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Determination of Total Antioxidant Capacity of Some Synthetic Antioxidants by a New Polarographic Method Based on Cerium (IV) Reducing Capacity Measurement

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Abstract

The nutritional and health relevance of antioxidants have been underlined by several studies and free-radical scavenging methods are commonly used to evaluate the capacities of antioxidants compounds or antioxidant potentials of plants. The present study employs a new method of assessing the relative potential of antioxidants. The total antioxidant capacity of 5 phenolic antioxidants including propyl gallate ,octyl gallate, dodecyl gallate, butylated hydroxyl toluene (BHT) and butylated hydroxyl anisole (BHA), which are considered true antioxidants were determined by the polarographic method in arbitrary units per milligram based on cerium (IV) Reducing Antioxidant Capacity (CERAC). The total antioxidant capacity of each phenolic antioxidant was calculated as the difference in peak height of the cerium (IV) before and after oxidation in arbitrary units per milligram of phenolic compound. The total antioxidant capacities calculated are as follow: propyl gallate (120,000), Octyl gallate (100,000), Dodecyl gallate (60,000) BHA (60,000) BHT (40,000). These values should serve as a guide for selecting phenolic compounds as antioxidants for use in processed foods in order to guarantee the safety of foods against autooxidation.

Keywords: *Antioxidant Capacity; Polaroraphic' Cerium (IV); Peroxidation*

Introduction

Reactive species, both of endogenous and exogenous origins are a continuous threat to human health and well-being. The mitochondrial electron transfer process associated with Adenosine triphosphate (ATP) production generates free radicals and reactive oxygen species (ROS) in aerobic life via donation of electron to molecular oxygen, leading to unstable intermediates which directly or indirectly cause oxidative damage to biological systems (Turrens, 2003). This phenomenon is unavoidable since the body cells indispensably require ATP for their continuous existence and functions. Moreover, autooxidation of foods rich in fats also leads to release of free radicals during storage [1-3]. Indeed, it has been reported that a rancid taste becomes noticeable when the peroxide value of stored food products is between 20 - 40 milli equivalent/kg [3]. Regular consumption of such foods contribute immensely to exposure of humans to free radical attack which is capable of directly inflicting damage on biomolecules (particularly lipids, proteins and DNA) or initiate chain reactions in which reactive oxygen species are passed from one molecule to another, resulting in extensive damage to cell structures and functions [4].

Free radical damage within cells has been linked to a range of disorders and diseases including Cancer, arthritis, atherosclerosis, diabetes and hypertension [4-6]. In view of this, the use of antioxidants especially synthetic antioxidant as preservative or fortifying agents in food processing is now popular. However, the choice of which antioxidants are most suitable for the purpose depends on a combination of factors but primarily on their antioxidant capacities.

Antioxidant capacity assay methods existing in the literature based on the measurement of radical scavenging activities of antioxidants are limited by the difficulties associated with the formation and stability of coloured radicals [7]. The evaluation of antioxidant activity by the changes of DPPH absorbance should be carefully interpreted since the absorbance of DPPH at 517 nm is decreased by light, oxygen, pH and type of solvent in addition to the antioxidant [8]. Measurement of oxygen radical scavengers with methods such as the ORAC (Oxygen Radical Absorbance Capacity) test may be interfered with by hydroxyl radical scavengers like benzoate which are not true antioxidants [9]. On the other hand, the ferric reducing ability (FRAC) assay of antioxidants which is based on ferric-ferrous reduc-

tion in the presence of Fe(II)-stabilizing ligand such as tripyridyluriazine is non-responsive to thio-type antioxidants like glutathione. The spectrophotometric method [9] and spectrofluorometric method [10] based on Ceric (IV) reducing capacity measurement have also been reported. The common drawback of the existing antioxidant activity assays for foodstuffs is that the measurement variable is either in the quantity or reactivity (or both) of antioxidant compounds [10].

It has been reported that there is no total antioxidant as a nutritional index available for food labeling due to lack of standard quantification methods [11,12]. There is obviously no single and widely acceptable assay method applicable to a reasonable variety of compounds and food materials. For this reason, researchers cannot easily compare the total antioxidant activity of various food plants, whereas, for example, moisture in plant materials or foods can be determined on a common standard basis. What previous studies have succeeded in doing is to develop an antioxidant capacity determination method effective for certain groups of antioxidants [9-11,13].

This study attempts to provide a standard basis for determining total antioxidant activity of five phenolic antioxidants commonly used in protecting foods from autooxidation. It is expected that the results obtained would provide a guide for selecting these compounds for use in different foods. The present study is based on the oxidation of the antioxidant compound with Ce (IV) sulphate at 55oC under control conditions of reagent concentration and pH, such that only the antioxidant compound and not the solvent used is oxidized. The unreacted Ce (IV) is then determined by polarography at a start potential of 0.15volts using Davis Differential Cathode Ray Polarograph.

Materials and Methods

Instruments and Reagents

Davis Differential Cathode Ray Polarographic stand, Corning Eeel Digital pH metre, Water with thermostat, Distilled water, Ceric sulphate (0.025M), Concentrated tetraoxosulphate (VI) acid (sulphuric acid), Propyl gallate, Octyl gallate, Dodecyl gallate, BHA, BHT, and Dioxan. All chemicals were of analytical grade and supplied by BDH.

Experiments and methods

A 100 microgram per milliliter standard solution of each of the following synthetic antioxidants :propyl gallate, octyl gallate, dodecyl gallate, BHA and BHT were prepared in purified dioxin by direct weighing of 10 mg of each antioxidant compound and dissolved in 100 mL purified dioxin in 100 mL volumetric flask. From each of the standard solution of antioxidant, aliquots of 0.1 mL, 0.2 mL, 0.3 mL, 0.4 mL, 0.5 mL and 0.6 mL were measured directly into 50 mL volumetric flask and the contents of each flask oxidized with 5.0 mL of 0.025M Ceric sulphate for 2 hours at temperature of 55oC with constant shaking using a flask shaker device. At the end of the oxidation period, the contents of each flask were diluted with 0.05M sulphuric acid. Polarographic Determination for the purpose of polarographic reading, unreacted Ce (IV) in each flask was placed in a polarographic cell, deaerated for 3 minutes with oxygen -free nitrogen and the dearated solution was then subjected to polarography at a start potential of 0.15 volt. The peak height of unreacted Ce (IV) in each flask was recorded against the corresponding weight of antioxidant. The difference in peak height of Ce (IV) before and after oxidation was used in calculating the total antioxidant capacity of each antioxidant compound.

Results and Discussion

	Peak Height of Cerium (IV) in Arbitrary Units				
Conc. of Antiox (µg/mL)	**PG**	**OG**	**DG**	**BHA**	**BHT**
0	9,800	9,800	9,800	9,800	9,800
10	8,600	8,800	9,200	9,200	9,400
20	8,000	8,200	8,700	8,500	8,800
30	7,400	7,600	8,200	8,000	8,300
40	6,900	7,000	7,700	7,400	7,600
50	6,400	6,500	7,100	6,700	7,000
60	6,000	6000	6,700	6,200	6,500
TAC/mg	120,000	100,000	60,000	60,000	40,000

Table 1: *Unreacted cerium (IV) and total antioxidant capacity for the selected antioxidants.*

TAC: Total Antioxidant Capacity; mg: milligram; Conc.: Concentration; Antiox: Antioxidant; PG: Propyl Gallate; OG: Octyl Gallate; DG: Dodecyl Gallate; BHA: Butylated Hydroxy Anisole; BHT: Butylated Hydroxy Toluene.

A comparison of the peak heights due to unreacted Cerium (IV) for each antioxidant at a particular antioxidant concentration reveals that the highest peak height was obtained for BHT, while the lowest peak height was obtained for propyl gallate. The relationship between antioxidant capacity and peak height in this method is inverse correlation. As such, the higher the peak height, the lower the antioxidant capacity. This implies that BHT has the lowest activity of all the synthetic antioxidants under study, while propyl gallate has the highest activity. The presence of substituents on the ortho and para positions of phenolic antioxidants like BHT and BHA has been reported to hinder their performance as antioxidants in foods. It is therefore recommended that these antioxidants be used in combination with other compounds capable of providing synergism.

Conclusion

The total antioxidant capacities of propyl gallate, octyl gallate, dodecyl gallate, butylated hydroxyl toluene (BHT) and butylated hydroxyl anisole (BHA), were successfully estimated using the polarographic method in arbitrary units per milligram based on cerium (IV) Reducing Antioxidant Capacity (CERAC). The principle underlining the new polarographic method allows it to effective determines the antioxidant capacity of other antioxidant molecules.

Bibliography

1. Shahidi F and Ambigaipalan P. "Phenolics and polyphenolics in foods, beverages and spices: Antioxidant activity and health effects–A review". *Journal of Functional Foods* 18 (2015): 820-897.
2. De Camargo AC., *et al*. "Fortification of cookies with peanut skins: effects on the composition, polyphenols, antioxidant properties, and sensory quality". *Journal of Agricultural and Food Chemistry* 62.46 (2014): 11228-11235.
3. Pearson D. "Rancidity". The Chemical Analysis of Foods, 7th Edition. Churchill Livingstone, London (1976): 494-495.
4. Disdaroglu M and Jaruga P. "Mechanism of Free Radical - Induced Damage to DNA". *Free Radical Research* 46.4 (2012): 382-419.
5. Hekimi S., *et al*. "Taking a Good Look at Free Radical in the Aging Process". *Trends in Cell Biology* 21.10 (2011): 569-576.
6. Droge W. "Free Radicals in the Physiological Control of Cell Function". *Physiological Review* 82.1 (2002): 47-95.
7. Amao Y., *et al*. "Optical oxygen detection based on luminescence change of metalloporphyrins immobilized in poly (isobutylmethacrylate-co-trifluoroethylmethacrylate) film". *Analytica Chimica Acta* 421.2 (2000): 167-174.
8. Ozcelik B., *et al*. "Effects of Light, Oxygen, and pH on the Absorbance of 2, 2-Diphenyl-1-picrylhydrazyl". *Journal of Food Science* 68.2 (2003): 487-490.
9. Ozyurt D., *et al*. "Determination of total antioxidant capacity by a new spectrophotometric method based on Ce (IV) reducing capacity measurement". *Talanta* 71.3 (2007): 1155-1165.
10. Ozyurt D., *et al*. "Determination of total antioxidant capacity by a new spectrofluorometric method based on Ce (IV) reduction: Ce (III) fluorescence probe for CERAC assay". *Journal of Fluorescence* 21.6 (2011): 2069-2076.
11. Apak R., *et al*. "Novel total antioxidant capacity index for dietary polyphenols and vitamins C and E, using their cupric ion reducing capability in the presence of neocuproine: CUPRAC method". *Journal of Agricultural and Food Chemistry* 52.26 (2004): 7970-7981.
12. Huang D., *et al*. "The chemistry behind antioxidant capacity assays". *Journal of Agricultural and Food Chemistry* 53.6 (2005): 1841-1856.
13. Erel O. "A novel automated direct measurement method for total antioxidant capacity using a new generation, more stable ABTS radical cation". *Clinical Biochemistry* 37.4 (2004): 277-285.

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