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ANTIOXIDANT ACTIVITY SCREENING MODELS AND THEIR MECHANISM: A REVIEW

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ABSTRACT

An antioxidant is a material that at low concentrations delays or prevents oxidation of a substrate. Antioxidant compounds act throughout several chemical mechanisms: hydrogen atom transfer (HAT), single electron transfer (SET), and the potential to chelate transition metals. The significance of antioxidant mechanisms is to understand the biological meaning of antioxidants, their possible uses, their production by organic synthesis or biotechnological methods, or for the standardization of the determination of antioxidant activity. In general, antioxidant molecules can react either by multiple mechanisms or by a predominant mechanism. The chemical structure of the antioxidant material allows understanding of the antioxidant reaction mechanism. This chapter reviews the *in vitro* antioxidant reaction mechanisms of organic compounds polyphenols, carotenoids, and vitamins C against free radicals (FR) and pro oxidant compounds under diverse conditions, as well as the most commonly used methods to evaluate the antioxidant activity of these compounds according to the mechanism involved in the reaction with free radicals and the methods of *in vitro* antioxidant evaluation that are used commonly depending on the reaction mechanism of the antioxidant.

Keywords: antioxidants, oxidative stress, reactive oxygen species, free radical, hydrogen atom transfer, single electron transfer

INTRODUCTION

Oxidative stress in biological systems is a complex process that is characterized by an inequity between the production of free radicals (FR) and the ability of the body to eliminate these reactive species through the use of endogenous and exogenous antioxidants. During the metabolic processes, a great variety of reactions take place, where the promoters are the reactive oxygen species (ROS), such as hydrogen peroxide (H_2O_2) and the superoxide radical anion (O_2 •−), among others. A biological system in the presence of an excess of ROS can present different pathologies, from cardiovascular diseases to the endorsement of cancer. Biological systems have antioxidant mechanisms to control damage of enzymatic and non-enzymatic natures that allow ROS to be inactivated. The endogenous antioxidants are enzymes, such as superoxide dismutase

(SOD), catalase (CAT), glutathione peroxidase, or non-enzymatic compounds, such as bilirubin and albumin. When an organism is exposed to a high concentration of ROS, the endogenous antioxidant system is compromise and, consequently, it fails to guarantee complete protection of the organism. To recompense this deficit of antioxidants, the body can use exogenous antioxidants supplied through food, nutritional supplements, or pharmaceuticals. Among the most significant exogenous antioxidants are phenolic compounds carotenoids and vitamins C and some minerals such as selenium and zinc. In the study of antioxidant compounds and the mechanisms involved, it is significant to differentiate between the concepts of antioxidant activity and capacity. These terms are often used interchangeably. Though, antioxidant activity refers to the rate steady of a reaction between an antioxidant and an oxidant. The antioxidant capacity is a measure of the amount of a certain free radical captured by an antioxidant sample (MacDonald *et al.,* 2006). Therefore, throughout the selection of a method, the response parameter must be considered to evaluate the antioxidant properties of a sample, which may be a function of the concentration of the substrate or concentration and the time required to slow down a defined concentration of the ROS. The reaction mechanisms of the antioxidant compounds are closely related to the reactivity and chemical structure of FR as well as the environment in which these reactive species are found. Therefore, it is very important to describe the ROS and, to a lesser degree, the reactive nitrogen species (RNS), which include both precursors and free radicals. In the literature, there are many *in vitro* methods to evaluate the effectiveness of antioxidant compounds present in a variety of matrices (plant extracts, blood serum, etc.) using lipophilic, hydrophilic, and amphiphilic media (emulsions). The *in vitro* methods can be divided into two main groups: (1) hydrogen atom transfer (HAT) reactions and (2) transfer reactions of a single electron (SET). These methods are widely used because of their high speed and sensitivity. When carrying out a study related to the antioxidant properties of a sample, more than one method is usually used to evaluate the antioxidant capacity/activity (Salazar *et al.,* 2008). This chapter describes the methods of *in vitro* antioxidant evaluation that are used frequently depending on the reaction mechanism of the antioxidant.

Oxidative strain

Oxygen is associated with aerobic life conditions (Davis,

Table 1: Free radicals (FR) generated in biological systems

Species	Sources	Function
O_2	Enzymatic process, autoxidation reaction, and non-enzymatic electron transfer reactions	It can act as reducing agent of iron complexes such as cytochrome-c or oxidizing agent to oxidize ascorbic acid and α-tocopherol
HO_2	Protonation of O_2*−	HO_2* initiates fatty acid peroxidation
HO	H_2O_2 generates HO• through the metal-catalyzed Fenton reaction	HO* reacts with both organic and inorganic molecules including DNA, proteins, lipids, and carbohydrates
NO^*_2	Protonation of ONOO− or homolytic fragmentation of $ONOOCO^{2-}$	This radical acts on the antioxidative mechanism decreasing ascorbate and α-tocopherol in plasma
ONOO*	Reaction of O_2 with NO*	ONOO• is a strong oxidizing and nitrating species of methionine and tyrosine residues in proteins and oxidizes DNA to form nitroguanine
CO^{3-}	The intermediate of reaction superoxide dismutase (SOD)- Cu^{2+}-OH• react with bicarbonate to generates CO_3•−	CO^{3-} oxidizes biomolecules such as proteins and nucleic acids
$ONOOCO^{2-}$	The peroxynytrite-CO_2 adduct is obtained by reaction of ONOO− with CO_2	This anion promotes nitration of tyrosine fragments of the oxyhemoglobin via FR

1995), representing the driving force for the maintenance of cell metabolism and viability and at the same time involving a potential danger due to its paramagnetic characteristics. These characteristics promote the formation of partially oxidized intermediates with a high reactivity. These compounds are known as reactive oxygen species (ROS). ROS are free radicals (FR) or radical precursors. In stable neutral molecules, the electrons are paired in their respective molecular orbitals, known as maximum natural stability. Therefore, if there are unpaired electrons in an orbital, highly reactive, molecular species are generated that tend to trap an electron from any other molecule to compensate for its electron deficiency. The oxygen triplet is the main free radical, since it has two unpaired electrons. The reaction rate of triplet oxygen in biological systems is slow. However, it can become highly toxic because it metabolically transforms into one or more highly reactive intermediates that can react with cellular components. This metabolic activation is favored in biological systems, because the reduction of O_2 to H_2O in the electron transport chain occurs by the transfer of an electron to form FR or ROS (Valko *et al.,* 2007). Free radicals in a biological system can be produced by exogenous factors such as solar radiation, due to the presence of ultraviolet rays. Ultraviolet radiation causes the homolytic breakdown of bonds in molecules. FR also occur during the course of a disease. In a heart attack, for example, when the supply of oxygen and glucose to the heart muscle is suspended, many FR are produced. Another exogenous factor is chemical intoxication, which promotes the formation of FR. The organism, because it requires the conversion of toxic substances to less dangerous substances, promotes the release of FR. The toxicity of many drugs is actually due to their conversion into free radicals or their effect on the formation of FR. The presence of contaminants, additives, pesticides, etc., in food can also become a source of FR. Inflammatory processes are due to endogenous factors that promote the presence of FR in the system. These FR, present inside the cleansing cells of the immune system, have the function of killing pathogenic microorganisms. Tissue damage is caused when FR are excessive during this process. Phagocytic cells (neutrophils, monocytes, or macrophages) use the NADPH oxidase system directly generating the superoxide ion (O_2 •−). O_2 •− is considered the primary ROS and when reacting with other molecules through enzymatic processes or catalyzed by metals generates secondary ROS. O_2 •− is protonated to produce H_2O_2 and HO_2 •. O_2 •− is produced from the irradiation of molecular oxygen with UV rays, photolysis of water, and by exposure of O_2 to organic radicals formed in aerobic cells such as NAD•, FpH•, semiquinone radicals, cation radical pyridinium or by hemoproteins. Likewise, it is produced by phagocytic leukocytes as the initial product of the respiratory explosion when consuming O_2. The radical O_2 •− does not react directly with polypeptides, sugars, or nucleic acids. As a defense mechanism cells generate •NO by the action of nitric oxide-synthase on intracellular arginine. The combination of O_2 with •NO results in the formation of ONOO•, which induces lipid peroxidation in lipoproteins. This happens in a very marked way in autoimmune diseases such as rheumatoid arthritis, systemic lupus erythematosus, primary biliary cirrhosis, type 1 diabetes, celiac disease, Graves' disease, Hashimoto's disease, inflammatory bowel disease, scleroderma, multiple sclerosis, psoriasis, and vitiligo. FR is necessarily present during metabolic processes because many of the chemical reactions involved require these chemical species. For example, the reactions of polymerization of amino acids to form proteins or the reactions of polymerization of glucose to form glycogen involve the participation of FR. FR are also involved in the catalytic activation of various enzymes of intermediary metabolism, such as hypoxanthine, xanthine oxidase, aldehyde oxidase, monoamine oxidase, cyclooxygenase, and lipoxygenase (Fridovich, 1976). Generally, antioxidant enzymes efficiently control these radicals.

Table 2: Methods most commonly used to evaluate antioxidant capacity/activity *in vitro.*

Method	Reaction Mechanism	Characteristics	References
Total radical-trapping antioxidant parameter(TRAP)	HAT	TRAP assay involves the initiation of lipid peroxidation by generating water-soluble ROO• and is sensitive to all known chain-breaking antioxidants	(Prior *et al.,* 2005)
Total oxyradical scavenging capacity total assay (TOSCA)	HAT	Evaluates inhibition oxidation of α-keto-γ-methiolbutyric acid (KMBA) by ROS. The antioxidant activity is measured through ethylene concentration, generated during decomposition of KMBA, relative to a control reaction monitored by headspace gas chromatography (HS-GC)	(Marco, 1968)
Crocin-bleaching assays (CBAs)	HAT	CBA is based on the abstraction of hydrogen atoms and/or addition of radical to the polyene structure of crocin and results in a disruption of the conjugated system accounting for crocin bleaching	(Fukumoto & Mazza 2000)
Oxygen radical absorbance capacity (ORAC)	HAT	ORAC assay is based upon the inhibition of peroxyl radical induced oxidation initiated by thermal decomposition of azo compounds such as AAPH	(Wayner *et al.,* 1985)
Inhibition of 2,2-diphenyl-1-picrylhydracyl radical (DPPH•)	SET or HAT	Colorimetric method based on the measurement of the scavenging capacity of antioxidants towards DPPH•	(Bors *et al.,* 1984)
Inhibition of 2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS•+) cation radical	SET or HAT	Colorimetric method to evaluate the decay of ABTS•+in the presence of an antioxidant agent	(Cao *et al.,* 1997)
Total phenols assay by Folin-Ciocalteu reagent	SET	A mixture of phosphomolybdate and phosphotungstate in highly basic medium oxidized phenolic compounds	(Brand *et al.,* 1995)
Ferric-reducing antioxidant power (FRAP)	SET	Colorimetric method that evaluates the reduction of Fe3+-tripyridyltriazine complex (Fe3+-TPTZ) by turning it into a ferrous form (Fe2+-TPTZ)	(Re *et al.,* 1999)
Total antioxidant capacity (TAC)	SET	This method is used to measure the peroxide level during the initial stage of lipid oxidation. Peroxides are formed during the linoleic acid oxidation, which reacts with Fe2+ to form Fe3+ and later these ions form a complex with thiocyanate	(Re *et al.,* 1999)

Another generating source of ROS is the structural alteration of essential macromolecules of the cell (DNA, protein, and lipids) by irreversible chemical reactions. These reactions generate derivatives, such as malonaldehyde and hydroperoxides that propagate oxidative damage. Additionally, there are also RNS, such as nitric oxide (NO•), nitrogen dioxide (NO_2 •), as well as peroxynitrite (ONOO−), nitrosoperoxycarbonate ($ONOOCO_2$ −), and nitronium ions (NO_2 +), and the neutral species, peroxynitrous acid (ONOOH) and dinitrogen trioxide (N_2O_3). These species are generated in small amounts during normal cellular processes such as cell signaling, neurotransmission, muscle relaxation, peristalsis, platelet aggregation, blood pressure modulation, immune system control, phagocytosis, production of cellular energy, and regulation of cell growth. Table 1 shows the most representative FR present during the process of energy production in aerobic biological systems.

Oxidative injure to bio-molecules

There are many ROS that act as biological oxidants, but the O_2 *− is the largest oxidant; the simple addition of a proton leads to the formation of HO_2*, becoming a very active oxidizing agent. Free radicals produce diverse actions on the metabolism of immediate principles, which can be the

Table 3: Some medicinal plants with antioxidant properties

Plant name	Common name	Family	Part used	References
Zingiber officinale	Ginger	Zingiberaceae	Rhizome	(Yoshida *et al.,* 1990)
Momordica charantia	Bitter gourd	Cucurbitaceae	Whole Plant	(Ranelletti *et al.,* 1992)
Cymbopogon citrates	Lemon grass	Gramineae	Aerial part, leaves	(Scambia *et al.,* 1990)
Adiantum capillus veneris	Southern maidenhair fern	Adiantaceae	Whole plant	(Yoshida *et al.,* 1992)
Datura metel	Datura	Solanaceae	Leaves	(Teofili *et al.,* 1992)
Teucrium polium	Felty germander	Lamiaceae	Aerial part	(Sakakibara *et al.,* 2003)
Polyalthia cerasoides	Cherry ashok	Annonaceae	Stem bark	(Wayner *et al.,* 1985)
Crocus sativus	Saffron	Iridaceae	Sepals	(Yoshida *et al.,* 1990)
Curcuma longa	Turmeric	Zingiberaceae	Rhizome	(Ranelletti *et al.,* 1992)
Azadirachta indica	Neem	Meliaceae	Leaf	(Scambia *et al.,* 1990)
Ocimum sanctum	Tulsi	Lamiaceae	Leaf	(Yoshida *et al.,* 1992)
Terminalia bellerica	Behda	Combretaceae	Fruit	(Teofili *et al.,* 1992)
Solanum tuberosum	Potato	Solanaceae	Tuber	(Sakakibara *et al.,* 2003)
Foeniculum vulgare	Saunf	Apiaceae	Seed oil	(Wayner *et al.,* 1985)
Cuscuta reflexa	Akashabela	Convolvulaceae	Stem	(Yoshida *et al.,* 1990)
Salvia officinalis	Common sage	Lamiaceae	Root	(Ranelletti *et al.,* 1992)
Litsea glutinosa	Soft bollygum	Lauraceae	Stem bark	(Scambia *et al.,* 1990)
Murraya koenigii	Curry tree	Rutaceae	Leaves	(Yoshida *et al.,* 1992)
Cinnamomum tamala	Tejpat	Lauraceae	Leave oil	(Teofili *et al.,* 1992)
Allium sativum	Garlic	Amaryllidaceae	Bulb	(Sakakibara *et al.,* 2003)
Allium cepa	Onion	Amaryllidaceae	Bulb	(Wayner *et al.,* 1985)
Costus pictus	Spiral ginger	Costaceae	Leaves	(Yoshida *et al.,* 1990)
Bacopa monnieri	Brahmi	Scrophulariaceae	Leaves	(Yoshida *et al.,* 1990)
Plantago asiatica	Chinese plantain	Plantaginaceae	Seed	(Ranelletti *et al.,* 1992)
Arnebia benthamii	Gaozaban	Boraginaceae	Whole plant	(Scambia *et al.,* 1990)
Aloe vera	Cactus/Indian aloe	Asphodelaceae	Leaves	(Yoshida *et al.,* 1992)
Daucus carota	Carrot	Apiaceae	Root	(Teofili *et al.,* 1992)
Mentha Pulegium	Pennyroyal	Lamiaceae	Leaves	(Sakakibara *et al.,* 2003)
Cotinus coggygria	Smoke tree	Anacardiaceae	Leaves	(Wayner *et al.,* 1985)
Tamus communis	Black Bryony	Dioscoreaceae	Root	(Yoshida *et al.,* 1990)
Aegle marmelos	Bengal quince	Rutaceae	Fruit	(Wayner *et al.,* 1985)

origin of cell damage (Limon *et al.,* 2009):

1. In the polyunsaturated lipids of membranes, producing loss of fluidity and cell lysis because of lipid peroxidation.
2. In the glycosides, altering cellular functions such as those associated with the activity of interleukins and the formation of prostaglandins, hormones, and neurotransmitters (Nakamura *et al.,* 1997).
3. In proteins, producing inactivation and denaturation (Nimse and Pal 2015).
4. In nucleic acids, by modifying bases, producing mutagenesis and carcinogenesis.

Physiological and physio-pathological processes connected to free radicals (FR)

The human body responds to oxidative stress with antioxidant defense, but in certain cases, it may be insufficient, triggering different physiological and physio pathological processes. Currently, many processes are identified related to the production of free radicals. Among them are mutagenesis, cell transformation, cancer, arteriosclerosis, myocardial infarction, diabetes, inflammatory diseases, central nervous system disorders, and cell aging (Dean *et al.,* 1997; Rice, 1995).

Function of antioxidants

Biological systems in oxygenated environments have developed defense mechanisms, both physiological and biochemical. Among them, at the physiological level, is a microvascular system with the function of maintaining the levels of O_2 in the tissues, and at a biochemical level, the antioxidant defense can be enzymatic or non-enzymatic, as well as being a system for repairing molecules.

Primary enzymatic system

Aerobic organisms have developed antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and DT-diaphorase. SOD is responsible for the dismutation reaction of O_2 to H_2O_2, which in subsequent reactions, catalyzed by catalase or by GPx, is converted into H_2O and O_2. SOD is the most important and most powerful detoxification enzyme in the cell. SOD is a metallo enzyme and, therefore, requires a metal as a cofactor for its activity. Depending on the type of metal ion required as a cofactor by SOD, there are several forms of the enzyme (Halliwell, 1996; Fridovich 1995). CAT uses iron or manganese as a cofactor and catalyzes the degradation or reduction of hydrogen peroxide (H2O2) to produce water and molecular oxygen, thus completing the detoxification process initiated by SOD (Dringen *et al.,* 2005). CAT is highly efficient at breaking down millions of H_2O_2 molecules in a second. CAT is mainly found in peroxisomes, and its main function is to eliminate the H_2O_2 generated during the oxidation of fatty acids. GPx is an important intracellular enzyme that breaks down H_2O_2 in water and lipid peroxides in their corresponding alcohols; this happens mainly in the mitochondria and sometimes in the cytosol (Marklund, 1984). The activity of GPx depends on selenium. In humans, there are at least eight enzymes GPx, GPx1–GPx8 (Goth *et al.,* 2004). Among glutathione peroxidases, GPx1 is the most abundant selenoperoxidase and is present in virtually all cells. The enzyme plays an important role in inhibiting the process of lipid peroxidation and, therefore, protects cells from oxidative stress (Moron *et al.,* 2012). Low GPx activity leads to oxidative damage of the functional proteins and the fatty acids of the cell membrane. GPx, particularly GPx1, has been implicated in the development and prevention of many diseases, such as cancer and cardiovascular diseases (Gill & Tuteja 2010). DT-diaphorase catalyzes the reduction of quinone to quinol and participates in the reduction of drugs of quinone structure (Rayman, 2005). DNA regulates the production of these enzymes in cells.

Non-enzymatic system

This system of antioxidants consists of antioxidants that trap FR. They capture FR to avoid the radical initiation reaction. Neutralize the radicals or capture them by donating electrons, and during this process, the antioxidants become free radicals, but they are less reactive than the initial FR. FR from antioxidants are easily neutralized by other antioxidants in this group. The cells use a series of antioxidant compounds or free radical scavengers such as vitamin E, vitamin C, carotenes, ferritin, ceruloplasmin, selenium, reduced glutathione (GSH), manganese, ubiquinone, zinc, flavonoids, coenzyme Q, melatonin, bilirubin, taurine, and cysteine. The flavonoids that are extracted from certain foods interact directly with the reactive species to produce stable complexes or complexes with less reactivity, while in other foods, the flavonoids

perform the function of co-substrate in the catalytic action of some enzymes.

Repair scheme

Enzymes that repair or eliminate the biomolecules that have been damaged by ROS, such as lipids, proteins, and DNA, constitute the repair systems. Common examples include systems of DNA repair enzymes (polymerases, glycosylases, and nucleases) and proteolytic enzymes (proteinases, proteases, and peptidases) found in both the cytosol and the mitochondria of mammalian cells. Specific examples of these enzymes are GPx, glutathione reductase (GR), and methionine sulfoxide reductase (MSR). These enzymes act as intermediaries in the repair process of the oxidative damage caused by the attack of excess ROS. Any environmental factor that inhibits or modifies a regular biological activity becomes a condition that favors the appearance or reinforcement of oxidative stress.

Uniqueness of antioxidants

The main characteristic of a compound or antioxidant system is the prevention or detection of a chain of oxidative propagation, by stabilizing the generated radical, thus helping to reduce oxidative damage in the human body (Chen *et al.,* 2000). Gordon (Gordon, 1990) provided a classification of antioxidants, mentioning that characteristic. There are two main types of antioxidants, the primary (breaking the chain reaction, free radical scavengers) and the secondary or preventive. The secondary antioxidant mechanisms may include the deactivation of metals, inhibition of lipid hydroperoxides by interrupting the production of undesirable volatiles, the regeneration of primary antioxidants, and the elimination of singlet oxygen. Therefore, antioxidants can be defined as "those substances that, in low quantities, act by preventing or greatly retarding the oxidation of easily oxidizable materials such as fats" (Chipault, 1962).

Mechanisms of action of antioxidants

A compound that reduces *in vitro* radicals does not necessarily behave as an antioxidant in an *in vivo* system. This is because FR diffuses and spread easily. Some have extremely short life spans, on the order of nanoseconds, so it is difficult for the antioxidant to be present at the time and place where oxidative damage is being generated. Additionally, the reactions between antioxidants and FR are second order reactions. Therefore, they not only depend on the concentration of antioxidants and free radicals but are also dependent on factors related to the chemical structure of reagents, the medium and the reaction conditions.

Phenolic compounds

The phenolic compounds constitute a wide group of chemical substances, with diverse chemical structures and different biological activities, encompassing more than 8000 different compounds which are a significant part of the human and animal diet (Martinez *et al.,* 2000). The phenolic compounds are important components in the mechanism of signaling and defense of plants. These compounds combat the stress brought about by pathogenic organisms and predators. The function of these compounds in plants is diverse: they are found as precursors of compounds of greater complexity or the intervention in the processes

of regulation and control of plant growth, as well as the defensive medium of plants. Phenolic compounds have the capacity to act as hydrogen donors or to chelate metal ions such as iron and copper, by inhibiting the oxidation of low-density lipoproteins (LDL). These characteristics in the phenolic compounds are associated with a decrease in risks of neurodegenerative diseases, such as cardiovascular diseases (paran *et al.,* 2009), gastrointestinal cancers (Yoshida *et al.,* 1990), colon (Ranelletti *et al.,* 1992), breast and ovarian cancers (Scambia *et al.,* 1990), and leukemia (Yoshida *et al.,* 1992; Teofili *et al.,* 1992; Sakakibara *et al.,* 2003). Phenolic compounds also have vasorelaxation and anti-allergenic activity. The phenolic compounds inhibit the oxidation of *in vitro* LDL. Phenolic compounds reduce or inhibit free radicals by transfer of a hydrogen atom, from its hydroxyl group. The reaction mechanism of a phenolic compound with a peroxyl radical (ROO•) involves a concerted transfer of the hydrogen cation from the phenol to the radical, forming a transition state of an H-O bond with one electron. The antioxidant capacity of the phenolic compounds is strongly reduced when the reaction medium consists of a solvent prone to the formation of hydrogen bonds with the phenolic compounds. For example, alcohols have a double effect on the reaction rate between the phenol and the peroxyl radical. On the one hand, the alcohols act as acceptors of hydrogen bonds. On the other hand, they favor the ionization of the phenols to anion phenoxides, which can react rapidly with the peroxyl radicals, through an electron transfer. The overall effect of the solvent on the antioxidant activity of the phenolic compounds depends to a great extent on the degree of ionization of the last compounds (Riemersma *et al.,* 2001; Foti, 2007). Leopoldini *et al.,* (Leopoldini *et al.,* 2004) conducted a theoretical study to determine the dissociation energy of OH bonds and the adiabatic ionization potentials of phenolic compounds of varied structure and polarity, among them tyrosol, hydroxytyrosol, and gallic and caffeic acids. These studies were performed simulating solvated and vacuum conditions. The results showed a clear difference in the behavior of these phenolic compounds. The compounds most likely to undergo a HAT were tocopherol, followed by hydroxytyrosol, gallic acid, caffeic acid, and epicatechin, while the phenolic compounds, which were better able to SET, were kaempferol and resveratrol. This undoubtedly gives us an indication that phenolic compounds can suffer both HAT and SET and that this depends mainly on the chemical structure of the phenolic compounds. The method based on the Folin-Ciocalteu reagent is commonly used to determine and quantify total phenols. This method evaluates the ability of phenols to react with oxidizing agents. The Folin-Ciocalteu reagent contains sodium molybdate and tungstate, which react with any type of phenol (Peterson, 1979). The transfer of electrons at basic pH reduces the sodium molybdate and tungstate in oxides of tungsten (W8O23) and molybdenum (Mo8O23), which have a bright blue color in solution. This color intensity is proportional to the number of hydroxyl groups of the molecule (Julkunen, 1985).

Carotenoids

Carotenoids are found in virtually all plants, animals, and microorganisms, and more than 700 carotenoids have been identified and characterized (Britton *et al.,* 2004). Most carotenoids have a characteristic symmetrical tetraterpene skeleton. The linear hydrocarbon skeleton is made up of 40 carbons and is susceptible to various structural modifications. These structural characteristics are related to degree of hydrogenation, *cis-trans* isomerization, presence of cycles at one or both ends of the linear skeleton, or the addition of side groups (which often contain oxygen) with their subsequent glycosylation. The most complex changes are related to the shortening or elongation of the resulting tetraterpene skeleton, to form carotenoids with chains of 50 carbons. It is also possible to find carotenoids with tetraterpene skeletons of 30 carbons, from the condensation of two units of farnesyl (Landrum, 2010). These compounds, in addition to conferring pigmentation on biological systems, fulfill other important functions. The most recent studies of these compounds are focused mainly on evaluating their function as antioxidants. The structural base fragment of the carotenoids is a conjugated polyunsaturated chain. This fragment is primarily responsible for the ability of these compounds to inhibit free radicals. Variations in the polyunsaturated chain from one carotenoid to another, together with the presence of hydroxyl groups, substantially modify the reactivity of the carotenoids. The reactivity of these compounds is also affected by the environmental conditions where they are found. For example, Edge and Truscott (Edge & Truscott 2018) found that carotenoids switch the antioxidant behavior to the prooxidant as a function of oxygen concentration. The study used a system that emulates a cell, to observe the protection effect induced by lycopene when exposing the system to high-energy radiation. Total protection is achieved in the absence of O_2, but in the presence of 100 % O_2, protection is completely lost. Carotenoids are characterized as excellent peroxyl radical scavengers. The polyunsaturated chains that make up the base structure of carotenoids give these compounds a lipophilic character. Carotenoids play an important role in the protection of cell membranes and lipoproteins against peroxyl radicals.

The carotenoids react as antioxidant agents through three mechanisms: the first is the SET, the second from the formation of one adduct, and the third by HAT. In general, the antioxidant properties of carotenoids are related to their high capacity for electron donation. Everett *et al.,* (Everett *et al.,* 1995) found that β-carotene reacts with NO_2• via SET. Carotenoid reactivity studies have also been carried out in the presence of the benzyl peroxyl radical, which has low reactivity, and it was concluded that in this case, the reaction mechanisms involved the formation of an adduct, while reactions by HAT are of little relevance (Mortensen, 2002).

Other studies have evaluated the effect of the chemical structure of carotenoids on the reactivity toward FR.

One of these studies found that carotenoids substituted with electrons are more susceptible to oxidation than carotenoids with withdrawn electron groups. A study of carotenoid reactivity with phenoxy radicals shows the order of reactivity to be lycopene > β-carotene > zeaxanthin > lutein > echinenone > astaxanthin (Mortensen & Skibsted 1997). The effect of the solvent on the reactivity of carotenoids in the presence of FR has also been evaluated, and it was found that in nonpolar solvents, the reactions are promoted via adduct formation; while in polar solvents, the formation of adducts takes place first and then the SET (El Agamey & Mcgarvey 2003).

Vitamin C

Vitamin C refers to a group of ascorbic acid analogs that can be both synthetic and natural molecules. Ascorbic acid is a water-soluble ketolactone with two ionizable hydroxyl groups. Anion ascorbate is the dominant form at physiological pH. Ascorbate is a potent reducing agent and undergoes two following losses of an electron, to form an ascorbate radical and dehydroascorbic acid. The ascorbate radical is comparatively stable because the unpaired electron is delocalized by resonance. The ascorbate concentration in plasma of healthy humans is around 10 μg/mL. At these concentrations, the ascorbate is a co-antioxidant with vitamin E to protect LDL from peroxyl radicals (Frei *et al.,* 1989). The ascorbate radical is poorly reactive and can be reduced to ascorbate by reductase-dependent NADH and NADPH (Linster & Van 2007). The ascorbate radical can alternatively undergo a disproportionation reaction that depends on pH, resulting in the formation of ascorbate and dehydroascorbic acid (Corti *et al.,* 2010). Vitamin C is chemically competent of reacting with most of the physiologically important ROS and acts as a hydrosoluble antioxidant. The antioxidant reaction mechanisms of vitamin C are based on the HAT to peroxyl radicals, the in activation of singlet oxygen, and the elimination of molecular oxygen (Abraham, 2014; Lee *et al.,* 2004). For example, ascorbic acid can donate a hydrogen atom to a tocopheroxyl radical at the rate of 2 × 105 mol/s (Buettner & Jurkiewicz 1996). Also, it has been proven that ascorbate can produce reactions with oxidizing agents through SET (Williams & Yandell 1985) or a concerted transfer of electron/protons (SET/HAT) (Aruoma, 1996).

Methods to evaluate antioxidant activity

The antioxidant activity of a compound can be evaluated *in vitro* or *in vivo* by means of simple experiments, and at the same time, the probable pro oxidant effect on different molecules can be evaluated. Antioxidant activity cannot be measured directly but is determined by the effects of the antioxidant to control the degree of oxidation. There are a variety of methods to evaluate antioxidant activity. Some methods involve a different oxidation step followed by the measurement of the response, which depends on the method used to evaluate the activity. When the antioxidant activity of a sample is studied, it is necessary to consider the source of ROS as well as the target substrate. An antioxidant can protect lipids against oxidative damage, while, on the other hand, it can promote the oxidation of other biological molecules (Huang *et al.,* 2005). Most assays of antioxidant activity involve inducing accelerated oxidation in the presence of a promoter and controlling one or more variables in the test system, for example, temperature, antioxidant concentration, pH, etc. However, the oxidation mechanisms can change when modifications are carried out on some of these variables. Therefore, it is important to evaluate the intervals in which the quantification of the antioxidant activity is done to generate reliable results.

The methods to determine the antioxidant capacity are divided into two general groups. This division is based on the reaction mechanisms involved in the RF reduction process. The first group of methods is based on the SET and the second group is based on the HAT. The result is the same: the inactivation of free radicals; however, the kinetics and secondary reactions involved in the process are different. The methods based on SET detect the capacity of a potential antioxidant for the transmission of a chemical species, including metals, carbonyls, and radicals. SET is shown through a change in color as the oxidant is reduced by antioxidant. The group of methods based on HAT measures the ability of an antioxidant to inactivate FR through the donation of a hydrogen atom. HAT reactions are theoretically independent of solvent nature and pH. These reactions are rapid and occur in no more than a few minutes. The presence of other reducing agents in samples, in addition to the antioxidants under study, makes HAT testing difficult and can lead to significant errors. Table 2 shows the methods of evaluation of the antioxidant activity *in vitro*. Here discuss some common plants having potent antioxidant activity (Table 3).

CONCLUSIONS

The reaction mechanisms involved in the antioxidant activity/capacity of different groups of compounds depend on several factors. Amongst these factors are the chemical structure of these compounds, the nature of the solvent, the temperature and pH, as well as the reactivity and chemical structure of free radicals. All these factors can also influence the reaction rate. Consequently, it is very important that, for studies of antioxidant properties, at least three evaluation methods are selected: one to exclusively evaluate the HAT, another SET, and a combined method, HAT/SET. Also, it is significant to perform reaction kinetics. In addition to this, it is necessary to believe that in mixtures of antioxidant compounds, possible synergistic effects are present and can enhance the activity/capacity or even modify their reaction mechanisms.

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