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A Review of Antioxidants and Autoxidation: Chemistry, Sources and Strategies

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ABSTRACT

Reactive species drive both product spoilage and human pathology, shifting the antioxidant paradigm from simple radical scavengers to context-dependent "physiological molecules". While existing literature treats industrial preservation and medical therapy in isolation, there is a need to potentially bridge this gap by establishing a cross-disciplinary framework to optimize stability, bioaccessibility, and redox safety. This synthesis review examines how radical mechanics align across consumer matrices and tissues, toxicological trade-offs with the shift to natural alternatives, and if advanced nano-delivery systems may overcome metabolic barriers without disrupting physiological signaling. Structural Roadmap: (1) Mechanisms & Pathology (Sec. 2–3): Chemical bases of autoxidation, radical scavenging mechanisms and macromolecular damage markers (8-OHdG, protein carbonyls, malondialdehyde). (2) Defenses & Spoilage (Sec. 4–6): Oxidative breakdown of food matrices and consumer product bases balanced against endogenous enzyme networks (SOD/CAT/GPx), dietary vitamins, and regulatory limits of synthetic phenolic stabilizers. (3) Delivery & Analytics (Sec. 7–9.2): Advanced manufacturing and nanocarriers (liposomes, solid lipid nanoparticles, micelles) alongside the methodological harmonization of conflicting in vitro antioxidant capacity assays. (4) Future Frontiers (Sec. 9.2–10): Gut microbiota-driven bioavailability variations, targeted stimuli-responsive delivery, and personalized, safety-aware redox therapies designed to mitigate prooxidant risks.

1 Introduction

Reactive oxygen species and related radicals are central to both oxidative spoilage of products and oxidative stress in biological systems, making antioxidant chemistry highly relevant to foods, medicines, cosmetics, and human health. Free radicals are highly reactive molecules with unpaired electrons that can damage lipids, proteins, and DNA, and an imbalance between their production and the body's defenses leads to oxidative stress implicated in cardiovascular, neurodegenerative, autoimmune diseases, cancer, and diabetes mellitus (Chandimali et al., 2025).

Endogenous enzymes such as superoxide dismutase, catalase, glutathione peroxidase, and glutathione reductase constitute a first line of defense against free radicals, while exogenous antioxidants from diet and supplements form a second line, acting through radical scavenging and stimulation of endogenous systems. These multiple levels of defense illustrate why antioxidant processes must be considered across both food matrices and the human body. Exogenous antioxidants obtained from food include vitamins, trace elements, and phytochemicals such as isoflavones, polyphenols, and flavonoids, all effective radical scavengers that donate electrons to reactive oxygen species and thereby reduce oxidative stress and molecular oxidation. Dietary antioxidants naturally present in foods have attracted interest because of their safety and potential nutritional and therapeutic effects, and they are widely distributed in fruits, vegetables, nuts, seeds, leaves, roots, and bark (Martemucci et al., 2022).

Polyphenolic raw materials with antioxidant properties occur in fruits, vegetables, fruit juices, herbs, spices, and oil seeds, and can be delivered as plant extracts in drugs, dietary supplements, nutricosmetics, or cosmetics, where their biological activity depends on bioavailability and ability to penetrate the skin barrier. Carotenoids, as fat soluble pigments such as α-, β-, and γ-carotene, lutein, zeaxanthin, lycopene, and others from common plant

sources, protect cellular lipids, proteins, and DNA from free radical attack, with their antioxidant power linked to conjugated double bonds and lipophilicity (Michalak, 2022). These data position plant derived antioxidants as key components of functional foods and topical products aimed at mitigating oxidative damage. Research into phenolic antioxidants has also clarified mechanistic aspects of autoxidation. Chain breaking phenolic antioxidants are governed by steric and electronic factors, and molecular orbital theory has been used to describe hydrogen abstraction mechanisms during autoxidation chains, treating electrons as molecular orbitals delocalized over entire molecules. Bromophenols exemplify phenolic structures with diverse biological activities, including antioxidant, antimicrobial, anticancer, anti inflammatory, and antidiabetic effects, and inhibition of several enzymes such as carbonic anhydrase, acetylcholinesterase, butyrylcholinesterase, aldose reductase, paraoxonase, α-amylase, and α-glycosidase (Gulcin, 2025).

Bioactive peptides from plant proteins represent another antioxidant class; sequences of 2–20 amino acids can supplement endogenous enzymatic and non enzymatic systems and reduce oxidative stress through free radical scavenging, electron or hydrogen transfer, metal chelation, ferric reducing power, and prevention of lipid peroxidation, with activity depending on amino acid composition, molecular size, hydrophobicity, and the nature of the reactive species (Michalak, 2022). Catechins from green tea chelate metal ions, preventing hydroxyl radical formation, and enhance endogenous antioxidant enzymes such as superoxide dismutase and catalase, further strengthening defense against oxidative stress (Chandimali et al., 2025).

Together, these studies demonstrate that both small phenolic molecules and macromolecular peptides contribute to antioxidant capacity relevant for autoxidation control in food and biological environments. Synthetic antioxidants are widely used as food additives for preservation, coloring, flavor enhancement, and sweetness, delaying nutrient degradation from bacterial, enzymatic, or chemical changes and extending shelf life. Compounds such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) are employed to stabilize fats and oils in foods, pharmaceuticals, cosmetics, plastics, rubber, and petroleum products, benefiting from heat stability and lipophilicity to provide "carry through" properties in baked and fried goods and to scavenge free radicals that would otherwise degrade product components. However, evidence of potential carcinogenic, mutagenic, and organ toxic effects has led to regulatory scrutiny and restricted use, with some synthetic antioxidants allowed in functional foods yet banned in certain countries due to harmful effects demonstrated in in vitro and in vivo studies (Gulcin, 2025). Concerns about toxicity, carcinogenicity, and hepatotoxicity have driven reduced reliance on synthetic antioxidants and increased demand for natural alternatives in human diets and health related products (Martemucci et al., 2022).

Despite these risks, antioxidant incorporation is still viewed as advantageous, because in their absence, oxidation products form in food and may pose greater health threats than the potential adverse effects of the antioxidants themselves. Antioxidants can themselves act as prooxidants under specific conditions. Many dietary antioxidants exhibit prooxidant behavior depending on concentration and the surrounding molecular environment, often by generating reactive oxygen or nitrogen species that damage cellular components when regulation fails. Vitamin C, usually regarded as a potent antioxidant, can reduce iron or copper ions and thereby catalyze H_2O_2 conversion to highly reactive hydroxyl radicals, while α-tocopherol at high concentrations may become a radical species that promotes lipid autoxidation if not efficiently regenerated by ascorbic acid. It has been noted that antioxidant concepts have shifted from "Miracle Molecules" to "Physiological Molecules," reflecting nuanced roles that include both protection and potential promotion of oxidative reactions depending on context (Gulcin, 2025). This duality is crucial for interpreting antioxidant effects in foods, cosmetics, and supplements as well as in human physiology.

2 Conceptual and Chemical Basis of Oxidation and Antioxidants

2.1 Oxidation, Autoxidation and Reactive Species

Oxidative processes in biological and food systems originate from the chemistry of reactive oxygen and nitrogen species and from radical chain reactions that propagate once initiated. Free radicals are molecular entities or fragments containing one or more unpaired electrons in an outer orbital, which makes them unstable and highly reactive toward nearby molecules that can donate or accept an electron. In oxygen-centered radicals, such as superoxide, hydroxyl, and peroxyl species, the unpaired electron predominately resides on an oxygen atom, and these radicals can act as oxidizing or reducing agents by abstracting hydrogen atoms or transferring electrons to attain a more stable configuration. Reactive oxygen and nitrogen species may be true radicals or non radical oxidants formed through partial reduction of oxygen or nitrogen oxides, and together they constitute a broad family of species capable of initiating oxidative damage in cells and materials. Within aerobic organisms, use of molecular oxygen for metabolism unavoidably generates reactive species. Free radicals arise as products of normal cell metabolism when redox processes involving oxygen

are only partially coupled to four electron reduction to water (Martemucci et al., 2022).

One electron reduction of oxygen yields the superoxide radical, which can be further converted by superoxide dismutase to hydrogen peroxide, a non-radical oxidant that can still participate in Fenton chemistry. In the presence of transition metals such as iron or copper, hydrogen peroxide undergoes Fenton or Haber–Weiss reactions that generate highly reactive hydroxyl radicals with extremely high one electron reduction potential and the capacity to react with virtually any nearby biological target at diffusion controlled rates (Chandimali et al., 2025; Martemucci et al., 2022).

These pathways illustrate how ordinary mitochondrial respiration and other enzymatic processes inadvertently contribute to a burden of reactive oxygen species that can initiate oxidative stress (Chandimali et al., 2025). Specific radical species display distinct reactivities and lifetimes that shape their roles in autoxidation. Hydroxyl radicals are considered the most harmful oxygen-centered radicals in vivo because of their very high reduction potential and their ability to react with DNA, proteins, lipids, amino acids, sugars, vitamins, and metals faster than they are generated. Reactions of hydroxyl radicals involve hydrogen abstraction from weaker C–H bonds in saturated compounds, addition to double bonds, and electron transfer, producing new radicals that can react with oxygen and propagate chain reactions (Martemucci et al., 2022).

Alkoxyl and peroxyl radicals are generated from the decomposition of hydroperoxides induced by heat, radiation, or redox-active metal ions, and they can abstract hydrogen from susceptible substrates, particularly bis-allylic positions in polyunsaturated fatty acids, thereby sustaining lipid peroxidation chains. Peroxyl radicals are relatively long lived compared with hydroxyl radicals and possess sufficient diffusion capacity to attack targets at some distance from their site of formation, sometimes cleaving to release superoxide or reacting together to yield singlet oxygen. Nonradical species such as hydrogen peroxide, ozone, and singlet oxygen also participate in oxidative processes, with singlet oxygen reacting preferentially with conjugated double bonds of polyunsaturated fatty acids and contributing to oxidative injury or therapeutic photodynamic effects depending on context (Jomova et al., 2023; Martemucci et al., 2022).

Autoxidation of lipids offers a central example of how reactive species translate into chemical and biological damage in foods and tissues. In membranes and bulk oils, peroxyl radicals formed by reaction of molecular oxygen with carbon-centered radicals initiate a chain reaction of lipid peroxidation (Gulcin, 2025; Jomova et al., 2023). Primary products are lipid hydroperoxides, which alter membrane fluidity and impair membrane protein function, and they can undergo iron-mediated one-electron reduction followed by oxygenation to yield epoxyallylic peroxyl radicals that further propagate peroxidation. Decomposition of lipid hydroperoxides generates reactive aldehydes such as 4 hydroxy 2 nonenal and malondialdehyde that are highly cytotoxic and can adduct to proteins and DNA, extending oxidative damage beyond the initial lipid targets. In food systems, these autoxidation reactions during harvesting, storage, and processing cause rancidity, off flavors, color changes, and degradation of nutritional and textural quality in dairy products, meats, vegetables, fruits, and pharmaceuticals. In biological membranes, the same radical driven chains disrupt cellular integrity and contribute to oxidative injury of lipids, enzymes, and nucleic acids. Because lipid hydroperoxides in membranes are key contributors to reactive oxygen species production in vivo, their detoxification is essential for cellular survival under oxidative stress, and the extent of peroxidation is often quantified using assays that measure hydroperoxide formation, such as the thiocyanate method based on ferrous thiocyanate generation from linoleic acid emulsions (Gulcin, 2025).

Across both food matrices and biological systems, these mechanistic insights into oxidation, autoxidation, and reactive species provide the chemical basis for understanding how antioxidants must operate to interrupt initiation, propagation, or secondary reactions of radical chain processes.

2.2 Definitions, Classes and Mechanisms of Antioxidant Action

Antioxidants are described as substances that, when present at low concentrations relative to an oxidizable substrate, markedly decrease or prevent the harmful effects of free radicals on human tissues. They encompass a chemically heterogeneous group that can be categorized according to structure, solubility, origin, enzymatic nature, and reaction mechanism. Fat-soluble representatives include α-tocopherol, β-carotene, lipoic acid, and ubiquinone, whereas water-soluble examples comprise glutathione and ascorbic acid. In addition, endogenous enzymatic systems such as superoxide dismutase, catalase, glutathione peroxidases, glutathione transferase, and glutathione reductase coexist with non-enzymatic species including glutathione, uric acid, melatonin, metal binding proteins, vitamins, carotenoids, and polyphenols (Kotha et al., 2022; Michalak, 2022).

Classification can proceed along multiple axes. Endogenous antioxidants originate within the body and are largely enzymatic, with superoxide dismutase, catalase,

glutathione reductase, and glutathione peroxidase converting superoxide and hydrogen peroxide to water and molecular oxygen and thereby forming a first line of defense. Non-enzymatic endogenous antioxidants such as glutathione and lipoic acid arise from metabolism and support these enzymes. Exogenous antioxidants include dietary vitamins, carotenoids, polyphenols, flavonoids, and bioflavonoids obtained from foods or supplements, which display in vivo activity and constitute a second line of defense. Antioxidants can further be grouped as natural or synthetic, polar or nonpolar, enzymatic or non-enzymatic, and by whether they act preventively, interrupt ongoing radical chains, or inactivate radical-derived products (Kotha et al., 2022; Martemucci et al., 2022).

Mechanistically, antioxidant activity is organized around hydrogen atom transfer, single electron transfer, and metal chelation. Preventive pathways involve enzymatic systems that limit formation of reactive oxygen species, while chain breaking and inactivation mechanisms rely on direct scavenging of radicals and transformation of reactive intermediates into less toxic products. Detailed mechanistic schemes identify single electron transfer from an antioxidant to an oxidant, direct hydrogen atom transfer, proton-coupled electron transfer, sequential electron transfer followed by proton transfer, and sequential proton loss electron transfer as routes by which phenolic structures and related molecules neutralize radicals. In these schemes, generic phenolic antioxidants (ArOH) undergo one-step hydrogen atom transfer to peroxyl radicals, or form cation radicals through electron loss, followed by deprotonation to yield relatively stable phenoxyl radicals that are less damaging than the initial reactive species. In the SPLET pathway, initial deprotonation to phenoxide anions is followed by electron transfer to the radical, again producing stabilized phenoxyl products (Gulcin, 2025; Kotha et al., 2022; Losada-Barreiro et al., 2022; Parcheta et al., 2021).

Specific molecular classes exemplify these general mechanisms. Phenolic compounds are described as primary antioxidants capable of hydrogen atom transfer, as in gallic acid, caffeic acid, and epicatechin, and single electron transfer, as in kaempferol and resveratrol. They also act as secondary antioxidants by binding potentially prooxidative metal ions, thus contributing to both radical scavenging and metal chelation. Carotenoids exhibit antioxidant activity through single electron transfer, adduct formation, and hydrogen atom transfer, and are particularly effective peroxyl radical scavengers. Vitamin C participates in reactions with oxidizing agents via hydrogen atom transfer, single electron transfer, or concerted electron proton transfer, reacting with superoxide and hydroxyl radicals in cytoplasmic compartments. Vitamin E prevents lipid peroxidation chain reactions and quenches singlet oxygen in lipid regions, reducing lipid peroxyl radicals by donating the phenolic hydrogen of its chroman ring. Uric acid, glutathione, and melatonin serve as electron donors and scavengers for superoxide, hydroxyl radical, and peroxynitrite, with glutathione additionally regenerating oxidized vitamin C and vitamin E (Kotha et al., 2022; Michalak, 2022).

Beyond small molecules, macromolecular antioxidants such as proteins, peptides, and polysaccharides are described as exogenous bioactive agents that regulate redox state, remove reactive oxygen species, and prevent lipid oxidation, particularly in polyunsaturated fat based products. Plant-derived bioactive peptides, defined as sequences of 2–20 amino acids, supplement endogenous enzymatic and non-enzymatic systems and reduce oxidative stress. They act through free radical scavenging, electron or hydrogen transfer, transition metal chelation, ferric reducing power, and inhibition of lipid peroxidation, and may additionally induce expression of protective proteins. Their activity depends on amino acid composition, molecular size, hydrophobicity, and the identity of the reactive species, with histidine and aromatic residues highlighted for metal chelation and hydroxyl radical scavenging (Michalak, 2022).

Taken together, these classifications and mechanistic frameworks show that antioxidant action encompasses a spectrum from enzymatic removal of primary oxidants to non-enzymatic radical quenching, metal chelation, and modulation of redox-sensitive signaling, with effectiveness governed by chemical structure, thermodynamic parameters such as bond dissociation enthalpy and ionization potential, and the capacity to reach relevant sites in biological or food matrices (Kotha et al., 2022; Losada-Barreiro et al., 2022; Parcheta et al., 2021).

3 Oxidative Processes in Biological Systems

3.1 Sources of Reactive Oxygen and Nitrogen Species

Reactive oxygen and nitrogen species arise from a combination of endogenous biochemical processes and exogenous environmental and pharmacological exposures, and they underpin the oxidative burden discussed previously in cellular and food-related contexts. Within cells, several organelles and enzyme systems continuously generate reactive species as part of normal metabolism, which can contribute to oxidative stress when their production exceeds antioxidant defenses. Mitochondrial respiration is a primary endogenous source. During oxidative phosphorylation, electrons flow through the electron transport chain complexes embedded in the inner mitochondrial membrane to reduce molecular oxygen to water, but a fraction of electrons leak prematurely and react

with oxygen to form superoxide radicals, predominantly at complexes I and III (Chandimali et al., 2025; Martemucci et al., 2022).

These mitochondria are estimated to reduce most of the cellular oxygen to water while converting a small portion to reactive species, and superoxide is then dismutated by superoxide dismutase to hydrogen peroxide, which can undergo Fenton chemistry in the presence of transition metals such as iron and copper to yield highly reactive hydroxyl radicals (Chandimali et al., 2025; Gulcin, 2025; Martemucci et al., 2022).

Beyond mitochondria, peroxisomes contribute substantially; their respiratory pathways transfer electrons from various metabolites to oxygen, generating hydrogen peroxide and other species such as superoxide, hydroxyl radical, and nitric oxide, largely coupled to fatty acid β-oxidation and the activity of oxidases including acyl CoA oxidase, D amino acid oxidase, urate oxidase, and xanthine oxidase (Kıran et al., 2023; Martemucci et al., 2022).

The endoplasmic reticulum further adds to intracellular reactive oxygen species through cytochrome P450 dependent redox reactions and thiol oxidases that convert dithiols to hydrogen peroxide, making this organelle the second major cellular source of reactive species after mitochondria (Martemucci et al., 2022).

Multiple oxidase enzymes and inflammatory processes also drive endogenous formation of reactive oxygen and nitrogen species. Enzymes such as NADPH oxidase, xanthine oxidase, nitric oxide synthases, lipoxygenases, prostaglandin synthase, cyclooxygenases, myeloperoxidase, glucose oxidase, and cytochrome P450 are all reported to generate superoxide, hydrogen peroxide, nitric oxide, and downstream oxidants during their catalytic cycles. NADPH oxidase in neutrophils and macrophages produces large amounts of superoxide during the respiratory burst, which is then converted to hydrogen peroxide and reactive products such as hypochlorous acid via myeloperoxidase, enabling pathogen killing but raising cytotoxic potential when regulation fails (Chandimali et al., 2025; Martemucci et al., 2022).

Nitric oxide synthases generate nitric oxide, which participates in physiological signaling yet can react with superoxide to form peroxynitrite, a potent oxidant capable of nitrating proteins and damaging lipids and DNA (Chandimali et al., 2025; Kıran et al., 2023; Martemucci et al., 2022).

Lipoxygenase and cyclooxygenase pathways produce unstable peroxides from arachidonic acid derivatives and other substrates, which can decompose to hydroxyl and alkoxyl radicals, contributing further to reactive species pools (Kıran et al., 2023; Martemucci et al., 2022).

Reactive species generation is not restricted to endogenous metabolism; numerous exogenous factors also induce reactive oxygen and nitrogen species. Ultraviolet radiation directly ionizes cellular molecules, producing radicals including singlet oxygen and superoxide, and can activate NADPH oxidase, while ionizing radiation such as X rays and γ rays breaks chemical bonds and drives radiolysis of water to generate reactive intermediates that evolve into hydrogen peroxide and other reactive oxygen species (Chandimali et al., 2025; Gulcin, 2025).

Environmental pollutants are prominent exogenous sources; cigarette smoke delivers both long-lived and short-lived radicals and prooxidants in tar and gas phases, initiating lipid peroxidation chains and oxidative processes, while heavy metals such as lead, cadmium, mercury, iron, and copper catalyze reactive oxygen species production through Fenton and Fenton like reactions or by depleting cellular antioxidants (Chandimali et al., 2025; Gulcin, 2025; Martemucci et al., 2022).

Additional exogenous contributors include air and water pollution, pesticides, polychlorinated biphenyls, dioxins, industrial solvents, exhaust gases, and ozone, all of which can generate reactive oxygen species or oxidize biomolecules such as lipids (Gulcin, 2025; Kıran et al., 2023; Martemucci et al., 2022).

Pharmacological agents and xenobiotics constitute another important category. Chemotherapeutic compounds like doxorubicin deliberately generate reactive oxygen species as part of their cytotoxic action against cancer cells, while acetaminophen overdose produces the reactive metabolite N acetyl p benzoquinone imine that depletes glutathione and induces oxidative stress in the liver (Chandimali et al., 2025).

Other drugs, anesthetic gases, and narcotics can similarly stimulate reactive oxygen species production, and metabolic processing of xenobiotics via cytochrome P450 in the endoplasmic reticulum introduces additional reactive oxygen species (Kıran et al., 2023; Martemucci et al., 2022).

Taken together, these findings map a network of sources in which mitochondrial and peroxisomal metabolism, specialized oxidase enzymes, immune cell activation, and diverse environmental and pharmacological exposures jointly produce reactive oxygen and nitrogen species that drive oxidative processes in biological systems (Chandimali et al., 2025; Gulcin, 2025; Kıran et al., 2023; Martemucci et al., 2022).

3.2 Molecular Targets and Consequences of Oxidative Damage

Oxidative damage in biological systems manifests through direct chemical modification of macromolecules, leading to structural disruption, loss of function, and downstream pathological processes. Excess reactive oxygen and nitrogen species oxidize proteins, lipids, nucleic acids, and carbohydrates, producing abnormal gene expression, disturbed receptor activity, altered cell proliferation, immune perturbation, mutagenesis, tissue injury, and a broad spectrum of clinical disorders (Kotha et al., 2022; Martemucci et al., 2022).

These macromolecular changes are central to the transition from transient oxidative insults to chronic disease states and are tightly linked to the imbalance between oxidant production and antioxidant defenses. Nucleic acids are highly vulnerable targets for reactive species such as hydroxyl radical and singlet oxygen. Hydroxyl radicals react with all components of DNA, including purine and pyrimidine bases and the deoxyribose backbone, causing base modifications, single and double strand breaks, DNA protein cross links, and changes in methylation and gene expression (Kıran et al., 2023; Martemucci et al., 2022).

Guanine oxidation to 8 hydroxy 2 deoxyguanosine (8 OHdG) and formation of thymine glycol exemplify such lesions and are associated with mutagenesis and carcinogenesis; 8 OHdG is widely regarded as a biomarker of DNA oxidative damage and oxidative stress and is more abundant in mitochondrial than nuclear DNA because of proximity to reactive oxygen species generation sites (Chandimali et al., 2025; Martemucci et al., 2022).

In cancer, 8 OHdG can mispair with adenine, driving GC to TA transversions in oncogenes and tumor suppressor genes, including p53, BRCA1, and APC, thereby promoting malignant transformation, genomic instability, and tumor progression (Chandimali et al., 2025; Tumilaar et al., 2024).

Reactive nitrogen species, including peroxynitrite, further contribute to DNA base alterations, strand breaks, and repair system impairment, compounding genomic damage and fostering carcinogenesis and neurodegeneration (Kıran et al., 2023; Kotha et al., 2022).

Proteins undergo extensive oxidative modification under conditions of elevated reactive oxygen and nitrogen species. Radical species such as superoxide, hydroxyl, peroxyl, alkoxyl, and hydroperoxyl, together with non radical oxidants including hydrogen peroxide, ozone, hypochlorous acid, singlet oxygen, and peroxynitrite, attack amino acid side chains to produce carbonyl derivatives and other oxidized residues (Martemucci et al., 2022).

Hypochlorous acid converts tyrosine to 3 chlorotyrosine, while metal associated histidine is oxidized to 2 oxohistidine; thiol groups in cysteine are transformed to sulfenic and sulfonic acids that cannot be reduced by thioredoxin or glutathione and thus represent irreversible modifications (Kıran et al., 2023; Martemucci et al., 2022).

Oxidative damage to lysine, proline, threonine, and arginine generates protein carbonyls, which denature proteins and abolish enzymatic, receptor, or transport functions; carbonyl groups and specific markers such as O tyrosine and 3 nitrotyrosine are widely used indicators of protein oxidation and have been linked to Parkinson's disease, muscle dystrophies, rheumatoid arthritis, atherosclerosis, progeria, Werner's syndrome, and aging (Kıran et al., 2023; Martemucci et al., 2022).

In neoplastic diseases, protein oxidation and carbonylation are particularly pronounced and alter signaling, cytoskeletal integrity, and cell cell interactions, supporting uncontrolled proliferation and invasion (Chandimali et al., 2025; Tumilaar et al., 2024).

Lipid peroxidation constitutes another major axis of oxidative injury. Reactive oxygen species initiate peroxidation of membrane and lipoprotein polyunsaturated fatty acids, producing lipid hydroperoxides that disrupt membrane fluidity, permeability, and biophysical properties and yield secondary products such as malondialdehyde, 4 hydroxy 2 nonenal, acrolein, prostanes, and hydroxyoctadecanoic acid (Kıran et al., 2023; Martemucci et al., 2022; Michalak, 2022).

Aldehydic end products are mutagenic and carcinogenic, can adduct to proteins and nucleic acids, and are implicated in the initiation of protein oxidation and DNA damage; malondialdehyde in particular is recognized as a key measure of lipid peroxidation in vivo (Martemucci et al., 2022; Michalak, 2022).

In cancer cells, lipid peroxidation leads to accumulation of reactive phospholipid derived aldehydes such as 4 hydroxy 2 nonenal, which are highly cytotoxic and influence matrix degradation, gene regulation, and metastatic behavior (Chandimali et al., 2025; Tumilaar et al., 2024).

Beyond carcinogenesis, lipid peroxidation contributes to regulated cell death pathways, including apoptosis induced by 4 hydroxy 2 nonenal and ferroptosis driven by extensive peroxidation, with products exhibiting both cytotoxic and cytoprotective, pro and anti inflammatory, and pro and anti atherogenic effects depending on context (Martemucci et al., 2022).

These molecular injuries translate into diverse disease phenotypes. Oxidative damage to DNA, proteins, and lipids

is associated with cardiovascular pathologies such as atherosclerosis and hypertension, neurodegenerative conditions including Alzheimer's, Parkinson's, Huntington's disease, amyotrophic lateral sclerosis, and multiple sclerosis, metabolic disorders like diabetes mellitus and metabolic syndrome, chronic kidney and pulmonary diseases, vitiligo, dermatitis, obesity related complications, and a wide spectrum of cancers affecting colorectal, breast, prostate, and lung tissues (Chandimali et al., 2025; Kıran et al., 2023; Kotha et al., 2022; Martemucci et al., 2022).

In many of these conditions, accumulation of reactive oxygen and nitrogen species, together with lipid peroxidation products and elevated malondialdehyde, reflects increased oxidative stress that drives inflammation, apoptosis, necrosis, mitochondrial dysfunction, and structural damage to membranes, collagen, and other extracellular components (Kıran et al., 2023; Martemucci et al., 2022).

The evidence thus delineates a mechanistic chain in which free radical and oxidant generation, macromolecular modification, and failure of antioxidant defenses converge to produce molecular lesions that underlie chronic disease and aging (Chandimali et al., 2025; Kotha et al., 2022; Michalak, 2022).

4 Oxidative Deterioration in Foods and Consumer Products

4.1 Mechanisms of Oxidative Spoilage in Food Matrices

Oxidative spoilage in food matrices is closely tied to lipid peroxidation and protein oxidation, both of which alter sensory attributes and nutritional quality. Lipid peroxidation proceeds through free radical chain reactions that generate lipid hydroperoxides as primary products. These hydroperoxides disrupt membrane fluidity and compromise membrane protein function, thereby affecting cellular integrity in biological tissues and functional properties in food systems. Iron mediated one electron reduction of lipid hydroperoxides followed by oxygenation yields epoxyallylic peroxyl radicals that further propagate peroxidation chains, amplifying oxidative deterioration. As peroxidation advances, reactive aldehydes such as 4 hydroxy 2 nonenal and malondialdehyde are formed. These aldehydes are highly cytotoxic and can adduct to proteins and DNA, extending oxidative damage beyond initial lipid targets and contributing to flavor defects and potential safety concerns in foods. In food matrices, lipid peroxidation occurs during harvesting, storage, and processing, leading to rancidity and degradation in nutritional value, flavor, color, safety, and texture across dairy products, meats, vegetables, fruits, and pharmaceuticals, making it a central mechanism of oxidative spoilage. The early stages of lipid oxidation can be monitored via the thiocyanate method, which quantifies hydroperoxides formed in a linoleic acid emulsion by measuring ferrous thiocyanate produced from the reaction of ferrous chloride and thiocyanate in the presence of hydroperoxides. This assay is widely applied to evaluate antioxidant activity in food systems and biological samples and thereby assess the extent of oxidative deterioration (Gulcin, 2025; Martemucci et al., 2022).

Beyond classical autoxidation, enzymic lipid peroxidation by lipoxygenase, cyclooxygenase, or cytochrome P450 generates regio, stereo, and enantio specific products. These pathways introduce molecular oxygen into polyunsaturated fatty acids such as arachidonic and linoleic acids, forming lipid hydroperoxides that act as unstable oxidative mediators. Their instability leads to further radical generation and oxidation reactions, which can be probed using fluorescent tools like diphenyl pyrenylphosphine (DPPP). In adapted assays, hydroperoxides formed by 15 lipoxygenase react with DPPP to give a fluorescent phosphine oxide, enabling simultaneous quantification of lipid and protein hydroperoxides and offering a sensitive means to track oxidative changes that do not necessarily impart odor or flavor but still degrade nutritional value and shelf life. These adaptations provide important analytical support for food quality control, where hydroperoxides and subsequent carbonyl formation mark early and advanced stages of oxidative spoilage (Mendonça et al., 2022).

Proteins in food systems also undergo oxidation, forming protein hydroperoxides that contribute to carbonylation and cross link formation. Such reactions lead to protein aggregation and loss of solubility, which interfere with sensory quality and nutritional value and can shorten product shelf life. Protein oxidation is partly independent of lipid oxidation, and elevated antioxidant concentrations can prolong the delayed phase of protein oxidation, thereby limiting dityrosine formation. This observation underlines that oxidative spoilage in foods encompasses both lipid and protein components and that managing oxidative processes requires attention to both classes of macromolecules. In complex matrices like milk, oxidation processes negatively impact quality by shortening shelf life and producing unpleasant aftertaste, while the antioxidant capacity reflects a balance between oxidants and endogenous or added antioxidants (Mendonça et al., 2022; Stobiecka et al., 2022).

Autoxidation of fats and oils in food products further illustrates spoilage mechanisms. Free fatty radicals, peroxides, and hydroperoxides generated during autoxidation lower oil quality and cause off flavors and off

odors. The rate and extent of autoxidation depend on the composition of the oil and storage conditions, including free fatty acid content, exposure to light, and temperature. These factors modulate the kinetics of oxygen consumption and radical formation, thereby controlling how quickly oxidative spoilage develops. Importantly, autoxidation can be slowed down or even completely prevented by the addition of antioxidants, which interrupt radical chains or chelate prooxidative metals, demonstrating a direct link between antioxidant strategies and mitigation of oxidative deterioration in food matrices (Gulcin, 2025; Kotha et al., 2022; Martemucci et al., 2022).

4.2 Oxidation and Stability in Medicines, Supplements and Cosmetics

Oxidative processes strongly influence the stability, safety, and efficacy of medicines, supplements, and cosmetic formulations, necessitating the strategic inclusion of antioxidants to protect active ingredients from degradation caused by exposure to oxygen and light. In fat-rich medicinal bases, supplements, and topical cosmetic matrices—such as blushes, eyeshadows, and lipsticks—the autoxidation of lipid components causes rancidity, unpleasant odors, and color shifts. Furthermore, oxidation can degrade sensitive active pharmaceutical ingredients (APIs), reducing product potency and shelf life. To mitigate these issues, formulations rely on a combination of natural and synthetic agents. Natural antioxidants like Vitamin E (tocopherols and tocotrienols) are widely used to safeguard hydrophobic phases by scavenging lipid peroxyl radicals. Water-soluble alternatives like ascorbic acid (Vitamin C) protect hydrophilic compartments, stimulate collagen synthesis, and chemically regenerate oxidized Vitamin E, although their own autoxidation can be problematically catalyzed by transition metal ions. Consequently, careful excipient selection and precise antioxidant balancing are essential to secure long-term product performance without compromising the structural integrity of the final commercial product (Gulcin, 2025; Parcheta et al., 2021).

4.3 Profiles of Synthetic Phenolic Antioxidants and Regulatory Limits

Synthetic phenolic antioxidants are widely used across the food, pharmaceutical, and cosmetic industries due to their high efficacy, chemical stability, and lipophilicity, which provide "carry through" properties in baked and fried goods and prevent nutrient degradation. However, evidence of potential carcinogenic, mutagenic, and organ toxic effects has led to regulatory scrutiny and restricted use. Butylated hydroxyanisole (BHA) is employed to stabilize fats and oils in foods, pharmaceuticals, cosmetics, plastics, rubber, and petroleum products. Butylated hydroxytoluene (BHT) is an aromatic compound that efficiently scavenges free radicals in hydrophobic environments. While BHT benefits from heat stability and lipophilicity to protect product components, high doses have demonstrated harmful effects in in vitro and in vivo studies, necessitating restricted use to balance preservation benefits against health risks.

Tert-butylhydroquinone (TBHQ) is highly effective at delaying nutrient degradation and preventing rancidity in frying oils and processed foods, retaining its capacity at temperatures reaching 180°C. However, high doses demonstrate toxic effects in animal models, restricting its acceptable limits due to potential organ toxic risks. Propyl gallate (PG) protects polyunsaturated fats and exhibits secondary antimicrobial activity by blocking microbial respiration, though its utility is balanced against distinct toxicological concerns. Finally, octyl gallate (OG) functions as an antioxidant excipient to preserve oxidation-sensitive active ingredients, though excessive intake can be toxic or induce adverse reactions. Regulatory guidelines generally restrict the concentration of these synthetic antioxidants, capping individual limits, and recommend proportionally reducing their use in combination to mitigate cumulative toxicity risks (Gulcin 2025; Michalak, 2022; Parcheta et al., 2021).

5 Endogenous Antioxidant Defense Systems

5.1 Enzymatic Antioxidants and Redox Enzyme Networks

Enzymatic antioxidants constitute a primary defense against reactive oxygen species, operating in tightly integrated redox networks that control hydrogen peroxide and lipid hydroperoxide levels and sustain cellular redox homeostasis. Key enzymes include superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase, glutathione S transferase, peroxiredoxins, thioredoxin reductase and paraoxonases, together with reduced glutathione as an essential cofactor and redox buffer (Kıran et al., 2023; Mendonça et al., 2022; Parcheta et al., 2021).

Superoxide dismutases convert superoxide anions to hydrogen peroxide and molecular oxygen, thereby preventing superoxide from reacting with nitric oxide to form peroxynitrite. In mammals, three isoforms are distinguished: Cu,ZnSOD (SOD1) in the cytosol, MnSOD (SOD2) in the mitochondrial matrix, and extracellular SOD3. Their catalytic reaction is $2O_2^- + 2H^+ \xrightarrow{SOD} H_2O_2 + O_2$, which places SOD at the gateway of the enzymatic antioxidant system and directly links it to downstream hydrogen peroxide detoxification (Kıran et al., 2023). Hydrogen peroxide removal is mainly carried out by catalase and glutathione peroxidase. Catalase is a hemoprotein localized predominantly in peroxisomes, with high activity in liver, kidney, myocardium, striated muscle

and erythrocytes. It catalyzes $2H_2O_2 \xrightarrow{CAT} 2H_2O + O_2$ and exhibits a very high turnover number, close to diffusioncontrolled limits, which allows rapid conversion of H_2O_2 to less reactive species under conditions of high hydrogen peroxide concentration (Kıran et al., 2023; Losada-Barreiro et al., 2022; Parcheta et al., 2021).

Human catalase operates through a twostep mechanism in which Catalase Fe(III) reacts with H_2O_2 to form compound I, followed by reaction of compound I with a second H_2O_2 molecule to regenerate Catalase Fe(III), releasing water and oxygen. At low H_2O_2 levels, catalase can also exhibit peroxidase activity, oxidizing organic substrates such as formic acid, ethanol, methanol, phenol or hydroperoxides according to $ROOH + AH_2 \rightarrow H_2O + ROH + A$, thereby coupling peroxide removal to organic oxidation (Parcheta et al., 2021).

Glutathione peroxidases form a family of multiple isozymes that use reduced glutathione as an electron donor to reduce both H_2O_2 and lipid hydroperoxides. Their general reactions are $H_2O_2 + 2GSH \xrightarrow{GPx} GSSG + 2H_2O$ and $ROOH + 2GSH \xrightarrow{GPx} ROH + GSSG + H_2O$, highlighting a central role in inhibiting lipid oxidation and detoxifying hydroperoxides. In mammals, six GPx isoforms are recognized, with GPx1, 2, 3 and 4 as selenoproteins and GPx5 and some nonmammalian forms as non-selenium enzymes. The catalytic center of selenium-dependent GPx contains selenocysteine, which enters a redox cycle in which selenol (ESeH) is oxidized to selenenic acid (ESeOH) by peroxides, then forms a selenyl sulfide adduct (ESeGH) with GSH, ultimately leading to GSSG formation and regeneration of ESeH. In humans, four major isoenzymes are described: extracellular GPx3, cytosolic and mitochondrial GPx1, cytosolic GPx2, and phospholipid hydroperoxide specific GPx4, with the liver containing particularly high levels because of its detoxification function (Kıran et al., 2023; Mendonça et al., 2022; Parcheta et al., 2021).

Glutathione is a tripeptide (γ glutamyl cysteinyl glycine) present in all plant and animal cells and acts as a direct antioxidant and a substrate for GPx. It protects against free radicals, peroxides and other toxic compounds, participates in detoxification of electrophiles, prostaglandin and leukotriene metabolism, amino acid transport and micronutrient absorption, but its predominant role is antioxidant. During regeneration of small molecule antioxidants such as vitamin C and vitamin E, GSH is oxidized to glutathione disulfide, which can be reduced back to GSH by glutathione reductase using NADPH as a cofactor. The GSH/GSSG ratio is widely used to evaluate intracellular oxidative stress, with increased GSSG indicating elevated oxidative burden. Glutathione reductase, a flavoprotein enzyme, restores GSH from GSSG and thereby supports neutralization of hydrogen peroxide and maintenance of protein sulfhydryl groups in the reduced state (Kıran et al., 2023; Martemucci et al., 2022; Mendonça et al., 2022; Parcheta et al., 2021).

Beyond SOD, CAT, GPx, GSH and GR, additional enzymes complement the redox network. Glutathione S transferases catalyze conjugation of GSH to xenobiotics and organic hydroperoxides, showing GPx like activity by forming alcohols and GSSG during peroxide reduction. Thioredoxin reductase, another NADPH dependent flavoprotein, maintains thioredoxins in the reduced state and contributes to protein repair, transcription factor activation and reduction of peroxiredoxins, which themselves detoxify small amounts of H_2O_2 and alkyl hydroperoxides in multiple cellular compartments. Paraoxonases associated with high density lipoproteins in plasma inactivate pro inflammatory and pro-oxidant mediators and participate in xenobiotic and drug metabolism, further linking enzymatic antioxidants to systemic protection against oxidative and electrophilic stress (Mendonça et al., 2022; Parcheta et al., 2021).

These coordinated enzymatic systems, supported by NADPH supply and small molecule antioxidants, exemplify endogenous defense networks that limit reactive oxygen species formation, remove primary oxidants, and prevent propagation of lipid and protein oxidation in biological tissues, thereby underpinning redox control relevant to the oxidative processes discussed for foods, medicines and other products in Section 4.2 (Kıran et al., 2023; Losada-Barreiro et al., 2022; Martemucci et al., 2022; Mendonça et al., 2022).

5.2 Non-Enzymatic Metabolites and Redox Buffering

Non enzymatic metabolites form a crucial complement to enzymatic antioxidants, acting as redox buffers and direct radical scavengers that sustain cellular homeostasis and modulate oxidative processes. Reduced glutathione (GSH), a low molecular weight tripeptide composed of glycine, cysteine and glutamic acid, is present in all plant and animal cells and functions as an intracellular reductant protecting against free radicals, peroxides and other toxic compounds (Mendonça et al., 2022; Parcheta et al., 2021).

GSH occurs in millimolar concentrations in cytosol, mitochondria and nuclei, and its thiol group participates in reversible disulfide bond formation that influences the structure and stability of water soluble proteins through equilibrium between 2R-SH and R-SS-R plus electrons and protons (Jomova et al., 2023; Parcheta et al., 2021).

Beyond direct antioxidant action, GSH is involved in detoxification of electrophilic xenobiotics, metabolization of prostaglandins and leukotrienes, amino acid transport, and intestinal absorption of micronutrients such as iron and selenium, yet its predominant role remains antioxidant, integrating metabolic and redox functions in one molecule (Mendonça et al., 2022).

GSH serves as a substrate for glutathione peroxidase (GPx), which reduces hydrogen peroxide and lipid hydroperoxides at the cost of oxidizing GSH to glutathione disulfide (GSSG), linking non enzymatic and enzymatic defenses. GSSG is then reduced back to GSH by glutathione reductase in the presence of NADPH, closing the glutathione cycle and maintaining high GSH levels that buffer oxidative insults (Gulcin, 2025; Mendonça et al., 2022).

The GSH/GSSG ratio is widely used to evaluate intracellular oxidative stress; an increased proportion of GSSG reflects elevated oxidative burden, and quantitative determination can be carried out enzymatically or by fluorometric methods described by Brehe and Burch (Mendonça et al., 2022). Age related declines in plasma GSH with concomitant increases in GSSG have been observed, and GSH depletion is associated with oxidative stress, while elevations in GSH may result from increased biosynthesis or enhanced GSSG reduction via glutathione reductase, underlining the dynamic nature of redox buffering in vivo (Gulcin, 2025; Mendonça et al., 2022).

Non enzymatic metabolites also act by regenerating other antioxidants and repairing oxidatively damaged biomolecules. GSH participates in the regeneration of oxidized small molecule antioxidants such as vitamins C and E, thus sustaining their radical scavenging capacity and extending protection against reactive oxygen species (Jomova et al., 2023; Mendonça et al., 2022).

It contributes to repair of proteins, nucleic acids and lipids damaged by peroxidation, and maintains protein sulfhydryl groups in the reduced state, preserving enzymatic activity and structural integrity under oxidative challenges (Mendonça et al., 2022). In the context of hydrogen peroxide metabolism, GSH, together with selenium dependent GPx, decomposes H_2O_2 and protects membranes and cellular components from oxidative damage, complementing catalase activity described previously in enzymatic defense networks (Mendonça et al., 2022; Parcheta et al., 2021).

Vitamin E and vitamin C illustrate how non enzymatic antioxidants cooperate within redox buffering schemes. α Tocopherol is a fat soluble vitamin anchored in lipid membranes, with its hydroxyl bearing aromatic ring oriented toward the aqueous phase where it scavenges reactive oxygen species and prevents membrane lipid peroxidation. Reaction of α tocopherol with radicals such as hydroxyl generates the α tocopheryl radical, which, although less reactive than initiating species, must be recycled to sustain antioxidant protection. Vitamin C, present predominantly as ascorbate anion under physiological conditions, reacts with radicals by hydrogen atom abstraction to yield a relatively stable ascorbyl radical (Jomova et al., 2023). Due to favorable one electron reduction potentials, ascorbate can regenerate vitamin E from its radical form, converting α tocopheryl radical back to α tocopherol while itself becoming oxidized, thereby forming an interlinked antioxidant pair that shuttles electrons and protons to buffer oxidative events in membranes and aqueous compartments (Jomova et al., 2023; Mendonça et al., 2022).

Coenzyme Q10 and other metabolic antioxidants extend this non enzymatic redox buffering capacity. Coenzyme Q10 is a natural lipophilic antioxidant localized primarily in mitochondria as a component of the electron transport chain, where it inhibits lipid oxidation and regenerates oxidized antioxidants including vitamins C and E (Losada-Barreiro et al., 2022).

Together with uric acid, bilirubin, transferrin, lipoic acid and nutrient antioxidants such as carotenoids and flavonoids, these metabolites participate in neutralizing reactive oxygen and nitrogen species, interrupting free radical reactions, and binding or inactivating metal ions that catalyze oxidative processes (Losada-Barreiro et al., 2022; Parcheta et al., 2021).

During postprandial metabolism, when production of reactive oxygen and nitrogen species increases, dietary phenolic compounds ingested with meals can augment endogenous non enzymatic defenses, enhancing plasma antioxidant capacity and helping to rebalance redox status by modulating cellular oxidative pathways (Losada-Barreiro et al., 2022).

Collectively, GSH, vitamins C and E, coenzyme Q10 and related small molecules form an intricate non enzymatic antioxidant network. They act as direct scavengers, redox couples, and co substrates for enzymatic systems, and their concentrations, redox states, and interconversion kinetics determine the buffering capacity that stabilizes reactive species generated by autoxidation and metabolic processes. This buffering underpins the ability of cells and tissues to withstand oxidative challenges that arise from normal physiology, environmental exposures, and nutrient derived stresses (Jomova et al., 2023; Losada-Barreiro et al., 2022; Mendonça et al., 2022; Parcheta et al., 2021).

6 Dietary and Supplemental Antioxidants

6.1 Vitamin-Based Antioxidants and Carotenoids

Vitamin E and carotenoids represent central vitamin based antioxidant families that operate primarily in lipid phases and are obtained through diet and supplements. Vitamin E comprises eight naturally occurring lipophilic compounds, four tocopherols and four tocotrienols, all sharing a chromanol ring and a 16 carbon phytyl like side chain, with tocopherols possessing saturated phytyl chains and tocotrienols unsaturated geranylgeranyl chains containing three double bonds (Mendonça et al., 2022; Parcheta et al., 2021).

Humans and animals cannot synthesize vitamin E, which is acquired from plant foods, with γ tocopherol most prevalent in seeds and α tocopherol predominant in plant tissues (Mendonça et al., 2022).

Among vitamin E constituents, α tocopherol exhibits the highest biological activity and is the most common form in the human organism, acting as a lipid phase chain breaking antioxidant that inhibits lipid peroxidation by scavenging lipid peroxyl radicals before they react with adjacent fatty acid chains or membrane proteins (Mendonça et al., 2022; Parcheta et al., 2021).

As part of cell membranes and lipoproteins, vitamin E protects lipids from oxidation, stops radical chains by forming low reactivity derivatives unable to attack lipid substrates, and appears effective in improving health outcomes, with diminished risk of cancer and cardiovascular disease reported in clinical trials. All tocopherols and tocotrienols show strong lipoperoxyl radical scavenging activity through hydrogen donation from the phenolic group on the chromanol ring, and forms with an unsubstituted 5 position, such as γ tocopherol, can additionally trap electrophiles including reactive nitrogen species, detoxifying NO_2 and peroxynitrite via 5 nitro γ tocopherol formation, whereas α tocopherol with a methyl group at the 5 position lacks this electrophile trapping capacity (Mendonça et al., 2022).

Trolox, a water soluble tocopherol derivative, extends vitamin E functionality by dissolving in aqueous phases and entering different cell structures from both hydrophilic and hydrophobic sides of membranes, protecting cells against hydrogen peroxide toxicity through reduced H_2O_2 uptake and activation of antioxidant enzymes such as glutathione peroxidase and catalase, and serving as a reference reagent to express total antioxidant capacity of plasma or serum as Trolox equivalents (Parcheta et al., 2021).

Vitamin C (ascorbic acid) complements vitamin E as a water-soluble antioxidant with well characterized bioavailability. Ascorbic acid is fairly available from foods and easily transported in the intestine; about 80–90% of an oral dose up to 100mg/day is absorbed, with absorption decreasing rapidly at higher intakes around 500mg/day (Mendonça et al., 2022).

In the human body it participates in hydroxylation reactions crucial for collagen and carnitine synthesis, contributes to prostacyclin and nitric oxide formation, and thereby maintains vascular function and reduces atherogenesis risk. Ascorbic acid also acts as a cofactor for enzymes such as dopamine hydroxylase and displays reducing and chelating properties that underpin its antioxidant role. Functionally, vitamin C regenerates vitamin E after it has been oxidized during free radical scavenging, with interactions between vitamin C and E radicals occurring in homogeneous solutions and liposomal membranes; ascorbate likely protects against lipid peroxidation by oxidizing reduced α tocopherol back to its active form, thus sustaining membrane antioxidant capacity. Stability of ascorbic acid in pharmaceuticals increases with the number of bound transition metal ions, which catalyze its autooxidation, and strong metal chelating ligands modulate this catalytic oxidation, indicating the importance of formulation chemistry for vitamin C containing medicines and supplements (Parcheta et al., 2021).

Experimental supplementation illustrates its protective effects: in streptozotocin induced diabetic rats, 100mg/kg ascorbic acid administered orally for two weeks before cerebral ischemia reperfusion significantly suppressed neuronal damage, reduced edema and infarct volume, and was interpreted as attenuating exacerbated cerebral ischemic injury via anti apoptotic and anti-inflammatory actions linked to improved oxidative stress (Mendonça et al., 2022).

Carotenoids form another major class of dietary antioxidants with pronounced roles in human health and product stability. They are fat soluble natural pigments responsible for the colors of many fruits, vegetables, and flowers and include two main structural classes: carotenes, such as α carotene, β carotene, γ carotene, and lycopene, containing only carbon and hydrogen, and xanthophylls, such as lutein, zeaxanthin, astaxanthin, β cryptoxanthin, and canthaxanthin, which are oxygenated derivatives of carotenes (Gulcin, 2025; Michalak, 2022). β carotene is widely distributed in yellow orange and dark green fruits and vegetables, with numerous plant sources listed for diet and supplement formulations, and is the most common carotenoid in foods (Michalak, 2022).

In the human diet, key carotenoids include β carotene, lycopene, lutein, β cryptoxanthin, zeaxanthin, and astaxanthin; their extended conjugated double bond

systems determine chemical properties and confer antioxidant potential, particularly through singlet oxygen quenching and free radical scavenging (Gulcin, 2025; Michalak, 2022).

These pigments protect cellular lipids, proteins, and DNA from attack by free radicals, and their antioxidant power is linked to the high number of conjugated double bonds and pronounced lipophilicity (Gulcin, 2025; Michalak, 2022).

Observational studies associate carotenoids and vitamin E with lower cardiovascular disease risk, and carotenoids are viewed as affordable preventive agents, though human intervention trials show mixed results, including positive, null, and occasionally harmful outcomes at high doses in risk populations (Gulcin, 2025). At the skin level, β carotene, lycopene, and lutein are present in significant amounts, and evidence summarizes their antioxidant effects, including direct scavenging of reactive oxygen species, enhancement of glutathione peroxidase, catalase, glutathione transferase and vitamin C, protection of lipid structures from ultraviolet radiation, inhibition of oxidant induced nuclear factor κB activation and pro inflammatory cytokine production, and suppression of matrix metalloproteinases involved in photoageing. Oral and topical administration of carotenoids such as lutein, zeaxanthin, β carotene, and α tocopherol reduce UV induced erythema, lipid peroxidation and sunburn cell formation, demonstrating synergistic roles of carotenoids and vitamins in photoprotection and maintenance of skin physiology parameters like hydration, elasticity and surface lipids (Michalak, 2022).

Carotenoids are sensitive to light, heat and oxygen, degrading under improper handling, which is critical for their use as food colorants and active ingredients in cosmetics and supplements where structural integrity is required to ensure antioxidant function (Gulcin, 2025).

7 Technological Use of Antioxidants in Preservation and Stability

7.1 Antioxidant Formulation, Processing, and Delivery Strategies

Translating antioxidant potential into practical preservation and clinical efficacy requires advanced processing and formulation strategies designed to optimize bioaccessibility, overcome low aqueous solubility, and shield labile compounds from degradation. Mechanical and thermal processing methods can actively modulate the bioaccessibility of antioxidants within a matrix; techniques such as grinding, milling, thermal pasteurization, sterilization, and encapsulation can disrupt cellular barriers, cleave hydrophobic forces, or alter conjugation patterns to facilitate the release of active molecules. Non-destructive methods, such as ultrasound-assisted extraction, utilize specialized frequencies to break micellar structures, maximizing the extraction and recovery of hydrophobic antioxidants without inducing alkaline saponification or thermal degradation (Kotha et al., 2022; Mendonça et al., 2022).

Nanotechnology-based delivery systems are increasingly deployed to overcome the limitations of conventional dosage forms, such as weak physicochemical stability, rapid first-pass metabolism, and poor gastrointestinal absorption. Liposomes and chitosan-coated liposomes utilize phospholipid bilayers that mimic cell membranes; the coating increases mucosal adhesion, apparent permeability, and C_{max} though they can be susceptible to structural leakage and physical aggregation over time. Solid lipid nanoparticles (SLNs) feature a core of lipids that remain solid at body temperature, allowing them to prolong blood circulation and accommodate varied cargos, though they suffer from relatively low cargo loading capacity and variable expulsion rates. Polymeric micelles, assembled from amphiphilic molecules in water, significantly improve the solubility of poorly water-soluble antioxidants but face constraints regarding scale-up manufacturing and poor hydrophilic cargo compatibility. Phytosomes, formed through direct chemical complexes between plant extracts and phospholipids, provide high entrapment capacity but exhibit lower standalone physicochemical stability and scaling challenges. Alternatively, proliposomes—dry granular formulations that seamlessly hydrate into liposomes in vivo—facilitate easy dosage filling and sterilization, though they require precise hydration kinetics to prevent premature drug leakage (Losada-Barreiro et al., 2022).

For all these carriers, particle size optimization is an absolute requirement, as formulations must be strictly engineered between 20 nm and 200 nm to avoid rapid renal clearance on the lower end and hepatic splenic sequestration on the higher end. Furthermore, active targeting and stimuli-responsive mechanisms allow for precision delivery; active targeting involves surface ligands like antibodies or aptamers that bind to overexpressed receptors on target cells to trigger endocytosis, while stimuli-sensitive systems are designed to release their payload only when triggered by localized shifts in pH, temperature, or elevated reactive oxygen species. These integrated technological strategies ensure that antioxidants remain stable during processing and are delivered precisely to their sites of action, maximizing their protective efficiency while preserving physiological redox signaling (Losada-Barreiro et al., 2022).8 Antioxidants, Human Health and Safety

8.1 Dietary Patterns, Lifestyle Factors and Oxidative Balance

Dietary patterns and lifestyle choices strongly modulate oxidative balance by shaping both endogenous defenses and the supply of exogenous antioxidants. Exogenous compounds obtained from food include vitamin E, vitamin C, carotenoids, trace elements such as selenium, copper, zinc, and manganese, and phytochemicals like isoflavones, polyphenols, and flavonoids (Martemucci et al., 2022).

These molecules act as strong electron donors that react with free radicals before major cellular targets are damaged, and they constitute a second line of defense complementing enzymatic systems such as superoxide dismutase, catalase, glutathione peroxidase, and glutathione reductase (Chandimali et al., 2025; Martemucci et al., 2022).

Evidence indicates active interactions between endogenous and dietary antioxidants; oral administration of vitamin E to humans leads to a significant increase in red blood cell glutathione, which may arise from stimulated glutathione synthesis via glutathione synthetase or reduced glutathione utilization for detoxification of free radicals (Martemucci et al., 2022).

These observations support the view that diet not only supplies scavengers but can also enhance enzymatic and non enzymatic antioxidant capacity. Natural antioxidants naturally present in foods have attracted attention because of their safety and potential nutritional and therapeutic effects on health. They are widely distributed across plant parts, including fruits, vegetables, nuts, seeds, leaves, roots, and bark, and can be ingested directly through fresh produce (Gulcin, 2025; Martemucci et al., 2022).

Dietary patterns rich in fruit, vegetables, fish, and whole grains have been associated with improved antioxidant system efficiency and reduced progression of oxidative stress related diseases, although detailed outcomes are not specified in the sources. In contrast, malnutrition and deficiency of antioxidant nutrients have been related to conditions such as chronic obstructive pulmonary disease and Crohn's disease, with lack of antioxidants increasing disease risk and worsening treatment outcomes (Martemucci et al., 2022).

Decreased intake or availability of vitamins C and E, carotenoids, and polyphenols can reduce the efficacy of antioxidant defenses and aggravate disease progression, illustrating how inadequate dietary patterns shift the oxidant–antioxidant balance toward damage (Chandimali et al., 2025; Martemucci et al., 2022).

Micronutrients that interact with endogenous enzymes further link diet to oxidative control. Selenium cooperates with glutathione peroxidase, while zinc interacts with superoxide dismutase, and combined selenium and vitamin E intake has shown protective effects against oxidative damage in the colon of rats with ulcerative colitis (Martemucci et al., 2022).

In the postprandial state, characterized by intense metabolic traffic and oxidative metabolism of absorbed nutrients, the imbalance between production of reactive oxygen and nitrogen species and their quenching by defenses can lead to biological dysfunction and cellular damage, particularly in obese or diabetic individuals consuming high amounts of carbohydrates and lipids. Hyperglycemia promotes non enzymatic glycation of proteins such as low density lipoprotein and increases susceptibility of LDL to oxidation, which can facilitate atherosclerosis progression (Losada-Barreiro et al., 2022).

Concomitant consumption of phenol rich fruits and vegetables during meals may counterbalance these oxidative and inflammatory effects by increasing plasma antioxidant capacity and modulating redox sensitive pathways, including nuclear factor κB activation and insulin signaling (Chandimali et al., 2025; Losada-Barreiro et al., 2022).

Lifestyle and environmental factors interact with diet in determining oxidative stress levels and the effectiveness of antioxidants. Unhealthy dietary patterns, exposure to pollutants, ultraviolet radiation, and tobacco smoke contribute to increased free radical production and can overwhelm endogenous defenses, making exogenous antioxidants more critical for maintaining redox balance (Chandimali et al., 2025).

Research shows that antioxidants derived from plant sources, including flavonoids, carotenoids, and polyphenols, can mitigate oxidative damage and reduce risks of cardiovascular, neurodegenerative, and metabolic diseases by scavenging radicals and modulating oxidative stress pathways (Chandimali et al., 2025; Gulcin, 2025; Losada-Barreiro et al., 2022).

At the same time, high dose antioxidant supplementation, even from natural sources, may exert prooxidant effects, disrupt physiological redox signaling, impair normal cellular processes such as tumor cell killing by chemotherapeutic oxidative mechanisms, or even promote tumorigenesis, underscoring the importance of appropriate dosing and avoiding uncontrolled use (Chandimali et al., 2025; Kotha et al., 2022; Martemucci et al., 2022).

Inappropriately high intake of supplements can act as prooxidants, whereas obtaining antioxidants from fruits and vegetables is regarded as a safer way to avoid overdosing (Martemucci et al., 2022).

Bioaccessibility and bioavailability of dietary antioxidants are further influenced by food processing, matrix interactions, and metabolism. Processing techniques such as drying, freezing, thermal treatment, pasteurization, ultrasound, milling, and encapsulation can increase or decrease the bioaccessibility of phytochemicals by modifying chemical bonds, disrupting cell walls, or protecting compounds until absorption, with effects depending on stability and matrix protective interactions (Kotha et al., 2022; Mendonça et al., 2022).

In vivo, structural transformations of flavonoids and other polyphenols, removal or addition of sugar moieties, and interactions with metabolites like uric acid alter antioxidant and prooxidant behavior, and large increases in plasma antioxidant capacity after polyphenol rich meals may partly reflect elevated urate rather than polyphenols themselves (Kotha et al., 2022; Losada-Barreiro et al., 2022).

These findings highlight that oxidative balance depends not only on what is consumed but also on how foods are processed, how compounds are metabolized, and how lifestyle and environmental exposures modulate the interplay between free radical generation and antioxidant defenses (Chandimali et al., 2025; Kotha et al., 2022; Losada-Barreiro et al., 2022; Martemucci et al., 2022).

8.2 Preventive and Therapeutic Potential of Antioxidant Strategies

Antioxidant based strategies for prevention and therapy rely on coordinated use of endogenous defenses and exogenous agents from diet and supplements to manage oxidative stress that contributes to chronic disease. Endogenous enzymatic antioxidants such as superoxide dismutase, catalase, and glutathione peroxidase convert superoxide and hydrogen peroxide into less reactive species, while non enzymatic glutathione directly detoxifies reactive oxygen species and regenerates other antioxidants. These systems provide a basal protective framework but are often overwhelmed by increased oxidative burden caused by environmental pollutants, unhealthy diets, and chronic pathologies, creating a rationale for exogenous antioxidant interventions (Chandimali et al., 2025).

Exogenous antioxidants obtained from foods or supplements include vitamins C and E, carotenoids, trace metals like selenium and zinc, and diverse polyphenols, which collectively form a second line of defense against free radicals. They act by scavenging reactive species, stimulating endogenous antioxidant enzymes, or both, and function as strong electron donors that react with radicals before major biomolecules are damaged (Chandimali et al., 2025; Martemucci et al., 2022).

Vitamin C neutralizes reactive oxygen species in aqueous compartments and regenerates oxidized vitamin E, while α tocopherol protects membranes by quenching lipid peroxyl radicals; their synergistic interaction ensures more comprehensive protection of cellular structures. Phytochemicals such as flavonoids, carotenoids, and polyphenols add further protective capacity by scavenging radicals, modulating oxidative stress pathways, and supporting eye and systemic health (Chandimali et al., 2025).

Dietary patterns rich in fruit, vegetables, fish, and whole grains have been associated with improved antioxidant efficiency and attenuation of disease progression, whereas malnutrition and deficiency of antioxidant nutrients such as vitamins C and E, carotenoids, and polyphenols are linked to chronic obstructive pulmonary disease, Crohn's disease, and poorer treatment outcomes. Foods providing α tocopherol and minerals like selenium and zinc are considered useful to alleviate reactive oxygen species related damage, with selenium and zinc interacting with glutathione peroxidase and superoxide dismutase, respectively, to combat oxidative stress. Combined selenium and vitamin E intake has demonstrated protective effects against oxidative damage in the colon of rats with ulcerative colitis, illustrating preventive and therapeutic potential in an inflammatory setting (Martemucci et al., 2022).

Postprandial metabolism represents a critical window where antioxidant strategies may offer benefit. After meals, particularly in obese or diabetic individuals consuming high amounts of carbohydrates and lipids, reactive oxygen and nitrogen species production increases, non enzymatic glycation of low density lipoprotein is promoted, and LDL becomes more susceptible to oxidation, contributing to atherosclerosis (Losada-Barreiro et al., 2022).

Concomitant intake of phenol rich fruits and vegetables can enhance plasma antioxidant capacity and modulate redox sensitive pathways, including nuclear factor κB and insulin signaling, suggesting that diet based antioxidant strategies may mitigate postprandial oxidative and inflammatory insults (Chandimali et al., 2025; Losada-Barreiro et al., 2022).

Therapeutic use of antioxidants must also account for dose dependent prooxidant behavior and interference with physiological redox signaling. High dose supplementation, even from natural sources, can act as prooxidants, worsening oxidative stress, impairing normal cellular signaling, or promoting tumorigenesis; excessive vitamins C and E may counteract the oxidative stress required for chemotherapy or radiotherapy to kill cancer cells,

potentially reducing treatment efficacy (Chandimali et al., 2025; Kotha et al., 2022; Martemucci et al., 2022).

Inappropriate use of dietary antioxidants can therefore be detrimental to physiological scavenging of reactive oxygen species, and obtaining antioxidants from fruits and vegetables is considered a safer route to avoid overdosing compared with concentrated supplements (Martemucci et al., 2022).

Evidence that combined vitamin C and E supplementation during exercise blunted improvements in insulin sensitivity and adiponectin that occurred without supplementation further underscores the need for cautious integration of antioxidants into therapeutic regimens (Losada-Barreiro et al., 2022).

Processing and formulation influence the preventive potential of antioxidant strategies by modulating bioaccessibility and bioavailability. Techniques such as drying, freezing, thermal processing, pasteurization, ultrasound treatment, milling, chemical and enzymatic treatments, and encapsulation can increase or decrease the release and stability of phytochemicals, with effects determined by compound stability, matrix protective interactions, and processing conditions (Kotha et al., 2022; Mendonça et al., 2022).

Ultrasound assisted extraction, for example, facilitates solvent penetration into hydrophobic regions without chemical degradation, improving recovery of antioxidant compounds for use in functional foods and supplements (Mendonça et al., 2022). Optimizing such processes is essential to ensure that preventive and therapeutic antioxidant strategies deliver bioavailable molecules capable of reaching relevant biological targets (Kotha et al., 2022; Losada-Barreiro et al., 2022).

Future directions emphasize development of targeted antioxidant therapies tailored to individual genetic, environmental, and lifestyle profiles, evaluation of antioxidant rich diets and supplements in long term clinical trials, and integration of non pharmacological interventions such as functional foods and lifestyle modifications into comprehensive oxidative stress management. Personalized dosing and careful assessment of interactions with other medications are necessary to enhance therapeutic outcomes while minimizing adverse effects, and validated biomarkers of oxidative stress will be critical for monitoring damage and guiding interventions (Chandimali et al., 2025; Martemucci et al., 2022).

9 Measurement, Bioavailability and Emerging Directions

9.1 Assessment of Oxidative Damage and Antioxidant Capacity

Reliable assessment of oxidative damage and antioxidant capacity is fundamental for interpreting how antioxidants function in foods, biological fluids, and tissues. Current approaches distinguish between antioxidant activity, a kinetic concept, and antioxidant capacity, a thermodynamic concept, yet these terms are often used interchangeably despite being measured differently (Kotha et al., 2022).

Antioxidant activity assays typically follow reaction rates with radicals or oxidants, whereas antioxidant capacity assays evaluate the overall ability of a sample to reduce or quench a probe at equilibrium. This conceptual distinction underpins assay design and data interpretation in both food and biomedical contexts (Kotha et al., 2022; Mendonça et al., 2022).

Chemical or biological test systems usually measure total antioxidant capacity by monitoring one of several elements of an oxidation reaction: substrate consumption, oxidant decay, intermediates, or final products. Methods based on hydrogen atom transfer and electron transfer dominate; mixed mode assays such as ABTS are widely applied to foods, plant extracts, processed products, and isolated compounds. These assays are used on plasma and cultured cells to relate total antioxidant capacity to altered redox status, oxidative stress, disease conditions, diets, supplements, or pharmacological treatments (Mendonça et al., 2022).

However, the concentration of specific antioxidant molecules cannot predict total antioxidant capacity because multiple compounds contribute, some only after metabolic transformation, and because antioxidants act synergistically in vitro and in vivo. As a result, focusing on single molecules can under or overestimate functional antioxidant behavior (Kotha et al., 2022; Mendonça et al., 2022).

Extensive use of in vitro assays has revealed major methodological limitations. Assays are non specific and respond to every component in a sample matrix, which means that changes in assay readouts cannot be unambiguously assigned to particular antioxidants or linked directly to health outcomes (Kotha et al., 2022).

Different assays operate with distinct mechanisms, pH, temperatures, and matrices, so their outputs are not inter convertible; FRAP, ORAC, DPPH, ABTS, and related methods each generate assay specific values without valid proportionality factors for conversion. AOAC and IUPAC concluded that no single universal antioxidant assay

accurately captures antioxidant activity or total capacity, and that results from different methods cannot be directly compared. Reflecting these concerns, the United States Department of Agriculture removed its ORAC database because accumulating evidence indicated no clear relationship between in vitro antioxidant activity values and the effects of bioactive compounds on human health (Gulcin, 2025; Kotha et al., 2022).

Additional analytical issues further complicate assessment. Reporting results in multiple units, for example expressing total phenolics as gallic acid, ascorbic acid, or ferulic acid equivalents, hampers comparison across studies (Kotha et al., 2022).

Chromophore or luminescent probe interactions with non target components may interfere with signal detection, while many protocols ignore reaction kinetics and thus fail to capture time dependent aspects of antioxidant behavior. Specific limitations include solubility constraints of antioxidants in extraction solvents, interference by colored substances and other reducers, and the frequent use of synthetic radicals that do not match physiological reactive species (Gulcin, 2025; Kotha et al., 2022).

In vivo assessment introduces additional layers of complexity. Whole plasma or serum based assays integrate contributions from exogenous dietary antioxidants such as ascorbic acid, vitamin E, and polyphenols and from endogenous molecules including enzymes (superoxide dismutase, glutathione peroxidase, catalase) and small macromolecules like albumin, bilirubin, ceruloplasmin, ferritin, and glutathione, without distinguishing their individual roles. Regulatory bodies such as EFSA have therefore criticized the use of such global antioxidant capacity measures for inferring human health effects. Moreover, in vivo antioxidant levels and activities depend on absorption, distribution, metabolism, and excretion profiles that are not captured in vitro; actual tissue concentrations are shaped by biological membranes, physicochemical properties, and gastrointestinal conditions (Kotha et al., 2022).

Bioaccessibility and bioavailability must thus be accounted for when interpreting capacity measurements, since only the fraction released from the matrix, absorbed into the bloodstream, and distributed to target sites can exert biological effects. Matrix effects and processing conditions are central determinants of both oxidative damage markers and measured capacity. Sample preparation steps such as grinding, drying, and extraction, together with the choice of solvent, strongly influence which antioxidants are recovered and how they appear in assays, particularly for phytochemicals with a wide polarity range (Kotha et al., 2022; Mendonça et al., 2022).

In bioavailability studies, the food matrix modulates the fraction of vitamins and polyphenols absorbed; lipid media enhance carotenoid uptake, whereas aqueous environments favor ascorbic acid, and simultaneous assessment of multiple antioxidants can complicate interpretation because of interactions such as ascorbate driven reduction of vitamin E phenoxyl radicals, which increases vitamin E levels but consumes ascorbate (Mendonça et al., 2022).

Metabolic transformations, including deglycosylation, methylation, and glycosylation of flavonoids, alter both antioxidant and prooxidant behavior in vivo, while elevation of uric acid after polyphenol rich meals may dominate increases in plasma total antioxidant capacity. Some analyses therefore argue that large postprandial rises in measured capacity reflect uric acid rather than the polyphenols themselves (Kotha et al., 2022).

Given these challenges, several recommendations have been articulated to improve assessment of oxidative damage and antioxidant capacity. Detailed matrix descriptions, optimized extraction methods spanning diverse polarities, and harmonized protocols with standard operating procedures, internal and external standards, and validated performance parameters are required. Distinguishing activity from capacity, using biologically relevant radical sources, and adopting methods suitable for both hydrophilic and lipophilic antioxidants with simple, robust instrumentation are also prioritized. Development of multi component standards, use of a single international system of units, and careful consideration of bioaccessibility, bioavailability, and delivery systems such as liposomes or nanoparticles are expected to enhance credibility and comparability of antioxidant measurements across nutrition and health research (Kotha et al., 2022; Losada-Barreiro et al., 2022; Mendonça et al., 2022).

9.2 Bioaccessibility, Bioavailability and Delivery Systems for Antioxidants

Bioaccessibility and bioavailability determine whether antioxidant molecules released from foods or formulations can reach biological targets and exert their effects. Bioaccessibility is defined as the fraction of bioactive compounds liberated from the food or dosage matrix during digestion so that they become available for absorption, whereas bioavailability represents the fraction that is actually absorbed into the bloodstream, distributed systemically, metabolized, and ultimately eliminated after exerting biological activity. For phytochemicals, including polyphenols and other antioxidant constituents, release from the matrix into the gastrointestinal lumen is a prerequisite, followed by passage across biological membranes whose permeability is governed by physicochemical properties such as molecular weight,

hydrogen bonding capability, and partition coefficient, often discussed in the context of Lipinski's Rule for optimal gastrointestinal absorption. Processing and matrix factors strongly condition bioaccessibility. Physical and chemical modifications of foods can cleave hydrophobic interactions, hydrogen bonds, and covalent linkages that bind phenolic compounds to macromolecules, disrupt cell wall barriers, or alter conjugation and derivatization patterns, thereby facilitating phytochemical release. Techniques including grinding, diverse drying and freezing protocols, thermal processing, sterilization, pasteurization, ultrasound treatment, milling, chemical and enzymatic treatments, and encapsulation are all reported to influence phytochemical availability, with beneficial or detrimental consequences depending on compound stability, protective effects of the matrix, and existing interactions among components. Careful adjustment of operating conditions can reduce degradation during processing, enhancing both bioaccessibility and subsequent bioavailability (Kotha et al., 2022).

Food matrices also modulate bioavailability of vitamins and carotenoids, with lipid media markedly increasing carotenoid uptake and aqueous environments favoring absorption of water soluble antioxidants such as ascorbic acid. Simultaneous assessment of multiple antioxidants can complicate interpretation; for example, ascorbic acid reduces vitamin E phenoxyl radicals, recycling vitamin E while lowering ascorbate levels, which affects measured bioavailability of both molecules (Mendonça et al., 2022).

Metabolism and structural transformation further shape in vivo antioxidant behavior. Flavonoids undergo extensive modification, including removal of glycosidic substituents by enzymes or gut bacteria, which is likely to increase their antioxidant activity, whereas methylation and glycosylation of hydroxyl groups can reduce prooxidant behavior through catechol O methyltransferase and related enzymes (Kotha et al., 2022).

Interactions with microbiota have been highlighted as a major source of inter individual variation in bioavailability and bioactivity of polyphenols, with differences in microflora causing larger variability than gender, age, body mass index or drug intake, and recovery of specific flavonoids and olive oil secoiridoids ranging from 2 to 60% across individuals (Losada-Barreiro et al., 2022).

Consumption of polyphenol rich foods increases plasma total antioxidant capacity, but evidence suggests that elevated uric acid, which rises postprandially, may contribute significantly, implying that changes in capacity are not solely driven by the ingested polyphenols themselves (Kotha et al., 2022).

Conventional dosage forms often fail to achieve adequate antioxidant bioavailability because many active ingredients display low aqueous solubility, weak physicochemical stability, extensive first pass metabolism, and enzymatic instability. These limitations hinder formulation as final products and restrict clinical utility, even when in vitro properties appear favorable. To address these problems, nano antioxidant delivery systems have been developed, reflecting broader nanotechnology advances in pharmaceutical research and development. Nano drug delivery systems can conjugate or encapsulate diverse active molecules, including drugs, biomolecules, and phytochemicals with variable solubility profiles, in biocompatible and biodegradable carriers that improve in vitro and in vivo properties of the loaded antioxidants. Their large surface area yields unique physicochemical characteristics, allowing prolonged circulation in the blood, carriage of one or more active molecules, tissue targeting, and enhancement of solubility and therapeutic efficacy. Multiple nanocarrier types have been investigated for antioxidants, including liposomes, protein based nanoparticles, oxide or metal based nanoparticles, polymer based nanoparticles, lipid based nanoparticles, micelles, solid lipid nanoparticles, proliposomes, polymeric micelles, and phytosomes. These systems can increase oral bioavailability through several mechanisms: solubility enhancement of poorly water soluble antioxidants, protection from enzymatic degradation in the gastrointestinal tract, modification of absorption pathways, inhibition of efflux transporters such as ABCB1 and metabolic enzymes such as cytochrome P450, and targeting to specific gastrointestinal regions. Intestinal epithelial cells possess a negative surface charge, so positively charged nano systems may be electrostatically attracted, potentially increasing intestinal absorption. Depending on surface functionality, nano carriers can be internalized via non specific endocytosis, pinocytosis, or receptor mediated endocytosis by enterocytes and M cells, and their particle size and distribution critically affect biodistribution, retention time in blood, and sequestration or clearance; systems larger than 200nm can be trapped in the liver, whereas those smaller than 20nm are rapidly cleared. Actively targeted nano systems employ antigen or receptor specific ligands such as antibodies, peptides, proteins, aptamers, or small molecules to bind receptors including transferrin receptor, folate receptor, epidermal growth factor receptor, and selected glycoproteins on cell surfaces. After receptor mediated endocytosis, these carriers internalize into target cells and release encapsulated antioxidants intracellularly, improving therapeutic effects while decreasing toxicity in healthy tissues. In addition, stimuli sensitive nano carriers responsive to $p\text{H}$, temperature, or reactive oxygen species have been designed to achieve site specific delivery and controlled content

release. Oral formulations based on chitosan coated liposomes, solid lipid nanoparticles, conventional liposomes, and proliposomes have increased apparent permeability, area under the curve, and maximum concentration of antioxidants such as catechin, hesperidin, liquiritin, berberine, and myricetin in animal models, with concomitant suppression of oxidative stress, reduction of apoptosis, improvement of hypoglycemic effects, and enhanced antioxidant and hepatoprotective activities (Losada-Barreiro et al., 2022).

More broadly, nano delivery approaches, together with encapsulation and other processing strategies, are recommended to raise antioxidant bioaccessibility and bioavailability, thereby maximizing in vivo efficacy of food derived and supplemental antioxidants in health related applications (Kotha et al., 2022; Losada-Barreiro et al., 2022; Mendonça et al., 2022).

9.3 Future Perspectives in Oxidative Stress Management and Product Design

Emerging directions in oxidative stress management and antioxidant based product design converge on three tightly linked axes: methodological refinement, enhanced bioavailability through advanced delivery systems, and personalized, safety aware therapeutic strategies. Improving assay methodology remains fundamental, because current antioxidant activity and capacity measurements are strongly affected by matrix effects, experimental parameters, and lack of harmonization, which complicate cross study comparisons and obscur interpretations of health relevance (Kotha et al., 2022; Mendonça et al., 2022).

Recommendations include detailed reporting of sample matrices and preparation steps, optimized extraction procedures that recover antioxidants across a broad polarity range, development of universal multicomponent standards, and strict distinction between antioxidant activity as a kinetic parameter and antioxidant capacity as a thermodynamic endpoint (Kotha et al., 2022).

Harmonized protocols with standardized operating procedures, validation, and a single system of units are expected to increase credibility and allow nutrition, clinical, and consumer communities to relate antioxidant measurements more reliably to nutrition and health outcomes. Future work on oxidative stress management will also depend on progress in understanding bioaccessibility and bioavailability. Processing techniques that induce controlled physical or chemical modifications, such as drying, freezing, pasteurization, ultrasound treatment, milling, and encapsulation, can cleave bonds, disrupt cell walls, or protect phytochemicals until absorption, thereby enhancing or degrading phytochemical availability depending on compound stability and matrix interactions (Kotha et al., 2022; Mendonça et al., 2022).

Evidence that removal of glycosidic substituents tends to increase in vivo antioxidant activity of flavonoids, while methylation and glycosylation reduce prooxidant behavior, underscores the need for careful evaluation of structural transformations during digestion and metabolism when designing functional foods and supplements (Kotha et al., 2022). Inter individual variation driven by gut microbiota composition, with recovery of flavonoids and olive oil secoiridoids ranging from 2 to 60%, points to microbiota targeted strategies as a possible route to modulate polyphenol bioavailability in future interventions. Nanotechnology based delivery systems have become a central focus for improving the clinical utility of antioxidant compounds with poor aqueous solubility, low physicochemical stability, extensive first pass metabolism, and enzymatic degradation. Nano antioxidant delivery systems encompass liposomes, protein based nanoparticles, oxide or metal based nanoparticles, polymer based nanoparticles, lipid based nanoparticles, micelles, solid lipid nanoparticles, proliposomes, polymeric micelles, and phytosomes, all capable of encapsulating or conjugating drugs, biomolecules, and phytochemicals with diverse solubility profiles. These carriers exploit large surface area and biocompatible, biodegradable materials to improve absorption, distribution, metabolism, and excretion characteristics, prolong blood circulation, and increase therapeutic efficacy while limiting side effects. Studies collated for catechin, hesperidin, liquiritin, berberine, and myricetin show that chitosan coated liposomes, solid lipid nanoparticles, liposomes, and proliposomes can increase apparent permeability, area under the curve, maximum concentration, and oral bioavailability, while enhancing antioxidant, hypoglycemic, cardioprotective, and hepatoprotective effects in animal models. These examples illustrate how future product design for foods, supplements, and medicines may increasingly rely on tailored nano carriers to secure both stability and targeted delivery of antioxidant payloads. Active and stimuli responsive targeting represent additional frontiers. Ligand modified nano systems that recognize transferrin, folate, epidermal growth factor receptor, or glycoproteins on cell surfaces can undergo receptor mediated endocytosis, internalize into target cells, and release encapsulated antioxidants, thereby enhancing therapeutic benefit and reducing toxicity in healthy tissues. Stimuli sensitive formulations responding to pH, temperature, or reactive oxygen species promise site specific drug release and refined control of redox interventions. Because particle size and charge strongly influence biodistribution and intestinal uptake, future design must carefully tune these parameters to balance liver sequestration, rapid clearance, and efficient absorption,

particularly in orally administered health products (Losada-Barreiro et al., 2022).

Parallel development of delivery systems such as liposomes and nanoparticles is specifically recommended to increase in vivo bioaccessibility and bioavailability of food antioxidants, which is essential to maximize their protective effects against oxidative stress in biological systems and products (Kotha et al., 2022).

On the clinical and public health side, future research agendas emphasize personalized antioxidant therapy, long term safety assessment, and integration with lifestyle based interventions. Key priorities include elucidating free radical mechanisms, developing novel antioxidant compounds and synthetic analogs with targeted actions, and optimizing delivery and bioavailability of existing antioxidants. Personalized approaches must account for genetic variability in oxidative stress response genes, inter individual differences in absorption and metabolism, and the modifying roles of diet, exercise, and pollutant exposure, which together complicate standardized dosing but open opportunities for tailored nutritional and pharmacological strategies. Long term trials assessing preventive effects of antioxidant rich diets and supplements on disease incidence and progression are needed, alongside studies on combinations of antioxidants with anti inflammatory or targeted therapies to explore synergistic management of oxidative stress (Chandimali et al., 2025).

Advancing biomarker discovery and validation will be crucial for monitoring oxidative damage and evaluating intervention efficacy, while public education on balanced antioxidant rich diets and lifestyle choices complements technological advances in product design to support overall oxidative stress management (Chandimali et al., 2025; Kotha et al., 2022).

10 Conclusion

This synthesis integrates the chemical mechanisms of reactive oxygen and nitrogen species, pathways of autoxidation in foods and tissues, and the molecular modes of antioxidant action within a unified framework that balances scientific convergence against critical translational challenges. A robust consensus exists that aerobic organisms rely on a multi-tiered, interconnected defense system where enzymatic networks and non-enzymatic metabolites work in tandem to maintain physiological redox signaling. Superoxide dismutase acts as the cellular gatekeeper, converting superoxide anions into hydrogen peroxide, which is then rapidly cleared at near-diffusion-controlled rates by peroxisomal catalase or glutathione peroxidases, thereby preventing the formation of highly destructive hydroxyl radicals via transition-metal-catalyzed Fenton chemistry. Intracellular non-enzymatic metabolites, including reduced glutathione, vitamin C, vitamin E, and coenzyme Q10, form a dynamic, fluid redox buffer system. These small molecules function through predictable hydrogen atom transfer or single electron transfer mechanisms, continuously neutralizing radicals and regenerating one another across aqueous and lipid phases to protect cell membranes and vital macromolecules from oxidative lesions.

However, when moving from dietary patterns to high-dose isolated supplementation, the literature presents a stark scientific conflict characterized by major prooxidant controversies. While diets rich in natural antioxidants systematically correlate with reduced chronic disease risk, clinical trials investigating concentrated supplements frequently show null or even deleterious health outcomes. At elevated concentrations, the chemical microenvironment dictates a dangerous shift toward prooxidant behavior. For example, excessive vitamin C can paradoxically catalyze Fenton reactions by reducing transition metal ions, generating highly reactive hydroxyl radicals. Similarly, alpha-tocopherol at high doses can itself transform into a radical species that accelerates lipid autoxidation if it is not efficiently recycled by ascorbic acid. Furthermore, extreme dosing of isolated antioxidants can actively blunt crucial physiological adaptations, such as exercise-induced improvements in insulin sensitivity, or interfere with the deliberate, reactive-oxygen-species-dependent mechanisms that therapies like chemotherapy use to destroy tumor cells.

This translational gap is heavily exacerbated by profound methodological limitations in how antioxidant behavior is historically measured and cataloged. The field has widely relied on non-specific in vitro chemical capacity assays, such as ABTS, DPPH, FRAP, and ORAC, which measure thermodynamic endpoints or the bleaching of synthetic radicals in a test tube. These chemical values completely fail to predict in vivo biological efficacy, a disconnect so severe that regulatory bodies have heavily criticized global capacity measures and the United States Department of Agriculture entirely retracted its public ORAC database. In vitro assays ignore vital pharmacokinetic factors, assuming a compound is fully bioavailable when its systemic utility is actually bottlenecked by bioaccessibility, gastrointestinal degradation, and tissue distribution. Moreover, large postprandial spikes in measured plasma total antioxidant capacity are frequently misattributed to ingested plant polyphenols, when they are actually driven by metabolic elevations of endogenous uric acid generated after a meal.

Translational challenges are further complicated by a significant overreliance on cell cultures and animal models, which cannot easily be extrapolated to human clinical realities. Cells cultured in vitro exist in hyperoxic

environments compared to native human tissues, artificially magnifying the baseline oxidative stress and the apparent rescue effect of added compounds. In vivo, the gut microbiota introduces massive inter-individual divergence in bioavailability and bioactivity. Microflora dictate the structural transformations, deglycosylation, and metabolism of complex dietary phytochemicals, causing the systemic recovery of active flavonoids to range wildly from 2 to 60% across different human individuals. Standardized animal models with uniform microbiomes and controlled environments simply cannot replicate this human diversity.

To guide future research effectively, the scientific community must move past basic descriptive compound screening and prioritize concrete, unresolved mechanistic questions. Future product design must focus on creating targeted nano-delivery systems, such as solid lipid nanoparticles, liposomes, and phytosomes, which can shield fragile antioxidant payloads from enzymatic degradation and enhance systemic absorption. Crucially, engineering must advance toward stimuli-responsive nanocarriers that achieve site-specific release, opening only when they encounter localized, pathologically high concentrations of reactive oxygen species. Clinical trials must also abandon generalized plasma capacity tests in favor of validated, specific biomarkers of macromolecular damage, explicitly tracking changes in protein carbonyls for tissue degradation, 8-hydroxy-2-deoxyguanosine (8-OHdG) for mitochondrial DNA lesions, and malondialdehyde for lipid peroxidation. Finally, future interventions must embrace personalized nutrition and medicine, tailoring antioxidant dosing to an individual's unique genetic background, environmental exposures, and gut microbiota profile to manage oxidative stress safely without disrupting crucial physiological redox signaling.

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