Chapter

Effect of Nanoparticles on Lipid Peroxidation in Plants

Shahla Hashemi

Abstract

The size of the nanoparticles is between 1 and 100 nm. Nanoparticles are widely used in consumer and medical products, as well as in agricultural and industrial applications. The excessive use nanoparticles increases its release into the environment. Plants are an important part of the environment that is affected by nanoparticles. Studies have examined the effect of nanoparticles on plants. The results showed that high concentrations of nanoparticles showed a negative effect. Reactive oxygen species generation is a toxicological mechanism of nanoparticles in plants. When the production of radicals is greater than its removal, oxidative stress occurs. The key indicator of oxidative stress is lipid peroxidation. The unsaturated fatty acids in the cell membrane are a major target for radicals. Radical absorbs hydrogen from unsaturated fatty acids to form water. Therefore, the fatty acid has a non-coupled electron, which is then able to capture oxygen and form a peroxyl radical. Lipid peroxyl radical can lead to a chain of radical production. Enzymatic and nonenzymatic systems exist for the removal of radicals in plants. Enzymatic systems include catalase, guaiacol peroxidase, ascorbate peroxidase, superoxide dismutase, glutathione reductase, and dehydroascorbate reductase. Nonenzymatic systems include ascorbate and carotenoids, glutathione, tocopherol, and phenolic compounds.

Keywords: nanoparticles, reactive oxygen species, malondialdehyde, catalase, ascorbate, glutathione

1. Introduction

The nanoparticles have a size of less than 100 nm in at least one dimension. Due to the specific properties of nanoparticles, in particular the high-surface-to-volume ratio, they have been used for several applications. For example, nanoparticles are used in the fields of biosensors and electronics, cosmetic industries, wastewater treatment, biomedicines, cancer therapy, and targeted drug delivery [1, 2]. The excessive use of nanoparticles results in the release of these materials into the environment. The environment includes plants, the main producers of the food chain, which are affected by nanoparticles. Nanoparticles are absorbed by plants and transmitted to various parts of the plants and affect them. Several factors such as physicochemical properties of nanoparticles, plant species, and exposure conditions contribute to the absorption and transfer of nanoparticles. Size, magnetic properties, surface charge, composition, crystalline state, and surface functionalization are some of the physical properties of nanoparticles that are important in their absorption

into the plant. Nanoparticles are introduced into the plant by various methods, for example, through penetration into the coating of seeds, during absorption of nutrient by the root, and entering the cuticle and stomata of the leaf. After absorbing nanoparticles, these materials can accumulate or move through the vascular system to the shoot. The first cell-level barrier to move nanoparticles is the cell wall. The size of the pores in the cell wall is 5–20 nm. Therefore, nanoparticles of less than 20 nm in size can easily pass through the pores. But nanoparticles with sizes larger than 20 nm through routes such as ion channels, endocytosis, and aquaporins and creation of new pores pass the cell wall of the barrier. The next barrier is the plasma membrane. The role of the plasma membrane is controlling the passage of materials in and out of the cell. Protein and lipids are two main parts of the plasma membrane structure. Plasma membrane lipids play an important role in determining cellular structures, regulating fluid membrane and signal transduction. Lipids are not only present in the plasma membrane but also in all parts of the plant. Plants have a diverse range of lipids including fatty acids, sterol lipids, glycolipids, sphingolipids, phospholipids, and waxes. In this chapter, we discussed the effects of nanoparticulate toxicity on the lipids of plants and plant defense mechanisms against this toxicity.

2. Interaction of nanoparticles with plants

There are reports that nanoparticles can lead to stress through release of reactive oxygen species (ROS) in plants. The lack of balance between the production and removal of ROS leads to the production of oxidative stresses with oxidative damage to DNA, proteins, and fats. There are two unpaired electrons in separate orbitals in the outer shell of oxygen. This oxygen structure makes it a candidate for the production of ROS. ROS are free radical species and non-free radical oxygen. Radicals can have neutral, negative, or positive charge. Free radical is an atom or group of atoms that have one or more unpaired electrons. Free radical oxygen species contains the hydroxyl radicals ($^{\bullet}OH$) and free radicals superoxide anion ($^{\bullet}O_2^-$). Non-free radical species containing hydrogen peroxide (H_2O_2) are various forms of activated oxygen resulted from oxidative biological reactions or exogenous factors (**Figure 1**). Radicals are naturally produced as intermediate biochemical reactions, but excess production of these radicals damages the plant and should be eliminated by the antioxidant system.

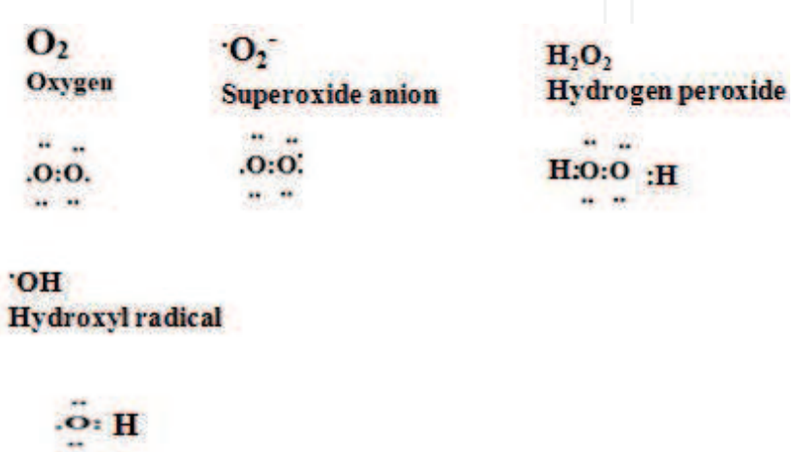

Figure 1.
Oxygen and some reactive oxygen species.

The antioxidant system in the plant contains an enzymatic and nonenzymatic system. The nonenzymatic antioxidant system contains alpha-tocopherol, flavonoids, ascorbate, glutathione and phenolic compounds, and carotenoids, while the enzymatic antioxidant system includes catalase (CAT), ascorbate peroxidase (APX), superoxide dismutase (SOD), peroxidase (POX), and glutathione reductase (GR).

3. Alpha-tocopherol

Alpha-tocopherol is a hydrophobic antioxidant that is produced by all plants (**Figure 2**). This compound is present primarily in the cell membrane and plays a key role in the collection of proxy lipid radicals from lipid peroxidation. One of the most prominent properties of tocopherol is their ability to turn off single oxygen, and it is estimated that a tocopherol molecule alone can neutralize about 120 molecules of single oxygen [3].

Alpha-tocopherol also acts as an end point for peroxidation reactions of unsaturated fats, which is converted to radical tocopheroxyl by reaction with lipid peroxyl radicals. Radical tocopheroxyl can be converted to tocopherol by reaction with ascorbic acid or other antioxidants [4] (**Figure 3**).

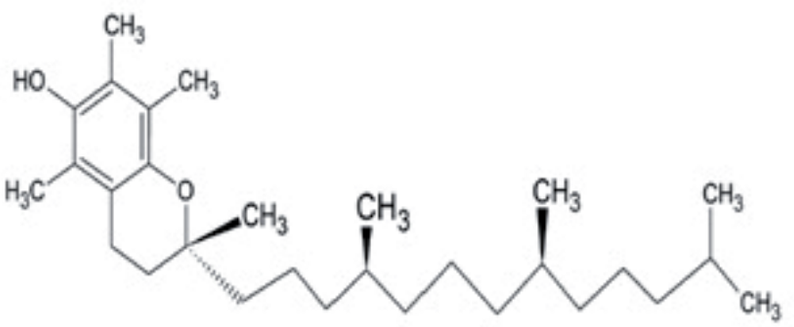

Figure 2.
Chemical structure of alpha-tocopherol [4].

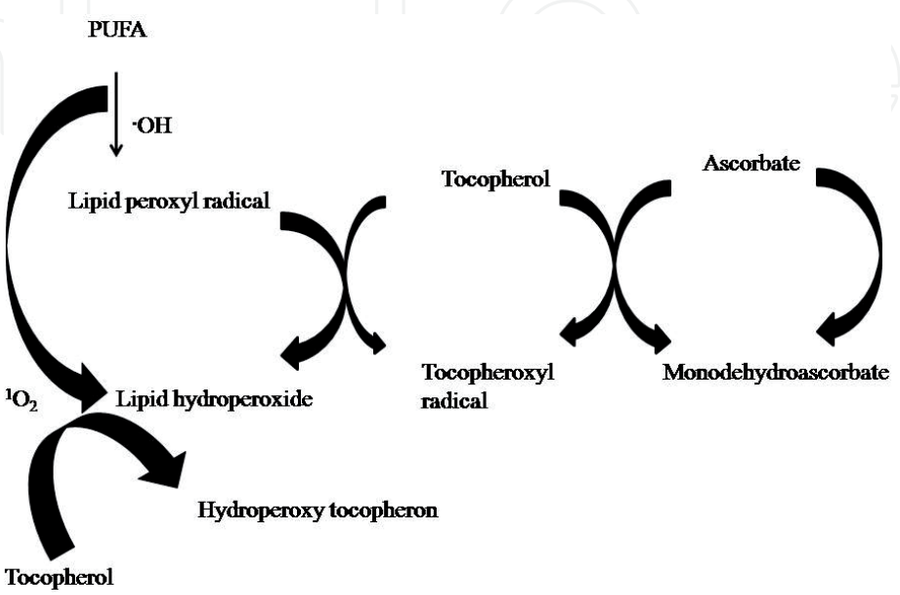

Figure 3.
The role of antioxidant tocopherol. Polyunsaturated fatty acids (PUFA).

4. Ascorbic acid

Ascorbic acid is one of the most powerful antioxidants that has been found in a variety of plant cells, organelles, and apoplastic space. In physiological conditions, ascorbic acid is often reduced. The ability of ascorbate to give the electron in a wide range of enzymatic and nonenzymatic reactions has transformed this substance into active oxygen species detoxification compound. Ascorbic acid plays a role in collecting superoxide, hydroxyl radicals, and singlet oxygen or converting hydrogen peroxide through the reaction of ascorbate peroxidase into water [5]. The conversion of hydrogen peroxide to water in ascorbate-glutathione cycle leads to the conversion of ascorbate to monodehydroascorbate. These compounds have a short life-span and convert into ascorbate by interfering with the enzymes of monodehydroascorbate reductase and NADPH (**Figure 4**).

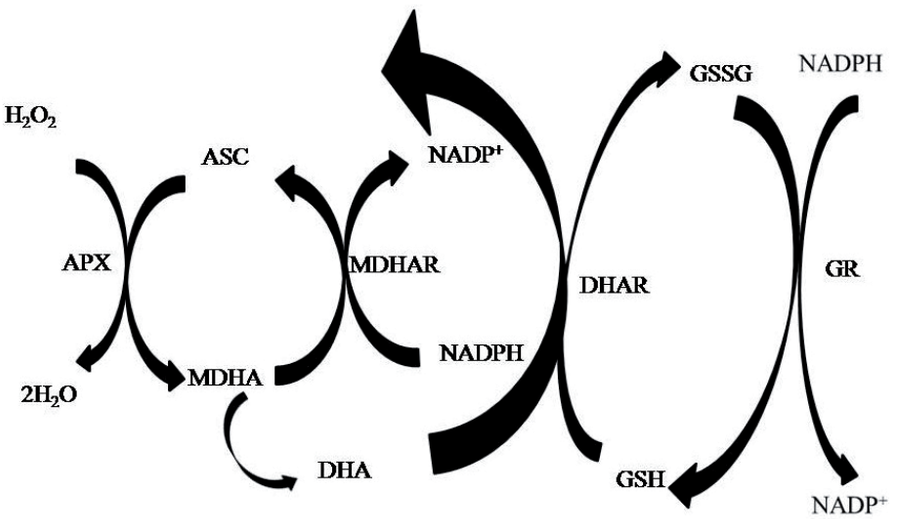

Figure 4.
Ascorbate-glutathione cycle. ASC, ascorbate; MDHA, monodehydroascorbate; DHA, dehydroascorbate; GSH, glutathione; GSSG, glutathione disulfide; APX, ascorbate peroxidase; MDHAR, monodehydroascorbate reductase; DHAR, dehydroascorbate reductase; GR, glutathione reductase [6].

5. Glutathione

Glutathione, γ-glutamyl-cysteinyl-glycine, is a tripeptide that has been found in all parts of the cell, such as cytosol, endoplasmic reticulum, chloroplast, vacuoles, and mitochondria [7]. Glutathione is one of the main sources of thiol in most plant cells. Due to the reactivity of the glutathione thiol group, this substance has been widely recognized for a wide range of biochemical reactions. The central cysteine in the glutathione molecule has created a high potential for reduction in this molecule. Reduced glutathione can remove the hydrogen peroxide [8]. The main role of glutathione in antioxidant defense is due to its ability to produce reduced ascorbic through the ascorbate-glutathione cycle. Some researchers have reported that glutathione protects the cell from oxidative stress by reacting thiol groups with singlet oxygen and radical hydroxyl [5, 9, 10].

6. Phenolic compounds

Phenolic compounds of a group of secondary metabolites include flavonoids and tannins, which are found in plant tissues abundantly. Most plants synthesize

phenolic compounds in natural conditions, but their synthesis and accumulation are induced by stresses of nanoparticles [11]. Phenolic compounds play a role in H_2O_2-scavenging, quenching of singlet oxygen, and reducing or inhibiting lipid oxidation [14, 15]. There are two main mechanisms for the protective role of phenolic compounds (POH).

In the first mechanism, the hydrogen atoms of phenolic compounds are eliminated by free radical ($R^{\cdot}$), and the phenolic compounds become radical:

$$R^{\cdot} + POH \rightarrow RH + PO^{\cdot}$$

In evaluating the phenolic compounds' action in this mechanism, the bond dissociation energy of the O–H bonds is an important parameter, because the weakening of the OH bond increases the activity of phenolic compounds for radical deactivation [12].

In the second mechanism, free radicals can take electrons from phenolic compounds and convert them into radical cation [12]:

$$R^{\cdot} + POH \rightarrow R^{-} + POH^{\cdot +}$$

According to the second mechanism, the lower ionization potential of phenolic compounds releases electrons more easily.

Therefore, the activity of phenolic compounds is easily estimated by calculating ionization potential and the bond dissociation energy of the O–H bonds [12]. Bendary et al. suggested that the phenolic compounds perform scavenging of H_2O_2 from the first mechanism. The number of hydroxyl groups and the aromatic ring substitution pattern are all important associated factors. The ortho and para position substitution with another hydroxyl group is another important factor that plays in H_2O_2-scavenging [13].

7. Carotenoids

Carotenoids are tetraterpenes that exist in the photosynthetic and non-photosynthetic tissues of the plants and synthesize from isoprenoid biosynthesis pathway. Carotenoids act as auxiliary pigment in chloroplasts, but their main role is the role of antioxidant activity [14, 15].

There are two types of carotenoids in plant tissues:

1. Carotenoids that only contain hydrocarbons (carotene)

2. Carotenoids which in addition to the hydrocarbon chain have oxygen atoms (xanthophyll).

These compounds carry the antioxidant role through the following routes:

1. Eliminating singlet oxygen and wasting energy in the form of heat (**Figure 5**)

2. The reaction with excite chlorophyll and gaining energy to prevent the formation of singlet oxygen (**Figure 5**)

3. Waste high energy of exciting through the xanthophyll cycle [14, 15]

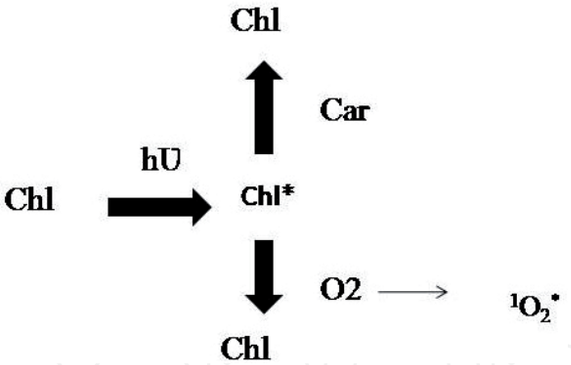

Figure 5.
*Schematic representation of singlet oxygen formation upon excitation of chlorophyll (Chl) and the role of carotenoids (car) in protection against photooxidative damage. The symbol * indicates excited states.*

8. The xanthophyll cycle

In green tissues, zeaxanthin epoxidase can be zeaxanthin converted to violaxanthin via the intermediate antheraxanthin. This is a reversible reaction; violaxanthin de-epoxidase converts violaxanthin to zeaxanthin by antheraxanthin. The relative concentration of zeaxanthin/violaxanthin is controlled by the xanthophyll cycle in plant photosynthetic tissues, which is a collection of light and dark control reactions.

Under high light conditions, violaxanthin de-epoxidase activated and converted violaxanthin to zeaxanthin. In dark conditions zeaxanthin is converted into violaxanthin [16] (**Figure 6**).

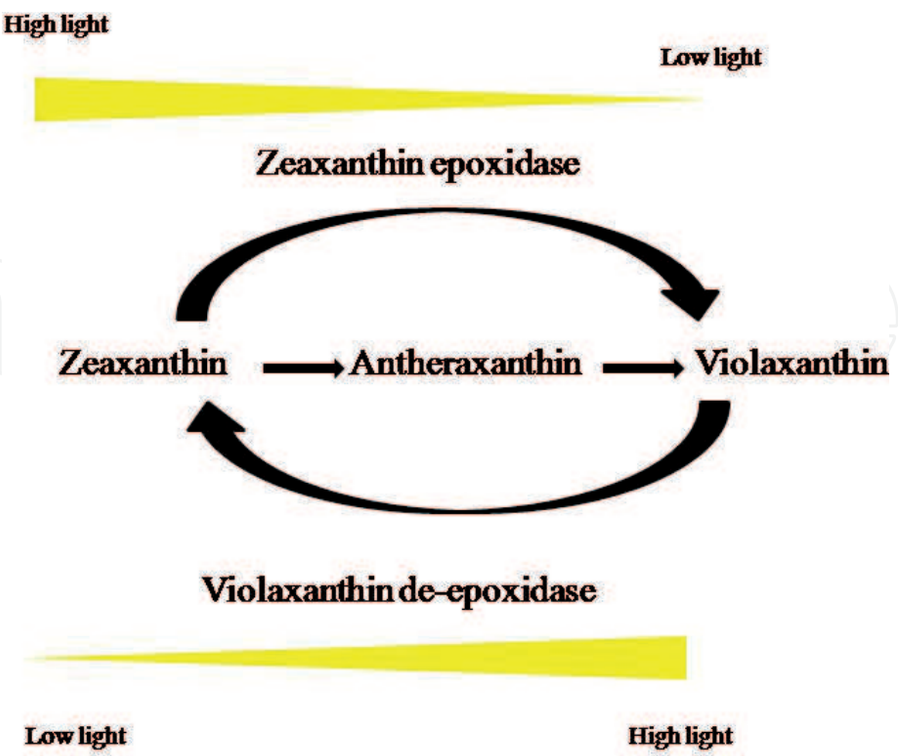

Figure 6.
The xanthophyll cycle.

9. Catalase

Catalase has a tetramer enzyme that breaks H_2O_2 into water and oxygen. Catalase has a lower affinity for H_2O_2, so it can remove H_2O_2 at high concentrations [17]. Hydrogen peroxide is very toxic to plant cells, especially in the chloroplast. Hydrogen peroxide at very low concentrations prevents the activity of the enzymes of the calvin cycle, especially the enzymes with *sulfhydryl* group such as glyceraldehyde 3-phosphate dehydrogenase and fructose 1,6 bisphosphatase [18, 19].

10. Peroxidases

Peroxides are a group of antioxidant enzymes that cause the decomposition of hydrogen peroxide with the oxidation of a substance. Peroxidases are located in the cytosol, vacuole, chloroplast, and extracellular space and are classified based on their combined composition.

11. Ascorbate peroxidase

Ascorbate peroxidase is an antioxidant enzyme that participates in the ascorbate-glutathione cycle, and its activity has been reported in the chloroplast, cytosol, peroxisome, and apoplast. This enzyme uses ascorbate as a reducing agent and decomposes hydrogen peroxide into water and oxygen [20]. The high concentration of ascorbate peroxidase to hydrogen peroxide shows that the ascorbate-glutathione cycle plays a vital role in controlling the level of radicals in cellular organs. In ascorbate-glutathione cycle with ascorbate peroxidase enzyme activity, ascorbate is oxidized to monodehydroascorbate, and ascorbate production is required to continue the cycle. In this cycle, the enzymes of monodehydroascorbate reductase (MADAR), dehydroascorbate reductase (DHAR), and glutathione reductase (GR) are active and reduce ascorbate using water and glutathione. Using NADPH, monodehydroascorbate reductase converts monodehydroascorbate to ascorbate. While dehydroascorbate reductase catalyzes dehydroascorbate to ascorbate using glutathione (GSH) oxidation.

12. Guaiacolperoxidase

Guaiacol peroxidase oxidize guaiacol. This enzyme is also present in the cytosol, vacuole, and cell wall [21].

13. Superoxide dismutase (SOD)

Superoxide dismutase is one of the enzymes that is located in all intracellular organs and apoplast and is very important in the defense against active oxygen species. This enzyme converts the radical superoxide to H_2O_2, which H_2O_2 should be detoxified during the next stages of antioxidant defense. In the presence of the superoxide dismutase enzyme, this reaction occurs 10,000 times faster [22].

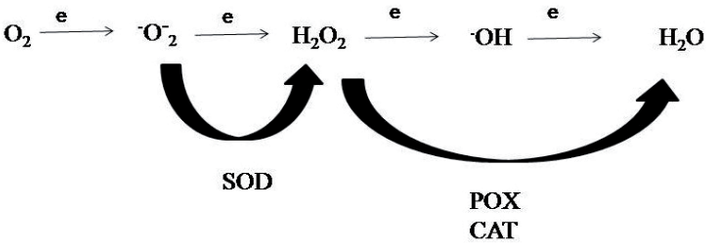

Figure 7.
Formation and elimination of reactive oxygen species.

This enzyme is divided into three groups based on its cofactor:

1. Cu/Zn SOD in the chloroplasts and cytosol

2. Fe/SOD in the chloroplasts of some plants

3. Mn/SOD in the mitochondrial matrix

There are genome types of SOD in the nucleus that are synthesized in the cytoplasm and then transmitted to different organs [23]. In the chloroplast, the superoxide dismutase enzyme is in two forms attached to the thylakoid membrane and is free in the stroma. The forms attached to the thylakoid membrane and the free enzyme, at the site of production and inward stroma, convert radical superoxide into H_2O_2 [24].

In summary, the activity of enzymes was shown in **Figure 7**.

14. Lipids and oxidation

Lipids are major constituents of prokaryotic and eukaryotic membranes. Besides serving as structural components of the plasma membrane and intracellular membranes, they provide diverse biological functions in energy and carbon storage, signal transduction, and stress responses. Plants contain a diverse set of lipids including fatty acids, phospholipids, glycolipids, sterol lipids, sphingolipids, and waxes. Polyunsaturated fatty acids (PUFAs) are lipid components commonly and easily oxidized by unbalanced ROS (mainly hydroxyl radical due to its indiscriminative reactive character).

15. Effect of reactive oxygen species on lipids

Cell membrane is one of the primary goals of many environmental stresses. Therefore, maintaining the integrity and stability of the membrane under stress is one of the signs of stress tolerance [25]. Polyunsaturated fatty acids are one of the most important membrane lipid compounds that are very sensitive to peroxidation. The main reason for the harmful effects of ROS is their ability to start the chain reaction of oxidation of unsaturated fatty acids, which leads to lipid peroxidation and membrane degradation.

The reaction is divided into three major steps: initiation, propagation, and termination.

Initiation

A radical fatty acid is produced at the initiation stage. Oxygen reactive species (ROS), such as OH, combines with hydrogen atom of unsaturated fatty acid to produce water and radical fatty acids (**Figure 8**).

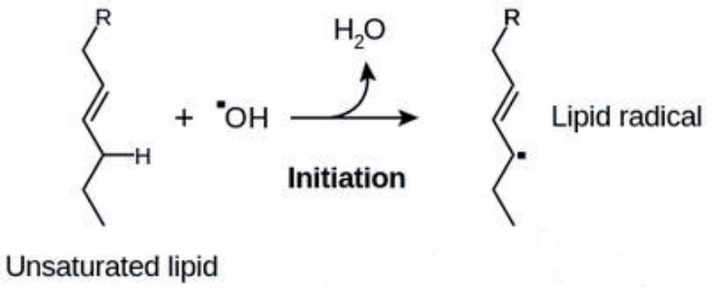

Figure 8.
The initiation phase of peroxidation of unsaturated fatty acids [26].

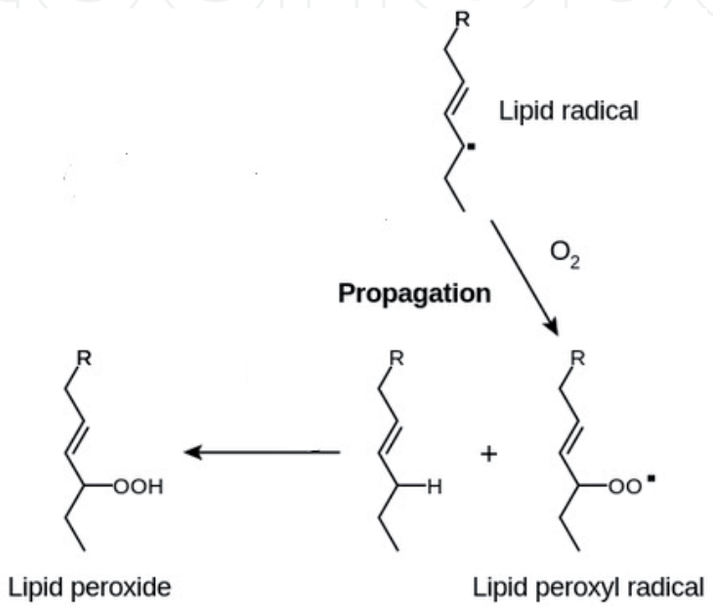

Figure 9.
The propagation phase of peroxidation of unsaturated fatty acids [26].

Propagation

The stability of the fatty acid radical is low, so that it reacts with molecular oxygen, resulting in the formation of peroxyl-fatty acid radical. This radical also has low stability, which reacts with another free fatty acid and produces a lipid peroxide and different radical fatty acid (**Figure 9**).

Termination

It always produces another radical, while a radical reacts with a non-radical. This process is called the "chain reaction mechanism." A non-radical species is produced when two radicals react together. So the radical reaction stops.

16. Formation malondialdehyde (MDA)

As previously mentioned, the polyunsaturated fatty acid (PUFA) acyl chain is attacked by free radicals and creates a radical fat that reacts easily with an oxygen molecule and forms a lipid peroxyl radical. The lipid peroxyl radical can attack neighboring PUFAs, propagating a chain reaction. A linolenic acid (18:3) peroxyl radical can also react internally, forming a cyclic peroxyl radical, which spontaneously reacts with a second oxygen molecule and is subsequently reduced to phytoprostane G1 (PPG1). Phytoprostane G1 (PPG1) either spontaneously decays, forming MDA and other alkanes and alkenes, or forms other phytoprostanes [27] (**Figure 10**).

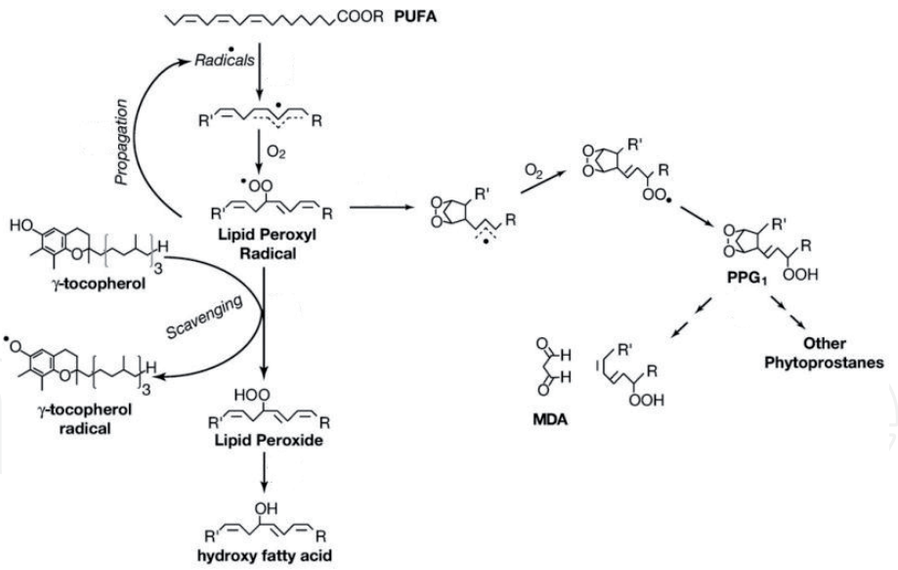

Figure 10.
Lipid peroxidation [27].

17. Malondialdehyde (MDA) assay

Lipid peroxidation was measured according to Heath and Packer (1968) by measuring the concentration of MDA. According to this method, 0.2 g of tissue was homogenized in 2 ml 0.1% (w:v) trichloroacetic acid (TCA) solution. The homogenate was centrifuged at 10,000 g (rcf) at 4°C for 10 min and 2 ml of supernatant transfer to new tube and then added 1 ml 20% TCA containing 0.5% (w:v) thiobarbituric acid (TBA). The reaction mixture was incubated in boiling water for 30 min at 95°C followed by placing the tubes on an ice bath to stop the reaction. The homogenate was centrifuged at 10,000 g for 15 min, and the absorbance was read at 532 nm [28]. The unspecific turbidity was corrected by A600 subtracting from A530. The amount of MDA–TBA complex (red pigment) was expressed as μmol/g FW and calculated by the extinction coefficient 155 mM − 1 cm − 1 using the formula (**Figure 11**).

$$MDA = \frac{(A532 - A600)}{155} \times \frac{Amount\ of\ reaction\ mixture}{tissue}$$

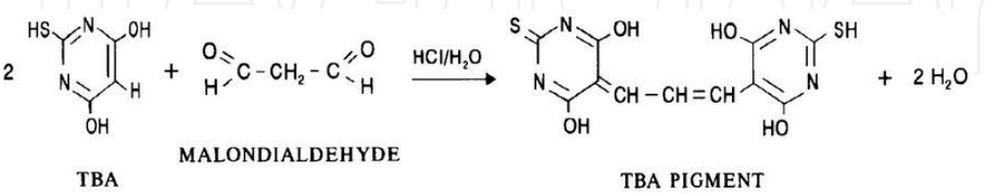

Figure 11.
The formation of MDA–TBA complex [29].

18. Radical scavenging assays

DPPH• is a stable free radical with purple color and a strong absorption band in the range of 515–520 nm. DPPH takes an electron or a hydrogen atom from an antioxidant accumulation molecule to become a stable DPPH molecule, in the presence of antioxidant compounds. The reduced form of DPPH is pale yellow. By studying spectrophotometric color changes, it may determine the antioxidant activity.

An antioxidant compound with a larger free radical scavenging capacity reduces DPPH further. Therefore, there is less purple color in the sample. The DPPH assay was performed according to the method developed by Blois (1958) slightly modified by Brand-Williams et al. [30, 31]. For 40 min, a solution of 1 mM $DPPH^{\bullet}$ in 80% (v/v) methanol was stirred. Then a standard or sample (50 mL) was added with 2.95 mL of $DPPH^{\bullet}$ solution and placed for 30 min in the dark.

Decrease of absorbance was read at 517 nm. DPPH scavenging effect is obtained from the following formula:

$$\text{DPPH scavenging effects}(\%) = \frac{A0 - A1}{A0} \times 100$$

A0: The absorbance is at 515 nm of the radical (DPPH) in the absence of antioxidant.

A1: The absorbance is at 515 nm of the radical (DPPH) in the presence of antioxidant.

19. $ABTS^{+}$ assay

Another important evaluation for antioxidant activity is the $ABTS^{+}$ test. In this assay, by peroxyl radicals or other oxidants, $ABTS^{+}$ is oxidized to its radical cation. $ABTS^{\bullet+}$ is intensely colored (dark green). Reduction of color $ABTS^{\bullet+}$ radical is used to measure the antioxidant capacity.

By using spectrophotometer, a decrease in absorbance by test compound and control is measured at 415 nm [32].

20. The role of enzymes in peroxidation of lipids

The lipoxygenase enzyme is one of the oxidative enzymes. This enzyme catalyzes the addition of molecular oxygen to unsaturated fatty acids, produces unsaturated fatty hydroperoxides, and accelerates lipid peroxidation. Free radicals produced by lipoxygenase cause irregularities in the selective membrane permeability. This irregularity leads to an increase in ion leakage, a decrease in the activity of ion pumps dependent on H^{+}ATPase, and changes in the cell membrane potential [33, 34].

21. Benefits of nanotechnology

Nanomaterials can offer many applications in mechanical industries especially in coating, lubricants, and adhesive applications. The magnetic nanoparticles such as Fe_3O_4 are employed in the biomedical and clinical fields. TiO_2 nanoparticles find an application in cosmetics, pigments, sunscreen products, solar cells, and photocatalysis [35]. However, human beings must take caution in using nanoparticles and nanotechnology.

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