Plant Antioxidants in Food Emulsions

Pascual García-Pérez, Eva Lozano-Milo,
Pedro P. Gallego, Concha Tojo,
Sonia Losada-Barreiro and Carlos Bravo-Díaz

Additional information is available at the end of the chapter

Abstract

Addition of free radical scavenging antioxidants (AOs) is one of practical strategies controlling the oxidative stability in food emulsions. Attention has been directed toward AOs derived from natural plant extracts with the capacity to improve health and well-being due to lack of consumers' trust toward synthetic antioxidant in food. Nevertheless, antioxidant efficiency varies widely from one compound to another and the most abundant AOs in our diet are not necessarily those that have the best availability profile at the reaction place with free radicals. In this book chapter, we will provide a state-of-the-art summary of the uses of plant AOs in colloidal systems, ranging from their main structural features to their benefits for the human health and their antioxidant role in controlling the oxidative stress and, particularly, the oxidation of lipid-based food emulsions.

Keywords: antioxidants, oxidative stress, lipid oxidation, emulsion stability

1. Introduction

Oxygen plays a controversial role in life: its presence is essential for aerobic organisms and, at the same time, it has been extensively reported as a toxic agent. Such toxicity derives from its capacity to form free radicals, considered highly energetic, unstable compounds with the ability of reacting easily with other molecules because they have unpaired electrons in the outermost orbitals. Whatever is the initiating mechanism, once the free radicals are formed, they can react with a biologically relevant molecule such as lipids, proteins, DNA, etc., leading to significant molecular blockage, degradative oxidation, and/or cell damage [1]. Polyunsaturated fatty acids, PUFAs (see **Figure 1**) are especially susceptible to chemical oxidation.
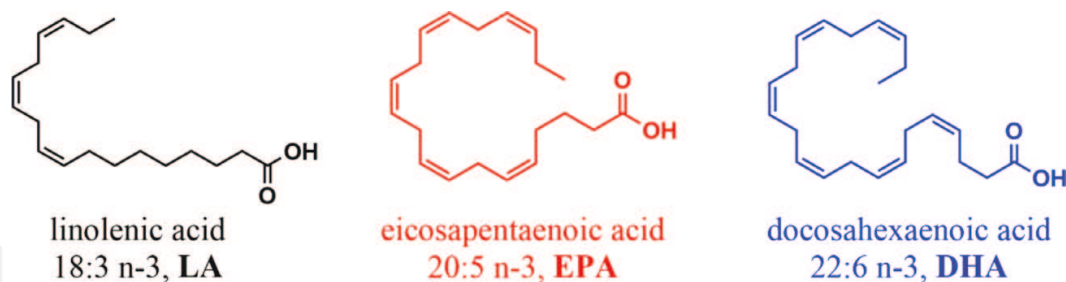
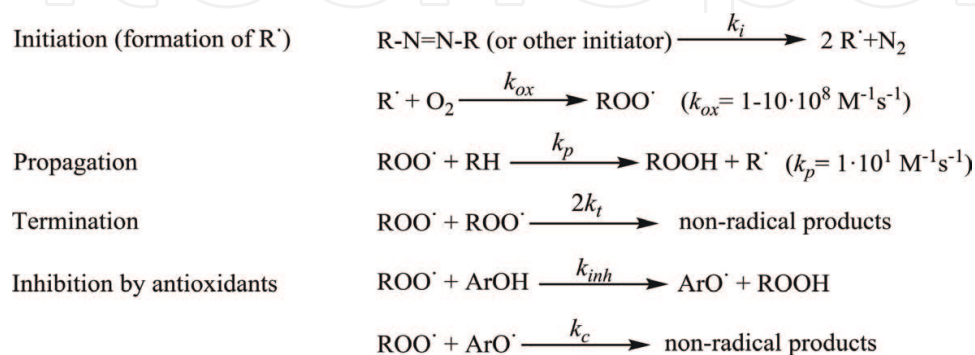**Figure 1.** Chemical structures of some PUFAs.

Figure 2. Main steps of the free radical oxidation of lipids and the inhibition by radical scavenger AOs. Average rate constant values for representative steps are also included. Constant abbreviations are found in the text. RH, lipid substrate; R•, lipid radical; ROO•, lipid peroxyl radical; ROOH, lipid hydroperoxide; ArOH, antioxidant; ArO• antioxidant radical.

In practical terms, lipid peroxidation (**Figure 2**) implies the reaction between a preexisting free radical and PUFAs during the initiation phase, whose reaction rate is given by the initiation constant, k_i, in which fatty acid radicals (R•) are generated. Subsequently, these radicals are able to react rapidly with molecular oxygen (O$_2$) to produce fatty acid hydroperoxyl radicals (ROO•), which constitutes the starting point for the subsequent propagation phase, driven by the rate of propagation constant, k_p, leading to oxidative stress. Finally, oxidative chain reactions may undergo a termination phase, characterized by its constant, k_t, where non-radical products are formed. Subsequently, ROO• radicals can be intercepted by AOs, throughout inhibition reactions, depending on their constant, k_{inh}. Furthermore, intercepted AOs may undergo termination reactions toward non-radical compounds production driven by the rate constant k_c, **Figure 2** [2].

Oxidative stress was first defined by Sies [3] as the lack of balance between the occurrence of reactive oxygen and nitrogen species (ROS and RNS, respectively) and the organism's capacity to counteract their action by the antioxidative protection systems. Since then, oxidative stress has been widely studied for decades, as it plays a key role on the etiology of several chronical diseases, i.e., diabetes, inflammation-related, neurodegenerative, and cardiovascular diseases, and cancer [1]. In order to overcome the deleterious effects attributed to oxidative stress, cells should maintain their redox homeostasis by enhancing *de novo* synthesis of AOs or by uptaking them from the diet or other exogenous sources.

2. Plant antioxidants

According to **Figure 2**, an efficient antioxidant is that molecule whose rate of trapping peroxyl radicals equals or overcomes the rate of formation of peroxyl radicals in the initiation step. Both rates depend on their intrinsic rate constants k and on the concentrations of reactants at the reaction site. Thus, the efficiency of a known amount of AO in a given reaction site depends on the value of the absolute rate constant for inhibition, k_{inh}, compared to the propagation rate constant, k_p, for reaction of the substrate with peroxyl radicals, e.g., the ratio k_p/k_{inh}. In general, AOs may protect against oxidation by a combination of various mechanisms. The predominant mechanism in a particular situation determines, to a great extent, the efficiency of the AO in inhibiting lipid oxidation.

Due to the high number of AOs reported to date and the plethora of reaction mechanisms that may be involved, the classification can be established according to multiple criteria. In a simple approach, AOs can be classified as follows: (1) according to their reactivity, (2) according to their origin, and (3) according to their structural features.

2.1. Functional classification of AOs

Based on functional features, AOs can be classified in two general groups: *primary* or *chain-breaking* AOs, responsible for the defense against ROS attack, by intercepting chain-propagating, O-centered, free radicals; and *secondary* or *preventive* AOs, that may prevent the attack of ROS on a substrate.

2.1.1. Primary or chain-breaking AOs

Primary (chain-breaking) AOs can trap two lipid radicals by donating a hydrogen atom to one radical and receiving an electron from another radical to form stable non-radical products. They inactivate free radicals by three main mechanisms, all playing important roles in determining radical scavenging activities depending on the particular environmental conditions: (1) transferring H-atoms to peroxyl radicals (hydrogen atom transfer mechanism, HAT), (2) electron transfer-proton transfer mechanism (SETPT), and (3) sequential proton loss-electron transfer mechanisms (SPLET) [4–6]. Probably the most common is the HAT mechanism, which involves the hemolytic cleavage of the O-H bond, converting them into harmless hydroperoxides and the oxidized antioxidant radical, which is less reactive with respect to $R^{\bullet}$, $RO^{\bullet}$, or $ROO^{\bullet}$ because of the delocation of the unpaired electron within their structures to form stable resonance hybrids.

2.1.2. Secondary or preventive AOs

Secondary AOs' function is closely related to lipid peroxidation, as it focuses on the interception of oxidative propagation processes after initiation. These agents may exert synergistic effects along with primary AOs as well, through several mechanisms [1]: (1) providing an acidic environment to stabilize primary AOs; (2) acting as hydrogen donors to regenerate

primary AOs; (3) promoting metal chelation activity; and (4) quenching molecular oxygen with the aim of intercepting its reaction with oxidation-sensitive compounds.

Oxidized transition metal ions (mainly iron and copper) are one of the leading causes of the oxidative burst since their reduction implies the participation of O_2 and hydrogen peroxide (H_2O_2). Consequently, oxygen is enrolled in the initiation of redox reactions and could give rise to oxygen-, lipid-, or protein-derived radical formation. In this sense, chelators are able to interfere with metal oxidized ions to avoid the implication of O_2. Catechol or galloyl moieties-containing AOs have the ability of forming complexes with metals due to the presence of adjacent hydroxyl groups (—OH) within their structures, conversely to AOs bearing unique —OH groups (e.g., vanillic, syringic, and ferulic acids). A well-known chelating agent is citric acid, widely found in plant-derived foods, which is able to form stable coordination complexes with transition metals (with a typical stoichiometry of 1:1). Citric acid-metal ion complexes (Fe^{2+}, Cu^{2+}, Al^{3+}, etc.) formation is driven by their respective equilibrium constants, K_1, ranging from 10^3 to 10^5 M^{-1}. Specifically, citric acid Fe^{2+} complex binding constant has been reported to be $K_1 \approx 1600$ M^{-1} [2]. Moreover, AOs such as caffeic acid can also chelate metal ions and its reported binding constants, K_2, range from 10^0 to 10^2 M^{-1}. In this sense, the binding constant for the formation of caffeic acid—Fe^{2+} complexes—is $K_2 = 8$ M^{-1}, as it has been reported elsewhere [7, 8].

2.2. Origin-based classification of AOs

AOs can be also classified, according to their origin, into endogenous and exogenous, since total antioxidant capacity in biological systems involves endogenous antioxidant systems (mainly enzymes) and exogenous antioxidant compounds, proceeding from the diet.

Endogenous AOs comprise all the inner compounds and enzymatic systems acting like antioxidant agents under physiological conditions. As a general rule, antioxidant enzymes not only catalyze the synthesis or regeneration of previously oxidized AOs but also develop additional activities as free-radical scavengers and peroxide decomposers (see **Figure 3**). The three major enzymatic systems acting as AOs are superoxide dismutases (SOD), catalases (CAT), and glutathione peroxidases (GPX) [6]. Particularly, these enzymes show an important structural feature that is closely related to their antioxidant properties since they all contain transition metal ions within their active structures.

Besides antioxidant enzymes, inner antioxidant agents, such as GSH, coenzyme Q, and uric acid, may act as indirect scavengers of free radicals, metal ion sequesters, and oxidation-repairing agents [9]. Nevertheless, in most cases, such features are correlated to enzymatic systems.

Exogenous AOs (especially polyphenols) are mainly obtained from the diet. Polyphenols are considered one of the major compounds proceeding from plant secondary metabolism, since their distribution in the plant kingdom is wide. These compounds have been categorized as nutraceuticals due to their presence in edible plants and antioxidant-rich foods and drug supplements; therefore, they have gained much attention as preventive agents of several
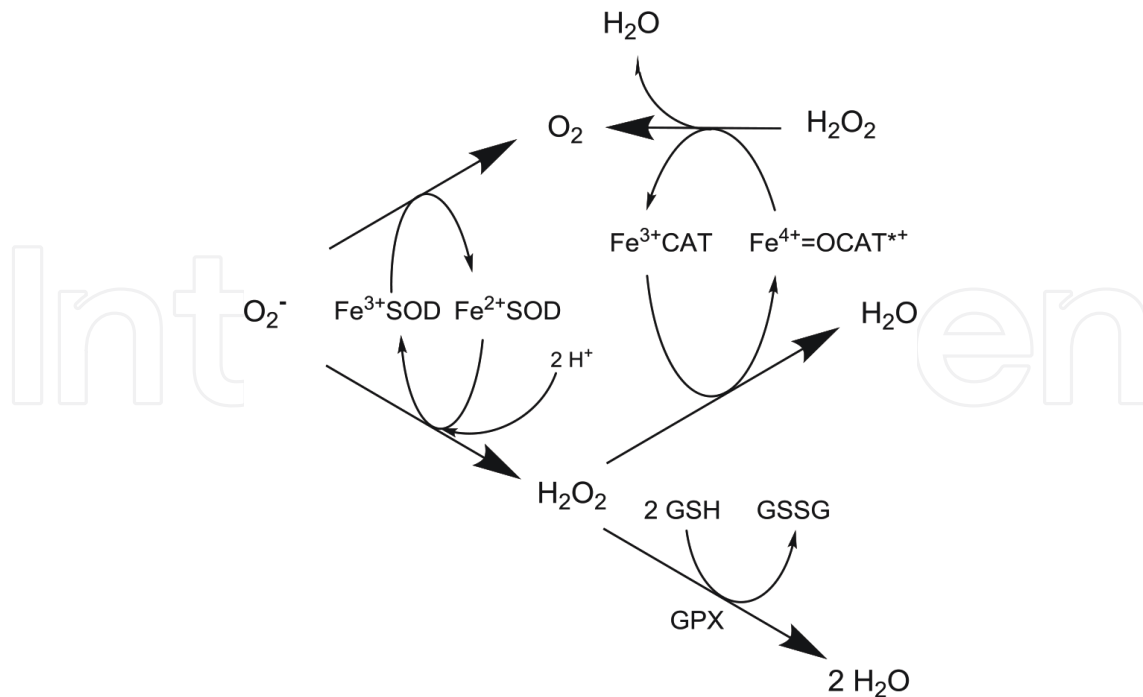
Figure 3. Overview of antioxidant enzymatic mechanisms: Fe-SOD: superoxide dismutase; Fe-CAT: catalase; GPX: glutathione peroxidase; GSH: glutathione; and GSSG: oxidized glutathione.

diseases worldwide. In this sense, neurodegenerative diseases, particularly Alzheimer's disease, and other oxidation-related diseases can be effectively prevented by polyphenol-rich foods intake. This way the regular consumption of polyphenols has been seen as an efficient antioxidant therapy against ROS due to the health benefits and anticancer effects attributed to such compounds [2].

2.3. Structural classification of plant-derived AOs

Plants have been used for centuries in traditional medicine for the effective treatment of several diseases. Such beneficial effects are a consequence of the biosynthesis of phytochemical compounds, derived from plant secondary metabolism in response to their environmental adaptation and protection. Thus, phytochemicals not only play a defensive role within plants but also many of them present additional features (acting as reproduction and environmental adaptation-related agents). Concerning dietary sources, the most common antioxidant phytochemicals found in vegetables and fruits are polyphenols and carotenoids [8]. Certain vitamins, which have also been isolated from several plant-based foods, show a strong antioxidant potential (see **Figure 4**).

2.3.1. Polyphenols

Polyphenols are structurally characterized by the presence of one or more aromatic rings bearing, and at least two hydroxyl groups in their chemical structures. As a consequence
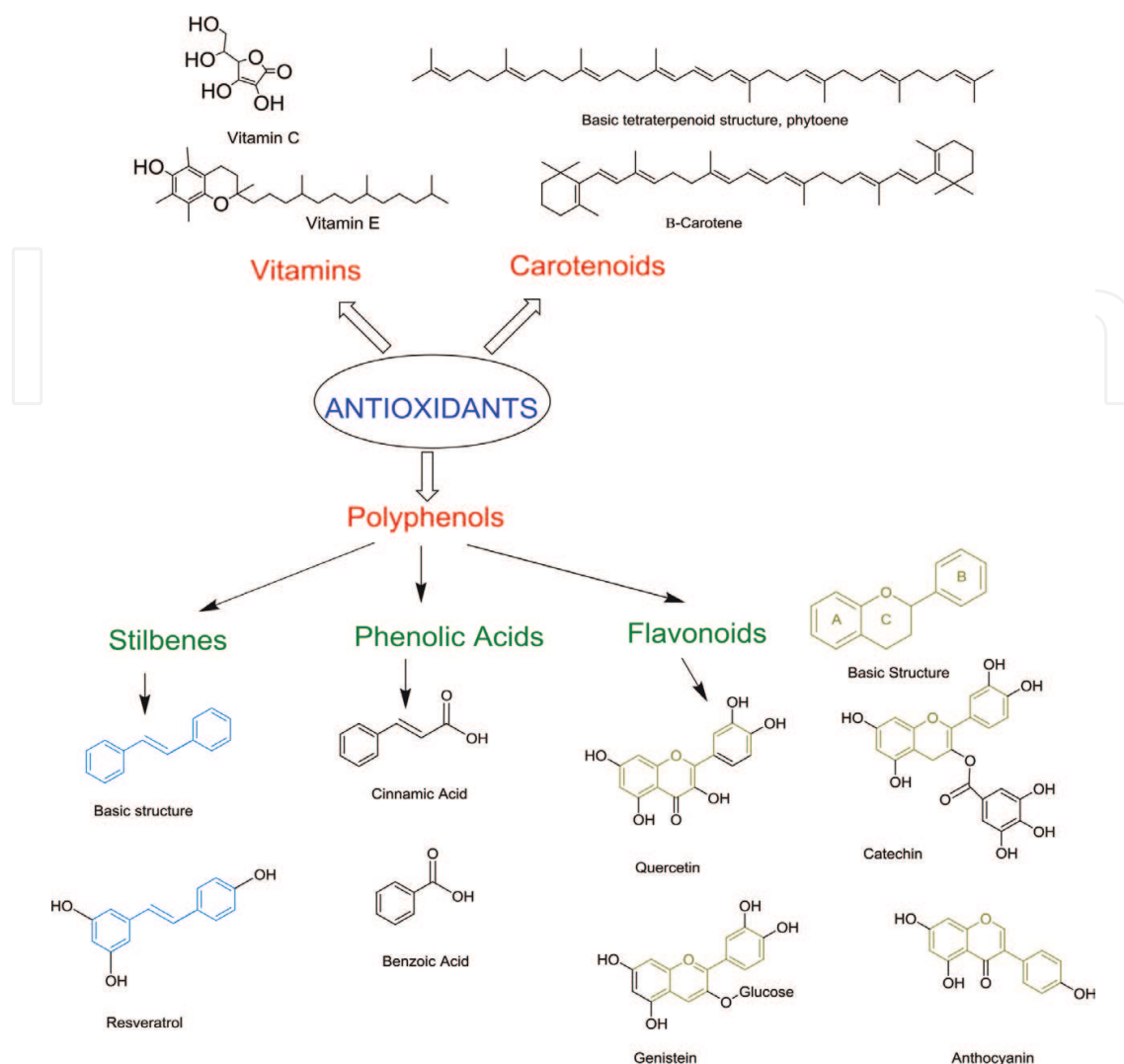
Figure 4. Typical classification of AOs and chemical structures of some representative AOs.

of their structural heterogeneity, polyphenols can be further subdivided into three groups, taking into account their presence in diet-derived products: flavonoids, phenolic acids, and stilbenes (see **Figure 4**).

2.3.1.1. Flavonoids

Flavonoids are plant secondary metabolites synthesized under oxidative conditions, due to their antioxidant properties as ROS scavengers, metal chelators, lipid peroxide decomposers, antioxidant enzymes inductors, and UV light absorbers [10]. Structurally, they are C6-C3-C6 benzo-γ-derivatives, containing phenolic and pyrane rings (**Figure 4**), and are broadly classified by the oxidation degree on their C-ring into: flavonols, flavones, flavanols (catechins), flavanones, isoflavones, and anthocyanins. However, only some of these groups achieve an effective antioxidant function throughout the diet.

2.3.1.2. Phenolic acids

Phenolic acids are plant secondary metabolites that contain at least one aromatic ring bearing one or more hydroxyl groups. They can be divided into two classes, according to the original acid they derive from: cinnamic acid (C6–C3) derivatives and benzoic acid (C6–C1) derivatives (**Figure 4**) [11]. The antioxidant activity attributed to these compounds is directly linked to the number of hydroxyl groups existing in their structure. Altogether, phenolic acids develop a pleiotropic antioxidant activity. The scavenging of oxygen and nitrogen-derived free radicals is the most significant feature which acts as chain-breaking AOs [12]. Just like flavonoids, phenolic acids are ubiquitous secondary metabolites found in various vegetables (artichoke and spinach), fruits (mainly citrus and berries), cereals, and coffee [11].

2.3.1.3. Stilbenes

Stilbenes are polyphenols containing a 1,2-diphenylethylene structural nucleus (**Figure 4**). Unlike other phenolic compounds, stilbene occurrence is limited within the plant kingdom and only discrete compounds have been studied in depth. In this sense, most of the studies concerning stilbenes have pointed at resveratrol (3,4′,5-trihydroxy-*trans*-stilbene, **Figure 4**) as the paramount compound belonging to this family. As an antioxidant agent, resveratrol possesses ROS and RNS scavenging activity and presents a strong influence upon the enhancement of antioxidant enzymatic systems [13]. Grapes, wine, and peanuts and their derived products are its principal sources [14].

2.3.2. Vitamins

For decades, many *in vitro* and *in vivo* studies highlighted the beneficial relation between the dietary consumption of vitamin-rich foods and the prevention of degenerative diseases, as a consequence of the antioxidant action of three vitamins: A, C, and E. Due to structural reasons, only vitamins C and E will be considered in this section, thus excluding vitamin A (a β-carotene-derived compound) to the next section.

Vitamin C (ascorbic acid, **Figure 4**) has been classically identified as a prominent antioxidant, due to its pleiotropic effects as free radical scavenger, metal chelator, and lipid oxidation inhibitor, with chain-breaking properties. An additional antioxidant feature of vitamin C is the ability of regenerating the oxidized form of vitamin E back to their active form, thus enhancing the synergistic effect between both compounds [15]. Fruits and vegetables, such as strawberries, papaya, kiwi, grapes, orange and their respective juices, pepper, tomato, and broccoli are considered their major sources [16].

Vitamin E covers a group of eight lipid-soluble molecules, derived from tocopherol and tocotrienol. Only α-tocopherol (**Figure 4**) has been found in significant amounts in dietary foods, such as edible oils and seeds [17]. The antioxidant efficiency of α-tocopherol is due to its chain-breaking properties, showing a strong specific affinity toward peroxyl radicals, exclusively [18].

2.3.3. Carotenoids

Carotenoids gained much interest in food chemistry as one of the major lipid-soluble groups of antioxidant compounds. Structurally, carotenoids are derived from phytoene, which is accepted as their tetraterpenoid precursor (**Figure 4**). However, only β-carotene (pro-vitamin A, **Figure 4**) and lycopene achieve a notable effect in dietary terms. They possess an enhanced effectiveness toward peroxyl radical scavenging and, additionally, their lipidic nature ensures an improved affinity to cell membranes, acting as protectors of these cell structures [15]. Due to their behavior as plant pigments, β-carotene is mainly found in highly pigmented fruits, such as apricots, carrots, and broccoli, while tomato is admitted as the major source of lycopene [19].

3. Food emulsions

Many natural and processed foods may be shown as examples of emulsion-type products. In the matter at hand, because of lipids that are highly hydrophobic and have a very low water solubility, they are usually incorporated into some kind of colloidal delivery system to make them dispersible in aqueous solutions. Emulsions are the main group of colloidal systems relevant for lipid-based foods [20]. Moreover, AOs are frequently added to lipid-based food emulsions because they are effective to hinder lipid oxidation. Over the past few years, there has been a growing emphasis on the understanding of the efficiency of AOs in emulsions, and it was found that antioxidant activity may vary largely depending on the composition of the emulsion [21]. Thereby, antioxidant activity is determined by a number of parameters such as interphase transport, surface accessibility, partitioning of AOs, and interaction of emulsifier with AOs. Advances in our understanding of the relationship between emulsion properties and antioxidant activity can be made through development of the principles and techniques of emulsion science, with the final purpose of improving the quality of the food production. The aim of this section is to give an introduction to the essential principles of emulsion science that are basic for understanding and manipulating the properties of food products.

Emulsion is a mixture of two immiscible liquids (usually oil and water), in which one liquid is dispersed as small spherical droplets in the other liquid. Foods such as milk, fruit juices, or mayonnaise, are composed by small droplets of oil dispersed in an aqueous solution (oil in water emulsion O/W). On the contrary, small droplets of water dispersed in a lipid medium are present in butter (water in oil emulsion W/O). They are all considered emulsions. The liquid present as discrete droplets is usually called disperse phase, and the another liquid is denoted by continuous phase.

The water phase in a food emulsion provides an unique environment, which not only supplies dissolving medium but also enhances interaction with many water-soluble components (protein, polysaccharide, vitamin, sugar, mineral, acid, base, preservative, etc.) [21, 22]. Also relevant is the oil phase since it contains aroma components at oil-water interface and dissolves several components, including AOs, oil-soluble vitamins, preservatives, and essential oils [20].
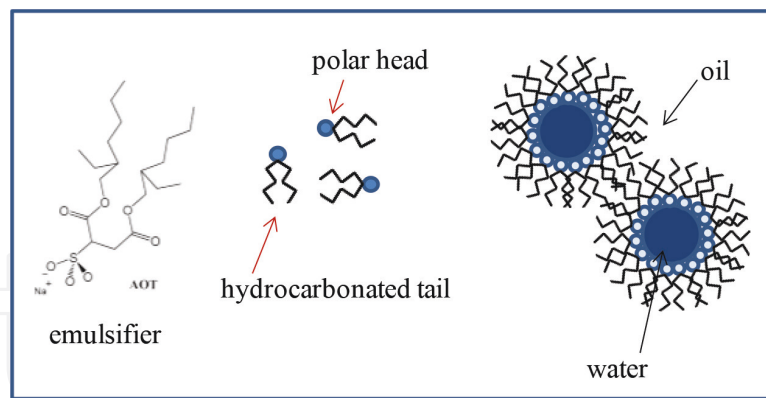
Figure 5. Representation of a location of the emulsifier at droplet surface.

3.1. Physical stability of emulsions

The molecules of the two immiscible liquids composing the emulsion are in direct contact with each other at the interface of each droplet. From a thermodynamical point of view, this arrangement is highly unfavorable. On the one hand, the entropy of the emulsion increases as the emulsion is formed due to increased entropy of mixing. However, this effect is not enough to compensate for the increased enthalpy, which is caused by the contact between hydrophilic and hydrophobic molecules. As a consequence, emulsions tend to separate the two liquids until the contact area between them is minimized (minimal free energy). Therefore, due to the presence of two immiscible phases, emulsions are thermodynamically unstable, that is, emulsions are vulnerable to break down over time by different process.

Although emulsions are thermodynamically unstable, it is possible to make it kinetically stable using an appropriate emulsifier. An emulsifier is usually a kind of molecule that consists of a water soluble hydrophilic part and an oil-soluble lipophilic part, as shown in **Figure 5**.

The addition of an emulsifier or surfactant to a mixture of water and oil stabilizes the emulsion because the emulsifier is arranged on the interface between the two phases [22]: the hydrophilic part of the emulsifier is anchored into water and its lipophilic part into oil. In this way, the emulsifier forms a film surrounding the surface of the droplets. It results in a reduction of interfacial tension, so emulsifiers or surfactants can be called surface-active compounds, which ensures kinetic stability in a certain period [23]. The choice of the emulsifier is crucial in the formation of the emulsion and its long-term stability (see **Figure 6**) [24].

3.1.1. Thermodynamic stability of emulsions

Droplet size and thermodynamic stability are one of the most characteristic features of an emulsion for classification as follows:

1. Macroemulsions: they constitute the most common emulsion type in foods, and they are thermodynamically unstable but kinetically stable. The droplet size ranges from 0.1 to 5 μm.
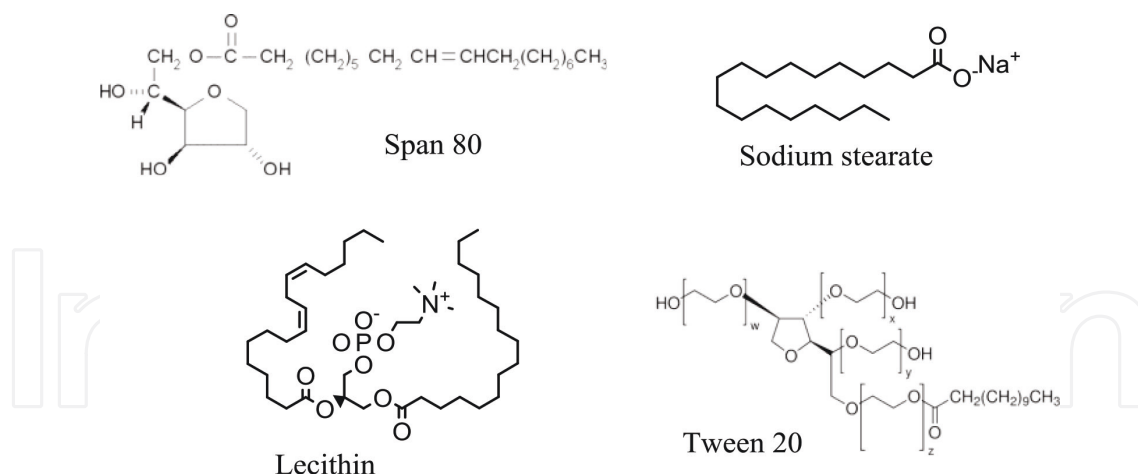
Figure 6. Common emulsifiers used to prepare food-grade emulsions.

2. Nanoemulsions: they are close to macroemulsions, but have a size range of 20–100 nm. They are only kinetically stable, so they are exposed to environmental degradation [25].

3. Microemulsions: droplet size is smaller than the previous ones (5–50 nm) and they are thermodynamically stable [26].

4. Multiple emulsions (W/O/W and O/W/O systems) can be described as emulsion of an emulsion. They are thermodynamically unstable dispersion systems [27].

Alternatively, emulsions may be classified according to HLB value (hydrophile-lipophile balance, see above) as hydrophilic (O/W type and HLB value of the water phase 9–18) or lipophilic (W/O type and HLB value of the oil phase 3–8).

3.1.2. Breakdown processes

One of the main goals of making food emulsions is to provide a stable and manageable source of food, whose properties do not significantly change until the product is consumed. Producing stable emulsions is already a challenge as it requires an in-depth understanding of interfacial physics, because emulsions are inherently unstable. To explain this, the fundamental nature of emulsions must be briefly considered.

Emulsions are usually prepared from the mixture of the two immiscible liquids by mechanical means, provided that the two liquids have no (or a very insufficient) mutual solubility. The average drop size in emulsions may grow with time until emulsions eventually separate into two liquid phases. The different breakdown processes are illustrated in **Figure 7**. These breakdown processes may occur on storage depending basically on the balance between attractive van der Waals forces and repulsive electrostatic (due to double layer) and steric forces.

The physical treatment involved in colloid stabilization is not simple, and it requires analysis of the various surface forces involved. DLVO[1] theory justifies why some colloids aggregate

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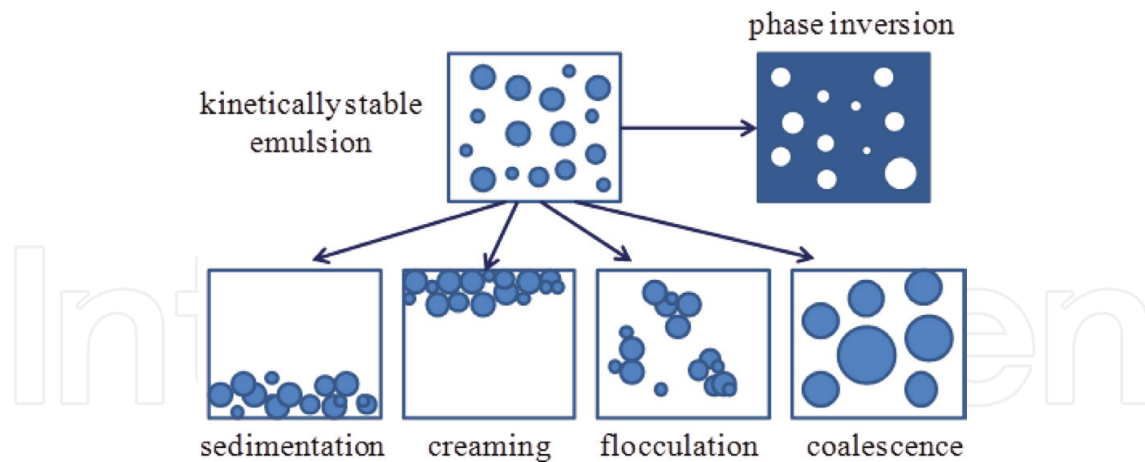[1]Derjaguin & Landau, Verwey & Overbeek.

Figure 7. Scheme of breakdown processes that may occur in emulsions.

and others remain separately. Colloidal particles have electric charge and they are surrounded by ions with opposite charge, so an electric double layer is generated. The double layer causes electrostatic repulsion between droplets, which hinders their approximation. So double layer plays as a shield that provides kinetic stability. Colloidal particles come together if collision between two droplets is enough energetic to break the double layer and solvatation. If the particles strongly repel each other, the colloidal system will be stable.

A summary of the breakdown processes, details of each process, and methods of its prevention are given as follows.

Creaming and *sedimentation* take place when gravitational or centrifugal forces exceed the thermal motion of the droplets. If droplet density is lower than that of the medium, heavier droplets move faster to the top. On the contrary, they will move to the bottom when their density is larger than that of the medium. The closeness of the droplets favors breakdown of the interface. Eventually, the droplets can build up a close-packed arrangement at the top or bottom, giving rise to creaming or sedimentation, respectively. For a deeper discussion, see Refs. [28, 29]. The recovery of a creamed emulsion may be made by simply shaking or prevented by the following ways: (1) reducing the droplet size because the gravitational force is proportional to the cube of the droplet size; (2) increasing the viscosity of the continuous phase because it causes a slowdown of droplet movement; and (3) adding thickening agents (high-molecular-weight polymers such as carrageenans, alginates, etc., that hinder droplets motion and increase the viscosity in the continuous phase).

Coalescence is a growth process during which the emulsified droplets join together to form larger ones. Contrary to creaming, coalescence implies the irreversible fusion of droplets into larger ones, which implies disruption of the interdroplet liquid film. So the driving force for coalescence is the film fluctuations. The high mobility of molecules at the interface gives rise to fluctuations in the interface film, which can cause the film to break [30], and thus the two droplets spontaneously merge, causing coalescence. For a deeper study of coalescence, see Ref. [29].

Coalescence occurs in colloidal systems in which electrical repulsive effect is negligible, so it takes place particularly in O/W systems containing nonionic surfactants. Repulsive

interactions can be modified by changing the charge at the surface, or by using a surfactant that provides a different thickness. Apart from that, coalescence is frequently caused by an incomplete covering of the droplets with surfactant molecules, so replace or increase surfactant can commonly avoid coalescence.

Flocculation is the process by which droplets (without changing droplet size) are aggregated into larger units. It occurs when there is not sufficient repulsion to keep the droplets apart to distances where the van der Waals attraction is weak. The van der Waals attraction is inversely proportional to the droplet-droplet distance of separation [29]. Flocculation is determined by the magnitude of attractive versus repulsive forces. On the contrary, coalescence is determined by the stability of the interdroplet film.

The van der Waals attractive forces depend on temperature, ionic strength, and charge of the interfacial layer [31], which strongly affects emulsion stability. Emulsions can be stabilized by electrostatic repulsion using ionic surfactants or by steric stabilization (adding large polymers to the surrounding aqueous phase).

Phase inversion is the process by which the dispersed phase and the medium are exchanged. As time goes by or due to a change in conditions, an O/W emulsion may turn to a W/O emulsion. Phase inversion usually takes place through a transition state including multiple emulsions. It can be minimized by choosing a suitable surfactant.

Finally, another process affecting emulsion stability is the *Ostwald ripening*, caused by the finite solubility of the liquid phases. Thus, liquids considered as being immiscible usually have no negligible solubilities. Specifically, in emulsions, curvature effects in the smaller droplets give rise to larger solubility than the larger ones. This difference in solubility between small and large droplets is the driving force for Ostwald ripening. The increase in solubility takes place when the droplet curvature increases, that is, when the droplet size decreases [32]. So the smaller droplets disappear and are deposited on the larger ones, resulting in larger droplets that grow at expenses of smaller ones.

There is a range of possibilities for modification in the properties of the emulsion which influence the stability and functional behavior of the colloidal system. Different protocols to ensure food storage stability can be found in Ref. [32]. All of them are based on the analysis of the following emulsion properties:

Droplet size distribution and *droplet concentration* are one of the most characteristic features of an emulsion not only due to the fact that most of the instability process are driven by droplet-droplet interactions, but also to the bulk properties (such as taste, color and texture), which depends mainly on these two parameters [22]. In particular, the speed of creaming depends on the effective particle size.

Composition of the stabilizing layer at the interface has an essential role to ensure emulsification and stability. The main component of the interfacial layer is the emulsifier or surfactant. The most used scale to classify emulsifiers is the hydrophilic-lipophilic balance (HLB), that is, a parameter relating molecular structure to interfacial packing and film curvature. The HLB value ranges from 0 to 20. An emulsifier with higher lipophilicity shows a lower HLB whereas

higher hydrophilicity has high HLB, and the behaviors and functions to water depend on this HLB. This parameter was introduced by Griffin [33] in order to obtain an empirical Eq. (1) for nonionic alkyl polyglycol ethers (C_iE_j) based on the surfactant chemical composition, where E_j wt% and OH wt% are the weight percent of ethylene oxide and hydroxide groups, respectively.

$$HLB = \left(E_j \text{ wt \%} + OH \text{ wt\%}\right)/5 \qquad (1)$$

Also relevant can be the presence of solid particles and cosurfactant at the interface layer. Specifically, food emulsions frequently carry particulate material which is located at oil-water interface favoring emulsion stabilization [34]. Pickering-type food emulsions are emulsions consisting of droplets coated by a layer of adsorbed solid particles at the interface. The formation of O/W or W/O emulsions is determined by whether the particles are predominantly hydrophilic or hydrophobic [34].

The addition of a cosurfactant usually allows to enhance the effectiveness of surfactant. A cosurfactant is added to break up the assembling at the liquid/liquid interface so that it allows to attain lower interfacial tension. Furthermore, cosurfactants can be used to fine-tune the formulation, for example, by expanding the temperature or salinity range of microemulsion stability.

3.2. Chemical stability of the emulsions

As it was mentioned, an AO is efficient in inhibiting lipid oxidation of the emulsified foods if $r_{inh} \geq r_p$, where r_{inh} is the rate of trapping of the lipid radicals $R^\bullet$ and r_p is the rate for propagation step. However, many factors can affect the both rates. Both rates depend on their intrinsic rate constants k and on the concentrations of AOs at the reaction site. On the one hand, the radical scavenging activity of an AO depends on its chemical structure. On the other hand, the concentration of the AOs at the reaction site (that is, interfacial region in emulsions [35]) depends on the distribution of AOs between the oil, interfacial and aqueous regions of the emulsions, which in turn, depend on the physicochemical features of AOs and other parameters such as the nature of oil, the oil/water ratio, the electrical nature and the hydrophilic-lipophilic balance (HLB) of emulsifier, acidity and temperature [2]. Therefore, all these factors and their effects need to be taken into account to enhance the AO efficiency in depth.

3.2.1. Substituent effects

Substituents play a key role on the hydrogen atom donating capacity of AOs and understanding on their conformational, electronic, and geometrical characteristics is of vital significance to comprehend the relationship among AO structure and AO activity [36, 37]. The presence of electron donor groups, particularly at the ortho and/or para positions of the ━OH group improve the scavenging activity of AOs due to lower the phenolic O━H bond dissociation enthalpy and higher reactivity with lipid radicals $R^\bullet$. For this reason, key structural features for a valuable radical scavenging activity are the position and number of hydroxyl groups attached to the aromatic ring and the presence of other functional groups such as alkyl chains

containing C—C double bonds and C=O carbonyl groups, alkyl hydrocarbon chains among others. In general, it was found that rates for the reaction of catechols, 1,2-dihydroxybenzene and derivatives, with lipid radicals R• are higher than those for ortho-methoxyphenols due to the stabilization of the semiquinone radical formed from catechol. Catechols constitute the skeleton of many natural AOs such as flavonoid compounds (**Figure 4**). Structure of phenolics that allows conjugation and electronic delocalization, as well as resonance effects also can improve the radial scavenging activity of AOs.

3.2.2. Partitioning effects of AOs

Efficiency of the AOs depends not only on the AO nature but also on its concentration at the reaction site because AOs can be transferred between different regions (aqueous, oil and inter-face) of the food emulsions (see **Figure 8**), affecting their radical scavenging activity. Though the chemical properties and reactivities of relevant AOs toward free radicals are becoming well comprehended, it remains less clear how these properties translate into multiphasic systems. Thus, prediction of the efficiency of AOs in multiphasic systems such as food emulsions can become unclear since their partitions between the different regions were not explored [2, 38].

The physical impossibility of separating the interfacial region from the aqueous and oil regions of emulsions makes that any attempt to determine antioxidant distributions needs to be done in the intact emulsions, that is, without sample pretreatment. Application of the pseudophase kinetic model to emulsions provides a natural explanation, based on molecular properties, of the effects of a variety of parameters (nature and type of the oil, HLB, temperature, acidity, etc.) on the distribution of components between the oil, interfacial, and aqueous regions of emulsions prepared with edible oils [39, 40]. The reaction of choice was the reduction of a hydrophobic arenediazonium ion, whose reactive group is located in the interfacial region of the emulsions, and that can be monitored by a sampling method.
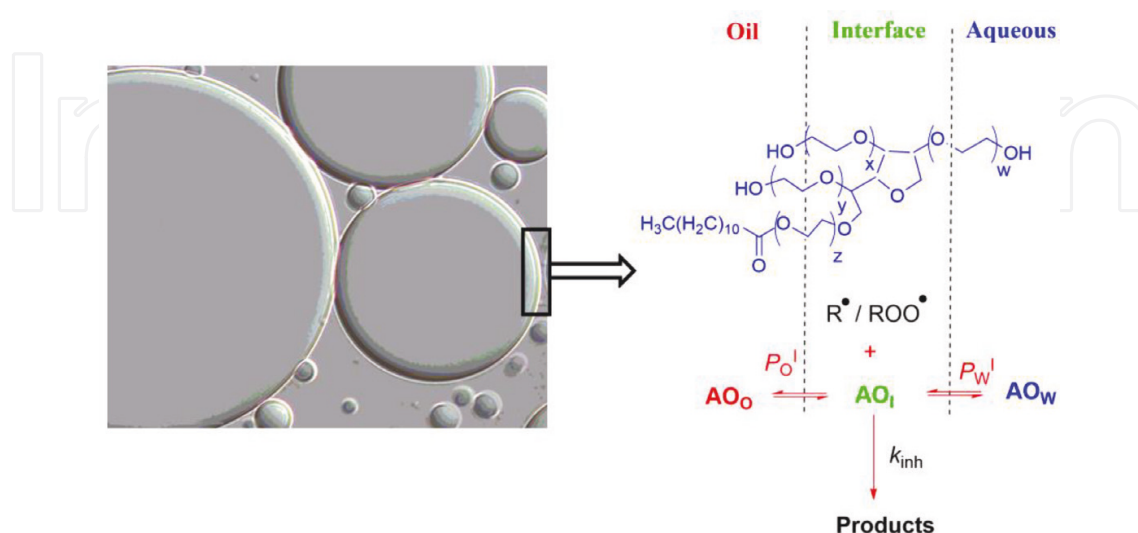

Figure 8. Left: optical microscope image of the droplets of an olive oil-in-water emulsion. Right: partition of AOs between the oil, interfacial (where lipid oxidation primarily occurs), and aqueous regions of the emulsion [38, 44].

Results obtained for a series of AOs (caffeic, gallic, protocatechuic acids, and hydroxytyrosol series) show that their distribution can be correlated with their antioxidant efficiency [35, 41–43]. This finding may have important consequences for the food industry because it opens the possibility of choosing the most efficient AOs for a particular food system on a scientific basis, resulting in an increase of the shelf-life of the product. Results should contribute to enhance current understanding of how antioxidant structure and physical location within the food system affect their efficiency and should provide basic information on the factors controlling antioxidant distributions and efficiencies, allowing a more rational selection of AOs and emulsifiers in food stabilization to maintain the organoleptic properties of foods.

3.2.3. Medium properties effects

The solvent properties of the reaction site affect both the intrinsic rate constant for the reaction between AO and free radicals, k_{inh}, and the partitioning of AOs between the different regions of the food emulsions. Among others:

- Emulsifier nature: the emulsifier electrical nature and HLB of the emulsifier can affect the concentration of AOs at the reaction site. Distribution results showed that the HLB of the emulsifier can modify the partition of moderate to high hydrophobicity AOs and the main parameter controlling the partition of AOs is the emulsifier concentration [45].

- Acidity of the aqueous region: the acidity of the medium can change substantially the partitioning of phenolic AOs. At the typical acidities of foods, phenolic AOs may be neutral or partially ionized and the ionic forms of the AOs are usually oil insoluble but much more aqueous soluble than the neutral forms, changing the partition of AOs and, as a consequence, the antioxidant efficiency [46].

- Oil nature: oxidation rates of monounsaturated fatty acids are much slower than those of polyunsaturated fatty acids. In this way, foods enriched with omega-3 can be seriously compromised by the oxidation of lipids due to their high degree of lipid unsaturation [35].

- Temperature: the temperature can affect lipid oxidation in many ways. It can produce not only variations in the rates for the reactions involved but also in the concentration of AOs in different regions of the emulsions due to changes in the solubility of the AOs [47].

- Oil/water (O/W) ratio: changing the O/W ratio of the emulsions significantly can affect the interfacial concentrations of very hydrophobic or hydrophilic AOs but not that of AOs of intermediate hydrophobicity [41].

4. Conclusions and future trends

Synthetic AOs have been employed for years, but because of the growing consumer interest in natural and health-promoting products, the industry is now challenged to offer new and efficient AOs derived from natural sources. Attention has, therefore, been directed toward

the isolation of extracts of spices, herbs, and other plants rich in AOs because they have the capacity to minimize/inhibit oxidative degradation of biomolecules and thereby improve the quality and nutritional value of food. However, a careful choice of the AOs and the delivery system employed is crucial because it strongly affects their bioavailability and chemical reactivity against ROS. Their evaluation requires a wide range of experimental models from the simplest antioxidant assays in homogeneous solution to the biologically more relevant cellular systems. Many emulsion-based delivery systems for lipophilic compounds are regarded as one of the most promising techniques for transporting AOs to the target areas, deserving further investigations on the topics.

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Author details

Pascual García-Pérez[1], Eva Lozano-Milo[1], Pedro P. Gallego[1], Concha Tojo[2], Sonia Losada-Barreiro[2]* and Carlos Bravo-Díaz[2]

*Address all correspondence to: sonia@uvigo.es

1 Plant Biology and Soil Science Department, Biology Faculty, University of Vigo, Vigo, Spain

2 Physical Chemistry Department, Chemistry Faculty, University of Vigo, Vigo, Spain

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