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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, pH 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	[14]
		• A synthetic antioxidant, used to prevent fats in foods from going rancid and as a defoaming agent for yeast	
3	Butylated Hydroxy Toluene (BHT, E321) 2,6-di-tert-butyl-4-hydroxytoluene	• Anticipated to be a human carcinogen	[15]
		• 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]
4	Octyl gallate (E 311) and dodecyl gallate (E 312)	BHT is tumour promoters, in high quantity	[15, 18]
		• 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 carotenenes.

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	[46]
		• α -tocopherol can also generate alkyl radicals, which can initiate the self-oxidation of unsaturated fatty acids	
		• 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]
2	Vitamin C (<i>ascorbic acid</i>)	• Can slowing the progression of age-related macular degeneration (AMD)	[51]
		• 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 flavylum 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.
Flavylum Cation (a) and general pyramidal oxonium ion (b).

Figure 15.
Basic Structure of Anthocyanidins (R_3' and $R_4' = -OH$).

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

(-)-Epicatechin

(+)-Catechin

(+)-Gallocatechin

(-)-Epigallocatechin

(-)-Epigallocatechin gallate

(-)-Epicatechin gallate

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), tangeritin (4', 5,6,7,8-pentamethoxyflavone), chrysin (5,7-dihydroxyflavone) and 6-hydroxyflavone are compounds that belong to the class of flavones [62].

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 $\text{HOOC-CH}(\text{NH}_2)\text{-CH}_2\text{-SH}$. 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)):

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.

and with free radicals:

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 heteroprotein nature from the group of aerobic dehydrogenases having as active groups derivatives of vitamin B2 (riboflavin or 7,8-dimethyl-10-ribityl-isoalloxazine), 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).

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.
Separation 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.

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Conflict of interest

The author declare no conflict of interest.

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