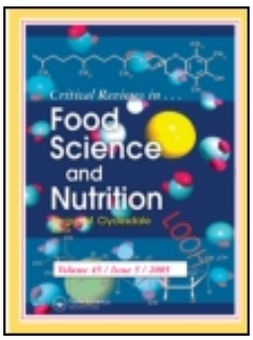

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What Makes Good Antioxidants in Lipid-Based Systems? The Next Theories Beyond the Polar Paradox

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The polar paradox states that polar antioxidants are more active in bulk lipids than their nonpolar counterparts, whereas nonpolar antioxidants are more effective in oil-in-water emulsion than their polar homologs. However, recent results, showing that not all antioxidants behave in a manner proposed by this hypothesis in oil and emulsion, lead us to revisit the polar paradox and to put forward new concepts, hypotheses, and theories. In bulk oil, new evidences have been brought to demonstrate that the crucial site of oxidation is not the air-oil interface, as postulated by the polar paradox, but association colloids formed with traces of water and surface active molecules such as phospholipids. The role of these association colloids on lipid oxidation and its inhibition by antioxidant is also addressed as well as the complex influence of the hydrophobicity on the ability of antioxidants to protect lipids from oxidation. In oil-in water emulsion, we have covered the recently discovered non linear (or cut-off) influence of the hydrophobicity on antioxidant capacity. For the first time, different mechanisms of action are formulated in details to try to account for this nonlinear effect. As suggested by the great amount of biological studies showing a cut-off effect, this phenomenon could be widespread in dispersed lipid systems including emulsions and liposomes as well as in living systems such as cultured cells. Works on the cut-off effect paves the way for the determination of the critical chain length which corresponds to the threshold beyond which antioxidant capacity suddenly collapses. The systematic search for this new physico-chemical parameter will allow designing novel phenolipids and other amphiphilic antioxidants in a rational fashion. Finally, in both bulk oils and emulsions, we feel that it is now time for a paradigm shift from the polar paradox to the next theories.

Keywords antioxidant, hydrophobicity, phenolipid, polar paradox, bulk oil, association colloids, emulsions, cut-off effect, critical chain length, self-aggregation hypothesis

INTRODUCTION

Unsaturated lipid substrates are prime targets of oxidation in foods and their oxidation leads to quality deterioration and could have harmful effects on health. This is especially important to the foods which have become more oxidatively liable in recent years with the removal of hydrogenated fats and addition of more unsaturated fatty acids, such as the highly oxidation-sensitive ω-3 fatty acids, to improve nutritional content. Thus, lipid oxidation is a major concern for agrifood industries more now than ever. A variety of mechanisms have been proposed to be responsible for the oxidation of lipid-based products during processing and storage, with photosensitized oxidation, metal-promoted and autoxidation being the most well-known. Some factors that impact the oxidative stability of lipid products include: oil extraction and processing conditions, light exposure, temperature, fatty acid composition, oxygen levels, presence of minor components and association colloids, morphology, size, surface charge and thickness of lipid-water interfaces, electrostatic repulsions/attractions, viscosity, partitioning and diffusion of oxygen or transient metals towards reaction centers, etc.

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(Laguerre et al., 2007; Waraho et al., 2011). Preventing or inhibiting the oxidation of lipid-based products by manipulating these factors is, therefore, of great importance to consumers and industry.

Besides these factors, one of the most effective ways of inhibiting lipid oxidation is to incorporate antioxidants. The very concept of antioxidant involves the notion of protecting a substrate (here lipids) from oxidation (Laguerre et al., 2010a). Antioxidant activity can be conveyed via many different pathways such as proxidant enzyme inhibition, singlet oxygen deactivation, UV filtration, enzymatic detoxification of reactive oxygen species (ROS), chelation of transition metals, as well as ROS stabilization through hydrogen radical transfer. In food, antioxidant molecules have been proven to efficiently prevent or delay lipid oxidation if they are utilized in proper conditions (concentrations ranges, low level of iron, etc.). However, due to regulatory issues, the number of antioxidants available to food manufacturers to control oxidative rancidity is limited and the approval of new antioxidants is challenging due to economic barriers in obtaining government approval for new food additives (Chaiyasit et al., 2007). To complicate matters further, there is a growing concern about the use of synthetic antioxidants, such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) since these compounds may be carcinogenic (Iverson, 1995). Therefore, to overcome the challenge of developing consumer acceptable foods (e.g., no *trans* fatty acids or synthetic food additives) with nutritionally significant amounts of unsaturated fatty acids, the food industry will have to develop new efficient antioxidant technologies derived from natural sources.

Nowadays, a myriad of natural antioxidant compounds are known such as carotenoids, tocopherols and tocotrienols, ascorbic acid, flavonoids, phenolic acids and alcohols, and stilbenes. These antioxidants mainly come from plants. The quest for natural antioxidants has become a major industrial and scientific research challenge. Extracts of aromatic herbs, tea, grapes and derivative products, citrus fruit peel, and seeds are amongst the most studied natural antioxidants. In the sole family of phenolic antioxidants, thousands of molecules may potentially act as antioxidants.

Thus, we are confronted by a paradoxical problem: the dilemma of choice. The tremendous number of available natural antioxidants, which should be considered as advantageous, is in fact rather challenging for food research and industries. *Which antioxidants among these myriads are best suited to protect lipid-based systems from oxidation*? That amounts to asking if we have a theory explaining *how antioxidants work in lipid-based system*. Even though great progress has been achieved, we are still unable to predict how the intrinsic chemical structure of antioxidants affects their behavior in different food systems. One striking example is the complex influence of hydrophobicity on antioxidant effectiveness which is still not understood. And yet, hydrophobicity is one of the most important parameters for antioxidant capacity. In recent years, lipophilization (or hydrophobation) of otherwise hydrophilic antioxidant molecules to synthesize antioxidants with a broad range of hydrophobicity has attracted broad interest (López Giraldo et al., 2007 Lecomte et al., 2010). The ability to fine-tune the hydrophobicity by the grafting of various alkyl chains should help us to better understand whether hydrophobicity enhances or decreases antioxidant properties.

In such context, the aim of this review is to cover the role of hydrophobicity on antioxidant properties in oil-in-water emulsions and bulk oils. A brief panorama of the sole existing theory (i.e., the polar paradox) is presented, as well as unexpected results which recently contradict this theory both in bulk oils and emulsions. These contradictory results profoundly changed our understanding of *what makes an antioxidant effective and how does it work in lipid-based systems such as foods and biological tissues*. This review, thus, paves the way for a paradigm shift from the polar paradox to the next theories which remain to be developed in the years to come.

A BIT OF RECENT HISTORY

As William W. Christies wrote in his lipid blog [http://lipidlibrary.aocs.org/news/blog.htm], "*we all like a nice simple theory to explain physical phenomena in relation to lipid.*" Such is the case with antioxidant capacity in lipid-based foods.

Before 1955, a large body of relative effectiveness data for antioxidants was generated, and very often it has been extrapolated uncritically from bulk lipids to emulsions, micelles, and membranes in whole tissue foods (Porter et al., 1989). Later, however, Chipault et al. (1956), Uri (1958, 1961), Simpson and Uri (1956), and Abbot and Waite (1962) started commenting on the unexpected contrast in relative effectiveness between the same antioxidant as used in bulk oil versus emulsion. In 1974, Scott et al. found that decarboxylation or esterification of Trolox (water-soluble derivative of α-tocopherol) resulted in increased antioxidant activity in emulsion and greatly decreases activity in bulk oils. In their seminal article, Porter et al. (1989) reported a lot of other studies showing differences of antioxidant behavior as a function of the lipid system used. For example, one can cite the work of Cort (1974, 1982) demonstrating that ascorbic acid was much more effective than ascorbyl palmitate in bulk soybean oil and much less effective than ascorbyl palmitate in the hemoglobin-safflower oil emulsion test. Even though these results gave hints, time to time, before 1980s, nobody tried to summarize the knowledge on the relationship between lipid oxidation and antioxidant characteristics to draw a general model.

To our knowledge, Porter and his colleagues were the first to propose a rational rule during the 1980s. Combining their own results and those previously reported, they postulated in a series of papers that polar antioxidants are more active than their nonpolar homologs in bulk oil, whereas nonpolar antioxidants are more active in oil-in-water emulsions, liposomes, or even whole tissues (Porter, 1980; Porter et al., 1989). Because at first sight this observation seemed paradoxical, they called it the "polar paradox."

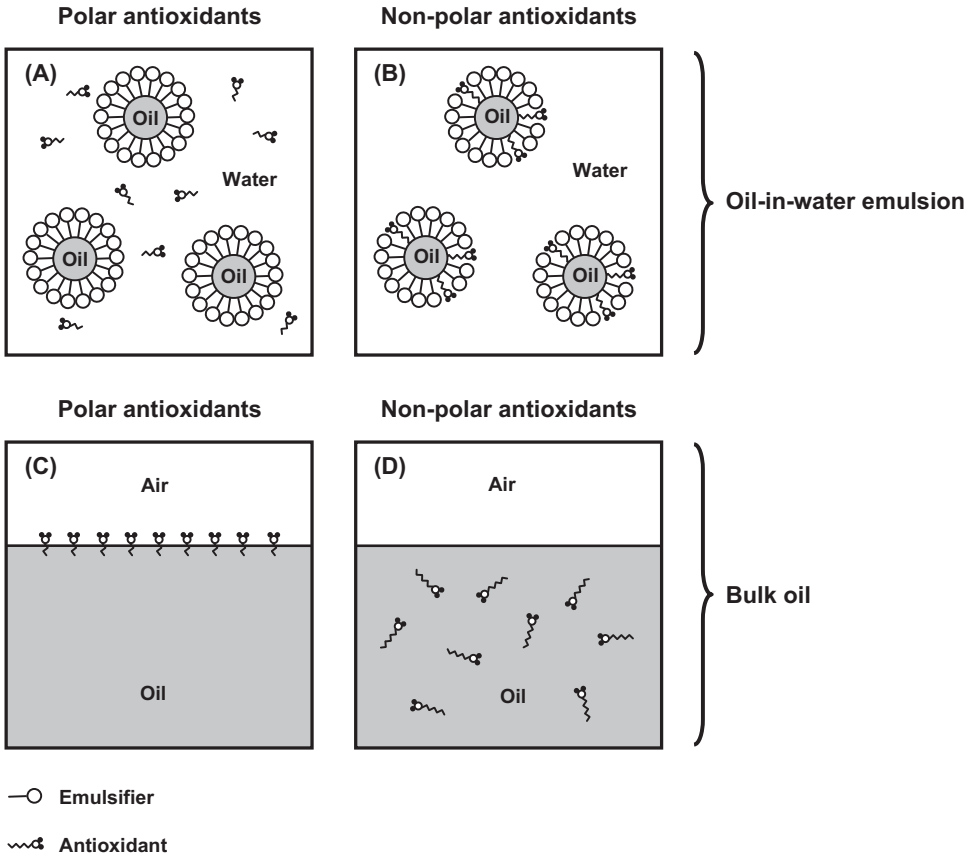

Figure 1 Interfacial phenomena as a possible mechanism of action of the polar paradox in oil-in-water emulsion (a and b) and in bulk oil (c and d). After Frankel et al. (1994).

The great achievement of the polar paradox is that it shows the irrelevancy of extrapolating uncritically effectiveness data for antioxidants from bulk oil to oil-in-water emulsions (or micelles, membranes, and whole tissues) as it was the case at that time (Laguerre et al., 2010b). Put in another way, they were the firsts to understand that the question of *which antioxidants are best suited to protect all types of lipid-based systems* is irrelevant and that there is no generic food-lipid system in which antioxidants behave universally in the same fashion. Instead, they dichotomized lipid-based systems in two smaller "behavioral systems": low surface-to-volume ratio systems such as bulk oil and high surface-to-volume ratio systems such as emulsions, membranes, and whole tissues. Antioxidants behave differently in one system compared to the other one. Because no mechanism of action was postulated by Porter et al. (1989), 1980s ends up with a nice empirical rule, but not yet a theory.

The shift from an empirical observation to a putative theory came in 1994 with the publication of a milestone article from Frankel et al. (1994) postulating that the polar paradox is due to an interfacial phenomenon. Accordingly, in oil-in-water emulsion, nonpolar antioxidants would concentrate in the oil-water interfaces (which are assumed to be the site where oxidation occurs) and inhibit oxidation more efficiently than polar antioxidants that partition into the water phase, where they would be less effective (Fig. 1a and b). In contrast, in bulk oils, they assumed that the increased effectiveness of hydrophilic antioxidants is due to their ability to migrate and concentrate at the air-oil interface where oxidation is prevalent, whereas lipophilic antioxidants are solubilized throughout the oily phase, where they would be less effective (Fig. 1c and d).

Experimental confirmations of the *polar paradox* in emulsions and bulk oils genuinely established it as the prevalent paradigm for more than one decade (Huang et al. 1996a, 1996b, 1997; Cuvelier et al., 2000). Regarding dispersed systems, Cuvelier et al. (2000) measured the antioxidant activity of 17 phenolic compounds in emulsified linoleic acid oxidized by iron/ascorbic acid and observed that the lipophilic antioxidants, α-tocopherol, BHA, and BHT were among the most efficient antioxidants and that the hydrophobicity was the strongest factor increasing their efficiency in dispersed medium.

As reported by Stöckmann et al. (2000), however, a lot of the studies supporting the polar paradox in dispersed systems like oil-in-water emulsions were based only on the difference in polarity of two antioxidant analogs, such as Trolox and α-tocopherol, carnosic acid and methyl carnosate, and ascorbic acid and ascorbyl palmitate (Huang et al. 1996a, 1996b, 1997). Consequently, these studies draw a binary representation instead having the overall picture of the influence of the polarity on

antioxidants. Besides these "binary" studies, others confirmed the polar paradox using more than two molecules, but in this case, these molecules were not always structurally related, and thus introduced a lot of new variables (number and location of OH groups, double bonds, side chain substitutions, etc.) which renders difficult the investigation of the sole effect of the polarity. For example, it is inappropriate to attribute the difference in efficiency of ascorbic acid and BHT solely to their polarity (Shahidi and Zhong, 2011). Although the antioxidant models used in these studies were relevant to establish an order of antioxidant capacity on an empirical point of view (this antioxidant is better than this one), these models were not relevant to accurately test the relationship between antioxidant properties and hydrophobicity. Finally, a third methodological problem could still arise with related compounds. In the case of phenolics, for instance, the antioxidant capacity is partly governed by the O-H bond dissociation enthalpy (BDE). Since O-H BDEs are dependent on the nature of ring substitutions (or side chain substitutions in conjugation with the phenolic ring), the modulation of hydrophobicity through addition or removal of such substitutions seems to be inappropriate to test the polar paradox. For example, α-tocopherol can not be considered more hydrophobic than β-tocopherol *all other things being equal*. In this case, the difference of hydrophobicity overlaps the difference of ring substitution, and hence, the difference on BDE, what makes challenging to ascribe any antioxidant difference to the sole hydrophobicity (Laguerre et al., 2009).

To prevent these biases and pitfalls, new phenolic antioxidant models have been used with the same active phenolic group, yet with differences in hydrophobicity. Such phenolic molecules with various alkyl chain lengths are known as "phenolipids" a term we adopted in 2010 to describe a phenolic compound conjugated to a lipid moiety (Fig. 2) (Laguerre et al., 2010b). With complete homologous series of phenolipids, new evidences have recently emerged that disagrees with the polar paradox both in bulk oil and emulsion.

ANTIOXIDANTS IN BULK OILS

The reaction mechanisms of lipid oxidation and its inhibition by antioxidant molecules, though extensively investigated since the beginning of the 20th century, are still not completely understood. In bulk oil, such knowledge is nevertheless highly desirable to develop new antioxidant strategies to protect nutritional lipids from oxidative alterations. As above-mentioned, the first rationale model suggested by Porter et al. (1989) states that polar antioxidants are more active in bulk oils than their nonpolar counterparts. Unlike nonpolar antioxidants, the tendency for polar antioxidants to accumulate at the air-oil interface where oxidation is supposed to be initiated is the mechanism proposed by Frankel et al. (1994) to account for their better activity in bulk oils. Although these hypotheses have been confirmed in numerous studies, some recent results shed doubts on the universality of the polar paradox and the interfacial concept in bulk oil and raise some crucial questions. Is there really a driving force to move polar antioxidant to the air-oil interface? Is the air-oil interface really the main site where oxidation of unsaturated lipids occurs in bulk oil? What if the observation that hydrophilic antioxidants are more active than lipophilic antioxidants in bulk oil (i.e., the polar paradox hypothesis) is not as general as expected? The present chapter will try to answer these questions.

Oil-Air or Oil-Water Interfaces?

Just one year after the milestone paper of Frankel et al. (1994), Koga and Terao (1995) postulated that the actual site of oxidation in bulk oil is not the air-oil interface but oil-water interfaces formed with traces of water. They based their interpretation upon the observation that the presence of phospholipids enhanced the antioxidant activity of α-tocopherol in model bulk oil systems containing a trace amount of water (1% v/v). In the presence of 2,2′-azobis(2-amidinopropyl) dihydrochloride (AAPH), a water-soluble azo compound that generates free radicals in the aqueous phase of the association colloids, the presence of phospholipids increased the degradation of α-tocopherol more than in the absence of phospholipids. Unlike AAPH, phospholipids did not enhance the interactions between α-tocopherol and lipid-soluble free radicals (2,2′-azobis(2-4-dimethylvaleronitrile, AMVN). These data strongly suggested that phospholipids exert no observable effect on the action of α-tocopherol when the radical chain reaction is initiated in

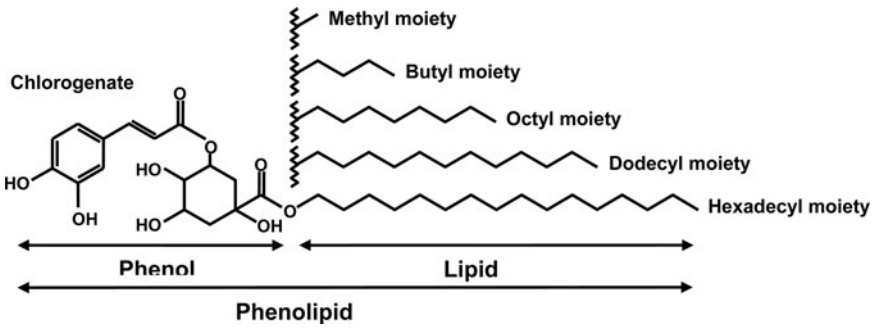

Figure 2 Example of a homologous series of phenolipids synthesized through chlorogenic acid transesterification by various alkyl chains.

the lipid phase, which is consistent with the hypothesis that α-tocopherol is positioned within physical structures known as association colloids in bulk oil and not at the oil-air interface.

It can also be argued that air is less polar than oil because the dielectric constant of air is 1.0 compared to approximately 3 for food oils (Chaiyasit et al., 2007). Consequently, there would not be a major driving force for polar antioxidants to migrate to the air-oil interface, and thus they would not be more likely to concentrate at the air-oil interface than hydrophobic antioxidants. Furthermore, it has been shown using Du Noüy ring method that most antioxidants are unable to decrease surface tension of hexadecane, meaning that the antioxidants do not concentrate at the air-oil interface. In contrast, they are able to accumulate at water-lipid interfaces, as can be seen by their ability to decrease interfacial tension (Chaiyasit et al., 2007). Another experimental data were brought by Chaiyasit et al. (2007) to show that the concentration of antioxidants such as α-tocopherol or Trolox (hydrophilic homologs of α-tocopherol) at the air-oil interface (collected by isolation of the top 1.5 mm of solidified oil and analysis of antioxidant concentrations) is not greater than the antioxidant concentrations in the bulk.

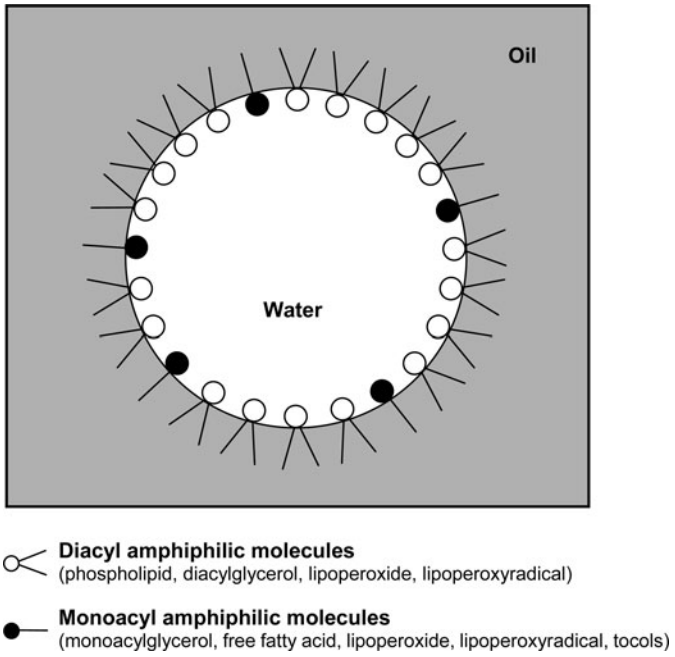

Figure 3 Simplified view of a spherical reverse micelle.

Existence of Physical Structures in "Bulk" Oil

Although often thought of as a simple homogeneous medium, oil is a complex multiphasic system; it contains small amounts of water and also various amphiphilic minor components such as mono- and diacylglycerols, phospholipids, sterols, and free fatty acids which are not completely removed by the refining process. Oils can also contain polar oxidation products (e.g., lipid hydroperoxides, aldehydes, ketones, and alcohols) that have higher polarity than their original lipid substrates due to the addition of oxygen. The presence of amphiphilic compounds in commercial bulk oils is observed when the oils are stripped (e.g., alumina (Al_2O_3) or silicic acid plus activated charcoal) of their minor components and the resulting stripped oil has a higher interfacial tension than the original refined oil (Chaiyasit et al., 2007).

These minor components are surface active and thus tend to entrap traces of water and to create oil-water interfaces within the so-called "bulk" oil. These structures, known as association colloids, are mainly reverse micelles and lamellar structures. Reverses micelles are structurally inverse analogs to normal micelles, containing a nanoscale aqueous core stabilized by a monolayer of surfactant molecules with the polar head groups of the surfactant extending into the water core and the aliphatic chains extending into the lipid medium (Fig. 3) (Xenakis et al., 2010). These dynamic aggregates are dispersed randomly following Brownian motion and are capable of exchanging their water content (Luisi and Straub, 1984; Pileni, 1989). Reverse micelles are efficient nano-reactors that allow increased interactions between lipid- and water-soluble components, and can greatly alter chemical reaction rates (Ghosh and Tiwary, 2001). In bulk oils, reverse micelles are typically formed by surfactants with low hydrophilic-lipophilic balance (HLB) values such as free fatty acids ($\sim$1), diacylglycerol ($\sim$1.8), and monoacylglycerol ($\sim$3.4–3.8) (24). Monoacylglycerols are known to form reverse micelles in triacylglycerol oils (Gulik-Krzywicki and Larsson, 1984). Phospholipids have intermediate HLB value ($\sim$8), which may explain why they form lamellar structures as well as reverse micelles (Gupta et al., 2001). Since oil contains a large variety of surface active components, it is likely that the association colloids are compositionally and structurally complex.

Research using X-ray diffraction has shown that lamellar structures exist in commercially available algal oil (Chaiyasit et al., 2007). Gupta et al. (2001) found that phospholipids in a nonaqueous media of hexane and vegetable oil in the presence of small amounts of water (less than 0.3 wt%) formed spherical reverse micelles with a diameter in the range 50–92 Å. More recently, Chen et al. (2010) observed a similar result since they evidenced with small angle X-ray scattering technique that dioleoylphosphatidylcholine (DOPC) forms spherical reverse micelles in stripped soybean oil and that this latter remained optically clear.

Experimental and theoretical approaches have shown that the key structural parameter of reverse micelles is the water/surfactant molar ratio (Mezzasalma et al., 2000; Gochman-Hecht and Bianco-Peled, 2005). Using fluorescent derivative of phospholipids (NBD-PE), it has been demonstrated that addition of water into bulk oil leads to water-phospholipid associations as indicated by a fluorescence intensity decrease and a red-shift of NBD-PE probe (Chen et al. 2010). This illustrates the fact that water serves to bridge the phosphate headgroups between neighboring phospholipids through hydrogen bond (Gupta et al., 2001). On the other hand, the water

content will determine structure's size as well as the amount of water that is strongly associated with the phopholipid headgroups (Chattopadhyay et al., 2002). For example, adding water to stripped soybean oil resulted in the 2-D X-ray pattern of the sample becoming aligned (Chen et al. 2010). This clearly indicates a transition from an isotropic to an anisotropic system, what suggests that the reverse micelles were loosing symmetry. According to Chen and co-workers (2010), this could be due to a stretching of the symmetrical structures of spherical reverse micelles into more oblong structures. Oil-rich phospholipids solutions with micellar morphology can also change to lamellar and hexagonal structures as the molar ratio between water and phospholipids is raised in the system (Zhang, 2003).

The Association Colloids Hypothesis

The impact of association colloids on lipid oxidation and its inhibition by antioxidant molecules has recently been the subject of numerous works. Measuring both lipid hydroperoxides and propanal, Chen et al. (2010) showed that the spherical reverse micelles formed by addition of DOPC (1000 μM) and water (200 ppm) in stripped soybean oil significantly increases lipid oxidation. Interestingly, below the DOPC CMC, where no reverse micelles are formed, DOPC does not promote lipid oxidation (Chen et al., 2010). 1,2-dibutyryl-*sn*-glycero-3-phosphocholine (DC_4PC) which does not form reverse micelles under the same condition as DOPC did not exhibit any prooxidative effect on stripped soybean oil. On the other hand, in stripped corn oil, an addition of 200 μM of DOPC and 400 ppm of water did not exert any influence on lipid oxidation (Laguerre et al., 2011a), which could indicate that a phospholipid concentration threshold is needed to be reached before exhibiting a prooxidative effect. These few examples illustrate the difficulty to predict the impact of association colloids on lipid oxidation because too many parameters play a role such as the temperature, the morphology and size of the association colloids, the partitioning of oxidation products within association colloids, the polarity of the phospholipids alkyl chain length, their surface charge, the volume of their inner water core, the presence of iron, etc.

Besides the role of association colloids on lipid oxidation, it is worth investigating their impact on antioxidant behavior. In 2007, Chaiyasit et al. (2007) introduced the concept of association colloids to provide insight on another factor that could impact antioxidant properties, especially in relation to the polar paradox. An early version of this *association colloids hypothesis*, assumed that *if the polar paradox is true*, then it can be explained by the fact that hydrophilic antioxidants are more active in bulk oil than their nonpolar homologs because they have a better affinity for the interface of association colloids. Increased effectiveness of the hydrophilic antioxidants would occur if the association colloids were a major site of oxidation due to their ability to concentrate transition metals and surface active lipid hydroperoxides. Therefore, oxidation is postulated to be likely to take place at the interfaces of these association colloids, rather than at the air-oil interface. At first sight, polar antioxidants, which are expected to be located at the interface or inside the inner water core of the association colloids, would therefore, counteract lipid oxidation more efficiently than nonpolar antioxidants which are expected to be located in the bulk lipid far from the actual site of oxidation. For instance, this seems to be the case for antioxidants such as α-tocopherol and Trolox. In accordance with the polar paradox, a lot of studies have indeed shown that hydrophilic Trolox is a more effective antioxidant than lipophilic α-tocopherol in bulk oil, even in presence of association colloids. In a recent study, it has been demonstrated that the fluorescence of NBD-PE (fluorescent derivative of phospholipids) was affected to a much greater extent by Trolox than α-tocopherol, suggesting that Trolox was partitioning into the DOPC reverse micelles more than α-tocopherol (Chen et al., 2011). This resulted in a better antioxidant activity for Trolox compared to α-tocopherol.

However, the question arises whether location at the interface or within the inner aqueous core of association colloids necessarily improves antioxidant properties compared to compounds localized in the bulk lipids. To answer this question, let us take into account that the antioxidant properties of a given compounds results from the balance between antioxidant and prooxidant properties and that "antioxidant" molecules may be prooxidant. For example, it is conceivable that polar antioxidants (located at the interface or within the aqueous core) reduce Fe^{3+} (also located within the aqueous core) into their more oxidative Fe^{2+} species. In this hypothetical case, polar antioxidants would be expected to be more prooxidant than their nonpolar homologs, which would be in contradiction with the polar paradox. Therefore, in the present review we extended and reformulated the *association colloids hypothesis* by including the possible negative effect of localization at the interface or within such physical structures.

Are Polar Antioxidants Really More Active than Nonpolar Ones?

Besides the legitimate questioning of the lipid-air interfacial oxidation concept, recent results show that in bulk oil, some antioxidants, especially phenolic compounds, do not behave in a manner dictated by the polar paradox. In other words, the more polar antioxidants are not necessarily the more active. For instance, it has been shown by Rancimat analysis that the stability of oil increases when the alkyl chain of the protocatechuyl alcohol is lengthened (Torres de Pinedo et al., 2007). For other phenolic compounds, hydrophobicity does not seem to exert any effect on antioxidant capacity especially for the methoxy-derivatives of protocatechuyl alcohol when CH_2 and CH_2-CH_2 groups were added. Such results can not be anticipated nor explained by the polar paradox hypothesis. One can nevertheless argue that the change of hydrophobicity for these derivatives is relatively modest with only three carbon atoms of difference, and that the polar paradox is a general rule

which apply on compounds with marked differences in polarity. And yet, Pereira-Caro et al. (2009) confirmed the results of Torres de Pinedo et al. (2007) using a complete homologous series of hydroxytyrosyl ethers containing the nonlipophilized hydroxytyrosol and its methyl, ethyl, propyl, butyl, hexyl, octyl, dodecyl, and octadecyl ethers. They found no significant effect of the alkyl chain length on antioxidant activity of hydroxytyrosyl ethers in stripped sunflower oil using the accelerated Rancimat method at 90°C. In 2002, Kikuzaki et al. already reported that the alkyl chain length does not influence the antioxidant capacity in bulk methyl linoleate (+ silicone oil) at 90°C for ferulate alkyl esters homologous series (from methyl to dodecyl alkyl esters). More recently, with chlorogenic acid and its butyl, dodecyl, and hexadecyl esters we reported another case contradicting the polar paradox (Laguerre et al., 2011a). Indeed, we found that there is no significant effect of the chain length of chlorogenate esters on their ability to inhibit the formation of both conjugated dienes and hexanal on stripped corn oil oxidized in the dark under natural conditions at 55°C.

Moreover, when water (∼400 ppm) and phospholipids (200 μM) were added above the critical micelle concentration, and that reverse micelles were formed, the lipophilic antioxidant (hexadecyl esters) was more active than hydrophilic chlorogenic acid, which contradicted the polar paradox proposal according to which polar antioxidants are more active in oil than their nonpolar counterparts. Formation of phospholipids reverse micelles decreased the antioxidant capacity of chlorogenic acid, while it did not change the activity of its hexadecyl counterpart. One possible cause of this observation could be the ability of phenolics to reduce iron (and/or other transient metal cations), thus increasing the prooxidant activity of the metals. Addition of water to stripped oil with phospholipids will produce reverse micelles with a water core (Chen et al., 2010). This water core could promote the partitioning of free chlorogenic acid into the reverse micelle, where it could interact with water-soluble metals solubilized in water and/or bound to the anionic phospholipids to increase the prooxidative activity of the metals. Hexadecyl chlorogenate could be too hydrophobic to partition into the water phase and thus might not increase the prooxidative activity of metals. Initially developed to rationalize the case where polar antioxidants were more efficient than nonpolar ones, the concept of association colloids can also explain why nonpolar antioxidants are in some conditions better antioxidants than their polar homologs. Moreover, these results showed that in absence of association colloids, hydrophobicity does not exert any noticeable effect on chlorogenate alkyl esters, probably because there were no water-lipid interfaces for phenolics of different chain length to partition into and, consequently, no physical means to discriminate them according to their hydrophobicity. In contrast, in presence of association colloids, hydrophobicity clearly exerted an effect by differentiating esters according to their chain length.

Other results contradicting the polar paradox have recently been reported in bulk oil. Indeed, Merkl et al. (2010) showed that esterification of caffeic acid by methyl, ethyl, propyl, butyl, and hexyl alcohols significantly improved antioxidant capacity toward sunflower oil (Rancimat method). These authors assessed other phenolipids such as protocatechuate, gentisate, ferulate, vanillate, and p-hydroxybenzoate esters with the same technique. For one series (gentisate esters), they found that the alkyl chain exerted negative effect on antioxidant capacity as suggested by the polar paradox, but for the others, the influence of esterification was either positive or negligible what disagrees with the polar paradox. Recently, Viskupicova et al. (2010) reported that esterification of rutin with short and medium alkyl chains (butyl, hexyl, octyl, and decyl) did not significantly modify the antioxidant capacity, while the grafting of medium to long alkyl chains (dodecyl, tetradecyl, hexadecyl, and octadecyl) resulted in a strong improvement of the antioxidant capacity toward sunflower oil at 100°C (Rancimat). Finally, using epigallocatechine gallate and its hydrophobic derivative containing four octadecyl chains (tetrastearate-EGCG), Shahidi and Zhong (2011) observed that at lower concentrations, tetrastearate-EGCG was more active than its hydrophilic counterpart EGCG, while at higher concentration, EGCG was the more active. It was hypothesized that, at low concentrations, the effect of solubility in oil dominates over the effect of interfacial phenomenon on antioxidant efficiency; thus, nonpolar antioxidants with better fat solubility have greater efficacies than their polar counterparts, whereas the reverse is true at higher concentrations. Put in another way, the antioxidant partition into the numerous interfaces present within oil starts only when a certain concentration is reached.

The First Rule in Bulk Oil is that There is No Rule (to Date)

The most important conclusion to make for bulk oil, is that polar antioxidants are not always more active than their nonpolar homologs nor nonpolar antioxidants are more active than their polar homologs. Both of these assumptions are equally wrong because experimental results have showed that in some conditions, with some compounds, sometimes, the effectiveness order goes in one way or the other. Consequently, we come to the conclusion that in bulk oil, to date, no general rule emerges. Only a case-to-case rule can be used to try to predict with more or less relevancy the impact of hydrophobicity on antioxidant capacity. But, why is there no rule to date?

Antioxidant effectiveness order depends on too many factors that can not be exhaustively known nor controlled. It depends on the presence of association colloids, the phospholipids and water content, and the presence of iron or other transition metals as we have recently suggested (Laguerre et al., 2011a). Indeed, with chlorogenic acid and its alkyl esters, intrinsic parameters such as hydrophobicity did not exert strong influence by itself on antioxidant capacity in bulk oil in absence of association colloids but became highly influential if potentialized by extrinsic factors such as reverse micelles. That illustrates the impact of the molecular environment and the physical structures on the behavior of antioxidants, especially phenolipids.

Furthermore, the effectiveness of an antioxidant in bulk oil is dependent on factors such as metal binding constants, redox potential, antioxidant radical stability and solubility. For example, chelators can inhibit metal-promoted lipid oxidation by preventing redox cycling and inhibiting metal-lipid interactions but can increase prooxidant activity by increasing metal solubility. Likewise, free radical scavengers can inhibit lipid oxidation by producing low energy oxidative species but can promote lipid oxidation when their redox potential is high enough to reduce metals into their more prooxidative states. To complicate matters further, antioxidants are often associated with other antioxidant molecules which can act as synergists or antagonists. The last but not the least parameter that may influence antioxidant capacity in bulk oil is the antioxidant concentration. Indeed, currently used methodologies to assess lipid oxidation (primary + secondary oxidation products) do not allow to analyze a lot of variables. Often, only one antioxidant concentration is tested for various antioxidants. However, we need to gain more insight on the role of the concentration on the antioxidant behavior in bulk lipid systems. Finally, since antioxidants have such a broad array of pathways in which they can impact oxidative reactions, it is not surprising that their ability to prevent lipid oxidation in bulk oils is difficult to predict on the sole basis of the polar paradox.

In the present paper, we did not review the numerous studies confirming the polar paradox which would have been beyond the scope of this contribution. Instead, we focused on results questioning this hypothesis not to show that the polar paradox in bulk oil is always false (what is wrong) but rather to show that to date no rule can already be drawn and that we need more experimental data acquired with varying conditions, especially concentration. Actually, predicting antioxidant capacity in bulk oil will only be possible when all the intrinsic and environmental parameters that affect antioxidant capacity will be known, which is far from the case to date since the heterogeneity of bulk lipid systems is so complex. Although the polar paradox failed to accurately predict antioxidant effectiveness in bulk oil, it succeeded in one important thing: it showed that a generic food-lipid system in which antioxidants behave in the same fashion does not exist and that we have to break it down in smaller "behavioral systems" in which more specific rules can be applied. Like Russian dolls, it is maybe time to decompose bulk oil system in smaller "behavioral systems" containing low, intermediate or high amounts of sterols, phospholipids, free fatty acids and other surface active lipids capable of forming physical structures, as well as transition metals and water.

ANTIOXIDANTS IN OIL-IN-WATER EMULSIONS

Lipid oxidation in emulsified systems plays an important role for food products, since many foods (milk, mayonnaise, dressings, dips, sauces, beverages, margarine, butter, ice cream, etc.) exist either partly or wholly as emulsion, or have been in an emulsified state at some time during their existence (McClements, 2008). Even nonemulsified foods can form emulsions in the digestive tract if they contain lipids thanks to the combined action of shearing forces and bile salts. The development of new antioxidants to counteract lipid oxidation in oil-in-water emulsions is hampered by the lack of a reliable theory describing and predicting the antioxidant properties in food dispersions. The polar paradox, which is the only rational model, assumes that hydrophobic antioxidants are more active in oil-in-water emulsion than their hydrophilic homologs, which can be implicitly interpreted as the fact that the relationship between hydrophobicity and antioxidant capacity follows a linear trend. In contrast, in a series of papers, we formulated an alternative hypothesis assuming that this relationship is not necessarily linear and that highly lipophilic antioxidants may display near-zero antioxidant capacity.

Does Hydrophobicity Always Enhance Antioxidants in Emulsion?

As pointed out by Stöckmann et al. (2000), before 2000, most of the studies supporting the polar paradox in dispersed systems, such as oil-in-water emulsions, were based only on the difference in polarity of two antioxidant analogs, such as Trolox and α-tocopherol, carnosic acid and methyl carnosate, and ascorbic acid and ascorbyl palmitate (Huang et al. 1996a, 1996b, 1997). Accordingly, very few homologous series have been assessed before Stöckmann et al. (2000) tested the polar paradox in stripped corn oil-in-water microemulsion stabilized by different surfactants using gallic acid and its alkyl esters. Measuring lipid hydroperoxides by the thiocyanate-ferric ion color reaction, they found different orders of antioxidant effectiveness according to the emulsifier used. In lecithin-stabilized emulsion (and although it is difficult to determine antioxidant capacity before the lag phase exit), the order was approximately propyl gallate $\sim$ butyl gallate $>$ octyl gallate $\sim$ ethyl gallate $\sim$ methyl gallate $\gg$ gallic acid. Thus, apart from octyl gallate, the polar paradox seems to be more or less confirmed in such dispersed system, as the most effective antioxidants were more or less the more hydrophobic molecules. On the contrary, in both sodium dodecyl sulphate (SDS)- and Brij 58-stabilized emulsions, results were not consistent with the polar paradox. Indeed, the order with SDS was methyl gallate $\sim$ ethyl gallate $>$ propyl gallate $\gg$ butyl gallate $\gg$ octyl gallate $\sim$ gallic acid, while with Brij 58 it was methyl gallate $\gg$ octyl gallate $\sim$ butyl gallate $>$ ethyl gallate $>$ propyl gallate $\gg$ gallic acid. Stöckmann et al. (2000) concluded that the polar paradox may be limited to emulsion containing emulsifiers with properties similar to phospholipids what considerably narrowed the applicability of the polar paradox in emulsion.

In another study contradicting the polar paradox in emulsions, Yuji et al. (2007) observed that, although butyl or dodecyl esters of *p*-hydroxyphenylacetic acid (HPA) concentrated at lipid/water interfaces more than free HPA, the latter antioxidant was more effective at inhibiting the oxidation of Brij 35-stabilized non stripped Menhaden oil-in-water emulsions. One

can also cite the work of Sasaki et al. (2010) who demonstrated in the same system that phenolic antioxidants conjugated with hydrocarbon chains are more highly associated with lipid emulsions droplets, but these changes in physical properties did not increase antioxidant activity.

These three studies showing that not all antioxidants behave in a manner proposed by the polar paradox suggest that antioxidant activity in such systems is governed by more complex phenomena. Although nonstripped oils were used (apart from Stöckmann et al. 2000) and antioxidant interactions could have made interpretation difficult, these inconsistencies led us to hypothesize that the relationship between the antioxidant capacity and the hydrophobicity in oil-in-water emulsion is not as linear as expected.

The Nonlinear Hypothesis

To test this hypothesis, the antioxidant capacity of a complete homologous series of chlorogenic acid and its alkyl esters (methyl, butyl, octyl, dodecyl, hexadecyl, octadecyl, and eicosyl) was evaluated using our newly developed *conjugated autoxidizable triene* assay (Laguerre et al., 2008) in its improved version using methanolic predissolution of antioxidants (Laguerre et al., 2009). We demonstrated for the first time in stripped tung oil-in-water microemulsion stabilized with Brij 35 that the antioxidant capacity increases as the alkyl chain is lengthened, with a maximum for the dodecyl chain, after which further chain extension leads to a collapse in antioxidant capacity (Laguerre et al., 2009) (Fig. 4a). This nonlinear effect was unexpected because it was generally thought that hydrophobic antioxidants are more active in emulsion than their nonpolar homologs as stated in the polar paradox hypothesis. To date, supporting evidence of this nonlinear trend has already been established using different phenolipid series such as rosmarinate (Laguerre et al., 2010b; Panya et al., 2011) and hydroxytyrosolate esters (Medina et al., 2009) in various oil-in-water emulsions (Brij 35/stripped tung oil, Tween 20/stripped soybean oil and lecithin/nonstripped cod liver oil). With a homologous series of rosmarinic acid and its alkyl esters (methyl, butyl, octyl, dodecyl, hexadecyl, octadecyl, and eicosyl) we have observed an even stronger nonlinear effect in stripped tung oil-in-water microemulsion. Indeed, the dependence between the alkyl chain length and the antioxidant capacity followed a parabolic shape with a maximum for the octyl ester, suggesting that the nonlinear trend can dictate the behavior of most phenolic antioxidants in emulsion (Fig. 4b) (Laguerre et al., 2010b). We confirmed this parabolic (or nonlinear) effect in 1% stripped soybean oil-in-water emulsion stabilized by Tween 20 using the same rosmarinate esters series (Panya et al., 2012). Both hexanal and hydroperoxide measurements showed that free rosmarinic acid and its octadecyl and eicosyl esters exhibited extremely low antioxidant capacity compared to medium chain esters such as butyl, octyl and dodecyl rosmarinates. Medina et al. (2009) also published a supporting evidence of our nonlinear hypothesis using nonstripped cod liver oil-in-water emulsion which has been made differently than ours and also using another way to assess lipid oxidation (conjugated diene measurement). In their study, they found that "*the presence of a short-medium lipophilic chain (acetate, butyrate or octanoate) improved the antioxidant efficiency of hydroxytyrosol [. . .], but longer alkyl chain (laurate) maintained or even decreased their antioxidant activity.*"

Some recent studies also tend to support the nonlinear hypothesis, however, only two or three homologs have been tested and thus it is risky to draw general conclusion. In oil-in-water emulsion, Sørensen et al. (2012) found in oil-in-water emulsion, that octyl dihydrocaffeate was more efficient at inhibiting lipid oxidation than oleyl dihydrocaffeate. Experiments with rutin and lipophilized rutin (rutin laurate and rutin palmitate) as antioxidants in oil-in-water emulsion also support the fact that highly lipophilic polyphenols display low antioxidant capacity, since the esters were consistently less effective compared to rutin (Lue, 2009).

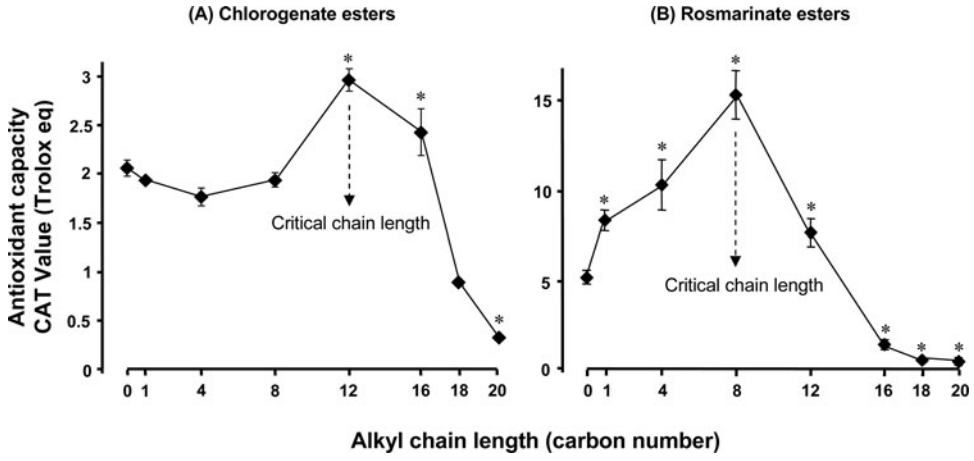

Figure 4 Influence of the alkyl chain length of chlorogenate (a) and rosmarinate (b) alkyl esters on antioxidant activity in tung oil-in-water microemulsions. After Laguerre et al. (2009, 2010b).

Beyond Emulsion: The Nonlinear Trend in Liposomes, Human Cells, and Bacteria

Regarding other dispersed lipid systems such as liposomes, Kikuzaki et al. (2002) showed a strong nonlinear effect of the alkyl chain of ferulate and gallate esters on their ability to inhibit the AAPH-induced peroxidation of egg yolk PC liposomes. The antioxidant capacity increases as the alkyl chain is lengthened with a threshold for hexyl-octyl ferulate and dodecyl gallate for which optimal activities have been measured. Beyond this threshold for a medium chain the antioxidant activities of the corresponding esters decrease in both ferulate and gallate ester families. One can also cite the pioneer work of Takahashi et al. (1992) who found on soybean phosphatidylcholine liposomes that diesters (distearate) of ascorbic acid were less efficient to counteract lipid oxidation mediated by AAPH than monostearate of ascorbic acid. Within the ascorbate diester series, dicaprilate (C8) was more effective than dipalmitate (C16) which was in turn more effective than distearate (C18).

More recently, we observed a sudden collapse of the antioxidant capacity after certain hydrophobicity with rosmarinate esters (Panya et al., 2010). This collapse is highly reminiscent of the nonlinear effect obtained in emulsions. Indeed, it has been observed in chitosan-coated liposomes that the order of antioxidant effectiveness toward lipid oxidation was: butyl rosmarinate > free rosmarinic acid > dodecyl rosmarinate ∼ octadecyl rosmarinate > eicosyl rosmarinate (Fig. 5). Note that, unfortunately, octyl ester (the *cut-off* molecule in oil-in-water microemulsion for the rosmarinate series) has not been assessed in this study. And yet, the optimal activity for butyl ester and the poor antioxidant values for long chain esters (octadecyl and eicosyl rosmarinates) are highly reminiscent of a nonlinear effect. This has to be linked to the fact the lipophilic eicosapentaenoic acid ester of epigallocatechin gallate (EGCG) had a higher rate of incorporation into liposomes but displayed a lower antioxidant activity than the EGCG itself (Shahidi and Zhong, 2011). Finally, another confirmation of the nonlinear effect has been published by An et al. (2011) and shows that the order of antioxidant effectiveness of alkoxy derivatives of isoflavonoid daidzein on phosphatidylcholine liposomes was: daidzein (prooxidant) < butyl derivative (prooxidant) < octyl derivative (antioxidant) < hexadecyl derivative (antioxidant) < dodecyl derivative (antioxidant). Once again, the hydrophobicity-activity relationship follows a non-linear trend with a critical chain length (CCL) for a medium chain (12 carbon atoms).

Probably the most significant and interesting aspect of the nonlinear effect is that it seems to takes also place in living cells, which suggests that it may have important biological consequences. Indeed, we recently observed in human dermal fibroblasts with chlorogenate esters (Laguerre et al., 2011b) the same antioxidant parabolic effect with a threshold for the dodecyl chain as already reported in microemulsion (Laguerre et al., 2009). More specifically, it turned out that lipophilization of chlorogenic acid by short fatty chains (1, 4, and 8 carbons) did not bring any noticeable modification of their ability to inhibit the oxidation of dichlorodihydrofluorescein (fluorogenic probe) upon oxidative stress, whereas the grafting of a dodecyl chain (12 carbons) led to a spectacular fourfold increase of antioxidant capacity compared to the parent molecule. Unexpectedly, it appeared that elongation of the aliphatic chain from 12 to 16 carbon atoms leads to a 45%-antioxidant capacity collapse (Fig. 6). In other words, we have observed an identical *cut-off* effect as the one we already reported in oil-in-water microemulsion with a threshold for a dodecyl chain (Fig. 4a) (Laguerre et al., 2009). A confirmation of this trend has been recently brought from antioxidant capacity measurements of rosmarinate esters on the same human dermal fibroblasts. Results showed that medium chain esters such as octyl ester, which was the best antioxidant in oil-in-water microemulsion (Bayrasy et al. 2013), were also the best phenolipids in fibroblasts (with decyl rosmarinate), and that beyond this medium chain threshold, antioxidant capacity suddenly collapse. Simultaneously to us, Tofani et al. (2010) showed that the relationship between the chain length of hydroxytyrosyl esters and their antioxidant capacity in L6 rat muscle cells oxidized by cumene hydroperoxides follows a sigmoid trend. For short

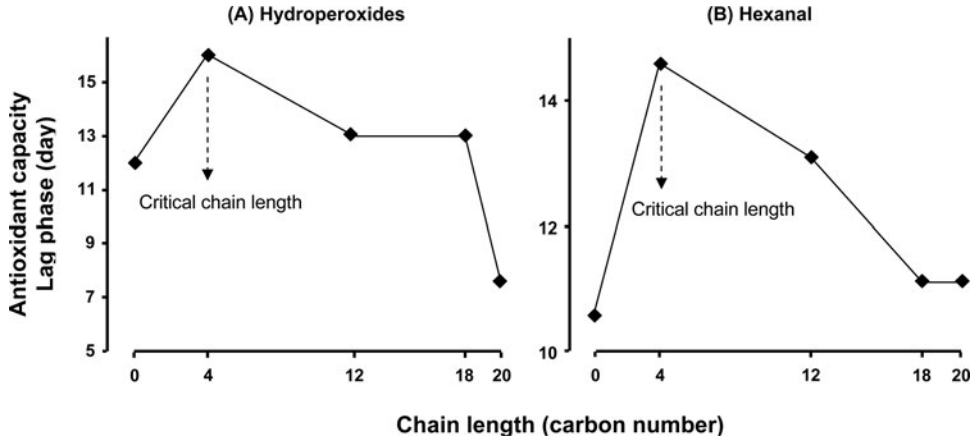

Figure 5 Influence of the alkyl chain length of rosmarinate esters on antioxidant capacity in chitosan-coated liposomes. Antioxidant activity is expressed as lag phase exit (day) for lipid hydroperoxides (a) and hexanal (b). Adapted from Panya et al. (2010).

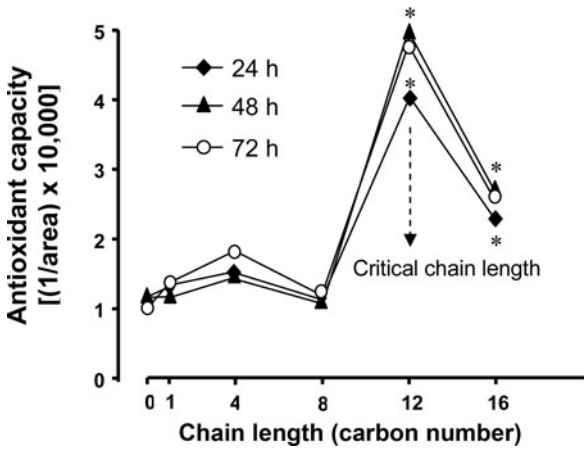

Figure 6 Influence of the alkyl chain length of chlorogenate esters on antioxidant activity in cancerous ROS-overexpressing human dermal fibroblasts. After Laguerre et al. (2011b).

to medium acyl chains (2–10 carbons) the antioxidant activity is not noticeably affected by lipophilicity, although giving values always higher than that of hydroxytyrosol itself, with a maximum for the butyrate hydroxytyrosolate. Of particular importance was the fact that the elongation over the decyl chain does not play a favorable role. Indeed, a constant antioxidant activity collapse was observed for long chain esters (12, 16, and 18 carbon atoms). Thus, they observed a similar phenomenon as us with a threshold at medium chain length (∼10 carbon atoms). Besides phenolic compounds, this nonlinear effect seems also to dictate the antioxidant behavior of acyl ascorbates (Kimura et al., 2003). As determined using the BODIPY 581/591 C11 membrane fluorescent probe oxidized by iron ($FeSO_4$) in Caco-2 cells, medium chain ascorbates (dodecyl and tetradecyl ascorbate) were much better antioxidants than ascorbic acid and its decyl, hexadecyl, and octadecyl esters. These results suggest that the nonlinear relationship between antioxidant capacity and hydrophobicity is not insulated to the sole phenolic family.

Surprisingly, an identical trend has also been observed for rosmarinate alkyl esters on their antimicrobial activities against *Staphyloccocus carnosus* (Suriyarak et al., 2013) with a threshold for medium chain (8–10 carbon atoms for rosmarinates). The nonlinear effect of the alkyl chain length was even sharper in both cases with an almost all-or-nothing fashion. It is worth mentioning that other studies have already been published on the nonlinear effect of hydrophobicity on antimicrobial activity of phenolipids. For instance, in 2004, Muroi et al. (2004) found that the antibacterial activity of anacardic acids possessing different alkyl chain lengths against the methicillin resistant *Staphylococcus aureus* strain was a parabolic function of their lipophilicity and maximized with the alkyl chain length of C10 and C12.

Bridging the Gap Between Nonlinear and Cut-Off Phenomena

In 2009, we bridged the gap between disciplines by reporting that besides the antioxidant properties, such a nonlinear influence of the chain length has been largely observed in a diverse range of biological activities (Laguerre et al., 2009). As early as 1932, Miescher described a nonlinear effect in local anesthesia in the homologous series of *N*-[2-(diethylamino)ethyl]-2-alkoxycinchonin amides (cinchocaines). Three years later, Meyer and Hemmi extended this observation to general anesthesia using a homologous series of *n*-alkanols (Meyer and Hemmi, 1935). At the same time as Meyer and Hemmi confirmed the occurrence of a parabolic effect in anesthesia, the Nobel Prize winner in Physiology or Medicine, Gerhard Domagk, discovered the same phenomenon for antimicrobial activity with a homologous series of quaternary ammonium salts (Domagk, 1935). Sometimes named the "parabolic case," this effect is now known under the name of the "cut-off effect," a term adopted by Ferguson in 1939 (Ferguson, 1939). It can be defined for a homologous series of hydrocarbon chain surface-active compounds, as the tendencies for various biological or physical-chemical activities to increase stepwise (or stay stable) with increasing chain length up to a critical point, beyond which these activities suddenly collapse. In this paper, we coin a new physico-chemical parameter in this field of critical chain length (*CCL*) to describe the cut-off point or the threshold in chain length. It is worth pointing out that the first part of the trend (below the *CCL*) does not always follow the same trend from a study to another one, while after such a threshold is reached, the trend does always follow a sudden collapse. This definition of the cut-off effect perfectly fit with the parabolic shape depicted in Fig. 4.

With decades went by, a growing number of other biological activities were found to be dictated by the cut-off phenomenon. To date, more than 50 studies (including ours) describe such an effect with homologous series of amphiphilic molecules as antimicrobials (Domagk, 1935; Lien et al., 1968; Tomlinson et al., 1977; Rucka et al., 1981, 1983; Daoud et al., 1983; Čižmárik et al., 1987; Devínsky et al., 1990 Kubo et al., 1993; Pavlíková-Mořická et al., 1994; Kopecky, 1996; Birnie et al., 2000; Vazquez et al., 2001; Muroi et al., 2004; Denny et al., 2005; Sugandhi et al., 2007; Hsu et al., 2009; Kanjilal et al., 2009), anesthetics (Miescher, 1932; Meyer and Hemmi, 1935; Čižmárik et al., 1976; Raines et al., 1993; Nakahiro et al., 1996), anti-proliferatives (Dasgupta et al., 2002; Salem et al., 2010), antivirals (Wong et al., 2002), cytotoxics (Locatelli et al., 2008), eye irritants (Cometto-Muñiz et al., 2007; Cometto-Muñiz and Abraham, 2008), alcohol intoxication inducers (McCreery and Hunt, 1978; Lyon et al., 1981), spermicidal agents (Wong et al., 2002), immunosuppressive drugs (Ashman et al., 1986), antiphotosynthetics (Králová et al., 1992, 2010; Sersen et al., 1992), phytotoxic (Murín et al., 1990), corneal drugs in rabbit (Mosher and Mikkelson, 1979), drugs in mice (Jeppsson, 1975) or in cultured cells (Wils et al., 1994; Bordeleau et al., 2005; Lakeram et al., 2007), inhibitors of enzymes (Karlovská et al., 2005) and receptors (Peoples and Weight, 1995), membrane perturbing (or destroying) surfactants (Devinsky et al., 1990; Sarapuk et al., 2000), antioxidants in emulsion (Laguerre et al., 2009, 2010b; Medina et al., 2009, Panya et al., 2012), in liposomes (Kikuzaki et al., 2002; Panya et al., 2010; An et al.,

2011), or in cultured cells (Kimura et al., 2003; Tofani et al., 2010; Laguerre et al., 2011b), environmental pollutants (Cravedi et al., 1983), hydrogen-bond breakers (Chiou et al., 1990), and finally protein binding agents (Eckenhoff et al., 1999) and enzyme inhibitors (Barla et al., 2009). According to Balgavy and Devinsky (1996), the cut-off effect is a general phenomenon which has been observed in various biological and toxic activities in practically every amphiphile homologous series tested so far. At the opposite, to the best of our knowledge, before our report (Laguerre et al., 2009), a cut-off effect has never been observed in *in vitro* antioxidant measurement involving emulsified system. This is possibly due to the fact that the homologous series currently used do not cover a large hydrophobic range, while in pharmacological studies the range and the number of compounds in a same series is generally much larger.

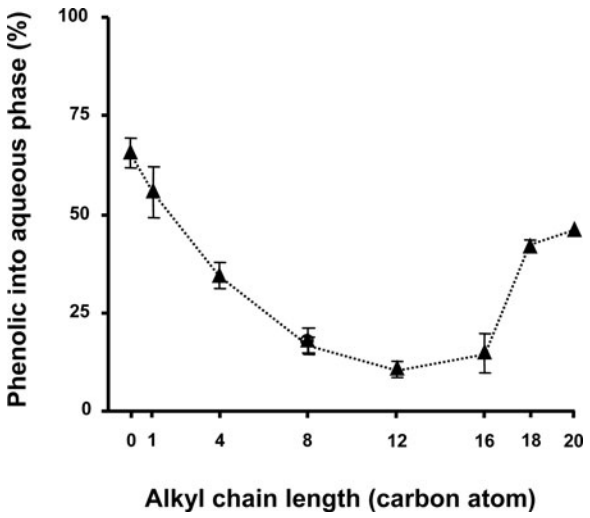

Figure 7 Alkyl chain length effect on the partition behavior of chlorogenic acid and its alkyl esters in a mixture of sunflower oil and phosphate buffer solution at pH 7.2 with 17 μM Brij 35 used as emulsifier. After Laguerre et al. (2009).

In Search for a Cut-Off Mechanism of Action

Criticizing existing theories such as the polar paradox is relatively easy compared to the challenging and risky effort to propose an alternative. In our case, this is all the more challenging if we try to rationalize the cut-off effect in all the above-mentioned biological activities and systems. Obviously, such a tour de force is beyond the scope of this contribution. Instead, we will put forward three putative mechanisms of action for the cut-off effect in oil-in-water emulsion: the "*reduced mobility,*" the "*internalization,*" and the "*self-aggregation*" hypotheses.

It has sometimes been reported that it is difficult to rationalize the cut-off effect, because most of the properties supposed to be involved in this behavior (partition coefficients, CMC) do not show maxima or minima at these chain lengths (Walters et al., 1993). This means that the cut-off theory lacks the physicochemical descriptor(s) needed to explain its nonlinear behavior. And yet, recently, the Morales's team and ours have unexpectedly shown that non-linear trends in parameters which are supposed to be highly influential on antioxidant capacity (oil-water distribution and surface tension at the CMC) can be observed if antioxidant phenolipids encompassing chain length long enough are assessed (Laguerre et al., 2009, 2010b; Lucas et al., 2010; Maldonado et al., 2011). In other words, simple potential physico-chemical explanations can be found to rationalize the cut-off effect in oil-in-water emulsion.

In 2009, we have found in sunflower oil-in-water emulsion, that the partitioning of chlorogenate alkyl ester into the water phase decreases with increasing chain length up to a dodecyl chain (corresponding to the *CCL* observed for antioxidant capacity) beyond which, the corresponding esters (hexadecyl, octadecyl, and eicosyl esters) unexpectedly accumulated into the water phase (Fig. 7) (Laguerre et al., 2009). Although the curves depicted in Figs. 7 and 4a are not perfectly symmetric, the partitioning behavior of chlorogenic acid and its esters in Fig. 7 showed the same threshold for a dodecyl chain length. This suggests that the lowest concentrated in the water phase, the best antioxidant.

One can also cite the work of Lucas et al. (2010) who showed using hydroxytyrosol alkyl esters that the surface tension at the CMC (γCMC) decreases as expected with the lengthening of the alkyl chain until a decyl chain is reached. Unexpectedly, beyond this *CCL*, there is a sudden increase of γCMC. This nonlinear tendency in γCMC fits the nonlinear effect observed on antioxidant capacity of hydroxytyrosol alkyl esters in oil-in-water emulsion (Medina et al., 2009). The Morales's group recently reported other similar trends with derivatives of gallic acid (Maldonado et al. 2011). This further extends the number of phenolipids homologous series which seems to behave in a manner proposed by our cut-off hypothesis (chlorogenates, rosmarinates, hydroxytyrosolates, gallates, glucosyl gallates, and glucuronosyl gallates). Taken together, these results from the Morales's group and ours, allow the proposal of new putative scenarios which may explain the cut-off influence of the alkyl chain length on the antioxidant capacity in oil-in-water emulsion. For clarity purpose, we have to divide the cut-off mechanism of action in two parts: what happens below the *CCL*, and what happens beyond.

Below the CCL, it can be hypothesized that the corresponding phenolipid antioxidants with short-medium chains are not close enough to the interface where oxidation is supposed to occur (Fig. 8a). Consequently, the weaker antioxidant capacity of the corresponding polar antioxidants may be seen as a consequence of not being at maximum concentration at the site where lipid oxidation is most prevalant (e.g., oil droplet surface). In contrast, when the *CCL* is reached (dodecyl chlorogenate, octyl rosmarinate, and decyl hydroxytyrosol), one can hypothesize that the medium chains concentrate the antioxidant at the site of oxidation, more than other alkyl chains along with an adequate orientation of the phenolic head toward the aqueous phase where the initiating free radicals are concentrated (Fig. 5b). In other words, only antioxidants with the appropriate hydrophobicity would be

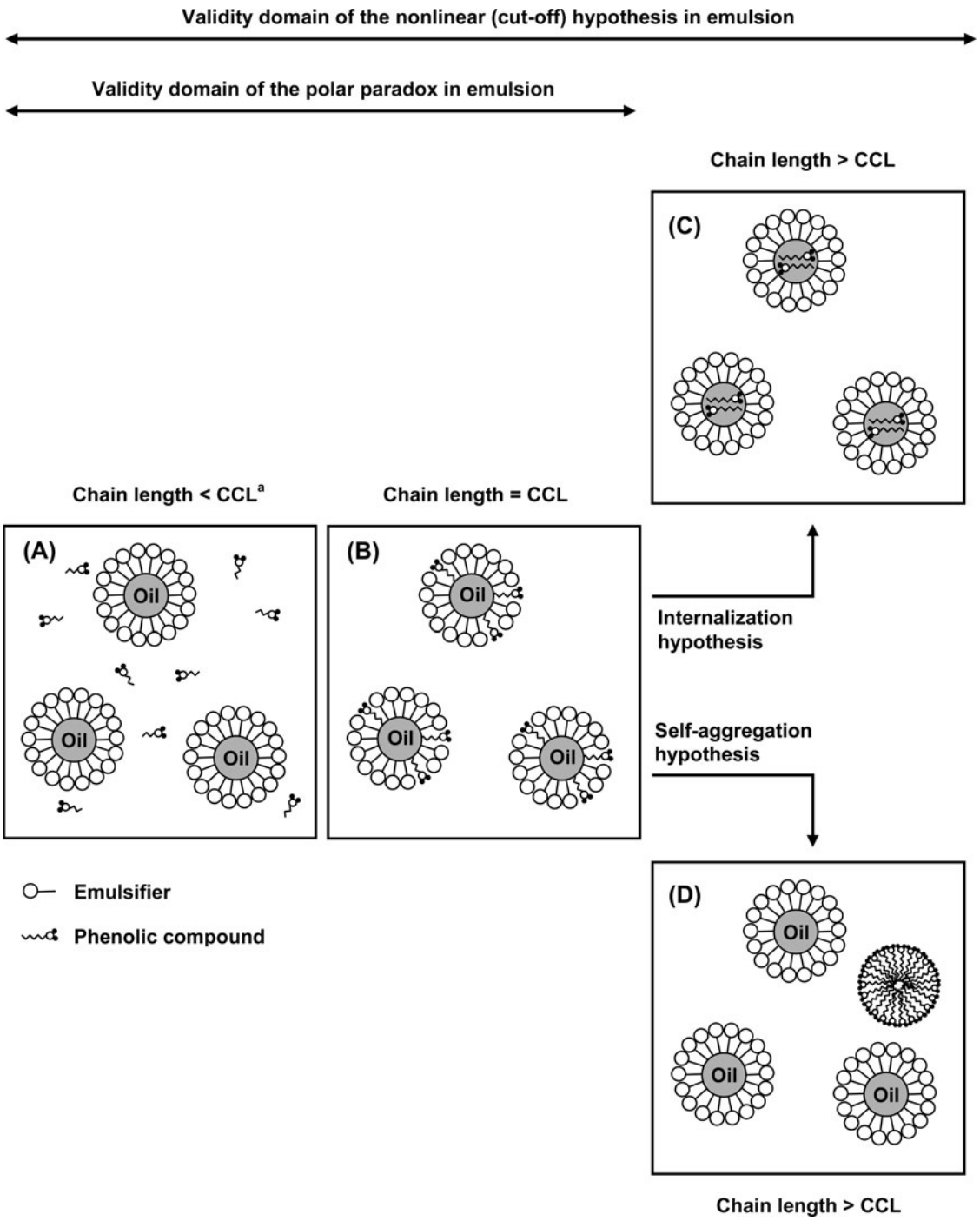

Figure 8 Putative scheme of the distribution of antioxidant in emulsified system. [a] CCL : critical chain length.

located at the water-oil interface where oxidation can be more efficiently counteracted. This paves the way for synthesis and evaluation of multifunctional emulsifier/antioxidant compounds.

One would have to acknowledge, that this explanation is exactly the same that the one Frankel et al. already put forward 15 years ago (Frankel et al., 1994), and that, to the best of our knowledge, nobody in the field has ever brought experimental data contradicting this scenario. *Supposing that the cut-off trend is largely encountered in oil-in-water emulsion for homologous series of antioxidants* (that is the cut-off hypothesis), the challenge is not the elucidation of what happens below the *CCL*, as everybody agree with the Frankel et al's scenario, but the description of what happens beyond the cut-off when the *CCL* is reached and that antioxidant capacity suddenly collapse. That is the main goal we now need to be focused on in order to develop the next theories.

Beyond the CCL, things get more complicated. How to explain that a chain lengthening of few carbon atoms increments leads to an almost total loss of antioxidant properties? What suddenly happens at the molecular level? We reviewed in this part the three different hypotheses which progressively emerge to rationalize the cut-off effect.

The Reduced Mobility Hypothesis

In this paper, we coin a new concept "*reduced mobility hypothesis,*" with the idea that the mobility of the lipophilic antioxidant decreases as its alkyl chain is lengthened, consequently decreasing its ability to move toward the numerous oxidation sites. Indeed, increased hydrophobic interactions between the alkyl chains of antioxidants and their molecular environment such as the hydrophobic tails of the emulsifier and the hydrophobic surface and core of the oil droplet, may lower the diffusion of long chain antioxidants to the reaction centers, i.e., oxidizable substrates, free radicals, and transition metals. Let us consider first that an antioxidant needs to move a lot at the interface to meet free radicals and, second, that each time antioxidant interacts with whatever molecules through hydrogen bonding or hydrophobic interaction is called contact. The reduced mobility hypothesis suggests that the frequency of the contact will decrease for long chain antioxidants compared to medium chains since long chain antioxidants are more strongly bound (by hydrophobic interactions) to the molecular environment and thus they have a lower degree of freedom. This may result in a reduced antioxidant activity. One can also notice that steric hindrance induced by increasing the chain length can render contact between long chain antioxidants and free radicals more difficult and may consequently be involved in the cut-off effect.

The putative effect of aliphatic chain length on the mobility of antioxidants in lipid dispersions has been put forward for decades now. In 1985, Niki et al. suggested that the intermembrane mobility of α-tocopherol containing a phytyl chain is reduced in liposomal system compared to 2,2,5,7,8-pentamethyl-6-chromanol, in which the phytyl chain has been replaced by a methyl group. According to the authors, the phytyl chain would enhance the retainment of α-tocopherol in liposomes and hence would suppress its ability to transfer between liposomal membranes. One year later, in 1986, Castle and Perkins pointed out the importance of the intermicellar diffusion rate in antioxidant capacity of hydrophobic antioxidant such as α-tocopherol in SDS micelles. In liposomal systems, Takahashi et al. (1992) concluded that the intramembrane and intermembrane mobilities of ascorbic acid alkyl esters decreased with increasing number and length of fatty acid chains. With gallate alkyl esters, Stöckmann et al. (2000) postulated that increased hydrophobic interactions (for longer alkyl chains) may lower the diffusion of gallates into the SDS-enriched environment resulting in a reduced antioxidant activity. In 2010, we used the same interpretation to explain why eicosyl rosmarinate ester exhibited much lower antioxidant capacity than their butyl and dodecyl homologs in chitosan-coated liposomes (Panya et al., 2010). The same year, Tofani et al. (2010) came to a similar conclusion to explain the cut-off effect of hydroxytyrosol alkyl esters on antioxidant capacity in L6 rat muscle cells. They assumed that at a certain level of lipophilicity, the easy diffusion of esters into the cells could be balanced (decyl hydroxyltyrosyl ester) or even made unproductive (dodecyl to octadecyl hydroxytyrosyl esters) by entrapment into the plasma membrane. This latter could be caused by the higher affinity of long acyl chains with the phospholipids or hydrophobic proteins inside the bilayer.

The Internalization Hypothesis

In this paper, we also coin the concept the "*internalization hypothesis,*" the assumption that increasing the hydrocarbon chain from medium to long chains could drive the antioxidant away from the interface (where oxidation is supposed to primarily occur) into the emulsion droplet core where the phenolipid would be a poor antioxidant. This putative effect has been suggested by numerous authors. Among other hypotheses, Medina et al. (2009) used the internalization hypothesis to explain why the octyl ester of hydroxytyrosol exhibits higher antioxidant capacity than the dodecyl one in fish oil-in-water emulsion. We also put forward this hypothesis to try to rationalize the sudden collapse of the antioxidant capacity of rosmarinate esters with long chains (dodecyl, hexadecyl, octadecyl, and eicosyl) (Laguerre et al., 2010b).

We can, however, argue that some results, including ours, show that the cut-off effect of chlorogenate esters can not be always explained by this hypothesis, since long chain chlorogenates (octadecyl and eicosyl) were equally or even more concentrated (depending on the emulsifier concentration) in the aqueous phase of an emulsion than medium chain esters (Laguerre et al., 2009). On the contrary, results on rosmarinate esters strongly support the internalization hypothesis by showing that eicosyl rosmarinate (20 carbon atoms) is mainly located in the oil droplet, while butyl and dodecyl rosmarinates are preferentially located at the interface of a stripped soybean oil-in-water emulsion (Panya et al., 2012). Addition of emulsifier in excess (more than is needed to saturate the emulsion droplet surface) leads to the formation of micelles which can solubilise eicosyl rosmarinate out of the emulsions droplet core and carry it to both the aqueous phase and the surfactant interface as it can be seen from front-face fluorescence, spectrophotometric analysis (trapping unreacted arenediazonium ion by *N*-(1-naphtyl)ethylenediamine)) and centrifuge/HPLC partitioning study. The addition of excess of emulsifier increases the antioxidant capacity of eicosyl rosmarinate in a dose-dependent manner, but only slightly decreases that of butyl and dodecyl rosmarinates. Consequently, the cut-off effect observed for medium chain esters (butyl, octyl, and dodecyl esters) in oil-in-water emulsion progressively disappears with the addition of excess of emulsifier which allows long chain esters moving from the oily core into the aqueous phase, and above all, at the interface where they would be more effective to counteract lipid

oxidation (Panya et al., 2012). Finally, *supposing that the cut-off effect is largely encountered in homologous series of antioxidant in oil-in-water emulsion*, more experiments are obviously needed to validate or invalidate the internalization hypothesis.

The Self-Aggregation Hypothesis

In this paper, we also coin "*self-aggregation hypothesis,*" the assumption made in 2009 (Laguerre et al., 2009) that beyond the *CCL*, the antioxidant capacity collapse is due to the antioxidant self-aggregation and that long chain phenolipids mainly exist as aggregates, possibly as micelles, in the water phase of an oil-in-water emulsion (Fig. 8). We assumed that this tendency to form aggregates for the longer chains becomes greater than the tendency to move toward the interfacial membrane, and thus the concentration of antioxidant at the site of oxidation decreased. Consequently, the micellization process could confer a major drawback in the ability of the long chain phenolipid antioxidants to counteract lipid oxidation: *the removal of the antioxidant from the interface where oxidation is most prevalent*. Another detrimental effect of the formation of aggregates is the decrease of the diffusion coefficient of long chain antioxidants when self-aggregation occurs (see Stokes-Einstein equation). Therefore, the self-aggregation hypothesis has to be combined with the reduced mobility hypothesis.

Often overlooked by scientists working on antioxidants such as tocopherol or other phenolipids is that these antioxidants have long alkyl chain and an adequate polar head to produce separate hydrophilic and hydrophobic portions of the molecule (Fig. 2). This could allow these amphiphilic molecules to self-assemble into micelles, lamellar structures, and other association colloids. Among natural phenolipids, resorcinolic lipids have the most precisely defined amphiphilic properties. They form stable monomolecular layers at the air-water interface (Kozubek, 1989, 1995), in which the dihydroxybenzene rings are oriented perpendicularly to the surface of the subphase (Kato et al., 1990). According to Stasiuk and Kozubek (2010), resorcinolic lipids show very low values for the critical micelle concentration. The CMCs determined for different homologs in neutral pH by solubilization of 1,6-diphenyl-1,3-5-hexatriene were in the range of 4.5–8.5 μM and depended on the length and saturation of the hydrocarbon chains. The CMCs obtained for long-chain homologs phenolipids by surface pressure measurement were lower (0.5–2.6 μM) (Gulati and Subba Rao, 1964). It has also been reported that under alkaline conditions, cardol and methylcardol form liposomal structures alone as well as in mixture with cholesterol, fatty acids, or phosphatidylethanolamine (Stasiuk and Kozubek, 2010). Moreover, these authors reported that the lipid aggregates of 3-pentadecylphenol in aqueous solution have a micellar character. Regarding synthetic phenolipids, Lucas et al. (2010) have recently determined by surface tension measurement the CMC of hydroxytyrosol alkyl esters. They found very low CMCs for long chains esters, namely, 2, 3.5, and 5.5 μM for hexadecyl, tetradecyl, and dodecyl esters, respectively. As expected, decreasing the chain length drastically increases the CMC: 90, 380, 1200, 1500, and finally 3000 μM for decyl, octyl, hexyl, butyl and ethyl esters, respectively (Lucas et al., 2010). Other phenolipids, such as tyrosolates (Lucas et al., 2010), gallates, glucosyl gallates, and glucuronosyl gallates (Maldonado et al., 2011) have been recently found to self-assemble into micelles.

The self-aggregation hypothesis is furthermore supported by some experimental data. On one hand, we have demonstrated using chlorogenate alkyl esters that beyond the *CCL* (for antioxidant capacity), long chain esters unexpectedly accumulate in the water phase of an oil-in-water emulsion (Laguerre et al., 2009). At low emulsifier concentration (below emulsifier CMC), the only physico-chemical process that can lead to accumulate oil-soluble antioxidant compound in water is a self aggregation of the antioxidant through micellization. On the other hand, on hydroxytyrosol alkyl esters, γCMC suddenly increases after the *CCL* is reached for an alkyl chain of 8–10 carbon atoms (Lucas et al., 2010). Interestingly, this corresponds to the *CCL* (octyl chain) in their antioxidant properties measured in fish oil-in-water emulsion. This sudden γCMC increase after a threshold is attained in hydrophobicity has also been observed for alkyl gallates, glucosyl alkyl gallates, and glucuronosyl alkyl gallates (Maldonado et al., 2011). Consequently, long chain phenolipids are no more located at the air-water interface, otherwise γCMC should have continued to decrease. Again, this suggests that long chain phenolipids self-aggregate and return in the water phase as micelles or other association colloids.

Study of the cut-off effect in emulsified systems also brings some challenges to the self-aggregation hypothesis. Indeed, some authors, including us, did not always find long chain phenolic esters in the aqueous phase of an oil-in-water emulsion. However, theoretically, if long chain phenolipids are self-assembled into micelles (the postulated basis for the discrimination), one would expect that these compounds are localized in the aqueous phase. Sørensen et al. (2012) did not detect octyl or oleyl dihydrocaffeate ester in the aqueous phase of an oil-in-water emulsion. The same result was observed in the study of the partitioning behavior of rosmarinic acid fatty esters. For the lowest concentration of emulsifier (17 μM Brij 35), no ester with a chain equal to or longer than 8 carbon atoms were detected in the water phase of a sunflower oil-in-water emulsion. This result incited us to state that although the self-aggregation hypothesis allowed to explain the cut-off effect for chlorogenate esters, it failed to explain the cut-off effect for rosmarinate esters (Laguerre et al., 2010b).

And yet, a recent re-examination of the partitioning methodology by Sørensen et al. (2012) pointed out that the absence of long chain phenolipids in the water phase may be due to an experimental bias rather than a failure of the self-aggregation hypothesis. Indeed, in their partitioning studies, as well as in our, the supernatant (aqueous phase) was filtered through 0.22 μm-filter prior to HPLC analysis. This is a reasonable explanation of the absence of observed long-chain phenolipids in the water phase. At that time, the phenolipids self-assembly properties well above micellization was neglected. We now know that

phenolipids such as long chain rosmarinate esters are able to self-assemble in much bigger objects than micelles with a size ranged between 200–1000 nm as measured by dynamic light scattering (to be published elsewhere). In case of rosmarinate esters, these aggregates should be stuck on the filter, and thus partitioning studies using filtration prior to HPLC may induce false negative regarding the concentration in the aqueous phase. Consequently, further partitioning studies in this field should use simple UV-visible or fluorescence spectrophotometry (for which no preliminary filtration is needed) instead of HPLC. Finally, the biggest source of errors in partitioning studies is that available methodologies are destructives and require breaking the emulsion. To overcome this pitfall, one can cite the novel, promising, and nondestructive method based on trapping unreacted arenediazonium ion (16-ArN_2^+) by *N*-(1-naphtyl)ethylenediamine to form an azo dye absorbing at 572 nm (Sánchez-Paz et al., 2008). Since 16-ArN_2^+ (which is localized at the interface) react with antioxidant, the detection of unreacted 16-$ArN_2{}^+$ by spectrophotometry allows evaluating the partitioning of antioxidants in emulsions at the interfacial level (Panya et al., 2012).

CONCLUSION

The polar paradox states that polar antioxidants are more active in bulk lipid than their nonpolar counterparts, whereas nonpolar antioxidants are more effective in oil-in-water emulsion than their polar homologs. However, recent results, showing that not all antioxidant behave in a manner proposed by this hypothesis in oil and emulsion, leads us to revisit the polar paradox and to put forward new vocabularies, ideas, hypotheses, and theories. Historically, the polar paradox was put forward to show that it is not relevant to extrapolate uncritically effectiveness data for antioxidants from bulk oil to emulsion (or micelles, membrane, and whole tissue). This review article does not put into question this part of the polar paradox which was, in the field of lipid oxidation, one of the greatest achievements of the last decades. Instead, we want to highlight that the polar paradox is a particular case of a more global phenomenon. Consequently, it failed to accurately predict the impact of hydrophobicity on antioxidant capacity in lipid-based foods. We feel that it is now time for a paradigm shift from the polar paradox to the next theories.

In bulk oil, it has been postulated, through the *association colloids hypothesis*, that the actual site of oxidation is not the air-oil interface but oil-water interfaces formed with traces of water and surface active compounds such as phospholipids, sterols, mono-, and diacylglycerols. Recent advances in this field have been covered in this review and experimental proofs of the existence of such physical structures in bulk oils have been reported. The role of these association colloids on lipid oxidation and its inhibition by antioxidants was also addressed as well as the complex influence of the antioxidant hydrophobicity on its ability to protect lipids from oxidation. We have also seen that to date no rule yet emerge to rationalize the antioxidant behavior in bulk oil. In some conditions which remained to be characterized, polar antioxidants are more active than their nonpolar counterparts as postulated in the polar paradox. However, in some other conditions, hydrophobicity does not exert any influence, which contradicts the polar paradox. Finally, cases have also been reported where nonpolar antioxidants are more active than their polar homologs. Clearly, if such contradicting results are confirmed, the polar paradox should be replaced by new rationales. More experimental data are thus needed to make further progress in our understanding of what makes good antioxidants in bulk oil in relation to their hydrophobicity.

In oil-in-water emulsion, we have observed a strong cut-off influence of the alkyl chain length on antioxidant capacity of phenolipids, which disagrees with the polar paradox. Three different mechanisms of action have been reported to account for this nonlinear phenomenon: the "*reduced mobility,*" the "*internalization,*" and the "*self-aggregation*" hypotheses. The question of the universality of the cut-off effect has also been addressed. Even though it is too early to conclude on that point, looking beyond our field at the numerous biological studies showing a cut-off occurrence, incite us to postulate that this effect may well be widespread. In terms of perspectives, since an increase of emulsifier concentration drastically affects the partition behavior of antioxidants, we predict that the cut-off effect may be partly hidden by an excess of emulsifier. We feel that this may be an important point since many oil-in-water emulsions contain more surfactant than is needed to completely saturate the emulsion droplet surface. Moreover, taking into account the strong effect of the emulsifier on antioxidant capacity (Stöckmann et al., 2000; Sørensen et al., 2008; Oehlke et al., 2010, 2011), a possible influence of this parameter directly on the cut-off effect needs to be evaluated in years to come, especially regarding the concentration, the charge, and the aliphatic chain length of the emulsifier. Study of the impact of pH, ionic strength, and the concentration of transition metal on the cut-off effect would also be highly desirable. Since real emulsified food systems contain many more molecules than in model emulsions, it remains to be seen whether the cut-off phenomenon is a robust model applicable to real systems. Finally, the recent work done on the cut-off effect paves the way for systematic investigation of the chain length effect to design new phenolipids and other amphiphilic antioxidants in a rational fashion.

REFERENCES

Abbot, J. and Waite, R. (1962). The effect of antioxidants on the keeping quality of whole milk powder. I. Flavones, gallates, butylhydroxyanisole and nordihydroquaiaretic acid. *J. Dairy Res.* **29**:55–61.

An, C. B., Li, D., Liang, R., Bu, Y. Z., Wang, S., Zhang, E. H., Wang, P., Ai, X. C., Zhang, J. P. and Skibsted, L. H. (2011). Chain length effects in isoflavonoid daidzein alkoxy derivatives as antioxidants: A quantum mechanical approach. *J. Agric. Food Chem.* **59**:12652–12657.

Ashman, R. B., Blanden, R. V., Ninham, B. W. and Evans, D. F. (1986). The interaction of amphiphilic aggregates with cells of the immune system. *Immunol. Today* **7**:278–283.

Balgavy, P. and Devinsky, F. (1996). Cut-off effects in biological activities of surfactants. *Adv. Colloid Interface Sci.* **66**:23–63.

Barla, F., Higashijima, H., Funai, S., Sugimoto, K., Harada, N., Yamaji, R., Fujita, T., Nakano, Y. and Inui, H. (2009). Inhibitive effects of alkyl gallates on hyaluronidase and collagenase. *Biosci. Biotechnol. Biochem.* **73**:2335–2337.

Bayrasy, C., Chabi, B., Laguerre, M., Lecomte, J., Jublanc, E., Villeneuve, P., Wrutniak-Cabello, C. and Cabello, G. (2013). Boosting antioxidants by lipophilization: A strategy to increase cell uptake and target mitochondria. *Pharm. Res.* 30:1979–1989.

Birnie, C. R., Malamud, D. and Schnaare, R. L. (2000). Antimicrobial evaluation of *N*-alkyl betaines and *N*-alkyl-*N*,*N*-dimethylamine oxides with variations in chain length. *Antimicrob. Agents Chemother.* **44**:2514–2517.

Bordeleau, J. L., Gailis, L., Fournier, D., Morissette, M., Di Paolo, T. and Daleau, P. (2005). Cut-off phenomenon in the protective effect of alcohols against lysophosphatidylcholine-induced calcium overload. *Pflugers Arch-Eur. J. Physiol.* **450**:292–297.

Castle, L. and Perkins, M. J. (1986). Inhibition kinetics of chain-breaking phenolic antioxidants in SDS micelles. Evidence that intermicellar diffusion rates may be rate-limiting for hydrophobic inhibitors such as α-tocopherol. *J. Am. Chem. Soc.* **108**:6381–6382.

Chaiyasit, C., Elias, R. J., McClements, D. J. and Decker, E. A. (2007). Role of physical structures in bulk oils on lipid oxidation. *Crit. Rev. Food Sci. Nutr.* **47**:299–317.

Chattopadhyay, A., Mukherjee, S. and Raghuraman, H. (2002). Reverse micellar organization and dynamics: A wavelength-selective fluorescence approach. *J. Phys. Chem. B* **106**:13002–13009.

Chen, B., Han, A., Laguerre, M., McClements, D. J. and Decker, E. A. (2011). Role of reverse micelles on lipid oxidation in bulk oils: Impact of phospholipids on antioxidant activity of α-tocopherol and Trolox. *Food Funct.* **2**:302–309.

Chen, B., Han, A., McClements, D. J. and Decker, E. A. (2010). Physical structures in soybean oil and their impact on lipid oxidation. *J. Agric. Food Chem.* **58**:11993–11999.

Chiou, J. S., Ma, S. M., Kamaya, H. and Ueda, I. (1990). Anesthesia cutoff phenomenon: Interfacial hydrogen bonding. *Science* **248**:583–585.

Chipault, J. R., Mizuno, G. R. and Lundberg, W. O. (1956). The antioxidant properties of spices. *Food Technol.* **10**:209–212.

Čižmárik, J., Švec, P. and Borovanský, A. (1976). Study of local anesthetics. LII. piperidinoethyl esters of alkoxyphenylcarbamic acids. *Acta Fac. Pharm. Univ. Comen.* **29**:53–80.

Čižmárik, J., Trupl, J. and Pesak, M. (1987). A correlation between the antimicrobial activity to staphylococcus aureus and selected physico-chemical parameters in the series of hydrochlorides of piperidinoethylesters of alkoxy-substituted phenyl-carbamix acids. *Československ. Farm.* **36**:345–348.

Cometto-Muñiz, J. E. and Abraham, M. H. (2008). A cut-off in ocular chemesthesis from vapors of homologous alkylbenzenes and 2-ketones as revealed by concentration-detection functions. *Toxicol. Appl. Pharm.* **230**:298–303.

Cometto-Muñiz, J. E., Cain, W. S., Abraham, M. H. and Sánchez-Moreno, R. (2007). Cut-off in detection of eye irritation from vapors of homologous carboxylic acids and aliphatic aldehydes. *Neuroscience* **145**:1130–1137.

Cort, W. M. (1974). Hemoglobin peroxidation test screens antioxidants. *Food Technol.* **28**:60–66.

Cort, W. M. (1982). Antioxidant properties of ascorbic acid in foods. **In**: Ascobic Acids: Chemistry, Metabolism, and Uses, p. 533. Seib, P. A. and Tolbert, B. M., Eds., Advances in Chemistry Series 200, American Chemical Society, Washington, DC.

Cravedi, J. P., Tulliez, J., Choubert, G. and Luquet, P. (1983). Digestibilité des hydrocarbures saturés chez la truite. *Environ. Pollut. A* **32**:39–49.

Cuvelier, M. E., Bondet, V. and Berset, C. (2000). Behavior of phenolic antioxidants in a partitioned medium: Structure-activity relationship. *J. Am. Oil Chem. Soc.* **77**:819–824.

Daoud, N. N., Dickinson, N. A. and Gilbert, P. (1983). Antimicrobial activity and physico-chemical properties of some alkyldimethylbenzylammonium chlorides. *Microbios* **37**:73–85.

Dasgupta, P., Singh, A. and Mukherjee, R. (2002). *N*-Terminal acylation of somatostatin analog with long chain fatty acids enhances its stability and anti-proliferative activity in human breast adenocarcinoma cells. *Biol. Pharm. Bull.* **25**:29–36.

Denny, B. J., Novotny, L., West, P. W. J., Blesova, M. and Zamocka, J. (2005). Antimicrobial activity of a series of 1-alkyl-2-(4-pyridyl)pyridinium bromides against Gram-positive and Gram-negative bacteria. *Med. Princ. Pract.* **14**:377–381.

Devínsky, F., Kopecka-Leitmanová, A., Šeršeň, F. and Balgavý, P. (1990). Cut-off effect in antimicrobial activity and in membrane perturbation efficiency of the homologous series of *N*,*N*-dimethylamine oxides. *J. Pharm. Pharmacol.* **42**:790–794.

Domagk, G. (1935). Eine neue klasse von disinfektionsmitteln. *Dtsh. Med. Wochenschr.* **61**:829–832.

Eckenhoff, R. G., Tanner, J. W. and Johansson, J. S. (1999). Steric hindrance is not required for *n*-alkanol cutoff in soluble proteins. *Mol. Pharm.* **56**:414–418.

Ferguson, J. (1939). The uses of chemical potentials as indices of toxicity. *Proc. Roy. Soc. London B.* **127**:387–404.

Frankel, E. N., Huang, S.-W., Kanner, J. and German, J. B. (1994). Interfacial phenomena in the evaluation of antioxidants: Bulk oils *vs* emulsion. *J. Agric. Food Chem.* **42**:1054–1059.

Ghosh, K. K. and Tiwary, L. K. (2001). Microemulsions as reaction media for a hydrolysis reaction. *J. Disp. Sci. Tech.* **22**:343–348.

Gochman-Hecht, H. and Bianco-Peled, H. (2005). Structure of AOT reverse micelles under shear. *J. Colloid Interface Sci.* **288**:230–237.

Gulati, A. S. and Subba Rao, B. C. (1964). Drug analogues from the phenolic constituents of cashewnut shell liquid. *Indian J. Chem.* **2**:337–338.

Gulik-Krzywicki, T. and Larsson, K. (1984). An electron microscopy study of the L2-phase (microemulsion) in a ternary system: Triglyceride/monoglyceride/water. *Chem. Phys. Lipids* **35**:127–132.

Gupta, R., Muralidhara, H. S. and Davis, H. T. (2001). Structure and phase behaviour of phospholipids-based micelles in nonaqueous media. *Langmuir* **17**:5176–5183.

Hsu, F. L., Chen, P. S., Chang, H. T. and Chang, S. T. (2009). Effects of alkyl chain length of gallates on their antifungal property and potency as an environmentally benign preservative against wood-decay fungi. *Int. Biodeterior. Biodegrad.* **63**:543–547.

Huang, S. W., Frankel, E. N., Aeschbach, R. and German, J. B. (1997). Partition of selected antioxidants in corn oil-water model systems. *J. Agric. Food Chem.* **45**:1991–1994.

Huang, S. W., Frankel, E. N., Schwarz, K. and German, J. B. (1996a). Effect of pH on antioxidant activity of α-tocopherol and Trolox in oil-in-water emulsions. *J. Agric. Food Chem.* **44**:2496–2502.

Huang, S. W., Hopia, A., Schwarz, K., Frankel, E. N. and German, J. B. (1996b). Antioxidant activity of α-tocopherol and Trolox in different lipid substrates: Bulk oils *vs* oil-in-water emulsions. *J. Agric. Food Chem.* **44**:444–452.

Iverson, F. (1995). Phenolic antioxidants: Health protection branch studies on butylated hydroxyanisole. *Cancer Lett.* **93**:49–54.

Jeppsson, R. (1975). Parabolic relationship between lipophilicity and biological activity of aliphatic hydrocarbons, ethers and ketones after intravenous injections of emulsion formulations into mice. *Acta Pharmacol. Toxicol.* **37**: 56–64.

Kanjilal, S., Sunitha, S., Reddy, P. S., Kumar, K. P., Murty, U. S. N. and Prasad, R. B. N. (2009). Synthesis and evaluation of micellar properties and antimicrobial activities of imidazole-based surfactants. *Eur. J. Lipid Sci. Technol.* **111**:941–948.

Karlovská, J., Hammel, M., Laggner, P., Lacko, I., Devinsky, F. and Balgavy, P. (2005). Effects of N-alkyl-N,N-dimethylamine-N-oxides on the activity of purified sarcoplasmic reticulum Ca^{2+}-transporting ATPase. *Pharmazie* **60**:135–137.

Kato, T., Seki, K. and Kaneko, R. (1990). Insoluble monolayers of irisresorcinol at the air/water interface. *Colloid Polym. Sci.* **268**:934–937.

Kikuzaki, H., Hisamoto, M., Hirose, K., Akiyama, K. and Taniguchi, H. (2002). Antioxidant properties of ferulic acid and its related compounds. *J. Agric. Food Chem.* **50**:2161–2168.

Kimura, Y., Kanatani, H., Shima, M., Adachi, S. and Matsuno, R. (2003). Antioxidant activity of acyl ascorbates in intestinal epithelial cells. *Biotechnol. Lett.* **25**:1723–1727.

Koga, T. and Terao, J. (1995). Phospholipids increase radical-scavenging activity of vitamin E in a bulk oil model system. *J. Agric. Food Chem.* **43**:1450–1454.

Kopecky, F. (1996). Micellization and other associations of amphiphilic antimicrobial quaternary ammonium salts in aqueous solutions. *Pharmazie* **51**:135–144.

Kozubek, A. (1989). Detergent-like effect of phenolic lipids on biological membranes. *Acta Univ. Wratisl.* **868**:27–32.

Kozubek, A. (1995). Interaction of alkylresorcinols with proteins. *Acta Biochim. Pol.* **42**:241–246.

Králová, K., Sersen, E. and Cizmárik, J. (1992). Inhibitory effect of piperidinoethyl esters of alkoxyphenylcarbamic acids on photosynthesis. *Gen. Physiol. Biophys.* **11**:261–267.

Králová, K., Sersen, E., Devinsky, F. and Lacko, I. (2010). Photosynthesis-inhibiting effects of cationic biodegradable gemini surfactants. *Tenside Surfact Det.* **47**:288–295.

Kubo, I., Muroi, H., Masaki, H. and Kubo, A. (1993). Antibacterial activity of long-chain alcohols: The role of hydrophobic alkyl groups. *Bioorg. Med. Chem. Lett.* **3**:1305–1308.

Laguerre, M., Chen, B., Lecomte, J., Villeneuve, P., McClements, D. J. and Decker, E. A. (2011a). Antioxidant properties of chlorogenic acid and its alkyl esters in stripped corn oil in combination with phospholipids and/or water. *J. Agric. Food Chem.* **59**:10361–10366.

Laguerre, M., Decker, E. A., Lecomte, J. and Villeneuve, P. (2010a). Methods for evaluating potency and efficacy of antioxidants. *Curr. Opin. Clin. Nutr. Metab. Care* **13**:515–525.

Laguerre, M., Lecomte, J. and Villeneuve, P. (2007). Evaluation of the ability of antioxidants to counteract lipid oxidation: Existing methods, new trends and challenges. *Prog. Lipid Res.* **46**:244–282.

Laguerre, M., López Giraldo, L. J., Lecomte, J., Baréa, B., Cambon, E., Tchobo, P. F., Barouh, N. and Villeneuve, P. (2008). Conjugated autoxidizable triene (CAT) assay: A novel spectrophotometric method for determination of antioxidant capacity using triacylglycerol as ultraviolet probe. *Anal. Biochem.* **380**:282–290.

Laguerre, M., López Giraldo, L. J., Lecomte, J., Figueroa-Espinoza, M.-C., Baréa, B., Weiss, J., Decker, E. A. and Villeneuve, P. (2009). Chain length affects antioxidant properties of chlorogenate esters in emulsion: The cut-off theory behind the polar paradox. *J. Agric. Food Chem.* **57**:11335–11342.

Laguerre, M., López Giraldo, L. J., Lecomte, J., Figueroa-Espinoza, M.-C., Baréa, B., Weiss, J., Decker, E. A. and Villeneuve, P. (2010b). Relationship between hydrophobicity and antioxidant ability of phenolipids in emulsion: A parabolic effect of the chain length of rosmarinate esters. *J. Agric. Food Chem.* **58**:2869–2876.

Laguerre, M., Wrutniak-Cabello, C., Chabi, B., López Giraldo, L. J., Lecomte, J., Villeneuve, P. and Cabello, G. (2011b). Does hydrophobicity always enhance antioxidant drugs? A cut-off effect of the chain length of functionalized chlorogenate esters on ROS-overexpressing fibroblasts. *J. Pharm. Pharmacol.* **63**:531–540.

Lakeram, M., Lockley, D. J., Sanders, D. J., Pendlington, R. and Forbes, B. (2007). Paraben transport and metabolism in the biomimetic artificial membrane permeability assay (BAMPA) and 3-day and 21-day Caco-2 cell systems. *J. Biomol. Screen* **12**:84–91.

Lecomte, J., López Giraldo, L. J., Laguerre, M., Baréa, B. and Villeneuve, P. (2010). Synthesis, characterization and free radical scavenging properties of rosmarinic acid fatty esters. *J. Am. Oil Chem. Soc.* **87**:615–620.

Lien, E. J., Hansch, C. and Anderson, S. M. (1968). Structure-activity correlations for antibacterial agents on gram-positive and gram-negative cells. *J. Med. Chem.* **11**:430–41.

Locatelli, C., Rosso, R., Santos-Silva, M. C., de Souza, C. A., Licínio, M. A., Leal, P., Bazzo, M. L., Yunes, R. A. and Creczynski-Pasa, T. B. (2008). Ester derivatives of gallic acid with potential toxicity toward L1210 leukemia cells. *Bioorg. Med. Chem.* **16**:3791–3799.

López Giraldo, L. J., Laguerre, M., Lecomte, J., Figueroa-Espinoza, M.-C., Barouh, N., Baréa, B. and Villeneuve, P. (2007). Lipase-catalyzed synthesis of chlorogenate fatty esters in solvent-free medium. *Enzyme Microb. Technol.* **41**:721–726.

Lucas, R., Comelles, F., Alcantara, D., Maldonado, O. S., Curcuroze, M., Parra, J. L. and Morales, J. C. (2010). Surface-active properties of lipophilic antioxidants tyrosol and hydroxytyrosol fatty acid esters: A potential explanation for the nonlinear hypothesis of the antioxidant activity in oil-in-water emulsions. *J. Agric. Food Chem.* **58**:8021–8026.

Lue, B. M. (2009). Enzymatic Lipophilization of Bioactive Compounds in Ionic Liquids. PhD thesis, Department of Molecular Biology, Aarhus University, Denmark.

Luisi, P. L. and Straub, B. (1984). Reverse Micelles. Biological and Technological Relevance of Amphiphilic Structures in Nonpolar Media. Plenum Press, London.

Lyon, R. C., McComb, J. A., Schreurs, J. and Goldstein, D. B. (1981). A relationship between alcohol intoxication and the disordering of brain membranes by a series of short-chain alcohols. *J. Pharmacol. Exp. Ther.* **218**: 669–675.

Maldonado, O. S., Lucas, R., Comelles, F., González, M. J., Parra, J. L., Medina, I. and Morales, J. C. (2011). Synthesis and characterization of phenolic antioxidants with surfactant properties: Glucosyl- and glucuronosyl alkyl gallates. *Tetrahedron* **67**:7268–7279.

McClements, D. J. (2008). Lipid-based emulsions and emulsifiers. **In**: Food Lipids. Chemistry, Nutrition, and Biotechnology, 3rd ed., pp. 63–97. Akoh, C. K. and Min, D. B., Eds., CRC Press, Boca Raton.

McCreery, M. J. and Hunt, W. A. (1978). Physico-chemical correlates of alcohol intoxication. *Neuropharmacology* **17**:451–461.

Medina, I., Alcantara, D., Gonzalez, M. J., Torres, P., Lucas, R., Roque, J., Plou, F. J. and Morales, J. C. (2010). Antioxidant activity of resveratrol in several fish lipid matrices: Effect of acylation and glucosylation. *J. Agric. Food Chem.* **58**:9778–9786.

Medina, I., Lois, S., Alcántara, D., Lucas, R. and Morales, J. C. (2009). Effect of lipophilization of hydroxytyrosol on its antioxidant activity in fish oils and fish oil-in-water emulsions. *J. Agric. Food Chem.* **57**:9773–9779.

Merkl, R., Hradkova, I., Filip, V. and Smidrkal, J. (2010). Antimicrobial and antioxidant properties of phenolic acids alkyl esters. *Czech. J. Food Sci.* **28**:275–279.

Meyer, K. H. and Hemmi, H. (1935). Beitrage zur theorie der narkose. III. *Biochem. Z.* **277**:39–71.

Mezzasalma, S. A., Koper, G. J. M. and Shchipunov, Y. A. (2000). Lecithin organogel as a binary blend of monodisperse polymer-like micelles. *Langmuir* **16**:10564–10565.

Miescher, K. (1932). Studien über lokalanästhetica. *Helv. Chim. Acta* **15**:163.

Mosher, G. L. and Mikkelson, T. J. (1979). Permeability of the *n*-alkyl *p*-aminobenzoate esters across the isolated corneal membrane of the rabbit. *Int. J. Pharm.* **2**:239–43.

Murín, A., Devínsky, F., Koleková, A. and Lacko, I. (1990). Relation between chemical structure and biological activity of N-alkyl dimethylaminoxcides series and some other related components. *Biológia (Bratislava)* **45**:521–531.

Muroi, H., Nihei, K. I., Tsujimoto, K. and Kubo, I. (2004). Synergistic effects of anacardic acids and methicillin against methicillin resistant Staphylococcus aureus. *Bioorg. Med. Chem.* **12**:583–587.

Nakahiro, M., Arakawa, O., Nishimura, T. and Narahashi, T. (1996). Potentiation of GABA-induced Cl^- current by a series of *n*-alcohols disappears at a cutoff point of a longer-chain *n*-alcohol in rat dorsal root ganglion neurons. *Neurosci. Lett.* **205**:127–130.

Niki, E., Kawakami, A., Saito, M., Yamamoto, Y., Tsuchiya, J. and Kamiya, Y. (1985). Effect of phytyl side chain of vitamin E on its antioxidant activity. *J. Biol. Chem.* **260**:2191–2196.

Oehlke, K., Heins, A., Stöckmann, H. and Schwartz, K. (2010). Impact of emulsifier microenvironments on acid-base equilibrium and activity of antioxidants. *Food Chem.* **118**:48–55.

Oehlke, K., Heins, A., Stöckmann, H., Sonnichsen, F. and Schwartz, K. (2011). New insights into the antioxidant activity of Trolox in o/w emulsions. *Food Chem.* **124**:781–787.

Panya, A., Laguerre, M., Bayrasy, C., Lecomte, J., Villeneuve, P., McClements, D. J. and Decker, E. A. (2012). An investigation of the versatile antioxidant mechanisms of action of rosmarinate alkyl esters in oil-in-water emulsions. *J. Agric. Food Chem.* **60**:2692–2700.

Panya, A., Laguerre, M., Lecomte, J., Weiss, J., McClements, D. J., Villeneuve, P. and Decker, E. A. (2010). Effects of chitosan and rosmarinate esters

on the physical and oxidative stability of liposomes. *J. Agric. Food Chem.* **58**:5679–5684.

Pavlíková-Mořická, M., Lacko, I., Devínsky, F., Masárová, L. and Mlynarčík, D. (1994). Quantitative relationships between structure and antimicrobial activity of new "soft" bisquaternary ammonium salts. *Folia Microbiol.* **39**:176–180.

Peoples, R. and Weight, F. F. (1995). Cutoff in potency implicates alcohol inhibition of *N*-methyl-*D*-aspartate receptors in alcohol intoxication. *Proc. Natl. Acad. Sci.* **92**:2825–2829.

Pereira-Caro, G., Madrona, A., Bravo, L., Espartero, J. L., Alcudia, F., Cert, A. and Mateos, R. (2009). Antioxidant activity evaluation of alkyl hydroxytyrosyl ethers, a new class of hydroxytyrosol derivatives. *Food Chem.* **115**:86–91.

Pileni, M. P. (1989). **In**: Structure and Reactivity in Reverse Micelles. Pileni, M. P., Ed., Elsevier, New York.

Porter, W. L. (1980). Recent trends in food applications of antioxidants. **In**: Autoxidation in Food and Biological Systems. pp. 295–365. Simic, M. G. and Karel, M., Eds., Plenum Press, New York.

Porter, W. L., Black, E. D. and Drolet, A. M. (1989). Use of polyamide oxidative fluorescence test on lipid emulsions: Contrast in relative effectiveness of antioxidants in bulk versus dispersed systems. *J. Agric. Food Chem.* **37**:615–624.

Raines, D. E., Korten, S. E., Hill, A. G. and Miller, K. W. (1993). Anesthetic cutoff in cycloalkanemethanols: A test of current theories. *Anesthesiology* **78**:918–927.

Rucka, M., Ooewiecimska, M. and Witek, S. (1981). New biocides for cooling water treatment. II. Alkoxymethyleneammonium chlorides. *Env. Protection Eng.* **7**:45–55.

Rucka, M., Ooewiecimska, M. and Witek, S. (1983). New biocides for cooling water treatment. III. Quaternary ammonium salts. *Env. Protection Eng.* **9**:25–30.

Salem, J. H., Humeau, C., Chevalot, I., Harscoat-Schiavo, C., Vanderesse, R., Blanchard, F. and Fick, M. (2010). Effect of acyl donor chain length on isoquercitrin acylation and biological activities of corresponding esters. *Process Biochem.* **45**:382–389.

Sánchez-Paz, V., Pastoriza-Gallego, M. J., Losada-Barreiro, S., Bravo-Díaz, C., Gunaseelan, K. and Romsted, L. S. (2008). Quantitative determination of α-tocopherol distribution in a tributyrin/Brij 30/water model food emulsion. *J. Colloid Interface Sci.* **320**:1–8.

Sarapuk, J., Kleszczynska, H., Radecka, H. and Oswiecimska, M. (2000). Potential pesticide efficiency of new bifunctional surfactants. *Cell. Mol. Biol. Lett.* **5**:349–356.

Sasaki, K., Alamed, J., Weiss, J., Villeneuve, P., Lopez Giraldo, L. J., Lecomte, J., Figueroa-Espinoza, M.-C. and Decker, E. A. (2010). Relationship between the physical properties of chlorogenic acid esters and their ability to inhibit lipid oxidation in oil-in-water emulsions. *Food Chem.* **118**:830–835.

Scott, J. W., Cort, W. M., Harley, H., Parrish, D. R. and Saucy, G. (1974). 6-Hydroxychroman-2-carboxylic acids: Novel antioxidants. *J. Am. Oil Chem. Soc.* **51**:200–203.

Sersen, F., Gabunia, G., Krejcirova, E. and Kralova, K. (1992). The relationship between lipophilicity of *N*-alkyl-*N*,*N*-dimethylamine oxides and their effects on the thylakoid membranes of chloroplasts. *Photosynthetica* **26**:205–212.

Shahidi, F. and Zhong, Y. (2011). Revisiting the polar paradox theory: A critical overview. *J. Agric. Food Chem.* **59**:3499–3504.

Simpson, T. H. and Uri, N. (1956). Hydroxyflavones as inhibitors of the aerobic oxidation of unsaturated fatty acids. *Chem. Ind. (London)* 956–957.

Sørensen, A. D. M., Haahr, A. M., Becker, E. M., Skibsted, L. H., Bergenståhl, B., Nilsson, L. and Jacobsen, C. (2008). Interactions between iron, phenolic compounds, emulsifiers, and pH in omega-3-enriched oil-in-water emulsions. *J. Agric. Food Chem.* **56**:1740–1750.

Sørensen, A. D. M., Nielsen, N. S., Yang, Z., Xu, X. and Jacobsen, C. (2012). Lipophilization of dihydrocaffeic acid affects its antioxidative properties in fish-oil-enriched emulsions. *Eur. J. Lipid Sci. Technol.* 114: 134–145.

Stasiuk, M. and Kozubek, A. (2010). Biological activity of phenolic lipids. *Cell. Mol. Life Sci.* **67**:841–860.

Stöckmann, H., Schwarz, K. and Huynh-Ba, T. (2000). The influence of various emulsifiers on the partitioning and antioxidant activity of hydroxybenzoic acids and their derivatives in oil-in-water emulsions. *J. Am. Oil Chem. Soc.* **77**:535–542.

Sugandhi, E. W., Macri, R. V., Williams, A. A., Kite, B. L., Slebodnick, C., Falkinham, J. O., Esker, A. R. and Gandour, R. D. (2007). Synthesis, critical micelle concentrations, and antimycobacterial properties of homologous, dendritic amphiphiles. Probing intrinsic activity and the "cutoff" effect. *J. Med. Chem.* **50**:1645–50.

Suriyarak, S., Bayrasy, C., Schmidt, H., Villeneuve, P. and Weiss, J. (2013). Impact of fatty acid chain length of rosmarinate esters on their antimicrobial activity against Staphylococcus carnosus LTH1502 and Escherichia coli K-12 LTH4263. *J. Food Protect.* 76:1539–1548.

Takahashi, M., Komuro, E., Niki, E. and Tanaka, K. (1992). Action of fatty acid esters of L-ascorbic acid as antioxidants in phosphatidylcholine liposomal membranes. *Bull. Chem. Soc. Jpn.* **65**:679–84.

Tofani, D., Balducci, V., Gasperi, T., Incerpi, S. and Gambacorta, A. (2010). Fatty acid hydroxytyrosyl esters: Structure/antioxidant activity relationship by ABTS and in cell-culture DCF assays. *J. Agric. Food Chem.* **58**:5292–5299.

Tomlinson, E., Brown, M. R. and Davis, S. S. (1977). Effect of colloidal association on the measured activity of alkylbenzyldimethyl-ammonium chlorides against *Pseudomonas aeruginosa*. *J. Med. Chem.* **20**:1277–1282.

Torres de Pinedo, A., Penalver, P. and Morales, J. C. (2007). Synthesis and evaluation of new phenolic-based antioxidants: Structure-activity relationship. *Food Chem.* **103**:55–61.

Uri, N. (1958). The mechanism of the oxidation of linoleic acid with particular reference to metal catalysis. Fourth International Conference on the Biochemical Problems of Lipids, p 30, Butterworths, London.

Uri, N. (1961). Mechanism of antioxidation. **In**: Autoxidation and Antioxidants. Lundberg, W. O., Ed., Interscience, New York.

Vazquez, J. L., Merino, S., Domenech, O., Berlanga, M., Vinas, M., Montero, M. T. and Hernandez-Borrell, J. (2001). Determination of the partition coefficients of a homologous series of ciprofloxacin: Influence of the N-4 piperazinyl alkylation on the antimicrobial activity. *Int. J. Pharm.* **220**:53–62.

Viskupicova, J., Danihelova, M., Ondrejovic, M., Liptaj, T. and Sturdik, E. (2010). Lipophilic rutin derivatives for antioxidant protection of oil-based foods. *Food Chem.* **123**:45–50.

Walters, K. A., Bialik, W. and Brain, K. R. (1993). The effects of surfactants on penetration across the skin. *Int. J. Cosmet. Sci.* **15**:260–270.

Waraho, T., McClements, D. J. and Decker, E. A. (2011). Mechanisms of lipid oxidation in food dispersions. *Trends Food. Sci. Tech.* **22**:3–13.

Wils, P., Warnery, A., Phung-Ba, V., Legrain, S. and Scherman, D. (1994). High lipophilicity decreases drug transport across intestinal epithelial cells. *J. Pharmacol. Exp. Ther.* **269**:654–658.

Wong, Y. L., Curfman, C. L., Doncel, G. F., Hubieki, M. P., Dudding, T. C., Savle, P. S. and Gandour, R. D. (2002). Spermicidal, anti-HIV, and micellar properties of di- and trihydroxylated cationic surfactants. *Tetrahedron* **58**:45–54.

Xenakis, A., Papadimitriou, V. and Sotiroudis, T. G. (2010). Colloidal structures in natural oils. *Curr. Opin. Colloid Interface Sci.* **15**:55–60.

Yuji, H., Weiss, J., Villeneuve, P., López Giraldo, L. J., Figueroa-Espinoza, M. C. and Decker, E. A. (2007). Ability of surface-active antioxidants to inhibit lipid oxidation in oil-in-water emulsion. *J. Agric. Food Chem.* **55**:11052–11056.

Zhang, S. (2003). Fabrication of novel biomaterials through molecular self-assembly. *Nat. Biotechnol.* **21**:1171–1178.