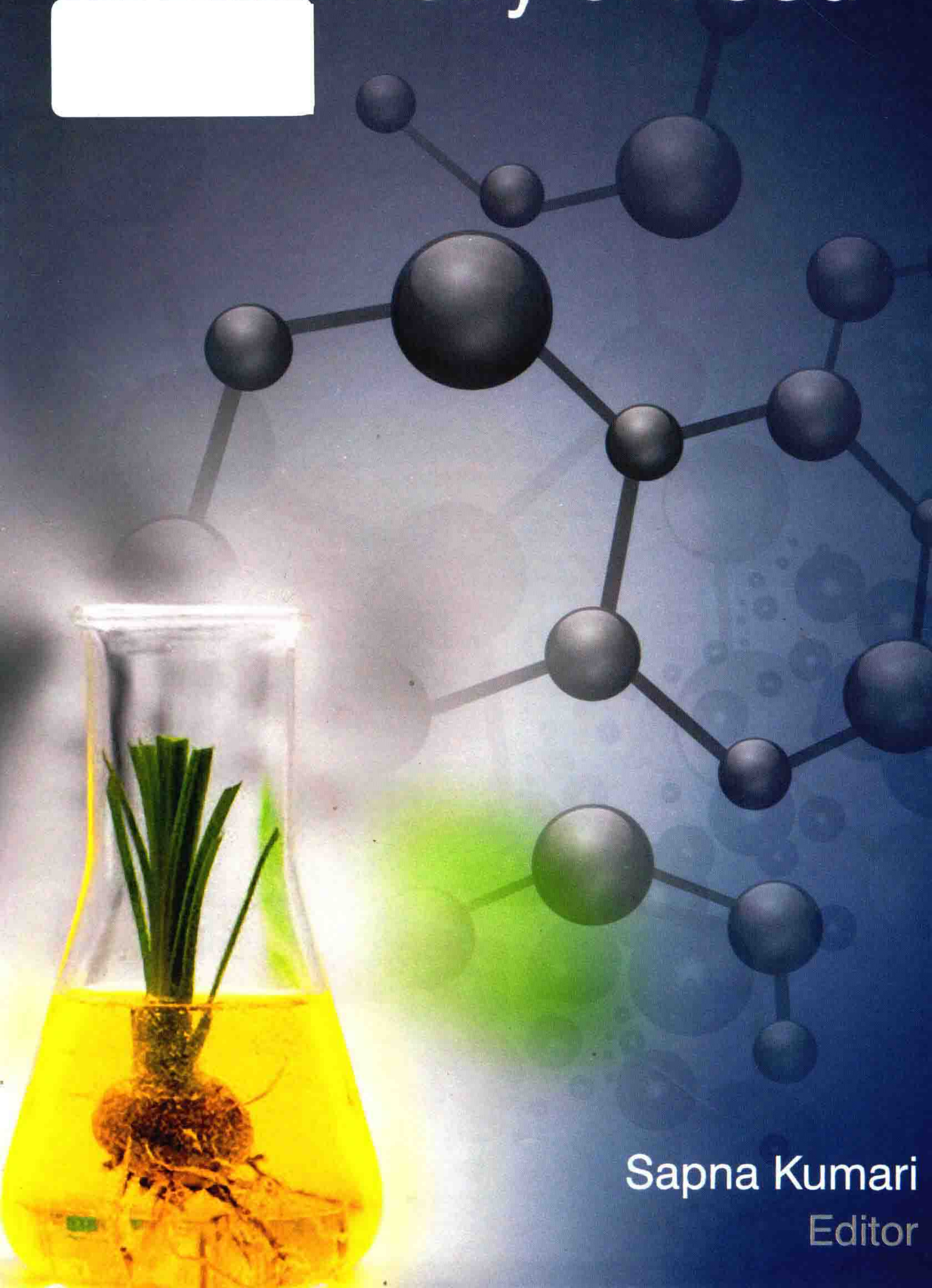
Chemistry and Biochemistry of Food



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Preface

The quality of the food we eat is determined by the quality of the raw materials, the processes used to produce the food and the storage conditions of raw materials and the final products. Food chemistry is a relatively new science and the development of modern biochemistry has been of great importance for food chemistry. Biochemistry is important to understand how enzymes affect different processes in foods, and to what degree these processes take place during production and storage of foods. If we look at it in this way we can say that food chemistry is applied biochemistry. Increased knowledge on the chemistry and biochemistry of foods and food raw materials and more knowledge on what happens in the different steps of food processing give us possibilities of controlling the processes so that we can make better products with longer shelf life and reduce the amount of food wasted. It will also make it possible to use the right raw material for the right process and product. Getting enough food for the still growing population on this earth will be a big challenge in the years to come. This Encyclopaedia delivers original text on the chemistry and biochemistry of the bioactive constituents of food, and substantiates their efficacy, safety, and potential uses.

A living system controls its activity through enzymes. An enzyme is a protein molecule that is a biological catalyst with three characteristics. First, the basic function of an enzyme is to increase the rate of a reaction. Most cellular reactions occur about a million times faster than they would in the absence of an enzyme. Second, most enzymes act specifically with only one reactant (called a substrate) to produce products. The third and most remarkable characteristic is that enzymes are regulated from a state of low activity to high activity and vice versa. Gradually, you will appreciate that the individuality of a living cell is due in large part to the unique set of some 3,000 enzymes that it is genetically programmed to produce. If even one enzyme is missing or defective, the results can be disastrous.

Much of the information about enzymes has been made possible because they can be isolated from cells and made to work in a test tube environment. Extensive work has also been done with X-Ray diffraction techniques to elucidate the three-dimensional structure of some enzymes. The ribbon and backbone form of carboxypeptidase is shown on the left. The substrate is shown in magenta.

The book examines in-depth study of the principles of food and fermentation biotechnology and recent advances and developments in the field of fermentation technology, focusing on industrial applications.

—Dr. Sapna Kumari

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Dietary Antioxidants

An antioxidant is a molecule capable of inhibiting the oxidation of other molecules. Oxidation is a chemical reaction that transfers electrons from a substance to an oxidizing agent. Oxidation reactions can produce free radicals. In turn, these radicals can start chain reactions that damage cells. Antioxidants terminate these chain reactions by removing free radical intermediates, and inhibit other oxidation reactions. They do this by being oxidized themselves, so antioxidants are often reducing agents such as thiols, ascorbic acid or polyphenols.

Although oxidation reactions are crucial for life, they can also be damaging; hence, plants and animals maintain complex systems of multiple types of antioxidants, such as glutathione, vitamin C, and vitamin E as well as enzymes such as catalase, superoxide dismutase and various peroxidases. Low levels of antioxidants, or inhibition of the antioxidant enzymes, cause oxidative stress and may damage or kill cells.

As oxidative stress might be an important part of many human diseases, the use of antioxidants in pharmacology is intensively studied, particularly as treatments for stroke and neurodegenerative diseases. However, it is unknown whether oxidative stress is the cause or the consequence of disease.

Antioxidants are widely used as ingredients in dietary supplements in the hope of maintaining health and preventing diseases such as cancer, coronary heart disease and even altitude sickness. Although initial studies suggested that antioxidant supplements might promote health, later large clinical trials did not detect any benefit and suggested instead that excess supplementation may be harmful. In addition to these uses of natural antioxidants in medicine, these compounds have many industrial uses, such as preservatives in food and cosmetics and preventing the degradation of rubber and gasoline.

As part of their adaptation from marine life, terrestrial plants began producing non-marine antioxidants such as ascorbic acid (Vitamin C), polyphenols, flavonoids and tocopherols.

Further development of angiosperm plants between 50 and 200 million years ago, particularly during the Jurassic period, produced many antioxidant pigments evolved during the late Jurassic period as chemical defences against reactive oxygen species produced during photosynthesis. The term antioxidant originally was used to refer specifically to a chemical that prevented the consumption of oxygen. In the late 19th century and early 20th century, extensive study was devoted to the uses of antioxidants in important industrial processes, such as the prevention of metal corrosion, the vulcanization of rubber, and the polymerization of fuels in the fouling of internal combustion engines.

Early research on the role of antioxidants in biology focused on their use in preventing the oxidation of unsaturated fats, which is the cause of rancidity. Antioxidant activity could be measured simply by placing the fat in a closed container with oxygen and measuring the rate of oxygen consumption. However, it was the identification of vitamins A, C, and E as antioxidants that revolutionized the field and led to the realization of the importance of antioxidants in the biochemistry of living organisms.

The possible mechanisms of action of antioxidants were first explored when it was recognized that a substance with anti-oxidative activity is likely to be one that is itself readily oxidized. Research into how vitamin E prevents the process of lipid peroxidation led to the identification of antioxidants as reducing agents that prevent oxidative reactions, often by scavenging reactive oxygen species before they can damage cells.

The Oxidative Challenge in Biology

A paradox in metabolism is that while the vast majority of complex life on Earth requires oxygen for its existence, oxygen is a highly reactive molecule that damages living organisms by producing reactive oxygen species. Consequently, organisms contain a complex network of antioxidant metabolites and enzymes that work together to prevent oxidative damage to cellular components such as DNA, proteins and lipids. In general, antioxidant systems either prevent these reactive species from being formed, or remove them before they can damage vital components of the cell. However, since reactive oxygen species do have useful functions in cells, such as redox signalling, the function of antioxidant systems is not to remove oxidants entirely, but instead to keep them at an optimum level.

The reactive oxygen species produced in cells include hydrogen peroxide (H_2O_2), hypochlorous acid (HOCl), and free radicals such as the hydroxyl radical (·OH) and the superoxide anion (O_2^-). The hydroxyl radical is particularly unstable and will react rapidly and non-specifically with most biological molecules. This species is produced from hydrogen peroxide in metal-catalysed redox reactions such as the Fenton reaction. These oxidants can damage cells by starting chemical chain reactions such as lipid peroxidation, or by oxidizing DNA or proteins. Damage to DNA can cause mutations and possibly cancer, if not reversed by DNA repair mechanisms, while damage to proteins causes enzyme inhibition, denaturation and protein degradation.

The use of oxygen as part of the process for generating metabolic energy produces reactive oxygen species. In this process, the superoxide anion is produced as a by-product of several steps in the electron transport chain. Particularly important is the reduction of coenzyme Q in complex III, since a highly reactive free radical is formed as an intermediate ($Q^{\cdot -}$). This unstable intermediate can lead to electron "leakage", when electrons jump directly to oxygen and form the superoxide anion, instead of moving through the normal series of well-controlled reactions of the electron transport chain. Peroxide is also produced from the oxidation of reduced flavoproteins, such as complex I. However, although these enzymes can produce oxidants, the relative importance of the electron transfer chain to other processes that generate peroxide is unclear. In plants, algae, and cyanobacteria, reactive oxygen species are also produced during photosynthesis, particularly under conditions of high light intensity. This effect is partly offset by the involvement of carotenoids in photoinhibition, which involves these antioxidants reacting with over-reduced forms of the photosynthetic reaction centres to prevent the production of reactive oxygen species.

Metabolites

Antioxidants are classified into two broad divisions, depending on whether they are soluble in water (hydrophilic) or in lipids (hydrophobic). In general, water-soluble antioxidants react with oxidants in the cell cytosol and the blood plasma, while lipid-soluble antioxidants protect cell membranes from lipid peroxidation. These compounds may be synthesized in the body or obtained from the diet. The different antioxidants are present at a wide range of concentrations in body fluids and tissues, with some such as glutathione or ubiquinone mostly present within cells, while others such as uric acid are more evenly distributed. Some antioxidants are only found in a few organisms and these compounds can be important in pathogens and can be virulence factors.

The relative importance and interactions between these different antioxidants is a very complex question, with the various metabolites and enzyme systems having synergistic and interdependent effects on one another. The action of one antioxidant may therefore depend on the proper function of other members of the antioxidant system. The amount of protection provided by any one antioxidant will also depend on its concentration, its reactivity towards the particular reactive oxygen species being considered, and the status of the antioxidants with which it interacts.

Some compounds contribute to antioxidant defence by chelating transition metals and preventing them from catalysing the production of free radicals in the cell. Particularly important is the ability to sequester iron, which is the function of iron-binding proteins such as transferrin and ferritin. Selenium and zinc are commonly referred to as *antioxidant nutrients*, but these chemical elements have no antioxidant action themselves and are instead required for the activity of some antioxidant enzymes.

Uric Acid

The antioxidant in highest concentration in human blood is uric acid, which provides about half of the total antioxidant capacity of human serum. Uric acid is an oxypurine produced from xanthine by the enzyme xanthine oxidase, and is a waste product of purine metabolism in primates, birds, and reptiles. An overabundance of this chemical in the body causes gout. The effects of uric acid in conditions such as stroke and heart attacks are still not well understood, with some studies linking higher levels of uric acid with increased mortality. This apparent effect might either be due to uric acid being activated as a defence mechanism against oxidative stress, or uric acid acting as a pro-oxidant and contributing to the damage caused in these diseases. Uric acid is released from tissues that are short of oxygen and elevated uric acid levels may be an important part of acclimatisation to high altitude.

Ascorbic Acid

Ascorbic acid or "vitamin C" is a monosaccharide oxidation-reduction (redox) catalyst found in both animals and plants. As one of the enzymes needed to make ascorbic acid has been lost by mutation during primate evolution, humans must obtain it from the diet; it is therefore a vitamin. Most other animals are able to produce this compound in their bodies and do not require it in their diets. Ascorbic acid is required for the conversion of the procollagen to collagen by oxidizing proline residues to hydroxyproline. In other cells, it is maintained in its reduced form by reaction with glutathione, which can be catalysed by protein disulfide isomerase and glutaredoxins. Ascorbic acid is redox catalyst which can reduce, and thereby neutralize, reactive oxygen species such as hydrogen peroxide. In addition to its direct antioxidant effects, ascorbic acid is also a substrate for the redox enzyme ascorbate peroxidase, a function that is particularly important in stress resistance in plants. Ascorbic acid is present at high levels in all parts of plants and can reach concentrations of 20 millimolar in chloroplasts.

Glutathione

Glutathione is a cysteine-containing peptide found in most forms of aerobic life. It is not required in the diet and is instead synthesized in cells from its constituent amino acids. Glutathione has antioxidant properties since the thiol group in its cysteine moiety is a reducing agent and can be reversibly oxidized and reduced. In cells, glutathione is maintained in the reduced form by the enzyme glutathione reductase and in turn reduces other metabolites and enzyme systems, such as ascorbate in the glutathione-ascorbate cycle, glutathione peroxidases and glutaredoxins, as well as reacting directly with oxidants. Due to its high concentration and its central role in maintaining the cell's redox state, glutathione is one of the most important cellular antioxidants. In some organisms glutathione is replaced by other thiols, such as by mycothiol in the Actinomycetes, or by trypanothione in the Kinetoplastids.

Melatonin

Melatonin is a powerful antioxidant and, unlike conventional antioxidants such as vitamins C and E and glutathione, it is both produced in the human body and is acquired in the diet (fruits, vegetables, cereals and herbs etc., contain melatonin). Melatonin easily crosses cell membranes and the blood-brain barrier Unlike other antioxidants, melatonin does not undergo redox cycling, which is the ability of a molecule to undergo repeated reduction and oxidation.

Redox cycling may allow other antioxidants (such as vitamin C) to act as pro-oxidants and promote free radical formation. Melatonin, once oxidized, cannot be reduced to its former state because it forms several stable end-products upon reacting with free radicals. Therefore, it has been referred to as a terminal (or suicidal) antioxidant.

Tocopherols and Tocotrienols (Vitamin E)

Vitamin E is the collective name for a set of eight related tocopherols and tocotrienols, which are fat-soluble vitamins with antioxidant properties. Of these, a-tocopherol has been most studied as it has the highest bioavailability, with the body preferentially absorbing and metabolising this form.

It has been claimed that the a-tocopherol form is the most important lipid-soluble antioxidant, and that it protects membranes from oxidation by reacting with lipid radicals produced in the lipid peroxidation chain reaction. This removes the free radical intermediates and prevents the propagation reaction from continuing.

This reaction produces oxidised a-tocopheroxyl radicals that can be recycled back to the active reduced form through reduction by other antioxidants, such as ascorbate, retinol or ubiquinol.

This is in line with findings showing that a-tocopherol, but not water-soluble antioxidants, efficiently protects glutathione peroxidase 4 (GPX4)-deficient cells from cell death. GPx4 is the only known enzyme that efficiently reduces lipid-hydroperoxides within biological membranes.

However, the roles and importance of the various forms of vitamin E are presently unclear, and it has even been suggested that the most important function of a-tocopherol is as a signalling molecule, with this molecule having no significant role in antioxidant metabolism. The functions of the other forms of vitamin E are even less well-understood, although g-tocopherol is a nucleophile that may react with electrophilic mutagens, and tocotrienols may be important in protecting neurons from damage.

Vitamin E

Vitamin E is a generic term for tocopherols and tocotrienols. Vitamin E is a family of a-, b-, g-, and d- (respectively: alpha, beta, gamma, and delta) tocopherols and corresponding four tocotrienols. Vitamin E is a fat-soluble antioxidant that stops the production of reactive oxygen species formed when fat undergoes oxidation. Of these, a-tocopherol (also written as alpha-tocopherol) has been most studied as it has the highest bioavailability.

α-Tocopherol

It has been claimed that a-tocopherol is the most important lipid-soluble antioxidant, and that it protects cell membranes from oxidation by reacting with lipid radicals produced in the lipid peroxidation chain reaction. This would remove the free radical intermediates and prevent the oxidation reaction from continuing. The oxidised a-tocopheroxyl radicals produced in this process may be recycled back to the active reduced form through reduction by other antioxidants, such as ascorbate, retinol or ubiquinol. However, the importance of the antioxidant properties of this molecule at the concentrations present in the body are not clear and it is possible that the reason why vitamin E is required in the diet is unrelated to its ability to act as an antioxidant. Other forms of vitamin E have their own unique properties. For example, g-tocopherol (also written as gamma-tocopherol) is a nucleophile that can react with electrophilic mutagens.

However, the roles and importance of all of the various forms of vitamin E are presently unclear, and it has even been suggested that the most important function of vitamin E is as a signalling molecule, and that it has no significant role in antioxidant metabolism.

So far, most studies about vitamin E have supplemented using only alpha-tocopherol, but doing so leads to reduced serum gamma- and delta-tocopherol concentrations. Moreover, a 2007 clinical study involving alpha-tocopherol concluded that supplementation did not reduce the risk of major cardiovascular events in middle aged and older men.

Tocotrienols

Compared with tocopherols, tocotrienols are sparsely studied. Less than 1% of PubMed papers on vitamin E relate to tocotrienols. Current research direction is starting to give more prominence to the tocotrienols, the lesser known but more potent antioxidants in the vitamin E family. Some studies have suggested that tocotrienols have specialized roles in protecting neurons from damage and cholesterol reduction by inhibiting the activity of HMG-CoA reductase[16-1];d-tocotrienol blocks processing of sterol regulatory element binding proteins (SREBPs)[16-1].

Oral consumption of tocotrienols is also thought to protect against stroke-associated brain damage in vivo. Until further research has been carried out on the other forms of vitamin E, conclusions relating to the other forms of vitamin E, based on trials studying only the efficacy of a-tocopherol, may be premature.

Recommended Daily Intake

The Food and Nutrition Board at the Institute of Medicine report the following dietary reference intakes for vitamin E:

Infants:

- 0 to 6 months: 4 mg/day
- 7 to 12 months: 5 mg/day.

Children:

- 1 to 3 years: 6 mg/day
- 4 to 8 years: 7 mg/day
- 9 to 13 years: 11 mg/day.

Adolescents and Adults:

- 14 and older: 15 mg/day.

Dietary Sources and Supplements

The following foods are rich in vitamin E:

- fortified cereals
- seeds and seed oils, like sunflower
- nuts and nut oils, like almonds and hazelnuts
- green leafy vegetables, like spinach, turnip, beet, collard, and dandelion greens
- tomato products
- pumpkin
- sweet potato
- blue crab
- rockfish
- mangoes
- asparagus
- broccoli
- papayas.

Health Effects

The consensus in the medical community is that there is no good evidence to support health benefits from vitamin E supplementation, yet there is strong evidence that taking more than 400 IU of vitamin E per day for extended periods increases the risk of death.

Pro-oxidant

Pro-oxidants are chemicals that induce oxidative stress, either through creating reactive oxygen species or inhibiting antioxidant systems. The oxidative stress produced by these chemicals can damage cells and tissues, for example an overdose of the analgesic paracetamol (acetaminophen) can cause fatal damage to the liver, partly through its production of reactive oxygen species.

Some substances can act as either antioxidants, or pro-oxidants, depending on the specific set of conditions. Some of the conditions that are important include the concentration of the chemical and if oxygen or transition metals are present. While thermodynamically very favoured, reduction of molecular oxygen or peroxide to superoxide or hydroxyl radical is fortunately spin forbidden. This greatly reduces the rates of these reactions, thus allowing aerobic life to exist. As a result, the reduction of oxygen typically involves either the initial formation of singlet oxygen, or spin-orbit coupling through a reduction of a transition-series metal such as manganese, iron, or copper. This reduced metal then transfers the single electron to molecular oxygen or peroxide.

Metals

Transition metals can act as pro-oxidants. E.g., chronic manganism is a classic "pro-oxidant" disease. Another disease associated with the chronic presence of a pro-oxidant transition-series metal is hemochromatosis, associated with elevated iron levels. Similarly, Wilson's disease is associated with elevated tissue levels of copper. Such syndromes tend to be associated with a common symptomology. This typically-includes various combinations of psychosis, dyskinesia (including Parkinsonian-like symptomology), pigmentary abnormalities, fibrosis, deafness, diabetes, and arthritis. Thus, all are occasional symptoms of (e.g) hemochromatosis, another name for which is "bronze diabetes". The pro-oxidant herbicide paraquat, Wilson's disease, and striatal iron have similarly been linked to human Parkinsonism. Paraquat also produces parkinsonian-like symptoms in rodents.

Fibrosis

Fibrosis or scar formation is another pro-oxidant-related symptom. E.g., interocular copper or vitreous chalcosis is associated with severe vitreous fibrosis, as is interocular iron. Liver cirrhosis is also a major symptom of Wilson's disease. The pulmonary fibrosis produced by paraquat and the antitumor agent bleomycin is also thought to be induced by the pro-oxidant properties of these agents. It may be that oxidative stress produced by such agents mimics a normal physiological signal for fibroblast conversion to myofibroblasts.

Pro-oxidant Vitamins

Vitamins that are reducing agents can be pro-oxidants. Vitamin C has antioxidant activity when it reduces oxidizing substances such as hydrogen peroxide, however, it can also reduce metal ions which leads to the generation of free radicals through the fenton reaction.

$$2\ Fe^{2+} + 2\ H_2O_2 \rightarrow 2\ Fe^{3+} + 2\ OH\cdot + 2\ OH^-$$

$$2\ Fe^{3+} + \text{Ascorbate} \rightarrow 2\ Fe^{2+} + \text{Dehydroascorbate.}$$

The metal ion in this reaction can be reduced, oxidized, and then re-reduced, in a process called redox cycling that can generate reactive oxygen species.