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REVIEW

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Understanding oxidants and antioxidants: Classical team with new players

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Abstract

The free radical oxidants such as reactive oxygen species, reactive nitrogen species, and reactive sulfur species are produced inside cells through various metabolic processes. The body is equipped with an antioxidant defense system that guards against oxidative damage caused by these reactive oxidants and plays a major role in protecting cells from oxidative stress and damage. Antioxidants such as glutathione (GSH), thioredoxin, ascorbic acid and enzymes, for example, superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT) counter the oxidative stress and protect lipids, proteins, and DNA. Antioxidants such as tocopherols, ascorbic acid, carotenoids, flavonoids, amino acids are also natural antioxidants present in foods. There is increasing demand and availability of designer foods fortified with antioxidants and probiotics that may be important in human health. The review article presents a brief overview of oxidants and antioxidant systems inside the human body including the role of probiotics and inflammation.

Practical applications

Antioxidants such as GSH, thioredoxin, ascorbic acid, etc. and protective enzymes, for example, SOD, GPx, CAT, etc. counter oxidative stress and protect cellular biomolecules. Antioxidants such as tocopherols, ascorbic acid, carotenoids, flavonoids, amino acids, phospholipids, and sterols are natural antioxidants found in consumed foods. They play a major role in scavenging free radical and non-radical oxidants, and protect cells from oxidative stress and damage. The importance of antioxidants can be understood from the fact that oxidative damage is now associated with a variety of diseases including cancer, neurodegeneration, diabetes, etc. Several approaches to improve human health and achieve longevity use dietary antioxidants as formulation in diet and fortified foods. Antioxidants also maintain freshness and prolonging the shelf life of food products. The fortified or designer foods that are added with antioxidant nutrients and the use of microorganisms as probiotics are increasingly available in the market as health foods and supplements.

KEYWORDS

antioxidants, enzymatic antioxidants, free radicals, functional foods, natural antioxidants, oxidants, probiotics

Abbreviations: CAT, catalase; GPx, glutathione peroxidase; H_2O_2, hydrogen peroxide; HO_2, perhydroxyl radical; HOCl, hypochlorous acid; $O_2^{-\cdot}$, superoxide anion; OH, hydroxyl radical; ONOO, peroxynitrite; R·, lipid alkyl radical; RH, lipid; ROO·, lipid peroxyl radical; ROOH, lipid hydroperoxide; SOD, superoxide dismutase.

1 | INTRODUCTION

Reactive species of oxygen, nitrogen, and the recently identified reactive sulfur species (RSS) are well known to induce oxidative damage to lipids, proteins, and DNA (Pallavi, Ambuj, Rama, & Mohammad, 2012). These reactive species could be free radicals or non-radical oxidants. Free radicals can be defined as potentially damaging chemical species containing an unpaired electron. Free radicals are generally electrically charged and they tend to neutralize themselves by reacting with other substances thereby causing oxidation (Cheeseman & Slater, 1993). Reactive oxygen species (ROS), reactive nitrogen species (RNS), and RSS are the three main classes of oxidants that are formed inside the body. Among ROS, the major players are free radicals such as superoxide radicals (O_2^{-}), hydroxyl radicals ($^{\cdot}OH$), and non-radical oxidant such as hydrogen peroxide (H_2O_2) and hypochlorous acid (HOCl). Major RNS are nitric oxide (NO) and peroxynitrite ($ONOO^{-}$) apart from others (Lu, Lin, Yao, & Chen, 2010). RSS include thiyl radical (RS) and RSS formed by the reaction of ROS with thiols. The main target of these oxidants are nucleic acids, sugars, lipids, and proteins (Carocho & Ferreira, 2013; Craft, Kerrihard, Amarowicz, & Pegg, 2002; Lu et al., 2010) (Figure 1). The deleterious effects of these ROS and RNS free radicals such as $O_2^{-\cdot}$, $^{\cdot}OH$, H_2O_2, and $ONOO^{-}$ occurs as a result of the alterations of organic biomolecules such as the polyunsaturated fatty acids in membrane lipids, oxidation of proteins, DNA strand breakage, RNA oxidation, mitochondrial depolarization, and apoptosis. Under normal conditions, reactive species are cleared by the antioxidants which are able to react directly with oxidants to reduce their oxidation capacity, for example, scavenging enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), etc., or chemicals inhibiting the activities of oxidant generating enzymes such as xanthine oxidase, for example, polyphenols (Figure 1). These molecules can be either natural or synthetic, either hydrophilic such as ascorbic acid or hydrophobic such as α-tocopherol. The antioxidants can prevent the generation of oxidizing species or reduce the effects of dangerous metabolic or xenobiotic oxidants and hence prevent the body from acute or chronic diseases and/or repair the cellular/tissue damage already sustained. However, a considerable number of antioxidant molecules have been found to have a prooxidant potential and promote oxidative reactions. It has also become obvious that free radicals are not only involved in pathological processes, but their existence is also necessary for many physiological functions of living organisms, including "healthy aging." It is now widely known that these biologically hyperactive molecules act as signaling agents in various cellular pathways known as "redox signaling." H_2O_2 and ONOO, in particular, have been implicated in a considerable number of cellular signaling cascades and due to their non-radical structure these molecules have a relative longer half-life than almost all other oxidants allowing them to migrate away from their production sites and to diffuse through membranes. Moreover, the transcription factors such as AP-1, NF-κB, and/or Nrf2 have been reported to be involved in these redox-modulated signaling pathways (Oter, Jin, Cucullo, & Dorman, 2012).

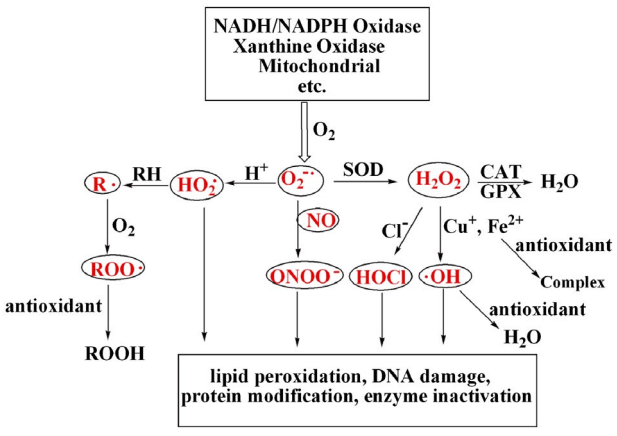

FIGURE 1 The type of ROS and action of antioxidants (adapted with permission from Lu et al., 2010)

Antioxidant in food sciences are compounds that block oxidative reactions, thereby maintaining freshness and prolonging the shelf lives of food products. Dietary antioxidants and antioxidant supplements quench ROS and may prevent different chronic diseases. In addition to the essential antioxidant nutrients such as vitamins E and C, there are several well-designed antioxidant and cytoprotective enzyme systems in the human body, which are more important than dietary non-nutrient antioxidants. At high concentrations, many antioxidants could act as prooxidants, increasing oxidative stress, and inducing toxicity (Yang et al., 2018). In recent years, the consumption of natural products or functional foods has increased and the food industry has expanded exponentially. Therefore, the functional foods or nutraceuticals are defined as a foods or ingredients that improve health. They contain dietary supplements like proteins, vitamins, minerals, and phytochemicals (Inan, 2019).

1.1 | Oxidants

ROS, RNS, and RSS include a variety of reactive molecules including free radicals and non-radical derivatives of oxygen, nitrogen, and sulfur that are capable of oxidizing substrates under appropriate conditions.

1.1.1 | Reactive oxygen species

The sequential one-electron reduction of molecular oxygen produces three ROS such as superoxide (O_2^{-}), hydrogen peroxide (H_2O_2), and hydroxyl radical (·OH) before terminating as water;

$$O_2 - e- \rightarrow O_2^{-\cdot}, O_2^{-\cdot} - e- \rightarrow H_2O_2, H_2O_2 - e- \rightarrow HO^{\cdot}, HO^{\cdot} - e- \rightarrow H_2O^{\cdot}$$

In addition to their toxicity, H_2O_2, and arguably $O_2^{-\cdot}$, are considered to be important regulatory molecules necessitating careful regulation of their titers. SOD and CAT are well known antioxidant enzymes,

the former catalyzes the dismutation of superoxide to oxygen and peroxide while the latter catalyzes peroxide dismutation to oxygen and water (Olson et al., 2018).

$$2\,O_2^{\cdot-} + 2H+ \rightarrow O_2 + H_2O_2,$$
$$2\,H_2O_2 \rightarrow O_2 + H_2O^{\cdot}$$

The production of superoxide radical begins with the consumption of oxygen and activation of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase located on the surface of phagocytes and non-phagocytes (John, 2008). The lifetime of $O_2^{\cdot-}$ in the water cellular environment is about 10^6 s (Pryor, 1986). The superoxide anion that is produced is quite reactive and unstable and is spontaneously converted to H_2O_2 and molecular oxygen (Alugoju, Dinesh, & Latha, 2015). When protonated, superoxide radicals exhibit much higher reactivity as compared to superoxide anion though physiologically it is unprotonated form that predominates (Aikens & Dix, 1991). Hydoxyl radicals constitute an important reactive ROS as they can react with most of the molecules present inside the cell (Esra, Umit, Cansin, Serpil, & Omer, 2012). They have very short life span of about 10^{-6} and short radius of action, that is, about 30Å from where they are produced (Devasagayam, Tilak, & Boloor, 2004). The main source of hydroxyls are metal catalyzed Fenton/Haber–Weiess reactions. H_2O_2 is one of the most stable oxidant. Though less reactive than superoxide anion it is more damaging as it can move too far off places from where it is produced as well as easily pass through the membranes (Alugoju et al., 2015). It can also easily convert into highly reactive $^{\cdot}OH$ under appropriate conditions such as presence metal ions (Jonah, 2013). Hypochlorite is a highly reactive non-radical ROS which can cause oxidative damage to cellular machinery of the organism (Marnett, Riggins, & West, 2003). Hypochlorite is generated under physiological conditions by activated neutrophils during respiratory burst in a reaction catalyzed by myeloperoxidases (Siddiqui et al., 2016). It reacts with a wide variety of biological molecules including DNA and proteins. Hypochlorite is a major oxidant which undergoes oxidation reactions (Winterbourn, 2002) and is a potent inactivator of anti-proteinases including α-2-macroglobulin (Siddiqui, Zia, Ali, Ahsan, & Khan, 2018; Wu & Pizzo, 2011). Hypochlorite modified proteins can be detected in diseases like atherosclerosis (Van der Veen, de Winther, & Heeringa, 2009) and are implicated in the pathology of human diseases such as Alzheimer's disease and arthritis (Wyatt et al., 2014).

1.1.2 | Reactive nitrogen species

One of the most important RNS is NO. It can be produced enzymatically via nitric oxide synthase (NOS) from arginine as shown in reaction 6 or nonenzymatically (Andrew, 1999). It is a free radical lipophilic diatomic gas, with small stokes' radius and neutral charge which allows its rapid diffusion across the membrane (Lamattina, García-Mata, Graziano, & Pagnussat, 2003). NO at a low concentration of 10 nmol/L is a signaling molecule while at higher concentrations is detrimental for health (Chen, Chen, Xu, & Shen, 2013). NO being free radical has an unpaired electron which allows high reactivity with oxygen, superoxide anion, nitrogen derivatives, as well as transition metals. NO can also be converted into nitrogen dioxide, nitrogen trioxide, nitrate, and several other RNS (Lamattina et al., 2003). NO can also react with thiols and generate RSS. NO can rapidly react with superoxide radical (rate ~ 6.7×10^9 $M.s^{-1}$) to form highly reactive ONOO. This RNS is a stronger oxidant and more stable as compared to $O_2^{\cdot-}$ and NO, and hence can attack and damage many more biological targets. ONOO formed is known to modify zinc finger motifs, thiols, iron-sulfur clusters, and is reported to be involved in etiology of number of diseases. At neutral pH the pernitrous acid can undergo hemolysis to generate nitrogen dioxide radical and $^{\cdot}OH$ which in turn can elicit more damage (Pacher, Beckman, & Liaudet, 2007).

1.1.3 | Reactive sulfur species

RSS is a newly classified broad group of sulfur containing reactive species that includes both radical and non-radical sulfur based moieties. This broad group includes radical species such RS, glutathionyl radical ($GSSG^{\cdot}$), as well as non-radical reactive sulfane species, reactive sulfur substances, etc. (Giles, Nasim, Ali, & Jacob, 2017) (Table 1). Unlike other reactive oxygen and nitrogen species, RSS are capable of both oxidation and reduction (Dhawan, 2014). It has now become apparent that molecules with sulfur-containing functional groups can also be stressors in their own right, with pivotal roles in cellular function and homeostasis. A key distinction for RSS is that, unlike oxygen or nitrogen, sulfur not only forms a plethora of specific reactive species, but sulfur also targets itself, as sulfur containing molecules, that is, peptides, proteins, and enzymes, preferentially react with RSS. RSS are omnipresent and sometimes even considered as important as ROS and RNS which for decades have dominated the redox field (Giles et al., 2017).

2 | OXIDATIVE STRESS AND CHRONIC INFLAMMATION

Oxidative stress has been implicated in the pathogenesis of many chronic diseases including the inflammatory process. Oxidative stress and inflammation are closely related pathophysiological processes and both processes are simultaneously found in many pathological conditions (Biswas, 2016). During inflammatory process the activated phagocytic cells like neutrophils and macrophages produce large

TABLE 1 Reactive Sulfur species (adapted from Giles et al., 2017)

Radicals	Non-radicals
Thiyl radical	Reactive sulfane species (RSR)
$GSSG^{\cdot}$	Reducing sulfur species (H_2S, GSH)
$RSR^{\cdot}$	Reactive sulfur substances (SO_2, SO_3)
	Sulfur secondary metabolites (Allicin)

amounts of ROS, RNS, etc. superoxide, H_2O_2, hydroxyl free radical, NO, ONOO, and HOCl to kill the invading agents. Under pathological inflammatory conditions there may be exaggerated generation of reactive species and some of those reactive species diffuse out of the phagocytic cells and thus they can induce localized oxidative stress and tissue injury. However, apart from the direct production of reactive species by the professional phagocytic cells, the nonphagocytic cells can also produce reactive species in response to pro-inflammatory cytokines (Li et al., 2015; Wu, Lu, & Antony, 2013). Recent finding also showed that the costimulation of Toll-like receptor produces oxidative stress with unbalance of pro-inflammatory and anti-inflammatory cytokine production. The NOX4 overexpression has also been found to enhance IL-6 production, and a positive reciprocal feedback loop has been found between IL-6 and NOX4, the two mediators of inflammation and oxidative stress, respectively (Wu et al., 2013). As the inflammatory process can induce oxidative stress, the oxidative stress can also induce inflammation through activation of multiple pathways. The reactive species, H_2O_2 can induce inflammation through activation of transcription factor NF-κB. Furthermore, oxidative stress plays an important role in the activation of NOD-like receptor protein 3 (NLRP3) inflammasome. The NLRP3 inflammasome is an oligomeric molecular complex that triggers innate immune defenses through the maturation of pro-inflammatory cytokines like IL-1β and IL-18. The ROS released from damaged mitochondria has been shown to activate NLRP3 inflammasomes leading to IL-1β secretion and localized inflammation. Oxidized mitochondrial DNA has also been found to activate NLRP3 inflammasomes during apoptosis. Furthermore, in conditions of oxidative stress the ROS causes the thioredoxin-interacting protein, an inhibitor of endogenous antioxidant thioredoxin, to dissociate from thioredoxin and to bind with NLRP3 leading to activation of NLRP3 inflammasome. Therefore, inflammation and oxidative stress are closely related and tightly linked interdependent pathophysiological processes (Biswas, 2016).

ROS accumulation may trigger oxidative stress involving many biological processes including apoptosis, necrosis, and autophagy. Autophagy may be induced by oxidative stress and lipid peroxidation. Autophagy is a highly conserved degradation pathway which can recycle nutrients and organelles to enable cells to adapt to undesirable surroundings. When cells sense nutritional stress, autophagy will be induced very quickly. One study revealed that oxidative stress is an essential element of autophagy induced by nutrient deficiency. However, nutrient excess will also induce autophagy mainly through ROS or endoplasmic reticulum (ER) stress (He et al., 2018). Moreover, autophagy protects cells against environmental stress through recycling limited nutrients or degrading damaged organelles. ROS derived from mitochondria can oxidize membrane lipids leading to damage of the mitochondrial membrane structure causing cell death. Studies have also reported that the induction of autophagy in tumor necrosis factor-alpha-treated cells via the NF-κB pathway requires the accumulation of ROS. The p38 mitogen-activated protein kinases (MAPK) are also involved in the generation of ROS. Studies have shown that oxidative stress can be elevated by the inhibition of p53 targeted gene expression inducing autophagy. Autophagy can be induced by nutritional stress, including nutritional starvation and excess nutrient stress. The homeostasis of nutrient metabolism is vital for the maintenance of cell survival and normal physiological functions (He et al., 2018).

3 | ANTIOXIDANTS

Antioxidants are those molecules that inhibit, decrease, delay, or completely scavenge the action of free radicals and oxidants, and protect the body from oxidative damage (Lobo, Patil, Phatak, & Chandra, 2010). The antioxidant defense is a universal mechanism that is present inside the cells and tissues of both plants and animals though their types and concentration vary. Halliwell and Gutteridge (1995) defined antioxidants as "any substance that, when present at low concentrations compared with that of oxidizable substrates, significantly delays or inhibits oxidation of that substrate." This definition however was later modified as "any substance that delays, prevents or removes oxidative damage to a target molecule" to include the molecules that repair the oxidative damage to the system (Halliwell, 1995a, 1995b; Halliwell & Gutteridge, 1995). Thus, effective antioxidants have the ability to delay oxidation reaction or obstruct the development of free radicals or break the generation of the autoxidation chain reaction that generates free radicals/oxidants. They also act as reducing agents and metal chelators which convert hydroperoxides into stable compounds. Some antioxidants act as metal chelators that transform metal prooxidants into stable form. Oxidative/nitrosative stress results from disequilibrium in oxidant-antioxidant balance in the favor of reactive species with the increase of reactive oxygen and/or RNS production, respectively (Kurutas, 2015). The body's defense mechanisms against oxidative damage are operative in two main systems. The first one includes removal of free radicals and reactive species by enzymes such as superoxide-dismutase, CAT, GPx, etc. and second one is scavenging of free radicals by electron donors, such as glutathione (GSH), tocopherols, ascorbic acid, thioredoxin, etc. (Devasagayam et al., 2004) (Figure 2). Other mechanisms include binding pro-oxidant metal ions, such as iron and copper by specific metal binding proteins such as transferrin, metallothionein, haptoglobin, ceruloplasmin (Table 2).

The antioxidants in foods help to prevent oxidative reactions that decrease their quality. Many dietary supplements have been promoted as antioxidants, but only a few, few antioxidant dietary supplements have been shown to promote health. The health effect of an antioxidant depends on the systemic bioavailability, the concentration of the compound that can be delivered to specific organ sites, and whether this antioxidant can perform the expected function. With the exception of antioxidant nutrients, many dietary antioxidants are generally less effective in combating ROS compared with the antioxidant and cytoprotective enzyme systems. Therefore, a non-pro-oxidative activator of Nrf2 may be more useful and less problematic, because antioxidant enzyme systems are better regulated in the body. Many studies have shown that taking antioxidants, especially at high doses, can lead to toxicity because of prooxidative activities. It would also be interesting to consider the issue of

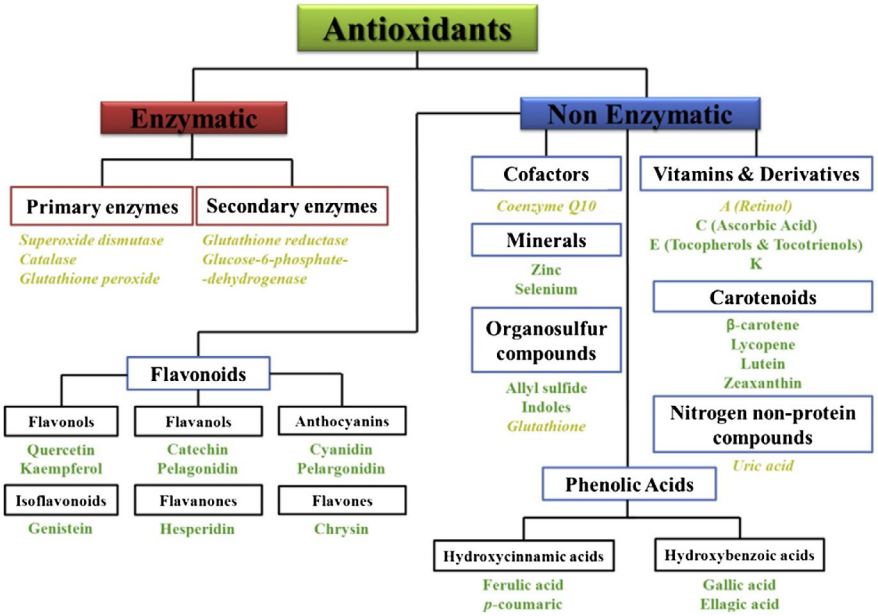

FIGURE 2 Different classes of antioxidants (Adapted with permission from Carocho & Ferreira, 2013)

TABLE 2 Major antioxidants and their functions (adapted from Zhang et al., 2019)

Antioxidants	Target	Presence	Functions
SOD1 (Cu/Zn)	$O_2^{\cdot-}$	Ubiquitous	Cytosolic and mitochondrial $O_2^{\cdot-}$ dismutation
SOD2 (Mn)	$O_2^{\cdot}$	Ubiquitous	Mitochondrial $O_2^{\cdot-}$ dismutation,
SOD3 (Cu/Zn)	$O_2^{\cdot}$	Vessel, lung, kidney	Extracellular space $O_2^{\cdot-}$ dismutation
CAT	H_2O_2	Liver, erythrocyte	H_2O_2 detoxification, Ethanol metabolism
GRx	GSSG	Ubiquitous	Reducing GSSG to GSH
GSH	GPX, PRDX1/4	Ubiquitous	GPX and PRDX1/4 reduction xenobiotic detoxification
Uric acid	$OH^{\cdot}$, HOCl, $ROO^{\cdot}$	Plasma	Major plasma ROS scavenger, Within the cell as prooxidant
Vitamin E	$OH^{\cdot}$, $LOO^{\cdot}$	Plasma	Membrane lipid peroxidation termination
Vitamin C	$TO^{\cdot}$	Plasma	Vitamin E reduction
Bilirubin	$O_2^{\cdot}$, H_2O_2, $ONOO^{\cdot-}$	Plasma, spleen	Protect lipids from oxidation

homeostasis in the redox states of tissues. ROS are also known to play physiological functions, such as being involved in killing infectious bacteria in monocytes. There are also suggestions that ROS play roles in the signal transductions of many physiological functions. The physiological health requires a proper balance between ROS generation and the antioxidant systems and when ROS overcomes the antioxidant-defense systems in the body resulting in oxidative stress (Yang et al., 2018). The effects of food on physiological antioxidant capacities and its measurement have limitations in predicting the health effects directly in animal models and in humans. A few methods and techniques employed to measure the oxidative and antioxidative properties have been shown in Table 3.

3.1 | Enzymatic antioxidants

Human system possesses a battery of enzymes that neutralize the reactive species formed. The important ones include CAT, SOD, GPx, and glutathione reductase (GRx). The activity of SOD, CAT, and GPx constitute the first line of antioxidant defense which plays a key role in the total defense mechanisms of the host biological system (Ighodaro & Akinloye, 2018).

3.1.1 | Superoxide dismutase

SOD was discovered by Irvin Fridovich in 1968. This enzyme catalyzes the transition $O_2^{\cdot-}$ into H_2O_2 (McCord & Fridovich, 1968, 1969). SODs are located in cytosol and mitochondria and belong to the family of multimeric metalloenzymes. They are categorized into different families: Cu-SOD, Mn-SOD, Cu-Zn-SOD, Fe-SOD, and Ni-SOD. These enzymes are found at different places, for example, Cu-Zn-SOD is predominantly present in the chloroplast and cytosol of eukaryoic cells, while MnSOD is mostly present in the matrix of mitochondria and cytosol of bacteria, and FeSOD is found in prokaryotes and some plants (Duke & Salin, 1985). Among these enzymes, Cu-Zn-SOD is

TABLE 3 Some methods and techniques for the analysis of oxidation and antioxidant activity (adapted from Carocho & Ferreira, 2013)

Method	Assay mechanism/ detection
ABTS (2,20-azinobis(3-ethylbenzothiazoline-6-sulfonic acid)	Scavenging activity
ACA (aldehyde/carboxylic acid)	Oxidation
Conjugated diene	Lipid peroxidation
DPPH (2,2-diphenyl-1-picrylhydrazyl)	Scavenging activity
ESR (electron spin resonance spectrometry)	Free radicals quantification
Fluorescence assay	Total aldehydes
FOX (ferrous oxidation-xylenol)	Lipid peroxidation
FRAP (ferric reducing antioxidant power)	Reducing power
FTC (ferric thiocyanate)	Lipid peroxidation
Gas chromatography (GC)	Lipid peroxides, Aldehydes, Sterols, Phenolic acids, Flavonoids
High performance liquid chromatography (HPLC)	Flavonoids, Aldehydes, Phenolic acids
Light emission	Excited-state carbonyls and singlet O_2
Spin trapping	Alkoxyl and peroxyl radicals
TBARS (thiobarbituric reactive substances)	Lipid peroxidation
TEAC assay (Trolox equivalent antioxidant capacity)	Antioxidant activity
TRAP (total radical-trapping antioxidant parameter)	Antioxidant activity

known to provide a strong defense against oxygen toxicity inside the cell, and according to studies it is accepted that Cu-Zn-SOD is an important enzyme for aerobic life and is irreplaceable (Peskin, Koen, & Zbarsky, 1977). Studies have shown that SODs play a role in protecting enzymes and proteins against oxygen toxicity in both prokaryotes and eukaryotes including higher plants (Fridovich, 1986; Bowler, Montagu, & Inzé, 1992; Gralla & Kosman, 1992; Hassan & Scandalios, 1990; Scandalios, 1990; Scandalios, 1992).

3.1.2 | Catalase

CAT is present in most cells and is considered as a major antioxidant enzyme. CAT (EC 1.11.1.6) is an enzyme which is present mainly in the peroxisomes of mammalian cells. Though found in mitochondria, animal CAT is largely localized in peroxisomes. It is a tetrameric enzyme consisting of four identical, tetrahedrally arranged subunits of 60 kDa each containing an active heme group and NADPH (Kirkman & Gaetani, 1984; Martfnez-Cayuela, 1995). CAT catalyzes

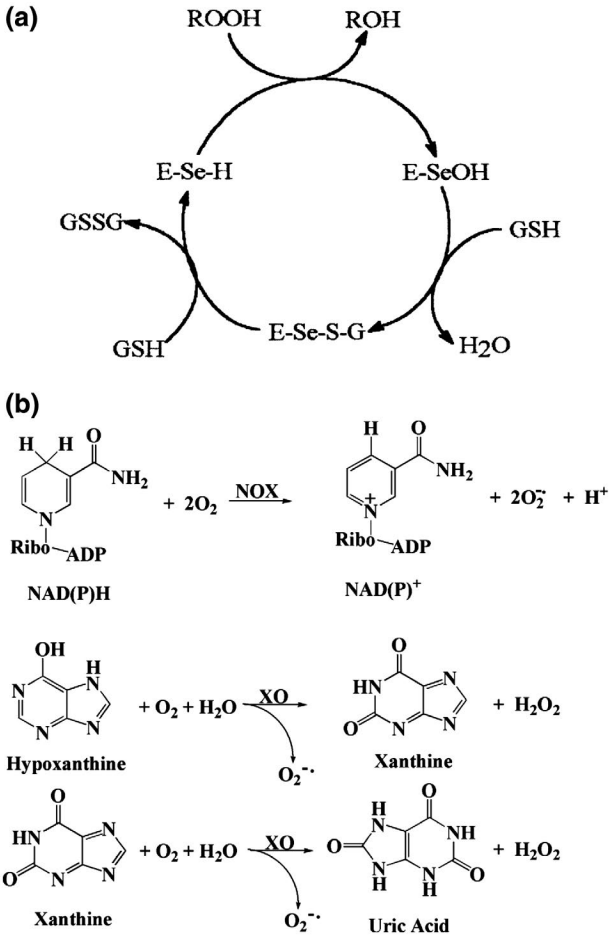

FIGURE 3 (a) Proposed catalytic mechanism of Glutathione (GSH). (b) Superoxide and hydroperoxide generation from NAD(P)H, oxidase (NOX), and XO (adapted with permission from Lu et al., 2010)

the decomposition of H_2O_2 to H_2O and O_2 (Aslani & Ghobadi, 2016). CAT has two enzymatic activities depending on the concentration of H_2O_2. If the concentration of H_2O_2 is high, CAT acts catalytically, that is, removes H_2O_2 by forming H_2O and O_2. However, at a low concentration of H_2O_2 and in the presence of a suitable hydrogen donor, for example, ethanol, methanol, phenol, and others, it acts peroxidically, removing H_2O_2, but oxidizing its substrate (peroxidatic reaction).

3.1.3 | Glutathione peroxidase and glutathione reductase

GPx is a family of enzymes that require reduced GSH as a substrate. They provide a second line of defense against oxidative stress. It is made up of four identical subunits of 21 kDa and each subunit contains a selenocysteine (Sec) residue (Lubos, Loscalzo, & Handy, 2011). GPx catalyzes the reduction of organic and inorganic H_2O_2 to H_2O and corresponding alcohols, using GSH as a cofactor (Birben, Sahiner, Sackesen, Erzurum, & Kalayci, 2012) (Figure 3a). It plays an important role in the protection of cell

membrane polyunsaturated fatty acids, where it functions as a multicomponent antioxidant defense system. GPx has a higher affinity for substrate than CAT and is known to reduce fatty acid hydroperoxides (Gathwala & Aggarwal, 2016). Most animal cells have both CAT and GPx (Sharma, Jha, Dubey, & Pessarkli, 2012). The GPx can be classified into two forms––selenium dependent and selenium independent. They are found in both cytosol and mitochondria. In mammalian tissues, four major types' selenium dependent GPx isozymes are present: (a) classical GPx (GPx1), (b) gastrointestinal GPx (GPx2), (c) plasma GPx (GPx3), and (d) phospholipid GPx (PHGPx4 or GPx4). GPx1 are mostly found in red blood cells (RBC), liver, lung, and kidney. GPx2 and GPx3 are ubiquitous but are mostly present in organs such as kidney, lung, epididymis, vas deferens, placenta, seminal vesicle, heart, and muscle. GPx4 are broadly dispersed in various tissues (Margis, Dunand, Teixeira, & Margis-Pinheiro, 2008). GPxs also play an important role in the detoxification of ROS and it was found to be first enzyme activated under high levels of ROS (Duggett et al., 2016; Halliwell & Gutteridge, 2015).

Glutathione reductase is a cytosolic protein that shows a similar distribution pattern as GPx in cells. It reduces oxidized glutathione (GSSG) by utilizing NADPH (Figure 3b). One of the important functions of GRx is to maintain the ratio of GSH/GSSG. When the concentration of GSSG (oxidized glutathione) increases inside the cell it often leads to DNA breakage, protein denaturation, and lipid peroxidation (Zitka et al., 2012).

3.1.4 | Glutathione

Glutathione or γ-glutamylcysteinylglycine is the major nonenzymatic antioxidant, involved in many cellular functions. It is a ubiquitous tripeptide that regulates intracellular redox homeostasis and is present either in reduced (GSH) or oxidized form (GSSG) (Figure 3). GSH is a tripeptide (cysteine, glycine, and glutamic acid) found in relatively high concentrations in many bodily tissues. It is found in almost all types of cell compartments: cytosol, ER, mitochondria, and vacuoles at millimolar concentration (Jimenez, Hernandez, Pastori, Rio, & Sevilla, 1998; Meister & Anderson, 1983). GSH scavenges many ROS such as H_2O_2, $O_2^{-\cdot}$ and $^{\cdot}OH$ (Misak et al., 2018). One of the basic role of GSH as an antioxidant is its ability to restore ascorbic acid via the ascorbate-GSH cycle (Noctor & Foyer, 1998). GSH is a potent detoxifier of xenobiotics and acts as a barrier against hydroperoxide induced oxidation. It plays a pivotal role in reducing oxidative stress, maintaining redox balance, enhancing metabolic detoxification, and regulating the immune system. Various chronic, age-related diseases such as those related to neurodegeneration, mitochondrial dysfunction, and even cancer, have been related to suboptimal or deficient GSH level. There is increasing awareness of its ability in mitigating toxin load through its ability to enhance hepatic conversion and excretion of compounds such as mercury and persistent organic pollutants (Minich & Brown, 2019).

4 | NONENZYMATIC ANTIOXIDANTS

Dietary micronutrients constitute many water and lipid soluble nonenzymatic antioxidants founds in the mammalian system. Important antioxidants include vitamin A (retinol and β-carotene as provitamin A), vitamin C (ascorbic acid), and vitamin E (tocopherol and tocotrienol). These antioxidants work as defense barrier against free radicals and non-radical oxidants and prevent the assault to DNA, proteins, lipids, and other molecules which are induced by oxidants. The most abundant cellular antioxidant is tripeptide GSH which prevents oxidation of thiols groups inside the cell. Another endogenous protein called thioredoxin which is involved in antioxidative reactions inside the cell is also discussed here.

4.1 | Ascorbic acid

Vitamin C is commonly called as ascorbic acid and it is mainly divided into two compounds, L-ascorbic acid and L-dehydroascorbic. Both possess antioxidant activity and can be enzymatically converted in vivo and may be absorbed through the gastrointestinal tract. Ascorbic acid is readily oxidized to dehydroascorbic acid (Figure 4). It is a vital water-soluble antioxidant found in biological fluids and required for normal metabolic functions of the body (Jaffe, 1984). It is a very potent free radical scavenger and conserves the integrity of low density lipoprotein (LDL) and also maintains the level of vitamin E in cell membranes (Harats et al., 1998; Niki, 1987). It prevents the macromolecules such as DNA, lipids, and proteins from oxidative damage by scavenging reactive oxygen and nitrogen species (Noctor & Foyer, 1998). Ascorbic acid mainly scavenges $ONOO^-$, NO, and HOCl but also quenches $O_2^{-\cdot}$, $^{\cdot}OH$, and O_2, and reduces H_2O_2 to H_2O via ascorbate peroxidase reaction (Noctor & Foyer, 1998). In addition to scavenging free radicals, ascorbic acid also helps to restore small molecules such as α-tocopherol, GSH, urate, and β-carotene so that they too can act as an antioxidant. It is an effective antioxidant that prevents lipid peroxidation and can reduce or prevent H_2O_2-induced lipid peroxidation and formation of 8-hydroxydeoxyguanosine (8-OHdG) in nucleic acids (Bayani, Singh, Zamboni, & Mahajan, 2009). Ascorbic acid is not synthesized inside the body because the glucuronic pathway required for the biosynthesis of vitamin C is defective due to mutation in the gene coding for L-gulonolactone oxidase (Woodall & Ames, 1997).

4.1.1 | Tocopherols

Naturally occurring four tocopherols are designated as alpha (α), beta (β), gamma (γ), and delta (δ)-tocopherols and out of these isoforms α-tocopherol (αT) being the most abundant is also called vitamin E. All the isoforms of tocopherols are composed of a chromanol ring and a 16-carbon phytyl like side chains (Jiang, 2014). While

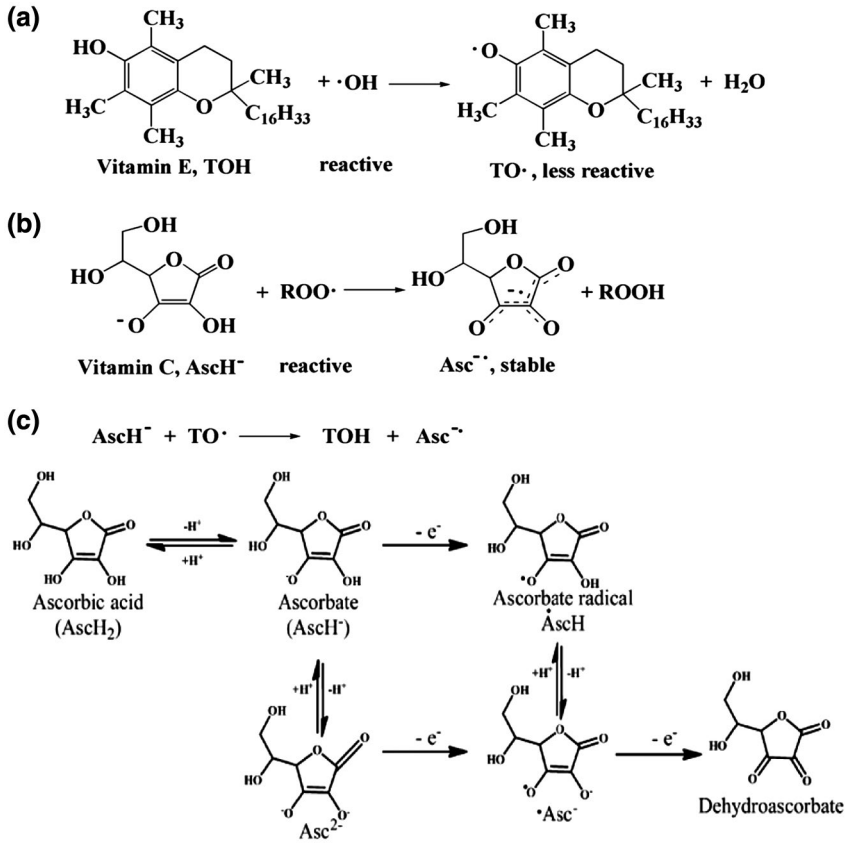

FIGURE 4 Radical scavenging mechanism of ascorbic acid (vitamin C). Direct reactions of vitamin E (TOH) with ·OH (a) and vitamin C (AscH) with ROO·(b) and regeneration of vitamin E from vitamin C (adapted with permission from Lu et al., 2010)

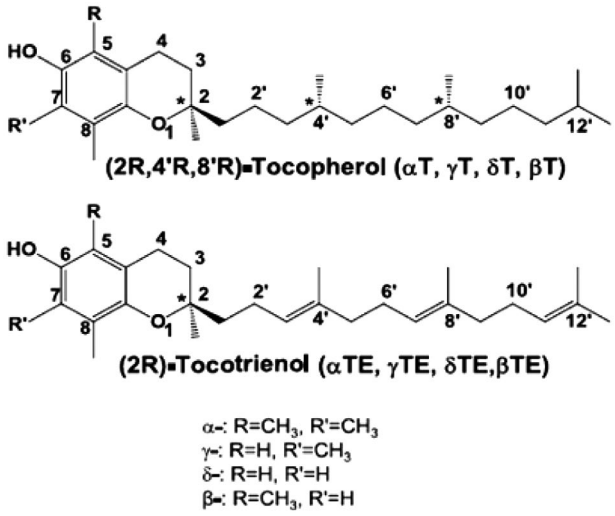

FIGURE 5 Natural forms vitamin E (tocopherols, tocotrienols)

α-tocopherol was the first vitamin E analoge to be recognized, eight chemically distinct analoges are now known, consisting of α, β, γ, and δ-tocopherols (T) and α, β, γ, and δ-tocotrienols (T3), all of them are now referred to as vitamin E. The tocopherols are saturated forms of vitamin E, whereas the tocotrienols are unsaturated and possess an isoprenoid side chain (Ahsan, Ahad, Iqbal, & Siddiqui, 2014, Ahsan, Ahad, & Siddiqui, 2015). As shown in Figure 5, the tocopherols are saturated while tocotrienols possess three double bonds. αT is not an endogenous antioxidant and is taken by diet in human body. Major source of vitamin E are nuts, seeds, whole grain, vegetable oil, etc. Vitamin E is an effective lipid soluble antioxidant found in plasma membrane where it works as chain breaker in lipid peroxidation of cell membranes (Bayani et al., 2009). It can directly scavenge lipoperoxyl radicals and protect cell membranes against lipid peroxidation by donating a hydrogen atom to the radical (Martfnez-Cayuela, 1995). The oxidized tocopheryl radical is then reconverted back via ascorbic acid facilitated pathway to tocopherol (Traber & Stevens, 2011). Though αT is the most abundant and the predominantly investigated form, the other isoforms such as γ-tocopherol, δ-tocopherol, and γ-tocotrienol also have antioxidative and anti-inflammatory activity and play a vital role in the pathophysiology of a number of diseases (Mathur, Ding, Saldeen, & Mehta, 2015).

4.1.2 | β-carotene

Since, β-Carotene has numerous biological functions in body but humans are not able to synthesize it, hence, it is necessary to supply these compounds with food or pharmaceuticals. Originally β-carotene was recovered from plants by physicochemical extraction mainly from carrots but nowadays β-carotene is mainly produced through chemical synthesis (Bogacz-Radomska & Harasym,

2018). Monaghan and Schmitt (1932) first described β-carotene as a fat-soluble vitamin A. This antioxidant protects lipids against rancidity and is known as the most efficient singlet oxygen radical scavenger.

4.1.3 | Thioredoxin

Thioredoxin is a pervasive cellular protein disulfide reductase which serves as an electron donor for several enzymes including ribonucleotide reductase, thioredoxin peroxidase, and methionine sulfoxide reductase (Arner & Holmgren, 2000). Thioredoxin is a small (12 kDa) multifunctional protein (Haendeler et al., 2002) consisting of 105 amino acid residues and acts as a powerful antioxidant (Kaimul, Nakamura, Masutani, & Yodoi, 2007). It is responsible for maintaining proteins in the reduced state thus regulating redox reactions in signal transduction pathways. It functions as an intracellular reductase through a dithiol–disulfide exchange reaction using two cysteine residues (Holmgren, 1985). Reduced thioredoxin forms dithiols and the oxidized form incorporates disulfide bonds in the active site. Reduced thioredoxin transfers a hydrogen ion (H^+) to the disulfide in the targeted oxidized protein to reduce it and itself becomes oxidized. Thioredoxin is also susceptible to nitrosylation (Engelman, Ziv, Arnér, & Benhar, 2016). Thioredoxin levels are high in some cancers such as hepatocelluler (Miyazaki et al., 1998), lung (Kim et al., 2003), and cervical cancers (Nishiyama, Masutani, Nakamura, Nishinaka, & Yodoi, 2001). Plasma concentrations of thioredoxin is a biomarker for oxidative stress-related disorders and metabolic syndrome. It stimulates hypoxia-inducible factor-1α (HIF-1α), which increases the production of vascular endothelial growth factor (VEGF) promoting tumor angiogenesis and drug resistance (Welsh, Bellamy, Briehl, & Powis, 2009). Thioredoxin is said to be a "moonlighting protein" (Jeffery, 1999) with several new functions being discovered.

5 | DESIGNER FOODS

The designer foods, also known as "functional food" and "fortified food," refers to the food fortified or enriched with nutrient content already present in them or other complementary nutrient. These are designed to have some health benefits other than its traditional nutritional value. The term was introduced in Japan in 1980s for referring processed food containing nutrient conferring of some additional health benefits apart from its own nutritional value, whereas in China, designer food (or health foods) is used in their traditional medicine. "A functional food is similar in appearance to, or may be, a conventional food that is consumed as part of a usual diet, and is demonstrated to have physiological benefits and/or reduce the risk of chronic disease beyond basic nutritional functions, i.e. they may contain bioactive compounds" (Health Canada, 1998). The Institute of Medicine's Food and Nutrition Board (IOM/NAS, 1994) defined functional foods as "any food or food ingredient that may provide a health benefit beyond the traditional nutrients it contains" (Rajasekaran & Kalaivani, 2013). Designer foods are normal foods that are enriched with one or more health promoting or disease preventing substances. Most of the time the processed food are fortified with health benefiting nutrients which are already present in them in small amounts or added with complementary nutrients. A variety of foods are now available in the market (Table 4) that are fortified with various health promoting nutrients and antioxidants that help in the maintenance of various aspects of health ranging from the immune, visual, inflammation systems, etc. (Rajasekaran & Kalaivani, 2013). Functional foods include a wide variety of foods and food components believed to improve the overall health and wellbeing, reduce the risk of specific diseases, or minimize the effects of other health concerns (IFIC, 2011). It can be produced by fortification or nutrification of conventional food. Genetically engineered foods containing higher than normal amounts of health promoting nutrients and fermented foods with live cultures are considered functional foods. Infant formula may be the first designer food as it contains nutrients for the development of brain and immune system. The addition of docosahexaenoic acid to health drinks for improving brain and visual development, the alteration or reduction of allergenic components in food, the use of probiotics and nucleotides to enhance immune response and sports nutrition are important examples of designer foods. Traditional and complementary medicine in various countries like China, Japan, and India has the tradition of using fermented food for its health benefits, which includes red wine, yogurt, tofu, cheese, etc. (Rajasekaran & Kalaivani, 2013).

TABLE 4 Designer food and their fortified contents

Food	Contents fortified/added	References
Chicken/meat	Selenium	Bennett & Cheng, 2010
Eggs	α-linolenic acid/vitamin E, vitamin D, omega-3 fatty acid	Bourre, 2006
Grains	Folic acid	Al-Hooti, Sidhu, Al-Sager, & Al-Othman, 2002
Milk	Linoleic acid, polyphenols	Axten, Wohlers, & Wegrzyn, 2008
Oil (vegetable, fish, nuts)	Omega-3 fatty acids, omega-6 fatty acids, polyphenols, tocopherols	Riediger, Othman, Suh, & Moghadasian, 2009
Rice	Vitamin A, iron	Kalaivani & Mathew, 2010
Yogurt	Probiotic microbes, vitamin D, vitamin B1, vitamin B2	Sleator, 2010

Probiotics are organisms or substances that contribute to intestinal microbial balance, in contrast to antibiotics that counteract microbial activity. However, a currently widely accepted definition is that "probiotics are live microorganisms which when administrated in adequate amounts confer a health benefit on the host." Humans have always ingested bacteria unintentionally together with food which could be adverse, but they could also be harmless "dietary bacteria" when fermented foods were consumed. In particular, lactic acid fermented foods such as yoghurt, cheese, olives may contain high concentration of live bacteria often of *Lactobacillus* species that are now used for probiotics. In search of strains with better resistance to pH of the stomach and digestive juices of intestine, *Lactobacillus acidophilus* was launched in USA and *Lactobacillus casei* (*L. paracasei*) in Japan in the 1930s as probiotics (Hakansson & Molin, 2011). The human gut microbiota has been the interest of research in recent years and the knowledge about their potential capacity is growing rapidly. Microorganisms have colonized throughout the gastrointestinal tract of human through a symbiotic relationship and influence physiology, metabolism, nutrition, and immune functions of an individual. The gut microbes are directly involved in conferring protection against pathogen colonization by inducing direct killing, competing with nutrients, and enhancing the response of gut-associated immune system. Damage to the microbiome (dysbiosis) is linked with several life-threatening pathophysiological conditions, that is, inflammatory bowel disease, cancer, obesity, allergy, and auto-immunity (Sokol et al., 2018; Viennois et al., 2019; Vitetta, Coulson, Thomsen, Nguyen, & Hall, 2017). Therefore, the manipulation of human gut microbiota is a potential target for therapeutic intervention in several human diseases (Mamantopoulos et al., 2017; Mamantopoulos, Ronchi, McCoy, & Wullaert, 2018; Mukherjee, Joardar, Sengupta, & Sinha Babu, 2018; Richard & Sokol, 2019).

6 | DESIGNER FOODS AND ANTIOXIDANTS

Due to growing interest in functional foods with antioxidative attributes, probiotics are known as potential sources of antioxidants (Mishra et al., 2015). The probiotic fermented milk prepared from cows, goats, and camels milk supplemented with bacteria *Pediococcus pentosus* showed radical scavenging antioxidant activity. Moreover, it has also been reported that different protein peptides present in fermented milk are responsible for increased radical scavenging activity. Some researchers worked on the effect of prebiotics (inulin, lactulose, raffinose) on multiplication of some probiotic strains of *Lactobacillus* and *Bifidobacterium*, to obtain a bread-like product (Mishra et al., 2015). Lactic acid bacteria (LAB) strains are the major representatives of probiotics both in the food and pharmaceutical industries. Probiotic *Bifidobacterium* is also a very commonly used probiotic bacterium and is able to promote antitumor immunity and relieve irritable bowel syndrome in women. In addition to the beneficial effects, studies have shed new light on the antioxidant capacity of probiotics. Further, the oxidative stress in patients with type 2 diabetes can be ameliorated by multispecies probiotics. It has been found that LAB can suppress oxidative stress and in humans, *Lactobacillus rhamnosus* exerted strong antioxidant activity in physical stress. Athletes exposed to oxidative stress might benefit from the ability of *L. rhamnosus* to increase antioxidant levels and neutralize the effects of ROS (Wang et al., 2017).

7 | CONCLUSION

The antioxidants play a major role in scavenging free radical and non-radical oxidants and protecting the cells from oxidative stress. Antioxidants both small molecules such as GSH, thioredoxin, ascorbic acid as well as enzymes, for example, SOD, GPx, CAT counter the stress caused by the reactive radicals and protect cellular biomolecules. The tocopherols, ascorbic acid, carotenoids, flavonoids, amino acids, phospholipids, and sterols are naturally occurring antioxidants. They inhibit the oxidation of foods by scavenging free radicals, chelating pro-oxidant metal ions, quenching photosensitizers, and inactivating non-heme containing lipoxygenase preventing lipid peroxidation. Antioxidant in food sciences describes compounds that block oxidative reactions, thus maintaining freshness and prolonging the shelf lives of food products. In addition to the essential antioxidant nutrients such as vitamins E and C, there are several well-designed antioxidant and cytoprotective enzyme systems in the human body, which are more important than dietary non-nutrient antioxidants. At high concentrations, many antioxidants could act as prooxidants, increasing oxidative stress and inducing toxicity. Newer approaches of designer foods that are fortified with antioxidants and nutrients are currently available in that may be important in human health including the use of different types of probiotics, which are live microorganisms and confer health benefit on the host. In particular, lactic acid fermented foods such as yoghurt, cheese, olives may contain live bacteria of the same *Lactobacillus* species that is now used in probiotics. Microorganisms have colonized the gastrointestinal tract of humans through a symbiotic relationship and influence the physiology, metabolism, nutrition, and immune functions of an individual.

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CONFLICT OF INTEREST

The authors declare that they have no competing financial interests.

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