DOI 10.1002/mnfr.200700395 Mol. Nutr. Food Res. 2008, *52*, 62 – 78

Review

Application of electron spin resonance (ESR) spectrometry in nutraceutical and food research

Liangli (Lucy) Yu and Zhihong Cheng

Department of Nutrition and Food Science, University of Maryland, College Park, MD, USA

Electron spin resonance (ESR) spectroscopy is able to directly measure the chemical species with unpaired electrons and has been widely used in a number of research fields. This review focused on its application in nutraceutical and food research. Current status of ESR in free radical scavenging capacity estimation, food oxidative stability evaluation, Cu^{2+} chelating capacity determination were summarized. Also discussed was the potential of ESR spin-label oximetry technique in examination of lipid peroxidation and oxygen diffusion–concentration products in liposomes, oxygen transport and depletion, and membrane structure and dynamic properties. In addition, ESR application in identifying and estimating irradiated foods including meat, fruits, vegetables, spices, cereal grains, and oil seeds was reviewed. Finally, the potential use of ESR technique in investigating microstructure change, phase transition and viscosity related properties during food formulation, processing, and storage was briefly mentioned, along with its potential in determination of radio-stability of food components. This review may provide some fundamental knowledge of ESR and its application in nutraceutical and food research.

Keywords: ESR / ESR oximetry / Food irradiation / Food stability / Radical scavenging capacity

Received: September 30, 2007; revised: November 5, 2007; accepted: November 7, 2007

1 Introduction

Electron spin resonance (ESR), also known as electron paramagnetic resonance (EPR), is a spectroscopic technique that directly detects chemical species with unpaired electron(s). These species include, but are not limited to, free radicals and transition metal ions. ESR is widely used in many research fields such as physics, chemistry, biology, life science, material science, medicine, nutrition, and nutraceutical and food science.

The principle of ESR spectroscopy is very similar to that of NMR spectroscopic technique. ESR measurements are based on the interaction between the unpaired electron and an applied magnetic field. The unpaired electron has a "spin" that is known as magnetic moment. When subjected to an applied magnetic field, the electron is aligned parallel or antiparallel to the direction of the magnetic field, which corresponds to a lower or an upper energy level, respectively. The energy difference between these two levels is positively proportional to the strength of the magnetizing field [1]. In ESR, the unpaired electron in the lower level state is excited to the upper level state upon absorbing a fixed frequency of microwave irradiation. Once the irradiation frequency is exactly matched by the strength of the applied magnetic field by continuous sweeping the magnetic field, the phenomenon is called ESR. Therefore, ESR is a direct and unambiguous measurement that is highly specific for detection of free radicals and other paramagnetic species such as transition metal ions (*e.g.*, Fe^{2+}, Cu^{2+}).

There are three major ESR methods, including direct measurement, as well as ESR spin-trapping and ESR spin-labeling techniques. ESR direct measurement is used to detect the stable radical species such as 2,2-diphenyl-1-pic-

Correspondence: Dr. Liangli (Lucy) Yu, Department of Nutrition and Food Science, 0112 Skinner Building, University of Maryland, College Park, MD 20742, USA
E-mail: lyu5@umd.edu
Fax:+1(301)-314-3313

Abbreviations: BMPO, 5-*tert*-butoxycarbonyl 5-methyl-1-pyrroline *N*-oxide; **CLA,** conjugated linoleic acid; **CTPO,** 3-carbamoyl-2,2,5,5-tetramethyl-3-pyrroline-1-yloxyl; **DMPC,** L-α-dimyristoyl-phosphatidylcholine; **DMPO,** 5,5-dimethyl-1-pyrroline *N*-oxide; **DPPH•**, 2,2-diphenyl-1-picryhydrazyl radical; **EPAEE,** eicosapentaenoic acid ethyl ester; **ESR,** electron spin resonance; **LA,** linoleic acid; **n-PCSL,** 1-palmitoyl-2-stearoyl-(*n*-doxyl)-*sn*-glycero-phosphorylcholine; **PBN,** *N*-*tert*-butyl-α-phenylnitrone; **PC,** phosphatidylcholine; **POBN,** pyridyl oxide *N*-*tert*-butylnitrone; **ROS,** reactive oxygen species; **TBARS,** thiobarbituric acid reactive substances; **TEMPO,** 2,2,6,6-tetramethylpiperidine-1-oxyl; **T-PC,** 1,2-dioleoyl-*sn*-glycero-3-phosphotempocholine

ryhydrazyl radical (DPPH•), while ESR spin-trapping method is often used to measure short-lived radicals. The ESR spin-trapping technique uses an exogenous spin-trapping molecule to intercept short-lived radicals and form relatively stable secondary radical, the radical-spin trap adducts, which can be easily detected by ESR [2]. This method is of great significance for investigating reactive oxygen/nitrogen species (ROS and RNS) in biological systems, which generally have a relatively short half-life ranging from nanoseconds to seconds [3]. Examples of commonly used spin-trapping agents include, but are not limited to, 5,5-dimethyl-1-pyrroline *N*-oxide (DMPO), 5-*tert*-butoxycarbonyl 5-methyl-1-pyrroline *N*-oxide (BMPO), 5-ethoxycarbonyl-5-methyl-1-pyrroline *N*-oxide (EMPO), and *N*-*tert*-butyl-α-phenylnitrone (PBN). These trapping agents are widely used for *in vitro* and *in vivo* studies to estimate the free radical scavenging capacities of antioxidants, and to better understand the role of free radicals in many biological mechanisms such as pathologies and diseases. It is noteworthy that the selection of a suitable spin-trapping agent is based on its solubility, compatibility with samples, stability of the radical adducts, spectrum complexity, and potential toxicity for *in vivo* studies [3].

ESR spin-labeling method uses a stable paramagnetic molecule (the spin label) to probe the structure or dynamic information of molecules in the specific region(s) of testing samples and to determine the type and change of a microenvironment in which the spin label is located. Most of the spin labels are nitroxide molecules that have ESR signals. The labeled molecules such as lipid, protein, nucleic acid, and cholesterol can be incorporated into the different depth of cell membranes to enable otherwise inaccessible systems to be studied [4]. The information of motion or orientation of biological molecules may also be obtained from the ESR spectra of the spin label. Moreover, ESR spin-label oximetry can be used to investigate local oxygen consumption, oxygen diffusion–concentration products, oxygen transport and depletion, and structure and dynamic properties in membranes and proteins [5, 6]. It is well noted that the quantitative ESR measurement may be achieved by: (i) double integration of the ESR peaks, (ii) determining the ESR signal intensity, and (iii) measuring the line width of the peak [5, 7, 8].

2 ESR in radical scavenging capacity examination

Most reactive oxygen species (ROS) including hydroxyl ($HO^{\bullet}$) and superoxide anion ($O_2^{\bullet -}$) radicals are free radicals. These ROS are formed under normal physiological conditions and may directly attack life important molecules such as membrane lipids, and initiate the pathogenic development of a number of aging-associated chronic human diseases such as cancers and coronary heart diseases [9, 10]. Growing evidence indicates that antioxidants may react with these free radicals and terminate their hazardous actions, and reduce the risk of human diseases [9]. This led to the increased demand of novel dietary antioxidative nutraceuticals for developing health beneficial functional foods and supplemental products. A number of rapid *in vitro* assays have been validated for discovering and investigating free radical scavenging properties of dietary antioxidants and for examining their changes during food formulation, processing, and storage [11–13]. ESR has been successfully employed to study the radical–antioxidant interaction and estimate free radical scavenging capacities of antioxidants [9, 14–21]. Stable radicals such as DPPH• or radical generation systems such as the Fenton Fe^{2+}/H_2O_2 system along with a spin-trapping agent are required for ESR estimation of free radical scavenging properties of a selected antioxidant sample.

2.1 ESR examination using a stable free radical

ESR examination using the stable DPPH• has been used to study the direct free radical–antioxidant interactions and to estimate the radical scavenging capacities of selected potential antioxidative samples [14, 16, 17, 22, 23]. In 2001, Yu [14] investigated the scavenging capacity of conjugated linoleic acids (CLAs) against DPPH• and provided the direct evidence for the first time that CLAs may react with and quench DPPH•. CLA are a group of nutraceutical fatty acids naturally present in foods. They have many reported health beneficial effects including anticarcinogenic and antiatherosclerotic activities [14]. Antioxidant activity was considered as a possible mechanism involved in their biological actions. A number of studies investigated the antioxidant activity of CLA during the years 1990–2000 and obtained conflicting results. It was almost concluded that CLA could not act as antioxidants but their derivatives might, until this research measured the reaction between CLA and DPPH• using ESR and provided direct evidence that CLA acted as free radical scavengers in a dose-dependent matter (Fig. 1) [14]. ESR was also used to determine the time-dependent property of antioxidant-DPPH• reactions (Fig. 2) [16, 17, 23] and to quantify the DPPH• scavenging capacity of selected antioxidants [17, 22]. DPPH• has reasonable stability and adequate solubility in both nonpolar organic solvents such as toluene and highly polar aqueous acetone or alcohol. It is an excellent simple radical system for ESR examination of direct antioxidant–radical interactions. Other stable radicals such as galvinoxyl radical may also be coupled with ESR for evaluating and comparing free radical scavenging capacities of potential antioxidants [24].

The primary advantages of ESR examination of stable radical–antioxidant interactions include its clear chemical mechanism, simple testing procedure, and no interference with pigment components in the sample. Pigments in the

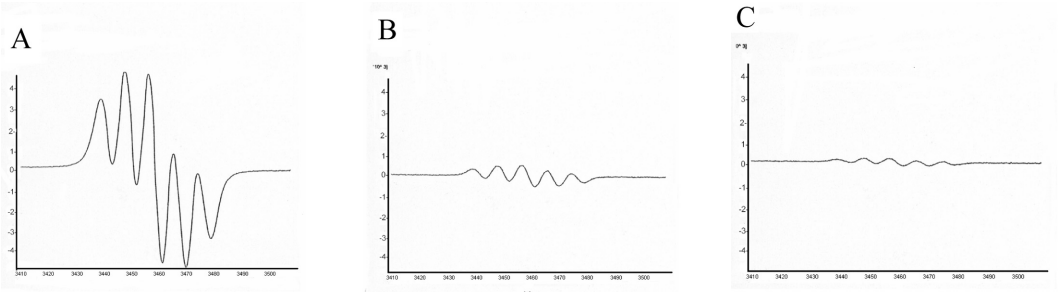

Figure 1. Free radical scavenging activity of CLA determined by ESR. The final concentration of DPPH• was 2.2 mM in all the three tested samples. (A), (B), and (C) represent initial CLA concentrations of 0, 5, and 10 mg/mL in the reaction mixtures, respectively. (Redrawn from ref. [14].)

sample are known to alter the DPPH• scavenging capacity estimation using spectrophotometric method. In addition, the ESR determination using DPPH• as the target free radical allows direct comparison of hydrophilic and lipophilic antioxidants for their radical scavenging capacities under similar conditions [11]. In general, a stable radical solution is mixed with an antioxidant solution or extraction to initiate the antioxidant–radical reaction, and the reaction mixture is recorded for ESR signal intensity under selected experimental conditions for a time point or for a time period. The disadvantage is that the assay is not conducted under physiological condition and the results may not truly reflect the beneficial actions of tested antioxidants *in vivo*.

2.2 ESR examination using radical generation systems

HO• and $O_2^{\bullet-}$ are highly reactive radicals and can be generated under physiological conditions. Estimation of HO• and $O_2^{\bullet-}$ scavenging capacities is important for discovery and development of novel antioxidant nutraceuticals and functional foods. ESR has been used to investigate the scavenging properties of nutraceutical antioxidants against HO• and $O_2^{\bullet-}$ using radical generating systems [10, 13, 18–21]. For ESR determination, HO• is commonly generated using Fenton Fe^{2+}/H_2O_2 and Fenton-like Fe^{3+}/H_2O_2 reactions [12, 13, 18, 19], while $O_2^{\bullet-}$ is generated by UV irradiation of a riboflavin/EDTA solution or through the xanthine/xanthine oxidase system [18, 19, 23]. A spin-trapping agent is required for ESR detection of HO• or $O_2^{\bullet-}$ because of their very short life time. Both BMPO and DMPO are commonly used spin-trapping agents to form BMPO-HO• or BMPO-$O_2^{\bullet-}$ and DMPO-HO• or DMPO-$O_2^{\bullet-}$ adducts which are detectable with an ESR spectrometer [18, 23]. The relative intensity of the radical-trap adducts has been used for quantitative estimation of scavenging capacity of a selected antioxidant sample against HO• or $O_2^{\bullet-}$.

ESR has been used to investigate the HO• scavenging capacity of a variety of potential antioxidant samples prepared from wheat bran, spices and herbs, natto (a kind of fermented soybean), synthetic peptides, wine anthocyanins and pyranoanthocyanins, chitosan-derived oligosaccharides, and pectin hydroxamic acids [10, 18–20, 23, 25, 26]. In 2007, 50% acetone extracts of black peppercorn, nutmeg, rosehip, cinnamon, and oregano leaf were evaluated and compared for their HO• scavenging capacities using ESR with DMPO as the spin-trapping agent and Fenton Fe^{2+}/H_2O_2 system to generate the radical [26]. The results indicated that these spice and herb materials contain significant levels of HO• scavenging components, which may time-dependently react with and quench HO• (Fig. 3). Cinnamon had highest HO• scavenging capacity among all tested botanicals on a *per* botanical material weight basis [26]. ESR analysis was also able to compare the HO• scavenging properties in bran samples of two hard wheat varieties [23] and in different water-soluble fractions of natto [18], as well as to demonstrate the dose-dependent HO• scavenging capacity of a chitosan-derived oligosaccharide [25].

HO• scavenging capacity of a possible antioxidant sample may also be quantitatively estimated by ESR analysis. In 2005, Garcia-Alonso *et al.* [20] quantitatively examined the HO• scavenging capacity for a group of wine anthocyanins using the relative ESR peak height. This research also quantitatively evaluated the dose-dependent effects of these wine anthocyanins. In 2003, ESR analysis was performed to measure the HO• scavenging capacity of a group of five chitosan-derived oligosaccharides in aqueous reaction mixtures using DMPO as the trapping agent and Fenton reaction to generate HO• [25]. As shown in Fig. 4, these chitooligosaccharides differed in their HO• scavenging capacities, although all of them were able to scavenge HO• in the testing system in a dose-dependent manner [25]. The limitation of this assay is that it is not suitable for lipophilic antioxidants due to their poor water solubility in the aqueous testing mixture. It should also be pointed out that chemicals can suppress HO• formation in the assay mixture, may also reduce the ESR signal intensity and lead to overestimation of HO• scavenging capacity.

Recently, an ESR protocol was developed to examine the HO• scavenging capacity of lipophilic antioxidants [13]. During this research, Fenton Fe^{2+}/H_2O_2 and Fenton-like Fe^{3+}/H_2O_2 systems were evaluated for their HO• purity and

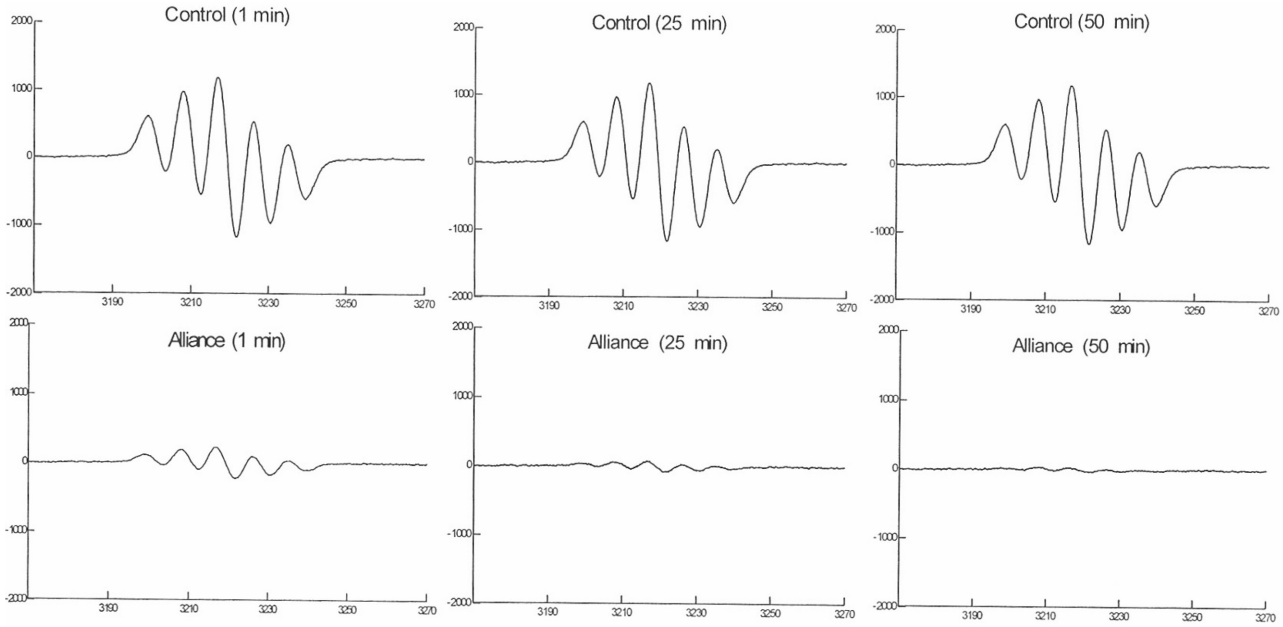

Figure 2. DPPH• scavenging activities of wheat bran extracts determined by ESR. Alliance stands for bran samples of Alliance wheat, while the Control represents the control reaction containing no antioxidant. The final concentrations were 250 μM for DPPH• and 50 mg of bran equivalents *per* milliliter for wheat bran in all reaction mixtures. ESR signals were recorded at 1, 25, and 50 min of each reaction at ambient temperature. (Redrawn from ref. [23].)

stability, as well as HO•-antioxidant reaction kinetics in the presence of commonly used organic solvents. The Fenton Fe^{2+}/H_2O_2 system was able to generate a constant flux of pure HO• in the presence of acetonitrile under a physiological pH. This radical generation system was further validated for its potential application in estimating relative HO• scavenging capacity for lipophilic antioxidants using a group of known lipophilic antioxidants including lutein, β-carotene, α-tocopherol, and BHT. The new assay exhibited excellent linearity, precision, and reproducibility. Also reported from this research was the potential interference of commonly used organic solvents in HO• scavenging capacity estimation using Fenton Fe^{2+}/H_2O_2 system [13].

$O_2^{\bullet-}$ is also a free radical species that may be generated in human body under physiological condition. ESR spin-trapping method has been widely employed in estimating $O_2^{\bullet-}$ scavenging capacity under physiological pH for potential antioxidants such as wheat antioxidants [23], wine anthocyanins and pyranoanthocyanins [20], chitooligosaccharides [25], natto extractions [18], and peptide [19]. In general, $O_2^{\bullet-}$ is generated in the assay mixture using the xanthine/xanthine oxidase system, with DMPO as a commonly used trapping agent [23, 25]. The advantage of this assay is that $O_2^{\bullet-}$ is physiologically relevant and ESR may measure the scavenging capacity against pure $O_2^{\bullet-}$. The primary problems with this assay are that (i) it is not suitable for lipophilic antioxidants, and (ii) it is not clear whether the antioxidants directly react with and quench $O_2^{\bullet-}$ or suppress the generation of the radical. For instance, xanthine oxidase inhibitors may result in a reduced ESR signal intensity for $O_2^{\bullet-}$.

2.3 Identification of radical species

As mentioned above, ESR is a unique technique to identify the nature of radical species. Radicals can be elucidated by two important parameters, *g*-value and hyperfine splitting pattern, which correspond to chemical shift and coupling constant in NMR, respectively. Every radical species has its own typical ESR spectrum and may be identified by ESR analysis. Elucidation of radical species using ESR spectra may be facilitated by computer simulation. For instance, typical six peaks and special hyperfine splitting in the ESR spectrum shown in Fig. 5A indicates the presence of DPPH•, whereas the four-line ESR spectrum with a signal height ratio of 1:2:2:1 (Fig. 5B) and the eight-line ESR signals (Fig. 5C) are typical for HO•-DMPO and $O_2^{\bullet-}$-DMPO adducts, respectively, suggesting the presence of HO• and $O_2^{\bullet-}$ [25]. DMPO is a commonly used spin-trapping agent. It has been wide accepted that ESR is a powerful tool to identify the radical species, as well as to investigate the chemical mechanism involved in free radical-mediated reactions [27].

In 1997, Roberts and Lloyd [27] conducted an ESR investigation on free radical formation in the Maillard reaction, which might give more insights of the reaction and

lead to a better understanding of the chemical mechanism(s) involved. Results showed that three series of free radicals were generated in the reaction between secondary amine and glycolaldehyde or glyceraldehyde. Furthermore, it was concluded that the species II radical was revealed to play the most important role in the Maillard reaction pathway [27].

In addition, ESR can be used to monitor the purity of radicals in the testing system, especially when a complicated radical generation system was employed. Many antioxidant

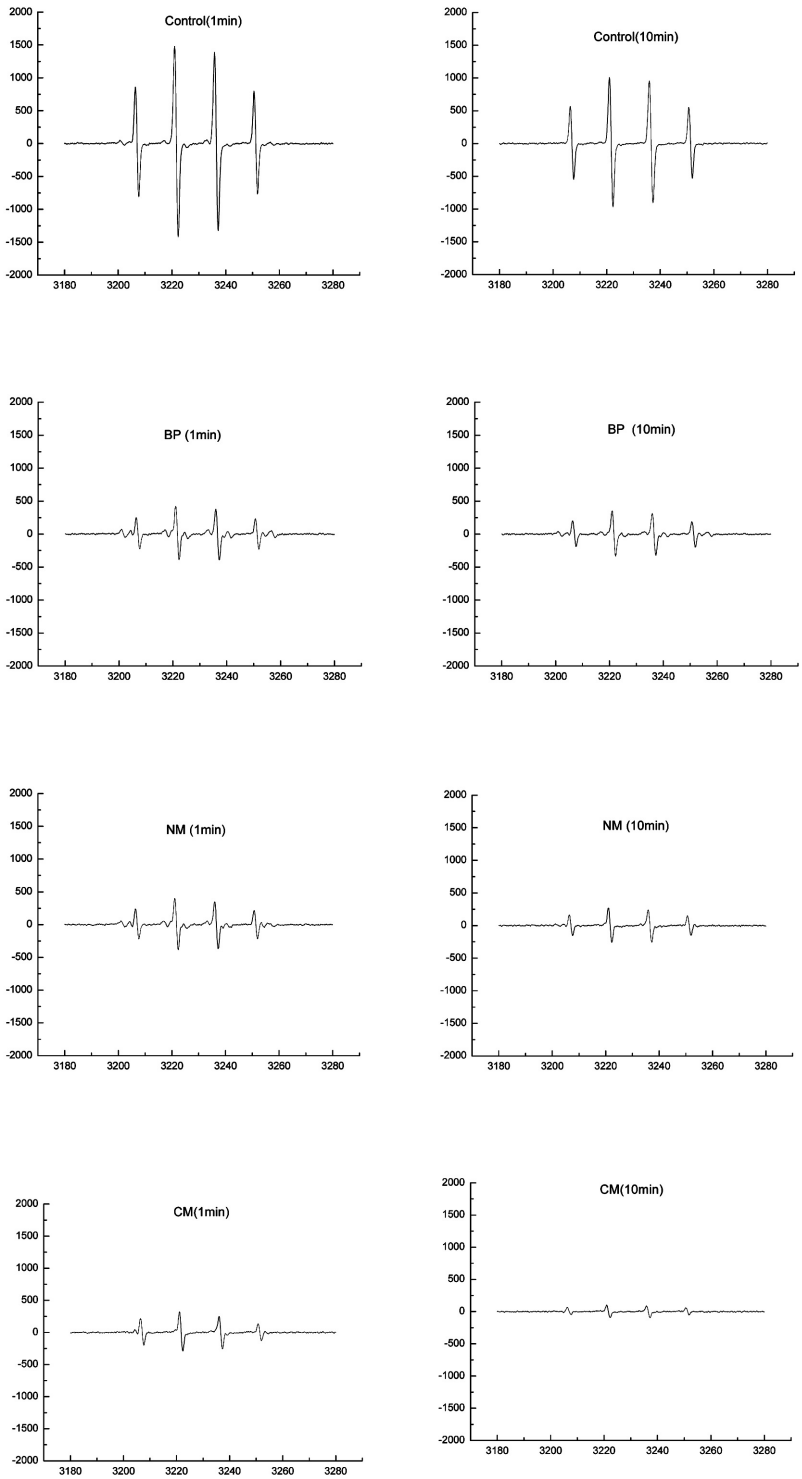

Figure 3. Hydroxyl radical ($OH^{\bullet}$) scavenging activity of black peppercorn, nutmeg, and, cinnamon. BP, NM, and CM stand for black peppercorn, nutmeg, and cinnamon, respectively, while the control represents the control reaction containing 50% acetone. Each reaction mixture contained 10 μL of freshly prepared 3 mM $FeSO_4$, 80 μL of 0.75 mM EDTA, 15 mL of 1 M DMPO, 15 μL of 0.5 mM H_2O_2, and 30 μL of 100 mg/mL spice extracts. ESR signals were recorded at 1 and 10 min of the reaction at ambient temperature. (Redrawn from ref. [26].)

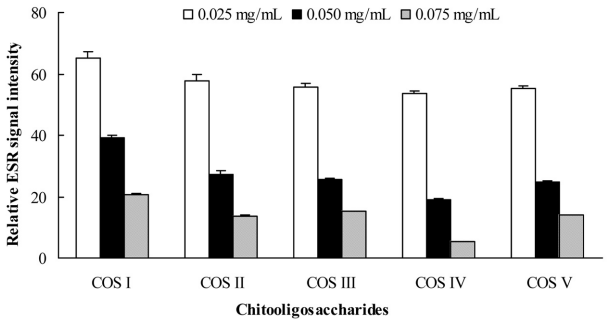

Figure 4. Hydroxyl radical (OH•) scavenging activity of chitooligosaccharides. Values represent the mean ± SD ($n = 3$). COS I, the oligosaccharides that did not pass out through molecular weight cutoff (MWCO) 10 kDa membrane. COS II, the oligosaccharides passed out through MWCO 10 kDa membrane but not passed out through 5 kDa membrane. COS III, the oligosaccharides passed out through MWCO 5 membrane but not passed out though 3 kDa membrane. COS IV, the oligosaccharides passed out through MWCO 3 membrane but not passed out through 1 kDa membrane. COS V, the oligosaccharides passed out through 1 kDa membrane. 0.025, 0.050, and 0.075 mg/mL were the initial concentrations of chitooligosaccharides in the reaction mixtures. (Redrawn from ref. [25].)

assays such as the ORAC, HOSC, and $O_2^{\bullet -}$ scavenging capacity assays, were performed in complicated reaction systems, and determined by spectrophotometric or fluorometric methods. Accurate estimation of scavenging capacity of a selected antioxidant sample against a selected radical highly depends on the purity and concentration consistency of the radical in the reaction mixture. Validation and confirmation of radical purity and concentration consistency are very important. Presence of additional free radical species may lead to overestimation or underestimation of free radical scavenging properties of a selected antioxidant sample. For instance, Moore *et al.* [12] evaluated a number of HO• generating systems for their capacities to form pure and consistent flux of HO• using ESR spin trap technique. In this study, ESR spin-trapping technique was also employed to evaluate the effects of several commonly used organic solvents for their potential interference with HO• generation reactions, as well as their effects on HO• concentration consistency. ESR examination was able to show that a few solvents including ethanol and DMSO acted as HO• scavengers and produced carbon-centered new radicals [12]. The carbon-centered radicals might lead to misestimate the HO• scavenging capacity of the selected antioxidants. In a recent study, Cheng *et al.* [13] systematically investigated the interaction between a number of common organic solvents and HO• using ESR spin-trapping technique. As shown in Fig. 6, ESR analysis was able to determine the carbon-centered radicals formed in the testing mixtures containing ethanol, methanol, DMSO, ethyl ether, ethyl acetate, or *n*-butanol. These two studies indicated that ESR spectroscopy may be employed in developing and validating new spectrophotometric and fluorometric assays for free radical scavenging capacity estimations.

3 Food stability evaluation

Lipid peroxidation during food processing and storage is one of the major factors affecting the quality, safety, and nutritional value of food products. The chemical mechanism of food lipid peroxidation is a free radical-mediated oxidative chain reaction involving initiation, propagation, and termination phases. The first a few free radicals are formed during initiation phase, which is the rate-limiting step in the chain reaction. Lipid oxidation is commonly measured by the formation of primary and secondary oxidation products, such as peroxides and thiobarbituric acid reactive substances (TBARS), respectively. These assays require multistep sample preparation and cannot detect the initial stage of lipid oxidation reaction. Recently, ESR spin-trapping and spin-labeling techniques have been successfully used to detect the free radical generation and accumulation in the very early stages of lipid peroxidation [28–30]. ESR signal intensity has been reported to correlate well with hexanal production in dehydrated chicken meat and the degree of oxidized flavor in dry milk powder [31], sup-

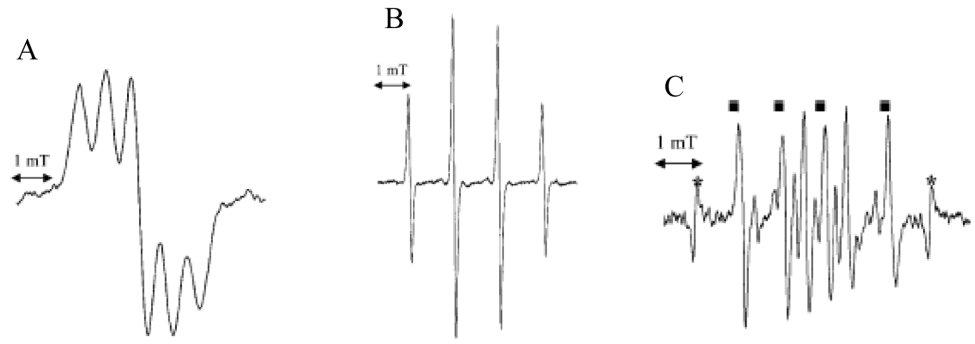

Figure 5. ESR spectra of (A) DPPH• in ethanol, (B) HO• generated by Fenton reaction with DMPO as the trapping agent, and (C) $O_2^{\bullet -}$ generated by hypoxanthine-xanthine oxidase system with DMPO as the trapping agent. (Redrawn from ref. [25].)

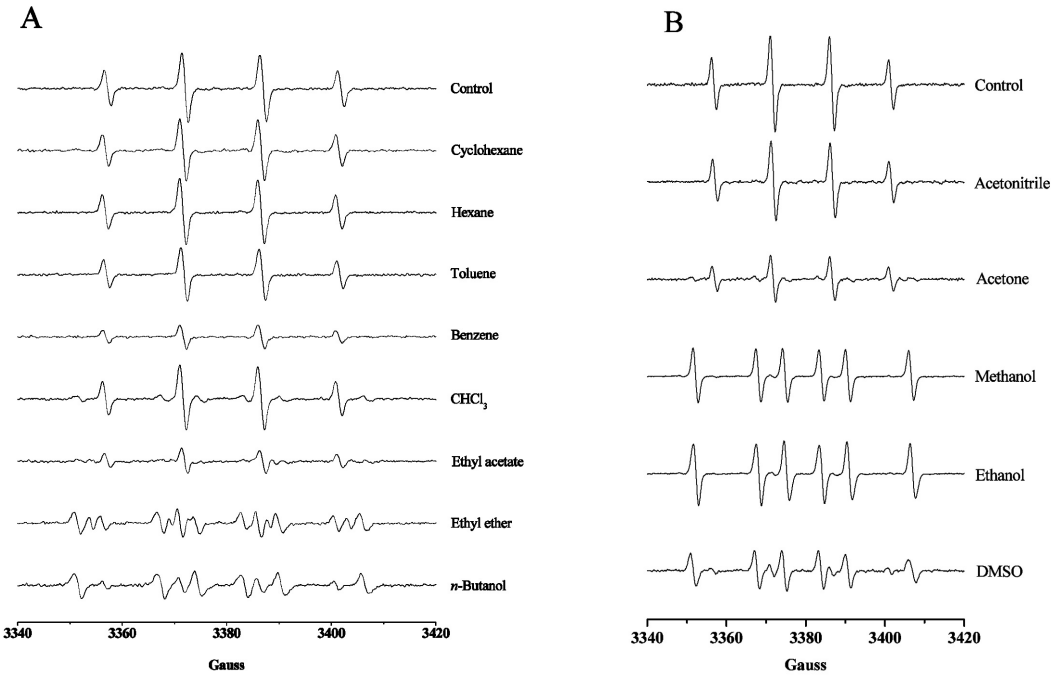

Figure 6. Effect of selected (A) water-immiscible and (B) water-miscible solvents on HO• generation and concentration using DMPO as the ESR spin-trapping agent. (Redrawn from ref. [13].)

porting its potential in estimation of oxidative stability of food products. ESR determination has been accepted as a rapid measurement of food oxidative stability with less sample preparation.

For instance, a recent study demonstrated the potential of ESR spectroscopy in determining oxidative stability of cooking oils with and without antioxidants [32]. The oil oxidative stability was measured as the formation of free radicals in the early stage of the oxidative chain reaction using PBN as the spin trap. Individual vegetable oils with and without selected antioxidants differed in their relative ESR signal heights during 60 min measurements under the experimental conditions [32], indicating that ESR spin-trapping method is a valuable approach to estimate oxidative stability of oil and fat samples. As shown in Fig. 7, ESR spin-trapping analysis was able to show the induction time for radical formation and the rate of radical accumulation in the partially hydrogenated palm oil samples containing different antioxidants. Earlier in 2002, ESR analysis was applied to investigate the influence of dietary fat and vitamin E supplementation on free radical production and on lipid and protein oxidative stability in turkey muscle extracts, with pyridyl oxide *N-tert*-butylnitrone (POBN) as the spin-trapping agent [33]. At the end of the dietary treatment period, tissue samples were extracted and oxidized at ambient temperature using an oxidative system containing NADPA, ADP, and $FeSO_4$. Free radical concentration was determined by ESR spin-trapping technique, whereas lipid oxidation was measured as TBARS. ESR analysis demonstrated that tissue extracts of animal with vitamin E supplementation had lower detectable amount of radical-POBN adducts, and dietary fat altered free radical formation in the turkey tissue samples with or without dietary antioxidant supplementation [33]. Same trend was observed for ESR signal intensity and TBARS value, indicating that ESR spin-trapping technique is a possible approach to evaluate food oxidative stability.

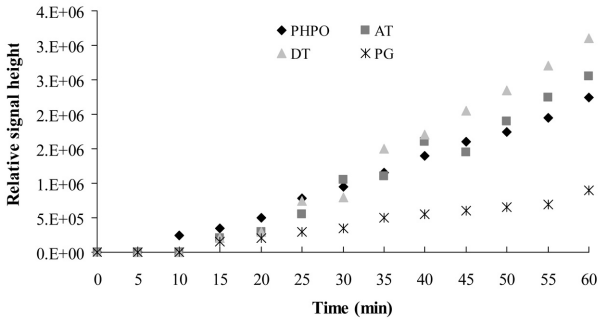

Figure 7. Determination of free radical content by ESR in partially hydrogenated palm oils containing different antioxidants. PHPO stands for partially hydrogenated palm oil without antioxidant, whereas AT, DT, and PG represent partially hydrogenated palm oils containing α-tocopherol, δ-tocopherol, and propyl gallate, respectively. The initial antioxidant concentration was 300 mg/kg for all tested antioxidants. ESR determination was conducted using PBN as the spin trap, and signal intensity was monitored by the peak-to-peak amplitude of the first line of the ESR spectrum. (Redrawn from ref. [32].)

Interestingly, ESR spin-trapping technique was employed to examine the oxidative stability of wort and beer in a few previous studies [34, 35]. Kocherginsky *et al.* [34] concluded that the lag phase of radical-PBN adduct formation is an indicator of flavor oxidative stability and shelf life of beer. This finding was in agreement with the observation in an earlier ESR study of beer oxidative stability with the addition of hexamethylenetetramine or sulfite during mashing [35]. PBN was the spin trap in beer in both studies, whereas POBN was a better spin-trapping agent used in wort. It was proposed that spin adducts in beer are formed by trapping 1-hydroxyl radicals because ethanol may prevent the direct reaction between PBN and the hydroxyl radicals generated due to the peroxide-transition metal reactions [35]. It was also discussed why POBN was a better spin-trapping agent used in wort, while PBN was the spin trap in beer.

In 2005, ESR spin-label technique was used to evaluate the oxidative stability of a powdery model system with the addition of amino acids and peptides [29]. The powdery model system contains maltodextrin encapsulated eicosapentaenoic acid ethyl ester (EPAEE). ESR data showed that addition of amino acids and peptides improved the oxidative stability of EPAEE in maltodextrin. Interestingly, the relative humidity altered the effectiveness of amino acids and peptides in suppressing lipid peroxidation. At a higher relative humidity of 75%, amino acids and peptide were more effective in improving EPAEE oxidative stability than at 10 or 40% relative humidity [29]. ESR spin-labeling technique was also compared with ESR spin-trapping or direct radical determination for its potential in evaluating oxidative stability of processed cheese [28]. DMPO was the spin trap agent, and 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO) was the spin label used in this research. Free radical concentrations were determined for cheese samples containing either DMPO or TEMPO during 2 wk of storage under four different conditions [28]. ESR spin-trapping technique detected the lag-phase in the cheese samples and showed the gradual increase in ESR signal intensity for DMPO-HO$^{\bullet}$ adducts, suggesting the potential of this technique in monitoring lipid peroxidation in cheese samples. Interestingly, ESR spin-labeling technique detected a decrease in ESR signal intensity from storage Day 0 to Day 1, but a signal increase from Day 1 to Day 2 in this study, indicating the presence of additional chemical reactions such as regeneration of stable radicals [28]. The conclusion from this study was that spin trapping with DMPO is a better technique than spin labeling with TEMPO for investigating oxidative stability of cheese samples. It needs to be pointed out that this conclusion may not be applicable to other spin-labeling agents.

It has to be kept in mind that spin-trapping agents may suppress free radical-mediated lipid peroxidation. Velasco *et al.* [30] investigated the effects of PBN, a commonly used spin-trapping agent, on lipid peroxidation by measuring the depletion of tocopherol and the formation of peroxides and TBARS. The results showed that addition of PBN in rapeseed, sunflower seed, and fish oils resulted in lower peroxide concentrations and smaller TBARS values than that detected in the corresponding control oils under the same experimental conditions. The results from this study also demonstrated that PBN was able to prevent depletion of tocopherols in all three testing oil systems. These data indicated that PBN possessed antioxidant activity and significantly inhibited lipid oxidation in the oil samples [30]. It was also pointed out that spin-trapping technique may not be a good method for oxidative stability study during a prolonged storage time period because of the spin adduct depletion, although it is an excellent assay to investigate the early events in free radical-mediated lipid peroxidation [30].

4 ESR spin-label oximetry determinations

ESR spin-label oximetry technique indirectly determines the local molecular oxygen concentration through measuring the alteration in ESR characteristics of spin-label due to the bimolecular collisions between oxygen and the spin-label agent [36, 37]. The physical principle and its application of measuring oxygen *in vivo* have been well reviewed by Swartz and Clarkson [38]. Recently, ESR spin-label oximetry method has been used in nutraceutical and food researches such as *in vitro* determination of membrane fluidity [36, 37], membrane structure [5], phase transition temperature of membrane lipid bi-layer [37], and lipid peroxidation and oxygen diffusion – concentration products in liposomes and membranes [5, 37, 39].

4.1 Determination of lipid peroxidation or oxygen diffusion – concentration products in membranes

In 1985, Subczynski and Kusumi [40] studied oxygen consumption of egg yolk phosphatidylcholine (PC) liposomes during the initial phase of lipid peroxidation using 3-carbamoyl-2,2,5,5-tetramethyl-3-pyrroline-1-yloxyl (CTPO) as the spin-label agent in a closed chamber. The study showed that ESR spectrum of CTPO consisted of three lines due to the hyperfine interaction of unpaired electron with the N nucleus. Each of the three lines has a group of lines because of proton superhyperfine interactions. Changes in molecular oxygen concentration might alter the bimolecular collisions of CTPO and oxygen, which consequently change the resolution of the proton superhyperfine lines because of Heisenberg spin exchange, which lead to ESR spectral change. This spectral change can be quantified to reflect the oxygen concentration in the closed chamber, which is the principle behind ESR spin-label oximetry measurements [36, 40]. The quantification using the calibration

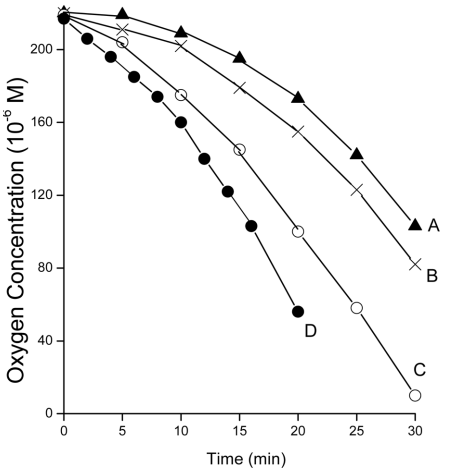

Figure 8. Oxygen consumption kinetics of liposomes prepared using egg yolk PC. The individual test systems included: (A) 5 mol% of PC (LA) in egg PC; (B) egg PC alone; (C) 5 mol% PC (*t*10,*c*12-CLA) in egg PC; and (D) 5 mol% PC (*c*9,*t*11-CLA) in egg PC. Aqueous liposome suspensions (30 mg/mL) were prepared at 37°C in 25 mM HEPES buffer (pH 7.2) containing CTPO (0.14 mM). Aliquots were taken for ESR determination from the (aerated) reaction mixture that had been incubated at 37°C for 45 min. (Redrawn from ref. [37].)

curves relating the *K* parameter, which could be obtained from the ESR spectrum, to oxygen concentration has been previously described in details [5, 39, 40]. The results from this study demonstrated that oxygen consumption determined using the ESR spin-label oximetry method was correlated with the progress of lipid peroxidation examined by measuring TBARS. It was demonstrated in this study that CTPO had no interference with the measurement of lipid oxidation in PC liposomes [40]. ESR oximetry was also used to investigate the effects of fumonisin B_1 on lipid peroxidation in membranes using CTPO as the spin-label agent [39]. Fumonisin B_1 is an amphiphilic mycotoxin compound produced by *Fusarium* spp. Lipid peroxidation in the presence of fumonisin B_1 in egg yolk PC and L-α-dimyristoylphosphatidylcholine (DMPC) under different conditions were successfully studied in closed capillaries. It was found out that incorporation of fumonisin B_1 increased oxygen consumption in membranes under the experimental conditions, suggesting its potential in promoting lipid peroxidation in cellular membranes [39].

ESR spin-label oximetry method may also be used to determine the antioxidant and prooxidant properties of chemicals. Recently, ESR oximetry with CTPO as the spin label was employed to evaluate the potential influence of CLA isomers on oxygen consumption in liposome suspensions prepared from a group of natural PCs [37]. CLA refers to a group of octadecadienoic acids (18:2) that contain conjugated double bonds. CLA has been demonstrated to have a number of health beneficial effects such as anticarcinogenesis, and antioxidant capacity was considered as a possible mechanism. The results showed that CLA isomers acted differently in suppressing or increasing oxygen consumption in the testing liposome systems. It was also observed that liposome composition may alter the effect of individual CLA isomers in their interactions with molecular oxygen in the liposome systems under the experimental conditions. Data in Fig. 8 showed that *c*9,*t*11-CLA isomer had a stronger antioxidant property than *c*10,*t*12-CLA, while linoleic acid (LA, 18:2*n*-6) had a pro-oxidant activity in egg yolk PC liposome suspensions [37]. It needs to be pointed out that CTPO is a water-soluble spin label and measures the oxygen concentration in the aqueous phase to reflect lipid peroxidation and oxygen diffusion–concentration products in the lipid bilayer of membranes. Earlier in 1999, effects of *c*9,*t*11-CLA on oxygen diffusion–concentration products and depletion in liposome suspensions containing different PCs were also investigated and compared with LA by ESR oximetry analysis using CTPO as the spin label [5]. ESR spin-label oximetry closed-chamber method may also be applied to study lipid peroxidation or oxygen diffusion–concentration product in other testing systems such as in human LDL and food systems, as well as cellular oxygen uptake [41]. In addition, this technique may be applied to investigate antioxidant activities. However, it is not possible to examine the oxygen transportation or permeability in membrane using a water-soluble spin label.

4.2 Determination of oxygen diffusion and transport in membrane

ESR oximetry method can be used to determine oxygen diffusion and transport in membrane using spin labels that may be implemented in the membrane lipid bilayers [5, 36, 42]. Heisenberg spin exchange due to bimolecular collisions of molecular oxygen and the spin label induces ESR line broadening. The change in ESR line width, which is often reported as $\Delta H(x)$, is proportional to the local oxygen diffusion–concentration products and can be measured at different depths in the membranes using the implemented spin labels to determine oxygen transport across the membrane. As shown in Fig. 9, $\Delta H(x)$ can be calculated using an equation $\Delta H(x) = H(x, O_2) - H(x, N_2)$, where $H(x, O_2)$ and $H(x, N_2)$ are the peak-to-peak ESR line widths of the first derivative spectra for testing sample(x) saturated with oxygen and nitrogen, respectively.

In 1996, ESR spin-label oximetry was used to study the effects of fumonisin B_1 on oxygen transport in egg yolk PC and DMPC membranes [36]. Five *n*-deoxyl stearic acid spin labels, containing ^{14}N-nitroxide moieties attached to carbon number 5, 7, 10, 12, and 16 positions (*n*-SASL), were employed along with another spin label 1,2-dioleoyl-*sn*-glycero-3-phosphotempocholine (T-PC) to investigate the effects of fumonisin B_1 on oxygen transport in the membranes prepared using egg yolk PC and DMPC [36]. T-PC is located at the water–lipid interface, and the nitroxide with

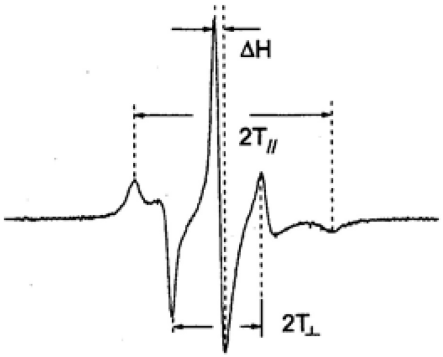

Figure 9. Typical conventional ESR spectrum of 7-SASL in egg yolk PC at 37°C, in 0.1 M HEPES buffer, pH 7.2. $\Delta H(x) = H_{pp}(x, O_2) - H_{pp}(x, N_2)$, where $H_{pp}(x, O_2)$ and $H_{pp}(x, N_2)$ are the peak-to-peak ESR line width of the first derivative spectrum. $H_{pp}(x, O_2)$ and $H_{pp}(x, N_2)$ are obtained at the center line position for the test sample saturated with O_2 and N_2, respectively. The order parameter S can be calculated according to the equation $S = 0.5407(T_{||} - T_{\perp})/\alpha_o$, where $\alpha_o = (T_{||} + 2T_{\perp})/3$. (Redrawn from ref. [36].)

a larger *n* number is located closer to the center of the lipid bilayer. In each membrane system, $\Delta H(x)$ at six different depths in the lipid bilayer, including T-PC, C5, C7, C10, C12, and C16 positions, were examined with and without fumonisin B_1. In both membrane systems, presence of fumonisin B_1 resulted in greater $\Delta H(x)$ values at C5 and C7 positions in the membranes, indicating that fumonisin B_1 was able to enhance the oxygen transport through the membrane and the oxygen diffusion–concentration products in the region near C5–C7 in the membranes [36]. Also noted was that the presence of fumonisin B_1 resulted in a higher $\Delta H(x)$ value at C12 position in egg yolk PC membrane compared to that of the control, but no effect on $\Delta H(x)$ value at C12 position was detected for fumonisin B_1 addition in DMPC membrane. This observation suggested the influence of membrane composition and structure on oxygen diffusion and transport estimation. Two or more sources of phospholipids are required to obtain a general conclusion for future studies.

In 1999, ESR oximetry analysis was conducted to compare the effects of *c*9,*t*11-conjugated linoleic acid (*c*9,*t*11-CLA) and LA on oxygen diffusion–concentration product in soy PC membranes using 1-palmitoyl-2-stearoyl-(*n*-doxyl)-*sn*-glycero-phosphorylcholine (*n*-PCSL) as the spin label agent [5]. Both CLA and LA, which were in the form of CLA-PC and LA-PC, were incorporated in synthetic 1-stearoyl-PCs. T-PC was also used in this study to represent the water–lipid interface position. The data showed that incorporation of 5 mol% CLA-PC resulted in greater $\Delta H(x)$ values at C12, C10, C7 positions and a slight increase in $\Delta H(x)$ value at C5 position in soy PC membrane compared to the control membrane containing 100% soy PC, whereas incorporation of 5 mol% LA-PC had no effect on $\Delta H(x)$ values under the same experimental conditions [5]. This result indicated that CLA may enhance oxygen transport across membrane and promote oxygen diffusion–concentration products in lipid phase of the membrane. The structure–activity relationship was also discussed in the article. These previous studies demonstrated the potential of ESR spin-label oximetry in investigation of oxygen diffusion and transport-related events in artificial membranes.

In addition, ESR spin-label oximetry method was used to determine the oxygen permeation through an oil-encapsulating glassy model food [43]. It was found out that oxygen permeation was increased upon temperature increase, and it might be possible to establish a kinetic model for estimation of the activation energy for oxygen permeation. These results indicated that ESR oximetry is a possible approach for examination of local oxygen concentration inside the intact food model [43]. It was pointed out that ESR spin-label oximetry analysis might be applied to determine oxygen depletion in low moisture food products.

4.3 Membrane structure change and dynamic property evaluation

Several parameters important for membrane structure and dynamic properties have been examined using ESR spin-label oximetry technique [5, 36, 42]. The lipid bilayer of membrane is a dynamic system. Changes in membrane structure or its dynamic properties may alter the bimolecular collision of oxygen and spin label compound, which may effectively alter the spin-lattice relaxation time and the line width of the ESR spectrum. As shown in Figs. 9 and 10, the order parameter S and the rotation correlation time τ can be estimated using the first derivative ESR spectrum. The rotation correlation time τ is a measurement of the rotational motion in membrane. An ESR oximetry analysis with *n*-PCSL as the spin label agents was conducted to evaluate the effects of CLA and LA on the order degree of the soy PC membrane [5]. ESR spectra of C5, C7, C10, and C12-PCSL labeled membranes were recorded to determine the S values according to the equation shown in Fig. 9. The S values were 0.438, 0.227, and 0.171 at C7, C10, and C12 positions of the soy PC membranes containing 5 mol% CLA, respectively, while S values were 0.481, 0.291, and 0.212 at C7, C10, and C12 positions of the soy PC membranes without CLA, respectively [5], indicating that incorporation of CLA perturbed membrane structure. This may be explained by the presence of the conjugated double bond in CLA. Under the same experimental condition, incorporation of LA had no influence on S values at the C5, C7, C10, or C12 position. The two double bonds in LA are separated by a CH_2 group and the molecule is more flexible compared to CLA.

A similar ESR oximetry analysis was performed to examine the influence of fumonisin B_1 on membrane order parameter S using *n*-SASL as the spin labels [36]. Egg yolk PC and DMPC membranes were used in the study. It is

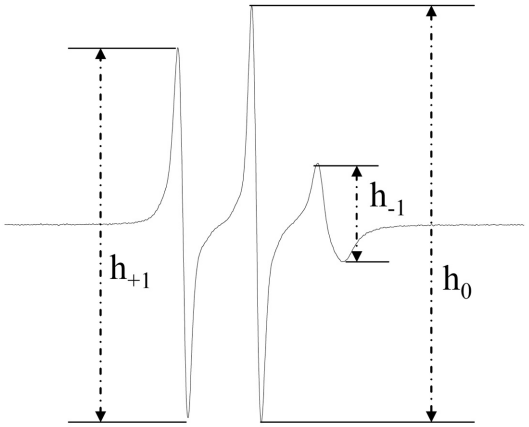

Figure 10. ESR spectrum of 16-SASL in DMPC at 37°C in 0.1 M HEPES buffer, pH 7.2. The rotation correlation time was calculated from the ESR spectra using the equation $\tau = 6.51 \times 10^{-10}\ \Delta H_o[(h_o/h_{-1})^{1/2} - (h_o/h_{+1})^{1/2}]$ s. ΔH_o is the center line width, h_o, h_{-1}, and h_{+1} were measured from the ESR spectra as indicated in the figure. (Redrawn from ref. [36].)

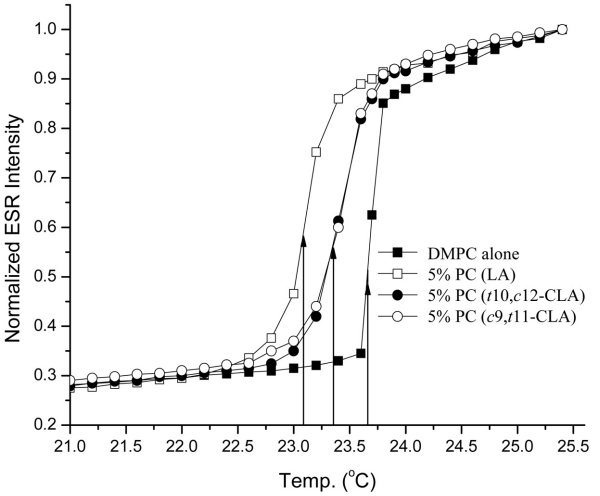

Figure 11. Phase transition curve of different DMPC membranes. The intensity of the center line of the ESR signal of 16-PCSL was normalized and plotted against temperature. Pure DMPC, and 5 mol% mixtures of PC (LA), PC (*c*9,*t*11-CLA), and PC (*t*10,*c*12-CLA) in DMPC were measured. PC (LA), PC (*c*9,*t*11-CLA), and PC (*t*10,*c*12-CLA) represent 1-stearoyl-2-octadec-*trans*9,*cis*12-dienoyl-*sn*-glycero-3-phosphorylcholine, 1-stearoyl-2-octadec-*cis*9,*trans*11-dienoyl-*sn*-glycero-3-phosphorylcholine, and 1-stearoyl-2-octadec-*trans*10,*cis*12-dienoyl-*sn*-glycero-3-phosphorylcholine, respectively. (Redrawn from ref. [37].)

believed that spin labels undergo anisotropic rotational motion at 37°C in the membranes. ESR spectra of synthetic yolk PC and DMPC membranes containing *n*-SASL (*n* = C5, C7, C10, C12, and C16) were recorded with and without fumonisin B_1 for estimating the *S* values. The results showed that fumonisin B_1 may increase *S* values at C5 and C7 positions in both membranes, but had no influence at C12 and C16 positions [36]. It was concluded that the spin label was in a fast motion near the center of the lipid bilayers, because the ESR spectrum of 16-SASL in DMCP membrane had three lines that were much narrower than that observed for 7-SASL in egg yolk PC membrane. These results suggested that fumonisin B_1 might be able to alter the order degree of membranes at the positions near the surface, which is the polar head area.

ESR spin-label technique may also be used to determine the rotation correlation time τ (Fig. 10), which is an important empirical measurement for studying the dynamic properties of membranes [5, 36, 42]. The τ values for 16-SASL in DMPC with and without fumonisin B_1 were 0.79 and 0.80 ns, respectively, at 37°C, and that were 0.78 and 0.77 ns in egg yolk PC membrane, respectively. Interestingly, no effect on τ values was detected using 12-SASL in both membranes [36]. It was concluded that fumonisin B_1 might perturb the hydrocarbon chain stronger at the region closer to the membrane surface and have minor interference at the region near the membrane center. The τ values for 16-PCSL in soy PC membrane containing 5 mol% CLA or LA were estimated in the other study [5]. The results showed that incorporation of 5 mol% CLA or LA had no significant difference on the τ value for 16-PCSL in the soy PC membrane [5].

In addition, ESR spin label oximetry has been employed to determine the phase transition temperature of membrane [37]. A recent research was conducted to evaluate the effects of the incorporation of LA, *c*9,*t*11-CLA, and *t*10,*c*12-CLA on DMPC membrane phase transition temperature. In this study, the phase transition temperature was determined by plotting the normalized intensity of the center line in the ESR signal of 16-PCSL against the testing temperature [37]. As shown in Fig. 11, the phase transition temperature was 23.6°C for DMPC membrane without addition of any fatty acid PCs, 23.3°C for DMPC membrane containing 5 mol% either *c*9,*t*11-CLA or *t*10,*c*12-CLA PC, and 23.1°C for DMPC membrane containing 5 mol% LA PC. Also noted was that incorporation of LA was associated with the greatest width of transition ($T_{1/2}$), which was followed by that from DMPC membranes containing *c*9,*t*11-CLA and *t*10,*c*12-CLA PC, while the pure DMPC membrane had lowest width of transition. It was concluded that incorporation of CLA isomers and LA may alter the membrane fluidity that may alter overall membrane functions and properties [37].

5 Chelating capacity assay

Free radical-mediated oxidative chain reaction is the chemical mechanism shared by a number of oxidation reactions in biological systems and food products, such as membrane lipid peroxidation and protein oxidation. It has been a continuous effort to develop antioxidative nutraceuticals for

suppressing these reactions to the risk of numerous chronic human diseases including cancer and cardiovascular diseases [44, 45]. Transition metals such as Fe^{2+} and Cu^{2+} may catalyze the formation of the first few radicals and accelerate the oxidative chain reactions. Chelating agents form complexes with these metal ions and reduce their catalytic activities in generating free radicals. Thus, assays for chelating activity determination are in high demand for developing new antioxidants for enhancing food shelf life and for suppressing radical generation in biological systems.

Fe^{2+} and Cu^{2+} are two life important transition metals. Cu^{2+} chelating activity has been determined by ESR method, although a spectrophotometric assay is widely accepted for rapid estimation of Fe^{2+} chelating capacity [23]. A number of previous ESR studies have demonstrated that the formation of chelating complexes will alter the ESR spectra of Cu^{2+} due to its interaction with the chelating agents [17, 46, 47]. ESR measurements were conducted to determine the possible Cu^{2+} chelating properties of selected phenolic acids including *p*-coumaric, ferulic, syringic, and vanillic acids [17]. As shown in Fig. 12, addition of ferulic and vanillic acids significantly altered the ESR spectra, indicating their capacities to form chelating complexes with Cu^{2+} [17]. The structure – activity relationships were also discussed [17].

ESR assay was also used to evaluate the potential chelating property for botanical extracts and wheat bran extracts [23, 26]. Black peppercorn, nutmeg, rosehip, cinnamon, and oregano leaf were extracted with 50% acetone and evaluated for their Cu^{2+} chelating activities [26]. ESR measurements showed that only the cinnamon extract exhibited significant Cu^{2+} chelating activity under the experimental conditions. In 2005, Zhou *et al.* [23] investigated the potential Cu^{2+} chelating activity for two hard red winter wheat bran extracts and found that both bran extracts were able to strongly interact with Cu^{2+} [23]. It needs to be pointed out that the ESR determination of Cu^{2+} chelating activity is qualitative, but not quantitative.

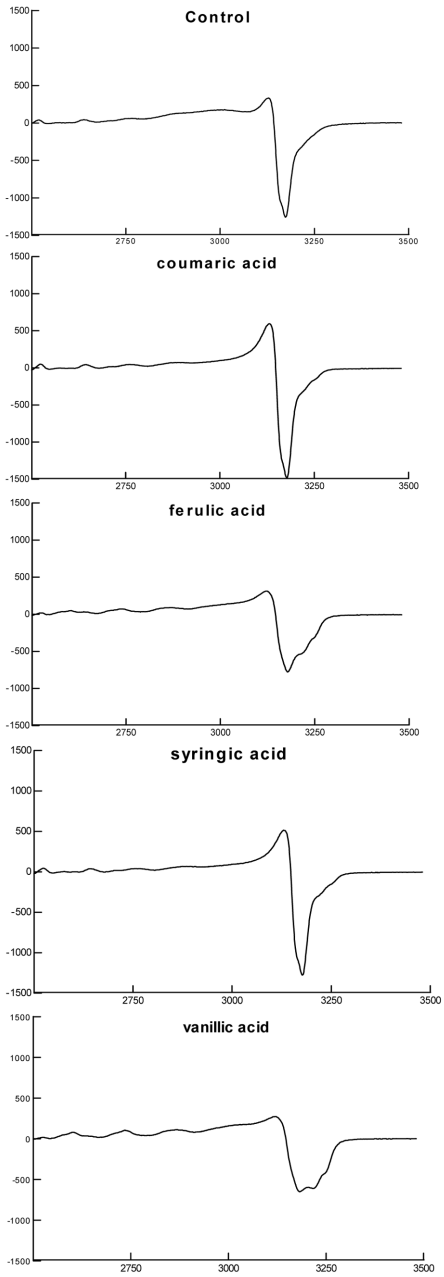

Figure 12. Interaction between Cu^{2+} and individual phenolic acids measured by ESR. The final concentrations were 5 mM for each phenolic acid and 1 mM for copper chloride ($CuCl_2$). The ESR spectra were recorded at 1 min of reaction with 40 mW incident microwave power and 100 kHz field modulation of 5 G at 77 K. (Redrawn from ref. [17].)

6 Food irradiation

6.1 Irradiated meat products

Irradiation is an effective and economic technique to reduce the risk of microorganism-related food safety and quality problems for food ingredients and food products [48 – 51]. Consumer concerns about the safety of food irradiation still affect the acceptability of irradiated food products although it has become legal in more than 40 countries [52]. Miyahara *et al*. [53] briefly reviewed the status of food irradiation and the development of ESR methods for its determination. For a number of reasons including the regulation and consumer acceptability of food irradiation, ESR has been intensively used to investigate the free radical generation and their concentrations in the irradiated food ingredients and food products, as well as to estimate the irradiation dose of these products [7, 52, 54, 55].

ESR is considered as one of the most promising and reliable techniques to determine whether a food ingredient or food product has explored to irradiation treatment [7, 54 – 57]. In 1999, a group of India scientists studied the effects of γ-irradiation at dose levels of 1, 2, 4, and 5 kGy on ESR

signal intensity in the lamb meat chunks with hind leg (femur) and rib bones [56]. They demonstrated that irradiation at 2.5 kGy generated detectable levels of free radicals in the lamb bones regardless of bone type, but the intensity of ESR signal was much higher in the hind leg bone than that in rib bone. They also showed that ESR signal intensity was positively associated with the degree of irradiation in the dose ranging 1–5 kGy in lame hind leg bone. Furthermore, the ESR signal intensity was stable for up to 7 months when 1 and 2 kGy irradiation was used to treat the lamb samples, whereas ESR signal intensity decreased at the storage time of 3 and 4 months for meat samples treated with 4 and 5 kGy irradiation. In addition, it was observed that ESR signal was still detectable after cooking and chilled storage, although conventional cooking procedures including boiling, pressure cooking, and microwaving, resulted in a reduction in ESR signal intensity in the cooked lamb meat products [56]. It was concluded that ESR spectroscopy is an effective technique for identifying irradiated lamb meat containing bone tissue, which were explored to an irradiation dose range of 2.5–5.0 kGy and might have been stored and/or cooked [56]. Later in 2004, Chawla and Thomas [55] reported two blind trials on ESR determination of irradiated meat containing bone tissues to demonstrate the reliability and usefulness of ESR technique in determination of the irradiated bone-in meat products. The first trial included 29 irradiated and nonirradiated bone-in lamb meat chunk samples, which were at different spoilage stages. The second trial involved total of ten chicken legs and 25 lamb meat chunks, which were divided in four lots, three of which consisted of ten samples each and explored to γ-irradiation at three different dose levels, and the fourth lot had five samples. Bone powders were prepared from all these meat samples and examined for the typical irradiation-induced ESR signals. For the first trial, 21 samples were identified as being irradiated and eight samples as nonirradiated according to ESR signal intensity measurements, which had a 100% success rate. ESR analysis of the 35 lamb and chicken leg samples showed that 30 samples were irradiated and the rest five were nonirradiated, and the identification was 100% accurate. In this study, a reirradiation method was employed to estimate the irradiation doses in samples, which were blind to the analysts. In general, a bone-in meat sample is reirradiated to several different dosages and measured for the ESR signal intensity at certain time points. These data are used to obtain a dose–response curve for the sample. Extrapolation of the curve to the negative axis provides an estimation of the initial absorbed irradiation dosage in the bone and the surrounding muscle tissue. In this study, linear, quadratic, and exponential equations were used to determine the original irradiation doses in the meat samples, and were found out that quadratic and exponential equations estimated the irradiation doses better whereas linear fit equation might overestimate the irradiation level [55]. It was then concluded that ESR might be a possible method for qualitatively identifying irradiated bone-in meat samples, and for estimation of the original irradiation doses in the bone-in meat samples using the reirradiation technique [55].

In 2004, ESR analysis successfully identified irradiated wing-tip bone of chicken, rib of black rockfish, frog leg bone, and clam shell [58]. It was observed that the ESR spectrum may be altered by the irradiation dosage, and the minimum detectable dose was believed approximately 0.5–1 kGy. Also noted was that the ESR spectrum shape might be altered by other components present in the meat samples [58]. In addition, ESR analysis has been used to identify irradiated fried anchovy and shrimp [59] and bone-in chicken [53].

6.2 Irradiated coffee beans

Microbial spoilage and insect proliferation are two major quality and safety problems for coffee beans during storage, processing, and transportation. Environmental hazardous fumigants such as ethylene dibromide have been used for controlling the microorganisms and insects [52, 57]. Irradiation was tested as an alternative approach to replace these chemicals. An ESR study was conducted to evaluate and compare γ-irradiation, storage, and thermal treatment for their effects on free radical concentration using two varieties of coffee beans [52]. The results showed that irradiation was able to dose-dependently increase the ESR signal intensity in whole beans of both coffee varieties, although two varieties differed in their ESR signal intensities. It was interesting that thermal treatment at 50°C for 16 h had similar effects on ESR signal intensity as that induced by γ-irradiation at 5 kGy in *Arabica* coffee and greater intensity than that induced by irradiation at 5 and 10 kGy in *Robusta* coffee beans [52]. It is known that 5 and 10 kGy are commonly used dosage level for microbial decontamination of coffee beans. Also noted was that ESR signal intensity of the irradiated coffee beans was significantly reduced after storage at 25°C for 24 h, but not in the nonirradiated samples [52]. These data suggested that irradiated coffee beans using a dose range of 5–10 kGy might be safe for human consumption. During this study, whole coffee beans and their silver skins from both coffee varieties were compared for their free radical formation induced by γ-irradiation. Similar as that observed in the whole coffee beans, free radicals were dose-dependently induced in the silver skins of both coffee varieties according to ESR analysis. Irradiation at 10 kGy resulted in six times stronger ESR signal intensity in the silver skins than that in the whole coffee bean portion [52]. Silver skin accounts only about 1.4% of the total coffee seed material, but may be a richer source of free radicals for ESR determination.

Another ESR study was also conducted in India to investigate the free radical status in irradiated "Indian Monsooned Malabar Coffee", which is a specialty coffee pre-

pared by exploring the beans of *Arabica* and *Robusta* coffee varieties to the high humidity winds of the monsoon season in open warehouses for about 6 – 7 wk [57]. This coffee has very unique taste and flavor, and has great market potential. Irradiation was considered as a possible approach to improve the safety and quality of Indian Monsooned Malabar Coffee with a reasonable cost. For consumer acceptability and confidence, the free radical generation during γ-irradiation was evaluated. The comminuted, comminuted and powdered, and roasted and cut coffee beans were subjected to γ-irradiation treatment at a dose range of 0 – 1 kGy and followed by ESR determinations. The results showed that irradiation alone at 0 – 1 kGy level did not generate detectable amount of free radicals in coffee bean samples, whereas both powdering and roasting processes induced detectable ESR signals under the experimental conditions. Furthermore, synergetic effect was observed between irradiation and powdering or roasting in the induction of free radicals in the coffee bean samples. It was concluded that irradiated Indian Monsooned Malabar Coffee at a dose up to 1 kGy might be safe for human consumption [57].

During the Indian Monsooned Malabar Coffee study, the role of phenols in free radical generation during irradiation was investigated [57]. The coffee bean powder was washed with methyl cellosolve to remove phenols. The phenol-free coffee powder and the original coffee powder were subjected to γ-irradiation at doses of 0, 0.5, and 1 kGy, and analyzed for their free radical levels using ESR. The results showed that ESR signal intensity was dose-dependently increased in the original coffee bean powder, but was not detectable in any phenol-free coffee powder samples under the same experimental conditions (Fig. 13). These data indicated that phenolic compounds may be a contributor for free radical formation and total free radical concentration in irradiated botanicals because they are ubiquitously present in the botanicals and have a high *G* value [57]. The *G* value is defined as the number of molecules destroyed or products formed for 100 eV energy absorbed. This observation also suggested possible effect of phenolics on long-term oxidative stability and safety of irradiated food products.

6.3 Irradiated cereal grains and oil seeds

ESR analysis has been employed to detect irradiated cereal grains and oil seeds including but not being limited to oat [7], maize [49], wheat [60, 61], rice [61], almonds [62], soybean [63], and sunflower seeds [61]. These previous researches reported typical ESR spectra for irradiated and nonirradiated cereal and oil seed samples, along with ESR parameters for determination, influence of irradiation dose on ESR signal intensity, stability of the induced free radicals or ESR signals, and the potential for estimating irradiation dosage in the samples [7, 49, 60, 61, 63]. For instance, an ESR analysis was conducted to study the typical ESR spectra of irradiated and nonirradiated ground maize cultivated in Turkey and the kinetic properties of these ESR signals using an irradiation dose range of 0 – 5 kGy [49]. The results showed that ESR peak area was positively correlated with irradiation doses using quadratic and exponential equations, although the radiation yield of maize was not high. The effects of irradiation temperature, kinetic properties of radicals at high temperature, and annealing time on individual free radical species were also evaluated. It was concluded that ESR technique might serve as a possible method for detecting irradiated maize.

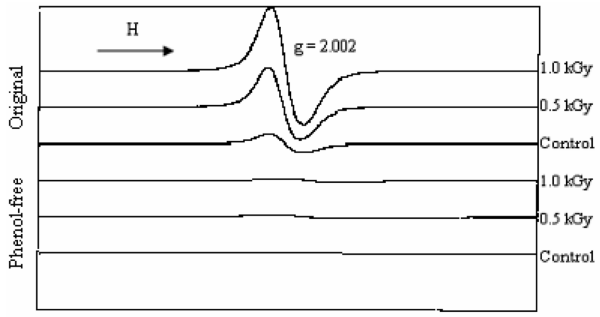

Figure 13. Free radical levels in *Arabica* monsooned coffee powder samples exposed to γ-radiation doses. Original represents coffee powder group without methyl cellosolve washings, whereas the phenol-free represents those washed with methyl cellosolve. (Redrawn from ref. [57].)

6.4 Irradiated fruits, vegetables, and spices

Irradiation is a very convenient approach to eliminate microorganisms in fruits and vegetables with less concern of altering their sensory and quality properties. ESR has been evaluated for potential in identifying irradiated fruits such as kiwi, papaya, tomato, grape, apple, cherry and other fruits [54, 64], and vegetables such as dried onion, green onion, and ginger [65], chestnut [66], pepper [67], and dried cabbage, carrot, garlic, onion, and green onion [51]. In 1999, pulps of kiwi, papaya, and tomato fresh fruits with and without 80% ethanol extraction were evaluated for their ESR spectra and signal stability [54]. It was observed that the ESR signal in the alcohol-treated pulp samples was stable and correlated with irradiation intensity, indicating that ESR analysis of ethanol-treated pulp might identify irradiated fresh fruits and possibly estimate the irradiation dose [54]. ESR analysis might also be used for determination of γ-irradiated vegetables [51, 65, 67]. In addition, ESR has been demonstrated for its potential application in identifying the irradiated spice samples [48, 50, 68, 69].

7 Other applications

Recently, ESR spin label technique was employed to study the kinetic physical changes in ice cream during freezing

and melting [70]. A lipophilic spin label, 1,1,3,3-tetramethylisoindolin-2-yloxyl (TMIO), was used to reflect the molecular motions in lipid phase, while a hydrophilic probe, sodium 1,1,3,3-tetramethylisoindolin-2-yloxyl-5-sulfonate (NaTMIOS), was used to observe the molecular collision in water phase. Lorentzian linewidths and rotation correlation times of both spin labels were calculated from ESR spectra. The results indicated that lipid phase contained both liquid and solid fat until the system was cooled to about −60°C, but water phase completely changed from liquid to solid state within temperature range of 1 to −18°C. These microstructure changes are important for improving the quality and stability of ice cream [70]. In addition, it was pointed out that ESR spin-label technique may be used to investigate phase transition and viscosity related properties in foods during formulation, processing, and storage [70].

In 2007, a study showed that ESR spectroscopy may be used to estimate the radio-stability of BHT, a commonly used synthetic antioxidant in food products [71]. It was concluded from this study that BHT has good radio-stability and may remain its antioxidant potential in irradiated foods. It was also suggested that BHT might be used for detection of irradiated food products with a dose range of 5–34 kGy [71]. In addition, radio-stability of vitamins including riboflavin, biotin, thiamine mononitrate, thiamine hydrochloride, folic acid, and pyridoxine were also analyzed using ESR spectroscopic method [72]. These previous studies suggest the potential of ESR technique in estimation of radio-stability of food components.

8 Conclusions

ESR spectroscopy has been widely used and will continuously serve as a powerful tool in nutraceutical and food research. ESR may be employed for estimating free radical scavenging capacities and Cu^{2+} chelating properties of potential antioxidant samples, and for studying the interaction between free radicals and antioxidative nutraceuticals. ESR may also be used to investigate oxidative stability and radio-stability of food and nutraceutical products, and to determine food irradiation. In addition, ESR spin-label oximetry technique can be used to evaluate lipid peroxidation, oxygen diffusion and transport, and structure and dynamic properties of cellular membranes. Developing new application of ESR in nutraceutical and food research may provide additional information/tool to advance our understanding of food systems and interactions among individual components during food/nutraceutical formulation, processing, and storage.

The authors have declared no conflict of interest.

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