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ANALYTICAL PROGRESS

Antioxidant Activity

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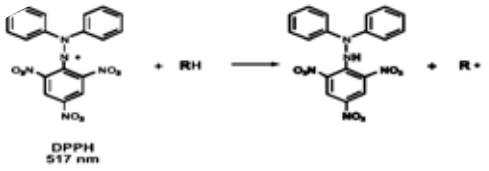
WHAT ARE ANTIOXIDANTS?

Antioxidant compounds in food play an important role as a health-protecting factor. Scientific evidence suggests that antioxidants reduce the risk for chronic diseases including cancer and heart disease. Primary sources of naturally occurring antioxidants are whole grains, fruits and vegetables. Plant sourced food antioxidants like vitamin C, vitamin E, carotenes, phenolic acids, phytate and phytoestrogens have been recognized as having the potential to reduce disease risk. Most of the antioxidant compounds in a typical diet are derived from plant sources and belong to various classes of compounds with a wide variety of physical and chemical properties. Some compounds, such as gallates, have strong antioxidant activity, while others, such as the mono-phenols are weak antioxidants.

The main characteristic of an antioxidant is its ability to trap free radicals. Highly reactive free radicals and oxygen species are present in biological systems from a wide variety of sources. These free radicals may oxidize nucleic acids, proteins, lipids or DNA and can initiate degenerative disease. Antioxidant compounds like phenolic acids, polyphenols and flavonoids scavenge free radicals such as peroxide, hydroperoxide or lipid peroxyl and thus inhibit the oxidative mechanisms that lead to degenerative diseases.

There are a number of clinical studies suggesting that the antioxidants in fruits, vegetables, tea and red wine are the main factors for the observed efficacy of these foods in reducing the incidence of chronic diseases including heart disease and some cancers. The free radical scavenging activity of antioxidants in foods has been substantially investigated and reported in the literature by Miller and Rigelhof et.al (1, 2).



METHOD CONSIDERATIONS

Various antioxidant activity methods have been used to monitor and compare the antioxidant activity of foods. In recent years, oxygen radical absorbance capacity assays and enhanced chemiluminescence assays have been used to evaluate antioxidant activity of foods, serum and other biological fluids. These methods require special equipment and technical skills for the analysis. The different types of methods published in the literature for the determinations of antioxidant activity of foods involve electron spin resonance (ESR) and chemiluminescence methods. These analytical methods measure the radical-scavenging activity of antioxidants against free radicals like the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical, the superoxide anion radical (O2), the hydroxyl radical (OH), or the peroxyl radical (ROO). The various methods used to measure antioxidant activity of food products can give varying results depending on the specific free radical being used as a reactant. There are other methods which determine the resistance of lipid or lipid emulsions to oxidation in the presence of the antioxidant being tested. The malondialdehyde (MDA) or thiobarbituric acid-reactive-substances (TBARS) (1) assays have been used extensively since the 1950's to estimate the peroxidation of lipids in membrane and biological systems. These methods can be time consuming because they depend on the oxidation of a substrate which is influenced by temperature, pressure, matrix etc. and may not be practical when large numbers of samples are involved. Antioxidant activity methods using free radical traps are relatively straightforward to perform. The ABTS [2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid)] radical cation (2) has been used to screen the relative radical-scavenging abilities of flavonoids and phenolics through their

Prior et al. (3) have used the Oxygen Radical Absorbance Capacity (ORAC) procedure to determine antioxidant capacities of fruits and vegetables. In the ORAC method, a sample is added to the peroxyl radical generator, 2,2'-azobis(2-amidinopropane)dihyd rochloride (AAPH) and inhibition of the free radical action is measured (4) using the fluorescent compound, B-phycoerythrin

or R-phycoerythrin. Phenolic and polyphenolic compounds constitute the main class of natural antioxidants present in plants, foods, and beverages and are usually quantified employing Folin's reagent. Vinson et al. (5) have measured phenolics in fruits and vegetables colorimetrically using the Folin-Ciocalteu reagent and determined the fruit and vegetable's antioxidant capacity by inhibition of low density lipoprotein oxidation mediated by cupric ions.

A rapid, simple and inexpensive method to measure antioxidant capacity of food involves the use of the free radical, 2,2-Diphenyl-1-picrylhydrazyl (DPPH). DPPH is widely used to test the ability of compounds to act as free radical scavengers or hydrogen donors, and to evaluate antioxidant activity of foods. It has also been used to quantify antioxidants in complex biological systems in recent years. The DPPH method can be used for solid or liquid samples and is not specific to any particular antioxidant component, but applies to the overall antioxidant capacity of the sample. A measure of total antioxidant capacity helps understand the functional properties of foods.

Antioxidant activity has been expressed in various ways including the percentage of the reagent used, the oxidation inhibition rate and so on. An easier way to present antioxidant activity of foods would be to reference a common reference standard. One common reference standard, (S)-(-)-6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid, also known as Trolox, serves this purpose.

THE DPPH METHOD

A simple method that has been developed to determine the antioxidant activity of foods utilizes the stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical. The structure of DPPH and its reduction by an antioxidant are shown above. The odd electron in the DPPH free radical gives a strong absorption maximum at 517 nm and is purple in color. The color turns from purple to yellow as the molar absorptivity of the DPPH radical at 517 nm reduces from 9660 to 1640 when the odd electron of DPPH radical becomes paired with a hydrogen from a free radical scavenging antioxidant to form the reduced DPPH-H. The resulting decolorization is stoichiometric with respect to number of electrons captured.

Antioxidant compounds may be water-soluble lipid-soluble, insoluble, or bound to cell walls. Hence, extraction efficiency is an important factor in quantification of antioxidant activity of foods. Trolox (as the reference standard) and the sample are reacted with DPPH solution in methanol/water for four hours at 35°C in a vessel mounted on a rotary shaker and the absorbance changes are measured at 517 nm. The quantity of sample necessary to react with one half of the DPPH is expressed in terms of the relative amount of Trolox reacted. Antioxidant activity of a sample is expressed in terms of micromole equivalents of Trolox (TE) per 100 grams of sample, or simply Trolox units per 100 gm or TE/100g.

RESULTS AND DISCUSSION

The reaction of DPPH with numerous antioxidants has been published and the stoichiometry characterized (6,7). As mentioned above, antioxidants in food may be water soluble, fat soluble, insoluble, or bound to cell walls and thus not necessarily freely available to react with DPPH, hence they react at different rates i.e. differing kinetics, and the reaction will often not go to completion in a reasonable assay time. Therefore, the sample size that can lower the initial absorbance of DPPH solution by 50% has been chosen as the endpoint for measuring the antioxidant activity. This change is compared to the change induced by Trolox, the reference standard, and the antioxidant activity of the sample is expressed in micromoles of Trolox equivalents (TE) per 100 gm of sample or Trolox units per 100 gm.

A difference between this method and other published methods is carrying out the reaction of the sample itself with DPPH in methanol/water. Reacting an aqueous-methanolic DPPH solution with the sample for 4 hours at 35 °C facilitates the extraction of antioxidant compounds from the sample thereby increasing the measured antioxidant activity of the sample. Determination of antioxidant activity of various types of foods using DPPH is comparable to other methods. It is probable each of these methods measure a somewhat different profile of antioxidant compounds. Antioxidant analysis by other published methods is limited to those compounds soluble in the selected solvent. Antioxidant activity of insoluble compounds is not accounted in a single extraction method. Extraction techniques using different solvents and concentrating the solvent is time consuming. In this method, DPPH is allowed to react with the whole sample. Sufficient time allows DPPH to react slowly with weak antioxidants.

TABLE 1

STANDARD ANTIOXIDANTS	ANTIOXIDANT ACTIVITY (TE/100 Grams)
Ascorbate	442,000
Trolox	400,000
Vitamin E	201,000
BHT	395,000

The kinetics of flavanones, flavanols and various phenolic compounds scavenging DPPH radicals have been studied by electron spin resonance spectrometry and a variety of other techniques. When one studies compounds with similar structures, these compounds follow similar kinetic trends no matter which of the various methods is used.

Antioxidant activity of grains, dry beans, fresh vegetables and fruits were analyzed using the DPPH method(See table 2). As a comparative basis, results for some standard, more commonly recognized, antioxidant compounds are presented in Table 1. DPPH results for standard phenolic compounds follow similar trends as observed by other methods. Antioxidant content of vegetables and fruits was previously reported using ORAC and other methods (8,9). Similar trends in antioxidant activity were observed for grains, vegetables and fruits comparing those results with those obtained using DPPH.

Antioxidant activity of dry beans increases with the red color of the beans, red kidney beans being the highest. Vinson et al. also observed that red kidney beans had higher activity than other beans (5). Similarly, red grapes have higher antioxidant activity than green grapes, and red cabbage is higher than green cabbage. Antioxidant capacity of blueberries and strawberries containing phenolic anthocyanins are high in the range of 3100-5100 TE/100gm. Dried fruits like raisins and dates have high antioxidant activities of 5900-6600 TE/100gm using the DPPH method, and these results are consistent with published ORAC data.

Antioxidant activities of various fractions of grain during milling process were studied and the results indicate that bran has the highest antioxidant activity and refined flour had the lowest activity. Antioxidant activities were also studied for ready to eat oat, wheat, corn, and rice cereals. In whole grain cereals, bran and germ are intact, hence antioxidant activity of whole grain cereals is higher than it is in refined grain cereals. Antioxidant activity of whole grain wheat and whole grain oat cereals is in the range 2200-3600 TE/100gm. Refined grain corn and rice cereals are in the range 1400 - 2000 TE/100gm, as a result of bran removal. Hence, the refined grain cereals have lower antioxidant activity. Similar trends have been observed with whole wheat bread, 2000 TE/100gm versus white bread, 1200 TE/100gm.

CONCLUSION

The antioxidant activity of various foods can be determined accurately, conveniently, and rapidly using DPPH testing. The trend in antioxidant activity obtained by using the DPPH method is comparable to trends found using other methods reported in the literature. This method can be used successfully for solid samples without prior extraction and concentration, which saves time. The reaction time of four hours and a

temperature of 35°C facilitates the extraction and reaction of antioxidant compounds with DPPH. Antioxidant activity measured using DPPH accounts partially for the bound and insoluble antioxidants. Relative antioxidant content provides an indication of importance of each of the foods. Antioxidant activity and nutritional labeling data including vitamins, fibers, minerals will aid in the interpretation of clinical results obtained as various food products are tested in biological models for chronic disease. It is reasonable to expect that high antioxidant foods have greater potential to reduce free radicals in the body than do low antioxidant foods. Thus it is important to know the antioxidant content of foods, in addition to knowing the basic nutritional information such as the protein, fiber, fat, mineral and vitamin contents.

TABLE 2

FOOD	ANTIOXIDANT ACTIVITY (TE/100 Grams)
Red Grapes	1350
Red Cabbage	1000
Broccoli Flowers	500
Spinach	500
Green Grapes	400
Tomato	300
Green Beans	175
Green Cabbage	150
Lima Beans	1055
Red Beans	11459
Blueberries	3300
Raisins	5900
Wheat Bran	4620
Wheat Flour (refined)	600

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