

Antioxidant Compounds in Food Matrices

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The generation of free radicals in our body occurs as consequence of exposure to different physiochemical conditions or pathological states. Free radicals are responsible for damaging biologically relevant molecules and lipids, nucleic acids, and proteins are the major targets. Antioxidants are capable of delaying or inhibiting the oxidation of a substrate by forming a new radical more stable to further oxidation.

Lactic acid bacteria

fungi

phenolic compounds

bioactive peptides

grains

1. Introduction

The increasing interest towards healthier food and lifestyles has steered the scientific community to pay great attention to the field of free radicals and antioxidant compounds. Free radicals are atoms, molecules, or ions containing an unpaired electron, which makes them unstable and highly reactive ^[1]. The generation of free radicals in our body occurs as consequence of exposure to different physiochemical conditions or pathological states. Free radicals are responsible for damaging biologically relevant molecules and lipids, nucleic acids, and proteins are the major targets ^[2]. They are generated continuously; however, they are also involved in inter-cellular and intra-cellular signaling systems and enzymatic reactions essential to intermediary metabolic processes of life ^[3], therefore, their daily production must be balanced. If free radicals overcome the body's ability to regulate them, and the ratio between free radical generation and antioxidant defenses is unbalanced, a condition known as oxidative stress ensues. Being the oxidative stress responsible for an enormous number of conditions, including cancer, cardiovascular and neurodegenerative diseases, atherosclerosis, inflammatory state, and many others ^{[2][4]}, preventing it by enhancing the intake of dietary antioxidants represents the most feasible way of protection against free radicals. Antioxidants need to be capable of delaying or inhibiting the oxidation of a substrate, yet an important property they should also have, is the ability to form a new radical that is stable through intramolecular hydrogen bonding on further oxidation ^[1]. Several mechanisms of action can be responsible for their activity. Antioxidants can (i) scavenge species that initiate peroxidation, (ii) donate a hydrogen or an electron, (iii) chelate metal ions preventing the generation of reactive species or lipid peroxides de-composition, (iv) quench the radical O_2^- preventing peroxides formation, (v) breaking autoxidative chain reaction, (vi) inhibit pro-oxidative enzymes, and/or (vii) reduce localized O_2 concentrations ^{[1][5]}. From a technological point of view, antioxidants are designed to prevent food from spoiling through oxidation, thus reducing loss of nutrients, and maintaining texture, color pigments, taste, freshness, functionality, and aroma ^[3]. Therefore, antioxidants are an important category of food preservatives and can be divided into natural or synthetic. Natural antioxidants include flavonoids, phenolic acids, carotenoids, and tocopherols ^[1]; however, other protein derived compounds, such as amino acids and bioactive peptides, have received great attention for their displayed antioxidant properties ^{[6][7][8]}. Both natural and synthetic

antioxidants, act by similar mechanisms and their chemical structure and polarity influence the antioxidant activity [5]. Antioxidants efficiency generally increases with the increase of their concentration; however, the dependence is not linear, and when the maximum activity is reached, it may also decrease [9]. Except for carotenes, tocopherols, and their esters, natural antioxidants are mostly much more polar than synthetic ones. They are also less active and substrate specific, and their antioxidant activity depends highly on synergists factors [9].

2. Phenolic Compounds

The antioxidant activity of phenolic compounds lies in their ideal chemical structure, facilitating the hydrogen or electron donation from hydroxyl groups positioned along the aromatic ring and conferring radical scavenging activities and metal-chelating potential. Phenolics have the ability of stabilizing and delocalizing the un-paired electron within their aromatic rings [10]. Phenolics are composed of at least one aromatic ring with at least one hydroxyl group and may be classified based on the number of phenol rings and the structural elements that are bound to the rings [11]. Phenolic acids, flavonoids, tannins, stilbenes, and lignans are the main groups of phenolics.

Phenolic acids are divided into hydroxybenzoic acids and hydroxycinnamic acids and usually act as antioxidants by trapping free radicals. Flavonoids, instead, can scavenge free radicals and chelate metals [5][10]. The common characteristic of flavonoids is the basic 15-carbon flavan structure. They are arranged in three rings (A, B, and C) and the different classes vary for the level of saturation of the C ring, whereas compounds within the same class differ for the level of substitution of A and B rings [5]. Polyphenols stability and free radical scavenging potential depend on both the number and location of the free OH group. The antioxidant activity increases with the increase of the hydroxyl groups, especially if positioned in ortho-3,4 [12].

Based on their chemical structure, tannins are defined as hydrolysable or condensed (proanthocyanidins). Condensed tannins are oligomers and polymers of flavan-3-ols, whereas hydrolysable tannins are glycosylated gallic and ellagic acid derivatives [13]. It was proven that free radicals scavenging activity of both hydrolysable and condensed tannins involves a fast and a slow step [14]. The fast scavenging reaction is inhibited by complexation of the tannin with protein, a very tight bond between the phenolic group of tannins and the NH group of proteins, which prevents their hydrolysis and digestion in the stomach [14]. Nevertheless, the overall capacity of the tannin-protein complex for scavenging seems to be similar to that of the free tannin [13][14].

3. Antioxidant Peptides and Protein Derivatives

Besides their nutritional, physicochemical, and sensory properties, proteins can be responsible for health promoting benefits, mainly attributed to biologically active peptides [15]. Bioactive peptides are produced by digestive enzymes during gastrointestinal digestion, or by proteolytic enzymes during food processing (ripening, fermentation, cooking), storage, or in vitro hydrolysis [7]. These peptides may play various roles (antimicrobial, antihypertensive, hypocholesterolemic, immunomodulatory, antioxidative, antithrombotic, antitumoral) and can be released from native proteins that derive from vegetable or animal matrices [7][8].

Peptides displaying antioxidant activity usually contain 5–16 amino acid residues [16]. The exact mechanism behind it has not fully been understood, yet several studies reported that they are inhibitors of lipid peroxidation, scavengers of free radicals and chelators of transition metal ions. [8][16]. Tyrosine, tryptophan, methionine, lysine, cysteine, and histidine are examples of amino acids displaying antioxidant activity. Synthesized peptides containing the active fragments have been proven to inhibit lipid peroxidation, while the tripeptides, Tyr-HisTyr, and Pro-His-His were found to be effective in stabilizing radical and non-radical oxygen species, including peroxynitrite and lipid peroxide [17]. Amino acids with aromatic residues can donate protons to electron deficient radicals, whereas histidine-containing peptides, thanks to the imidazole group, have been found to have hydrogen-donating, lipid peroxyl radical trapping, and metal ion-chelating abilities [8]. On the other hand, sulphur containing amino acids, have antioxidant action due to the direct interaction of thiol group with radicals. Cysteine and homocysteine inhibit LDL (low-density lipoprotein) oxidation by hemin and copper, methionine residues, instead, scavenge oxidizing agents [6]. Besides the presence of the proper amino acids, their specific positioning in the sequence plays an important role in antioxidant activity of peptides as well as other factors, such as the structure, amino acids configuration, hydrophobicity, and concentration [8].

4. Synthetic Antioxidants

Synthetic antioxidants were developed to prolong food shelf life but also because of the need to have a standard measurement system to compare with natural antioxidants. There are numerous compounds used in food, animal, and cosmetic applications to prevent oxidation; some also have antifungal properties and possess at least one phenolic ring in the structure. Butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), tert-butylhydroquinone, propyl gallate, octyl gallate, 2,4,5-Trihydroxy butyrophenone, nordihydroguaiaretic acid, and 4-Hexylresorcinol are some examples [1]. Among these, BHT and BHA, alone or in combination with others, are the most commonly employed [2] and synergistic effects were also reported [18]. Today, almost all processed foods contain synthetic antioxidants and, despite being reported safe in the past, several studies have addressed their danger for human health, among which liver, kidney, and lungs damages, mutagenesis, carcinogenesis, and many others [14]. For this reason, between 2011 and 2012, the European food safety authority re-evaluated their maximum levels of intake in adults and children [19][20]. Considering the increasing risk factors related to synthetic antioxidants, there has been a global trend toward the use and the search for effective natural substance as therapeutic antioxidants.

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