

The Impact of Physical Processes and Chemicals of the Antioxidants (Bioactivity Compounds)

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Executive Summary

Nature has selected and included, in an evolutionary manner, in the design of living bodies, reactions generating free radicals with complex roles: functional, inter-cellular communicational or destructive, etc. The focus of this review is highlighting the effects of physical and chemicals processes on different antioxidants. These changes affect the structures, properties and qualities on activity compounds. The repercussions of these transformations on antioxidants functionality are discussed.

Keywords: Free radicals; Antioxidant activity; Physical and chemicals processes

Background

Free radicals (pro-oxidants) are compounds belonging to the radical groups; they are active biochemically and biologically, and they destroy cell membranes, nuclei, and cytoplasm, producing and maintaining an intense oxidative stress. This is a consequence of the presence of one or several free electrons on the last layer of an atom in the molecule. Free radicals are oxygenated electron-deficit anions that do not make up salts, acids, or bases, but keep this reactive (free) form. The effect of an antioxidant is determined by its availability in a "compartment" to combine with receptors on which it acts to produce a specific effect. The availability of an antioxidant is influenced by transport of molecules through biological membranes, by degree of bonding between plasmatic proteins and tissues, by blood flow at level of action site, and by metabolising and elimination of antioxidant [1]. To produce the desired effect, an antioxidant needs to cross cell membranes, i.e. two phospholipid layers. Glycoproteins, lipoproteins, as well as different ion or polar groups on surface of membranes make up membranes. As a result of the interaction of different endogenous or exogenous substances with the receptors on the biological membranes, they can undergo alterations through alterations of spatial orientation of resulting compounds and, consequently, channels/pores measuring up to 8 Å at the level of cell membrane and up to 60–80 Å at the level of capillaries, open up. Besides the opening of ion channels, the effect of interaction mediator-receptor can consist in mobilisation of enzymes mobilising secondary messengers. The potential necessary for transfer through the membranes depends on different factors (Figure 1).

The crossing of the biological membranes by the antioxidant substances depend on:

Factors depending on biological membranes, i.e.: lipid content; existence of specialised transport systems; polarisation of the membrane; presence of pores; membrane physic-pathological state.

Factors depending on the antioxidant substance: molecular mass; chemical structure; ionisation constant (pKa); rate; lipo/hydro solubility of antioxidant substance, etc.

Factors depending on the environment on the surface of the biological membranes: pH; protein bonding; vascularisation; blood flow, etc.

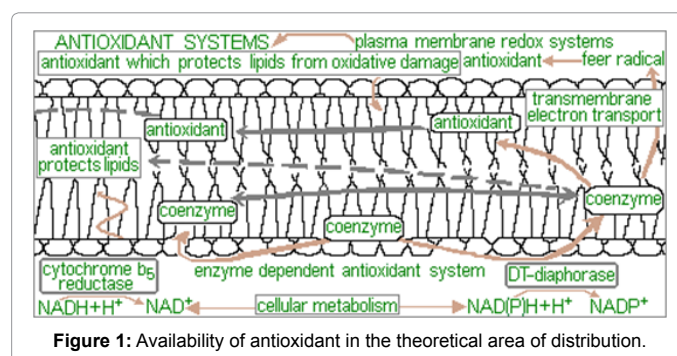


Figure 1: Availability of antioxidant in the theoretical area of distribution.

Antioxidant substances can cross biological membranes in two ways: passive transfer and specialised transfer [2]. The physiological role of antioxidants is to prevent the destruction of cell components that occur as a result of chemical reactions involving free radicals. Antioxidant activity depends on that part of molecule with important electron donor properties.

The Impact of Physical Processes

Until recently, antioxidants were considered instruments in fight against oxidation; now that antioxidants are known to maintain and improve health, we need to protect antioxidant nutritional value [3]. High temperatures trigger quick chemical processes. When temperature rises with 10°C, there is doubling or tripling of the chemical reaction speed. Thus, when temperature increases with 1°C,

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reaction speed increases with 10%. Even lower temperature increases result in a shortening of reaction speed.

Structural integrity

Structural integrity plays a role in prevention of contact between antioxidants and O_2 thus destroying oxidative potential. Antioxidant is encapsulated in their own protective liposomes or in cell membrane structures and, cannot get into contact with O_2 . Intact oil seeds are stable but, once crushed, extracted, warmed in process of refining, oil resistance to oxidation decreases. There is a negative nutrition consequence—the loss of structural integrity, but there is also a positive nutrition consequence—antioxidants become more bioactive [4]. Gastrointestinal absorption of carotenoids from vegetables is inversely proportional with the size of panicle. The treatment procedure of lycopene at high temperature improves absorption; thus, the amount of lycopene in plasma increases. Ascorbic acid from broccoli becomes more bioactive if boiled than if consumed raw.

Humidity

Humidity should be carefully controlled. Antioxidant content should be optimised since the diagram of oxidation is U-shaped: it represents the quick rates of oxidation when humidity content is low and when it becomes too high. Most dehydration processes destroy lipooxidases, and when water content decreases below the level allowing the formation of a protective water layer on product surface, self-oxidation potential increases [5]. The high level of water acts as a solvent that mobilises catalysts and reactants towards oxidation. Water can interact chemically through hydrogen bonds with other molecule species.

Thermal treatment

Thermal treatment plays an important role due to numerous benefits it plays in preserving foods. It has both positive and negative effects on antioxidants. Positive effects include inactivation of oxidase and breaking of food structures that lead to improved bioactivity. Thermal treatment has an important negative impact on antioxidants in foods. Carotenoids and antocyanins are sensitive to warming; components degrade gradually with time and temperature. Water soluble antioxidants (ascorbic acid) are sensitive to temperature [6]. Another effect of warming is the increase of carotenoid isomerisation in foods. The relevance of this fact is that *cis* isomers from β -carotene are less absorbed by gastro-intestinal tract and less carried in body (*cis* and *trans* isomers are not biological equivalents).

Storage temperature

Antioxidant losses in fresh fruits and vegetables during storage are significant. At room temperature, 90% of the vitamin C in spinach leaves is lost in three days. At fridge temperature, this loss is about 50%. Freezing can stop and decrease vitamin C losses in peas, broccoli, green beans, and spinach for a period of over three months [7]. Temperature fluctuations have a negative impact on balance between air humidity and products humidity resulting in drying or moisturizing of products. According to the Vant'Hoff Law (dependence of chemical reactions on temperature), metabolic activity products decreases at low temperatures to reduce the intensity of biochemical reactions [8,9]. Air temperature oscillations are influenced by environmental air temperature oscillations which are determined by a complex of physical processes.

Oxygen reduction

There are processes that allow the removal or reduction of O_2 in

foods. This is very important because, for instance, L-ascorbic acid degradation rate in orange juice and canned green peas depends on amount of O_2 in sample. Fruit juices that contain air entrapped are subjected to a vacuum removal that minimise potential of destructive changes on ascorbic acid and on other oxidable components due to the due to molecules to oxygen (O_2) [10,11]. A new approach is use of some enzymes: glucose oxidase (EC 1.1.3.4), thiol oxidase (EC 1.8.3.2), galactose oxidase (EC 1.1.3.9), pyranose oxidase (EC 1.1.3.10) and hexose oxidase (EC 1.1.3.5) related to (EC 1.15.1.1), glutathione transferase (EC 2.5.1.18), catalase (EC 1.11.1.6) and glutathione peroxidase (EC 1.11.1.9) that act by removing O_2 and ROS or by decreasing hydroperoxide lipids [12]. Oxygen lies at basis of many changes as a result of oxidation, which it favours (in presence of light and temperature): it oxidised fats, phenols (colour alterations), liposoluble vitamins (loss of vitamin values), resins, balms, aldehydes, ketones, etc.; it favours polymerisation and resinification of essential oil; it favours rancidisation of vegetal fats; it favours inactivation of antiradical substances acting on active substance.

Protection against light

At wave lengths below 500 nm, light generates lipid oxidation and vitamin destruction. Some components behave like photosynthesizers: riboflavin, chlorophyll, myoglobin, haemoglobin pigment, as well as some double-bond systems [13]. Solar rays, particularly those in UV range, produce physical, chemical, etc., alterations. They alter structure and physic-mechanic properties, thus having a negative impact. Under impact of radiations, methionine from protein, together with vitamin B_2 , is turned into methylmercaptpropionic aldehyde. A lower or higher permeability to radiations—depending on colour of wrapping, determines destruction of vitamins B_2 and C.

Irradiation

The effects of irradiation vary depending on antioxidant compounds subjected to irradiation and on strength and length of irradiation time. Irradiation of bell-peppers showed that there is no alteration in content of vitamin C at rates up to 300 Gy. Storage post-irradiation results in increases of amount of vitamin C in fruits and vegetables. At 3 kGy (maximum amount recommended by the Food and Drug Administration, there was a loss of 15% β -tocopherols and 30% α -tocopherols [14]. At 3 gGy and 2°C, there is a decrease of 6% in α -tocopherols and no alteration in β -tocopherols.

Impact of Chemical Processes

Chemical alterations occur as a result of the action of singular or conjugated factors such as temperature, catalysts, or activation energies [15]. They occur in products as components/substances with properties different from those of the initial products.

Chemical alterations (reactions)

Light radiation trigger photochemical reactions that lead to destruction of some valuable elements in products. The effects of photochemical reactions depend on radiation intensity, as well as lighting time. The most frequent destructions consist in a decrease of content or even in total reduction of vitamin content, in fat rancidness, in decolouring, and in product ageing [16].

Photochemical reactions occur as photolysis and photo-oxidation. Photolysis is photochemical decomposition produced by action of radiations in visible or ultra-violet spectrum. Reaction speed is high in photolytic process. Photo-oxidation takes place in a longer time because, since in photo-oxidative reactions, radiation energy is not

enough for chemical alterations, it must be completed with energy resulted from outer oxidation processes [17]. There are a series of damages, among which corrosion, in which temperature and radiations come after other factors (dry gases) in the triggering and amplification of the damages they produce. Oxygen molecule is the enemy of antioxidants: protecting foods against the interaction with O₂ is the principle on which rely protective antioxidative technologies.

Enzymes

Chemico-biological reactions occurring in body are catalysed by enzymes. In living bodies, chemical reactions cannot occur without enzymes. Another characteristic of enzymes is fact that they do not change during reaction. Enzymes are the best known substances of antioxidant protection system (catalase, glutathione peroxidase, superoxide dismutase) and they achieve the alteration of free radicals. Enzymes are important components of the protection/defence mechanisms. Enzymes eliminate toxic substances from body together with biomolecules, mainly hereditary genetic material and so-called regulation-repair enzymes that repair damage of DNA structure.

The existence of living organisms is conditioned by the activity of some resistance and immunity mechanisms capable of protecting their chemical individuality through recognition and differentiation mechanisms of self-substances from non-self-substances [18,19].

These mechanisms are tolerant to self-molecules, but they activate and react more or less rigorously to remove, neutralise or destroy non self-substances. Controlling polyphenol oxidation has long been a priority in food science because oxidation phenols are responsible for unwanted brown colour of cut fruits and vegetables and for development of improper colours and flavours in frozen foods. Inactivating these enzymes can be done by a quick treatment of warming (boiling) with preservation of freshness in foods. Quick inactivation of oxidase after apple pressing is a must in establishing apple juice stability [20]. Extrusion, due to the conditions of temperature and pressure, destroy oxidase and favour the bonding of lipids with proteins and carbohydrates in foods, which protects lipid oxidation. Fruit juices that contain a large amount of pulp are more stable than juices with smaller amounts of pulp, due to content of polyphenols and glycoside [21,22].

In the preparation of apple or strawberry juices with less pulp, they often use pectinases. These enzymes hydrolyse the lateral glycosidic chains of flavonoids that become harder to oxidated. Glycosidic chains of flavonoids and isoflavonoids are not completely absorbed by the small intestine, unlike aglycons. The nutritional impact of using pectinases is difficult to quantify: on one hand, polyphenols become more active and, thus, influence health; on the other hand, there are less polyphenols in the products, which can be easily oxidized since they are less stable.

Supplementing

For technological reasons, antioxidant nutrients can be added supplementary to foods. Ascorbic acid is used more to increase food resistance to oxidation. Cut fruits and vegetables are saturated in ascorbic acid which is oxidized, thus preventing oxidation of catechin-tannins, and inhibit browning enzymatic reactions. In hydrophobic foods, we use vitamin E is used to protect antioxidants. Since vitamin C "recycles" vitamin E, it is useful to use simultaneously these antioxidants [23,24]. Despite all this, since vitamin C is hydrophilic antioxidants and vitamin E is lipophilic antioxidants lipophilic antioxidants, it is difficult to use them simultaneously in food systems. A solution could be to use ascorbyl-6-palmitate (AP), hydrophobic

form of vitamin C which proved to be useful in lipid food systems [25]. The mixture of vitamin E, vitamin C, and phospholipids is an efficient mixture since phospholipids act as emulsifiers allowing contact of two vitamins; phospholipids themselves participate actively in process of anti-oxidation. Both vitamins can be incorporated in liposomes and, thus, they can be used as a feeding system in "oil-in-water" emulsions. Non-nutrient antioxidants such as sulphur dioxide can be used to protect those antioxidants that are nutritionally beneficial [26,27]. Sulphur dioxide prevents carotenoid oxidation but it causes the bleaching of antocyanins.

Supplementary antioxidants from natural sources such as plants and condiments are efficient in protection of foods against oxidation. Plant extracts that have antioxidative properties is: rosemary, sage, thyme, oregano, ginger, cloves, laurel etc. [28] are active in decrease of oxidant impact. Antioxidant mixtures can be obtained from natural sources mixing with oil and pressing, or using extracts in CO₂ from antioxidants. This latter technique is of interest since solvent involved is not toxic; it is flammable and it has a low price.

Final Remarks

Free radical action is an inevitable, continuous process, but when it becomes excessive, it is destructive. When the organism's cells are not protected from oxidative attack, cell damage can be disastrous. Antioxidants are important because they maintain cell and organism systems health. To play a role in destruction of free radicals, antioxidants need: to be present in the proper place and in proper amounts (free radicals have a short life span); to have a non-specific character to react with radical species; to be distributed widely in the intracellular space or to be trans-locatable; to be synthesised by the cells or to be part of nutrition and to be regenerable.

Antioxidant action is due to fact that these substances have an increased ability of bonding oxygen, compared to glycerides, unsaturated fatty acids that bond with more difficulty (rancidity induction period is increased), for instance, α -tocopherol and synthesis substances: propyl gallate, octyl gallate, dodecyl gallate. Certain chemical substances increase the antioxidant effect of these substances (synergic substances): citric acid and ascorbic acid. The synergic effect is due to blocking of metals favouring fat rancidity. Organisms have natural ways of preventing and correcting occurrence of errors and cell atypia—fight against free radicals is carried out with the help of antioxidants who bring cell back to a neuter condition from a redox point of view.

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