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Chapter 3

Antioxidant Compounds and Nutraceutical Benefits of Mediterranean Red Fruit

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Abstract

Today, the responsibility of reactive oxygen species (ROS) on the onset of non-transmissible chronic degenerative diseases has been clearly recognized by the scientific community. Epidemiological studies show that eating habits can significantly influence the incidence of neurodegenerative diseases. A conspicuous part of the protective effects of the Mediterranean diet is due to the presence of antioxidants in foods and beverages obtained from red fruits. The protective effect of dietary antioxidants from red fruits is supported by data obtained from animals, showing that the supplementation of the diet with red fruits can have beneficial effects against different types of cancer, neurodegenerative diseases and cardiovascular damage. This chapter aims to contribute to a better understanding of the abilities of the antioxidant compounds of red fruits to counteract the damaging effects of ROS and will focus on the relevance of their antioxidant action.

1. Introduction

Today, the responsibility of reactive oxygen species (ROS) on the onset of non-transmissible chronic degenerative diseases has been clearly recognized by the scientific community. Indeed, ROS react with amino acid residues (in particular cysteine, proline, arginine and histidine), lipids and DNA, so inducing the structural and functional alterations capable of inhibiting the biological activity of cells [1]. The chemical species ROS are very unstable and highly reactive intermediates that are generated as a result of external stimuli (ionizing radiation, chemicals, drugs, stress) and of normal cellular metabolic reactions [2]. It is well known that during cellular respiration, which occurs at the level of the inner membrane of mitochondria, cells perform the complete oxidation of organic substrates at the expense of oxygen to obtain carbon dioxide (CO_2) and water. In the mitochondrial respiratory processes, the main substrate is pyruvate, which comes from glycolysis, but fatty acids and organic acids are also used.

Within the mytochondrial respiratory electron transport chain, the reduction of molecular oxygen (O_2) in superoxide ($O_2\bullet^-$) occurs. This latter is the precursor of many other ROS, such as H_2O_2 (hydrogen peroxide), HO• (hydroxyl radical), $HO_2\bullet$ (hydroperoxide radical), $^1O_2$ (singlet oxygen), and nitric oxide NO•. The presence of the unpaired electron in the last orbital make these chemical species highly unstable and able to bind with biological macromolecules, in turn converted into free radicals. Approximately 3 to 10% of the oxygen used by the tissues is converted to its reactive intermediates, which damages the functioning of cells and tissues [3]. These indirect negative effects of ROS can cause lipid oxidation, that inevitably leads to inefficiencies in trans-membrane processes, protein oxidation (with particular regard to thiol groups) and consequent change in the kinetics and inactivation of many enzymes [4], alteration of the mechanisms involved in cellular regulation, signal transduction and gene transcription [5, 6], oxidative damage at the level of DNA and telomeres with consequent cell death apoptosis, cell degeneration and neoplastic transformation [7]. These negative effects on cell metabolism are further increased when an imbalance between the amount of ROS produced and the control systems for their scavenging occurs.

It is certain and scientifically proved the involvement of ROS in the processes of the pathogenesis of many forms of disease, ranging from inflammations to immunosuppression, from liver cirrhosis to atherosclerosis, from diabetes to Alzheimer's disease (AD) and various types of cancer [1, 8]. However, epidemiological studies have clearly shown that the adoption of the Mediterranean diet, based on fruits and vegetables rich in specific micronutrients, vitamins and plant pigments (phytochemicals) helps to restore a balance between the production of free radicals and control ROS-scavenging systems, impacting positively on the reduction of the oxidative damage [8 - 16].

2. Oxidative Stress in Humans

The term oxidative stress refers to a condition, which is characterized by the accumulation of non-enzymatic oxidative damage to molecules that threatening the normal function of the cell or the organism [17]. Several authors have shown that there is a close relationship between oxidative damage, cell aging and the onset of many diseases [18 - 20], ranging from various forms of cancer, neurodegenerative and cardiovascular damage. Observational studies on vegetarians, regular consumers of natural, fresh and simple food, rich in bioactive compounds, highlighted the low incidence rate of the onset of cancer and dementia [1 - 3, 21]. Likewise, women with breast cancer, have shown reduced recurrence rates, as well as less risks of mortality, following a antioxidant-based diet [4, 22]. However, it should be recalled that the production ROS is a condition absolutely physiological and in normal cases not related to cytotoxic effects. In fact, they are molecules that play essential roles for cell metabolism acting, in some cases, as second messengers [23]. The mechanisms of action of ROS have been highlighted not only in eukaryotic cells, but also in bacteria and in plants [22, 24]. It seems that ROS are mainly involved in the regulation of some transcription factors [24 - 28], cellular signal transduction [29 - 37], and cell growth, death by apoptosis, differentiation and immune response.

Indeed, high concentrations of ROS are a source of damage to all biological macromolecules. The imbalance between the production of molecules and the control systems can cause modifications of proteins, lipids and DNA. Proteins undergo fragmentation of the aminoacidic chains, especially in correspondence with arginine, proline and histidine residues. The oxidative damage appears also to induce the formation of crosslinks between two or more proteins by the oxidation of sulfhydryl groups of cysteine [38]. Overall, these alterations result in a premature degradation of damaged proteins and the consequent reduction of their biological function. In this regard, some enzymes are very sensitive to the oxidative damage. These include mitochondrial aconitase [39], all metalloenzymes [40], the enzymes involved in the Calvin cycle (in particular, fructose bisphosphatase and glyceraldehyde-3-phophate dehydrogenase) [41]. Another indication of protein oxidation is the production of nitrotyrosine from peroxynitrite, which could be a useful clinical parameter of the presence of oxidative stress in neurodegenerative diseases, as high levels of nitrotyrosine have been found, after autopsy, in patients with Parkinson's disease (PD) and Amyotrophic Lateral Sclerosis (ALS) [42 - 47].

Lipid peroxidation is considered the main source of damage by ROS. This induces a series of chain reactions (initiation, propagation and termination), which destabilize the structure of the phospholipid bilayer of cellular membranes, with oxidation of polyunsaturated fatty acids (PUFA), formation of toxic species, such as isoprostanes and aldehydes. These include propanal, butanal, pentanal, hexanal and 4-hydroxy-2-trans-nonenal (4-HNE). This last product appears to be involved in neuronal death, and in particular in the pathogenesis of PD [48, 49] and in the consequent complete destruction of the lipid bilayer of cellular membranes.

Oxidative damage to DNA consists in the rupture or rearrangement of the double helix following the formation of hydantoins, glycols, deaminated products and rearrangements in the pyrimidine ring [50].

2.1. Damage Caused by Oxidative Stress and Apoptosis

Any damage to the cells, which have been originated by chemical agents or toxic stimuli, can trigger a complex series of events culminating in cell death. This process can follow two distinct pathways, called the accidental necrosis and the programmed cell death. The former generally appears as a result of pathological processes, such as loss of membrane integrity, cell lysis and consequent release of cytoplasmic components in the extracellular space, inflammatory state, and damage amplification. The latter does not take place only after to pathological events, but is a physiological mechanism occurring during normal body development and growth.

From a morphological point of view, the cells destined to apoptosis lose contact with the surrounding cells and undergo condensation and fragmentation. During apoptosis, cell cytoplasmic organelles assume a compact form and chromatin condenses, resulting in DNA destruction. The resulting DNA fragments are surrounded by the plasma membrane, looking like bubbles (blebbing), which tend to weld together and give rise to "apoptotic bodies", without any damage to neighboring cells. However, particular attention should be placed on the nerve cells, highly sensitive to this type of mechanism. Indeed, a vast scientific literature reports that there is a relationship between ROS production, apoptosis and pathogenesis of neurodegenerative disorders [51 - 53].

2.1.1. Neurodegenerative Damage

If we consider that the human brain is only 2% of body weight, but it consumes an amount of oxygen significantly higher than that of all other organs (about 20% of the quantity available), and it has a high metabolic activity combined with a reduced capacity for cell regeneration and a lower level of antioxidant defenses, it is easy to understand why the structures of the central nervous system (CNS) are particularly susceptible to oxidative damage. In particular, the dopaminergic neurons are strongly susceptible, as the metabolism of dopamine (DA) generates hydrogen peroxide and peroxide radicals, and dopamine oxidation generates dopamine-quinone [54], a protein capable of damaging other proteins and with an essential role in the onset of a Parkinsonian symptomatology.

Also, excessive exposure to ROS seem to be implicated, as demonstrated in experiments of neurotoxicity *in vitro* and *in vivo*, in the production of amyloid peptides, responsible for the onset of AD [55 - 58].

It seems that ROS are eliminated from the cellular antioxidant systems, that includes enzymes as glutathione peroxidase (GPX), ascorbate peroxidase (APX), catalase (CAT), and superoxide dismutase (SOD). Indeed, it has been shown that brain tissues of PD-affected patients, the concentration and activity of these enzymes is significantly lower if compared to normal patients. This suggests that oxidative stress, which is generated as a result of the reduction of cellular antioxidant defenses, not only may be an incidental damage to the cells, but could also trigger intracellular signaling pathways that lead to their death.

In PD, the oxidative damage of neurons, which are highly sensitive to ROS [59], also determines the oxidation of proteins and their subsequent precipitation. It has been demonstrated that α-synuclein, a small presynaptic protein whose physiological role is not yet known, shows increased ability to aggregation and precipitation when oxidized, and it also appears to exist a relation between the oxidative damage to this protein and exposure to herbicides [60 - 62].

Several studies have proved that alterations in the functionality of mytochondrial complex I can expose the cells not only to an energy deficit, but also to oxidative damage [63]. The increase of ROS could amplify the presence of structurally abnormal proteins, which require an increased functionality of the ubiquitin-proteasome system to be eliminated. The alterations of this complex, reducing the availability of energy, may limit the vesicular storage of dopamine, whose level could increases in the intercellular spaces and interact with other cellular proteins. This would explain why dopaminergic neurons are so sensitive to oxidative damage. While the alterations of the mitochondria have been deeply documented, it is impossible to verify with autopsy, as dopaminergic neurons die before the analysis.

However, at present, it is unclear whether there is a final common pathway or if different stimuli lead to cell death. The most important type of programmed cell death is apoptosis. In PD, has not yet been established whether the process of apoptosis is responsible for the primitive cell death, or whether it simply performs its original function, namely to remove irreversibly damaged cells.

2.1.2. Cardiovascular Diseases

Atherosclerosis is a multifactorial disease that represents the leading cause of death in the world. High levels of LDL (low density lipoprotein) in plasma and low levels of HDL (High density lipoprotein) are associated with a high risk of atherosclerosis, following oxidation of LDL. Specifically, LDL is a spherical particle composed of fats and proteins, whose central nucleus contains triglycerides and cholesterol esters. It has been seen that these particles also contain antioxidants, such as vitamin E. The LDL represents a serious danger to the arterial walls, especially when an oxidative process modifies them. The oxidation of LDL, or peroxidation, is a chain reaction caused by free radicals. By means of *in vitro* experiments, it has been shown that the oxidation of these lipoproteins occurs less frequently in the presence of antioxidants. Therefore, flavonoids appear to have the ability to block LDL oxidation and platelet aggregation, so reducing the risk of atherosclerosis.

Many epidemiological studies correlate a moderate consumption of red wine and green tea, particularly rich in flavonoids, with a lower risk of cardiovascular diseases. In addition, olive oil appears to strongly reduce LDL oxidation, and its antioxidant effect seems to be due to the presence of polyphenolic compounds such as hydroxytyrosol and its precursor oleuropein.

The high content of monounsaturated fatty acids has an important protective role on human health. In fact, it was tested that substitution of saturated fats with monounsaturated or polyunsaturated fats in the diet reduces the levels of LDL, which are therefore less available for oxidative processes. Experimental studies have also shown that the LDL rich in oleic acid, the most abundant fatty acid of olive oil, are much more resistant to oxidation.

2.1.3. Endothelial Damage

Several authors have shown that oxidative damage greatly influence vascular function by modulating the activity of contraction and expansion, as well as cell growth, apoptosis, migration, and inflammation [64 - 67]. The mechanism of action by which ROS act at the level of the vascular endothelium is strongly related to their ability to modulate the expression and function of some important genes, including the vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF) and platelet-derived growth factor (PDGF), which appears to play an important atherogenic role in the regulation of cell growth and differentiation [68].

2.1.4. Metabolic Disorders

According to Reitman et al. [69], obesity, a disease with a complex etiopathogenesis, seems to be related to a decrease in the concentration of plasma antioxidants. Furthermore, fat increase in adipocytes appears to be closely related to the markers of oxidative stress [70] and is widely correlated with body mass index (BMI) [71]. On this basis, it is possible to define obesity as a situation of chronic inflammation [72].

Generally, obesity is frequently associated with metabolic diseases like diabetes, and it has been found that the increase of free fatty acids (FFA) in the pre-diabetic phase induces oxidative stress due to increased mitochondrial activity (for example, in the case of increased beta oxidation and mitochondrial uncoupling), with a significant increase in ROS production. In knockout mice, it has also been demonstrated that ROS alter the metabolism of methionine, involved in the enhancement of insulin resistance, and that hyperglycemia is itself an activator of oxidative stress.

These phenomena increase the risk of the onset of a common disease called NAFLD (non-alcoholic fatty liver disease), which in turn can lead to hepatocarcinome (HCC). Probably, the mechanisms responsible of this disease are multiple and include mitochondrial dysfunction, oxidative stress, lipid peroxidation, inflammation and alterations in methionine metabolism [73 - 75].

2.1.5. Oxidative Damage and Cancer

If the concentration of ROS is controlled, their action on cells can be protective, for example inducing phenomena as stasis of the cell cycle, senescence, apoptosis or necrosis of the altered cells. On the other hand, it has been shown that during a condition of ROS imbalance, the action of ROS promotes the proliferation, invasiveness, and metastasis formation of tumor cells.

In addition to directly cause oxidative damage, ROS appear to influence the behavior of cells in many ways. Depending on the types and levels of ROS, ROS exposure time, ROS-removing cellular antioxidant defenses, and activities of cell repair systems, the responses or cells exposed to ROS can include increased proliferation, dysfunctions in cell cycle, senescence, apoptosis or necrosis. The responsibility of ROS in promoting initiation and progression of the tumor may be due to their effects on cell cycle, gene expression, direct or indirect damage to DNA, and other forms of apoptosis and cell death.

It was observed that knockout mice lacking Cu/Zn-SOD, a strong antioxidant enzyme defined as the main scavenger of superoxide radical ($O_2{\bullet}^-$), have a high chance of developing liver cancer. However, at mitochondrial level, Mn-SOD plays a key role, and its deficiency in mice is lethal. Heterozygous animals (50% of normal mitochondrial Mn-SOD) survive, but show increased risk of developing lymphomas, adenocarcinomas and pituitary adenomas with age.

3. Main Types of Antioxidants

An antioxidant is defined as "any substance that, present in very low concentration compared to that of an oxidisable substrate, is able to significantly retard or inhibit the oxidation of that substrate" [76]. An oxygen atom has eight external electrons. The metabolic reactions can deprive the atom of an electron, turning it into a free radical. When the free radical subtracts an electron from a molecule located in the cell membrane, a new free radical is formed and a chain reaction starts. The chain of "kidnapped electrons" damages the cell membrane, leading to the disintegration of the cell and opening the way for cancer and other diseases.

On the basis of their origin, antioxidants can be divided into endogenous or exogenous, depending if they are produced by the body or are obtained from the diet, respectively. In relation to their mechanism of action, antioxidants are distinguished in:

a) preventive or primary antioxidants that, through the chelation of transition metals or the "quenching" of the oxygen singlet, prevent the upstream ROS generation. In this way, the sequence of chain reactions of radical production is not just triggered.
b) the secondary antioxidants or the chain-breaking type antioxidants (i.e. polyphenols), that are agents able to block free radicals, transforming them in less reactive compounds from the starting ones. In this way, these antioxidants are able to act in the phases of initiation and propagation of radical reactions thereby slowing down their speed.

However, in nature, the limits between these classes of antioxidants are not so clear either because some mechanisms of action of certain antioxidants have not been well defined or because there are substances, such as phenolic compounds, which can act simultaneously as first and second type antioxidants.

3.1. Endogenous Antioxidants

The main endogenous enzymes arrayed against the excess of oxygen free radicals include SOD, GPX, APX, CAT, other peroxidases using different substrates (PDX), and a series of water-soluble or liposoluble agents distributed both inside the cells and in extracellular fluids, such as, among others, ascorbate, glutathione, tocopherols, lactoferrin and transferrin.

The antioxidant enzymes cited above act synergically to reduce the $O_2^{\bullet -}$ to H_2O_2, a less reactive chemical species. Therefore, this transformation allows limiting the concentration of ROS within the cell.

Among the non-enzymatic endogenous antioxidants, we have referred to glutathione, able not only to neutralize free radicals, but also to regenerate in their active reduced forms other antioxidants, such as vitamins C (ascorbate) and E (α-tocopherol). Lactoferrin and transferrin play a preventive antioxidant function, expressed in terms of iron chelation. In this way, iron is prevented to react with peroxides and these latter are not transformed into strongly harmful free radicals, such as alkoxy and peroxyl, by Fenton reaction.

3.2. Exogenous Antioxidants

Exogenous antioxidants, widely present in fruits and vegetables, can be divided into three main classes:

a) Vitamins
b) Carotenoids
c) Phenolic compounds

3.2.1. Vitamins

Among vitamins, vitamin C (ascorbic acid) and vitamin E (α-tocopherol) play an important role. Indeed, vitamin C is the most powerful water-soluble antioxidant present in blood plasma and also acts as a regenerator of vitamin E [77] and cationic species radical of carotenoids [78]. Block et al. [79] observed that vitamin C may reduce the levels of C-reactive protein (CRP), a marker used to monitor the inflammation of the cardiovascular diseases. It is well known that vitamin C is capable of removing $O_2^{\bullet -}$ and HO•, and regenerating tocopherols [80].

Vitamin E, a generic term to define the family of derivatives of α-tocopherol (tocopherols and tocotrienols), is a fat-soluble vitamin that extrinsic its function through the donation of a hydrogen atom with the following formation of tocopheroxyl radical [81]. Vitamin E has strong protective effects against cardiovascular and coronary diseases due to its inhibiting action toward the oxidation of low-density lipoprotein-cholesterol (LDL-cholesterol) [82].

3.2.2. Carotenoids

Carotenoids are a family of yellow, orange and red pigments produced in plant tissues. They are compounds resulting from the head-to-tail condensation (with 1,4 bounds) of isoprenic units (C_5H_8). All the double bonds present in a carotenoid may be in *cis* or *trans* form.

The hydrocarbon carotenoids, formed only by carbon and hydrogen, are called carotenes (tetraterpenes), while those containing also oxygen are called xanthophylls. Many carotenoids have been isolated and the most important ones are lycopene, β-carotene, α-carotene, phytoene, phytofluene and lutein. While carrots, papaya, and mango are rich in β-carotene, tomatoes contain particularly high levels of lycopene.

The carotenoids scavenge singlet oxygen ($^1O_2$) by a "quenching" effect, acting on the transfer of energy between molecules. In fact, carotenoids are able to receive the molecular energy of $^1O_2$ by producing stabilized oxygen (O_2) and excited carotenes at the triplet state. Instead of generating further chemical reactions, carotenoids dissipate the excess energy in the medium in which they are located. Furthermore, as in the course of these reactions carotenoids do not change, they are again available for other cycles of $^1O_2$-quenching [83]. The effectiveness of carotenoids as quenchers is related to the number of double bonds, so the N-carotene, zeaxanthin, cryptoxanthin, W-carotene and, especially lycopene molecules are particularly active from this point of view.

Generally, all carotenoids, and in particular β-carotene, being liposoluble are localized in the lipids of the cell membrane, so providing a protective barrier against ROS. Several studies have shown a lower incidence of lung, prostate and stomach cancer, as well as cardiovascular diseases in individuals subjected to a carotenoid-rich diet. In particular, the consumption of foods rich in lycopene was associated with a lower incidence of prostate cancer [84] and infarct [85].

It was observed that carotenoids and in particular lycopene and β-carotene, are associated with various classes of lipoprotein; specifically, 75% of carotenoids are associated to low density lipoprotein (LDL) and 25% to high-density lipoprotein (HDL) [86]. Carotenoids also have a high antioxidant capacity, due to the ability of their conjugated double bonds to delocalize unpaired electrons. Therefore, their antioxidant action is of chain-breaking type. Human body enacts a series of reactions, through which the correct balance of oxidants/antioxidants is maintained: on the one hand, $O_2^{\bullet -}$ gives rise to secondary ROS that can interact with proteins, lipids and DNA, and on the other hand, the permanent of antioxidants tend to limit such damage, detoxifying and blocking ROS, so bringing lipids and vitamins in their original un-damaged conditions.

3.2.3. Polyphenols

Epidemiological studies have clearly demonstrated that consumption of fruits and vegetables, rich in phytochemicals with a high phenolic content, is strongly correlated with a reduction in cardiovascular and cerebrovascular diseases, as well as with a decrease in cancer mortality [87].

Polyphenols are the most abundant antioxidants of Mediterranean diet and are found in fruits, fresh and dried vegetables, cereals, olives, chocolate and beverages, such as tea, coffee and wine. Polyphenols are products of the secondary metabolism of plants, whose function is to protect the plant from the attacks of pathogenic parasites, and also to give color to some plant organs (e.g., fruits, flowers and leaves), making them attractive to insects and birds (pollination and seed dispersal).

Despite their wide distribution, studies on the effects of polyphenols have begun only in recent years. Several hydroxyl groups placed on aromatic rings characterize the basic structure polyphenol, but molecules with a single phenolic ring can also be considered polyphenols (e.g., phenolic acids and phenolic alcohols). The natural polyphenols are divided into several classes, depending on the number of phenolic rings and to the structural elements that bind each ring with one another.

3.2.3.1. Phenolic Acids

Both grapes and wines contain simple phenolic acids like benzoic acid and cinnamic acid, whose concentration varies from 100 to 200 mg L^{-1} in red wines and from 10 to 20 mg mL^{-1} in white wines. There are seven known types of benzoic acids (C6-C1). Two of them, salicylic acid (*o*-hydroxybenzoic acid) and gentisic acid (2,5-hydroxybenzoic acid) are present only in traces. Benzoic acids differ for the types of substituents of the benzene ring and are found in grapes, especially in the form of glycosides, which are liberated by acid hydrolysis, or in the form of esters (gallic and ellagic tannins), from which they are liberated by alkaline hydrolysis. The free forms are mostly present in red wines, because of the hydrolysis of their combined forms and of degradation reactions of more complex molecules, as it happens for anthocyanins under the action of heat. In grapes and wines, cinnamic-type phenolic acids, with a C6-C3 structure, are also present. They are located essentially as esters of tartaric acid or as glycosides of glucose, while the free forms are poorly represented.

3.2.3.2. Flavonoids

Flavonoids belong to a group of natural substances with variable phenolic structures and particularly abundant in fruits, vegetables, cereals, flowers, tea and wine. Over 4000 varieties of flavonoids have been identified, and many of them are responsible for the attractive colors of flowers, fruit and leaves. Structurally, they are formed by three phenolic rings (two of them, called A and B, are aromatic,) joined by a heterocyclic one (ring C) formed by three carbon atoms and one atom of oxygen [87, 88].

They are divided according to the structure of the ring C in: flavonols (e.g., quercetin, myricetin), flavones (e.g., luteolin and apigenin), flavanols (e.g., catechin and epicatechin), flavans, anthocyanidins and isoflavones [89, 90]. The functional properties of the flavonoids are influenced by a number of structural characteristics, here reported:

a) the presence of a hydroxyl group in the position 3 of the ring C: flavonoids aglycons having a 3-OH (e.g., quercetin, myricetin) are antioxidants more powerful than those having the same group substituted (e.g., diosmentin, apigenin, hesperetin and naringenin);
b) the presence of a double bond between C2 and C3 increases their antioxidant capacity;
c) their antioxidant activity increases with increasing the number of hydroxyl groups (-OH) on the ring B;
d) the presence of glycosidic groups causes a steric hindrance that reduces their antioxidant capacity.

Among flavonoids, anthocyanins are glycosides having a sugar, usually glucose, bonded to the C-3 position (anthocyanin = anthocyanidin aglycon + sugar). The presence of this sugar determines an increase of water solubility and allows anthocyanins to diffuse into the must and wine during maceration, contributing to the color intensity and properties of red wines. Based on the position of hydroxyl and methyl groups on the second phenyl ring, anthocyanin pigments are classified into five groups: cyanins, petunins, peonins, malvins, and delphinins.

Generally, flavonoids inhibit the activity of many enzymes, including lipoxygenases, cyclooxygenase, monooxygenase, xanthine oxidase, NADH oxidase, phospholipase A2, some protein kinases, and transcription factors such as NF-kB [91]. Because of the acidic character of the hydroxyl groups and of the nucleophilic properties of the phenolic rings, flavonoids are highly reactive. They appear to have antiviral, antibacterial, immunostimulant, anti-ischemic, anti-neoplastic, anti-inflammatory, anti-allergic, anti-lipoperoxidative and gastroprotective properties [92-94].

3.2.3.3. Resveratrol

Resveratrol (3,5,4'-trihydroxystilbene) belongs to the family of stilbenes. These are phenolic compounds, with low molecular weight, characterized by the presence of two aromatic rings joined by an ethane or by an ethenic bridge (C6-C2-C6). They are widely distributed in some Bryophytes and in higher plants, where they usually act as phytoalexins and as growth regulators. For example, lunularic acid is a dihydroxystilbene, which acts as a growth inhibitor in a manner analogous to abscisic acid (ABA). Resveratrol is a natural phytoalexin synthesized by plant in response to fungal attacks, or to abiotic agents, such as exposure to ultraviolet rays and drought. It is mainly synthesized in the berry skin of red grapes, and is transferred into wine [95 - 99].

4. Antioxidants in Mediterranean Red Fruits

4.1. Red Grape and Wine

4.1.1. Bioactive Compounds in Red Grapes

The main bioactive compounds extracted from the processes of grape transformation are essentially resveratrol, anthocyanins, proanthocyanidins (tannins), flavonols, and catechins [100]. Wine marc contains significant amounts of substances that have beneficial effects on human health, such as fibers (17-21%), tannins (16-27%) and other polyphenolic compounds (2-6.5%), fats (7-12%), sugars (3%), and salts of tartaric acid. In particular, polyphenols (mainly ellagic acid and quercetin) and resveratrol have relevance for their antioxidant properties and their ability to scavenge ROS [101]. Therefore, the use of residues of grape processing as a raw material for the production of bioactive compounds may constitute an interesting option.

4.1.2. Metabolic Effects of Grape Antioxidants

Grape polyphenols have antioxidant characteristics, with potential beneficial effects on human health, such as the reduction in the risk of cardiovascular diseases and cancer. Polyphenols have also been studied as an additional source of benefits of organic products, even though no conclusive evidence has come yet. They can bind the non-heme iron, a less absorbable form of iron (approximately 60% of the iron in meat and 100% of the iron in vegetables, fruits, milk and dairy products are in this form), so increasing iron absorption in the body. The recommended daily dose of polyphenols from red wine is 100 to 300 mg, which corresponds to about two glasses of red wine day^{-1}.

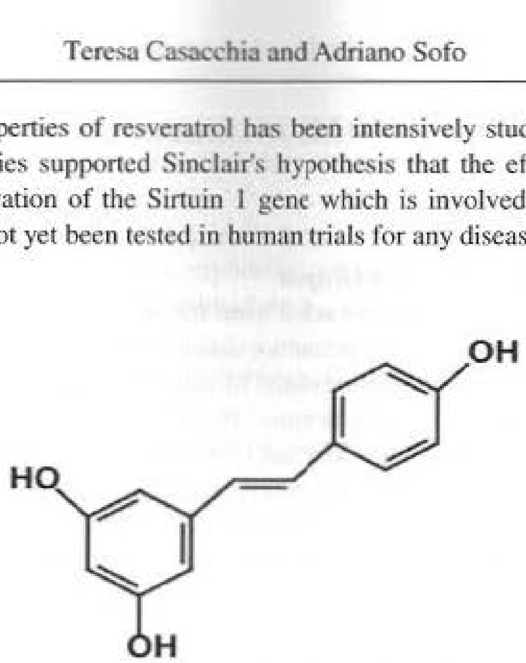
The wide range of health-promoting antioxidant compounds in grape and wine suggests that several different and interrelated mechanisms of action are involved. The study of the components present in grapes and wine, as in the case of flavonols and anthocyanins, may also contribute to winegrape taxonomic characterization [102 -104] and to certificate wine quality and origin.

4.1.2.1. Resveratrol

Red wines contain high levels of resveratrol (Figure 1), as do grapes, raspberries, peanuts, and other cultivated plant species [105]. This compound is located in the skin of red grapes and is a constituent of red wine.

On the basis of the results obtained with tests on animals, the average level of resveratrol in wine seems to be not sufficient to justify the "French paradox". Several plants produce Resveratrol, perhaps due to its antifungal properties. It is one of the most important grape polyphenols. Natural Resveratrol can be extracted from red grapes and wine but also by their roots.

Numerous beneficial effects of resveratrol on health, such as anti-cancer, anti-viral, neuroprotective, anti-aging, anti-inflammatory and "stretch-life" activity were found in animals (e.g., rats). Resveratrol was effective against dysfunction and death early detection of nerve cells, and it could also act against diseases such as Huntington's and Alzheimer's diseases. Resveratrol has been shown to reduce tumor incidence in animals by affecting one or more stages of cancer development. For all these reasons, the strong antioxidant and radical-scavenging properties of resveratrol has been intensively studied in both grapes and wines. Interesting studies supported Sinclair's hypothesis that the effects of resveratrol are indeed due to the activation of the Sirtuin 1 gene which is involved in life extension [106, 107]. Resveratrol has not yet been tested in human trials for any disease.



trans-resveratrol

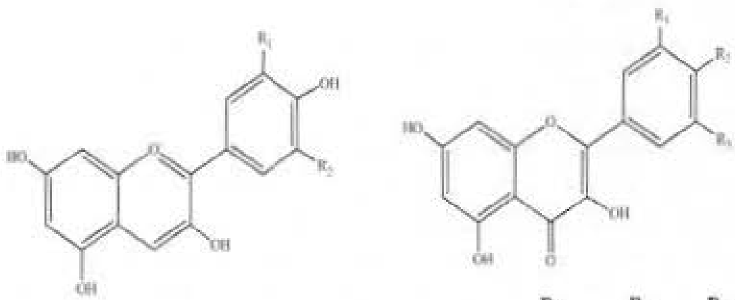
Figure 1. Molecular structure of trans-resveratrol.

Resveratrol has a wide range of pharmacological properties and it has been invoked to explain the "French paradox". This paradox dates back to the epidemiological studies conducted in the '70s that revealed an inverse correlation between red wine consumption and cardiovascular disease in France, a country where the consumption of saturated fats is particularly high. Other clinical studies have demonstrated that a moderate consumption of red wine determines a reduction of the risk factors for atherosclerosis. In red wine consumers, a reduction of platelet aggregation, an increase in plasma levels of HDL-cholesterol (responsible for the disposal of the excess cholesterol in peripheral tissues) and a lower oxidation of LDL occur. These events are associated with a lower formation of atherosclerotic plaques in blood vessels [95] and thus to a reduction of cardiovascular events, making resveratrol also a strong cardioprotective agent. Resveratrol, like many phytoalexins, possesses many activities, such as inhibition of platelet aggregation and lipid peroxidation, anti-inflammatory and vessel-relaxing properties, inhibition of the damage induced by ROS, and agonist properties for estrogen receptors. In the last ten years, it has been shown that resveratrol, flavonoids (e.g., genistein, quercetin) and other polyphenols are able to induce apoptosis of cancer cells [96, 97, 98]. In 1997, Jang et al. [99] reported that resveratrol possesses a strong antitumoral capacity, reducing tumor in rats. These authors demonstrated that resveratrol blocks the in vivo the three stages of carcinogenesis: initiation, promotion and progression. Other studies on cell lines deriving from human tumors confirmed that resveratrol is a chemopreventive agent.

However, the average amount of resveratrol present in any wine is negligible if compared to the amount necessary to obtain beneficial effects on health: 1-10 mg L^{-1} of wine against hundreds of thousands of mg day^{-1}. The amount of resveratrol in food varies greatly. The skins of fresh grapes contain from 50 to 100 µg g^{-1} of resveratrol. European red wines contain from 0.2 to 30.88 mg L^{-1} of total resveratrol, depending on the variety of grape, while white wines contain much less amounts. This is due to the fact that red wine is obtained by the fermentation of must deriving from grape skins rich in resveratrol. On the contrary, white wine is obtained by fermenting the must after berry skin removal, and thus the resulting wine is particularly poor of resveratrol.

4.1.2.2. Anthocyanins and Flavonols

Anthocyanins are a very large group of red-blue polyphenol plant pigments, located within vacuoles of the skin cells of red grape berries, characterized by a positive charge on the molecule [101, 108, 109] (molecular formula in Figure 2). They are also synthesized by some bacteria. In plants, they are responsible for the coloration of leaves, buds, roots, flowers and fruits.



	R_1	R_2
Cyanidin	OH	H
Peonidin	OCH_3	H
Delphinidin	OH	OH
Petunidin	OCH_3	OH
Malvidin	OCH_3	OCH_3

	R_1	R_2	R_3
Kaempferol	H	OH	H
Quercetin	OH	OH	H
Isorhamnetin	OCH_3	OH	H
Myricetin	OH	OH	OH
Laricitrin	OCH_3	OH	OH
Syringetin	OCH_3	OH	OCH_3

Figure 2. The anthocyanins and flavonols commonly found in red grape berry skin and wine.

Blueness and redness levels of these compounds are directly related to the number of free hydroxyl groups of methoxy groups. An example of this phenomenon is malvidine, the main anthocyanin of red grapes, that has the greatest degree of methylation and thus a reddest color.

The polyhydroxylated anthocyanins are able to react with oxidants, such as ROS, thus reducing the damage that these molecules may lead to cells and tissues. On the basis to this ROS-scavenging activity, such substances can be very important for their uses in medicine. For instance, anthocyanins appear to protect against capillary fragility and various processes of aging due to cellular changes caused by ROS, such as inflammatory processes and cancerous changes. Some of these activities have been also demonstrated for red wine consumption.

Anthocyanins have strong anti-inflammatory properties [110]. These compounds are used to fight coronary heart disease [111] and they are a dietary supplement for the prevention and treatment of metabolic disorders, in particular obesity, weight gain, insulin resistance syndromes, diabetes, fasting hyperlipidemia and osteoarthritis [112, 113].

In particular, cyanidin-3-glucoside, delphinidin-3-glucoside, pelargonidin-3-galactoside are the major anthocyanins that promote insulin secretion (1.4-fold at 4 mM glucose concentration) [114, 115]. In red wines, the free anthocyanin fraction is the main contributor to its total antioxidant capacity [116, 117]. Glycosilated and methoxy-derivatives of anthocyanins, like malvidin-3-glucoside, are the most effective as inhibitors at both the initiation and promotion-propagation stages of tumor promotion-carcinogenesis, and protect cells against oxidative damage. These antioxidant properties are due to the flavylium ion (Figure 3).

The presence and concentration of each of the five groups of anthocyanins strongly depend on the environmental conditions and viticultural practices [118 - 121]. Moreover, the pattern of anthocyanin accumulation appears to be closely related to genetic characteristics of the grape itself, and these pigments can be considered as chemical markers to differentiate grape cultivars [122 - 123].

Anthocyanins are also used as food additives and in particular constitute the die anthocyanin red (E163), used in jams and other normally acid food, such as yogurt. Anthocyanins are extracted from red grape skin, as by-product of wine production and processing. The extraction takes place with dilute acids and the product is a liquid containing sugars, acids, salts and pigments originally present in the berry skins. With drying, a water soluble powder relatively rich in these pigments is obtained.

In plants, anthocyanins act as powerful anti-oxidant, helping to protect the plant from ROS produced by the action of UV radiation and during metabolic processes (e.g., electron transport chain during mitochondrial respiration and chloroplastic photosynthesis). The antioxidant power of anthocyanins is maintained even after their consumption by other organisms, which provides a clear explanation of why fruits and vegetables with a red peel are an excellent source of nutrition. It is possible to extract from 30 to 75 mg of anthocyanins from 100 g of red grapes, and 24 to 35 mg from 100 mL of red wine.

On the other hand, flavonols (molecular formula in Figure 1) are pigments that vary in colour from white to yellow distributed in the cellular vacuoles of the epidermis and the first sub epidermal cell layers of berry skins and leaves of white and red grapes.

Noteworthy, flavonols play a protective role against UV radiations and their biosynthesis has found to be light induced in many plant species [101, 104]. In combination with anthocyanins, flavonols are involved in the long-term color stability of red wines by forming intermolecular co-pigments and pigmented polymers with tannins, also precipitating in colloidal coloring matter during wine aging, so positively affecting the aging of red wines [124, 125]. Furthermore, flavonols are considered beneficial because of its antioxidant protective role in the pathogenesis of multiple diseases associated to oxidative stress such as cancer, CHD and arteriosclerosis.

Quercetin, myricetin, laricitrin, kaempherol, isorhamnetin, syringetin and their derivatives are the most commonly flavonols found in grape and wine. Particularly, quercetin resulted to be the most active flavonoid, and high quercetin content is responsible for most of antioxidant activity of medicinal plants. Quercetin has demonstrated significant anti-inflammatory activity by inhibiting the beginning of inflammatory processes. For example, inhibits both the production and the release of histamine and of other allergic/inflammatory mediators.

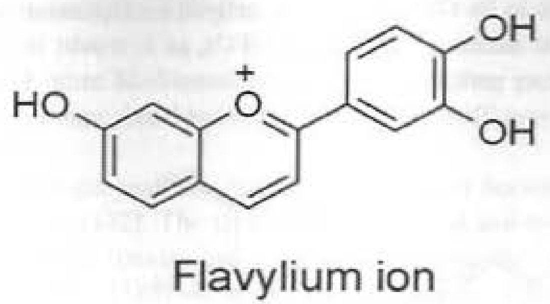


Figure 3. Molecular structure of the flavylium ion.

In addition, it exerts a potent antioxidant and protective action against vitamin C. Quercetin may have positive effects in combating or helping to prevent cancer, prostatitis, heart disease, cataracts, allergies/inflammations, and respiratory diseases, such as bronchitis or asthma. Kaempherol is able to prevent arteriosclerosis by inhibiting the oxidation of low-density lipoprotein and the formation of platelets in the blood. Current evidence indicates that kaempherol not only protects LDL from oxidation but also prevents atherogenesis through suppressing macrophage uptake of oxLDL. Numerous studies showed kaempferol might have health benefits for people at risk of cancer [126, 127, 128]. Finally, isorhamnetin inhibits adipogenesis through down-regulation the genes PPAR-gamma and C/EBP-alpha and represses adipogenesis in 3T3-L1 cells [129].

Although relevant advances in the knowledge of the structures of wine compounds, no systematic research on the qualitative and quantitative determination of the anthocyanins and flavonols has been carried out and the chemistry of wine phenolics subjected to different irradiance levels is still largely a matter of speculation [119]. It is known that the level of light can alter the chemical composition of the fruit both directly, through the activation of specific metabolic pathways or indirectly through control of the berry size [130, 131] or by the control of gas exchange or modification of the source-sink relationships [121]. At the same time, the qualitative and quantitative profiles of grapevine anthocyanins and flavonols also depend on berry skin characteristics, such as thickness and total surface per unit of volume, that in turn are affected by berry size [130].

4.1.2.3. Proanthocyanidins (Tannins)

Proanthocyanidins, another class of grape flavonoids, are polymers of catechin derivatives (Figure 4). They are also known as oligomeric proanthocyanidins (OPCs), tannins or condensed flavan-3-ols.

Jacques Masquelier, who was the first to develop techniques for their extraction from certain plant species, discovered proanthocyanidins in 1936. Because of incorrect food habits, most of the people do not assume sufficient amount of these compounds.

Proanthocyanidins can be found in many plants, particularly in the cortex of the pine, in grape skins and seeds, and in red wines. Since high concentrations of OPCs are present only in some parts of plants such as bark, seeds and skin, is practically impossible to assume the necessary amount of OPCs from our daily diet.

In particular, seeds, containing high amounts of OPCs, are rarely present in food, as sold table grapes are usually seedless. A conspicuous part of the beneficial effects of red wine

(French paradox) is due to its OPC content. A daily consumption of wine, however, is not sufficient to provide the necessary amount of OPCs, as it would be necessary to consume daily two liters of wine per person.

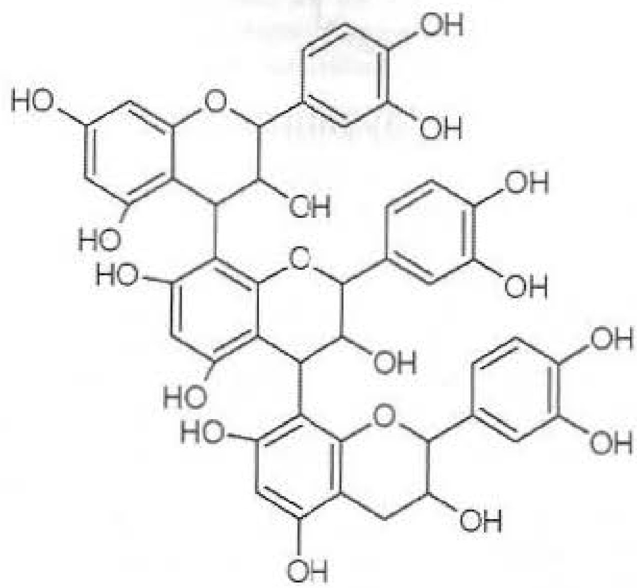


Figure 4. Building blocks of a proanthocyanidin.

The OPCs are powerful antioxidants, able to reduce ROS within the organism. These free radicals are produced by the normal oxygen metabolism, as well as by exposure to sunlight, chemicals, nicotine, alcohol, bacteria, parasites, some fatty acids, and many other substances and conditions. ROS can destroy cell membranes, damage connective tissues, disrupt important physiological processes, and create mutations within the DNA. In particular, collagen and elastin are the main proteins of the so-called connective tissue. With age, these tissues are oxidized, becoming wrinkled and stiff and losing their elasticity. In combination with the vitamin C, OPCs create a favorable environment for the biosynthesis of new collagen and elastin. The antioxidant effects of OPCs and their affinity for connective tissues are the reasons that justify their use, as they stabilize collagen and elastin, making skin young and smooth.

The oxidation of LDL-cholesterol is one of the main responsible for the hardening of the arteries and heart disease. The OPCs have proved very effective antioxidant properties and for this reason different doctors prescribe OPC-based products for the treatment of vascular diseases. Several studies have shown that OPCs are able to reduce tumor activity and propagation. However, these antioxidants cannot be considered effectively a cure for cancer, but rather have a preventive effect and strengthen the immune system by balancing the daily diet. It is believed that the ideal dosage of OPC is from 1 to 3 mg kg^{-1} of body weight day^{-1}.

4.2. Other Mediterranean Red Fruits

The consumption of other Mediterranean red fruits and their juices (e.g., pomegranate, watermelon strawberry, orange, small fruits) have received insufficient attention in relation to their antioxidant activity.

For example, pomegranate juice has become very popular because of the attribution of important biological actions [132]. The strong antioxidant and anti-tumor activity of tannins in the bark of pomegranate (punicacortein) [133, 134] and the antioxidant activity of pomegranate-fermented juice [135] were reported. However, detailed investigations of phenolic compounds and the antioxidant activity of pomegranate juice has not yet been fully clarified. In the next paragraphs, the antioxidant properties of these Mediterranean red fruits will be discussed in detail.

4.2.1. Pomegranate

It is not easy to explain in details all the antioxidant and health-promoting properties of pomegranate [136], so we decided to summarize the main benefits caused by their consumption (as fruits or juices), listed as follows:

- reduction of the risk of breast cancer [137 - 140];
- slowing of prostate cancer: studies on mice show that when fed with red fruits or juices, a slowing of prostate cancer occurs [137, 138, 141]. In a study on 50 men who regularly consumed a glass of pomegranate juice a day, PSA (Prostate Specific Antigen) levels remained stable, reducing the need for further treatments, such as chemotherapy or hormonal therapy. The protective activity against cancer pathologies is due to the high content of flavonoids, powerful ROS-scavenging antioxidants;
- protection of neonatal brain: studies show that their consumption is capable of protecting a child from the disorder that affects the neural tube [142];
- prevention of osteoarthritis: several researches show that they may prevent cartilage deterioration [143];
- protective action for arteries and heart. They combat hardening of the arteries and related diseases, like heart attacks and strokes [144];
- reduction in the blood levels of LDL-cholesterol (bad cholesterol) and increase in HDL-cholesterol (good cholesterol) [145];
- reduction of blood pressure: for instance, a study showed that drinking 50 mL of pomegranate juice day^{-1} can lower systolic blood pressure by 5% [146];
- prevention of AD. After a diet with red fruits or their juice, mice reduce or delay the manifestation of this disease, compared to the control that had instead followed a normal diet [147];
- reduction of the problems of erectile dysfunction, a disorder that affects 1/10 men worldwide and 10 million to 30 million of men in the U.S. Alone. Erectile dysfunction can be caused by several factors: high blood pressure, heart disease, diabetes, neurological damage, endocrine imbalance or depression [148]

The recommended dose of pomegranate juice is 250 mL day^{-1}, capable of supplying about 50% of the Recommended Daily Allowance (RDA) of vitamins A, C and E, and 13% RDA of potassium for adults. It is absolutely recommended the consumption for all women who want to prepare for pregnancy. For instance, pomegranate juice adds up to 100% RDA of folic acid, whose role is crucial for preventing serious diseases in children, such as the disorder of the neural tube.

4.2.2. Watermelon

In watermelon, an important protective role is played by citrulline (Figure 5), a non-essential α-amino acid synthesized by the human body but whose levels are significantly higher in this fruit. It is not encoded by mRNA codons, although it is part of some proteins, such as histones. Thus, the citrulline contained in proteins derives from a post-translational process called "citrullination", or deimination of arginine.

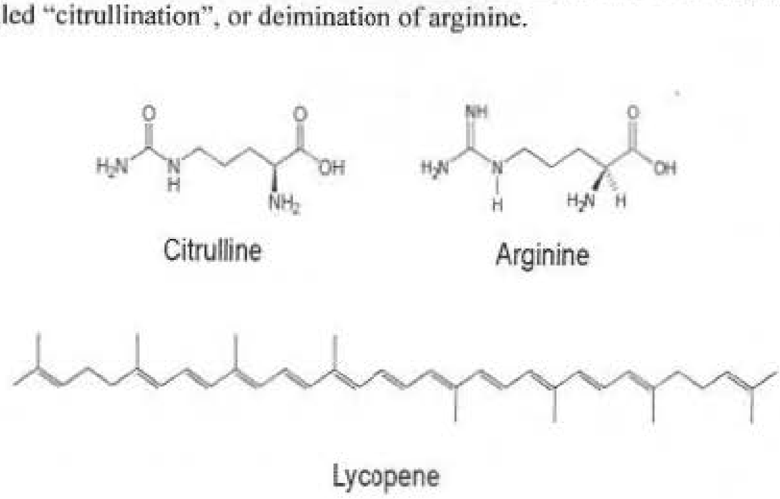


Figure 5. Main antioxidant compounds of watermelon.

This latter falls into the category of "conditionally essential amino acids" together with glycine, glutamine, proline and taurine. Citrulline is used in the human body to produce arginine (Figure 5), an amino acid essential for cell multiplication and the removal of ammonia from the body. Some authors suggest that a diet rich in watermelon can be a real remedy for the prevention of atherosclerosis and of other vascular complications, and for the control of high blood pressure and high levels of glucose in the blood [149 - 152].

Furthermore, according to a study conducted at the University of Texas, the arginine which originates from citrulline increases the concentration of nitric oxide, which prevents the hardening of blood vessels with a Viagra-like effect [153]. For sure, it has not the same effect of drugs, but may bring some benefits without side effects. Finally, it has been discovered the antibody against citrulline, which is a highly specific index for rheumatoid arthritis [154].

Watermelon is also a good dietary source of lycopene (Figure 5), an interesting antioxidant which seems to be implicated, due to its high number of conjugated dienes, in reducing the risk of various chronic degenerative diseases [155, 156]. In particular, research suggests that high blood levels of lycopene, are associated with a considerable reduction of risk of cardiovascular diseases [157].

These benefits appears to be related to the concomitant presence of other interesting phytonutrients, such as the glycoside esculeoside A, the flavonoids chalconaringenin, naringenin, rutin, and kaempferol, and the fatty acid 9-oxo-octadecadienoic acid. These phytonutrients seem to be implicated in the regulation of lipid metabolism and thus contribute to the decrease in total cholesterol, LDL-cholesterol and triglycerides.

4.2.3. Strawberry

Significant levels of antioxidants in the blood caused by consumption of strawberries [158, 159] have been observed in many studies, and their beneficial effects include the decrease of atherogenic risk [160 - 162], diabetes [163], multiple sclerosis [164], and different forms of cancer [165 -167], as well as a higher neuroprotective capacity [168]. The antioxidant capacity of strawberries appears to be the highest among other fruits, such as apples, peaches, pears, grapes, tomatoes, oranges and kiwifruit.

The benefits of the consumption of strawberries can be attributed to the very high content of vitamin C (Figure 6). For instance, in the summer, 5-6 strawberries can substitute one orange fruit, as 100 of strawberries contain 54 mg of vitamin C vs 50 mg of vitamin C in 100 g of oranges.

Furthermore, the antioxidant activity of strawberries is associated with the presence of ellagic acid (Figure 7), which is the most prevalent polyphenol and whose levels strongly depend on storage conditions, and of *p*-coumaric acid (Figure 5) [169 - 172].

The former is found in mean concentrations of 39.6 and 52.2 mg 100 g^{-1} of strawberries and decreases during the process of fruit ripening; the latter, whose concentration depends on climatic conditions, has average values ranging around 0.9 to and 4.1 mg 100 g fruits [169, 170].

Among flavonoids, ripe strawberries is almost absent in kaempferol (Figure 2). This likely depends on the extraction methods and times, and on storage temperatures. According to D'Antuono et al. [173], organic strawberries have higher antioxidant power, total phenolic content and ellagic acid levels if compared to those deriving from integrated cultivation. The anthocyanin content greatly affects the antioxidant capacity of strawberries, as these compounds represent between 52 and 92% of the total content of flavonoids [169].

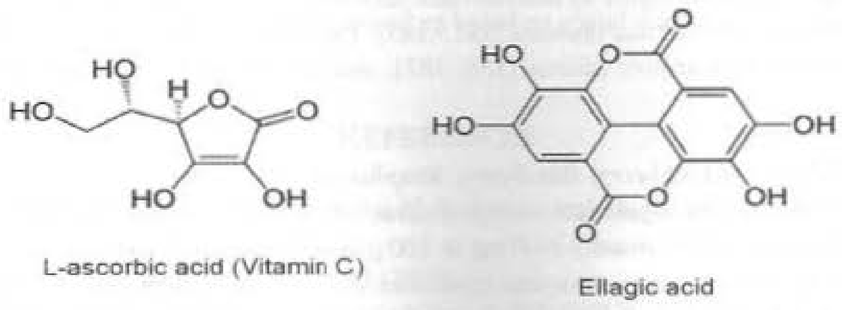


Figure 6. Main antioxidant compounds of strawberry.

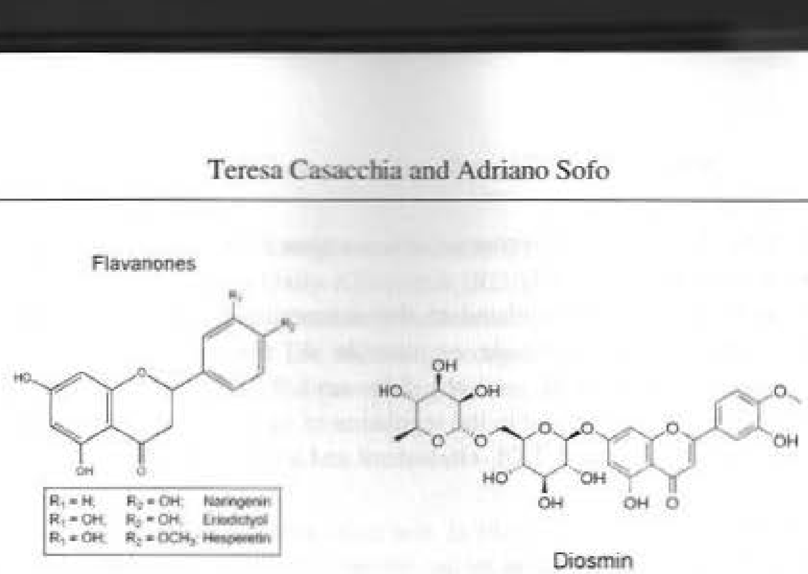


Figure 7. Main antioxidant compounds of orange.

4.2.4. Orange

Oranges are a rich source of antioxidants and nutraceutical activities if consumed regularly during the winter season. They have higher levels of vitamin C (Figure 7), whose concentration in the freshly pressed orange juice ranges between 35-56 mg 100 mL^{-1} [174]. Oranges also contain important ROS-scavenging anthocyanins, responsible for the red color of the varieties Sanguinella, Moro and Tarocco, such as cyanidin-3-glucoside, peonidin-5-glucoside, delphinidin-3-glucoside and petunidin-3-glucoside (Figure 2). In particular, cyanidin-3-glucoside has antioxidant properties higher than those of resveratrol and vitamin C, which results in a greater inhibition of LDL oxidative process [175] with consequent demonstrated protection of the myocardium [176]. Some studies [177, 178] examined the uptake of physiological concentrations of cyanidin-3-glucoside into human vascular endothelial cells. The experimental observations were carried out in isolated plasma membrane vesicles and intact endothelial cells from human endothelial cells and on an ischemia–reperfusion model in isolated rat hearts. Cyanidin-3-glucoside was transported via bilitranslocase into endothelial cells, where it acted as a powerful intracellular antioxidant and a cardioprotective agent in the reperfusion phase after ischemia [179]. Furthermore, recent studies demonstrated the anticancer activity of cyanidin-3-glucoside [180].

In oranges, phenolic acids (ferulic, coumaric, synapic, and ceffeic), flavanones (hesperidin, neohesperdin, hesperetin, poncirin, eriocitrin, neoeriocitrin, naringenin, eriodictyol, and narirutin; Figure 8) and flavcnes (diosmin; Figure 7) play significant roles in the prevention of cardiovascular diseases [181 - 185]. These molecules seem to have powerful anticancer and neuroprotective actions [186, 187], and are able to induce apoptosis [188 - 190].

4.2.5. Small Fruits (Blackberry, Blueberry, Raspberry)

Small fruits contain significant concentrations of several antioxidants [191 - 194], including vitamin C (approximately 19.0 mg in 100 g of edible portion), procyanidins, ellagic acid derivatives, and the very complex molecules ellagitannins (Figure 8). For instance, recent studies suggest that a diet rich in raw blackberry seems to have anticancer [195, 196] and anti-inflammatory actions [197].

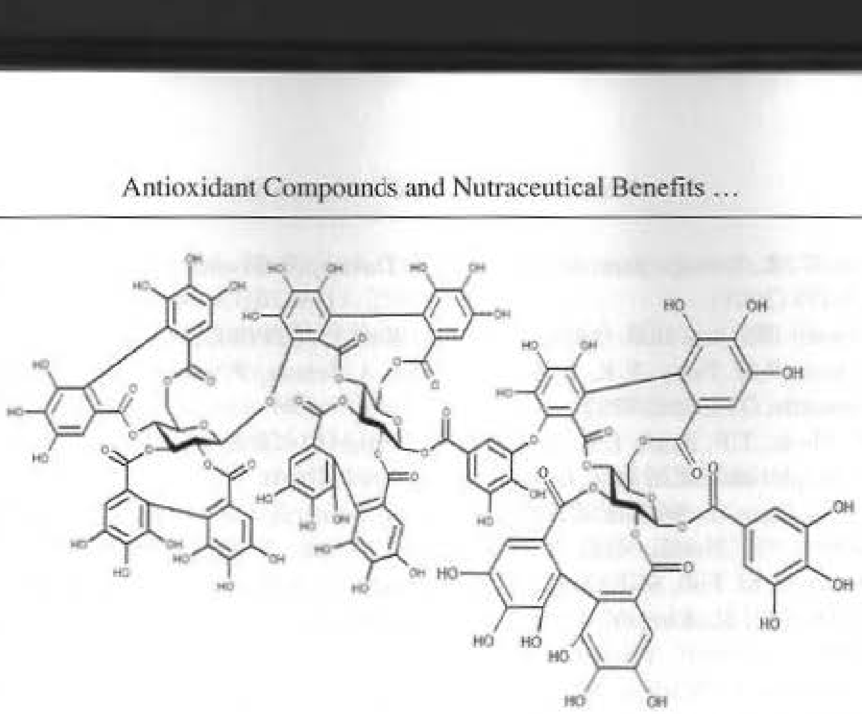


Figure 8. Raspberry ellagitannin containing 6 ellagic acid-type components and two additional monomeric phenolics, for a total of 14 gallic acid units.

CONCLUSIONS

Although the available data are still limited, epidemiological studies show that eating habits can significantly influence the incidence of neurodegenerative diseases such as dementia (Including AD) and PD. For example, data on AS incidence obtained from the so-called "Agees Personnes Quid" (PAQUID) studies showed that people who drink three to four glasses of wine a day (or red fruit juice equivalent) for three years have a decrease by 80% of dementia and AD, if compared to those who drink less or do not drink at all.

A conspicuous part of the protective effects of the Mediterranean diet is due to the presence of antioxidants in foods and beverages obtained from red fruits. Many epidemiological studies have also found an inverse association between the intake of vitamin E from red fruits and the appearance of PD. The protective effect of dietary antioxidants against neurodegenerative and cardiovascular diseases and different types of cancer is supported by data obtained from animals. Furthermore, the supplementation of the diet with red fruits can have beneficial effects on the age-related decline of neuronal and cognitive functions. The ability of these compounds to counteract the damaging effects of ROS and the relevance of their antioxidant action is therefore based on sound science.

REFERENCES

[1] M. Valko, C.J. Rhodesb, J. Moncola, M. Izakovica and M. Mazura, *Chem-Biol Interact,* 160, 1 (2006).

[2] JM. McCord, *Am. J. Med.* 108, 652 (2000).

[3] R. Noor, S. Mittal, J. Iqbal, *Med. Sci. Monit.* 8, 210 (2002).

[4] B. Ames, *J. Biol. Chem.* 278, 4369 (2003).

[5] Squier T.C. and D.J. Bigelow, *Front. Biosci.* 5: D504 (2000).

[6] Grune T, R. Shringarpure, N. Sitte and K. Davies, *J. Gerontol. A Biol. Sci. Med. Sci.* 56: B459 (2001).

[7] P. Jansen-Dürr and H.D. Osiewacz, *EMBO Rep.* 3, 1127 (2002).

[8] L. Lyras, R.H. Perry, E.K. Perry, P.G. Ince, A. Jenner, P. Jenner and B. Halliwell, *J. Neurochem.* 71, 302 (1998).

[9] J.M. Thole, T.F. Kraft, L.A. Sueiro, Y.H. Kang, J.J. Gills, M. Cuendet, J.M. Pezzuto, D.S. Seigler and M.A. Lila, *J. Med. Food,* 9, 498 (2006).

[10] K. de la Torre-Carbot, J.L. Chávez-Servín, O. Jaúregui, A.I. Castellote, R.M. Lamuela-Raventós, T. Nurmi, H.E. Poulsen, A.V. Gaddi, J. Kaikkonen, H-F. Zunft, H. Kisewetter, M. Fitó, M-I. Covas, C. López-Sabater, *J. Nutr.* 140, 501 (2010).

[11] J-K. Moon, J-R. Kim, Y.-J. Ahn and T. Shibamoto, *J. Agric. Food Chem.* 58, 6672 (2010).

[12] Y. Allouche, F. Warleta, M. Campos, C. Sanchez-Quesada, M. Uceda, G. Beltarn and J.J. Gaforio, *J. Agric. Food Chem.* 59, 121 (2011).

[13] N. Bendinelli, G. Masala, C. Saieva, S. Salvini, C. Calonico, C. Sacerdote, C. Agnoli, S. Grioni, G. Frasca, A. Mattiello, P. Chiodini, R. Tumino, P. Vineis, D. Palli and S. Panico, *Am. J. Clin. Nutr.* 93, 275 (2011).

[14] C.E. Guerrero-Beltrána, M. Calderón-Olivera, J. Pedraza-Chaverria and Y.I. Chirino, *Exp. Toxicol. Pathol.* Dec 1 2010, Epub ahead of print, http://www.sciencedirect.com/science/article/pii/S0940299310001831 (2010).

[15] C.E. Guerrero-Beltrána, P. Mukhopadhyaya, B. Horvátha, M. Rajesha, E. Tapiad, I. García-Torrese, J. Pedraza-Chaverrib and P. Pachera, *J. Nutr. Biochem.* Jun 16 2011, Epub ahead of print, http://www.sciencedirect.com/science/article/pii/S0955286311000775 (2011).

[16] M. Ruiz-Canela and M.A.Martínez-González, *Maturitas,* 68, 245 (2011).

[17] R. Blomhoff, *Curr. Opin. Lipidol.* 16, 47 (2005).

[18] T. Finkel and N.J. Holbrook, *Nature,* 408, 239 (2000).

[19] G. Lighthall, B.P. Hamilton and M. Hamlyn, *J. Hypertens,* 22, 1487 (2004).

[20] C. Xu, S. Goodz and E.M. Sellers, Advanced Drug Delivery Review 54, 1245 (2002).

[21] P. Giem, W.L. Beeson, G.E. Fraser GE, *Neuroepidemiology,* 12, 28 (1993).

[22] S. Nechuta, W. Lu, Z. Chen, Y. Zheng, K. Gu, H. Cai, W. Zheng and X.O. Shu, *Cancer Epidemiol. Biom. Prev.* 20, 262 (2011).

[23] E.Y. Dimova, A. Samoylenko and T. Kietzmann, *Antioxid Redox Sign* 4, 777 (2004).

[24] J.L. Rosner and G. Storz, *Curr. Top Cell. Regul.* 35, 163 (1997).

[25] M.B. Toledano and W.J. Leonard, *Proc. Natl. Acad. Sci. USA,* 88, 4328 (1991).

[26] K. Shirasu, R.A. Dixon and C. Lamb, *Curr. Opin. Immunol.* 8, 3 (1996).

[27] H. Kamata and H. Hirata, *Cell Signal,* 11, 1 (1999).

[28] T. Finkel, *Curr. Opin. Cell. Biol.* 10, 248 (1998).

[29] D. Hecht and Y. Zick, *Biochem. Biophys. Res. Commun.* 188, 773 (1992).

[30] G.L. Schieven, R.S. Mittler, S.G. Nadler, J.M. Kirihara, J.B. Bolen, S.B. Kanner and J.A. Ledbetter JA, *J. Biol.Chem.* 269, 20718 (1994).

[31] K.S. Yao, M. Clayton, P.J. O'Dwyer, *J. Natl. Cancer Inst.* 87, 117 (1995).

[32] H. Kamata, C. Tanaka, H. Yagisawa, S. Matsuda, Y. Gotoh, E. Nishida and H. Hirata, *J. Biol. Chem.* 271, 33018 (1996).

[33] S.G. Rhee, S.W Kang, L.E. Netto, M.S. Seo and E.R. Stadtman, *Biofactors,* 10, 2 (1999).

[34] M. Saitoh, H. Nishitoh, M. Fujii, K. Takeda, K. Tobiume, Y. Sawada, M. Kawabata, K. Miyazono, H. Ichijo, *EMBO J.* 17, 2596 (1998).
[35] W.C. Barrett, J.P DeGnore, Y.F. Keng, Z.Y Zhang, M.B. Yim and P.B. Chock, *J. Biol. Chem.* 274, 34543 (1999).
[36] P. Chiarugi, T. Fiaschi, M.L. Taddei, D. Talini, E. Giannoni, G. Raugei and G. Ramponi, *J. Biol. Chem.* 276, 33478 (2001).
[37] T.J. Preston, W.J. Muller and G. Singh, *J. Biol. Chem.* 276, 9558 (2001).
[38] E.R. Stadtman and C.N. Oliver, *J. Biol. Chem.* 266, 2005 (1991).
[39] D.H. Flint, J.F. Tuminello and M.H. Emptage *J. Biol. Chem.* 268, 22369 (1993).
[40] W.F. Beyer Jr, I. Fridovich. *Anal. Biochem.* 161: 559 (1987).
[41] K. Asada and M. Takahashi, *Photoinhibition*, Elsevier, Amsterdam (1997).
[42] K. Abe, L.H. Pan, M. Watanabe, H. Konno, T. Kato and Y. Hoyama, *Neurosci. Lett.* 19, 124 (1997).
[43] M.F. Beal, R.J. Ferrante, S.E. Browne, R.T Matthews, N.W. Kowall and R.H. Brown Jr, *Ann. Neurol.* 42, 644 (1997).
[44] M.A. Smith, P.L. Richey Harris, L.M. Sayre, J.S. Beckman and G. Perry, *J. Neurosci.* 17, 2653 (1997).
[45] K. Hensley, M.L. Maidt, Z. Yu, H. Sang, W.R. Markesbery and R.A. Floyd, *J. Neurosci.* 18, 8126 (1998).
[46] P.F. Good, P. Werner, A. Hsu, C.W. Olanow and D.P. Perl, *Am. J. Pathol.* 149, 21 (1996).
[47] P.F. Good, A. Hsu, P. Werner, D.P. Perl and C.W. Olanow, *J. Neuropathol. Exp. Neurol.* 57, 338 (1998).
[48] Yoritaka, N. Hattori, K. Uchida, M. Tanaka, E.R. Stadtman and Y. Mizuno, *Proc. Natl. Acad. Sci. USA*, 93, 2696 (1996).
[49] M.L. Selley, *Free Radic. Biol. Med.* 25, 169 (1998).
[50] B.P. Yu, *Physiol. Rev.* 74, 13 (1994).
[51] M.F. Beal, *Neuroscientist*, 3, 21 (1997).
[52] Y. Ishikawa, T. Satoh, Y. Enokido, C. Nishio, T. Ikeuchi and H. Hatanaka, *Brain Res.* 824, 71 (1999).
[53] R.F. Castilho, M.W. Ward and D.G. Nicholls, *J. Neurochem.* 72, 1349 (1999).
[54] D.G. Graham, *Molec. Pharmacol.* 14, 633 (1978).
[55] J.N. Keller, Z. Pang, J.W. Geddes, J.G. Begley, A. Germeyer, G. Waeg and M.P. Mattson, *J. Neurochem.* 69, 273 (1997).
[56] Q. Guo, L.S. Sebastian, B.L. Sopher, M.W. Miller, C.B. Ware, G.M. Martin, M.P. Mattson, *J. Neurochem.* 72, 1019 (1999).
[57] W.R. Markesbery and J.M. Carney, *Brain Pathol.* 9, 133 (1999).
[58] W.R. Markesbery, *Free Radic. Biol. Med.* 23, 134 (1997).
[59] S. Przedborski and V. Jackson-Lewis, *Marcel Dekker Inc., New York*, 273 (2000).
[60] B.I. Giasson, R. Jakes, M. Goedert, J.E. Duda, S. Leight, J.Q. Trojanowski and V.M-Y. Lee, *J. Neurosci. Res.* 59, 528 (2000).
[61] A.B. Manning-Bog, A.L. McCormack, J. Li, V.N. Uversky, A.L. Fink and D.A. Di Monte, *J. Biol. Chem.* 277, 1641 (2002).
[62] M.K. Lee, W. Stirling, Y. Xu, X. Xu, D. Qui, A.S. Mandir, T.M. Dawson, N.G. Copeland, N.A. Jenkins and D.L. Price, *Proc. Natl. Acad. Sci. USA*, 99, 8968 (2002).

[63] J.T. Greenamyre, T.B. Sherer, R. Betarbet and A.V. Panov, *IUBMB Life* 52, 135 (2001).
[64] K.K. Griendling and D.G. Harrison, *Circ. Res.* 85, 562 (1999).
[65] K.K. Griendling , D. Sorescu, B. Lassegue and M. Ushio-Fukai, *Arterioscler. Thromb. Vasc. Biol.* 20, 2175 (2000).
[66] J. Chandra, A. Samali and S. Orrenius, *Free Rad. Biol. Med.* 29, 323 (2000).
[67] T. Finkel and N.J. Holbrook, *Nature*, 408, 239 (2000).
[68] M. Eyries, T. Collins and L. M. Khachigian, *Endothelium*, 11, 133 (2004).
[69] Reitman, I. Friedrich, A. Ben-Amotz and Y. Levy, *Isr. Med. Assoc. J.* 4, 590 (2002).
[70] S. Furukawa, T. Fujita, M. Shimabukuro, M. Iwaki, Y. Yamada, Y. Nakajima, O. Nakayama, M. Makishima, M. Matsuda and I. Shimomura, *J. Clin. Invest.* 114, 1752 (2004).
[71] S.O. Olusi, *Int. J. Obes.* 26, 1159 (2002).
[72] H. Xu, G.T. Barnes, Q. Yang, G. Tan, D. Yang, C.J. Chou, J. Sole, A. Nichols, J.S. Ross, L.A. Tartaglia and H. Chen, *J. Clin. Invest.* 112, 1821 (2003).
[73] H. Tilg and G.S. Hotamisligil, *Gastroenterology*, 131, 934 (2006).
[74] M. Cave, I. Deaciuc, C. Mendez, Z. Song , S. Joshi-Barve and C. McLain. *J. Nutr. Biochem.* 18, 184 (2007).
[75] M. Wortham, L. He, M. Gyamfi, B.L. Copple and J.Y. Wan, *Dig Dis Sci.* 53, 2761 (2008).
[76] B.H. Halliwell and J.M.C. Gutteridge, *Free Rad. Biol. Med., 2nd edn*, Clarendon Press, Oxford, UK.
[77] E. Niki , N. Noguchi, H. Tsuchihashi and H. Gotoh, *Am. J. Clin. Nutr.* 62, 1322S (1995).
[78] Mortensen, L.H. Skibsted and T.G. Truscott, *Arch. Biochem. Biophys.* 385, 13 (2001).
[79] G. Block, C. Jensen, M. Dietrich, E.P. Norkus, M. Hudes, L. Packer, *J. Am. Coll. Nutr. 23*, 141 (2004).
[80] M.W. Davey, M. van Montagu, D. Inze, M. Sanmartin, A. Kanellis, N. Smirnoff, I.J.J Benzie, J.J. Strain, D. Favell and J. Fletcher, *J. Sci. Food Agr.* 80, 825 (2000).
[81] A-M. Lampi, A. Kamal-Eldin and V. Piironen, *Functional Food - Biochemical and Processing Aspects*, CRC Press LLC, Boca Raton, FL, USA, 1 (2002).
[82] M.J. Stampfer and E.B. Rimm. *Am. J. Cli. Nutr.* 62, 1365 (1995).
[83] W. Stahl and H. Sies, *Mol. Aspects Med.* 24, 345 (2003).
[84] E. Giovannucci, A. Ascherio, E.B. Rimm, M.J. Stampfer, G.A. Colditz abnd W.C. Willett, *J. Natl. Cancer Inst.* 87, 1767 (1995).
[85] L. Kohlmeier, J.D. Kark, E. Gomez-Gracia, B.C. Martin, S.E. Steck, A.F.M. Kardinaal, J. Ringstad, M. Thamm, V. Masaev, R. Rieserma, J.M.Martin-Moreno, J.K. Huttunen, F.J. Kok, *Am. J. Epidemiol.* 146, 618 (1997).
[86] S. Goulinet and M.J. Chapman, *Arterioscler. Thromb. Vasc. Biol.* 17, 786 (1997).
[87] Y. Arai, S. Watanabe, M. Kimira, K. Shimoi, R. Mochizuki and N. Kinae, *J. Nutr.* 130, 2243 (2000).
[88] J.B. Harborne, *Encyclopedia of Plant Physiology, Volume 8, Secondary Plant Products*, Springer-Verlag, Berlin, 329 (1980).
[89] Z. Liu , W. Li, J. Sun, C. Liu, Q. Zeng, J. Huang, B. Yu and J. Huo, *Asia Pac. J. Clin. Nutr.* 13, 204 (2004).

[90] L.H.Yao, Y.M. Jiang, J. Shi, F.A. Tomás-Barberán, N. Datta, R. Singanusong and S.S. Chen, *Plant Food Hum. Nutr.* (Formerly Qualitas Plantarum) 59, 113 (2012).

[91] N. Skottova, R. Vecera, K. Urbanek, P. Vana, D. Walterova and L. Cvak, *Pharmacol. Res.* 47, 17 (2003).

[92] G. Mojzisovà and M. Kuchta, *Physiol. Res.* 50, 529 (2001).

[93] P.M. Kris-Etherton, K.D. Hecker and A. Bonanome, S.M. Coval, A.E. Binkoski, K.F. Hilpert, A.E. Griel and T.D. Etherton, *Am. J. Med.* 113, 71 (2002).

[94] S. Parthasarathy, E. Wieland and D.A. Steinberg, *Proc. Natl. Acad. Sci. USA*, 86, 1046 (1989).

[95] E.N. Frankel, J. Kanner, J.B. German, E. Park, J.E. Kinsella, *Lancet*, 43, 454 (1993).

[96] Y.J. Surh, Nat Rev Cancer 3, 768 (2003).

[97] T. Richard, A.D. Pawlus, M-L. Iglésias, E. Pedrot, P. Waffo-Teguo, J-M. Mérillon, J-P. Monti, *Annals of the New York Academy of Sciences* 1215, 103 (2011).

[98] M. Minakawa, A. Kawano, Y. Miura and K. Yagasaki, *J. Clin. Biochem. Nutr.* 48, 237 (2011).

[99] M. Jang, L. Cai, G.O. Udeani, K.V. Slowing, C.F. Thomas, C.W. Beecher, H.H. Fong, N.R. Farnsworth, A.D. Kinghorn, R.G. Mehta, R.C. Moon and J.M. Pezzuto, *Science*, 275, 218 (1997).

[100] L. Casas, C. Mantell, M. Rodríguez, A. Torres, F.A. Macías and E.J. Martínez de la Ossa. *J. Supercrit. Fluid*, 45, 37 (2008).

[101] M. Manfra, M. De Nisco, A. Bolognese, V. Nuzzo, A. Sofo, A. Scopa, L. Santi, G.C. Tenore and E. Novellino E, J Sci Food Agr 91, 2749 (2011).

[102] R.S. Jackson, *Wine Science: Principles, Practice, Perception. Second Edition.* Academic Press, San Diego, CA, USA (2000).

[103] P.Y. Ribéreau-Gayon, Y. Glories, A. Maujean and D. Dubourdieu, *Handbook of Enology, Volume 2: The Chemistry of Wine and Stabilization and Treatments*, John Wiley and Sons Ltd., Chichester, UK (2006).

[104] N. Terrier, C. Poncet-Legrand and V. Cheynier. *Wine Chemistry and Biochemistry.* Springer, New York, USA, 463 (2009).

[105] D.M. Goldberg and G.J. Soleas, *Wine. A scientific exploration*, Taylor and Francis, New York, USA 160 (2003).

[106] K.T. Howitz, K.J. Bitterman, H.Y. Cohen, D.W. Lamming, S. Lavu, J.G. Wood, R.E. Zipkin, P. Chung, A. Kisielewski, L.L. Zhang, B. Scherer and D.A. Sinclair, *Nature*, 425, 191 (2003).

[107] J.G. Wood, B. Rogina, S. Lavu, K. Howitz, S.L. Helfand, M. Tatar and D. Sinclair, *Nature*, 430, 686 (2004).

[108] J.M. Kong, L.S. Chia, N-K. Goh, T-F. Chia and R. Brouillard, *Phytochemistry*, 64, 923 (2003).

[109] Sofo, V. Nuzzo, G. Tataranni, M. Manfra, M. De Nisco and A. Scopa, *J. Plant Physiol.* Accepted for publication. In press.

[110] H. Wang, G. Cao, and R.L. Prior, *J. Agr. Food Chem.* 5, 304 (1997).

[111] S. de Pascual-Teresa, D.A. Moreno and C. García-Viguera, *Int. J. Mol. Sci.* 11, 1679 (2010).

[112] G. Mazza, *Acta Hortic.* 754, 577 (2007).

[113] E.I. Meja-Meza, J.A. Yanez, C.M. Remsberg, J.K. Takemoto, N.M. Davies, B. Rasco, C. Clary, *J. Food Sci.* 75, H5 (2010).

[114] M. Pinent, M.C. Bladé, M.J. Salvadó, M.J., Arola and L. Ardévol, *J. Agric. Food Chem.* 53, 5932 (2005).
[115] B. Jayaprakasam, S.K. Vareed, L.K. Olson and N.G. Nair, *J. Agr. Food Chem.* 53, 28 (2005).
[116] M.D. Rivero-Pérez, P. Muñiz and M.L. González-Sanjosé, M.L., *Food Chem. Toxicol.* 46, 2815 (2008).
[117] B. Radovanović and A. Radovanović, *Molecules*, 15, 4213 (2010).
[118] A.B. Bautista-Ortína, J.I. Fernández-Fernández, J.M. López-Roca, E. Gómez-Plaza, *J. Food Compos. Anal.* 20, 546 (2007).
[119] C. Santos-Buelga and V. de Freitas, *Wine Chemistry and Biochemistry. Springer, New York*, 529 (2009).
[120] J. Carreño, L. Almela, A. Martínez and A. Fernández-López, *Ebensmittel-Wissenschaft und-Technologie*, 30, 259 (1997).
[121] M.O. Downey, N.K Dokoozlian, M.P. Krstic, *Am. J. Enol. Viticult.* 57, 257 (2006).
[122] Hall, D.W. Lamb, B.P. Holzapfel and J.P. Louis, *Prec. Agric.* 12, 103 (2010).
[123] P.K. Boss and C. Davies. *Grapevine Molecular Physiology and Biotechnology*, Springer, Netherlands, 263 (2009).
[124] R. Boulton, *Am. J. Enol. Viticult.* 52, 67 (2001).
[125] Zimman and A.L. Waterhouse, *Am. J. Enol. Viticult.* 55, 139 (2005).
[126] Y. Zhang, A.Y. Chen, M. Li, C. Chen and Q. Yao, *J. Surg. Res.* 148, 17 (2008).
[127] V. Sharma, C. Joseph, S. Ghosh, A. Agarwal, M. Kumar Mishra and E. Sen, *Mol. Cancer Ther.* 6, 2544 (2007).
[128] C. López-Sánchez, F.J. Martín-Romero, F. Sun, L. Luis, A.K. Samhan-Arias, V. García-Martínez and C. Gutiérrez-Merino, *Brain Res.* 1182, 123 (2007).
[129] J. Lee, E. Jung and J. Lee, *Obesity*, 17, 226 (2009).
[130] G. Roby, J.F. Harbertson, D.A. Adams, M.A. Matthews, *Austr. J. Grape. Wine Res.* 10, 100 (2004).
[131] F. Roby and M.A. Matthews, *Austr. J. Grape. Wine Res.* 10, 74 (2004).
[132] E.P. Lansky and R.A. Newman, *J. Ethnopharmacol.* 109, 177 (2007).
[133] J.D. Su, T. Osawa, S. Kawakishi and M. Namili, *Phytochemistry*, 27, 1315 (1988).
[134] Y. Kashiwada, G.I. Nonaka, I. Nishioka, J.J. Chang and K.H. Lee, *J. Nat. Prod.* 55, 1033 (1992).
[135] S.Y. Schubert, E.P. Lansky and I. Neeman, *J. Ethnopharmacol.* 66, 11 (1999).
[136] Faria and C. Calhau, *Crit. Rev. Food Sci. Nutr.* 51, 626 (2011).
[137] Malik, F. Afaq, S. Sarfaraz, V.M. Adhami, D.N. Syed, and H. Mukhtar, *Proc. Natl. Acad. Sci. USA*, 102, 14813 (2005).
[138] D.N. Syed, A. Malik, N. Hadi, S. Sarfaraz, F. Afaq and H. Mukhtar H, *Photochem. Photobiol.* 82, 398 (2006).
[139] V.M. Adhami, N. Khan and H. Mukhtar, *Nutr. Cancer*, 61, 811 (2009).
[140] S.A. Khan, *Pak. J. Pharm. Sci.* 22, 346 (2009).
[141] A.J. Pantuck, J.T. Leppert, N. Zomorodian, W. Aronson, J. Hong, R.J. Barnard, N. Seeram, H. Liker, H. Wang, R. Elashoff, D. Heber, M. Aviram, L. Ignarro and A. Belldegrun, *Clin. Cancer Res.* 12, 4018 (2006).
[142] D.J. Loren, N.P. Seeram, R.N. Schulman and D.M. Holtzman, *Pediatric Res.* 57, 858 (2005).
[143] M. Hadipour-Jahromy and R. Mozaffari-Kermani , *Phytotherapy Res.* 24, 182 (2010).

[144] F. de Nigris, S. Williams-Ignarro, L.O. Lerman, E. Crimi, C. Botti, G. Mansueto, F.P. D'Armiento, G. De Rosa, V. Sica, L.J. Ignarro and C. Napoli, *Proc. Natl. Acad. Sci. USA*, 102, 4896 (2005).

[145] C.B. Stowe, *Compl. Ther. Clin. Pract.* 17, 113 (2011).

[146] M. Aviram and L. Dornfeld, *Atherosclerosis*, 158, 195 (2001).

[147] R.E. Hartman, A. Shah, A.M. Fagan, K.E. Schwetye, M. Parsadanian, R.N. Schulman, M.B. Finn and D.M. Holtzman, *Neurobiol. Dis.* 24, 506 (2006).

[148] K. Azadzoi, R. Schulman, M. Aviram, M. Siroky, *J. Urol.* 174, 386 (2005).

[149] A.J. Edwards, B.T. Vinyard, E.R. Wiley, E.D. Brown, J.K. Collins, P. Perkins-Veazie, R.A. Baker, B.A. Clevidence, *J. Nutr.* 133, 1043 (2003).

[150] P. Lucotti, E. Setola, L.D. Monti, E. Galluccio, S. Costa, E.P. Sandoli, I. Fermo, G. Rabaiotti, R. Gatti and P. Piatti, *Am. J. Physiol. Endocrinol. Metab.* 291, E906 (2006).

[151] J.K. Collins, G. Wu, P. Perkins-Veazie, K. Spears, P.L. Claypool, R.A Baker and B.A. Clevidence, *Nutrition*, 23, 261 (2007).

[152] University of Kentucky, Watermelon reduces atherosclerosis, animal study finds. *ScienceDaily*, http://www.sciencedaily.com/releases/ 2011/10/111027125153.htm (2011).

[153] Texas AandM University, Watermelon May Have Viagra-effect. *ScienceDaily*, http://www.sciencedaily.com/releases/2008/06/ 080630165707.htm (2008).

[154] S. Agrawal, R. Misra and A. Aggarwal, *Clin. Rheumatol.* 26, 201 (2007).

[155] G.W. Comstock , A.J. Iberg, H.Y. Huang, K. Wu, A.E. Burke, S.C. Hoffman, E.P. Norkus, M. Gross, R.G. Cutler, J.S. Morris, V.L. Spate and K.J. Helzlsouer , *Cancer Epidem. Biomar. Prev.* 6, 907 (1997).

[156] B.B. Freeman and K. Reimers, *Am. J. Lifestyle Med.* http://ajl.sagepub.com/content/early/2010/11/10/1559827610387488.abstract (2010).

[157] T.H. Rissanen, S. Voutilainen, K. Nyyssönen, R. Salonen, G.A. Kaplan, J.T. Salonen, *Am. J. Clin. Nutr.* 77, 133 (2003).

[158] S.M. Henning, N.P. Seeram, Y. Zhang, L. Li, K. Gao, R-P. Lee, D.C. Wang, A. Zerlin, H. Karp, G. Thames, J. Kotlerman, Z. Li and D. Heber, *J. Med. Food*, 13, 116 (2010).

[159] F. Giampieri, S. Tulipani, J.M. Alvarez-Suarez, J.L. Quiles, B. Mezzetti and M. Battino, *Nutrition*, 28, 9 (2012).

[160] J. Salas-Salvado, M. Bullo, A. Pérez-Heras and E. Ros, *Brit. J. Nutr.* 96, S45 (2006).

[161] Burton-Freeman, A. Linares, D. Hyson, T. Kappagoda, *J. Am. Coll. Nutr.* 29, 46 (2010).

[162] S.J. Zunino, M.A. Parelman, T.L. Freytag, C.B. Stephensen, D.S. Kelley, B.E. Mackey, L.R. Woodhouse and E.L. Bonnel, *Brit. J. Nutr.* 9, 1 (2011).

[163] J. Montonen, P. Knekt, T. Härkänen, R. Järvinen, M. Heliövaara, A. Aromaa and A. Reunanen, *Am. J. Epidemiol.* 161, 3 (2005).

[164] Y. Gilgun-Sherki, E. Melamed and D. Offen, *J. Neurol.* 251, 261 (2004).

[165] N.P. Seeram, L.S. Adams, Y. Zhang, R. Lee, D. Sand, H.S. Scheuller and D. Heber, *J. Agric. Food Chem.* 54, 9329 (2006).

[166] Y. Zhang, N.P. Seeram, R. Lee, L. Feng and D., *J. Agric. Food Chem.* 56, 670 (2008).

[167] D.E. Wedge, K.M. Meepagala, J.B. Magee, S. Hope Smith, G. Huang and L.L. Larcom, *J. Med. Food*, 4, 49 (2001).

[168] S. Fortalezas, L. Tavares, R. Pimpão, M. Tyagi, V. Pontes, P.M.Alves, G. McDougall, D. Stewart, R.B. Ferreira and C.N. Santos, *Nutrients*, 2, 214 (2010).

[169] S.H. Häkkinen, S.O. Kärenlampi, H.M. Mykkänen, I.M. Heinonen and A.R. Törrönen, *Eur. Food Res. Technol.* 212, 75 (2000).

[170] M. Kosar, E. Kafkas, S. Paydas and K. Hüsnü, *J. Agric. Food Chem.* 52, 1586 (2004).

[171] M. da Silva Pinto, F.M. Lajolo, M.I. Genovese, *Food Chem.* 107, 1629 (2008).

[172] R. Bobinaitė, P. Viškelis, P. Rimantas Venskutonis, *Food Chem.* 132, 1495 (2012).

[173] L.F. D'Antuono, M.L. Maltoni, S. Foschi, S. Elementi, E. Borgini, G. Baruzzi, W. Faedi, *Riv. Fruttic. Ortofloric.* 66, 64 (2004).

[174] H-G. Yan, W-H. Zhang, J-H. Chen and Z-E. Ding, *Afri. J. Biotechnol.* 11, 3623 (2012).

[175] MP. Khknen and M. Heinonen , *J. Agric. Food Chem.* 51, 628 (2003).

[176] A.M. Amorini, G. Fazzina, G. Lazzarino, B. Tavazzi, D. Di Pierro, R. Santucci, F. Sinibaldi, F. Galvano and G. Galvano, *Free Radic. Res.* 35, 953 (2001).

[177] A.M. Amorini, G. Lazzarino, F. Galvano, G. Fazzina, B. Tavazzi and G. Galvano , *Free Radic. Res.* 37, 453 (2003).

[178] L. Ziberna, F. Tramera, S. Mozec, U. Vrhovsekd, F. Mattivid, S. Passamontia, *Free Radic. Res. Med.* 52, 1750 (2012).

[179] J. Azevedo, I. Fernandes, A. Faria, J. Oliveira, A. Fernandes, V. de Freitas, N. Mateus, *Food. Chem.* 119, 518 (2010).

[180] X. Luo, S. Fang, Y. Xiao, F. Song, T. Zou, M. Wang, M. Xia and W. Ling, *Mol. Cell. Biochem.* 362, 211 (2012).

[181] K.J. Joshipura, F.B. Hu, J.E. Manson, J. Stampfer, E.B. Rimm, F.E. Speizer, G. Colditz, A. Ascherio, B. Rosner, D. Spoegelman, W.C. Willett, Ann Intern Med 134, 1106 (2001).

[182] P.M. Kris-Etherton, K.D. Hecker, A. Bonanome, S.M. Coval, A.E. Binkoski, K.F. Hilpert, A.E. Griel and T.D. Etherton, *Am. J. Med.* 113, 71S (2002).

[183] N.R. Song, H. Yang, J. Park, J.Y. Kwon, N.J. Kang, Y.S. Heo, K.W. Lee and H.J. Lee, *Food Chem.* 133, 658 (2012).

[184] E. Karimi, E. Oskoueian, R. Hendra, A. Oskoueian and H.Z.E. Jaafar, *Molecules*, 17, 1203 (2012).

[185] H. Li, Z. Deng, H. Zhu, C. Hu, R. Liu , J.C. Young, R. Tsao, *Food Res. Int.* 46, 250 (2012).

[186] N. Nalini, S. Aranganathan and J. Kabalimurthy, *Toxicol. Mech. Methods*, Epub ahead of print, http://informahealthcare.com/doi/abs/ 10.3109/15376516.2012.673092 (2012).

[187] S-L. Hwang, P-H. Shih and G-C. Yen, *J. Agric. Food Chem.* 60, 877 (2012).

[188] A.A. Alshatwi, G. Shafi, T.N. Hasan, A.A. Al-Hazzani, M.A. Alsaif, M.A. Alfawaz, KY Lei and A. Munshi, *Asian Pac. J. Cancer. Prev.* 12, 1555 (2011).

[189] C.A. Camargo, M.C.C. Gomes-Marconed, N.C. Wutzki and H. Aoyama, *Anticancer Res.* 32, 129 (2012).

[190] Ghorbani, M. Nazari, M. Jeddi-Tehrani and H. Zand, *European J. Nutr.* 51, 39 (2012).

[191] J. Reyes-Carmona, G.G. Yousef, R.A. Martinez-Peniche and M.A. Lila, *J. Food Sci.* 70, S497 (2005).

[192] Elisia, C. Hu, D.G. Popovich and D.D. Kitts, *Food Chem.* 101, 1052 (2007).

[193] W-Y. Huang, H-C. Zhang, W-X. Liu and C-Y. Li, *Journal of Zhejiang University - Science B* 112, 94 (2012)

[194] S.Y. Wang and H. Lin, *J. Agric. Food Chem.* 48, 140 (2000).

[195] F. Aqil, A. Gupt, R. Munagala, J. Jeyabalan, H. Kausar, R.J. Sharma, I.P. Singh and R.C. Gupta, *Nutr. Cancer,* 64, 428 (2012).

[196] M. Kähkönen, P. Kylli, V. Ollilainen, J-P. Salminen and M. Heinonen, *J. Agric. Food Chem* 60, 1167 (2012).

[197] L. Kaume, W.C. Gilbert, C. Brownmiller, L.R. Howard and L. Devareddy, *J. Funct. Foods*, Available online 12 March 2012, http://www.sciencedirect.com/science/article/pii/S175646461200031X (2012).