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Review Article

Oxidative stress and plant deriving antioxidants

Aneta Popova

Department of Catering and tourism, Economics Faculty, University of Food Technologies, 26 Maritza Blvd. BG-4002 Plovdiv, Bulgaria

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ABSTRACT

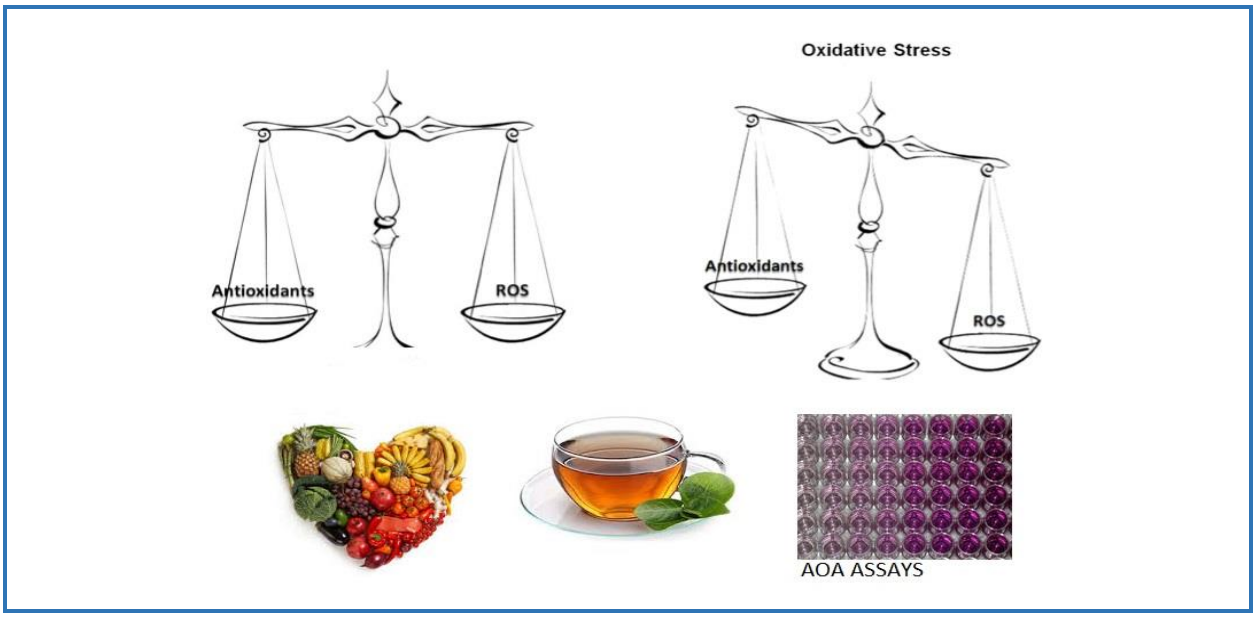
Antioxidant activity is strongly influenced by the amount of total phenolics, natural habitat and the specific growing conditions (climate, soil minerals, humidity external contaminants), flowering period and other factors. A number of degenerative diseases *i.e.* cancer, autoimmune diseases, rheumatoid arthritis, cardiovascular *etc*. are due to oxidative stress.

The human body has several mechanisms to counteract oxidative stress, by producing natural antioxidants or by submitting them to the outside through food and/or supplements. Once the antioxidant enters the body, it does not necessarily pass unchanged through the gastro-intestinal tract. It must therefore be considered bioavailability and bioactivity. The bioavailability of phenolic and polyphenolic compounds was studied in detail over the past two decades, whether by examining the kinetic models of polyphenol absorption in body fluids of healthy free radicals or by enhancing the immune system and reducing the risk of cancer and degenerative diseases.

In recent years, there has been an increased interest in finding natural antioxidants because they can interfere with the distribution of reactions of free radicals. The most effective components are phenolic compounds contained in many vegetable raw materials.

Corresponding author, email: popova_aneta@yahoo.com (A. Popova).
Tel.: +359 3260 3784.

Graphical Abstract



Biographies

Aneta Popova, PhD is currently working at the Department of Catering and tourism of the University of food technologies, Plovdiv. Her research interests are focused on food technology, healthy lifestyle and biological activity of plants. She is working on several projects and has recently finished one concerning new data on phytochemical extracts of fruit and herbs obtained by a multi-disciplinary approach needed to raise consumer awareness.

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Introduction

Food provides the energy needed by every human body and serves as a source of protein, fat, carbohydrates, vitamins and minerals. The exchange of substances for the proper functioning of organs and systems, tissues and cells depends on the diet, which determines the interest in food as a source, not only of structural components, but also as biologically active compounds protecting against the development of diseases.

Since ancient times, wild plants are present in the human diet. In culinary, they are used in combination or separately, as spices or major components of dishes, and their combination determines the peculiarities of national cuisine.

In the process of food provision, man discovered that some plants, not only have nutritious value, but also manifest healing properties. By enriching the knowledge and experience, they are currently used as tinctures, decoctions or conventional drugs in the treatment of various diseases.

The interest of the scientific community in wild plants grows with the establishment of compounds showing biological activity and positive effects on the human body. The involvement of free radicals in the aging process and a number of chronic degenerative diseases like cancer, cardiovascular diseases, *etc.* has been proven. The preventive and curative role of antioxidants makes it an interesting subject for research base to create food with healthy and functional effects. By enriching the knowledge of plants, as an indispensable source of biologically active components, ways to use natural ingredients in foods with expected health effects are being looked for.

Plant antioxidants

Antioxidants slow down or prevent oxidative damage to biological structures [1]. They interact with free radicals and terminate the chain reaction before a failure of life-essential cell activity [2]. Depending on their participation in the inhibition of oxidative processes, antioxidants can be defined as "primary" (actively blocking oxidation reactions) or "secondary" (indirectly influencing oxidation) [3]. It is believed that phenols act as secondary oxidants due to their ability to connect with potential pro-oxidative metal ions [4]. Antioxidants act on different levels: preventative, removing radicals, restorative and adaptive. Preventive antioxidants inhibit the formation of free radicals by reducing hydro peroxides and hydrogen peroxide to alcohol and water without forming free radicals [5]. Endogenous antioxidants "scavengers of radicals" are hydrophilic (ascorbic acid) and lipophilic

(tocopherols). They block the responses to initiate the formation free radical or and/or interrupt the chain reaction [6]. Proteolytic enzymes, proteinases, proteases and peptidases, present in cytosol and mitochondria of cells recognize, break down and eliminate oxidative modified proteins.

Some antioxidants (glutathione, uric acid, *etc*.), are produced in normal metabolic body processes [7]. The body also needs antioxidants that it cannot produce alone, and supplements them with food. Such are α-tocopherol, ascorbic acid, β-carotene, polyphenols *etc*., which are contained in herbaceous products [8].

In the event of oxidative stress, the body activates redox signaling mechanisms that mobilize the antioxidants contained in the cells or their reserves. Upon restauration of the antioxidant protection the normal physiological condition of the body is preserved. In case of antioxidant deficiency, tissue/organ damage occurs in/directly, leading to their respective malfunction [9]. The mobilization of antioxidants in response to oxidative stress, is a dynamic process in which, food intake can significantly affect the health [10].

According to some *in vivo* studies and biomarkers, high amounts of dietary antioxidants are not always effective in preventing disease, probably because they do not limit the damage from the harmful oxidation effects [11]. The antioxidant potential does not always correlate with the *in vivo* efficiency, possibly due to the chemical changes of the antioxidant compounds in the digestive tract [12]. Antioxidants act as anti-inflammatory, anti-cancer agents and radical scavengers [13], which possess antimicrobial properties and are used as preservatives to extend the shelf life of food products [14].

Antioxidants are divided into 3 main groups: enzymes, vitamins and phytochemicals [15]. Other authors [16] group them as carotenoids; allyl sulfides, and polyphenols (Phenolic acids, flavonoids, stilbenes, lignans).

Phenolic acids derivatives of benzene and hydroxy-cinnamic acid (*p*-coumaric, coffee, ferulic acid, salicylic acid, gallic acid, *etc*.) have proven health effect. The antioxidant activity of rosmarinic acid is seen as compatible with this of quercetin [16]. Tannins contribute with their antibacterial, antioxidant, and antiviral properties [17]. Flavonoids, contained in juniper, color elderberry, hawthorn, *etc.*, increase the resistance of capillary blood vessels, increase their elasticity; stimulate cardiac activity, lower blood pressure, and have antioxidant effects [18, 19].

Antioxidant properties

Researchers exhibit strong scientific interest in the antioxidant properties of polyphenols and the possibility of increasing the antioxidant defense system by including plants rich in them in the diet. It has been shown *in vitro*, that flavonoids' influence on the inflammatory cells and the activity of

many enzyme systems are quite a few facts about the *in vivo* effects [20]. Much of the evidence for the preventive effects of polyphenols on many diseases are *in vitro* assays or animal experiments. They are often carried out in doses higher than those of human intake through food [21].

Polyphenols improve the condition of the various biomarkers of oxidative stress [22]. The data is yet not very convincing regarding the applicability of these biomarkers as indicators of disease risk and appropriateness of the various used methods [23]. Substantial progress is reached in the field of cardiovascular disease. By biomarkers closely linked to the risk of cardiovascular disease, it has been found that certain polyphenols, used as an additive or as a food, improve the health status [24].

Epidemiological studies confirm the preventive effects when consuming rich in polyphenolic compounds products against cardiovascular disease [25]. In contrast, evidence of the role of polyphenols in the prevention of cancer and neurodegenerative diseases as well as against the deterioration of brain function is based mainly on animal experiments and *in vitro* studies [26].

One of the main difficulties to clarify the effects of polyphenols on health, is their great diversity with regard to the structure and construction, yielding compounds differing in biological activity [27]. It is possible that the active ingredients are not native polyphenols, which are found in foods, and their metabolites.

In vivo studies on humans have shown that dietary polyphenols undergo a substantial modification in the metabolism, and the formulation that reaches the blood and tissues in most cases differs from the primary compounds [28]. Studies have shown that dietary polyphenols (Flavonoids, flavonols and isoflavones) are partially absorbed in the body and have the potential for biological effects [29].

Polyphenols, depending on their structure, may have different biological properties and models of diffusion and effects in tissues and cells [30].

The quality of natural extracts and their antioxidant activity depend on the type of plant, source part (Leaf, stem, flower, seeds), geographical origin, climatic conditions, harvesting time and storage [31].

Influence on human health

Most of the studies focus on the amount, type and antioxidant properties of dietary polyphenols, as well as methods for their determination. There still remains unclear the specific mechanism by which these compounds act on health [32–34]. There is epidemiological evidence on the relationship of high intake of ingredients with antioxidant capabilities and a lower incidence of various hurt tubs or mortality. Known antioxidant activity of natural products (Culinary herbs, spices, vegetables,

fruit), stimulates scientists to create synthetic antioxidant enzymes, and develop extracts or functional foods enriched with biologically active substances [35].

It has been found that the beneficial influence on human health of the plants is associated with the presence of specific biologically active substances. It is important to determine the type of these compounds in different representatives, as well as the influence of the type of farming, storage and processing. Antioxidants can be measured as separate compounds or as a total capacity. Over the past two decades, methods for determining the content and antioxidant capacity of phenolic compounds in plant foods and food ingredients have been developed [36]. There are methods for determining the efficacy of phenolic antioxidants in lipid food models [37], and the antioxidant activity of the phenolic compounds in vitro and *in vivo* [34].

There is indisputable scientific evidence that eating significantly affects the health status of people, which is largely due to compounds that are found in plants [35]. Research confirms that free radicals play an important role in the development of cancer, cardiovascular disease, aging and damage to the immune system [38]. They are generated constantly in the body in the natural metabolism and under the action of infections, pollution, UV rays, smoking, radiation, pesticides, tension, stress, and others [39]. According to Stadtman [40] there is "good" and "bad" oxidative stress. During infections, the organism stimulates the "production" of free radicals, causing oxidation of various substrates, leading to the modification of organic molecules and degradation of the microbe cell structures present in human body [41]. Plant antioxidants limit the harmful effects of "bad" oxidative stress on health [42]. They stabilize the unpaired electrons and chelate transition metal ions, occurring in as donors of protons and electrons and alter the kinetics of the peroxidation of membrane lipids [43]. This determines the growth of scientific interest in natural antioxidants that may protect cells from changes resulting from oxidative stress [44]. Plants, with antioxidant properties, are used as aqueous, oily or alcoholic extracts; herbal infusions or decoctions [45], ointments, pharmaceuticals or spices, ingredients of food or beverages [46].

In many *in vitro* and *in vivo* studies it was found that polyphenols, due to their antioxidant activity, exhibit pro-inflammatory properties and have a beneficial effect on blood pressure, lipid balance and insulin resistance, and may reduce the risk of coronary artery disease of the heart, myocardial infarction, stroke, heart failure, atherosclerosis, diabetes, cancer and other diseases [47].

Carcinogens, mutagens and toxins are indicators of oxidative stress. They exist in the body and daily, in each cell, more than 10000 free radicals are produced, each of which exists for a split second, but still leaves considerable damage. In order to deal with the excess of free radicals produced in the oxidative stress, the human body has endogenous and exogenous mechanisms to maintain the redox

homeostasis. Naturally occurring dietary plant polyphenols exhibit antioxidant properties and influence the regulation of cellular activities [48].

The human body contains a very complex system of chemical and enzymatic defense mechanisms. Once the antioxidant enters the body, it is possible in the gastrointestinal tract to undergo modification. It is assumed that deconjugation occurs in the cells. It must therefore be considered the bioavailability and bioactivity of antioxidants. The bioavailability of phenolic and polyphenolic compounds is an object of researching the past two decades, by studying the kinetics of absorption of substances in the body fluids of healthy volunteers [49], and epidemiological studies in hospitals [50].

The researchers interest has been directed to the study and elucidation of the availability, structure and interaction of the phenolic antioxidants.

Studies have shown that a diet rich in fruit, vegetables, herbs, spices and other sources of phenols can lead to an increase in the amount of antioxidants in the body [51], and improvement of the overall health status [52]. This not only promotes research to identify the biologically active components contained in plants but also to study the factors that influence their preservation. Along with providing the potential health benefits, ingredients rich in phenols are used as antioxidants in various foods [53].

Within the actual food systems, the connection between the structural elements of the polyphenol compounds and their antioxidant activity is highly dependent on the conditions of the system such as substrates, temperature, light, oxygen pressure, physical characteristics, polarity and metals.

Plants and food

Plants (Herbs and culinary spices, and vegetables) contain biologically active substances, which are used as the main or additional components of dishes, salads, infusions, decoctions and others [48]. In the diet of primitive man, they constitute a major part of the daily intake it is characterized by a large amount and variety of biologically active substances. Now one uses three times more limited variety of natural plant food and is placed within stressful factors affecting adversely the health status. With scientific progress and replacing natural with artificial functional compositions many foods lose biologically active substances and to a considerable extent their physiological role.

Phenolic compounds (*e.g*. phenolic acids, flavonoids, quinones, coumarins, lignans, stilbenes, tannins), nitrogen compounds (Alkaloids, amines, betalaines), vitamins, terpenoids (Including carotenoids), and some other endogenous metabolites which possess antioxidant activity ar some of the free radical scavenging molecules contained in plants [38].

Polyphenols determine the color and taste characteristics of raw vegetable materials, increase the shelf life of food products displaying antioxidant properties [53]. Studies show that they have: anti-inflammatory, antitumor, antimutagenic, antibacterial and antiviral effect [54]. Recently, more manufacturers' attention and consumer's preference are focused on the natural resources due to the properties of synthetic antioxidants (High volatility, instability at elevated temperature, *etc.*), evidence of their carcinogenic nature, higher prices and stricter legislation. It has been shown that there is an inverse relationship between the consumption of foods rich in antioxidants (*e.g.* plants) and the incidence of disease [6]. Many antioxidant compounds naturally occurring in plant sources are defined as "scavengers" of free radicals or acyive oxygen. The antioxidant potential of different plant species has been investigated, including leafy vegetables and culinary herbs [32]. The use of natural rich in biologically active substances traditional herbs in the diet is an alternative to synthetic flavors and enhancers, and is in keeping with the natural diet.

In cooking, water and ethyl alcohol are the natural habitat of processing raw materials. Determining the most effective solvent extraction conditions, ensuring maximum extraction of biologically active substances will allow more efficient plant usage. Water, the natural environment for culinary preparation, is widely used as an extraction agent because of its properties as solvent, which may vary according to temperature and pH. Most of the medicinal herbs are applied as decoctions or infusions. The duration of the heat treatment plant is crucial for their properties. Microwave heating is a popular way to cook dishes and is used as an extraction method for the selective extraction of compounds from different raw materials.

Herbs, traditionally used in cooking, are subject to a number of technological operations (Cutting, grinding, crushing, drying, blanching, boiling, frying, freezing, baking, microwave heating), which leads to changes in the chemical structure and properties [31, 32]. There is still limited data on the impact of technological factors (Temperature, duration of exposure, *etc.*) on the biologically active substances of culinary spices.

Antioxidant methodologies

The spectrophotometric measurements are most commonly used in the definition of radical scavenging power of plant samples. Many attempts have been made to identify active components in plant material using colorimetric measurements. Gas chromatography (GC) and high performance liquid chromatography (HPLC) showed sensitivity and specificity necessary for accuracy in identifying the components. Except for HPLC and GC, extracts are also used for thin layer chromatography (TLC) and supercritical fluid chromatography mass spectrometry (SFC-MS) analysis [53]. The method and medium extraction influence the values of the antioxidant capacity of

the plant samples [54]. Slightly polar solvents such as ethyl acetate, create more active extracts than aqueous solutions of ethanol or methanol [31].

Given the diversity of the active species in the antioxidant compounds and various radical scavenging reactions, the choice of method may affect the obtained results. Not all established methods and antioxidants are compatible. It is possible that the same antioxidants give different values of the individual assays. This entails the need to use several methods for assessing antioxidant potential sources of phenolic compounds, taking into account the course and the conduct of the reaction. Properly selected and implemented analysis give valuable *in vitro* data and reveal the potential of antioxidant compounds *in vivo*. There are compounds (*e.g.* reducing sugars) which are not antioxidants, but affect the determination of the redox potential [48].

The lack of unified standards used for calibration and modification of methodologies, makes it difficult to compare results reported in the literature. *Bhagwat* et al. [55] come to similar conclusions when ORAC values of the same food sample tested in two different laboratories showed significant differences [56]. Efforts are being made to standardize and unify the methods for determining antioxidant activity (AOA) [57], but so far unsuccessfully.

AOA-TPC correlation

Good correlations between total phenolic content (TPC) and antioxidant activity (AOA) have been reported for cocoa [58], some herbal liqueurs [59], and cognacs [60], *while Heinonen* et al. [61] found low correlation in berry fruit wines and liqueurs. Several authors have outlined a positive correlation between TPC and AOA in studies with corn [62], wheat [63], bean [64] and rice [65]. These results show the relationship between phenolic content and antioxidant capacity of raw materials. Many studies establish a significant relationship between the content of phenols in plant extracts and antioxidant activity. Some authors suggest that the AOA of flavonoids is due to the presence of an aromatic hydroxyl group [66]. According to Mokgope [67] the antioxidant efficacy of phenolic compounds depends on many factors such as the number of hydroxyl groups, binding site of the aromatic ring, the mutual position of the hydroxyl groups in the aromatic ring and their ability to act as hydrogen or electron donating agents. For example, rutin and quercetin flavonols have higher AOA than ascorbic acid, which is considered to be a powerful reducing agent [68].

Al-Duais et al. [69] documented that the phenolic compounds of *Cyphostemma digitatum* (Forssk.) are not responsible for their antioxidant capacity after studying the TPC using different four (DPPH, ORAC, TEAC and FRAP) reliable antioxidant methods.

The difference between the TPC and antioxidant analyses correlations shows the variety of phenolic compounds and their different responses to the different methods for determining the

antioxidant activity. The reason for these differences could be the fact that the method of folin-ciocalteau determines the general phenolic compounds, while individual phenolic compounds are affected differently by the reagent Folin-Ciocalteau and contribute differently to antioxidant activity [70].

Conclusion

During the age of functional foods and healthy lifestyle, plant components are a major source of radical scavengers. Higher intake of natural antioxidants is gaining significant importance. Future modern research in combination with traditional health principles should continue with the aim of investigating the antioxidants bioavailability, bioactivity and understanding their impact on human health. Not only cost and time efficiency are needed when it comes to antioxidant assessment methods, but there is also an enourmous need for standatization of methodologies in order to promote infirmity and better comparison in scientific results.

Orcid

Aneta Popova 0000-0002-1290-503X

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