ANALYTICAL METHODS FOR DETERMINING THE ANTIOXIDANT ACTIVITY

METODE ANALITICE DE DETERMINARE A ACTIVITĂŢII ANTIOXIDANTE

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Abstract. *Importance of improving quality of human life in the idea of conservation of natural genetic fund a rational and safe food is a subject of concern the world of science. Alongside the problem of food as a source of health, may be located and the problem „succesful aging" concept that defines individuals ability to survive to old age in good physical shape, many factors of aging can be prevented or treated. There are numerous studies and research showing that intake of vegetables and fruits help to combat oxidative stress. Antioxidants thus the effects of free radicals. To determine the antioxidant capacity of horticultural products, researchers have used different methods. One of these methods is the ORAC method (Oxygen Radical Absorbance Capacity). The method consists of measuring antioxidant capacity to absorb free radical. Is the only test wich measure the time and inhibition degree of free radicals.*

Key words: ORAC method, antioxidant capacity

Rezumat. *Importanţa ameliorării calităţii vieţii umane în ideea conservării fondului său genetic natural printr-o alimentaţie raţională şi inofensivă constituie o temă care preocupă lumea ştiinţei. Alături de alimentaţie ca sursă de sănatate, poate fi menţionată şi abilitatea indivizilor de a supraveţui până la vârste înaintate într-o formă fizică bună, numeroşii factori ai îmbătrânirii putând fi preveniţi sau chiar trataţi. Există numeroase studii şi cercetări care demonstrează că aportul de legume şi fructe contribuie la combaterea stresului oxidativ. Antioxidanţii contraatacă efectele radicalilor liberi. Pentru determinarea capacităţii antioxidante a produselor horticole cercetătorii au folosit diferite metode. Una dintre aceste metode este metoda ORAC (capacitatea de absorbţie a radicalilor de oxigen). Metoda constă în măsurarea capacităţii antioxidanţilor de a absorbi radicalii liberi. Este singurul test în care se iau în considerare atît timpul cît şi gradul de inhibare a radicalilor liberi*.

Cuvinte cheie: metoda ORAC, capacitatea antioxidantă

INTRODUCTION

The development of many chronic and degenerative diseases, such as cancer, heart disease and neuronal degeneration such as Alzheimer's and Parkinson's disease has been theorized to be caused, in part, by oxidative stress. Oxidative stress has also been implicated in the process of aging. It is known that reactive oxygen species can damage biological molecules such as proteins, lipids, and DNA. While the human body has developed a number of systems to eliminate

free radicals from the body, it is not 100% efficient. (Takayuki Shibamoto şi colab., 2008)

Antioxidants are compounds that protect biological system against the harmful effects of process or reactions caused by excessive oxidation. Dr. Lester Packer, known as the "father of antioxidants", he wrote in "The antioxidant miracle" that the antioxidants have an incredible potential to prevent hundreds of diseases. (Lester Packer, 1999). Determination of antioxidant capacity of products is put in value through methods as:

- ORAC - oxygen radical absorbance capacity;
- TEAC - trolox equivalent antioxidant capacity;
- FRAP - ferric reducing ability plasma;
- DPPH - 1,1 diphenil- 2- picrilhidrazil;
- AEAC - ascorbic acid equivalent antioxidant capacity.

Antioxidant capacity may be expressed as: ORAC units (as μmol TE/100 g), total phenolic content (expressed as gallic acid equivalent - GAE mg%), anthocyanin content (mg%), Trolox equivalent (TEAC – Trolox equivalent antioxidant capacity, μmol TE/g), Fe (II), (FRAP - ferric reducing ability plasma) DPPH - 1,1 diphenil- 2- picrilhidrazil, ascorbic acid equivalent (AEAC - ascorbic acid equivalent antioxidant capacity, mg%). (www.sciencedirect.com)

High performance chromatography (HPLC) is a usual method used for the separation of antioxidants such as catechines.

The total content of polyphenols: blue formed between phenolic compounds and Folin – Ciocalteu reagent being indepndents of phenolic compounds structure, thereby building complex between the metal center and phenolic compounds. The absorption is recorded at a particular wavelength. Total phenol content is expressed as a gallic acid.

Method of Trolox equivalent antioxidant capacity (TEAC), is based on the ability of antioxidants to neutralizate the anion ABTS + radical. ABTS is oxidized by radicals and other peroxil oxidants to its ABTS cation radical, intense color (λmax = 734 nm). Antioxidant capacity is expressed as potential test compounds to bleach ABTS+ radical by direct reaction with it. Antioxidant capacity of tested compounds was expressed as Trolox equivalents.

MATERIAL AND METHOD

In preparing of this bibliographical synthesis it has been consulted a number of treaties, books, articles published or available on the Internet. The references are mentioned only partially, given that some information is repeated and that some sources treated in a very general way the studied problem.

Oxygen Radical Absorbance Capacity method determines the ability of inhibition of peroxil radical, inducing oxidation, highlighting the issue of classical radical, H atom transfer orac values were reported as Trolox equivalents, were expressed as μmol TE/DW. Fluorescence intensity, ex.485nm, em. 525 nm was monitored for 35 min.

RESULTS AND DISCUSSIONS

Healty human body synthesizes under conditions of oxidative stress, large amounts of endogenous antioxidant active substances (products of their own body). The main endogenous antioxidants are: uric acid, albumin, bilirubin, various enzymes (catalase, gluthatione peroxidase). The biosynthesis may decrease greatly in terms of psychological stress in some disease when immunity is low or if a long - term oxidative stress, as happens, for example smokers.

Exogenous antioxidants (those placed in body from outside sources) supplemented endogenous deficit, having a very positive action on the body. Several classes of compounds carries antioxidants, as can be seen in the teble below. (www.elsevier.com)

Table 1

Exogenous antioxidants substances

Groupe of substances	REPRESENTATIVES
Minerals	Selenium
	Copper
	Zinc
	Iron
Vitamins	Vitamin A (retinol)
	Vitamin C (L – ascorbic acid)
	Vitamin E (tocopherols)
	Vitamin P (rutina/bioflavone)
	PABA (para – aminobenzoic acid)
	Folic acid
Protide and its precursors	Betaine
	Histidine
Lipids and fatty acids	Conjugated linoleic acids (CLA)
Glycosides	Flavonoids
	Glucobrasiscin
Enzymes	Peroxidase
Terpenes	Intibina

The ORAC (Oxygen Radical Absorbance Capacity) assay is a relatively simple but sensitive method suitable for quantifying the antioxidant capacity of a number of products including whole fruits and vegetables, beverages such as fruit juices and wines, and supplement products. The ORAC assay is used primarily for water-soluble antioxidants. It can also be used to measure the antioxidant capacity of biological samples such as human plasma, blood serum or organ tissue. (Determining antioxidant capacity. Food Technology, 2008)

The chemical assay at the core of the ORAC method was developed by Guohau Cao at the Nutritional Science Department at the University of Connecticut. In 1994, Dr. Cao brought the method to the USDA Human Nutrition Research Center on Aging to work with Dr. Ronald Prior.

Most measurements of antioxidant activity use either the inhibition time at a fixed degree of inhibition or the extent of inhibition at a fixed time for a basis of

quantifying the results. The ORAC method is unique in that it measures both: the degree to which a sample inhibits the action of an oxidizing agent; and how long it takes to do so. These measurements are integrated into a single measurement called the ORAC Value.

Trolox, a non-commercial water-soluble derivative of tocopherol, is used as the control standard of antioxidant activity and the units of an ORAC value are expressed as micromoles Trolox equivalents per gram of a substance (mmole TE/g). Trolox it is used in other many methods for antioxidant activity determination.

The assay measures the oxidative degradation of the fluorescent molecule (either beta-phycoerythrin or fluorescein) after being mixed with free radical generators such as azo-initiator compounds. Azo-initiators are considered to produce the peroxyl radical by heating, which damages the fluorescent molecule, resulting in loss of fluorescence. Antioxidant is able to protect the fluorescent quantified using a fluoremeter. Fluorescein is currently used most as a fluorescent probe. Equipment that can automatically measure and calculate the capacity is commercially available (Biotek, Roche Diagnostic).

The fluorescent intensity decreases as the antioxidant degeneration proceeds, abd this intensity is typically recorded for 35 minutes after the addition of the azo-initiator (free radical generator). The degeneration (or decomposition) of fluorescein is measured as the presence of the antioxidant slows the fluorescence decay. Decay curves (fluorescence intensity vs. time) are recorded and the area between the two decay curves (with or without antioxidant) is calculated. Subsequently, the degree of antioxidant-mediated protection is quantified using the antioxidant trolox (a vitamin E analogue) as a standard. Different concentrations of trolox are used to make a standard curve, and the test sample are compared to this. Results for test samples (foods) have been published as “trolox equivalents”or TE.

Although the method is straightforward, it was time consuming to run many samples at once. In 1995, Dr. Cao and Dr. Prior automated this method in order to analyze large numbers of samples. The automation of the method resulted from adapting the chemical assay to work in a COBAS FARA II analyzer and linking the analyzer to a computer to store the data. Unfortunately, the COBAS FARA II analyzer is no longer produced and therefore, there are very few laboratories that run this method regularly. (ORAC: Oxygen radical absorbance capacity, 2002)

Today, the ORAC assay has become commonplace in research and in the marketing of antioxidant products, keeping these laboratories considerably busy. A small number of supplement companies have managed to procure this obsolete piece of equipment for measuring ORAC value of their products for quality control and research and development purposes.

In 2001, Dr. Prior and Brunswick Laboratories made improvements to the ORAC assay. The original ORAC assay used b-phycoerythrin (B-PE) as the fluorescent probe. However, B-PE is isolated from a natural source and the purity varied from lot to lot, which often caused poor reproducibility. In addition, B-PE

was found to interact with phenolic compounds, usually the very compounds believed to have the antioxidant activity in many samples, and therefore, the values reported were often understated. The improvements made to the assay eliminate the problems associated with using B-PE. By using fluorescein, a synthetic compound, the variability and the phenolic-interference problems were solved. However the newer method reports ORAC values 2-3 times higher than the original method.

ORAC method is considered most suitable due to its biological relevance in vivo antioxidant effectiveness.

Prof. Rui Hai Liu of Cornell University says that there is a food group with high antioxidant capacity, but growing conditions, variety, genetic origin, may affect the antioxidant capacity measured in vitro. (ORAC: Oxygen radical absorbance capacity, 2002)

Ronald L. Prior Research Chemist/ Nutritionist, USDA/ARS Arkansas Children's Nutrition Center, Little Rock, Ark., said that antioxidant phytochemicals in fruits and vegetables are effective in protecting against free-radical damage in vitro but that additional research is needed on factors affecting their absorption and metabolism.

Prior said that different radicals can give different test results, depending on their structure. ORAC uses the peroxyl radical, the most common free radical in the human body. A lot of methods give the same relative ranking of antioxidants, he said, but there's some variation depending on how the radicals react with components in food. (ORAC Antioxidant assay kit, 2008).

The measurement of the antioxidant capacity of products is a matter of growing interest because it nay provide a variety of information, such as resistance to oxidation, quantitative contribution of antioxidant substances, or the antioxidant activity that may be present inside the organism after ingestion.

Ana Zubuta *et al*. compared oxygen radical antioxidant capacity (ORAC) and trolox equivalent antioxidant capacity (TEAC) assays to estimate the total antioxidant capacity (TAC) of orange juice, milk, and an orange juice-milk beverage. When the TEAC method was used with this beverage, an increase in the concentration of orange juice corresponded to an increase in TAC, but increasing the percentage of milk did not increase the TAC value. When the ORAC method was applied, it was seen that increased concentrations of juice or milk corresponded to greater antioxidant capacity. An evaluation was also made of the influence of certain compounds (ascorbic acid, gallic acid, b-carotene, lutein, zeaxanthin and albumin) with antioxidant capacity that were present in the samples studied. (ORAC and Teac assays comparison to measure the antioxidant capacity of food products, Food Chemistry, 2009)

The antioxidant capacity of foods depends on many factors, including the colloidal properties of the substrates, the conditions and stages of oxidation, and the localization of antioxidants in different phases. Moreover, the measured antioxidant capacity of a sample depends on which technology and which free radical generator or oxidant is used in the measurement.

Consequently, comparison of different analytical methods for determining TAC is a key factor in helping investigators to choose a method and to understand the result obtained.

Although the TEAC method is simpler and cheaper than the ORAC method, it gives an underestimate of the antioxidant capacity of foods or beverages of a more complex nature.

Table 2

Advantages and disadvantages of the TEAC and ORAC methods

Methods	Advantages	Disadvantages
ORAC	-Uses biologically relevant free radicals -Integrates both degree and time of antioxidant reaction -Standardised: allows for data comparison across laboratories	-Normally requires use of expensive equipment -pH-sensitive -Requires long times to quantify results
TEAC	-Inexpensive and easy to use -Stable to pH, hence can be used to study pH effect on activity -Fast reaction	-Extra step to generate free radical from ABTS salt necessary -Generated free radical not stable for long periods of time

CONCLUSIONS

1. ORAC assay is the only test which measure the time and the inhibition degree of free radicals.

2. It is used to determine the hydrophilic and lipoliphific antioxidants.

3. The method is effective for in vitro and in vivo determinations.

4. Most researchers use this method when it comes to clinicals trials.

5. It is used to control the antioxidant content of various foods, cosmetics, and product with therapeutic potential.

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