Chapter

Natural Compounds with Antioxidant Activity-Used in the Design of Functional Foods

Petre Săvescu

Abstract

This chapter is intended to describe the main antioxidants used in the design and construction of functional foods. Defining the role of antioxidants, in the main redox processes in which certain oxidoreductases are involved, the best way of monitoring the activity of certain coenzymes of these oxidoreductases, will be established the main criteria in the design of sustainable functional foods. In addition, the importance of some coenzymes (FMN, $FMNH + H^+$, NAD, $NADH + H^+$) in preserving the activity of some valuable bio-compounds (with the role of antioxidants) in functional foods will be highlighted. Antioxidants are good disease-fighters, protecting our bodies from free radicals' attacks that would otherwise damage of the human cellular structures. Knowing and supporting the activity of the main compounds (with antioxidant activity) are operations that improve the reaction mechanisms of redox processes and can significantly contribute to achieving good functional foods - able to regulate the acid–base balance of the body and improve the metabolic processes from the consumer body.

Keywords: main antioxidants, functional foods, bio-compounds, coenzymes NAD and FMN, oxidoreductases, healthy products

1. Introduction

The modern man, limited by time resources is often attracted to fast food, regardless of the side effects generated by this diet. These foods prepared and served quickly are the result of previous scientific research - a process aimed at obtaining finished products with minimal effort, from extremely limited natural resources and carefully studied transfer phenomena (heat, mass, impulse). However, the results obtained are in contradiction with a normal and healthy diet and, over time, produce major changes in metabolism for consumers, till severe diseases. Today, our foods must be enriched in antioxidants; these are important in combating free radicals and decreasing of diseases such as cancer, type 2 diabetes, and chronic fatigue syndrome [1].

Consumer education, directing their attention to natural and high-energy diets, personalizing diets according to genetic characteristics, personal, acquired, and developed throughout life are the main goals of any nutritionist. A special role in the development of a safe, healthy diet is associated with the food industry specialist able to study and improve both the quality of raw materials entered in the manufacturing process and all stages of this process.

In order to develop synergetic collaboration between farmer, processor and nutritionist, consumer and all other factors interested in the integrated process "from farm to fork", is necessary a good professional and complementary training, good communication on the production and consumption chain, good promotion of good production practices, hygiene, laboratory, good dissemination of the results of scientific research in the field, promotion of natural bionic, biotechnological, bio-nanotechnological practices, a revaluation of food resources (to combat food waste and encourage the use of all components of the chain in innovative biotechnological sequences) [2].

Antioxidants are among the best natural disease-fighters, protecting our bodies from everyday stresses that would otherwise attack the human cellular structures.

Free radicals are primarily a by-product of oxygen. Through aerobic metabolism, every cell in the body utilizes oxygen to make energy so that it can live. The body creates by-products called oxidants, or free radicals when cells burn oxygen. These unwanted free radicals cause to damage cells in the body as they react to molecules in and outside of cells. The thermodynamics make a moving free radical to seeks another molecule which will be whole, for stability. Unfortunately, when it binds to another molecule, it tears cell walls, these free radicals can rip pieces of DNA, or can changes the chemistry of cell structures [3]. The antioxidants can change these phenomena through blocked the active energy of free radicals. They can neutralize the reactivity of unwanted free radicals and the consumer body will be protected. The formation of free radicals in the body, especially in the catabolism mechanism, is a normal process; it can happen as a result of breathing [4].

Plus, the following factors contribute to the increased level of free radicals in the body: stress, pollution, radiation, the unknow and ultra-processed food, the excess of drugs, the unwanted metals, the weak mentality, and a low level of consciousness. All of these must be changed. The first results come through the use of antioxidants, innocuity foods, functional foods, nutraceuticals, organic products - in the consumers' nutrition.

Very important - on the production chain "from farm to fork" (regardless of the size of the production chain) are the processes that take place with electron exchange (redox processes) - which include extremely complex mechanisms in which participates one of the most important classes enzymatic (oxidoreductases) [5]. The role of functional foods and dietary supplements in supporting and regulating metabolic functions in conditions of a daily life affected by stress and pollution is well known. In plants, there is an important category of compounds with high values of nutritional density and therefore, it is desirable to use as many recognized bioactive compounds as possible, in order to design and develop various functional foods [6]. An important problem arises in the case of preserving the active properties, in the conditions of advanced processing and therefore, it is extremely important to study the application of new protective technologies in the construction of such foods.

Antioxidants - used as food additives - can extend the shelf life and protect food from damage caused by the oxidation process. The oxidation reaction occurs due to the presence of oxygen. Atmospheric oxygen comes into contact with certain foods and can produce a significant number of unwanted compounds. After oxidation, a number of unwanted processes can also occur oxidation and rancidity of fats, peroxidation with changes in color, taste, smell of food.

Antioxidants - as food additives are widely used in the food industry, and additives can be classified into two broad groups.

The first group comprises compounds (acids and their derivatives), which block or delay the colour change in fruits or meat products. These substances include Ascorbic Acid (E 300) and Citric Acid (E330). Although a natural antioxidant occurring in most fruits and vegetables, E300 (for Australia or New Zealand only "300", without "E") can also be produced in a synthetically way, from the fermentation and oxidation of glucose. It is an acid that is most commonly used in the manufacture of bread, by acting as a flour-treating agent [7].

According to FDA (Food and Drug Administration), citric acid is generally considered safe (GRAS) and can be used in food with no limitations other than current good manufacturing practice [8]. It can be used as an antimicrobial agent, antioxidant, flavouring agent, *p*H control agent, sequestrant in food.

According to EFSA (European Food Safety Authority), citric acid anhydrous and monohydrate (E330) are authorized as food additives in Commission Regulation (EU) No 231/2012 and categorized as "additives other than colours and sweeteners" [9].

The second group of antioxidants is composed of substances that prevent the oxidation of fats and oils. This oxidation leads the rancidity of food by changing its appearance and becoming inedible. In this group of antioxidants, can find Butylated Hydroxy Anisole (BHA, E320), Butylated Hydroxy Toluene (BHT, E321), and Galat (E 310, E 311, E312). However, they are chemicals obtained by synthesis, they are not recommended for use as antioxidants in functional foods (**Table 1**).

No	Food additives (antioxidants)	The characteristic activity	References
1	Citric Acid (E 300)	Important for the healthy development of bones, teeth, and blood vessels	[7]
		An act to reduce wrinkles (support the production of collagen in the skin)	[9]
		Acidulant, preservative, antioxidant and chelating agent in food (can prevent or slow down the oxidation process in foods)	[10]
		Prevents oesophageal cancer cell growth (inhibition of cell proliferation and induction of cell apoptosis)	[11, 12]
		Citrate can suppress tumours growth	[13]
2	*BHA* or *butylated hydroxy anisole* (1,1-dimethylethyl)-4-methoxyphenol)	• Not recommended for use as antioxidants in functional foods • A synthetic antioxidant, used to prevent fats in foods from going rancid and as a defoaming agent for yeast	[14]
		• Anticipated to be a human carcinogen	[15]
3	*Butylated Hydroxy Toluene* (*BHT*, E321) 2,6-di-tert-butyl-4-hydroxytoluene	• Synthetic phenolic antioxidants (SPAs), not recommended for use as antioxidants in functional foods	[16, 17]
		• BHT exposure is linked to cancer, asthma, and behavioural issues in children	[16, 17]
		BHT is tumour promoters, in high quantity	[15, 18]
4	Octyl gallate (E 311) and dodecyl gallate (E 312)	• Are substances authorized as antioxidants in foods as well as in food flavourings	[Annexes II and III to Reg.(EC) No 1234/ 2007. 1333/2008]
		• Required for a proper assessment of the safety of octyl gallate as a food additive	Reg. UE 2018/1481. [19]

Table 1.
Antioxidants – Food additives.

2. Antioxidants: compounds with antioxidant activity

2.1 Antioxidants: vitamins, provitamins with antioxidant activity

Vitamin A - Vitamin A is a group of unsaturated nutritional organic compounds that includes retinol, retinal, and several provitamins A carotenoids (most notable beta-carotene) [20–22] (**Figure 1**).

Generally, the three major antioxidant vitamins are beta-carotene (precursor of vitamin A), vitamin C, and vitamin E. We will find them in colourful fruits and vegetables, especially those with purple, blue, red, orange, and yellow hues [23].

The active form (retinol) comes from animal sources such as milk, eggs, meat, and fatty fish, all of which may be high in fat and cholesterol. But it also comes from plants, in the form of beta-carotene and other carotenoids, which are converted into vitamin A in the body [24].

Depending on the environment, vitamin A can be converted to an ester (a) or oxidized to aldehyde (b). The chain can continue to oxidize the aldehyde of vitamin A to the specific acid [25] (**Figure 2** and **Table 2**).

The main carotenes are showed in **Figure 3** (right side) (**Figure 4**).

The group of *xanthophylls* includes (among many other compounds) lutein, zeaxanthin, neoxanthin, violaxanthin, flavaxanthin, and α- and β-cryptoxanthin (**Figures 5** and **6**).

Vitamin E is a fat-soluble vitamins group with 4 tocopherols and 4 tocotrienols. The tocopherol content of animal and vegetable fats (oils) is strictly influenced by animal feed (**Figure 7**).

For alpha(α)-tocopherol each of the three "R" sites has a methyl group (CH3) attached. For beta(β)-tocopherol: R1 = methyl group, R2 = H, R3 = methyl group. For gamma(γ)-tocopherol: R1 = H, R2 = methyl group, R3 = methyl group. For delta (δ)-tocopherol: R1 = H, R2 = H, R3 = methyl group. The same configurations exist for the tocotrienols, except that the hydrophobic side chain has three carbon–carbon double bonds whereas the tocopherols have a saturated side chain [43]. For alpha(α)-tocotrienol each of the three "R" sites has a methyl group (CH3) attached.

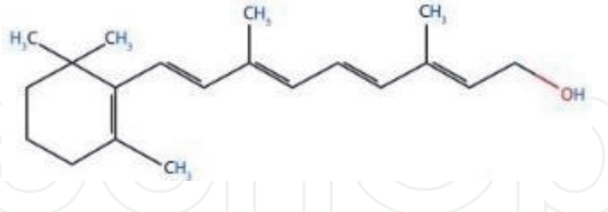


Figure 1.
Structure of vitamin A (Retinol).

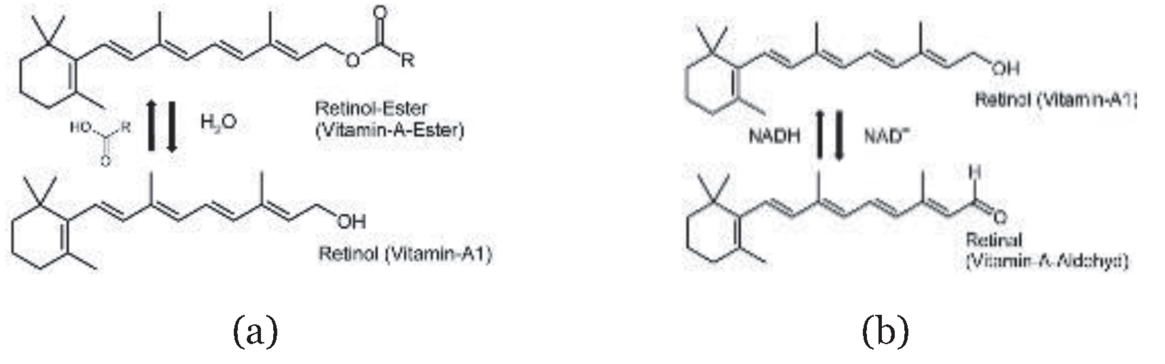


Figure 2.
Changes of vitamin A (to ester (a) or to aldehyde (b)).

No	Vitamins A, Provitamins	The characteristic activity	References
1	*Vitamin A (Retinol or retinyl ester – in tissues)*	• Important for growth, for the maintenance of the immune system, and for good vision	[23, 26]
		• In the food classical technology, produced and administrated as esters such as retinyl acetate or palmitate	[27]
		• Fat-soluble vitamin that maintains healthy soft tissue, bones, and mucous membranes, and produces pigment in the retina of the eye.	[24]
		• Retinol promotes healthy reproduction in women, fights cancer, and prevents premature aging	[27]
		• No pieces of evidence that beta-carotene or vitamin A supplements increase longevity in healthy people or in people with various diseases	[21]
2	*The carotenes:* alpha-carotene, beta-carotene, gamma-carotene; and the xanthophyll beta-cryptoxanthin (*all of which contain beta-ionone rings*)	• Function as provitamin A in organisms which possess the enzyme beta-carotene 15,15′-dioxygenase in the intestinal mucosa	[24, 28]
		• Cleave and convert provitamin A to retinol	[29].
		• β-carotene supplements may increase the risk of lung cancer for smokers	[30, 31]
		• β-carotene is a true antioxidant	[31]
		• The synthetic β-carotene can increase mortality by 1–8%	[32]
		• Lutein and zeaxanthin protect the body's proteins, fats, and DNA from stressors and can even help recycle glutathione	[33]
		• Consumption of lutein and zeaxanthin may protect against AMD (Age-related macular degeneration) progression to blindness	[34]
3	Other carotenoids, including lycopene (without *beta-ionone rings*),	• Have antioxidant activity and thus biological activity in other ways	[32]
		• Encapsulation increases the chemical and thermal stability of carotene molecules (and preserve the antioxidant activity)	[35, 36]
		• Lycopene having antioxidant effects in humans, particularly in the skin, heart function, or vision protection from ultraviolet light	[37]
		• Lycopene is a key intermediate in the biosynthesis of many carotenoids	[38]
		• During the processing of fruits, increases the concentration of bioavailable lycopene	[39–42]

Table 2.
The Vitamins A, provitamins with antioxidant activity.

R1 = methyl group, R2 = H, R3 = methyl group in beta(β)-tocotrienol. R1 = H, R2 = methyl group, R3 = methyl group – in gamma(γ)-tocotrienol. R1 = H, R2 = H, R3 = methyl group, in delta(δ)-tocotrienol. Palm oil is a good source of alpha and gamma tocotrienols (**Figures 8** and **9** and **Table 3**) [58].

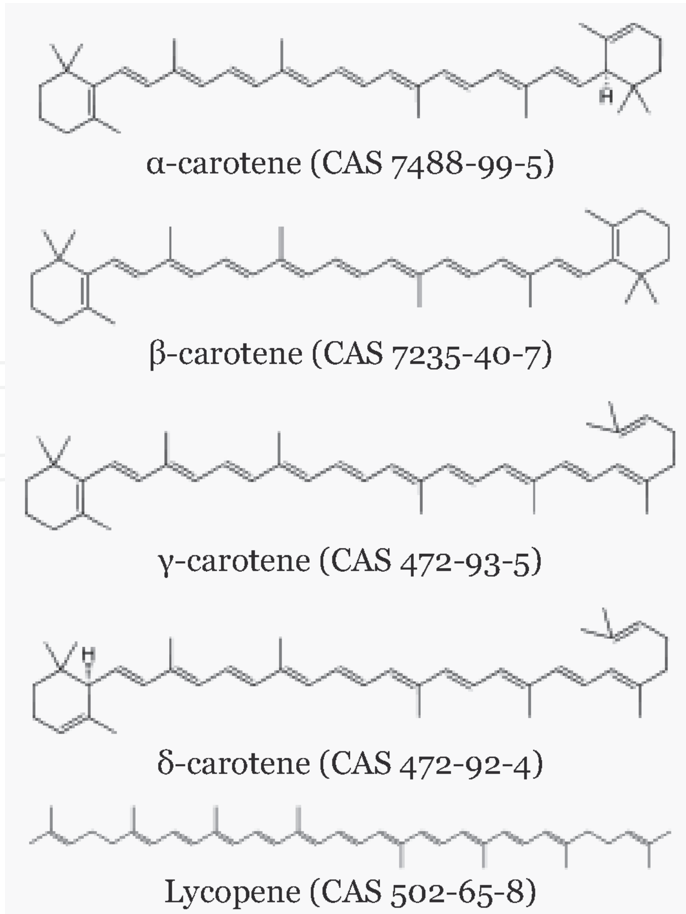


Figure 3.
The main carotenes.

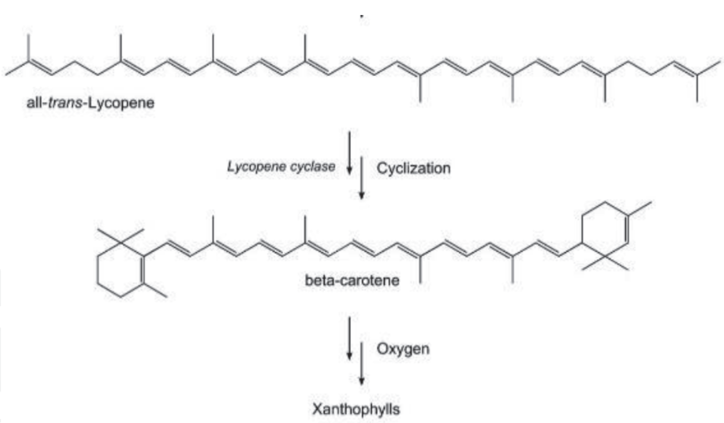


Figure 4.
Lycopene is a key intermediate in the biosynthesis of many carotenoids.

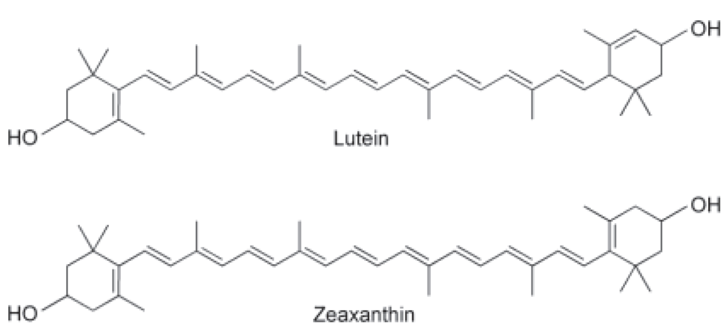


Figure 5.
The Chemical Structure of Lutein and Zeaxanthin.

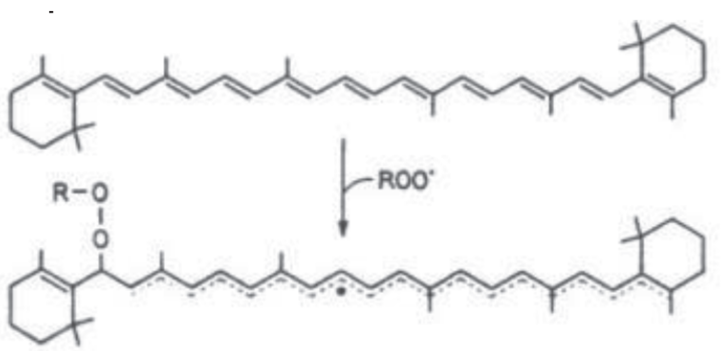


Figure 6.
The action of carotenoids on free radicals.

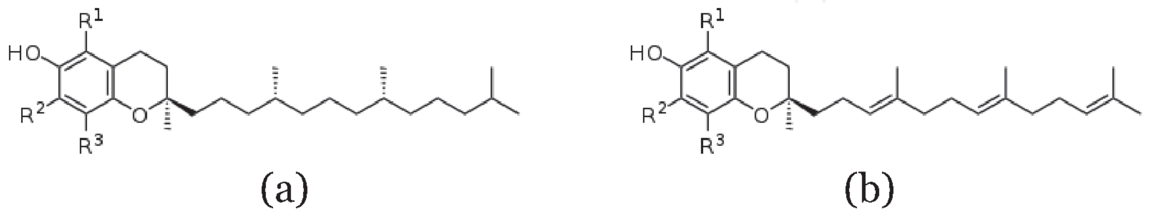


Figure 7.
General chemical structure of tocopherols (a) and tocotrienols (b).

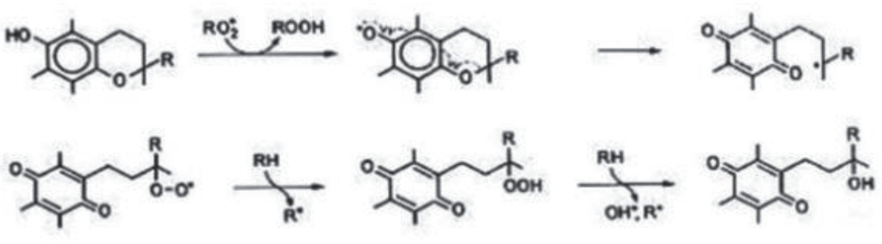


Figure 8.
Conversion of α-tocopherol to hydroxy alkyl quinone [44].

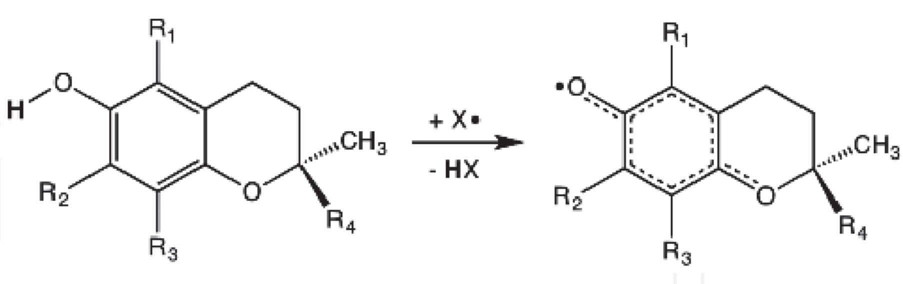


Figure 9.
Tocopherols function by donating H atoms to radicals (X) [45].

Oxidation of L-ascorbic acid to dehydroascorbic acid (**Figures 10** and **11**) depends on several parameters: oxygen partial pressure, pH, temperature and the presence of metal ions (**Figure 12**). Traces of metal ions - especially Cu^{2+} and Fe^{3+} – result from losses or transfers of substances (insufficiently controlled reaction media, insufficiently protected packaging).

2.1.1 Vitamins K

Actively participates in cellular oxidations, by reversing the transition from oxidized to reduced form, ensuring the transport of hydrogen non-enzymatically (**Figure 13**).

No	Vitamins E, Vitamins C	The characteristic activity	References
1	Vitamins E (tocopherols and tocotrienols)	• Fat-soluble antioxidant protecting cell membranes from reactive oxygen species	[46]
		• The antioxidant activity of tocopherols increases from alpha to delta; α-tocopherol is a good inhibitor of peroxide radicals formed during oxidation than γ-tocopherol • α-tocopherol can also generate alkyd radicals, which can initiate the self-oxidation of unsaturated fatty acids	[46]
		• Alpha-tocopherol is a lipid-soluble antioxidant functioning within the glutathione peroxidase pathway	[47]
		• Protect cell membranes from oxidation by reacting with lipid radicals produced in the lipid peroxidation chain reaction	[48]
		• The oxidized α-tocopheroxyl radicals produced in the lipid peroxidation chain may be recycled back to the active reduced form through reduction by other antioxidants, such as ascorbate, retinol or ubiquinol.	[49]
		• Vitamin E is implicated in the maintenance of normal cell function of cells lining the inner surface of arteries and generates anti-inflammatory activity and inhibition of platelet adhesion and aggregation	[50]
		• Can slowing the progression of age-related macular degeneration (AMD)	[51]
2	Vitamin C *(ascorbic acid*	• Protect of connective tissue, bones, cartilage, and blood vessels, as well as in healing injuries and forming collagen	[52]
		• The main biochemical role of vitamin C is to act as an antioxidant (a reducing agent) by donating electrons to various enzymatic and non-enzymatic reactions	[53]
		• Ascorbic acid acts as an antioxidant, thereby reducing the adverse effects of chemotherapy and radiation therapy	[54, 55]
		• Ascorbic acid functions as a cofactor for enzymes involved in photosynthesis, synthesis of plant hormones, as an antioxidant and also regenerator of other antioxidants	[56, 57]

Table 3.
The Vitamins E and C activity.

A number of other vitamins (B vitamins, vitamin K) together with their precursors are active in redox processes in the body and can be very good antioxidants, especially in reduced forms (hydrogenated forms). These bio-compounds are the first to oxidize, protecting the cellular environment from free radical attack.

2.2 Other antioxidants

2.2.1 Phenols and Polyphenols with antioxidant activity

Flavonoids (or bioflavonoids; from the Latin word flavus, meaning yellow, their colour in nature) are a class of polyphenolic secondary metabolites found in plants, and thus commonly consumed in diets. Flavonoids are a well-known family of plant polyphenolic compounds. Flavonoids are represented by 6 major subclasses, present in the basic diet in humans: anthocyanidins, flavan-3-ol, flavonols, flavanones, flavones and isoflavones, flavonols.

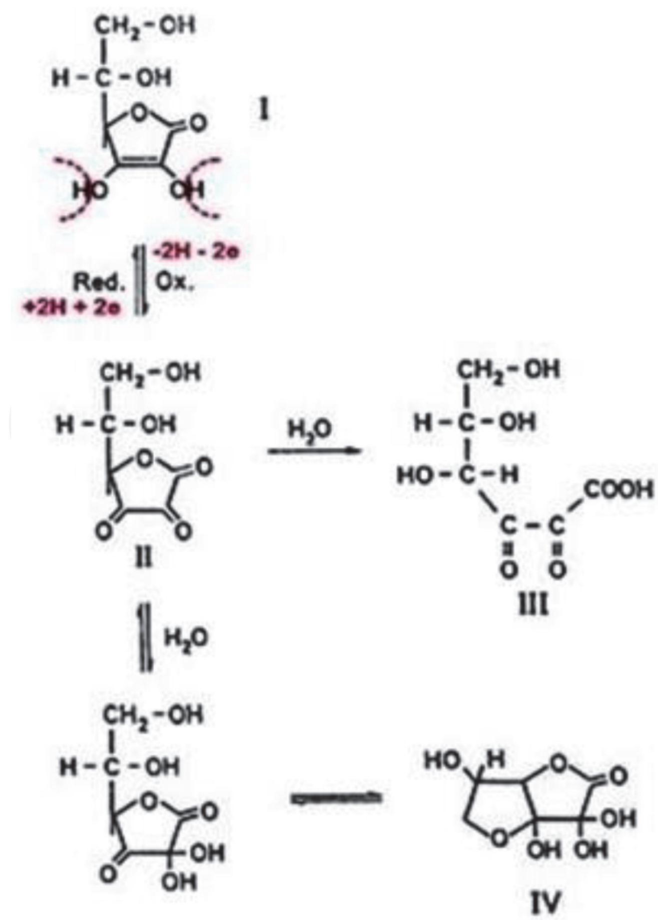


Figure 10.
Forms of ascorbic acid: I-L-Ascorbic acid, II-Dehydroascorbic Acid, III-2,3-Dicetogulonic acid; Hydrated IV-hemiacetal.

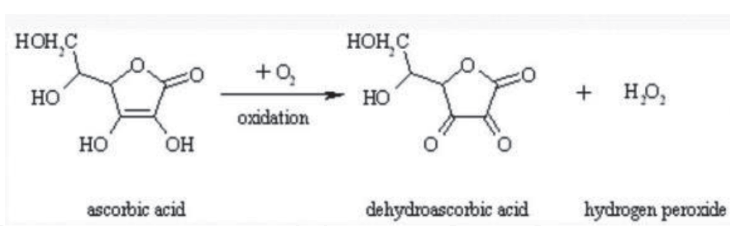


Figure 11.
L-ascorbic acid is a powerful reducing agent.

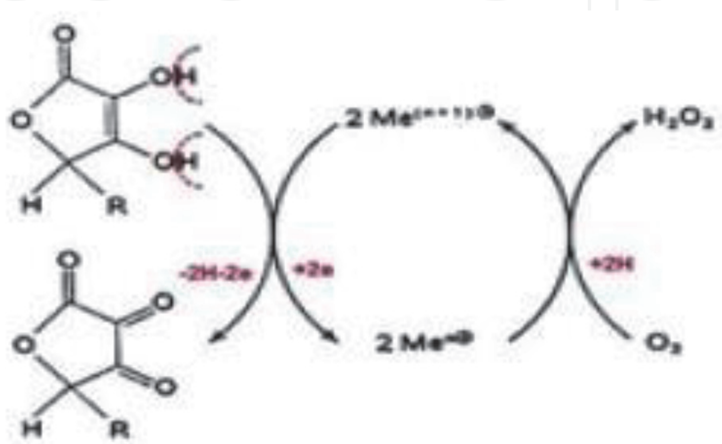


Figure 12.
The electron transfer during metal catalysis.

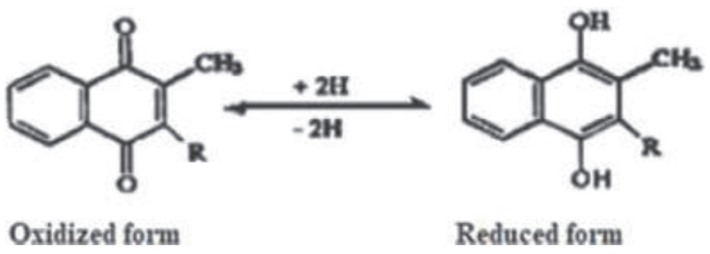


Figure 13.
The redox system of vitamin K.

Anthocyanidins are vegetable pigments, similar to anthocyanins but lacking in the carbohydrate side (**Table 4**). Their activity is based on that of the flavylium cation and the oxonium ion, which have various replacement groups of hydrogen atoms. Depending on the pH, these pigments can have various colours: red, purple, blue, and bluish green [59] (**Figures 14** and **15**).

Flavan-3-ols (sometimes referred to as flavanols) are derivatives of flavans that possess a 2-phenyl-3,4-dihydro-2H-chromen-3-ol skeleton.

These compounds include catechin, epicatechin-gallate, epigallocatechin, epigallocatechin gallate, pro-anthocyanidins, theaflavins, thearubigins (**Figure 16**).

Until 2013, both the Food and Drug Administration and the European Food Safety Authority did not issue restrictions on the use of catechins, nor did they approve any catechin-based medicines. [60] (**Figure 17**).

Flavonols are a class of flavonoids that have the 3-hydroxyflavone backbone (IUPAC name: 3-hydroxy-2-phenylchromen-4-one). Their diversity stems from the different positions of the phenolic -OH groups. Flavonols are present in a wide variety of fruits and vegetables. In Western populations, estimated daily intake is in the range of 20–50 mg per day for flavonols. Individual intake varies depending on the type of diet consumed [61]. The most used flavonols: Isorhamnetin, Kaempferol, Myricetin, Quercetin (**Figure 18**).

Anthocyanidin	R3′	R5′	R5	R6	R7
Cyanidin	−OH	−H	−OH	−H	−OH
Delphinidin	−OH	−OH	−OH	−H	−OH
Malvidin	−OCH3	−OCH3	−OH	−H	−OH
Pelargonidin	−H	−H	−OH	−H	−OH
Peonidin	−OCH3	−H	−OH	−H	−OH
Petunidin	−OH	−OCH3	−OH	−H	−OH

Table 4.
The main anthocyanidins and their substitution radicals.

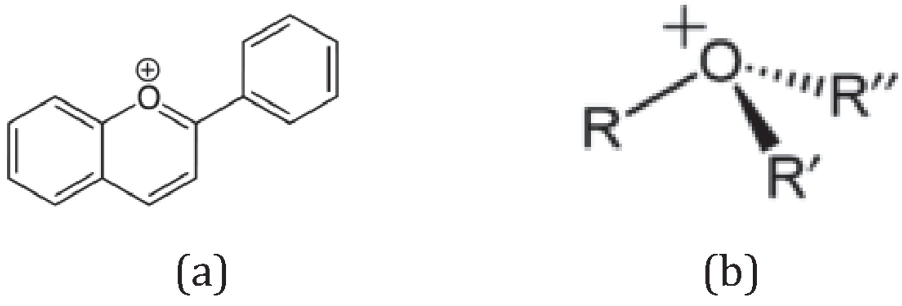


Figure 14.
Flavylium Cation (a) and general pyramidal oxonium ion (b).

DOI: http://dx.doi.org/10.5772/intechopen.97364

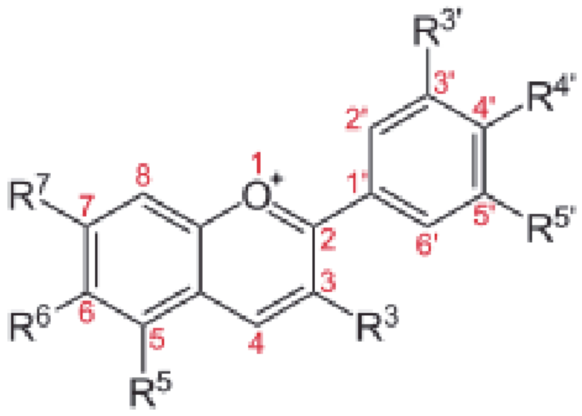


Figure 15.
Basic Structure of Anthocyanidins (R3' and R4' = -OH).

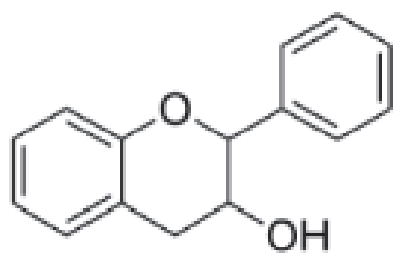


Figure 16.
Chemical Structure of Flavan-3-ols.

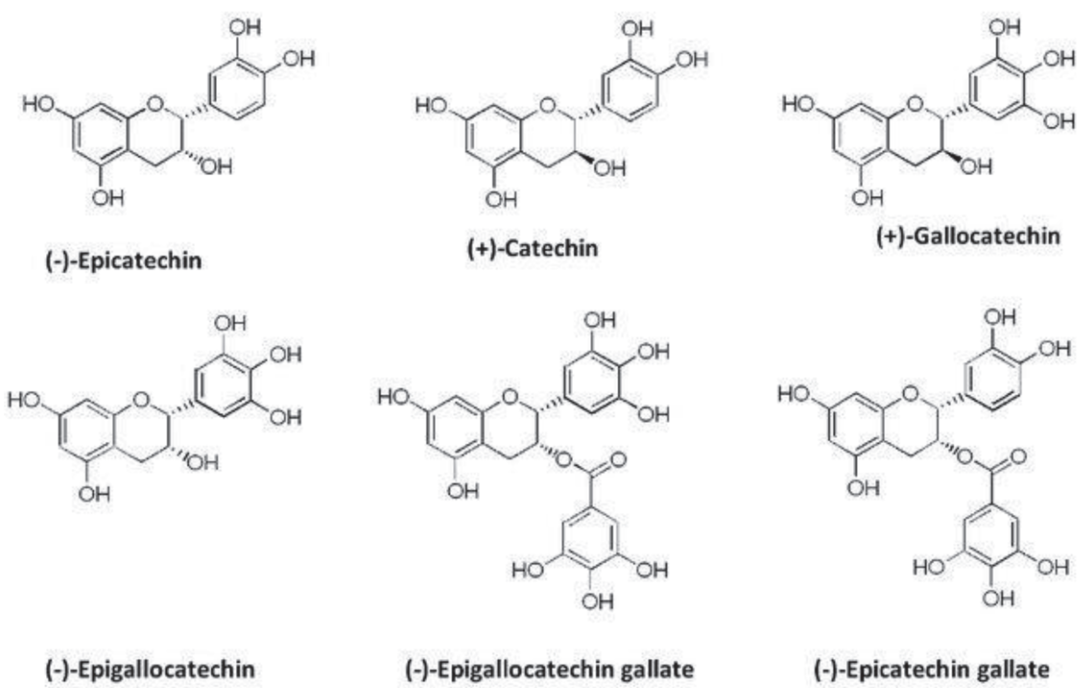
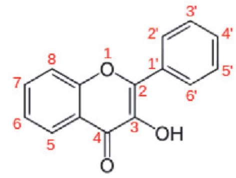


Figure 17.
Chemical Structure of Catechins.

Flavones (derived by the Latin flavus "yellow") are a class of flavonoids based on the nucleus of 2-phenylchromen-4-one (2-phenyl-1-benzopyran-4-one). Apigenin (4 ', 5,7-trihydroxyflavone), luteolin (3', 4 ', 5,7-tetrahydroxyflavone), tangeritine (4', 5,6,7,8-pentamethoxyflavone), chrysin (5,7-dihydroxyphlavone)) and 6-hydroxyflavone are compounds that belong to the class of flavones [62].



Flavonols	The substitution radicals								
Name	5	6	7	8	2′	3′	4′	5′	6′
Isorhamnetin	OH	H	OH	H	H	OCH3	OH	H	H
Kaempferol	OH	H	OH	H	H	H	OH	H	H
Myricetin	OH	H	OH	H	H	OH	OH	OH	H
Quercetin	OH	H	OH	H	H	OH	OH	H	H

Figure 18.
Chemical Structure of the main Flavonols and their substitution radicals.

In plants, a number of flavonoid glycosides often appear, which are in fact colourless aromatic ketones, derived from flavone (flavanone). [63].

Isoflavones are substituted derivatives of isoflavone, a type of naturally occurring isoflavonoids [64] many of which act as phytoestrogens in mammals [65]. Isoflavones are produced almost exclusively by the members of the bean family, Fabaceae (*Leguminosae*) (**Figures 19** and **20**).

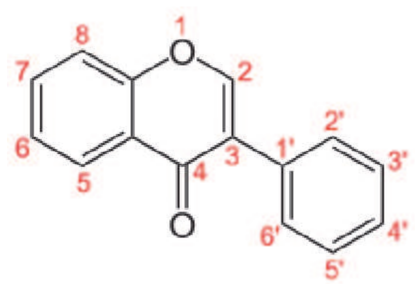


Figure 19.
Isoflavone, numbering. Genistein (5-OH, 7-OH, 4'-OH) or daidzein (7-OH, 4'-OH).

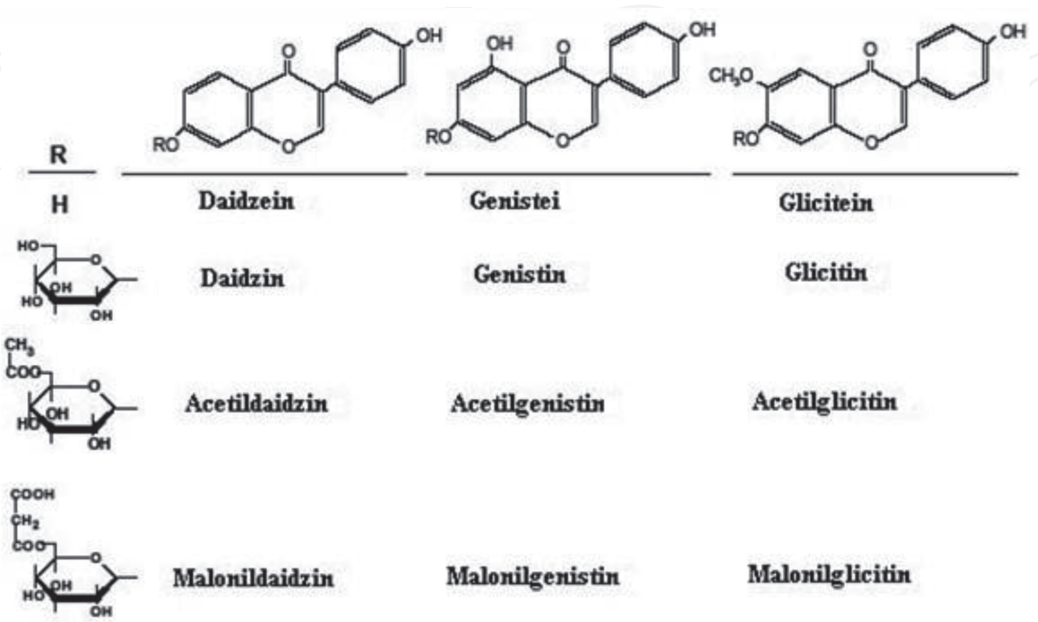


Figure 20.
Chemical Structure of the main isoflavones.

The consumption of isoflavones-rich food or dietary supplements is under preliminary research for its potential association with lower rates of postmenopausal cancer [66, 67] and osteoporosis in women [68]. Use of soy isoflavone dietary supplements may be associated with reduction of hot flashes in postmenopausal women [67, 68] (**Figure 21**).

2.3 Antioxidants: acids, amino acids and other compounds with antioxidant activity

2.3.1 Lipoic acid

Lipoic acid (LA) is an organo-sulfurized compound of caprylic acid (octanoic acid). It is also known - in the technical literature and as α-lipoic acid (ALA) and thioctic acid [69] (**Figures 22** and **23**).

In cells, α-LA can be reduced to dihydrolipoic acid, the more bioactive form of LA, involved in antioxidant processes that lead to decreased redox activities of iron and copper ions in solutions. [70]. Recent research has shown that the anti-aging and cellular disease prevention effects are mainly due to genetic mechanisms that improve the antioxidant state of the cell. However, this likely occurs via pro-oxidant mechanisms, not by radical scavenging or reducing effects [71–73]. α-Lipoic acid is an antioxidant that acts in both forms (both oxidized and reduced) on tissues and lipo- and water-soluble substances. It can be easily reduced by breaking the disulfide bridge with the formation of sulfhydryl groups. The di-hydrolipoic form of α-lipoic acid is regenerated by the redox mechanisms of vitamins C and E.

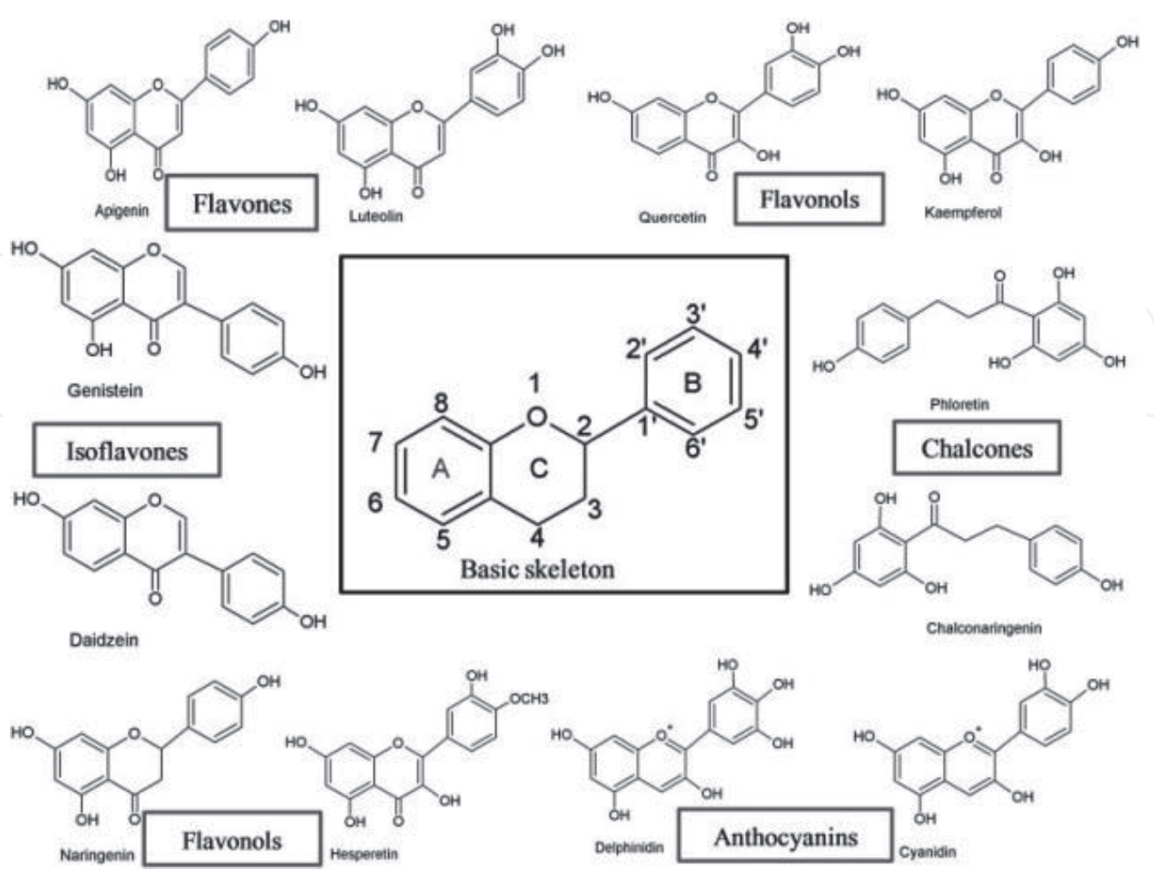


Figure 21.
The main structures of flavonoids.

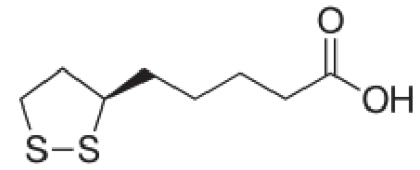


Figure 22.
Structure of α-Lipoic Acid.

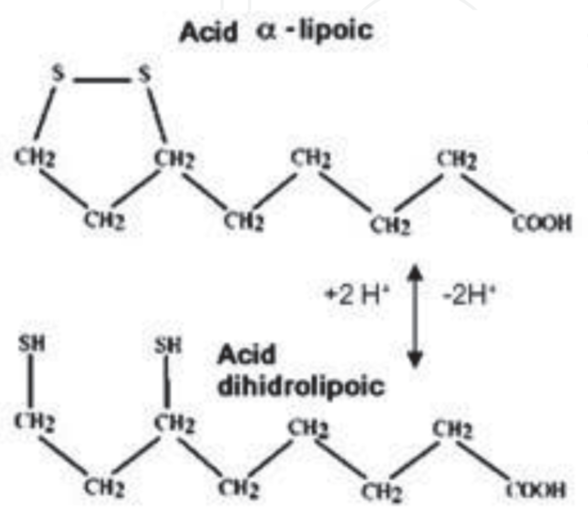


Figure 23.
α-lipoic acid (α-LA) and the two forms in which it is found (oxidized and reduced).

2.3.2 Folic acid

The tetrahydrofolate (II) derivative of folic acid (I) is the enzymatic cofactor that can transfer a carbon unit in various oxidation states (such as in formyl or hydroxymethyl residues) (**Figure 24**).

Folate contributes major to spermatogenesis. In women, folate is important for oocyte quality and maturation, implantation, placentation, fetal growth and organ development [74].

2.3.3 Cysteine

Cysteine (symbol Cys) [75] is a semi essential [76] proteinogenic amino acid with the formula HOOC-CH-(NH_2)-CH_2-SH. The thiol side chain in cysteine often participates in enzymatic reactions, as a nucleophile. Due to the ability of thiols to undergo redox reactions, cysteine has antioxidant properties. Its antioxidant properties are typically expressed in the tripeptide glutathione, which occurs in humans and other organisms. The systemic availability of oral glutathione (GSH) is negligible; so, it must be biosynthesized from its constituent amino acids, cysteine, glycine, and glutamic acid [77]. While glutamic acid is usually sufficient because amino acid nitrogen is recycled

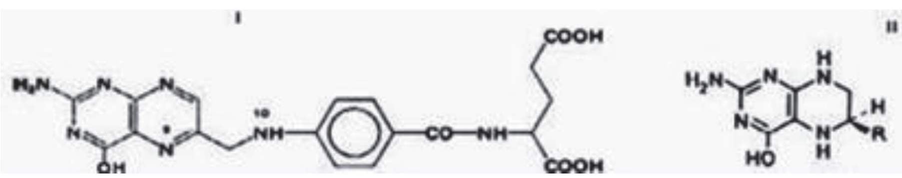


Figure 24.
Folic acid (I) and tetrahydrofolate derivative (II).

through glutamate as an intermediary, dietary cysteine and glycine supplementation can improve synthesis of glutathione [78]. Cysteine and cystine - form an important redox system, whose steady-state depends on oxidation conditions (**Figure 25**).

2.3.4 Glutathione

Glutathione (γ-L-glutamyl-L-cysteinyl-glycine) is found in both animals, plants, and microorganisms (**Figure 26**).

The active group of glutathione is -SH, through which glutathione can participate in redox reactions, having a reduced form marked with G-SH and an oxidized one (with disulfide bridge, G-S-S-G, according to the Eq. (1)):

$$\begin{array}{ll} 2GSH \longleftrightarrow & G\text{-}S\text{-}S\text{-}G + 2H \\ \text{Reduced form} & \text{Oxidized form} \end{array} \quad (1)$$

GSH protects cells by neutralizing single reactive oxygen species [79–81]. This transformation is found in the reduction of peroxides:

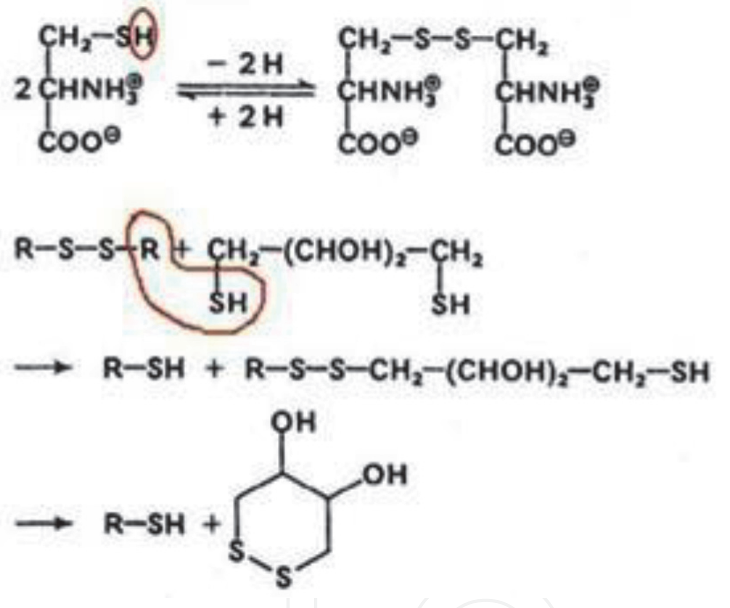


Figure 25.
The redox mechanism Cysteine-Cystine.

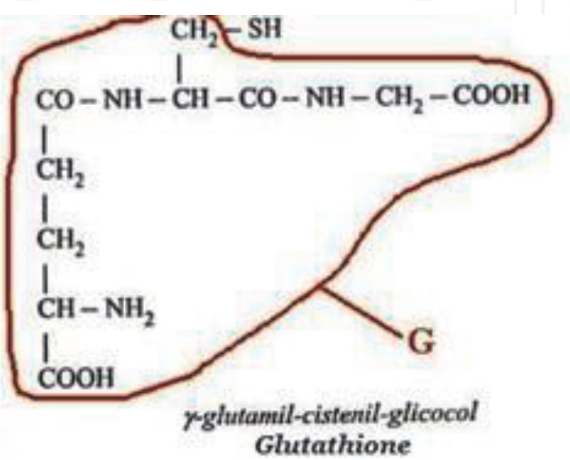


Figure 26.
Structure of Glutathione.

$$2\ GSH + R_2O_2 \rightarrow GSSG + 2\ ROH\ (R = H, alkyl) \quad (2)$$

and with free radicals:

$$GSH + R. \rightarrow 0.5\ GSSG + RH \quad (3)$$

It maintains exogenous antioxidants such as vitamins C and E in their reduced (active) states [81].

2.4 The main oxidoreductases with antioxidant activity

a. *FAD-dependent oxidoreductases* are enzymes of a heteroproteinic nature from the group of aerobic dehydrogenases having as active groups derivatives of vitamin B2 (riboflavin or 7,8-dimethyl-10-ribithyl-isoaloxazine), namely: flavin adenine mononucleotide (FMN) and flavin dinucleotide (FAD). Flavin enzymes (FMN, FAD) are involved in electron and proton transfer reactions mediated by the isoalloxazine nucleus. They accept either an electron or a pair of electrons (unlike NAD and NADP which only accept electron pairs) (**Figure 27**).

$$FAD\ (FMN) + 2\ H + +2e - \leftrightarrows FADH_2\ (FMNH_2) \quad (4)$$

Flavin-enzymes have the standard redox potential E_o between +0.19 V (oxidants stronger than NAD +) and − 0.49 V (reducing agent stronger than NADH), which shows a wide range of variation of redox properties depending on environmental conditions and the nature of the substrate (**Figure 28**). For some flavin -enzymes that also contain a metal (molybdenum or iron) in their molecule, it can stabilize the semi-quinone form by pairing the electron alone with unpaired electrons existing in metal ions; the metal can transport electrons to the respective flavin enzymes.

b. *NAD-dependent oxidoreductases* are enzymes from the class of anaerobic dehydrogenases and have as coenzymes, Nicotinamide Adenine Dinucleotide (NAD+) or reduced (NADH + H+) and Nicotinamide Adenine Dinucleotide Phosphate Oxidate (NADP+) or reduced (NADPH). These coenzymes consist of a derivative of vitamin PP, nicotinamide and an adenine-derived nucleus (**Figure 29**).

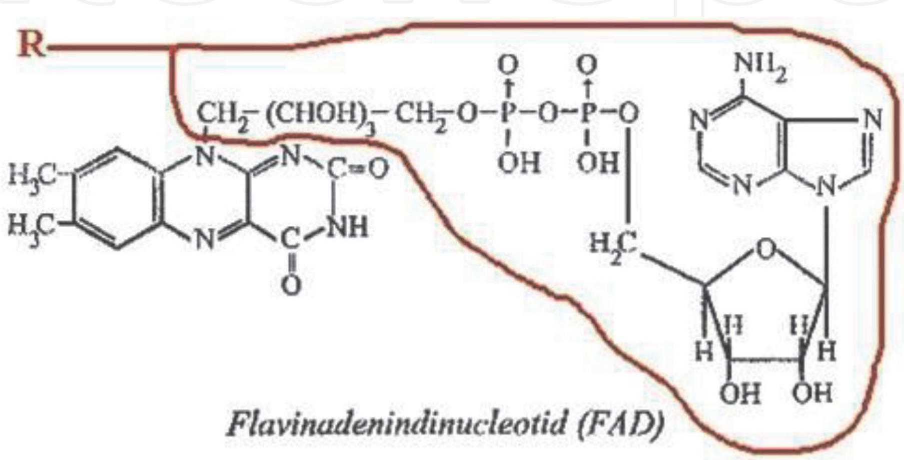


Figure 27.
Structure of Flavin Adenine Dinucleotide (FAD).

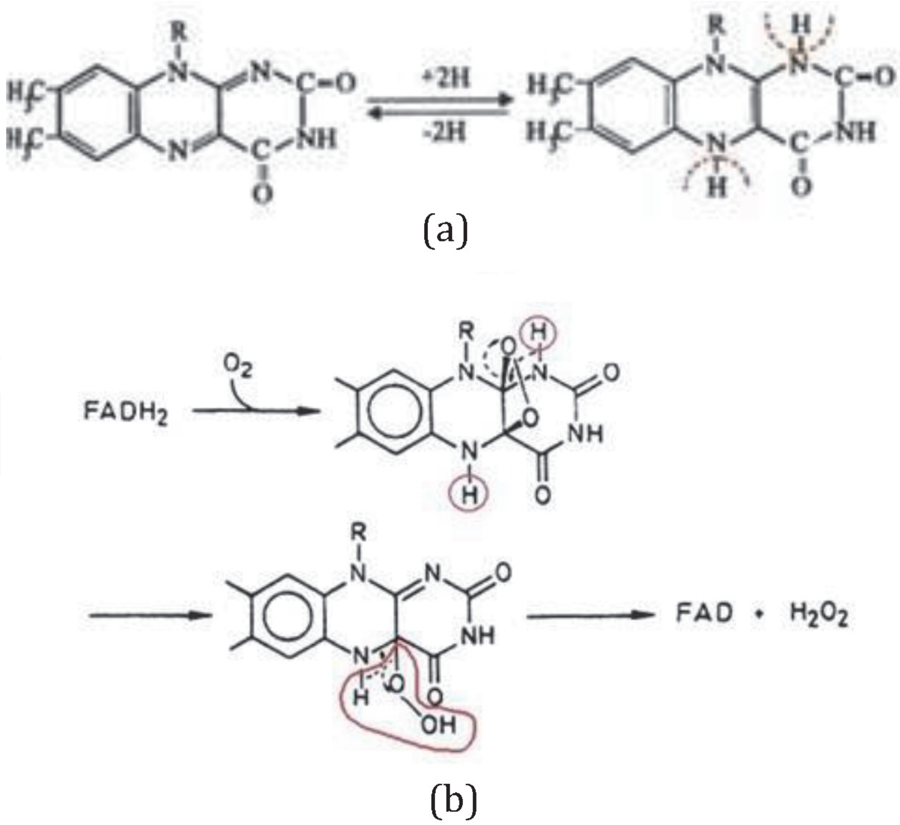


Figure 28.
Mechanisms of FAD (a-left) and (b-down).

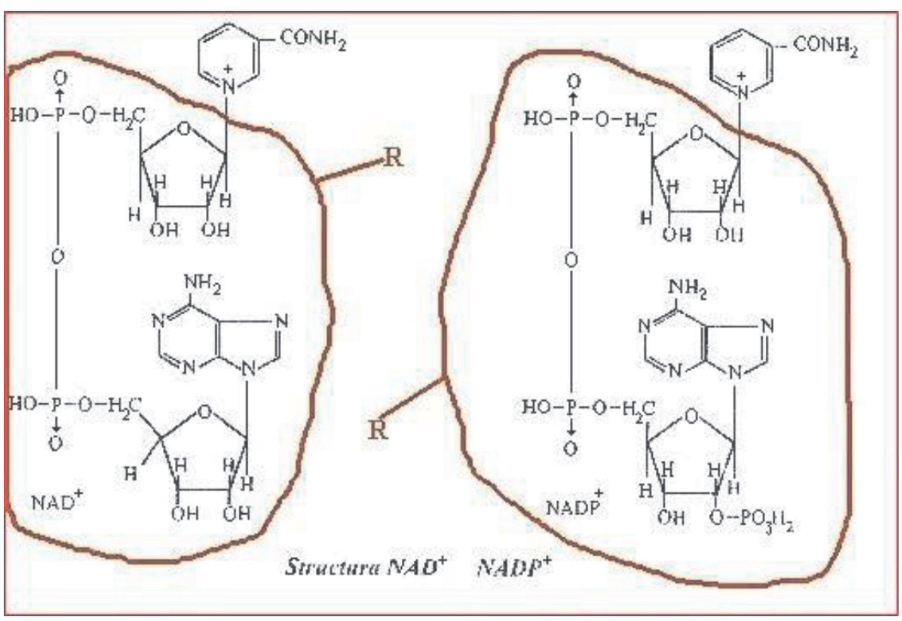


Figure 29.
Structure of NAD and NADP.

NAD^+ and $NADP^+$ are anaerobic, because the transferred hydrogen acceptor is not oxygen, but another element. They catalyze redox reactions by the generally reversible transfer of protons. The transfer of hydrogen in the redox reactions catalyzed by NAD^+ and $NADP^+$ is carried out at the level of the nicotinamide component in the structure of these coenzymes (**Figure 30**).

Preservation of antioxidant characteristics can be achieved by using special techniques: Mild Food Processing, Supercritical Fluid Extraction (SFE), separation in active plasma field, separation in magnetic and gravitational field.

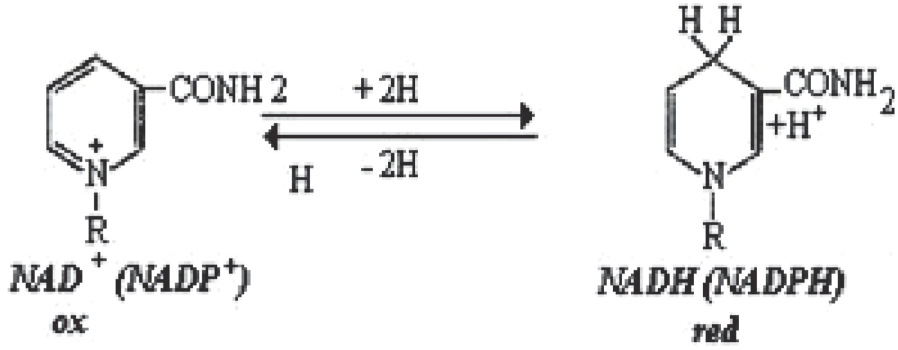


Figure 30.
NAD (P) – redox mechanism.

Figure 31.
Sepparation a synthetic food preservative from a liquid food using, nano-plasma field, SFE and antioxidant agent (©).

Using the properties of compounds with antioxidant activity (from certain redox systems in food), an improved SFE process at the nanomolecular level - with the help of a nano-plasma field, Professor Savescu Petre succeeded in separating (in the form of crystals) a synthetic food preservative from a liquid food (**Figure 31**). The advanced separation was performed by a personal technique (under innovative patent by PhD. Habil. Professor Petre Săvescu), within the INCESA Research Hub of the University of Craiova, Romania.

3. Conclusions

Antioxidants are valuable bio compounds that can increase both the nutritional value of the functional food and the therapeutic value of this important product.

For dietary supplements and functional foods, it is important to use only natural antioxidants. Synthetic antioxidants can cause a number of consumer health problems. In the design and construction of a functional food it is important to use only inoculated and even organic raw materials. All used raw materials, food additives, and technological adjuvants must be analysed before processing the food supplement - to avoid unwanted reactions and the appearance of compounds with a potential risk to the health of the consumer.

It is forbidden to use raw materials, food additives, technological auxiliaries which can contain traces of antibiotics, plant or animal hormones, pesticides, heavy metals. For their analysis will be used complex chromatography techniques (GC, LC), advanced separation techniques (using supercritical fluids and plasma fields), optical methods of analysis (UV-Viz, NIR, FT-IR) with Certified Reference Materials and Pure Analysis Substances and modern standardized methods of electrochemistry. Antioxidants can have the functions of immune-modulatory compounds, food preservatives, and food colouring, sequestering/chelating agents for heavy metals.

Acknowledgements

This work was supported by a grant of the Romanian National Authority for Scientific Research and Innovation, CNCS/CCCDI – UEFISCDI, project number PN-III-P1-1.2-PCCDI-2017- 0566 (contr. nr. 9 PCCDI/2018-2021/P4 - Complex system of full capitalization of some agricultural species with energy and food potential). "Valuing the Food Potential of Selected Agricultural Crops (artichoke tubers, sorghum seeds, hemp pomace - resulting from cold pressed hemp oil)". Responsible Project: Petre Savescu. Project Code: PN-III-P1-1.2-PCCDI-2017-0566.

Conflict of interest

The author declare no conflict of interest.

Author details

Petre Săvescu
Department of Agricultural and Forest Technologies, Food Control and Expertise, Faculty of Agronomy, University of Craiova, Romania

*Address all correspondence to: petre.savescu@edu.ucv.ro; psavescu@gmail.com

References

[1] Bayani Uttara, Ajay V. Singh, Paolo Zamboni, R.T Mahajan, 2009, Oxidative Stress and Neurodegenerative Diseases: A Review of Upstream and Downstream Antioxidant Therapeutic Options, *Curr Neuropharmacol*.; 7(1): 65–74. doi: 10.2174/157015909787602823

[2] Savescu, P.; Iacobescu, F.; Poenaru M.M. Study on the use of biomaterials as protective membranes for certain functional foods, Book: Advances in Bionanomaterials II – Selected Papers from the 3rd International Conference on Bio and Nanomaterials, BIONAM 2019, Book Series: Lecture Notes in Bioengineering. Publisher: Springer International Publishing, Print ISBN: 978-3-030-47704-2, Springer Nature Switzerland AG 2020, S. Piotto et al. (Eds.): BIONAM 2019, LNBE, 2020, 178–190.

[3] Di Meo, S.; Venditti, P. Evolution of the knowledge on free radicals and other oxidants. In Oxidative Medicine and Cellular Longevity; Hindawi: London, UK, 2020; p. 32-36.

[4] Forman, H.J.; Davies, K.J.; Ursini, F. How do nutritional antioxidants really work: Nucleophilic tone and para-hormesis versus free radical scavenging in vivo. Free Radic. Biol. Med. 2014, 66, 24–35.

[5] Savescu P., Badescu G., Milut M., Ciobanu A., Apostol L., Vladut V., 2019. Healthy Food – Through Innovative Technologies. Vol. ISB-INMA-TEH 2019 INTERNATIONAL SYMPOSIUM Bucharest. 2019, p.2048-2053.

[6] Belenky, P.; Bogan, KL.; Brenne,r C.,2007. NAD^+metabolism in health and disease. Trends Biochem. Sci. 32 (1): 2007, 12–19.

[7] Apleblat, Alexander (2014). Citric acid. Springer. ISBN 978-3-319-11232-9.

[8] https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/ CFRSearch.cfm?fr=184.1033, accessed in dec,12,2020

[9] https://efsa.onlinelibrary.wiley.com/doi/pdf/10.2903/j.efsa.2015.4010

[10] Citric acid, FDA, 21CFR184.1033, https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfCFR/CFRSearch.cfm?fr=184.1033, Commission Regulation (EU) No 1129/2011 of 11 November 2011 amending Annex II to Regulation (EC) No 1333/2008 of the E.P. and of the Council by establishing a Union list of food additives,

[11] Chen, X., Lv, Q., Liu, Y., Deng, W. (2017). Effect of Food Additive Citric Acid on The Growth of Human Esophageal Carcinoma Cell Line EC109. Cell journal, 18(4), 493–502. https://doi.org/10.22074/cellj.2016.4716

[12] Citrate Suppresses Tumor Growth in Multiple Models through Inhibition of Glycolysis, the Tricarboxylic Acid Cycle and the IGF-1R Pathway, Nature, 2017, (https://www.nature.com/articles/s41598-017-04626-4)

[13] Report on Carcinogens, USA, Fourteenth Edition, USA, 2016

[14] Wang W, Kannan K. Quantitative identification of and exposure to synthetic phenolic antioxidants, including butylated hydroxytoluene, in urine. Environ Int. 2019 Jul;128:24-29. doi: 10.1016/j.envint.2019.04.028. Epub 2019 Apr 25. PMID: 31029976; PMCID: PMC6526070

[15] Williams GM, Iatropoulos MJ, Whysner J. Safety assessment of butylated hydroxyanisole and butylated hydroxytoluene as antioxidant food additives. Food Chem Toxicol. 1999 Sep-Oct;37(9-10):1027-38. doi: 10.1016/s0278-6915(99)00085-x. PMID: 10541460

[16] Helmenstine, Anne Marie, Ph.D. "Chemistry of BHA and BHT Food Preservatives." ThoughtCo, Aug. 28, 2020, thoughtco.com/bha-and-bht-food-preservatives-607393

[17] Ito N, Fukushima S, Tsuda H. Carcinogenicity and modification of the carcinogenic response by BHA, BHT, and other antioxidants. Crit Rev Toxicol. 1985;15(2):109-50. doi: 10.3109/10408448509029322. PMID: 3899519

[18] https://eur-lex.europa.eu/legal-content/RO/TXT/PDF/?uri=CELEX:32018R1481&from=EN

[19] Blaner WS (2020). "Vitamin A". In BP Marriott, DF Birt, VA Stallings, AA Yates (eds.). Present Knowledge in Nutrition, Eleventh Edition. London, United Kingdom: Academic Press (Elsevier). pp. 73–92. ISBN 978-0-323-66162-1.

[20] Vitamin A. Micronutrient Information Center, Linus Pauling Institute, Oregon State University, Corvallis. January 2015.

[21] Fennema O (2008). Fennema's Food Chemistry. CRC Press/Taylor & Francis. pp. 454–455. ISBN 9780849392726.

[22] Blaner WS (2020). "Vitamin A". In BP Marriott, DF Birt, VA Stallings, AA Yates (eds.). Present Knowledge in Nutrition, Eleventh Edition. London, United Kingdom: Academic Press (Elsevier). pp. 73–92. ISBN 978-0-323-66162-1.

[23] Vitamin A. MedlinePlus, National Library of Medicine, US National Institutes of Health. 2 December 2016.

[24] Bjelakovic G, Nikolova D, Gluud LL, Simonetti RG, Gluud C (March 2012). "Antioxidant supplements for prevention of mortality in healthy participants and patients with various diseases". The Cochrane Database of Systematic Reviews. 3 (3): CD007176. doi:10.1002/14651858.CD007176.pub2. hdl:10138/136201. PMID 22419320.

[25] DeMan J (1999). Principles of Food chemistry (3rd ed.). Maryland: Aspen Publication Inc. p. 358. ISBN 978-0834212343.

[26] Tanumihardjo SA (August 2011). "Vitamin A: biomarkers of nutrition for development". The American Journal of Clinical Nutrition. 94 (2): 658S–65S. doi: 10.3945/ajcn.110.005777. PMC 3142734. PMID 21715511.

[27] Meschino (2013), Health. "Comprehensive Guide to Vitamin A"

[28] Vitamin A. MedlinePlus, National Library of Medicine, US National Institutes of Health. 2 December 2016.

[29] British Cancer Organization Calls for Warning Labels on Beta-Carotene. 2000-07-31

[30] The Alpha-Tocopherol, Beta Carotene Cancer Prevention Study Group (1994). "The effect of vitamin E and beta carotene on the incidence of lung cancer and other cancers in male smokers". N Engl J Med. 330 (15): 1029–35

[31] Bjelakovic G, Nikolova D, Gluud LL, Simonetti RG, Gluud C (2007). "Mortality in randomized trials of antioxidant supplements for primary and secondary prevention: systematic review and meta-analysis". JAMA. 297 (8): 842–57. doi:10.1001/jama.297.8.842

[32] *Yanagi, Kazuhiro; Iakoubovskii, Konstantin; Kazaoui, Said; Minami, Nobutsugu; Maniwa, Yutaka; Miyata, Yasumitsu; Kataura, Hiromichi (2006).* Light-Harvesting Function of β-Carotene Inside Carbon Nanotubes (PDF). *Phys. Rev. B. 74 (15): 155420.*

[33] Ma L, Dou HL, Wu YQ, Huang YM, Huang YB, Xu XR, Zou ZY, Lin XM. Lutein and zeaxanthin intake and the risk of age-related macular degeneration: a systematic review and meta-analysis. Br J Nutr. 2012 Feb;107(3):350-9. doi: 10.1017/S0007114511004260. Epub 2011 Sep 8. PMID: 21899805.

[34] Traber MG, Bruno RS (2020). "Vitamin E". In BP Marriott, DF Birt, VA Stallings, AA Yates (eds.). Present Knowledge in Nutrition, Eleventh Edition. London, United Kingdom: Academic Press (Elsevier). pp. 115–36. ISBN 978-0-323-66162-1.

[35] Saito, Yuika; Yanagi, Kazuhiro; Hayazawa, Norihiko; Ishitobi, Hidekazu; Ono, Atsushi; Kataura, Hiromichi; Kawata, Satoshi (2006). "Vibrational Analysis of Organic Molecules Encapsulated in Carbon Nanotubes by Tip-Enhanced Raman Spectroscopy". Jpn. J. Appl. Phys. 45 (12): 9286–9289. Bibcode: 2006JaJAP.45.9286S. doi:10.1143/JJAP.45.9286.

[36] Scientific Opinion on the substantiation of health claims related to lycopene and protection of DNA, proteins and lipids from oxidative damage protection of the skin from UV-induced (including photo-oxidative) damage, contribution to normal cardiac function, and maintenance of normal vision pursuant to Article 13(1) of Regulation (EC) No 1924/2006". EFSA Journal. 9 (4): 2031. 2011. doi:10.2903/j.efsa.2011.2031.

[37] *Ilahy, R; Piro, G; Tlili, I; Riahi, A; Sihem, R; Ouerghi, I; Hdider, C; Lenucci, M. S. (2016). "Fractionate analysis of the phytochemical composition and antioxidant activities in advanced breeding lines of high-lycopene tomatoes". Food Funct. 7 (1): 574–83.* doi:10.1039/c5fo00553a

[38] Carotenoids: α-Carotene, β-Carotene, β-Cryptoxanthin, Lycopene, Lutein, and Zeaxanthin. Micronutrient Information Center, Linus Pauling Institute, Oregon State University, Corvallis, OR. July 2016.

[39] Perdomo F, Cabrera Fránquiz F, Cabrera J, Serra-Majem L (2012). "Influence of cooking procedure on the bioavailability of lycopene in tomatoes". Hospital Nutrition (Madrid). 27 (5): 1542–6. doi:10.3305/nh.2012.27.5.5908

[40] Kamiloglu, S.; Demirci, M.; Selen, S.; Toydemir, G.; Boyacioglu, D.; Capanoglu, E. (2014). Home processing of tomatoes (*Solanum lycopersicum*): Effects on in vitro-bio-accessibility of total lycopene, phenolics, flavonoids, and antioxidant capacity. Journal of the Science of Food and Agriculture. 94 (11): 2225–33. doi:10.1002/jsfa.6546

[41] Yamaguchi, Masayoshi (2010). Carotenoids: Properties, Effects and Diseases. New York: Nova Science Publishers. p. 125. ISBN 9781612097138

[42] Maria Alessandra Gammone, Graziano Riccioni, Nicolantonio D'Orazio (2015). Carotenoids: potential allies of cardiovascular health? Food Nutr Res. 2015; 59: 10.3402/fnr.v59.26762. DOI: 10.3402/fnr.v59.26762

[43] Shahidi F, de Camargo AC (October 2016). Tocopherols and Tocotrienols in Common and Emerging Dietary Sources: Occurrence, Applications, and Health Benefits. International Journal of Molecular Sciences. 17 (10):1745. doi: 10.3390/ijms17101745. PMC 5085773.

[44] *Reboul E, Richelle M, Perrot E, Desmoulins-Malezet C, Pirisi V, Borel P (November 2006). "Bioaccessibility of carotenoids and vitamin E from their main dietary sources". Journal of Agricultural and Food Chemistry. 54 (23): 8749–55.* doi:10.1021/jf061818s

[45] Wefers H, Sies H (June 1988). The protection by ascorbate and glutathione against microsomal lipid peroxidation is dependent on vitamin E. European

Journal of Biochemistry. 174 (2): 353–7. doi:10.1111/j.1432-1033.1988.tb14105.x.

[46] Manolescu B, Atanasiu V, Cercasov C, Stoian I, Oprea E, Buşu C (2008). So many options but one choice: the human body prefers alpha-tocopherol. A matter of stereochemistry. Journal of Medicine and Life. 1 (4): 376–82. PMC 5654212. PMID 20108516.

[47] Traber MG, Atkinson J (July 2007). Vitamin E, antioxidant and nothing more. Free Radical Biology & Medicine. 43 (1): 4–15. doi:10.1016/j.freeradbiomed.2007.03.024.

[48] Wang X, Quinn PJ (July 1999). "Vitamin E and its function in membranes". Progress in Lipid Research. 38 (4): 309–36. doi:10.1016/S0163-7827(99)00008-9. PMID 10793887.

[49] Kirmizis D, Chatzidimitriou D (2009). "Antiatherogenic effects of vitamin E: the search for the Holy Grail". Vascular Health and Risk Management. 5: 767–74. doi:10.2147/vhrm.s5532. PMC 2747395. PMID 19774218.

[50] Evans JR, Lawrenson JG (July 2017). "Antioxidant vitamin and mineral supplements for slowing the progression of age-related macular degeneration". The Cochrane Database of Systematic Reviews. 7: CD000254. doi:10.1002/14651858.CD000254.pub4. PMC 6483465

[51] Vitamin C. Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium, and Carotenoids. Washington, DC: The National Academies Press. 2000. pp. 95–185. ISBN 978-0-309-06935-9.

[52] Vitamin C. Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium, and Carotenoids. Washington, DC: The National Academies Press. 2000. pp. 95–185. ISBN 978-0-309-06935-9.

[53] Fritz H, Flower G, Weeks L, Cooley K, Callachan M, McGowan J, Skidmore B, Kirchner L, Seely D (July 2014). "Intravenous Vitamin C and Cancer: A Systematic Review". Integrative Cancer Therapies. 13 (4): 280–300. doi:10.1177/1534735414534463. PMID 24867961.

[54] Du J, Cullen JJ, Buettner GR (December 2012). "Ascorbic acid: chemistry, biology and the treatment of cancer". Biochimica et Biophysica Acta (BBA) - Reviews on Cancer. 1826 (2): 443–57. doi:10.1016/j.bbcan.2012.06.003. PMID 22728050.

[55] Ye Y, Li J, Yuan Z (2013). "Effect of antioxidant vitamin supplementation on cardiovascular outcomes: a meta-analysis of randomized controlled trials". PLOS ONE. 8 (2): e56803. Bibcode:2013PLoSO...856803Y. doi:10.1371/journal.pone.0056803.

[56] Gallie DR., 2013. "L-ascorbic Acid: a multifunctional molecule supporting plant growth and development". Scientifica. 2013: 1–24. doi:10.1155/2013/795964. PMC 3820358. PMID 24278786.

[57] Flavonoids: chemistry, biochemistry, and applications. Andersen, Øyvind M., Markham, Kenneth R. CRC, Taylor & Francis. 2006. ISBN 0849320216.

[58] Galli F, Azzi A, Birringer M, Cook-Mills JM, Eggersdorfer M, Frank J, et al. (January 2017). "Vitamin E: Emerging aspects and new directions". Free Radical Biology & Medicine. 102: 16–36. doi: 10.1016/j.freeradbiomed.2016.09.017.

[59] EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA)2, 3 European Food Safety Authority (EFSA), Parma, Italy (2010). Scientific Opinion on the substantiation of health

claims related to various food(s)/food constituent(s) and protection of cells from premature aging, antioxidant activity, antioxidant content and antioxidant properties, and protection of DNA, proteins and lipids from oxidative damage pursuant to Article 13 (1) of Regulation (EC) No 1924/20061. EFSA Journal. 8 (2): 1489. doi:10.2903/j.efsa.2010.1489

[60] Cermak R, Wolffram S (October 2006). "The potential of flavonoids to influence drug metabolism and pharmacokinetics by local gastrointestinal mechanisms". Curr. Drug Metab. 7 (7): 729–44. doi:10.2174/138920006778520570. PMID 17073577

[61] Flavone. ChemSpider, Royal Society of Chemistry. 2015.

[62] Flavanones, Webster's New World College Dictionary (Fifth ed.), Houghton Mifflin Harcourt Publishing Company, 2014

[63] Kaufman PB, Duke JA, Brielmann H, Boik J, Hoyt JE (1997). "A comparative survey of leguminous plants as sources of the isoflavones, genistein and daidzein: implications for human nutrition and health". J Altern Complement Med. 3 (1): 7–12. CiteSeerX 10.1.1.320.9747. doi:10.1089/acm.1997.3.7.

[64] Heber, D (2008). "Plant Foods and PhyTOChemicals in Human Health". In Berdanier, C.D; Dwyer, J.T.; Feldman, E.B. (eds.). Handbook of Nutrition and Food, Second Edition. CRC Press. pp. 176–181. doi:10.1201/9781420008890.ch70. ISBN 978-0-8493-9218-4.

[65] Soy. MedlinePlus, US National Library of Medicine.

[66] Varinska L, Gal P, Mojzisova G, Mirossay L, Mojzis J (2015). "Soy and breast cancer: focus on angiogenesis". Int J Mol Sci (Review). 16 (5): 11728–49. doi:10.3390/ijms160511728. PMC 4463727

[67] Wei, P; Liu, M; Chen, Y; Chen, D. C. (2012). "Systematic review of soy isoflavone supplements on osteoporosis in women". Asian Pacific Journal of Tropical Medicine. 5 (3): 243–8. doi: 10.1016/S1995-7645(12)60033-9

[68] Lipoic acid. Micronutrient Information Center, Linus Pauling Institute, Oregon State University, Corvallis.

[69] Haenen, GRMM; Bast, A (1991). "Scavenging of hypochlorous acid by lipoic acid". Biochemical Pharmacology. 42 (11): 2244–6. doi:10.1016/0006-2952(91)90363-A.

[70] Shay, KP; Moreau, RF; Smith, EJ; Hagen, TM (June 2008). "Is alpha-lipoic acid a scavenger of reactive oxygen species in vivo? Evidence for its initiation of stress signaling pathways that promote endogenous antioxidant capacity". IUBMB Life. 60 (6): 362–7. doi:10.1002/iub.40. PMID 18409172.

[71] Shay, KP; Moreau, RF; Smith, EJ; Smith, AR; et al. (October 2009). "Alpha-lipoic acid as a dietary supplement: Molecular mechanisms and therapeutic potential". Biochimica et Biophysica Acta. 1790 (10): 1149–60. doi: 10.1016/j.bbagen.2009.07.026. PMC 2756298.

[72] *Shay, KP; Shenvi, S; Hagen, TM. "Ch. 14 Lipoic Acid as an Inducer of Phase II Detoxification Enzymes Through Activation of Nr-f2 Dependent Gene Expression". Lipoic Acid: Energy Production, Antioxidant Activity and Health Effects. pp. 349–71.* In Packer & Patel 2008.

[73] Ebisch IM, Thomas CM, Peters WH, Braat DD, Steegers-Theunissen RP (March–April 2007). "The importance of folate, zinc and antioxidants in the pathogenesis and prevention of

subfertility". Human Reproduction Update. 13 (2): 163–74. doi:10.1093/humupd/dml054

[74] Nomenclature and symbolism for amino acids and peptides (IUPAC-IUB Recommendations 1983), Pure Appl. Chem., 56 (5): 595–624, 1984, doi: 10.1351/pac198456050595

[75] The primary structure of proteins is the amino acid sequence. The Microbial World. University of Wisconsin-Madison Bacteriology Department.

[76] Belitz, H.-D; Grosch, Werner; Schieberle, Peter (2009-02-27). Food Chemistry. ISBN 9783540699330.

[77] Sekhar, Rajagopal V; Patel, Sanjeet G (2011). "Deficient synthesis of glutathione underlies oxidative stress in aging and can be corrected by dietary cysteine and glycine supplementation". The American Journal of Clinical Nutrition. 94 (3): 847–853. doi:10.3945/ajcn.110.003483. PMC 3155927. PMID 21795440

[78] Michael Brownlee (2005). The pathobiology of diabetic complications: A unifying mechanism. Diabetes. 54 (6): 1615–25. doi:10.2337/diabetes.54.6.1615. PMID 15919781

[79] Guoyao Wu, Yun-Zhong Fang, Sheng Yang, Joanne R. Lupton, Nancy D. Turner (2004). Glutathione Metabolism and its Implications for Health. Journal of Nutrition. 134 (3): 489–92. doi:10.1093/jn/134.3.489. PMID 14988435.

[80] Dringen R.,2000. "Metabolism and functions of glutathione in brain". Progress in Neurobiology. 62 (6): 649–71. doi:10.1016/s0301-0082(99)00060-x. PMID 10880854. S2CID 452394.

[81] Scholz, RW. Graham KS. Gumpricht E. Reddy CC. (1989). "Mechanism of interaction of vitamin E and glutathione in the protection against membrane lipid peroxidation". Ann NY Acad Sci. 570 (1): 514–7. Bibcode: 1989NYASA. 570..514S. doi:10.1111/j.1749-6632.1989.tb14973.x.