Antioxidant Activity of Essential Oils: A Mechanistic Approach

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Bijendra Kumar Singh and Akash Maurya

Abstract

Essential oils (EOs) have long been extracted using a variety of techniques from different aromatic and therapeutic plants. Because of their antioxidant quality and attractive fragrances, they have found broad utilisation in various fields as antioxidant, antibacterial, and antifungal agent in pharmaceutical, cosmetic, and food sectors. Antioxidants are a group of compounds that can prevent oxidation by acting as reducing agents, pro-oxidant inactivators, free radical scavengers, and radical species quenchers. Reactive oxygen species (ROS) interact with any material that may be oxidised and modify the structural makeup of the material as a result, causing oxidative damage. There are plenty of approaches available for the estimation of antioxidant activities of Eos; ABTS (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) and DPPH (1,1-diphenyl-2-picrylhydrazine) assays are frequently used methods. Since EOs contain a variety of active components, their antioxidant mode of action cannot be achieved by a single method of action. The EOs and its nanoencapsulated products are more effective as free radical scavengers in nature due to slow release, longer shelf life, and easily available in the medium without direct interference with food matrix. The application of EOs as antioxidants in real food systems have been associated with some hurdles, which could be overcome using the encapsulation technology and advanced food packaging systems. In this chapter, the antioxidant properties of EOs, estimation of antioxidant activities, mechanisms involved in antioxidant activity, encapsulation process with enhanced activity and applicability, its application in real food systems, and future possibilities have been discussed.

B. K. Singh (✉)
Department of Botany, Feroze Gandhi College, Raebareli, Uttar Pradesh, India

A. Maurya
Department of Botany, Shri Murli Manohar PG College, Ballia, Uttar Pradesh, India

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3.1 Introduction

A class of substances known as antioxidants are capable of inhibiting the oxidation process by acting as free radical scavengers, reducing agents, inactivators of pro-oxidants, and quenchers of radical species. Antioxidants are substances, when present in foods or the body in very small amounts, which slow down, stop, or stop oxidative processes that result in food quality deterioration or the development and spread of degenerative illnesses in the organisms. Numerous organic antioxidants, such as polyphenols (anthocyanins, flavanols, and flavones), carotenoids, phenolic acids, tannins, lignin, and vitamins, are found in plants (Loi and Paciolla 2021; Arias et al. 2022). Due to its capacity to lower oxidative stress, several studies demonstrate that antioxidants are crucial for preserving human health as well as for preventing and treating illnesses. In order to ensure the quality of functional foods and, more critically, to determine how effective food antioxidants are in preventing and treating illnesses associated with oxidative stress, it is crucial to measure the antioxidant activity/capacity of foods and biological samples (Munteanu and Apetrei 2021; Siano et al. 2023).

There are numerous origins of ROS production. Different processes to control ROS production and availability, such as localised and compartmentalised creation as well as the activation of sinks (detoxifying factors) and redox relaying, are in place to keep their levels at physiological concentrations. The presence of free Fe^{2+} ions, which are engaged in the Fenton reaction, which produces the most reactive ROS form hydroxyl radicals with a single unpaired electron and the ability to react with almost all biomolecules, has a substantial impact on ROS reactivity. Due to the high reactivity of hydroxyl radicals, cellular problems such as altered protein structures, lipid peroxidation, and membrane disintegration occurs (Demidchik 2015; Acosta-Casique et al. 2023). One of the primary factors in food degradation is oxidation. For instance, the oxidation of oil results in both poisonous and low-molecular-weight molecules with little taste. It also has other negative consequences, such as vitamin deterioration and important fatty acid loss, which reduces nutritional values (Umaraw et al. 2020). The pursuit of efficient antioxidants from natural resources as alternative solutions to suppress food deterioration is now centred on edible plants because they have fewer side effects than the synthetic chemicals used in today's food products, which is associated with growing consumer interest in natural food additives (Valdivieso-Ugarte et al. 2019). EOs are liquid compositions of volatile chemicals that are frequently derived from aromatic plants. Natural antioxidants including polyphenols, carotenoids, and vitamins may come from plants and various plant components like flowers, stems, and roots. The biological benefits of EOs have been recognised, including their antiviral, antibacterial, anti-inflammatory, and antioxidant activities (Tit and Bungau 2023). The use of natural and safer preservatives is on the rise, giving food an impression of being natural origin. EOs have received a lot of interest for usage as food preservatives. The popularity of EOs as a natural preservative is increasing since last decade due to growing concern of health-related issues caused by synthetic preservatives (Burt 2004; Wu et al. 2019a, b; Falleh et al. 2020).

There are numerous techniques that may be used to assess the transfer of the hydrogen atom or the transfer of electrons from antioxidants to free radicals directly. The antioxidant activities described for this technique category are typically linked to their ability to scavenge certain radical species, some of which may be synthetic and insignificant to biology. Some of these approaches have the drawback of not accurately reflecting the circumstances in oxidised food or in vivo cases (Munteanu and Apetrei 2021). ROS interact with other components that are susceptible to oxidation and undergo structural changes to cause oxidative degradation. The category of ROS includes hydroxyl radicals (OH^-), superoxide radicals (O_2^{2-}), hydrogen peroxide (H_2O_2), and alkoxyl (RO^-). EOs slow down the beginning of antioxidant chains, free radical scavengers, hydrogen abstraction, and quenchers of singlet oxygen production by a variety of direct and indirect mechanisms (Rodriguez-Garcia et al. 2016).

For EOs to be used as food preservatives, precise knowledge of how food matrix components affect Eos' antibacterial activity is needed. Despite their remarkable preservation and antioxidant efficacy, EOs have limited employment in the food and agricultural industries because of their volatility, hydrophobicity, and negative impacts on food organoleptic properties (Prakash et al. 2015; Falleh et al. 2020; Singh and Dubey 2023). It has been consistently justified to use encapsulation as a technical substitute to mask the extremely intense aroma of some of the EOs and increase its shelf life. By guaranteeing their equal dispersion in the media, EO encapsulation provides an effective method to boost their bioactivity and bioavailability. The exterior protective layer utilised in the encapsulation functions as a hydrophilic barrier to EOs to get over their hydrophobic restriction (Nehme et al. 2021; López-Gómez et al. 2023). By preventing exposure and degradation of EOs and their bioactive ingredients by forming a physical barrier and by facilitating their regulated release, nanoencapsulation looks to be a promising method to eliminate these restrictions. This results in increased bioavailability and efficacy. More focus should be placed on these particular parameters in order to achieve better nanoencapsulation and ultimately to their application into the food systems because a large number of intrinsic factors can affect the successful nanoencapsulation of EOs and their bioactive constituents into polymeric systems (Chaudhari et al. 2021; Singh et al. 2022a, b; Chaudhari et al. 2023). A lot of research has been done regarding the encapsulation materials, factors influencing the process, and behaviour of nanoencapsulated EOs in the real food systems. But there is need of more research to be carried out regarding the hurdles involved in the application of natural antioxidants such as EOs in real food systems and their large-scale practical applications, which is the demand of present-day generations and surely going to increase several folds in future.

3.2 Reactive Oxygen Species (ROS) and Antioxidants

The term 'ROS' refers to a class of molecules generated via reduction-oxidation (redox) processes or electronic excitation and derived from molecular oxygen. Several kinds of 'reactive species' have piqued attention in biology and medicine (Hawkins and Davies 2019). Their names reflect the characteristics of the reactive atoms. There are several origins of ROS production. Different strategies to control ROS production and availability, such as localised and compartmentalised creation as well as the activation of sinks (detoxifying factors) and redox relays, are used to keep their levels at physiological concentrations (Sies and Jones 2020; Zeng et al. 2023). ROS are a normal by-product of cellular aerobic metabolism in a biological setting and are primarily produced by mitochondrial respiration (Mailloux 2020). Free radicals are made up of atoms or molecules that have an unpaired electron in their outer shell, which makes them unstable and extremely reactive, or, to put it another way, makes them prone to 'stealing' electrons from other molecules (Pham-Huy et al. 2008). In addition, environmental pollution, exposure to xenobiotic compounds, pesticides, and heavy metals, drug and alcohol usage, cigarette use, extended use of some medicines, and a number of other lifestyle choices adversely influence ROS production. Recently, free radicals have become more prevalent due to pollution, stress, and eating commercial food products (Al-Gubory 2014; Khalid et al. 2023).

There are plenty of research material available depicting ROS as a signal factor taking part in defence mechanism and signal transduction, and considered as core contributors in complex signalling cascade. In reaction to stressors or other environmental perturbations, physiological targets of oxidants operate as molecular redox switches in signal transduction at different levels of cell control (Mittler et al. 2011; Sies and Jones 2020). Generally, the functions of ROS in signalling are correlated with tightly controlled site- and time-specific modifications of ROS levels, whereas unregulated ROS accumulation as a result of either consisted or defective catabolism, or their combined effect, is correlated with harmful oxidative effects that can result in cellular damage or even cell death (Mittler 2017). Through oxidative processes, namely those involving protein thiol groups or iron-containing clusters, ROS can alter the structures and behaviours of proteins. However, the harmful effects of accumulating ROS are also used as chemical defence within immunological repertoires of various species against pathogens, such as the ROS burst that causes plant cell death during the plant hypersensitive response to biotrophic infections (Eckardt 2017; Janků et al. 2019).

Food rotting mostly results from chemical reactions that happen when food is exposed to air or when spoilage bacteria are present. ROS interact with any substance that can be oxidised and undergo structural changes as a result to produce oxidative damage (Gómez-Estaca et al. 2014). Additionally, ROS cause a number of unwanted and substantial changes in food, including a reduction in nutritional content and a loss of colour, taste, and odour. This process in packaged foods is typically thought to be started by a change in the oxidation state of the metals already present in the food and/or exposure to ultraviolet (UV) light, like sunlight. This causes subsequent hydrogen extrapolation from fats, which in turn triggers subsequent reactions in nearby fat molecules (Shahidi and Ambigaipalan 2015). Moisture can contribute ROS formed from the food product's water content, which is a determining factor in oxidation. Additionally, when oxidative reactions develop over time, a number of compounds (such as sugars and short-chain fatty acids) are made more accessible to spoilage microbes, making the food surface more inviting to them. Foods are also sensitive to oxidation, enzymatic activity, and the release of ROS (Carpena et al. 2021).

Antioxidants are substances that have beneficial antioxidant effects due to their ability to provide electrons to neutralise free radicals and stop oxidation processes. Through a variety of processes, antioxidants scavenge ROS and help to convert it to less reactive and more stable forms (Pateiro et al. 2018). In order to be close to the places where ROS are produced, enzymatic antioxidants are localised in a variety of subcellular locales. Non-enzymatic antioxidants can come from inside the body or be obtained from food (Shields et al. 2021). There are around more than 6000 naturally occurring chemicals that belong to the huge and varied family of secondary metabolites known as polyphenols, which have important biological functions linked to their antioxidant capabilities and free radical scavenging abilities (Buchanan et al. 2015; Tomás-Barberán et al. 2000). The highly reactive superoxide anion is changed into a less reactive hydrogen peroxide by the enzyme superoxide dismutase (SOD). Using transition metals in its active site, the enzyme catalyses the transfer of electrons through two redox processes. The enzyme known as superoxide dismutase (SOD) has a specific function in reducing the consequences of oxidative stress caused by the presence of free radicals. SOD is involved in catalysing the recombination reaction of the oxygen radicals (Getzoff et al. 1983; Munteanu and Apetrei 2021)).

In order to deal with hydrogen peroxide-producing processes, including those of the SODs, catalase (CAT) catalyses the conversion of hydrogen peroxide to water and molecular oxygen (Ighodaro and Akinloye 2018). The glutathione peroxidase (GPx) and glutathione reductase (Grx) systems must function at optimal glutathione (GSH) levels because GSH works to restore each enzyme to its active state. Additionally, GSH itself has the ability to function as an antioxidant, donating electrons via the sulfhydryl group to lower and detoxify ROS (Birben et al. 2012). In addition to being a precursor molecule for GSH, N-acetyl cysteine (NAC) also possesses its own thiol group, which enables it to take part in redox processes and give its electrons for the detoxification of ROS and the defence of sulfhydryl-containing proteins against oxidative damage.

The secondary metabolism in plants produces volatile aromatic molecules known as essential oils (EOs). Between a dozen and several hundred components make up each EO, the majority of which are terpenes and terpenoids, including oxygenated derivatives such as aldehydes, ketones, alcohols, ethers, esters, and epoxides (Bajpai and Baek 2016). Another biological characteristic of significant interest is the antioxidant activity of EOs, which may help protect food from the harmful effects of oxidants (Valdivieso-Ugarte et al. 2019). Antioxidants have the function of neutralising free radicals in biological cells, which have a detrimental effect on living things. Bioactive EOs have been included into active food packaging due to their distinctive scent, tastes, and natural antibacterial properties to increase the shelf life and safety of perishable food. They have been categorised as natural preservatives, culinary flavourings, and therapeutic treatments because of plants' antiviral, antimicrobial, and insecticidal properties (Bonda et al. 2020; Ni et al. 2021).

3.3 Antioxidant Activity of EOs

Recently, numerous studies have investigated the antioxidant activities of EOs due to their chemical compositions. Moreover, Phenols and other secondary metabolites are linked with double bonds that are responsible for substantial antioxidant activities (Prasad et al. 2022; Tiwari et al. 2022). Therefore, aromatic plant EOs and their major constituents are widely used as an alternative food additive to avoid the degradation of the food products instead of suspected synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and propyl gallate (PG) that pose potential adverse effects on human health (Maurya et al. 2021a; Al-Maqtari et al. 2021). EOs also exhibit important role in preventing ageing and variety of lifestyle-related diseases such as cancer, dysfunction, immune system decline, and heart disease due to closely associated with active oxygen and lipid peroxidation (Bhavaniramya et al. 2019). Thus, the EOs were recommended as good substitute of synthetic antioxidants, because the majority of EOs are generally recognised as safe (GRAS) (Singh et al. 2022a, b). In addition, most of the plant EOs are rich in oxygenated monoterpenes such as aldehydes (*Galagania fragrantissima*), alcohols (*Achillea fillipendulina*), ketones (*Artemisia rutifolia*, *Anethum graveolens*, and *Mentha longifolia*), and esters (*Salvia sclarea*). However, phenolic terpenoids (*Origanum tyttanthum* and *Mentha longifolia*) such as thymol and carvacrol exhibited strongest antioxidant activities in EOs, due to their phenolic structure (Bhavaniramya et al. 2019). Phenolic component has redox potential, thus, exhibited significant role in free radicals' neutralisation and peroxide decomposition. Additionally, some other EO components such as alcohols, ethers, ketones, aldehydes, and monoterpenes like citronellal, isomenthone, 1,8-Cineole, geranial/neral, menthone, linalool also play important role in the antioxidant activities (Angane et al. 2022).

Maurya et al. (2022) investigated the dose-dependent free radicals scavenging activity of *Carum carvi* EO (CcEO) by using DPPH radicals, and they revealed that, IC_{50} value of CcEO was 10.564 μL/mL. The free radical scavenging activity presented by CcEO was found better than some of the formerly reported EOs such as *Gaultheria fragrantissima* (13.09 μL/mL) and *Zingiber officinale* (14.44 μL/mL) (Ramsdam et al. 2021). Similarly, El-Baroty et al. (2010) reported the antioxidant activity of 45 EOs of various plant species against the DPPH radical. In this work, they evaluated that EOs of clove bud and cinnamon exhibited the higher inhibition rates (approx. 96%), and these EOs have more than 80% of eugenol in their chemical composition. Moreover, Chaudhari et al. (2020) measured the radical scavenging activity of *Pimenta dioica* EO (PDEO) by DPPH and ABTS assay. They found that, PDEO showed dose-dependent scavenging of free radicals and its IC_{50} value was 0.82 and 0.31 μL/mL for DPPH and ABTS, respectively. Thus, the lower IC_{50} value of PDEO strengthens its strong antioxidant potential and plays as natural eco-friendly antioxidant for food preservation by reducing the oxidative burden of food products. In addition, Singh et al. (2021a, b) evaluated the highest DPPH and ABTS radical scavenging activity that was exhibited in the sequence *Salvia sclarea* EO (SSEO) and Linalyl acetate (LA) combination (0.0024, 0.00082 μL/mL) > LA (0.0047, 0.00093 μL/mL) > SSEO (0.0051, 0.00109 μL/mL), respectively. In this study, they found that combined mixture of SSEO and LA revealed significant IC_{50} value as compared to some other previously investigated EOs such as *Stachys inflate*, *Satureja montana*, and *Satureja subspicata* EOs. The strong antioxidant potential corresponds to phenolic group in LA promoting the hydrogen donation and stability of phenoxyl radicals. Semeniuc et al. (2018) examined various EOs belonging to family Lamiaceae and Apiaceae, and found that thyme EO showed greater antioxidant activity due to the presence of phenolic rich compounds. Thus, the other plant species' EOs and their variety of bioactive components with strong antioxidant capacities are relevant to use as a natural substitute of synthetic antioxidant to reduce the oxidative deterioration of food and food items.

3.4 Estimation of Antioxidant Activity

Although ROS are challenging to detect precisely, especially in vivo, due to their short lifespan and strong reactivity, several techniques have been developed to quantify ROS directly (Griendling et al. 2016). Because it is challenging to measure ROS levels directly, researchers frequently measure ROS indirectly by evaluating oxidative damage to cell components or oxidative stress-related survival rates (Katerji et al. 2019). This consequence might also be caused by lower quantities of antioxidant or repair enzymes. Larger levels of oxidative damage are typically taken to mean increased levels of ROS. Increased amounts of ROS may be indicated by decreased tolerance to oxidative stress, but excessive levels of ROS can also promote the overexpression of antioxidant enzymes (Anaissi-Afonso et al. 2018; Ahmad and Suzuki 2019).

There are various analytical methods like DPPH (1,1-diphenyl-2-picrylhydrazine), ABTS (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid), phosphomolybdenum, reducing power and some other tests are known in identifying the antioxidant activity of EOs. Despite of merits and demerits of all analytical methods, it has been found that the most common and reliable methods are DPPH, ABTS, and reducing power assay methods (Munir et al. 2018; Vorobyova et al. 2019).

3.4.1 Reducing Power Assay

According to Oyaizu's approach, the reducing power of the samples is assessed. A phosphate buffer (5 ml, 0.2 M, pH 6.6) and potassium ferricyanide (5 ml, 1%) are combined with the sample in 1 ml of methanol, and the combination is then incubated at 50 °C for 20 min. The reaction mixture is then mixed with 5 ml of 10% trichloroacetic acid, centrifuged for 10 min at 3000 rpm. The solution's top layer (5 ml) is combined with ferric chloride (1 ml, 1%) and distilled water (5 ml), and is then tested for absorbance at 700 nm. An enhanced reducing power is shown by a greater absorption. In the process of developing corrosion inhibitors based on plant material, using an indicator of antioxidant activity as an indirect assessment of the inhibitory effect would save a lot of time and, more importantly, allow a greater proportion of the various types of plant material to be explored systematically (Maheshwari et al. 2011; Vorobyova et al. 2019).

3.4.2 DPPH Assay

The stable free radical DPPH interacts with substances that may give off an atom of hydrogen. This technique works by scavenging DPPH by including an antioxidant or radical species that changes the colour of the DPPH solution. One of the prominent techniques for determining a compound's capacity to scavenge free radicals is DPPH reactivity, which has been widely used to the antioxidants in fruits and vegetables. For plant samples, it is one of the most often used antioxidant tests. For this, the subsequent drop in absorption at 517 nm is used to calculate the antioxidant activity. It involves preparing a 0.1 mM DPPH solution in methanol and adding 4 ml of this solution, at different concentrations, to 1 ml of the sample solution in methanol ((Munir et al. 2018).

3.4.3 ABTS Assay

One of the most often used antioxidant tests for plant materials is the ABTS radical scavenging technique. The oxidation of ABTS with potassium persulfate produces the ABTS radical cation, and its decrease in the presence of antioxidants that donate hydrogen is detected spectrophotometrically at 734 nm. The enhanced procedure uses ABTS and potassium persulfate to react, producing a blue/green $ABTS^{+}$ chromophore. Radical scavenging technique 2,2′-azinobis(3-ethylbenzthiazoline-6-sulphonic acid), also known as ABTS, was created by Rice-Evans and Miller and then improved by Re et al. (1999). Metmyoglobin is activated by hydrogen peroxide in the presence of $ABTS^{+}$ to create a radical cation, which is the basis for the modification.

3.4.4 Hydroxyl Radical Scavenging Assay

The Fenton reaction, which takes place in the presence of reduced transition metal ions (such as $Fe^{2+)}$ and H_2O_2, can result in the formation of hydroxyl radicals. Since the hydroxyl radical is highly reactive with sugars, amino acids, lipids, and even nucleotides, the antioxidant action of removing this radical is crucial. In this experiment, ascorbate-hydrogen peroxide produces hydroxyl radicals. Typically, gallic acid is employed as a control solution. In an ultraviolet-visible (UV-Vis) spectrophotometer, the mixture is heated for one hour at 37 °C before the absorbance is recorded at 510 nm (Diniz do Nascimento et al. 2020).

3.4.5 Cellular Antioxidant Activity Assay

This technique assesses the antioxidant activity using cell lines including Caco-2, HepG2, IPEC-J2, and MCF-7 cells as well as a biosensor (Wu et al. 2019a, b; Diniz do Nascimento et al. 2020). Dihydrodichlorofluorescein (DCFH2, fluorescence probe dye) is used in certain research as a redox sensor. When peroxyl ($ROO^{\bullet}$), produced by the breakdown of 2,2-azobis(2-methylpropionamidine) hydrochloride, is present, it oxidises to fluorescent dichlorofluorescein (DCF). The technique assesses a substance's capacity to prevent the oxidation of intracellular DCFH2, which may be detected by fluorescence. The amount of quercetin equivalents per mole is used to measure antioxidant activity. For one hour, the emitting fluorescence is measured every 5 min using absorbances of 485 nm and 535 nm (Amorati and Valgimigli 2015; Nascimento da Silva et al. 2016).

3.5 Mechanism of Antioxidant Activity of EOs

Preservation of food and food products faced a challenge due to oxidative deterioration, directly bound to the chemical composition of the food and food products (Pateiro et al. 2018). Oxidative deterioration is performed by the interaction of reactive oxygen species (ROS) with other component vulnerable to be oxidised and suffer structural changes in the process. Hydroxyl radicals (OH^-), superoxide radicals (O_2^{2-}), hydrogen peroxide (H_2O_2), and alkoxyl (RO^-) are classified under the category of ROS (Carpena et al. 2021). These ROS exhibited various undesirable and significant changes in foods such as reducing nutritional component, lowering their shelf life, loss of flavour, colour, and odour (Borges et al. 2019).

In these references, EOs and their active components act as promising natural antioxidant due to presence of various secondary metabolites, have inherent ability to reduce or stop the oxidation of lipids, and enhance the shelf life of food products at relatively low concentration. Indeed, EOs have multiple mechanism of direct and indirect actions to slow down the antioxidant chain initiation, free radical scavengers, hydrogen abstraction, and quenchers of singlet oxygen formation (Rodriguez-Garcia et al. 2016). In addition, due to the presence of various active component in EOs, their antioxidant mode of action cannot be performed in only single mode of action (Maurya et al. 2021b). Antioxidant mode of action of EOs occurs in three stages such as initiation, propagation, and termination. Hydroxyl group of EOs are generally the key site of hydrogen donation, inactivating the lipid peroxyl and lipid alkoxyl radicals (free radicals) produced from unsaturated fatty acids' oxidation

(Fig. 3.1) (Pateiro et al. 2018). Moreover, their redox activities are due to their property of electron donation to the free radicals, and reducing the other components from oxidising. In this way, phenolic compounds such as eugenol, carvacrol, and thymol can be used for the retardation of lipid oxidation of food and food products due to their redox potential (Jayasena and Jo 2014: Maqsood et al. 2014).

3.6 Antioxidant Activity of Nanoencapsulated Eos, Edible Films, and Coatings

EOs have an excellent source of phenolic components that exhibit antioxidant activity; however, their direct (free EOs) utilisation in food products has some limitations such as high volatility, lower antioxidant capacity compared to synesthetic, and also changes the organoleptic properties (Jamróz et al. 2018). Therefore, nanoencapsulation of EOs and their bioactive constitutes has been proven to be an effective and promising technique to enhance the chemical stability of components by preserving them from volatilisation, organoleptic properties, and extending their effectiveness (Ozogul et al. 2022). Thus, the nanoemulsions applied in food packaging have range of advantages such as enhancing the antioxidant and antimicrobial activity, minimising the contact with food ingredients, decreasing the transfer of important elements, and uniformity of food packaging (Al-Tayyar et al. 2020; Kocira et al. 2021). Moreover, antioxidant capacity of EOs can be demonstrated by their ability to act as oxygen scavengers and permit the diffusion of bioactive components into coated food items.

In this context, recently, Zheng et al. (2019) investigated that incorporation of eugenol and acorn starch in edible chitosan-based film significantly enhanced the antioxidant activity (approx. 86.77%). Su et al. (2020) reported that cinnamon EO (0.1–0.38%) is encapsulated into chitosan nanoparticles via oil-in-water emulsification and ionic gelation technique to develop biodegradable films. In this work they found that, percentage of radical scavenging activities of DPPH and hydroxyl radicals was in the ranges of 20.6 to 56.9% and 21.0 to 53.4%, respectively. However, DPPH and hydroxyl radicals' scavenging activities of free cinnamon EO were only 19.5 and 22.6%, respectively. In a similar way, Wu et al. (2019a, b) demonstrated the antioxidant activities of chitosan-based coating with liposomes that consist

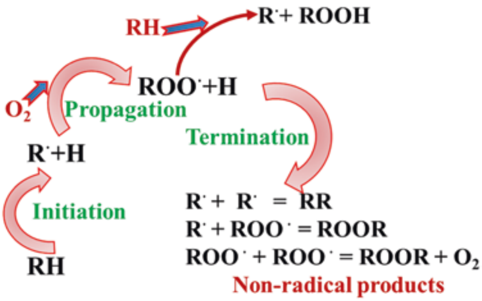

Fig. 3.1 Schematic diagram showing antioxidant mechanism of action

laurel EO (lip-LEO) and silver nanoparticle (AgNPs). They found that Lip-LEO-AgNPs (32.95 µg FeSO4/0.1 mL) had excellent antioxidant activity, which showed no important difference in redox potential in comparison with Lip-LEO (31.31 µg FeSO4/0.1 mL). In comparison to blank liposome, Lip-LEO-AgNPs and Lip-LEO had good antioxidant activity due to chemical composition of laurel EO such as eugenol, cajeputol, terpinene, and geraniol, which have significant ability to capture free radicals. In addition, Doost et al. (2019) examined the free radical scavenging ability of thymol by incorporation into quillaja saponin (QS) stabilised nanoemulsions that was ameliorated for the purpose of depressing chicken breast meat oxidation. Thus, the thymol molecules are more stable and soluble in delivery systems due to improved radical scavenging activity. Singh et al. (2022a, b) reported both the free and chitosan encapsulated nanoparticle antioxidant activity of *Aniba rosaeodora* EO (AREO) against DPPḤand $ABTS^{\cdot+}$ radicals. They found that IC50 value of free AREO showed 4.468 µL/mL against DPPḤ and 2.370 µL/mL against ABTS· + assay, while nanoencapsulated AREO (AREO-CsNe) exhibited enhanced free radical activity with IC50 3.792 µL/mL and 1.706 µL/mL against DPPH and $ABTS^{+}$ respectively. In this way nanoencapsulated EOs improved the stability of number of oxidative-sensitive substances and reduce the volatilisation of their bioactive ingredients. Thus, the antioxidant activity of active components could be significantly protected by encapsulation technique (Zhaveh et al. 2015).

3.7 Antioxidant Activity of Essential Oils in Real Food Systems

One of the most significant factors in food deterioration is oxidation. It is a multilateral reaction that results in unfavourable modifications to the nutritional value, organoleptic standards, food value, and the production of potentially hazardous chemicals. Food that has experienced significant oxidation has serious flaws and cannot be consumed. While oxidation may be detected by colour changes and the emergence of bad odours in food during processing and/or storage, the modification of the food's primary constituents, such as lipids, is not always noticeable (Prakash et al. 2015; Falleh et al. 2020). At relatively low concentrations, antioxidant chemicals can stop, modify, or even completely stop oxidative processes. Accordingly, EOs and their components are crucial in promoting antioxidant activity.

The majority of the legal food additives are used for their preservation abilities, which are related to their acknowledged bioactivities. Acetic, malic, lactic, sorbic, and propionic acids, potassium and calcium acetates, carbon dioxide, benzoates, sorbates, propionates, nitrites, nitrates, and parabens are a few examples of additives with antimicrobial properties that can control food spoilage and/or prevent contamination by foodborne pathogens. Sulphites are the most common additives used to stop food from browning due to chemical or enzymatic processes (Wu et al. 2022). Despite the fact that all of these substances are legal to use in the food industry, the current fad is to swap out synthetic chemicals with natural ones. In this regard, using EOs is viewed as an alternative to using artificial additives (Carocho et al. 2014; Ribeiro-Santos et al. 2017).

Consumers' growing health consciousness has generated unfavourable attitudes of artificial food additives. As a result, the food business began using a large number of natural and secure EOs as part of an 'organic and green approach', as a new barrier to ensure the removal of pathogens from a particular food matrix (Falleh et al. 2019). EOs can function directly or indirectly in a variety of ways, such as by stopping the beginning of chains and scavenging free radicals (Rodriguez-Garcia et al. 2016). Both the European Commission (EC) and the Food and Drug Administration (FDA) in the United States authorised a number of EOs and EO components, classifying them as generally recognized as safe (GRAS) to be used as flavourings and/or preservatives in food items. Lavandin, menthol, rose, sage, oregano, cinnamon, basil, clove, coriander, nutmeg, ginger, and thyme EOs are among the list of crude EOs with the GRAS designation. The recognised EO components also contained substances that are all regarded as safe within the parameters of the recommended daily consumption, such as thymol, carvacrol, eugenol, linalool, carvone, vanillin, cinnamaldehyde, citral, and limonene (Hyldgaard et al. 2012; Falleh et al. 2020).

Free EOs have been blended with various biodegradable substances, including paper and edible films. Paper and cardboard have historically been utilised as packaging materials, but novel packaging solutions are constantly being developed, such as active cardboard trays covered with emulsions that include encapsulated EOs. These are frequently used as food and vegetable packaging materials (with waterproof layers) and are viewed as a replacement for the packaging industry's heavy reliance on plastics (Buendía et al. 2019; Derbassi et al. 2022). To create an aqueous dispersion for edible films, EOs are included into a hydrophilic matrix. As a result, the hydrophilic components of the film undergo structural rearrangement as the drying process causes the lipid droplets to get lodged in the matrix (Sánchez-González et al. 2011). Adding particular EOs (such as oregano, coriander, thyme, and clove) to meat as preservatives helps to eradicate numerous viruses and native flora responsible for deterioration. After ingestion, EO components, especially thymol and carnosic acid, may build up to sufficient amounts in the animal's muscle to operate as an efficient antibacterial agent (Burt 2004).

It is important to note that the source and makeup of EOs affect their impact on food preservation. Precise information about the impact of food matrix components on Eos' antibacterial activities is required for the application of EOs as food preservatives. The volatility, hydrophobicity, and detrimental effects on food organoleptic qualities drive EOs' limited employment in the food and agriculture industries despite their outstanding preservation effectiveness (Weisany et al. 2022; Polat and Aygun 2023). For a slow release of EO aroma suitable with food-based applications, particular delivery mechanisms are thus needed. Common EOs delivery methods include edible coating, active packaging, and nanoencapsulation. These methods facilitate EO dispersion while maintaining constant antibacterial activity and extending food shelf life. Additionally, regulated release behaviour exhibits improved diffusion kinetics and lessens the influence of EO on the organoleptic qualities of food systems (Singh et al. 2021a, b; Maurya et al. 2021a, b; Lim et al. 2023).

3.8 Conclusion and Future Perspectives

Growing health consciousness among consumers has led to negative views against artificial food additives. It has been widely researched and found to be promising that EOs might be used in active packaging technology. Because of their accessibility and vast variety of biological activity, EOs and their constituents are significant. Another benefit is that when employed in the precise quantities, they do not alter the flavour and intrinsic properties of stored food products, prolonging its shelf life. Food storage stability is determined by the amount of antioxidant present, which may be assessed using both direct and indirect approaches. To measure the antioxidant activity of EOs, a variety of techniques have been developed, and each technique employs a different strategy, indication, and analytical target. Using the most appropriate analytical techniques for each food product or antioxidant would be crucial for providing more precise information about the oxidation status and antioxidants' capacity. EOs embedded in nanoparticles are a relatively recent use in food packaging. With the regulated release of EOs, nanoencapsulation offers a longer and more efficient usage of the antibacterial and antioxidant impact while also limiting their strong aroma.

A fresh viewpoint on the organoleptic qualities of food may be offered by the use of EO blends that are created in line with the features of the stored products. Novel methods may be useful to safeguard and enhance EOs' features and biological activities due to their flimsiness under environmental challenges like temperature and light. Further research should concentrate on the mechanisms of action and synergistic effects between various EOs and their components. The EOs and their nanoencapsulated outcomes might be very effective as natural antioxidants in the present scenario of changing choices of the people and their heavy interest towards the natural products and preservatives. Thus, the antioxidant properties of EOs could be further enhanced by more suitable active packaging, synergism between involved components, and their more appropriate formulations using advanced techniques.

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