

Comparing Antioxidant Values with the ORAC Method

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When pro-oxidant molecules are produced in the body at a rate that exceeds its ability to neutralize them a condition of oxidative stress arises. This condition is widely believed to contribute to disease and natural aging.¹ Among these pro-oxidant molecules are the reactive oxygen species (ROS). These include peroxide (H_2O_2), superoxide radical anion ($\bullet O_2^-$), hydroxyl radical ($HO\bullet$), hypochlorite anion (OCl^-), and singlet oxygen (1O_2).

ROS Formation

The respiratory process is the source of a large portion of the reactive free radicals. The mitochondria shuttle molecular oxygen through the system in a four-electron reduction to form two molecules of water. This process operates at about a 98-percent efficiency. Occasionally, the oxygen is reduced by only one electron forming the superoxide radical. Normally, this species is converted to hydrogen peroxide by the enzyme superoxide dismutase (SOD).

However, the species may also undergo a reaction with trace metal ions and hydrogen peroxide to produce the hydroxyl radical. This very reactive species can react, at essentially diffusion-controlled rates, with the unsaturated fatty acids of a cell's lipid bi-layer to produce lipid radicals. These, in turn, react with molecular oxygen to form peroxy radicals. (Fig. 1) These lipid peroxy species are not as reactive as the hydroxyl radical, but this stability can allow them to pass on unpaired electrons to other lipids and cause more oxidative damage. This chain reaction goes on until a chain-breaking reaction occurs between two radicals.

Defense Against ROS Damage

Defense against ROS damage is provided by antioxidants: the body's enzymes SOD and catalase; macromolecules such as albumin, ceruloplasmin, and ferritin or small molecules such as ascorbic acid, α -tocopherol, beta-carotene, ubiquinol, reduced glutathione, methionine, uric acid, and bilirubin.² Other dietary intakes are also likely to contribute to the body's total antioxidant capability. The extent of this contribution is a matter of strong current interest in nutritional science.

Determining Antioxidant Capacity

For some time, epidemiologic studies have suggested that certain foods may be therapeutic for treating the major diseases: cancers; heart diseases; diabetes; et cetera. The "antioxidant properties" of these foods are often cited as the source of the beneficial effects. It is not surprising then that many research groups in nutritional medicine have sought a reliable measure of antioxidant capacity. Several candidates have emerged over the last 15 years. Most of these methods have been based on the ability of antioxidants to inhibit the fluorescence quenching of peroxy radicals or other ROS.³⁻⁷ One method relies on the ability of antioxidants to reduce iron III in solution.⁸

The ORAC Measurement

The past 5 years has seen the improvement and automation of some of these techniques⁹ particularly the oxygen radical absorbance capacity assay (ORAC) of Cao et al.⁷

The most recent version of the ORAC method relies on the ability of peroxy radicals to quench the fluorescence of fluorescein dye and measures the ability of antioxidants in food samples or sera to protect the dye from the radical damage.¹⁰

The peroxy radicals used in ORAC measurement are generated in aqueous solution from the hydrochloride of 2,2'-azobis-2-methyl-propanimidamide. The compound quickly produces two mols of peroxy radical. In the absence of an inhibitor, these radicals will rapidly destroy the fluorescence of the fluorescein dye. By following the time course of the fluorescence decay, with and without added test substances, a measure of the radical trapping ability of the test substance can be estimated.

Figure 2 shows plots of fluorescent intensity versus time for blank and sample runs. The difference between the area under the curve (AUC) for each parameter is the raw datum for the measurement.

ORAC values are reported in comparison to the very efficient quenching of a water-soluble analogue of α -tocopherol called trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid). The number of μ mol equivalents of trolox (TE) that produces the same AUC as one g of the test substance (or mL in the case of liquid samples such as fruit juices) is the ORAC value (μ mol TE/g (mL)). Sometimes the value is called the "ORAC unit," which is defined as the net protection produced by 1 μ mol of trolox.¹¹ The numbers are equivalent.

Because the ORAC measurement is made in aqueous solution fat-soluble antioxidants must be extracted and analyzed separately. The lipophilic fraction of a sample is extracted with hexane. The hexane is then removed and the residue redissolved in acetone and diluted with a solution of a solubilizing agent, randomly methylated β -cyclodextrin, in a 50/50 acetone–water mixture.¹²

ORAC is primarily a measure of peroxy radical quenching by hydrophilic antioxidants. However, the adaptation discussed above for lipophilic antioxidants and other recent variations of the method may extend its sensitivity to other radicals¹³ and to the analysis of more specific antioxidants.¹⁴

ORAC Values of Foods

Measurement of food ORAC values is of interest of course, but the end result, and of more interest, is the effect of the foods on the antioxidant levels or the radical-quenching ability of the body. Several studies reporting ORAC measurements of blood plasma and other body fluids have appeared in the past few years.^{15–17} Because the proteins in blood plasma and other body fluids comprise the largest contributor to the ORAC measurement, they must be precipitated from solution prior to fractionation and analysis.

ORAC is, of course, not the only method of estimating the antioxidant value of foods or the antioxidant status of the body, but this technique has become widely, almost routinely, quoted

in the scientific literature as the method of choice in the last few years. It has also become a marketing tool for foods and supplements. A World Wide Web search of the terms ORAC foods or High-ORAC foods or High-ORAC supplements returns up to 3000 sites, mostly commercial ones.

There is some information available on the ORAC values of various foods from commercial sites on the Web. One example is shown in Table 1. Although less precise, the per serving units used in these compilations are more useful to consumers than the standard units discussed above ($\mu\text{mol TE/g (mL)}$). As with supermarket unit pricing, it is essential to pay attention to the comparison of “apples with apples” when looking at commercial information. Such factors as serving size, cooked/raw, et cetera, must be comparable.

ORAC Values of Dietary Supplements

ORAC information on dietary supplements is not easily available from sources apart from the manufacturers. Some manufacturers' Web sites quote ORAC values in the thousands, but often leave out the units (see, for example, www.askbillsardi.com/reports/orac_report.pdf) This confusion may be corrected soon, if the results of a comprehensive study at the U.S. Department of Agriculture Agricultural Research Center's Center for Aging Research at Tufts University, Boston, are published, as promised.¹⁸

Table 1. ORAC Values of Super Antioxidant Fruits and Vegetables

Fruit or vegetables	ORAC value (per 5 g)	Serving size	ORAC value (per serving)
Prunes	288.50	1 pitted prune	462
Raisins	141.50	1/4 cup	1019
Blueberries	111.70	1/2 cup	1620
Blackberries	101.80	1/2 cup	1466
Garlic	96.95	1 clove	58
Kale	88.50	1/2 cup, cooked	1150
Cranberries	87.50	1/2 cup	831
Strawberries	76.80	1/2 cup	831
Spinach (raw)	60.50	1 cup	678
Raspberries	61.35	1/2 cup	755
Plums	47.45	1 plum	626
Alfalfa sprouts	46.55	1 cup	307
Spinach (steamed)	45.45	1/2 cup, cooked	1089
Broccoli florets	44.40	1/2 cup, cooked	817
Beets	42.05	1/2 cup, cooked	715
Avocados	39.10	1/2 avocado	149
Oranges	37.50	1 orange	982
Grapes (red)	36.95	10 grapes	177
Peppers (red)	36.55	1 medium-sized pepper	540
Cherries	33.50	10 cherries	455
Kiwifruit	30.25	1 kiwifruit	458
Beans (baked)	25.15	1/2 cup	640

Source: www.newspirit.com/graphics/techbulletinpdfs/orac.pdf

ORAC, oxygen radical absorbance capacity assay.

Clinical Studies

Looking into the peer-reviewed publications involving ORAC measurements has shown that the field is in its infancy, at least with regard to medically focused research. A total of seventeen clinical studies has been reported since 1997.

Given the widely accepted relationship between diet and disease and the supposed effects of oxidative stress on health, it is not surprising that of most current interest in this area are studies on the direct relationship between dietary intake and antioxidant status. Twelve studies have addressed this subject directly in the past few years.

In an early, uncontrolled study (by the developers of the ORAC method), a group of 36 healthy nonsmoking subjects consumed diets with 10 servings of fruits and vegetables each day for 15 days in a controlled residential setting. The subjects' ORAC baselines closely correlated with the reported dietary intake of antioxidants from fruits and vegetables during the previous year and was significantly increased after the period of the controlled diet.¹⁹

More recently, the dry solids of red wine have been shown to improve blood ORAC values significantly when taken with food. The plasma of 10 healthy subjects showed reduced ORAC after subjects ate a light meal compared to baseline (5.0 ± 0.5 reduced to 4.4 ± 0.4) and remained low for 6 hours. If the meal was served with lyophilized red wine, equivalent to 350 mL of the beverage, the ORAC values increased (4.8 ± 0.4 increased to 6.4 ± 0.6 after 90 minutes) then reduced to 5.0 ± 0.3 after 6 hours.²⁰

A study on the effect of postprandial consumption of wild blueberry (*Vaccinium myrtillus*) powder on 8 healthy middle-age men showed a significant rise in the ORAC value in the water-soluble fraction of serum. In this single-blinded crossover study the men ate a high-fat meal followed by a placebo supplement to establish a baseline. One (1) week later, they consumed the same meal followed by 100 g of freeze-dried wild blueberry powder. The increase in serum ORAC was 8.5 percent.¹⁶ Other clinical studies on blueberry powder²¹ and on honey²² have yielded similar positive results.

A recent study on the effect of high-ORAC diet on the plasma ORAC status of smokers undergoing fish-oil supplementation showed significant increases during the 3 weeks of added fruit and vegetables in their diets. Eighteen (18) healthy male smokers volunteered to participate in the 9-week study. They each consumed 4 g per day of fish oil during the entire 9 weeks. After week 3, the men included an extra five servings of fruits and vegetables in their daily diets. After a 3-week period the subjects went back to their normal diets. During the 3 weeks of enhanced diet, the men's plasma ORAC levels increased significantly ($P < 0.001$) although the men's levels of antioxidants such as α -tocopherol, retinol, and uric acid did not change.²³

The effects of antioxidant dietary supplements on ORAC of plasma are not as clear as those of whole foods. An 8-week, double-blinded, placebo-controlled clinical trial with 80 older adults found no effect of 100-percent Recommended Daily Allowance multivitamin treatment on ORAC values of blood plasma.¹⁷

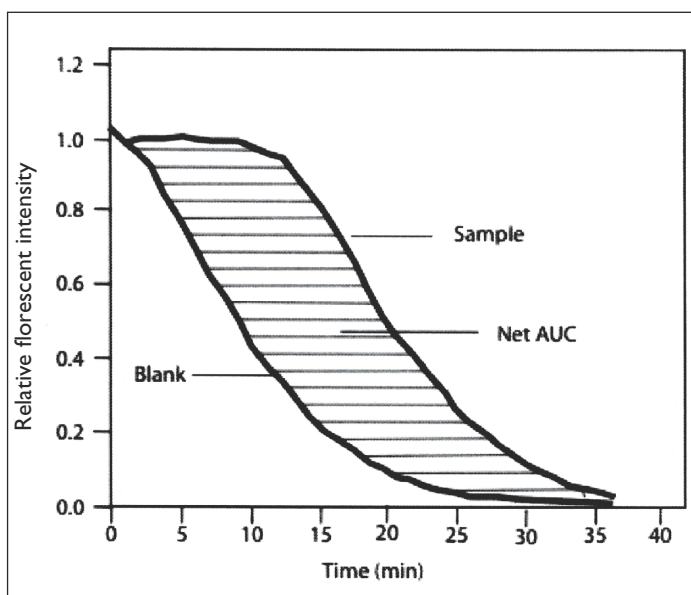


Figure 1. Formation of peroxy radical.

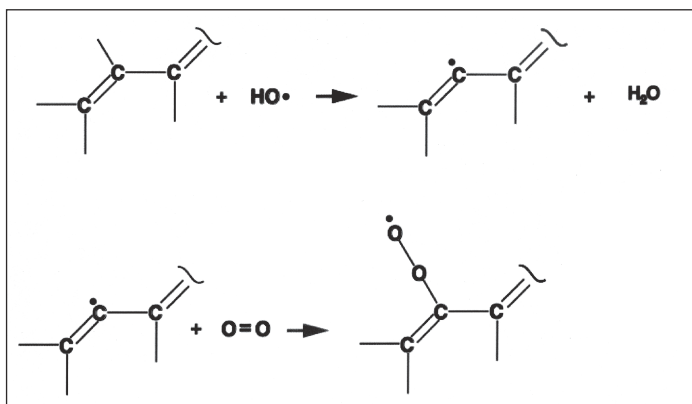


Figure 2. Plots of fluorescent intensity versus time for blank and sample runs to measure radical trapping ability of test substances.

Two studies on a popular botanical supplement came to opposite conclusions about its effect on plasma antioxidant status.

One study assessed the effect of the pine bark extract pycnogenol (PYC) on the ORAC of human plasma during a 6-week supplementation period. This noncontrolled study involved 25 healthy subjects who received 150 mg per day of the extract. Blood plasma taken at 0, 3, and 6 weeks and after a 4-week washout period showed a significant increase in ORAC values throughout the supplementation period. ORAC returned to baseline after the 4-week washout.²⁴

A second, more controlled study seemed to contradict these results directly.²⁵ In what was described as a nonrandom intervention, 27 subjects took a placebo dose twice daily, with meals, for 2 weeks to establish a baseline. This was followed by 2 weeks of 200 mg per day of PYC under the same regimen. On days 15 and 29, fasting blood samples were taken from the subjects. After an hour's delay, each subject was given either a placebo or a 200-mg dose of PYC with a beverage. After 1 hour, a second blood

sample was taken. The ORAC values of the blood plasma actually declined significantly after the 2-week supplementation ($P = 0.005$). After the extra dosage of PYC or placebo on days 15 and 29 the ORAC increased by 15–19 percent but there was no significant difference between the PYC and placebo groups.

Caveats and Suggestions.

The emergence of simple and reliable tools to estimate both the antioxidant potential of foods and supplements and the antioxidant status of body fluids and tissue could be a powerful spur to research in nutrition science. Researchers are now taking the first steps to assess the strength of ORAC measurements as such a tool. However, several caveats should be mentioned.

At the moment ORAC is not a measure of total antioxidant capacity (TAC), as it is sometimes referred to, even when both hydrophilic and lipophilic fraction analyses are taken into account. ORAC is still only sensitive to peroxy radical quenching although some progress is being made to extend the technique's sensitivity to hydroxyl radicals as mentioned above.

Sheldon S. Hendler Ph.D., M.D. (clinical professor of medicine (voluntary), at the University of California, San Diego, La Jolla, and Editor-in Chief of the *Journal of Medicinal Food*) remarked, regarding ORAC as a measure of oxidative stress: "It does not measure a whole universe of radicals." Dr. Hendler noted that other markers of oxidative stress have a longer history and are perhaps more reliable as a gauge of oxidative stress in the body. In particular, he mentioned isoprostanes and 8-epiprostaglandin.

Because the work has only just begun on the relationship between the ORAC of foods and supplements and the oxidative status of the body's tissue and sera, it would be wise not to take at face value the claims of ORAC numbers by manufacturers. There is still a long way to go before knowing how the body absorbs such antioxidants and what role their metabolites might play.

None of this is to say that we should be cautious when advising patients to add good foods with high-ORAC values to their diets. It is hard to be too liberal with such foods as broccoli, kale, and spinach or, indeed, with pleasing fruits such as blueberries and raspberries.

Much has been made of the adverse role of ROS in the body but it should be kept in mind that they are necessary for a healthy immune response; too much defense is not a proper strategy for the body. □

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