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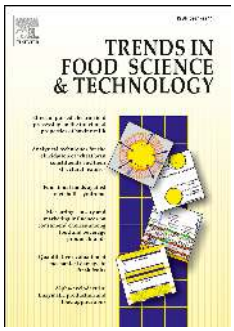

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Hurdles in Predicting Antioxidant Efficacy in Oil-in-Water Emulsions

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Abstract

Numerous compounds exist in nature that can scavenge free radicals and thus have the potential to act as antioxidants in foods. Interest in natural free radical scavengers has resulted in tens of thousands of publications on various molecules and extracts but only an extremely small number have actually been used in commercial applications. The gap between research interest and commercial application is mainly due to the lack of bench top methods that can predict the efficacy of antioxidants in complex food matrices. This disconnection seems to be due to the extremely complex nature of lipid oxidation and antioxidant activity in even relatively simple food systems such as oil-in-water emulsions. This review highlights a number of areas where lack of knowledge is currently holding back our ability to predict which free radical scavengers will be good antioxidants in emulsions: non-free radical scavenging reactions of antioxidants; the existence of different types of oil-water interfaces; difficulties in characterizing lipid droplet surfaces; and differences in oxidation kinetics in different lipid droplets. Further research is needed to identify the key factors that determine antioxidant efficacy in complex heterogeneous systems. This knowledge would then increase our ability to predict how antioxidant structure and properties relate to their activity in food emulsions.

Introduction

Lipid oxidation continues to be a major challenge in the food industry's efforts to minimize food spoilage and waste, and therefore improve the sustainability of the food supply. Much of the early research focused on chemical mechanisms of lipid oxidation in bulk oils. More recently, research has focused on understanding lipid oxidation mechanisms in heterogeneous lipid dispersions, which often has different factors influencing oxidation kinetics than in bulk oils (for reviews see Waraho *et al.*, 2011; Berton-Carabin, *et al.*, 2014). Studying lipid oxidation in dispersed food systems is particularly important from a commercial point of view because most oils are not consumed in their bulk form but are instead dispersed into foods as colloidal particles in products such as dairy foods, processed meats, dressings, dips, beverages, desserts, yogurts, and sauces. When lipids are dispersed as small particles in aqueous-based food products, there is a huge increase in the lipid-water surface area. The specific surface area (A_s, area per unit mass) of a spherical lipid particle is related to its particle size by the following relationship: $A_S = 6/\rho d$, where ρ is the particle density and d is the particle diameter. This expression shows that the specific surface area is proportional to the reciprocal of the particle size. The size of the lipid particles in many food dispersions can be very small (less than a micron), which means that the surface area of an emulsified lipid is much greater than that of a bulk lipid. For example, the specific surface area of lipid particles with diameters of 100 nm, 1 μm, 10 μm and 100 μm are 65,220, 6,522, 652, and 65.2 m^2/kg, respectively. The impact of increased lipid surface area on lipid oxidation is highlighted in **Figure 1**, which shows that the oxidation of an algae oil-in-water emulsion stabilized by Tween 20 is much faster than algae oil in its bulk form.

Reducing the dimensions of the lipid particles in foods may also promote lipid oxidation because reactants such as hydroperoxides are surface active molecules that tend to accumulate at oil-water interfaces (**Figure 2**). The surface activity of hydroperoxides is dependent on their molecular structure, with hydroperoxides on free fatty acids being more surface active than those on methylated fatty acids or triacylglycerols (Nuchi *et al.*, 2002). Lipid hydroperoxides at the surfaces of lipid droplets can interact with transition metals in the surrounding aqueous phase, causing them to decompose into free radicals that propagate the lipid oxidation reaction. The importance of transition metals in this reaction scheme is demonstrated by the ability of metal ion chelators to strongly inhibit oxidation in oil-in-water emulsions (Mancusco *et al.*, 1999; Frankel *et al.*, 2002). The importance of the interface of the lipid particles in emulsions is further supported by the fact that particle characteristics alter the ability of hydroperoxides to interact with transition metals at the droplet surfaces thereby altering lipid oxidation reactions. In simple oil-in-water emulsions, factors that increase metals at the interface increase oxidation rates (e.g. negatively charged emulsifiers) and factors that remove metals from the interface (e.g. positively charged emulsifiers and metal chelators) decrease oxidation rates (for review see Waraho *et al.*, 2011). However, transition metals associated with lipid droplet surfaces are not always prooxidative. For example, studies with mayonnaise showed that transition metals bound to adsorbed proteins did not promote lipid oxidation because binding inactivated them (Jacobsen *et al.*, 2001). However, when solution conditions were altered so as to release the protein-bound metals (*e.g.* pH reduction or ascorbic acid addition) the reactivity of the metal ions increased thereby promoting lipid oxidation.

The important role of free transition metals in promoting lipid oxidation in emulsions, means that metal chelators are particularly effective at controlling oxidation. Indeed, EDTA is a

highly effective, low cost, and flavorless secondary antioxidant that is widely used in the food industry. However, the synthetic nature of EDTA has led to some consumer concerns, which have led the food industry to search for alternative metal chelators. Potential alternatives include proteins/peptides, anionic polysaccharides, organic acids and certain phenolics (for reviews see Elias *et al.*, 2008; Jacobsen, 2015; Shahidi and Zhong, 2011). These compounds all have lower binding constants than EDTA and more importantly often have pK_a values that are generally greater than pH 3.0-4.0 making them protonated and thus ineffective chelators in low pH foods, such as salad dressings and beverages. Recently, metal binding packaging has shown to be another promising EDTA replacement but commercial production of such packaging is currently not feasible (Tian *et al.*, 2013).

Efficacy of free radical scavenging antioxidants in oil-in-water emulsions

Lack of consumer acceptable and effective metal chelators has required food manufacturers to utilize free radical scavenging antioxidants to control lipid oxidation in oil-in-water emulsions (Waraho *et al.*, 2011). Free radical scavengers inhibit lipid oxidation by rapidly reacting with lipid radicals and forming low energy antioxidant radicals which are ineffective at promoting fatty acid oxidation. However, free radical scavenging activity is not always a predictor of the efficacy of an antioxidant in foods. For example, Alamed and coworkers reported that ascorbic acid was twice as effective at scavenging peroxyl radicals as propyl gallate in the oxygen radical absorbance capacity assay while propyl gallate was a much better antioxidant in oil-in-water emulsions (Alamed *et al.*, 2009). This highlights the issue of antioxidant location in a food and its ability to inhibit lipid oxidation.

In 1980, Porter proposed the antioxidant polar paradox, which stated that polar antioxidants work best in bulk oils while non-polar antioxidants work best in lipid dispersions such as oil-in-water emulsions and liposomes (Porter, 1980). The explanation for the antioxidant polar paradox in lipid dispersions was attributed to the fact that polar antioxidants with significant water solubility would partition into the aqueous phase of oil-in-water emulsions where they cannot interact with non-polar fatty acid radicals, while non-polar antioxidants would be retained in the emulsion droplet where they can scavenge lipid soluble free radicals. Porter's hypothesis went further than proposing that the non-polar antioxidants just needed to be retained in the lipid but instead need to be at the interface when he stated that "the action again appears to be at the surface, but here the surface is nearly the whole phase". Therefore he suggested that oxidation chemistry is at the lipid droplets interface and that antioxidants that reside there would be the most effective. Porter and Frankel both reemphasized the importance of the interface in lipid oxidation chemistry in the late 1980's and early 1990's (Porter *et al.*, 1989; Frankel *et al.* 1994). As discussed above, research, since the proposal of the antioxidant polar paradox, have supported the hypothesis that oxidation occurs at the surface of lipid droplets where both lipid hydroperoxides and transition metals can accumulate and come into close proximity (for review see Waraho *et al.*, 2011, Laguerre *et al.*, 2015).

Evidence has continued to mount that effective non-polar antioxidants in oil-in-water emulsions not only need to be retained in the droplets but also must be surface active. Most initial studies utilized antioxidants of varying polarities (for review see Frankel, 2005). However, these studies were hampered by the fact that the antioxidants had potentially different free radical scavenging activity and thus it was difficult to tell if differences in antioxidant effectiveness was due to differences in antioxidant partitioning or differences in chemical

reactivity. To overcome this problem, research was conducted with antioxidants esterified to fatty acids ranging from 2-20 carbons (Laguerre *et al.*, 2015). This strategy allowed the comparison of a single antioxidant (e.g. chlorogenic or rosmarinic acid) with different polarities with the advantage of all the esters having similar free radical scavenging activity. This research showed that antioxidant esters had maximal activity in the 8-12 carbon chain length with esters with shorter and longer hydrocarbon chains being less effective (Laguerre *et al.*, 2009, 2010). Antioxidants with shorter hydrocarbon chains are more water soluble and therefore primarily partition into the continuous phase where they cannot interact with lipid radicals, but could interact with radicals generated in the aqueous phase. Conversely, antioxidants with longer hydrocarbon chains are more lipid soluble and less surface active and therefore tend to be located in the interior of the lipid droplets. Consequently, there is a relatively low antioxidant concentration at the oil-water interface where lipid hydroperoxides are decomposing into free radicals.

Complications with using the antioxidant polar paradox to predict antioxidant efficacy

The Antioxidant Polar Paradox was originally thought to have greater promise for predicting the efficacy of antioxidants in complex food systems. Unfortunately, it has turned out to be more limited in its range of application because antioxidants can impact lipid oxidation kinetics in multiple ways and so knowledge of only their physical location is not always an accurate predictor of their efficacy. For example, hydrophilic antioxidants such as ascorbic and gallic acids are strong free radical scavengers that have been observed to be prooxidative in emulsions. This is thought to be due to their ability to reduce metals into their more prooxidative state thus accelerating hydroperoxide decomposition into free radicals. Increasing the hydrophobicity and

thus surface activity of these antioxidants by esterification to palmitic acid (ascorbyl palmitate) has mixed results on increasing their antioxidant activity. For example, ascorbyl palmitate is strongly prooxidative in mayonnaise, but antioxidative in milk (Jacobsen, 2015). This suggests that the ascorbyl group may still promote lipid oxidation in some instances, even when is located at the droplet surfaces. The ability of ascorbyl palmitate to be antioxidative in some food systems could be due to the low reactivity of metals as would occur in milk where casein is a strong metal chelator. Gallic acid can also reduce metals and be prooxidative especially at pH 3.0 (Gonzalez-Maria *et al.*, 2015; Schwarz *et al.*, 2000; Mei *et al.*, 1999; Arouma *et al.*, 1993). Gonzalez-Maria and coworkers (2015) reported that esterification to increase the hydrophobicity and surface activity of gallic acid increased its antioxidant activity suggesting that it was not able to reduce metals when it is associated with the lipid droplets. However, Barreiro *et al.* (2013) found that gallic acid and propyl gallate were better antioxidants than C8 and C12 esters in olive oil-in-water emulsions even though the C8 and C12 esters were more highly associated with the lipid droplet. Not all antioxidants will reduce metals equally and it is unknown how this metal reducing activity is influenced by physical location. Since several factors can potentially influence the metal reducing/prooxidant activity of antioxidants, this again makes it hard to just use physical location as an indicator of antioxidant efficacy.

The need for antioxidants to be associated with the lipid droplets is also not entirely clear. For example, some highly polar antioxidants, such as catechins, are effective in oil-in-water emulsions even though their lipid solubility is very low (Liu *et al.*, 2016; Yin *et al.*, 2012; Zhou and Elias, 2012; Di Mattia *et al.*, 2009; Kahkonen and Heinonen, 2003; Shibusawa *et al.*, 2005). It is unclear how these polar antioxidants are protecting the lipids. One possibility is that the aqueous phase antioxidants are still able to scavenge free radicals at the interface assuming that

the fatty acid radicals are surface active. However, this seems like it would be inefficient since most emulsions used in these studies only contain 5-10% lipid and thus >90% aqueous phase meaning that any water-soluble antioxidants tend to be fairly dilute in the continuous phase and thus their reactivity could be limited by molecular diffusion processes. Another possibility is that aqueous phase antioxidants could regenerate oxidized antioxidants at the lipid droplet surfaces. However, this again seems unlikely since most phenolic antioxidants do not have reduction potentials low enough to regenerate antioxidants such as tocopherols (Decker *et al.*, 2010), and since antioxidants like catechin also work in emulsions with methyl linoleate which does not contain lipid phase antioxidants. Another possibility is those polar antioxidants are scavenging free radicals generated in the aqueous phase. For example, hydrogen peroxide can be generated by metal promoted oxidation of polyphenols and decomposition of hydroperoxide can produce highly reactive hydroxyl radicals in the aqueous phase (Zhou and Elias, 2012). Aqueous phase hydroperoxides could also originate from surfactants such as Tweens and phospholipids that are not at the interface of the lipid droplets but can decompose into free radicals and promote lipid oxidation (Nuchi *et al.*, 2001; Mancuso *et al.*, 1999). Finally, some of these hydrophilic antioxidants may be able to chelate transition metals, and therefore retard lipid oxidation.

Knowledge of the polarity of an antioxidant might not be sufficient to predict its physical location in an oil-in-water emulsion. As mention previously, catechin has very low solubility in oil (Shibusawa *et al.*, 2005). However, Martínez-Aranda and coworkers (2014) reported that a large majority of catechins in Tween 20-stabilized oil-in-water emulsions react with a surface active probe (4-hexadecylbenzenediazonium) suggesting that catechin is associated with the lipid droplet interfaces which could help explain its ability to be an effective antioxidant. Unfortunately, interactions with surface active probes do not always predict antioxidant efficacy

as this same group found that gallic acid and propyl gallate were more effective antioxidants in oil-in-water emulsions than 8 and 12 carbon esters of gallic acid even though the 8 and 12 carbon esters interacted more strongly with the surface active probe.

To provide some insight into the importance of the physical location of antioxidants on inhibiting lipid oxidation in emulsions, the fraction of lipid droplet surfaces potentially occupied by antioxidant molecules was predicted theoretically. As mentioned previously, the surface area of food emulsions is relatively large ranging from 65 to 65,000 m^2/kg for droplet diameters of 0.1 to 100 µm. To determine the fractional coverage of antioxidants in food emulsions, we reviewed 180 publications from the Web of Science that studied purified antioxidants in oil-in-water emulsions and that reported droplet size, lipid concentration, and antioxidant concentration. First, the total surface area of the lipid droplets in the emulsions was calculated. Next, maximal antioxidant size was determined using ACD/ChemSketch 2015.2.5 software assuming that the antioxidant is a sphere and where the maximal molecular surface area of the antioxidant was $\pi r^2 \times n \times N_A$; with r being the molecular radius; n being the amount of antioxidant present (moles); and N_A, being Avogadro's constant. It was then assumed that all antioxidant would be present at the lipid droplet surfaces. Thus, this would allow for the determination of the maximal antioxidant coverage of the droplet interface. In this exercise, only antioxidant concentrations that were shown to effectively inhibit lipid oxidation were used. These calculations showed that antioxidant coverage ranged from 0.04 to 344 % but with 76 % of the examples presenting antioxidant coverage lower than 11 % (Table 1).

Antioxidant coverage greater than 100% where obtained for the research of Abdalla and Roozen (1999) and Di Mattia *et al.* (2009). The emulsion droplets in these papers were 2.5 and 17.6 µm, respectively, and thus their smaller surface areas resulted in calculations of surface

coverage from 70 to 438%. Obviously the surface coverage cannot be > 100%, so in these emulsions much of the antioxidant would partition into the lipid or aqueous phase. Other papers with high emulsion droplet surface coverage used high antioxidant concentrations (e.g. Huang *et al.*, 1996 a, b and c ; Met *et al.*, 1999; Riberio *et al.*, 2003). However, there were a series of papers where maximal emulsion droplet coverage of a variety of antioxidants (tocopherols, Trolox, gallic acid and derivatives, hydroxytyrosol, TBHQ, BHT, carnosic acid and derivatives, rosmarinic acid and quercetin) was less than 10% (Malassagne, and Roozen, 1999; Alamed *et al.*, 2009; Huang *et al.*, 1996a, b and c; Mei *et al.*, 1999; Richards *et al.*, 2002; Chaiyasit *et al.*, 2005; Let *et al.*, 2007; Medina *et al.*, 2009; Belhaj *et al.*, 2010). In these studies, noticeable antioxidant activity was observed even though surface coverage was less than 10% and in some cases <1%. Calculations used in this exercise would be for the maximum potential coverage of an antioxidant in an emulsion droplet. In reality the coverage would be lower since molecular sizes could be smaller, the antioxidants would be competing for the surface with the surfactants used in these studies to stabilize the oil in water emulsion and the antioxidants would also have solubility in the oil and aqueous phases. The fact that such low levels of antioxidant coverage on the emulsion droplet interface calculated in this exercise can still provide substantial inhibition of lipid oxidation is somewhat surprising because free radical lifetime is very short (e.g. hydroxyl radicals ½ life = 10-6 to 10 -9 sec, Karogodina et al., 2009) and the antioxidants would have to diffuse and react with the free radical before it oxidizes fatty acids. While this could be possible if the majority of free radical production is at the emulsion droplet interface, it is also likely that inhibition of lipid oxidation is due to the ability of the remaining antioxidants to scavenge free radicals at other locations (e.g. lipid and/or water phases).

Partitioning of antioxidants in oil-in-water emulsions

Further insights into the impact of antioxidant properties on their efficacy can be obtained by considering emulsion composition and structure. In its simplest view, an oil-in-water emulsion can be considered to consist of at least three phases: oil, aqueous, and interfacial phases. The oil phase consists predominantly of hydrophobic molecules (such as triacylglycerols, cholesterol, and oil-soluble vitamins), whereas the aqueous phase consists mainly of water molecules and some polar solutes (such as hydrophilic salts, acids, bases, carbohydrates, or proteins). The interfacial layer mainly consists of surface-active molecules such as emulsifiers, which includes surfactants, proteins, polysaccharides, and phospholipids (McClements, 2015). In some systems the interfacial layer is predominantly located at the surface of the oil droplets, *e.g.,* for protein and polysaccharide emulsifiers. For other systems, the interfacial layer may be partly at the oil droplet surfaces but also partly in micelles or liposomes dispersed in the aqueous or lipid phases, *e.g.,* for surfactant or phospholipid emulsifiers (**Figure 3**). The partitioning of an antioxidant between water and lipid phases is described by an equilibrium partition coefficient (K), which depends on the thermodynamic affinity of the molecules for the two different phases. At relatively low antioxidant concentrations, the oil-water partition coefficient can conveniently be expressed as: $K_{OW} = c_O/c_W$, where c_O is the concentration of the antioxidant in the oil phase and c_W is the concentration in the water phase (McClements, 2015). The thermodynamic affinity of an antioxidant for the two phases depends on both entropy and enthalpy effects (Israelachvili, 2011). The entropy of mixing of an antioxidant favors an even distribution of the molecule throughout the system. On the other hand, an antioxidant may preferentially accumulate in a particular phase if there is a sufficiently large gain in the free energy associated with the molecular interactions in the system (i.e., reduction in unfavorable interactions and increase in favorable interactions). In emulsions, the most important type of molecular interaction is the

hydrophobic effect, which is associated with the fact that water-water interactions are much stronger than water-oil or oil-oil interactions (McClements, 2015). The system will try to adopt a configuration that minimizes the number of unfavorable contacts between non-polar groups and water molecules. If the magnitude of the hydrophobic effect is large enough to overcome the entropy of mixing effect, then the antioxidant may partition into a particular phase (such as the interface or oil phase). However, these simple oil-water partitioning tendencies are limited in emulsions because they do not account for the droplet interface.

The fraction of an antioxidant that accumulates at the oil-water interface in an emulsion can be described by the following equations, which are derived from a mass balance of the system:

$$(AO_I)/[AO_T] = \frac{K_{IW}K_{IO}}{K_{IW}\varphi_O + K_{IW}K_{IO}\varphi_I + K_{IO}\varphi_W} \quad (1)$$

or, after rearrangement:

$$(AO_I)/[AO_T] = \frac{K_{IW}}{K_{OW}\varphi_O + K_{IW}\varphi_I + \varphi_W} \quad (2)$$

Here, (AO_I) is the interfacial antioxidant concentration (mass per unit volume of interface), and [AO_T] is the total antioxidant concentration (mass per unit volume of emulsion), K_{IW}, K_{IO} and K_{OW} are the interface-water, interface-oil and oil-water equilibrium partition coefficients, and ϕ_O, ϕ_W and ϕ_I are the oil, water, and interfacial volume fractions ($\phi_O + \phi_W + \phi_I = 1$). It should be noted that the oil-water partition coefficient is given by: $K_{OW} = K_{IW} / K_{IO}$. Here it is assumed that the molecular species partitioning between the different phases are in a monomer form (rather than micelles). This equation predicts that the percentage of the antioxidant located at the interface will increase as the total antioxidant concentration increases, the interface-water

partition coefficient increases, or the volume fraction of the interface increases. As an example, the percentage of antioxidant located at the oil-water interface is predicted for a system that contains 10% oil, 1% interface, and 89% water, assuming antioxidants with different interface-water and oil-water partition coefficients (**Figure 4A**). The percentage of antioxidant at the interface was calculated using the expression: $\%\text{interface} = (AO_I)/[AO_T] \times \phi_I \times 100$. As expected, the predictions show that the fraction of antioxidant at the interface increases as K_{IW} increases. In addition, they show that at a fixed K_{IW} value the amount of antioxidant at the interface decreases as the K_{OW} value increases, which is because the antioxidant has a stronger tendency to go into the oil phase. In reality, the K_{IW} and K_{OW} values will both depend on the molecular structure of the antioxidant (and its tendency to form micelles), and therefore they are likely to be related. For a surface-active antioxidant, the concentration of antioxidant in the interfacial region (expressed as mass of antioxidant in the interfacial region per unit volume of interface) will decrease as the volume fraction of the interfacial region increases, even though the overall fraction of antioxidant in the system that is present in the interfacial region increases (expressed as mass of antioxidant in the interfacial region per unit volume of emulsion).

Experimentally, the oil-water partition coefficient of an antioxidant can be estimated by measuring the concentration of antioxidant in a bulk oil phase (c_O) and a bulk water phase (c_W) that have been left in contact for an extended period. On the other hand, the interfacial partition coefficients in the above equation are usually more difficult to measure directly because it is difficult to accurately determine the amount in the thin interfacial layer. Nevertheless, they can sometimes be measured using appropriate radioactive (Sengupta and Damodaran, 1998) or spectrophotometric active interfacial probes using the pseudo phase model (Losada-Barreiro *et al.*, 2015; Romsted and Bravo-Diaz, 2013). An alternative approach is to use the expression

309 $$K_{IW} = \frac{c_I}{c_W} = \frac{(AO_I)}{c_W} = \frac{\Gamma_I}{c_W \times \delta_I} \quad (3)$$

310 Where, c_I (=(AO_I)) is the antioxidant concentration in the interfacial region (expressed as mass
311 per unit volume of interface), c_W is the antioxidant concentration in the water phase (expressed
312 as mass per unit volume of water phase), Γ_I is the antioxidant concentration per unit surface area
313 (surface load) and δ_I is the thickness of the interface (approximately equal to the length of the
314 emulsifier molecules). The value of Γ_I can be estimated by measuring the interfacial tension
315 *versus* bulk antioxidant concentration curve (**Figure 4B**) (McClements, 2015). Nevertheless,
316 this may be difficult to do in the presence of an emulsifier because then the antioxidant may have
317 little impact on the measured interfacial tension because the emulsifier is much more surface
318 active.

319 *Adsorption of Antioxidants to Interfaces*

320 An alternative approach to describing the adsorption of antioxidants to interfaces is to
321 consider that there is an equilibrium between the adsorbed and non-adsorbed states of an
322 antioxidant. Consider a system that consists of a bulk liquid phase in contact with a surface. The
323 antioxidant may adsorb to the surface or it may be dispersed within the bulk liquid phase (**Figure**
324 **4B**). When the antioxidant level in the bulk liquid is increased, so does its surface concentration,
325 which causes a decrease in interfacial tension. The presence of an antioxidant at the surface
326 decreases the interfacial tension by reducing the thermodynamically unfavorable contacts
327 between the surface and bulk liquid molecules (Norde, 2011). As the antioxidant level is
328 increased, the interfacial tension decreases until it reaches a constant value because the surface
329 becomes saturated with antioxidant molecules. The tendency for a surface-active molecule (such
330 as some antioxidants) to adsorb to surfaces can be described using thermodynamic approaches,

such as the *Langmuir Adsorption Isotherm* (Hunter, 1986; Hiemenz and Rajagopalan, 1997; Norde, 2011). The *Langmuir adsorption isotherm* relates the level of a surface active species present at a surface to its concentration in the bulk liquid:

$$\theta = \frac{\Gamma}{\Gamma_\infty} = \frac{c/c_{1/2}}{1 + c/c_{1/2}} \quad (4)$$

where, θ is the fraction of adsorption sites for the antioxidants at the surface that are occupied, Γ_∞ is the surface excess concentration of the antioxidant when the surface is completely saturated, and $c_{½}$ is the antioxidant level in the bulk liquid when half the adsorption sites are occupied, i.e., θ = ½. The equilibrium constant for adsorption ($K = 1/c_{½}$) provides a convenient indication of the *surface activity* or *binding affinity* of an antioxidant to a surface: the greater $1/c_{½}$ the higher the binding affinity. The surface activity of an antioxidant is related to the free energy of adsorption (McClements, 2015):

$$K = \frac{1}{c_{1/2}} = \exp\left(-\frac{\Delta G_{ads}}{RT}\right) \quad (5)$$

where ΔG_{ads} corresponds to the free energy change associated with exchanging a solvent molecule with an antioxidant molecule at the surface. The more negative is the free energy change associated with antioxidant adsorption (ΔG_{ads}), the higher is the affinity of the antioxidant for the surface. The adsorption free energy is mainly comprised of molecular interaction and entropy of mixing contributions. The molecular interaction contributions are associated with net changes in the overall magnitude of the interaction energies associated with adsorption, *e.g.,* van der Waals, electrostatic, steric, hydrogen bonding, and hydrophobic interactions. The entropy of mixing contributions are mainly due to changes in the number of ways the antioxidant molecules

can be arranged in the system in the non-adsorbed and adsorbed states, *e.g.*, configuration and orientation entropies (Norde, 2011). Typically, the entropy of mixing effect opposes the adsorption of an antioxidant to a surface, since this reduces the number of configurations it can have in the system. Hence, a compensating molecular interaction (such as the hydrophobic effect) is needed to overcome this effect.

Predictions made using the Langmuir adsorption equation for antioxidants with low and high binding affinities (surface activities) are shown in **Figure 5A**. Initially, the amount of adsorbed antioxidant (Γ) increases with increasing antioxidant concentration in the bulk solution (c), but then reaches a constant level (Γ_∞) when the surface becomes saturated with antioxidant. In practice, saturation may not occur because there is not enough antioxidant present, or it is not surface-active enough. These predictions show that antioxidants vary in their tendency to adsorb to surfaces depending on their molecular characteristics, such as molecular weight and polarity.

An advantage of this approach is that the surface activity ($1/c_{½}$) of an antioxidant can often be determined from measurements of the interfacial tension versus antioxidant concentration in the bulk phase (**Figure 5A**). These values are then converted into a plot of surface pressure versus antioxidant concentration, where surface pressure is the difference in interfacial tension in the absence and presence of antioxidant. The $c_{½}$ value is taken to be the antioxidant concentration where the surface pressure is half of its value at the saturation level.

Using this approach an antioxidant can be described by three parameters: (i) the surface activity; (ii) the surface pressure at saturation; and, (iii) the surface load. The surface activity is an indication of how strongly the antioxidant binds to the surface, whereas the surface pressure at saturation is a measure of how effective the antioxidant is at reducing the interfacial tension.

373 The surface load is a measure of the mass of antioxidant per unit surface area, and can be derived
374 from measurements of the interfacial tension versus bulk antioxidant concentration (**Figure 5A**).

375 *Competitive Adsorption of Antioxidants and Emulsifiers*

376 One of the problems with predicting the amount of antioxidant that is present at an oil-water
377 interface is that there may be numerous different kinds of surface-active species present in an
378 emulsion that all compete for the same interface. In this case, the interfacial composition will
379 depend on the relative concentrations and surface activities of the different species. In particular,
380 a surface-active antioxidant may compete at the oil-water interface with an emulsifier. If it is
381 assumed that the antioxidant and emulsifier molecules compete for the interface, and that they
382 both have similar molecular dimensions, then the relative concentrations of the antioxidant and
383 emulsifier can be given by the following expressions (McClements, 2015):

384 $$\frac{\Gamma_1}{\Gamma_{TOT}} = \frac{c_1 / c_{1,1/2}}{c_1 / c_{1,1/2} + c_2 / c_{2,1/2}} \quad (6a)$$

385 $$\frac{\Gamma_2}{\Gamma_{TOT}} = \frac{c_2 / c_{2,1/2}}{c_1 / c_{1,1/2} + c_2 / c_{2,1/2}} \quad (6b)$$

386 Here c_1 and c_2 are the concentrations of the antioxidant and emulsifiers in the bulk solution, Γ_1
387 and Γ_2 are the surface excess concentrations of the antioxidants and emulsifiers at the interface,
388 $\Gamma_{1,\infty}$ and $\Gamma_{2,\infty}$ are the surface excess concentrations at saturation, Γ_{TOT} is the surface excess
389 concentration (antioxidants + emulsifiers) at the interface, and $c_{1,½}$ and $c_{2,½}$ are the antioxidant
390 and emulsifier concentrations where $\Gamma_1/\Gamma_{1,\infty}$ and $\Gamma_2/\Gamma_{2,\infty}$ = ½. It is assumed that solvent,
391 antioxidant, and emulsifier molecules have the same dimensions in deriving these expressions.
392 The above equations predict that the concentration of the antioxidant at the interface will
393 decrease as its concentration and surface activity decrease relative to that of the emulsifier

394 molecules. An example of this effect is shown in **Figure 5B**. As more emulsifier is added to
395 the system the amount of antioxidant present at the interface decreases.

396 In reality, the solvent, antioxidant, and emulsifier molecules do not have similar
397 molecular dimensions, and so the above equations will not be accurate. In particular, small
398 antioxidant molecules may be able to penetrate between the head or tail groups of the emulsifier
399 molecules, and therefore not directly compete with the emulsifiers. Moreover, the presence of
400 the antioxidants may change the optimum curvature and interfacial tension of the droplet
401 surfaces. This competition between antioxidants and emulsifiers is currently poorly understood,
402 and often ignored in studies of the role of antioxidants in emulsions. This is an important area of
403 research for future studies.

404 Another factor that may influence the level of antioxidants at the surfaces of the droplets
405 in emulsions is when an antioxidant can bind to other molecules in the system, such as proteins
406 or polysaccharides. This binding can either increase or decrease the concentration of the
407 antioxidant at the interface depending on whether the proteins or polysaccharides are at the
408 interface or in the aqueous phase.

409 ## Are all emulsion interfaces equal?

410 *Lipid droplet surfaces*

411 Little is actually known about the composition and structural organization of the
412 interfacial regions surrounding most lipid droplets. Textbook representations of an emulsion
413 droplet interface show a very simple monolayer of surfactants surrounding the lipid phase.
414 However, in commercial food oils there are many surface active components (*e.g.* proteins,
415 polysaccharides, phospholipids, free fatty acids, mono- and diacylglycerols, plant sterols and
416 antioxidants) and all of these surface active components will compete for the lipid droplet

417 interface. This could mean that while an antioxidant is associated with the interface, its location
418 and properties may vary considerably depending on the nature of the emulsifiers and other
419 surface active components present. For example, the antioxidants could be located on the oil
420 side, the water side, or within the emulsifier layer itself depending on emulsifier type. Moreover,
421 the chemical reactivity of the antioxidant may be altered depending on the precise nature of its
422 local molecular environment. The physiochemical properties of the interfacial region, and
423 therefore the activity of an antioxidant located there, may therefore vary appreciably depending
424 on the type of emulsifiers used. As an example, polysorbates have large neutral hydrophilic head
425 groups, phospholipids have zwitterionic head groups, and proteins contain a variety of positive,
426 negative, and neutral amino acids (some of which have antioxidant properties). The impact of
427 emulsifier type on antioxidant efficacy is further complicated by the fact that emulsifiers also
428 impact the activity of prooxidants. For instance, negative emulsifiers can bind and increase the
429 reactivity of transition metals (Mancusco *et al.*, 1999) and emulsifiers that create thick interfacial
430 layers can decrease lipid oxidation rates by forming steric barriers (Silvestre *et al.*, 2000). By
431 altering the activity of prooxidants, emulsifiers could also impact the activity of antioxidants by
432 decreasing the ability of an antioxidant to reduce metals or by changing the location of where
433 lipid hydroperoxides decompose thus altering the ability of an antioxidant to scavenge free
434 radicals. Mei and coworkers (1999) found that gallate derivatives were less effective at inhibiting
435 lipid oxidation in SDS- than Brij-stabilized emulsions, presumably due to the higher reactivity of
436 iron in the anionic SDS-stabilized emulsions. Stockman *et al.* (2000) found that both the charge
437 of the emulsifier and the ability of the emulsifier to bind to methyl gallate and gallic acid altered
438 antioxidant partitioning in oil-in-water emulsions. Di Mattia and coworkers (2011) found that
439 syringic acid, tyrosol and oleuropein had similar ability to inhibit lipid hydroperoxide formation

in Tween 20 or whey protein concentrate (WPC) stabilized emulsions while these antioxidant were more effective in WPC stabilized emulsions at inhibiting the formation of thiobartiburic acid reactive substances. Further work is clearly needed to systematically study how emulsifier type impacts interfacial properties and antioxidant efficacy.

Micelles

Another complicating factor to using the antioxidant polar paradox and the pseudo phase model to predict antioxidant efficacy in emulsions is the impact of surfactant micelles on antioxidant activity. Emulsions formed using small molecule surfactants often contain a considerable amount of non-adsorbed surfactants in the aqueous phase, and these can assemble into small colloidal particles (micelles) when their concentration exceeds a critical value (**Figure 3**). The surfactant molecules in micelles organize so that their non-polar tails are located in the interior (away from water) and their polar head groups are located at the exterior (in contact with water). Amphiphilic or non-polar antioxidants can be solubilized within surfactant micelles due to the hydrophobic effect. Micelles are highly dynamic structures that can rapidly exchange components (such as lipid hydroperoxides or antioxidant molecules) in an oil-in-water emulsion by interacting with other micelles, the aqueous phase, or the lipid droplets (McClements, 2015). The rates of these exchanges are fast (seconds or minutes) when compared to the time it takes most foods to oxidize (days). Interdroplet molecular exchanges have been evaluated using 2 different Tween 20-stabilized emulsions made with octadecane and hexadecane. Upon mixing of the two different emulsions, surfactant micelles were able to transport hydrocarbon molecules between droplets with mixing observed after 24 h of incubation (McClements et al., 1992). Even more rapid exchanges (<10 min) have been observed with hydrophobic fluorescent dyes in toluene or octane emulsions stabilized by Triton X-100 (Malassagne-Bulgarelli and McGrath

2013). However, not all lipids can be transferred between lipid droplets. Coupland *et al.* (1996) found that while hydrocarbons (hexadecane or octadecane) can be transferred between lipid droplets by surfactant micelles, triacylglycerols (corn oil) cannot, presumably because the larger molecular dimensions of triacylglycerols do not allow them to be incorporated into micelles. In addition, saturated and unsaturated oils did not mix together in mayonnaises containing surfactant micelles (Raudsepp *et al.*, 2014), confirming the fact that triacylglycerol molecules cannot be rapidly transferred from one droplet to another. However, the ability of micelles to solubilize non-polar components would be dependent on the system being studied.

The ability of surfactant micelles to transfer components between lipid droplets could theoretically both increase and decrease lipid oxidation rates. Increased oxidation rates could be caused by surfactant micelle transfer of prooxidative factors between droplets. For example Raudsepp *et al.* (2014) showed that radicals generated in the interior of lipid droplets can contribute to the initiation of autoxidation in neighboring droplets in the presence of surfactant micelles. In addition, surfactant micelles can solubilize lipid hydroperoxides (Nuchi *et al.*, 2002) and metals (Cho *et al.*, 2002) out of lipid droplets. Solubilization of prooxidants out of lipid droplets could inhibit lipid oxidation by partitioning them away from the unsaturated fatty acids or it could promote oxidation by allowing an individual lipid droplet that is undergoing oxidation to transfer prooxidative factors to a second droplet.

Surfactant micelles can also impact antioxidant activity. Rosmarinic acid esters have been reported to have maximal antioxidant activity in oil-in-water emulsions when attached to a hydrocarbon tail with 8 carbons (Laguerre *et al.*, 2010). The 18 and 20 carbon esters have almost no antioxidant activity despite having essentially the same partitioning in the lipid droplets as the 8 carbon ester (Panya *et al.*, 2012). Since all the esters have similar free radical

scavenging activities due to having the same rosmarinic acid head group, this suggested that the most non-polar esters where not partitioning in the same location as the 8 carbon ester thus decreasing their efficacy (Laguerre *et al.*, 2010). When excess surfactant was added to the emulsion to produce aqueous phase surfactant micelles, the concentration of all esters in the aqueous phase increased indicating that the micelles were able to solubilize the esters out of the lipid droplets (Panya *et al.*, 2012). One would expect this to decrease antioxidant activity as the antioxidants would be partitioning away from the unsaturated triacylgycerols which are thought to be the source of free radical formation. However, when surfactant micelles were added to an emulsion containing 20 carbon esters, the aqueous phase C20 ester concentrations increased as expected but unexpectedly antioxidant activity dramatically increased even though less antioxidant was in the lipid droplets (Panya *et al.*, 2012). Similar trends were also observed for tocopherols with increasing Tween 20 concentration, increasing aqueous phase tocopherol concentrations and antioxidant activity (Kiralan *et al.*, 2014). Increasing surfactant micelle concentrations in oil-in-water emulsions, where the oil had been stripped of its endogenous antioxidants, had minimal impact on lipid oxidation rates. Oehlke and coworkers (2010) also found that partitioning of ferulic acid and isoferulic acid into surfactant micelles seemed to increase antioxidant activity in oil-in-water emulsions produced with SDS and Brij 58. Earlier research showed that the addition of Brij micelles to oil-in-water emulsions decreased lipid oxidation rates (Richards *et al.*, 2002). Originally, this was thought to be due to removal of prooxidant factors such as hydroperoxides (Nuchi *et al.*, 2002) and metals (Cho *et al.*, 2002) from the lipid droplets but in hindsight, this was likely due to solubilization of tocopherols into the aqueous phase micelles since the oils in this research were not stripped of their tocopherols. While antioxidants could be at the interface or interior of a micelle, it is unclear how this would

improve their activity compared to when they are partitioned into the lipid droplets. One potential hypothesis is that the antioxidants in the micelles could act as a reservoir to replace oxidized antioxidants in the lipid droplets since micelles can rapidly exchange their contents with lipid droplets (Laguerre *et al.*, 2015). Another possibility is that antioxidants in surfactant micelles could inactivate aqueous phase free radicals originating from hydrogen peroxide or surfactant peroxides. However, very little is known about the exact structure of micelles and other physical structures that reside outside of the emulsion droplets. More work is need to study these structures by isolation via molecular sieving techniques or noninvasive techniques such as NMR and fluorescent probes.

Reverse Micelles

Refined oils contain surface active compounds such as phospholipids, free fatty acids, mono- and diacylglycerols, sterols and antioxidants. These surfactants in combination with water have been shown to form association colloids in bulk oils, such as reverse micelles and bilayers (for review see Chen *et al.*, 2011; Chaiyasit *et al.*, 2007). These structures have a water-lipid interface that can influence the partitioning and activity of both prooxidants and antioxidants in bulk oils. For example, the more polar Trolox can partition into association colloids more than α-tocopherol which could explain its strong antioxidant efficacy in bulk oils (Chen et al., 2011).

It is unknown if association colloids maintain their structure once the oil is converted to an oil-in-water emulsion. In oil-in-water emulsions, the association colloids could be lost as the surface active compounds migrate to the surfaces of the lipid droplets, conversely they could persist and change their structures if the emulsifier used to produce the emulsion prevents them from migrating to the lipid droplet surfaces or if some of the emulsifier and water migrate to the interior of the lipid droplets. Almost nothing is known about the existence and influence of

association colloids on antioxidant activity in oil-in-water emulsions but theoretically, association colloids could change the physical location of antioxidants thereby altering their efficacy.

Droplet size effects

An additional factor that has not been widely studied in lipid oxidation is the fact that not all emulsion droplets are the same in food dispersions. Commercially and experimentally, oil-in-water emulsions are made by a variety of techniques including high pressure homogenizers, microfluidizers, sonicators, colloidal mills and simple blenders (McClements, 2015). These different homogenizers, how they are operated (e.g. pressure, time) and variations in emulsifier type and concentrations can produce emulsions with a wide variety of droplet sizes and polydisperities (**Figure 6**). Most studies have shown that lipid droplet size only has a minimal impact on lipid oxidation rates (Gohtani *et al.*, 1999; Hegenauer *et al.*, 1979; Lethuaut *et al.*, 2002, Nakaya *et al.*, 2005, Imai *et al.*, 2008; Coupland *et al.*, 1996; Osborn and Akoh, 2004; Dimakou *et al.*, 2007; Kiokias *et al.*, 2007; Paraskevopoulou *et al.*, 2007; Sun and Gunasekaran, 2009). However, the majority of these papers used homogenization techniques that produce polydisperse emulsions and thus contain both large and small droplets. In polydisperse emulsions, the heterogeneity of the droplets could impact lipid oxidation differently. Since the small droplets have much larger surface area, it is possible that they could oxidize faster than the large droplets if surface area is limiting the ability of reacts (e.g. hydroperoxides and transition metals) to interact. However, one of the above papers used a membrane homogenizer which produces very monodisperse emulsion droplets (Nakaya *et al.*, 2005). Interestingly, in this research the small droplets (0.83 and 1.1 µm) were more oxidatively stable than the large droplets (9.8 µm) suggesting that surface area was not the predominant factor in determining

555 oxidative stability. The increased oxidative stability of the small droplets could be due to factors
556 such as different interfacial properties than the large droplets as the curvature of the droplet
557 surface and the surfactant packing could be different. This could impact the ability of
558 antioxidants and hydroperoxides to partition at the interface thus either increasing antioxidant
559 efficacy or decreasing prooxidant activity. Alternatively, there may have been less prooxidants
560 per droplet in the smaller droplets or the large surface area could dilute the concentration of
561 reactants at the interface making interactions between hydroperoxides and metals less likely.
562 Consequently, once oxidation starts in one droplet it may rapidly propagate through an emulsion
563 containing fewer larger droplets, but not through one containing many smaller droplets. More
564 research is required to establish the potential differences in the physicochemical properties of
565 small versus large emulsions and to determine whether this is an important factor in lipid
566 oxidation and antioxidant effectiveness.

567 ## Conclusions

568 In the antioxidant polar paradox approach, an emulsion is considered as having a lipid core (1),
569 surfactant interface (2) and aqueous phase (3) as shown in **Figure 3**. This approach does not
570 consider differences that might impact antioxidant activity if oxidation mechanisms are different
571 in droplets of different sizes (1 vs 4) or if antioxidants partition into other interfacial structures
572 such as aqueous phase surfactant micelles (5) and lipid phase association colloids (6). In an
573 attempt to improve upon the antioxidant polar paradox, a pseudo phase approach has been taken
574 to directly measure antioxidants location and then attempt to link the location to antioxidant
575 efficacy (Romsted and Bravo-Diaz, 2013; Costa et al., 2016; 2017; Almeida et al., 2016). This is
576 an interesting technique to measure the location of different antioxidants in emulsions since
577 measurement can be made directly in an opaque emulsion without need to physically separate the

578 lipid droplets and aqueous phases, which could alter droplet properties. However, this technique
579 is limited in that it cannot exactly determine if the antioxidant is embedded in the droplet
580 interface (2) or could be in other locations such as just below the water-droplet interface or
581 physically absorbed to the surface of the emulsion droplet interface. In addition, it cannot
582 differentiate antioxidants in the interfacial layer (2) of an emulsion droplet or in lipid phase
583 aqueous phase surfactant micelles (5) or lipid phase association colloids (6) since the probe will
584 partition in all emulsion phases that form physical structures. The pseudophase method is a large
585 improvement over other methods in that direct measurement of antioxidants associated with
586 surfactants can be made. However, as suggested by Genot (2015), it must be correlated with
587 antioxidant efficacy in interfaces with different properties (e.g. mixed emulsifiers, interfacial
588 layers of different thicknesses and viscosity, interfacial layers in small vs large emulsion
589 droplets). In addition, more work is need to determine where interfacial antioxidant
590 concentrations determined by the pseudophase method correlate with antioxidant efficacy. If the
591 method is versatile enough to measure antioxidant interfacial location in real food emulsions, this
592 could provide important information of how complex emulsion droplet interfaces with multiple
593 emulsifiers impact antioxidant efficacy.

594

595 While compounds with potential antioxidant activity are widespread in nature, it continues to be
596 surprising that many more natural solutions to control spoilage by rancidity are not available. To
597 successfully identify effective antioxidants that can be commercialized, knowledge is needed on
598 their free radical scavenging activity and physical partitioning in addition to other factors that
599 impact lipid oxidation kinetics and antioxidant efficacy. Thus more research is needed in areas
600 such as:

1. How do surfactant micelles alter the effectiveness of antioxidants?

2. Do reverse micelles/association colloids exist in the lipid phase of oil-in-water emulsions, and if they do what is their structure and how do they impact antioxidant efficacy?

3. How do different emulsifiers and emulsifier combinations impact the physiochemical properties of the lipid droplet interface and how do these properties impact the location and reactivity of both prooxidants and antioxidants?

4. In polydisperse oil-in-water emulsions, do small droplets oxidize at a different rate than large droplets? Also, do small droplets have different physicochemical properties that can alter the ability of antioxidants and prooxidants to impact lipid oxidation kinetics?

5. It would seem unlikely that lipid oxidation would occur simultaneously in all droplets. If this is true, is rancidity due to the oxidation of a subset of the entire emulsion droplet population? Alternately, when oxidation occurs in one droplet, can these reaction products be transferred to other droplets eventually causing oxidation in the entire population? Depending on which occurs, can antioxidants be targeted to the most susceptible droplet population or can the transfer of oxidation products between droplets be inhibited to slow oxidation?

Due to the complex nature of lipid oxidation in emulsions, it is highly unlikely that one free radical scavenging antioxidant will be effective in all foods. With expanded knowledge of the factors that influence lipid oxidation and antioxidant activity in research areas such as those describe above, it will be easier to predict which type of antioxidant properties relate to efficacy in real food systems. This in turn could provide more opportunities to utilize natural antioxidants to prevent spoilage in foods.

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Table 1. Calculated surface coverages of antioxidants (AO) in oil droplets in oil-in-water emulsions using examples from the literature.

AO name	AO concentration (µM)	Particle size (µm)	Fat content (wt %)	Size AO molecule (nm2)	ratio AO/oil surface (%)	References
hydroxytyrosol acetate	5	0.1–0.25	0.1	0.82	0.07	Medina et al. (2009)
octyl gallate	4	0.1–0.25	0.1	0.43	0.04	Medina et al. (2009)
hydroxytyrosol acetate	51	0.1–0.25	0.1	0.43	0.59	Medina et al. (2009)
octyl gallate	35	0.1–0.25	0.1	0.43	0.41	Medina et al. (2009)
hydroxytyrosol acetate	102	0.1–0.25	0.1	0.43	1.19	Medina et al. (2009)
octyl gallate	71	0.1–0.25	0.1	0.43	0.83	Medina et al. (2009)
α-tocopherol	151	0.1-0.25	10	0.66	1.74	Huang et al. (1996a)
trolox	15	0.1-0.25	10	0.66	0.18	Huang et al. (1996a)
α-tocopherol	302	0.1-0.25	10	0.66	3.49	Huang et al. (1996a)
trolox	30	0.1-0.25	10	0.66	0.35	Huang et al. (1996a)
α-tocopherol	302	0.17-0.25	10	1.65	10.50	Huang et al. (1996b)
trolox	304	0.17-0.25	10	1.65	10.56	Huang et al. (1996b)
α-tocopherol	1161	0.17-0.25	10	1.65	40.37	Huang et al. (1996b)
trolox	1175	0.17-0.25	10	1.65	40.85	Huang et al. (1996b)
quercetin	331	0.2	10	1.09	7.46	Belhaj et al. (2010)
methyl carnosate	144	0.2-0.25	10	0.70	1.67	Huang et al. (1996c)
α-tocopherol	151	0.2-0.25	10	1.65	8.20	Huang et al. (1996c)
carnosic acid	301	0.2-0.25	10	1.26	6.25	Huang et al. (1996c)
methyl carnosate	289	0.2-0.25	10	0.70	3.48	Huang et al. (1996c)
α-tocopherol	302	0.2-0.25	10	1.65	8.20	Huang et al. (1996c)
alpha or delta tocopherol	25	d3,2=0.26-0.28	5	1.65	1.12	Chaiyasit et al. (2005)
butylated hydroxyltoluene (BHT)	25	d3,2=0.26-0.28	5	0.43	0.29	Chaiyasit et al. (2005)
alpha or delta tocopherol	140	d3,2=0.26-0.28	5	1.65	6.26	Chaiyasit et al. (2005)
butylated hydroxyltoluene (BHT)	140	d3,2=0.26-0.28	5	0.43	1.63	Chaiyasit et al. (2005)
tertiary butylhydroquinone (TBHQ)	116	diameter 0.3-0.4	5	0.28	1.14	Richards et al. (2002)
gallic acid	50	d43=0.38 ± 0.1	5	0.33	1.24	Alamed et al. (2009)
propyl gallate	50	d43=0.38 ± 0.1	5	0.64	2.43	Alamed et al. (2009)
tertiary butylhydroquinone (TBHQ)	50	d43=0.38 ± 0.1	5	0.28	1.06	Alamed et al. (2009)
rosmarinic acid	50	d43=0.38 ± 0.1	5	2.41	9.18	Alamed et al. (2009)
propyl gallate	20	d[3,2]=0.48-1.36	1 wt % milk fat; 0.5 wt % fish oil	0.64	2.42	Let et al. (2007)
gallic acid	5	0.94-1.01	2	0.33	7.95	Mei et al. (1999)
methyl gallate	5	0.94-1.01	2	0.40	9.71	Mei et al. (1999)

gallamide (3,4,5-trihydroxybenzamide)	5	0.94-1.01	2	0.35	8.60	Mei et al. (1999)
Gallic acid	500	0.94-1.01		0.33	79.51	Mei et al. (1999)
Methyl gallate	500	0.94-1.01	2	0.40	97.07	Mei et al. (1999)
gallamide (3,4,5-trihydroxybenzamide)	500	0.94-1.01	2	0.35	89.05	Mei et al. (1999)
tocopherols	232		18	1.65	30.33	Serfert et al. (2009)
ascorbyl palmitate	241		18	1.80	34.45	Serfert et al. (2009)
lecithin	136		18	0.56	6.04	Serfert et al. (2009)
tocopherols	2322	Various Oil droplet size, 50th percentile max toco=1.42	18	1.65	303.33	Serfert et al. (2009)
ascorbyl palmitate	2412		18	1.80	344.52	Serfert et al. (2009)
lecithin	1362		18	0.56	60.39	Serfert et al. (2009)

Figure Legends:

Figure 1. Differences in propanal formation (mmol/kg lipid) in emulsified and bulk algae oil.

Figure 2. Proposed lipid oxidation mechanism in oil-in-water emulsions consisting of an oil phase, water phase, and interfacial region (which is usually covered with emulsifier).

Figure 3. The physical location of water-oil interfaces in an oil-in-water emulsion. 1 = oil phase; 2 = emulsion droplet interface; 3 = aqueous phase; 4= small emulsion droplet; 5 = aqueous phase surfactant micelles; 6 = lipid phase surfactant reverse micelles.

Figure 4. A: The calculated amount of antioxidant at a surface for emulsions containing 10% oil, 1% interface, and 89% water as a function of interface-water and oil-water interface. B: Schematic diagram of the change in interfacial tension with increasing concentration of a surface-active antioxidant or emulsifier.

Figure 5. A: Comparison of the change in fractional surface coverage with the concentration of a highly surface active (c½ =0.25) and weakly surface active (c½ =10) substance. B: Schematic diagram of the change in interfacial composition at the oil-water interface when two different surface-active substances at present at different levels.

Figure 6. An example of an oil-in-water emulsion consisting of oil droplets (red) dispersed in an aqueous medium (black).

Figure 1.

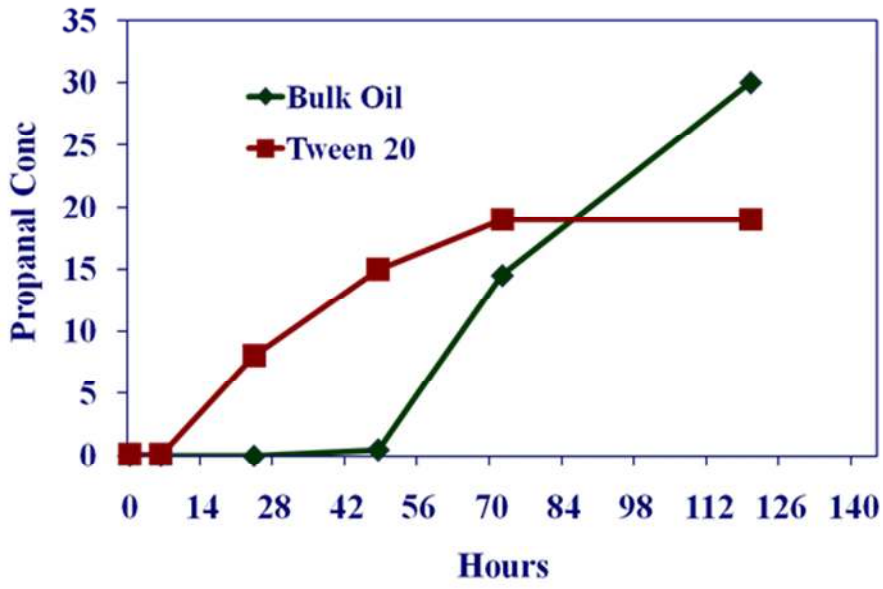


Figure 2

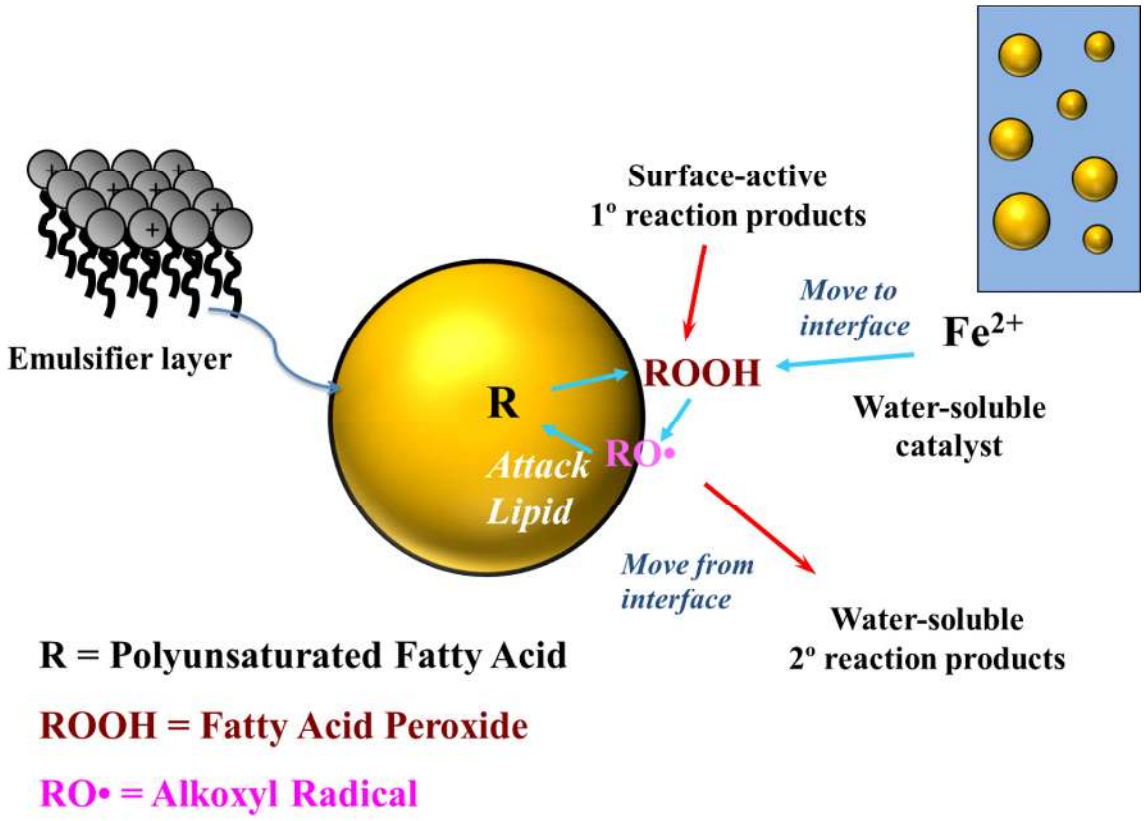


Figure 3

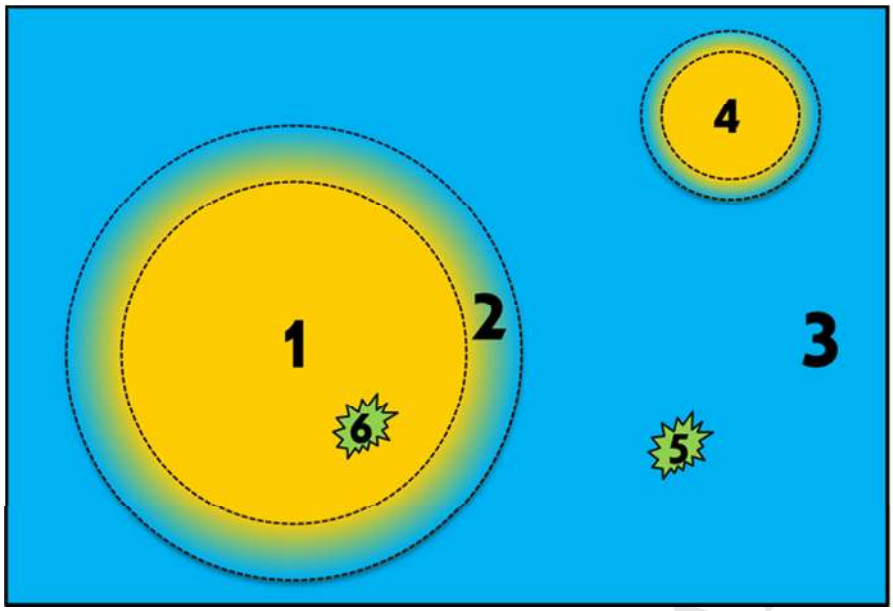
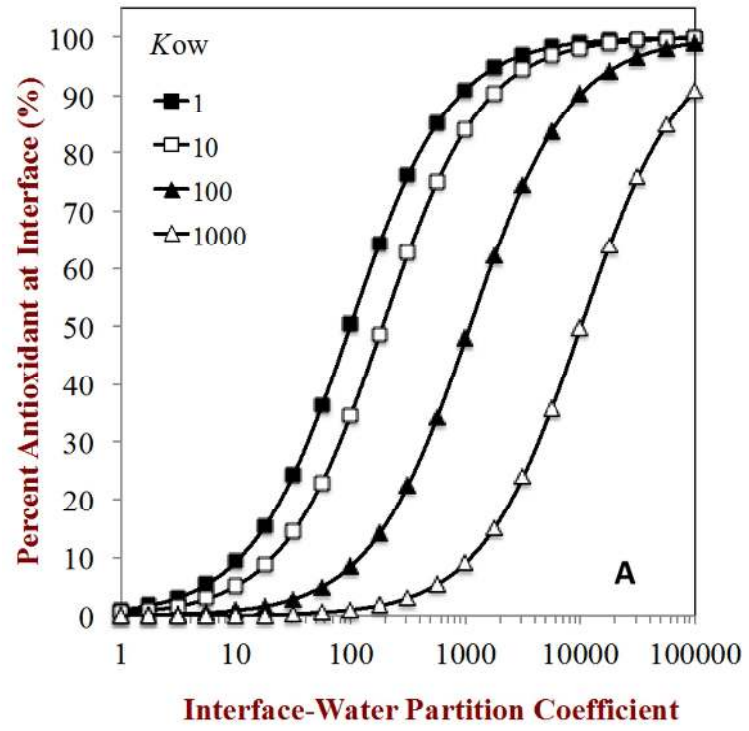


Figure 4



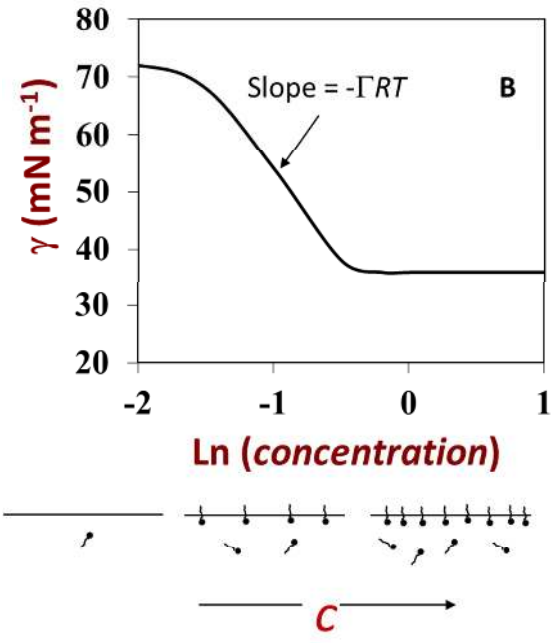


Figure 5

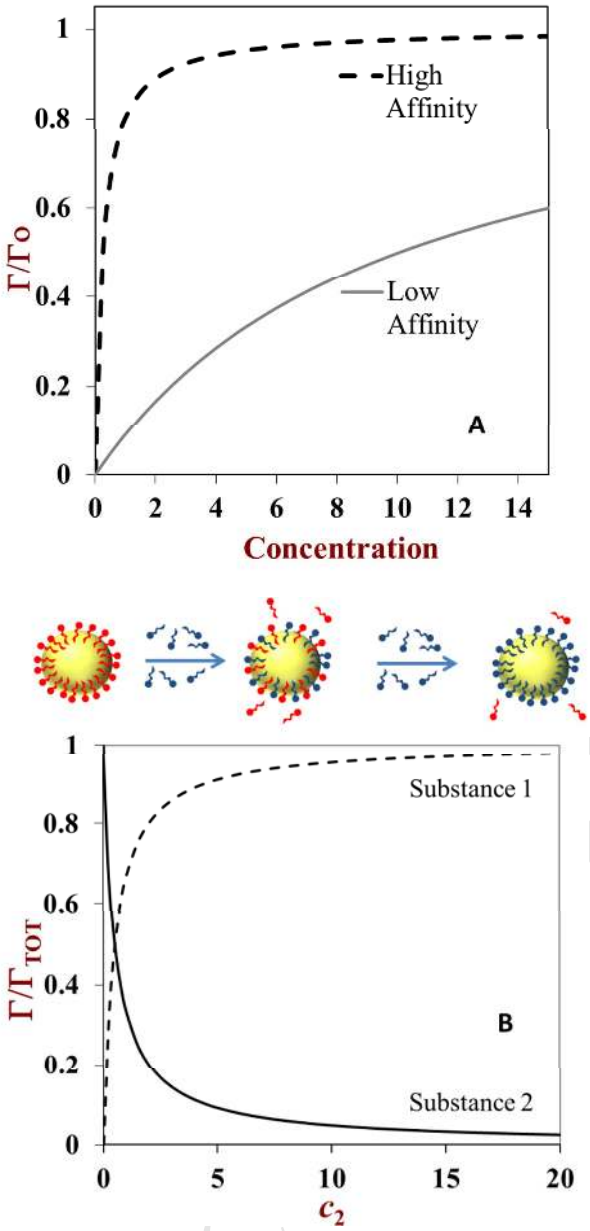
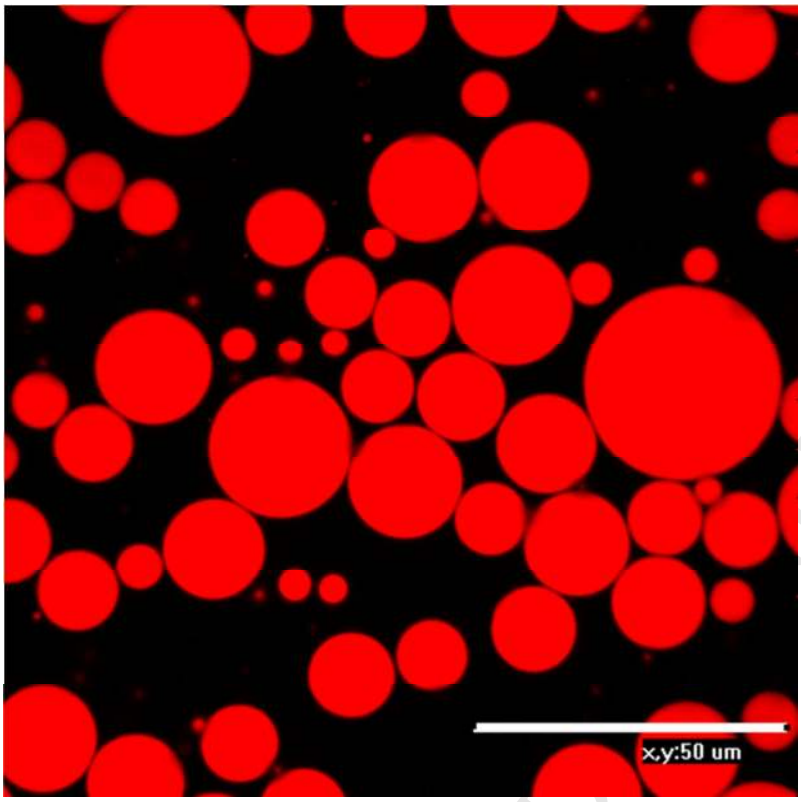


Figure 6



Highlights for “Hurdles in Predicting Antioxidant Efficacy in Oil-in-Water Emulsions”

1. Despite thousands of published papers on antioxidants, very few new antioxidants have actually be commercialized. This is because many research protocols evaluate antioxidants in manners that do not predict their efficacy in foods.

2. Discussion on factors besides free radical scavenging that can impact the efficacy of antioxidant in foods emulsions. For example, interfacial coverage of antioxidants, antioxidant interaction with other food components and antioxidant partitioning into multiple physical locations.

3. Knowledge gaps were identified and presented as future research directions including oxidation of different sized droplet in multidisperse emulsions, transfer of antioxidants and prooxidant between emulsion droplets, role of surfactant micelles on antioxidant activity and impact of emulsifiers on antioxidant location and prooxidant activity.