	0.16±0.04	0.22±0.04	0.19±0.04	1.10±0.11	1.37±0.07*	1.23±0.17	1.4	13.4
Kiwi	83.1	0.34±0.04	0.14±0.03*	0.24±0.12	8.84±2.52	8.31±1.38	8.58±1.90	9.2	2.6
Navel orange	85.8	0.18±0.02	0.39±0.10*	0.29±0.13	15.10±1.93	20.61±3.08*	17.85±3.79	18.1	1.6
Grapefruit	88.3	0.41±0.09	0.29±0.07	0.35±0.10	12.19±0.90	18.06±1.58*	15.13±3.36	15.5	2.3
Strawberry	90.6	0.57±0.13	0.14±0.06*	0.36±0.25	33.69±3.95	37.13±4.29	35.41±4.24	35.8	1.0
Blueberry	83.9	0.52±0.10	0.21±0.05*	0.36±0.18	62.29±6.01	61.38±10.18	61.84±7.75	62.2	0.6
Raspberry	85.1	2.01±0.13	0.79±0.39*	1.62±0.66	48.85±8.79	45.24±2.92	47.65±7.18	49.3	3.3
Avocado	71.5	4.20±0.92	6.77±1.69*	5.52±1.85	13.41±5.06	14.22±1.98	13.81±3.58	19.3	28.6
<i>Vegetables</i>									
Tomato	94.5	0.26±0.07	0.22±0.07	0.24±0.01	2.95±0.21	3.26±0.94	3.13±0.69	3.4	7.1
Radish	95.6	0.36±0.09	0.18±0.06*	0.26±0.12	8.43±1.38	9.92±0.94	9.28±1.31	9.5	2.7
Lettuce, Iceberg	95.7	0.29±0.10	0.36±0.11	0.33±0.10	7.12±0.45	1.98±0.67*	4.18±2.80	4.5	7.3
Celery	95.0	0.45±0.04	0.36±0.05*	0.41±0.07	5.37±0.85	5.29±3.01	5.33±2.05	5.7	7.1
Potato, Russet	78.3	0.42±0.16	0.60±0.02	0.51±0.14	11.86±2.42	13.57±2.61	12.72±2.28	13.2	3.9
Carrot	88.0	0.59±0.14	N/A ^c	0.59±0.14	11.56±1.79	N/A	11.56±1.79	12.2	4.9
Baby carrot	90.0	0.74±0.14	0.86±0.28	0.81±0.22	4.50±1.84	2.84±0.74	3.55±1.48	4.4	18.6
Broccoli	90.5	1.68±0.13	1.77±0.33	1.72±0.24	13.85±2.45	14.51±1.86	14.18±2.04	15.9	10.8
<i>Nuts</i>									
Almond	N/C ^d	2.06±0.18	1.38±0.49*	1.72±0.50	41.64±11.23	44.01±6.88	42.82±8.71	44.5	3.9
Macadamia	N/C	2.15±0.53	2.88±0.37	2.52±0.57	14.86±3.37	14.01±0.75	14.43±2.31	17.0	14.9
Pecan	N/C	4.26±1.34	4.07±0.64	4.16±0.98	172.39±7.62	178.26±13.08	175.24±10.36	179.4	2.3
Pistachio	N/C	3.01±0.40	5.18±1.21*	4.25±1.46	72.00±4.80	78.26±13.51	75.57±10.50	79.8	5.3
Cashew	N/C	4.16±1.82	5.17±1.02	4.74±1.38	15.08±2.96	15.34±1.56	15.23±2.04	20.0	23.7
<i>Dried fruits</i>									
Date	N/C	0.46±0.14	0.22±0.04*	0.32±0.16	37.47±2.11	39.50±3.91	38.63±3.21	39.0	0.8
Raisin	N/C	0.28±0.09	0.42±0.14	0.35±0.13	33.34±4.98	26.69±3.09	30.02±5.23	30.4	1.2
Prune	N/C	2.28±0.27	1.30±0.11*	1.79±0.56	82.01±14.68	85.98±20.35	83.99±16.56	85.8	2.1
<i>Grain brans</i>									
Rice bran	N/C	154.70±3.58	N/A	154.70±3.58	88.17±1.52	N/A	88.17±1.52	242.9	63.7

*Differences between Pass 1 and Pass 2 (sampling times) were significantly different ($P<0.05$).

^aData were expressed as fresh weight (FW) or usual form of consumption.

^bMean values for Pass 1 and Pass 2 represent duplicate analyses of samples from 4 different regions of the US.

^cN/A, not available.

^dN/C, not calculated.

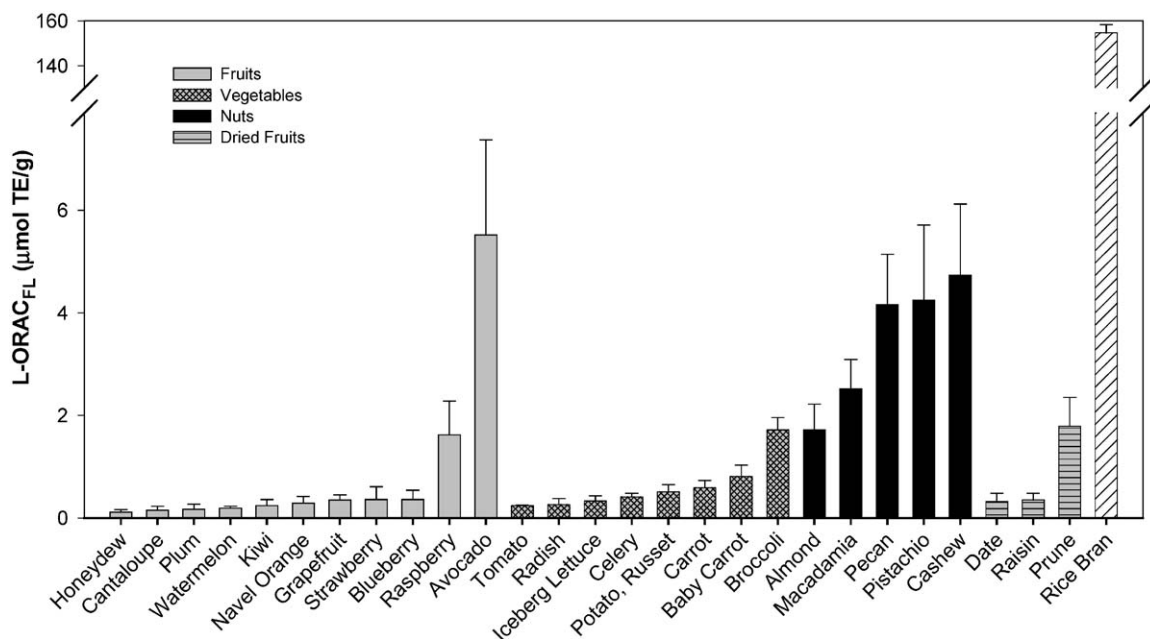


Fig. 1. Lipophilic ORAC_{FL} values of 28 selected foods. Results are expressed as μmol Trolox Equivalents (TE) per gram fresh weight (FW) or usual form of consumption. Samples of the same group of foods were clustered for comparative purposes.

foods. Kurilich et al. (2002) used DMSO to reconstitute the lipophilic extracts of broccoli and then the samples were diluted further with potassium phosphate buffer/DMSO (93:7, v/v) for L-ORAC_{FL} analysis. When we tried this method, we found that for certain samples containing large amounts of oil in the lipophilic extracts, potassium phosphate buffer/DMSO did not dissolve them well (e.g., samples from some nuts). Our data are based upon the validated method developed by Huang et al. (2002) using the ORAC_{FL} assay for lipophilic antioxidants. Using this method, we can measure the L-ORAC_{FL} and H-ORAC_{FL} based on the same principle using a peroxy radical generator.

The ORAC values that we determined previously for “total antioxidant capacity” came from extracts obtained using hydrophilic solvents (Wang et al., 1996; Cao et al., 1996). Before we began the current studies on lipophilic ORAC_{FL} analysis, we picked several representative samples to determine if the hydrophilic solvent alone would be able to extract hydrophilic and most lipophilic components from food samples. From Table 1, of all four samples, the H-ORAC_{FL} for strawberry, cashew and lettuce were very similar with that obtained with direct extraction with AWA. The ORAC_{FL} value of avocado (52.63 ± 1.57 μmol TE/g) from direct extraction was about 10% higher than that after lipophilic extraction (47.81 ± 0.07 μmol TE/g), but much lower than the TAC (79.03 μmol TE/g). Thus, some lipophilic antioxidants might have been extracted with AWA in this food. However, it is clear that the ORAC_{FL} values obtained by hydrophilic extraction alone did not represent total antioxidant capacity. In order to get more accurate results of total antioxidant capacity, it is important to measure the lipophilic and hydrophilic ORAC_{FL} separately and use the value of the sum of these two numbers.

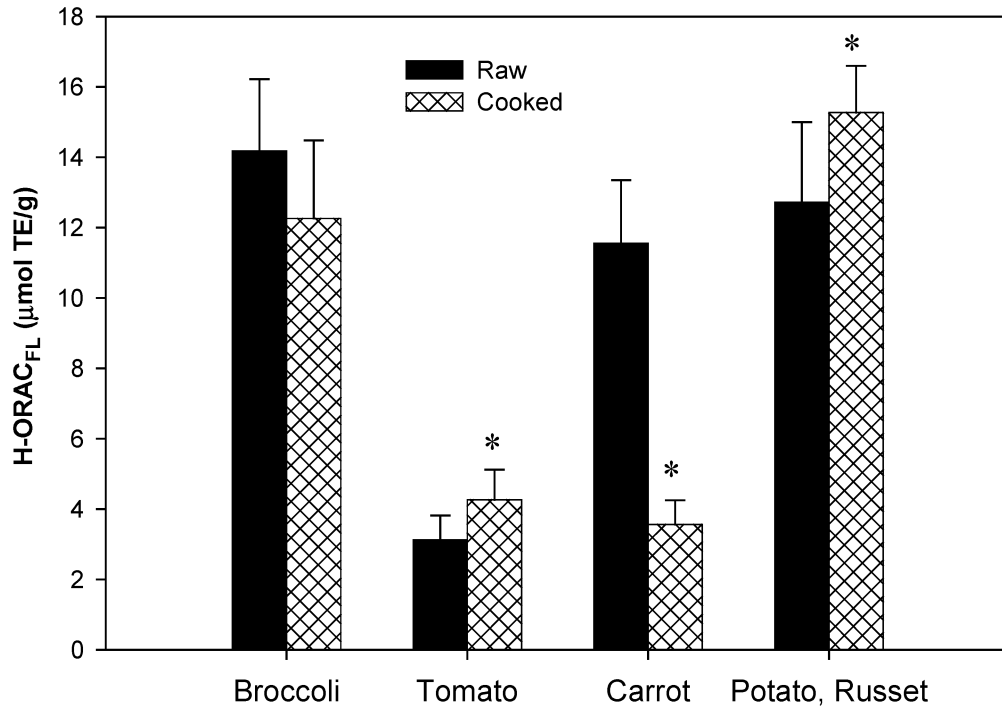


Fig. 2. Effects of cooking on hydrophilic antioxidant capacity. Results were expressed as μmol Trolox Equivalent (TE) per gram fresh weight (FW). Potato was baked for 50 min and the other samples were cooked 3–4 min by boiling. Tomato and Russet potato had significant increases and carrot decreases in antioxidant capacity in the cooked form (marked with $*P < 0.05$).

Acetone was used to reconstitute the lipophilic extracts after they had been dried under nitrogen flow because (1) acetone is a good solvent for dissolving lipophilic components with a relatively wide polar range and (2) the solvent used to dilute samples and blanks for lipophilic ORAC_{FL} analysis was 7% RMCD in acetone/water (1:1, v/v). Thus, by using the same solvent, we reduced the impact of solvent on the assay. However, we did observe that some white undissolved substance was left after acetone dissolving. We selected four foods (strawberry, plum, cashew and lettuce) with undissolved material to determine if any antioxidant activity was remaining that did not dissolve in acetone. DMSO was able to dissolve these substances. Data in Table 2 indicate that the antioxidant capacities contained in these materials were all less than 5% of the total, indicating that acetone was a satisfactory solvent for dissolving the lipophilic antioxidants.

Extraction of lipophilic components followed by the hydrophilic components was carried out on an ASE[®] 200 Accelerated Solvent Extractor (Dionex Corporation, Sunnyvale, CA) using extraction solvents at elevated temperature and high pressure. The main advantage was that the whole extraction process was carried out in a small sealed cell in a nitrogen environment, thereby avoiding interactions with light and oxygen. These two factors are well known to be responsible for degradation of organic compounds. One study (Palma et al., 2001) demonstrated that even with temperatures as high as 150°C, the stability of phenolic compounds in methanol extracts

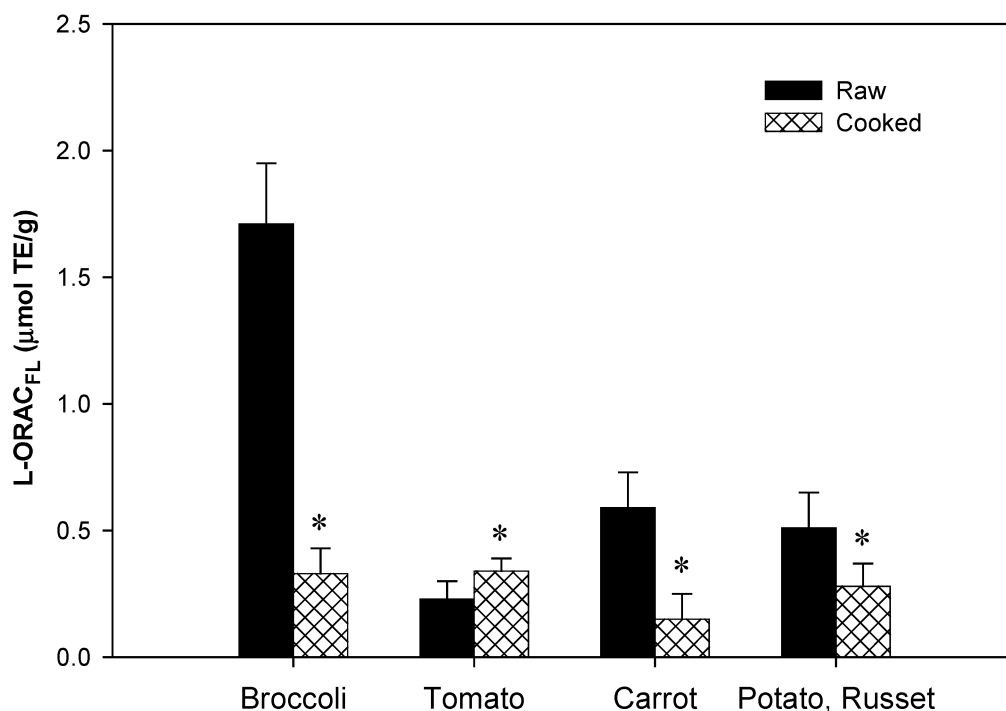


Fig. 3. Effects of cooking on lipophilic antioxidant capacity. Results were expressed as μmol Trolox Equivalents (TE) per gram fresh weight (FW). Potato was baked and the other samples were cooked by boiling. Broccoli, carrot and Russet potato had significant decreases and tomato had significant increases in antioxidant capacity in the cooked forms compared to the uncooked form (marked with $*P < 0.05$).

using the ASE-200 Accelerated Solvent Extractor was much better than that from boiling methanol (65°C) in contact with air. Our studies (unpublished data) showed that our methods (70°C for lipophilic solvent extraction and 80°C for hydrophilic solvent extraction) can increase antioxidant extraction rates and, at the same time, not change significantly the antioxidant profiles. Additional advantages of the ASE method are that the extraction time is much shorter (15–20 min for each sample with one solvent) and extractions with multiple solvents can be easily performed.

Results of L-ORAC_{FL} on twenty-eight food samples are shown in Fig. 1. Table 3 lists L-ORAC_{FL}, H-ORAC_{FL} and TAC, and the percentage of L-ORAC_{FL} relative to TAC. Most fruits and vegetables except raspberry and avocado had very low L-ORAC_{FL} (less than $0.5 \mu\text{mol TE/g}$). The L-ORAC_{FL} as a percentage of TAC for fruits in general is quite low, with most values being less than 5%. Avocado had a very high L-ORAC_{FL} and a high percentage of L-ORAC_{FL} to TAC. The variation in the L-ORAC_{FL} of vegetables is large, ranging from $0.24 \pm 0.01 \mu\text{mol TE/g}$ (tomato) to $1.72 \pm 0.24 \mu\text{mol TE/g}$ (broccoli). The percentages of L-ORAC_{FL} relative to TAC varied from 3.85% (Russet potato) to 18.58% (baby carrot). Our data on some foods are different from other published data (Pellegrini et al., 1999; Buratti et al., 2001; Cano et al., 2002, 2003) using different methods. Since different oxidant or radical sources were used, it is not possible to compare absolute values. The percentage of L-ORAC_{FL} to TAC was used as a comparison

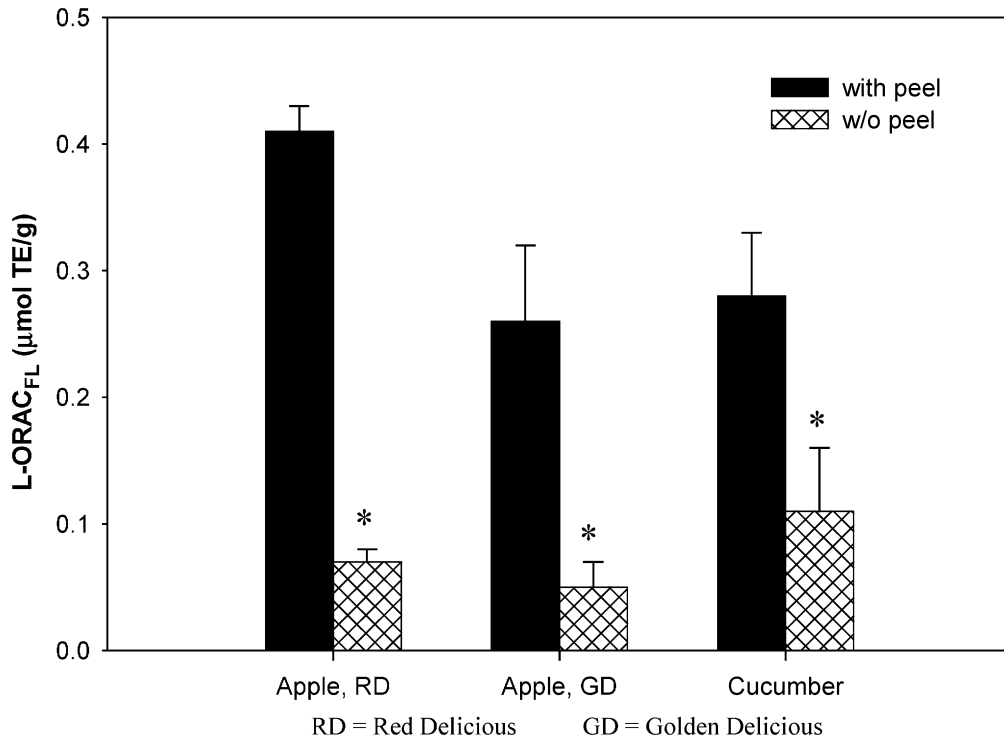


Fig. 4. Effects of peel on lipophilic antioxidant capacity. The results were expressed as μmol Trolox Equivalents (TE) per gram fresh weight (FW). All three samples had significant decreases in antioxidant capacity in the peeled form (marked with $*P < 0.05$).

parameter. One study showed the percentage of $\text{L-ORAC}_{\text{FL}}$ to TAC from tomatoes in different ripening stage ranged from 14.5% (stage I) to 29.2% (stage III) (Cano et al., 2003), which was much higher than our result of 7.1%. Theoretically, there are a lot of factors that may impact this percentage. One of them is the method. Different methods utilize different free radical generators and usually different standards, all of which can produce a difference. The second possible reason is the extraction solvent being used. In the work by Cano et al. (2003), ethyl acetate was used to extract lipophilic components from the aqueous phase. The polarity of ethyl acetate is higher than that of hexane and dichloromethane. This solvent may be capable of extracting some hydrophilic components from the aqueous phase. From our results in Table 1, it is clear that Hex/De extraction did not reduce the ORAC_{FL} value of the hydrophilic fraction significantly compared to direct hydrophilic extraction. The third reason is that different varieties and/or cultivars may significantly change the results. $\text{L-ORAC}_{\text{FL}}$ values for nuts are higher than most foods but still relatively low. Rice bran has a $\text{L-ORAC}_{\text{FL}}$ that is higher than its $\text{H-ORAC}_{\text{FL}}$ value. The main constituents in the lipophilic extract of rice bran are likely vitamin E and γ -oryzanol compounds (Shin and Godber, 1994; Xu and Godber, 1999). Xu et al. (2001) found that vitamin E and γ -oryzanol compounds in rice bran exhibited significant antioxidant activity against cholesterol oxidation.

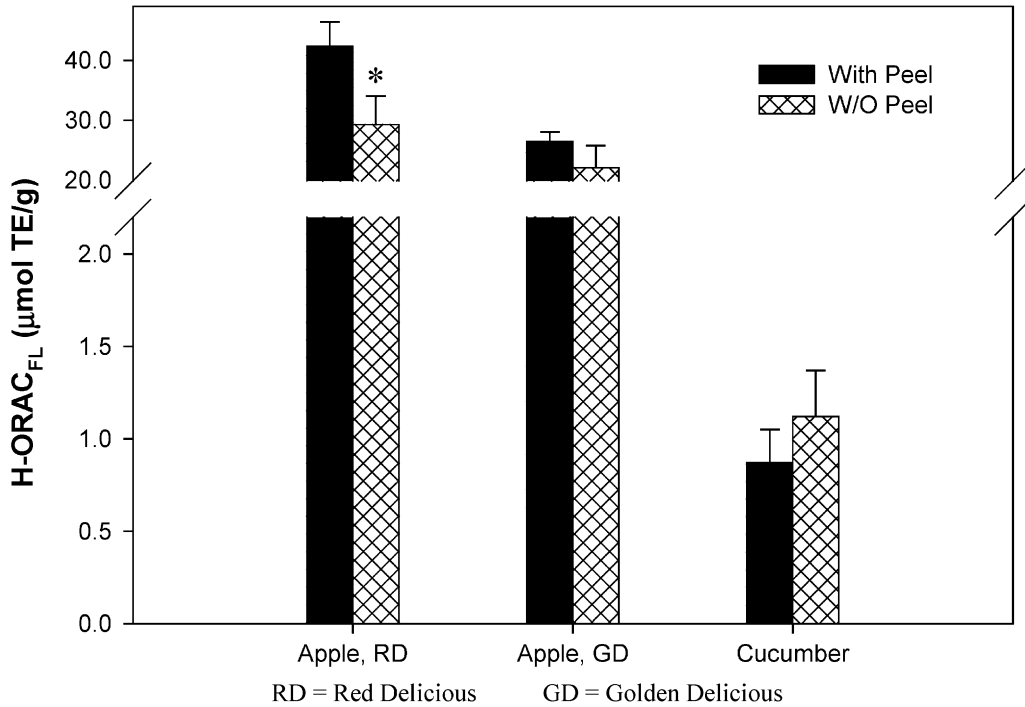


Fig. 5. Effects of removing the peel on hydrophilic antioxidant capacity. The results were expressed as μmol Trolox Equivalents (TE) per gram fresh weight (FW). Peeled Red Delicious apples had a significantly lower antioxidant capacity compared to non-peeled apples (marked with $*P < 0.05$).

Better measurement and understanding of total antioxidant capacity on common foods may help nutritionists and other investigators in studies of dietary factors and the etiology of diseases. Heart diseases and cancer, the leading causes of health related deaths in the US, have been shown to have free radical involvement in their etiology. It is recognized that foods with high antioxidant capacity might provide some protection. Here the concept “total antioxidant capacity” reflected the integrated effects of all the antioxidants and if any, synergic effects of them. Well-known antioxidants, such as vitamins, carotenoids and flavonoids may contribute partly to this value. But each different antioxidant has its own antioxidant properties for the diverse structures. Recently, some fairly large-scale analyses were done to evaluate the antioxidant capacity of foods (Miller et al., 2000; Halvorsen et al., 2002; Ou et al., 2002; Pellegrini et al., 2003). Unfortunately, the methods used in most of these studies were different. The radical source used in the assay can have dramatic effects on the antioxidant capacity observed because of the differential response of different types of antioxidant compounds to the radical source (Cao et al., 1996). Because of this variation, use of radical sources that are relevant to human biology becomes important in the analysis of food sources. The peroxy radical is the most common free radical in human biology, but the hydroxyl radical, singlet oxygen, superoxide radical, and reactive nitrogen species all are present in biological systems. ORAC_{FL} uses the peroxy free radical and is the only method combining the inhibition degree and time into one value. For these reasons, we think ORAC_{FL} is the preferable method for studies such as this.

A number of factors, including genetics, and growing conditions (i.e., fertilization, moisture, pest and disease burden, etc.), are known to affect the levels of what are considered 'plant secondary metabolites'. Many of these secondary metabolites have antioxidant capacity and may have important health consequences. The foods we tested were sampled at two different time points in an attempt to account for some of this variation that might exist in the US market. For the different foods, the two sampling times (termed 'passes'), were planned to be 'in season' and 'out of season' for the fresh produce market in most US markets. However, this differential sampling could not be maintained because of variation in production cycles within different localities within the US. Of the chosen samples, fruits and vegetables displayed significant variation in the different sampling times, but nuts and dried fruits did not. The second observation from these data is that the variation in L-ORAC_{FL} and H-ORAC_{FL} is quite different. L-ORAC_{FL} is much more variable than H-ORAC_{FL}, perhaps due in part to the much lower concentrations. Furthermore, L-ORAC_{FL} and H-ORAC_{FL} were not always similarly affected. Significant sampling period changes were observed for H-ORAC_{FL} in six samples (honeydew, cantaloupe, watermelon, navel orange, grapefruit and iceberg lettuce) and for L-ORAC_{FL} in 13 samples (cantaloupe, kiwi, navel orange, strawberry, blueberry, raspberry, avocado, radish, celery, almond, pistachio, date and prune). These differences were as large as three-fold in magnitude. Thus, it is clear that time of sampling is a factor that must be considered in developing the database. These data also point to the importance of measuring both the hydrophilic and lipophilic separately.

Processing, especially cooking of food, is another factor that can impact antioxidant capacity (Papas, 1996). Some vegetables are commonly eaten in cooked form (i.e., potatoes and asparagus), while others are consumed in either raw or cooked forms (i.e., broccoli, carrots, and tomatoes). Thus, cooking is an important issue that needs to be considered in estimating the daily total antioxidant capacity intake. However, few studies have considered this relative to antioxidants. Cooking is generally regarded as being destructive to antioxidant compounds (Krishnaswamy and Raghuramulu, 1998). In our studies, both L-ORAC_{FL} and H-ORAC_{FL} of raw broccoli and carrots were significantly higher than that of their cooked forms (Figs. 2 and 3). Baked Russet potatoes showed a significantly increased H-ORAC_{FL} (Fig. 2) but a significantly decreased L-ORAC_{FL} (Fig. 3) compared to the raw forms. For tomatoes, both lipophilic and hydrophilic ORAC_{FL} values of the cooked forms were significantly higher than those of their raw forms (Figs. 2 and 3). Ascorbic acid and lycopene are the major hydrophilic and lipophilic antioxidants in tomatoes, and they are well correlated with hydrophilic and lipophilic antioxidant activities, respectively (Cano et al., 2003). The effects of processing on the lycopene content and bioavailability have been studied by several investigators (Weisburger, 1998; Shi and Le Maguer, 2000; Takeoka et al., 2001). Studies have shown that lycopene degrades during cooking due primarily to isomerization and oxidation. However, we observed a significant increase in both L-ORAC_{FL} and H-ORAC_{FL} in tomatoes, which is in agreement with the results of Takeoka et al. (2001). Other studies have demonstrated an increased bioavailability of carotenoids from processed compared to raw tomatoes (Shi and Le Maguer, 2000).

Cucumbers and two apple samples were prepared by removing the peel which significantly decreased the L-ORAC_{FL} by two- to five-fold (Fig. 4). Removing the peel from Red Delicious apples reduced H-ORAC_{FL} by about 30%, but changes were not significant in the Golden Delicious apple and cucumber (Fig. 5). Our results with apples are similar to those of a study by

Wolfe et al. (2003). The distribution of lipophilic and hydrophilic antioxidants in fruit appears to be different, with a large portion of the lipophilic antioxidants concentrated in the peel of apples and cucumbers but not necessarily the hydrophilic antioxidants.

From our results it seems clear that a number of factors including all of those that are included in season of sampling (environmental, genetics, etc.) as well as processing and cooking impact the levels of lipophilic and hydrophilic antioxidants present in fruits and vegetables. These differences need to be considered in developing a database of food antioxidant capacity.

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