Lipid Oxidation in Water-in-Olive Oil Emulsions Initiated by a Lipophilic Radical Source

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Received: November 27, 2009; Revised Manuscript Received: January 31, 2010

The lipophilic 2,2′-azobis(2,4-dimethylvaleronitrile) (AMVN) was used to study thoroughly the oxidation reaction in a model water-in-olive oil emulsion system. This radical species decomposes thermally generating a constant flux of radicals in the oil phase. The dissociation constant k_d in olive oil at 40 °C for AMVN was calculated as 2.5×10^{-4} min^{-1} and the rate of initiation of the oxidation reaction, R_i was calculated by using vitamin E as antioxidant. The olive oil oxidation in emulsion was monitored by measuring the hydroperoxide concentration by a sensitive fluorimetric method. The DPPP (diphenyl-1-pyrenylphosphine) was used as a probe because it reacts stoichiometrically with hydroperoxides to yield a fluorescent product, the diphenyl-1-pyrenylphosphine oxide (DPPP-O). Oxidation data together with emulsion droplet size data showed that in the presence of radical initiator and a large interface, the oxidation reaction is accelerated in W/Olive oil emulsion with respect to whole oil. The mediation of the surface area of water droplets is surely involved in this process because the addition of saturated solutions of ascorbic acid (AA) dispersed in the oil brings about the strong reduction of the oxidation rate even in the presence of the highest AMVN quantity.

Introduction

Emulsions represent a class of colloidal systems widely found in foods, cosmetics, and pharmaceuticals.[1] As far as food emulsions are concerned, they owe their characteristics of texture, taste, and aroma to their microstructure and ingredient composition. Some examples of common food emulsions are mayonnaise, spreads, creams, and chocolate. The oil-in-water (O/W) type prevails in foods but there are also examples of water-in-oil (W/O) emulsions, such as low fat spreads and chocolate, butter, and veiled extra virgin olive oils.[2,3] The common feature of all these different emulsions is the fat or oil component, which is very important because it determines both the structure and the stability of the food. In fact, the main problem to face when a food emulsion formulation is being created is the limited stability inherent of these systems that can be affected by the emulsifiers composition and by the occurrence of degradation reaction to be charged to emulsion components. In this context, lipid oxidation is a major problem for the stability of fat-containing foods.[4] It not only does affect the stability and shelf life of these emulsions but also their safety for consumption, because the products of oxidation are known to be responsible of severe pathological events such as tumorigenesis, liver disease, and atherosclerosis.[5] For this reason, it is important to have thorough knowledge of the mechanisms of oxidation in complex heterogeneous systems. The importance of the colloidal properties of the oil phase has been recognized and several investigators have demonstrated differences in the efficiency, for example, of antioxidants between emulsions and bulk oils.[6] The presence of a large interface between oil and water may affect the oxidation pathways because it affects the partition of antioxidants/prooxidants between phases. The role of interfaces in the oxidation reaction is undoubted and some authors have stressed the significance of W/O or O/W surfaces on the oxidation process.[7−9] Chain reactions taking place in compartmentalized systems can follow kinetics and mechanisms quite different than those observed in homogeneous solution. The in-depth study of the oxidation reaction in these systems is complicated by the complexity of the oxidation reaction which consists of initiation, propagation and termination steps proceeding autocatalytically and at once. In oxidation studies it is convenient to use radical initiators that decompose thermically to give a constant flux of radicals with time.[10,11] In this way the initiation step is controlled and the corresponding rate can be easily calculated. Besides the elimination of an unknown of the oxidation reaction, another advantage of using radical azo-initiators is that the oxidation rate is accelerated at lower temperatures (∼40 °C) with respect to other accelerated oxidation methods such as rancimat and oxygen bomb. These conditions of oxidation are so mild that the initial properties of emulsions are preserved. The use of azo-initiators instead of high temperatures or UV light allows to draw a more realistic comparison with the oxidation conditions occurring during the normal food shelf life.[2] In this work the apolar initiator 2,2′-azobis(2,4-dimethylvaleronitrile) (AMVN) is used to study lipid oxidation in W/Olive oil emulsion oxidation at 40 °C. The aim was to understand the role of the W/Oil interface in the oxidation reaction when the radicals are formed in the continuous oil phase. There are many examples of papers in which AMVN has been used as initiator but most of these deal with O/W emulsions and liposomes dispersion.[12] Up to now, few are the works on the oxidation of biocompatible W/O emulsions and this work tries to add a piece in the puzzle describing the oxidative properties of this class of food emulsions, which achieves more importance as it builds up the demand of healthy low fat foods. To do this, a fluorescence method for the quantitative measurement of hydroperoxides and emulsion droplet size data gathered through optical microscopy were used.

Experimental Methods

Materials. Polysorbate 80 (Tween 80) and vitamin E were from Fluka analytical. The Olive oil (*Olivae oleum virginale*)

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10.1021/jp911288e
Published on Web 02/22/2010

of composition and purity according to the European Pharmacopoeia was from Fluka. The radical initiator 2,2′-azobis(2,4-dimethylvaleronitrile) was from Cayman Chemical Company. The fluorescence probe diphenyl-1-pyrenylphosphine was from Probior. Sorbitan monoleate (Span 80) and L-ascorbic acid were from Sigma Aldrich.

Choice of Emulsion Formulation for Oxidation Studies. The choice of the right emulsion formulation is of crucial importance since the oxidation experiments have to be carried out at temperatures higher than RT. It is well-known that high temperatures may accelerate emulsion degradation. So the choice was driven by the need to have an emulsion stable for the experimental period. Different W/O emulsions were prepared with a water content ranging from 0.5 to 1 wt % and a total surfactant content from 0.1 to 1 wt %. The surfactant used were nonionic sorbitan fatty acid esters, namely Span80 and Tween 80 in different combinations, to have a certain final hydrophile−lipophile balance (HLB) of the mixture. In particular, the hydrophilic Tween 80 was added to the aqueous phase while the lipophilic Span 80 was added to the oil phase and left to equilibrate overnight under magnetic stirring. Then, the aqueous phase was added to the Span containing oil phase drop by drop during mixing with Ultraturrax T25 S25N-8G (IKA Werke Gmbh & Co. KG, Janke & Kunkel, Staufen, Germany) at 24×10^3 rpm for 2 min. Emulsion stability was evaluated by visual inspection of samples left at room temperature. The presence of creaming layers or sediment was taken as an instability indicator. Some formulations that gave best results from macroscopic observations were further tested by turbidity measurements. Turbidity measurements of W/O emulsions were carried out according to Song et al.[13] Spectral absorbance was measured after dilution of the emulsions 1:2 with olive oil containing Span 80 to meet the same final concentration of the original emulsions. The turbidity ratio R was defined as the ratio of turbidity at wavelengths 850 and 450 nm and it was followed for two hours at 40 °C. According to reference,[13] the slope of the curves at $t \rightarrow t_0$ is well related to emulsion stability.

Determination of Rate Constants for the Azo-Initiator Decomposition. The k_d values were determined both in ethanol and in olive oil by following the loss of azo chromophore at 348 nm. Ethanol experiments were carried out at 25, 40, 50, and 60 °C with an initiator concentration of 0.4 wt %. Olive oil experiments were carried out versus olive oil as a reference at 40, 50, and 60 °C with AMVN concentration of 0.4 wt % and at three different initiator concentrations, 0.4, 0.8, and 1.2 wt %, at 40 °C. In this way it was possible to follow the degradation of the natural pigments present in olive oil such as carotenoids. The decomposition of carotenoids was monitored by following the decrease in absorbance at 418, 452, and 480 nm corresponding to, respectively, norbixin, β-carotene, and lutein. The concentration of carotenoids was determined according to Minguez-Mosquera et al.[14]

UV−vis Spectrophotometry of Olive Oil Enriched with Vitamin E. The spectra of olive oil were recorded in whole oil containing the azo initiator in different concentration versus olive oil in a first set of experiments and olive oil plus initiator and vitamin E in a second step to calculate the rate of initiation of the oxidation in homogeneous system. Since vitamin E is a very viscous oil, it was added as an ethanolic solution in order to improve the solubilization and to reach the desired final concentration. A 10 μL of vitamin E stock solution 0.36 M in ethanol was then added directly in 1 g of olive oil.[12] The vitamin degradation was followed by monitoring the absorption peak at 311 nm.[15] The linear range of concentration was verified by a calibration curve.

Emulsion Oxidation. The azo initiator was added to the oil phase before emulsification but after the addition of surfactants. Emulsions were kept at 40 °C in a water bath in a capped vial. Aliquots of sample were collected at fixed data interval and used for hydroperoxides analysis.

Optical Microscopy. Optical micrographs were obtained by means of an optical microscope Zeiss Axioplan 2, at 25 °C. To get representative results from the system video enhanced microscopy (VEM) was used.[16] Such a technique combines the magnification power of the microscope with the digital image acquisition capability of a video camera (Panasonic, model GP-KR222). A series of images (9−10 pictures, 1000−1500 particles) were examined to determine the size polydispersity that was estimated by counting the "average number" of droplets at different radii in micrographs of a Thoma grating. Image analysis software (Sigma Scan Pro, SPSS Science Software Products), which provides a wide range of analytical features in addition to image enhancement, was used to digitize the images.

Monitoring the Oxidation: Fluorescence Spectroscopy. Oil oxidation was monitored with time by using a fluorimetric method adapted from Akasaka et al.[17] A 20−100 mg emulsion sample, depending on the expected extent of oxidation, was weighed exactly and filled up to 10 mL of chloroform/methanol (1:1, v/v). A 20 μL aliquot was then put in a reaction vial together with 10 μL of diphenyl-1-pyrenylphosphine (DPPP) solution 3 mM (in chloroform/methanol 1:1, v/v) and taken to a final volume of 100 μL with the chloroform/methanol solvent mixture. The vial was capped tightly and left for 60 min in a water bath at 60 °C in the dark. Then it was cooled to room temperature and taken to a final volume of 2 mL with the solvent mixture before the measurement of fluorescence emission at 380 nm ($\lambda_{ex} = 352$ nm). DPPP is reported to be a fluorescent reagent for the measurements of hydroperoxides with high selectivity and sensitivity.[18,19] DPPP itself is not fluorescent but its oxide, DPPP oxide, shows a strong fluorescence. So the concentration of hydroperoxides present in the sample can be calculated as the concentration of DPPP oxide that forms from the stoichiometric reaction with hydroperoxides. The concentration of hydroperoxides was calculated using a calibration curve obtained by the reaction of DPPP with known amounts of H_2O_2. The experimental uncertainty was estimated by calculating the SD of repeated measurements ($n \geq 3$).

Results and Discussion

Homogeneous System. Apolar azo-initiators have a good potential to mimic the natural oxidation process by increasing the rate of oxidation in relatively mild conditions. By making comparisons with other accelerated test of oxidation like oxidative stability instrument (OSI), oxygen bomb (OB), and Rancimat in the presence of azo initiators, the temperature can be lowered to values closer to those at which the lipidic product is actually stored. Consequently, the results obtained by using azo initiators can be correlated to some extent to what occurs during the real shelf life of food emulsions. The main advantage of using apolar azoinitiators is that they will form radicals directly in the lipid matrix producing lipid radicals through the reaction with lipids in their surroundings.[20] Such initiators are thought not to interfere with other stage of the oxidation process and provide a constant flux of radicals for the initiation of the autoxidation reaction. In this way, one can measure the rate of

initiation of the reaction and so an important unknown of the oxidative process may be eliminated.

First, radical generation was monitored in different homogeneous systems, AMVN (2,2′-azobis(2,4-dimethylvaleronitrile)) dissolved in ethanol 96% and AMVN dissolved in olive oil plus the nonionic emulsifier Span 80. These experiments were carried out at different AMVN concentrations and temperatures for each homogeneous system.

Kinetics for initiator decomposition were determined by measurements of the disappearance of the azo group absorption in the UV at 348 nm. Experiments in ethanol with AMVN gave good first order kinetics. Once the kinetic order has been verified, the calculation of the rate constants is straightforward. Rate constants for AMVN decomposition in ethanol, k_d, were determined at 60 °C, 6.0×10^{-3} min^{-1}; 50 °C, 1.6×10^{-3} min^{-1}; 40 °C, 3.0×10^{-4} min^{-1}; and 25 °C, 4.0×10^{-5} min^{-1}. These data followed the Arrhenius law and E_a and the pre-exponential factor were determined as $E_a = 1.19 \times 10^5 \pm 2 \times 10^3$ J/mol, $A = 2.8 \times 10$.[16] These values are similar to those reported by other author for the decomposition in toluene.[21]

It is well-known that the solvent may affect the decomposition rate of azo initiators. In fact, initiators radicals initially formed in solution are held together briefly in a cage of solvent molecules. This cage effect causes radical molecules to recombine and slows down their diffusion through the solvent. Therefore, the rate of the decomposition of the initiator depends, above all, on the viscosity of the solvent and on the characteristic of the system. For this reason, the decomposition of the azo group of AMVN was followed in olive oil. The model emulsion that will be used for oxidation studies is composed mainly of olive oil with a low-water content to best resemble the conditions found in veiled olive oils. So the aim of this experiment was to find the best conditions to oxidize the W/O emulsions to keep intact emulsion structure for a long time.

Several experiments were carried out to find the best initiator concentration. In particular, oxidation kinetics in olive oil were followed at 40 °C and at three different AMVN content (0.4, 0.8, and 1.2 wt %). Good first order kinetics ($R^2 > 0.99$) were obtained for the dissociation of AMVN. Rate constants for the decomposition of 0.8 wt % of AMVN in olive were determined at 60 °C, 3.0×10^{-3} min^{-1}; 50 °C, 9.0×10^{-4} min^{-1}; and 40 °C, 3.2×10^{-4} min^{-1}, $t_{1/2} = 36$ h. These data followed the Arrhenius law and the calculated parameters are $E_a = 9.5 \times 10^4 \pm 3 \times 10^3$ J/mol and $A = 1.5 \times 10$.[14] By comparing the E_a calculated for AMVN in ethanol and olive oil, it is clear that the dissociation reaction in olive oil is less dependent on temperature than in ethanol. These data were used to choose the concentration of AMVN for oxidation studies as 0.2, 0.4, and 0.8 wt % to obtain a general picture of the oxidation reaction in a short time course.

Figure 1 shows the spectra of olive oil with 0.8 wt % AMVN versus olive oil as reference at $t = 0$ and $t = 122$ h of oxidation at 40 °C. By following AMVN containing olive oil spectrum with time, it is possible to follow the disappearance of compounds during the oxidation reaction. In the time course of the oxidation experiment, the negative spectrum of olive oil appears with the characteristic pigments bands while the azo group absorbs in the wavelength range between 300 and 350 nm.

The wavelength range between 400 and 480 nm is the zone of the carotenoids where each peak corresponds to a particular molecule (norbixin 419 nm, β-carotene 452 nm, and lycopene 480 nm).[22] The zone between 650 and 700 nm refers to chlorophylls. During the oxidation, the pigments present in olive oil fade away because they are very susceptible to radical attack. In particular, carotenoids and especially β-carotene are reported to be powerful antioxidants in olive oil and they undergo bleaching during the oxidation reaction. As a result, the olive oil loses its characteristic yellow-green color (Figure 1).

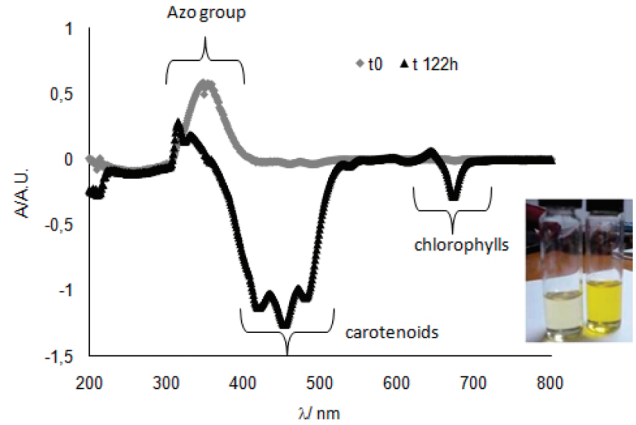


Figure 1. Absorption spectrum of olive oil with AMVN 0.8 wt % vs olive oil as reference at two oxidation times. The color of olive oil fades away during the oxidation reaction.

TABLE 1: Initial Rates of Degradation of Carotenoids As a Function of AMVN Concentration in Olive Oil

	initial rate of degradation[a]/ $\times 10^6$ s^{-1}		
	AMVN/ mmol g^{-1} $\times 10^3$		
	16	32	48
β-carotene	6.0 ± 0.3	0.12 ± 0.01	0.18 ± 0.01
lycopene	5 ± 0.3	0.10 ± 0.01	0.16 ± 0.01
norbixin	3 ± 0.2	0.75 ± 0.04	0.13 ± 0.01

[a] Each value is expressed as a mean ± SD ($n = 3$).

As far as carotenoids are concerned, they behave as radical scavengers due to the extensive system of conjugated double bonds that makes them very susceptible to radical addition. According to this mechanism, β-carotene is able to scavenge peroxyl radicals as follows[23]

$$\beta\text{-carotene} + \text{ROO}^\bullet \rightarrow \text{ROO}-\beta\text{-carotene}^\bullet \quad (1)$$

$$\text{ROO}-\beta\text{-carotene}^\bullet + \text{O}_2 \leftrightarrow \text{ROO}-\beta\text{-carotene}-\text{O}^\bullet \quad (2)$$

$$\text{ROO}-\beta\text{-carotene}^\bullet + \text{ROO}^\bullet \rightarrow \text{nonradical products} \quad (3)$$

The resulting carbon-centered radical reacts rapidly with oxygen to give a new peroxyl radical or, alternatively, with a peroxide to give nonreactive products. In Table 1 are reported the initial rates of degradation of carotenoids as a function of AMVN concentration in olive oil.

It is clear that the rates of degradation of the different carotenoids molecules are proportional to AMVN concentration, so indicating a direct relationship between AMVN dissociation and carotenoids degradation.[22,23] The rate of degradation of carotenoids increases as the concentration of AVMN increases and this indicates that the rate of carotenoids degradation is directly related to the rate of the initiation step of oxidation. In other words, the kinetic is of first order with respect to AMVN. An apparent kinetic constant can be calculated as $k_{app} = k_{inh} \times$

[Car] where k_{inh} is the kinetic constant of the antioxidant reaction (eq 9, see below). When $v = v_0$, [Car] is $\approx$ [Car]$_0$ and is 1.86 $\times 10^{-6}$ mmol g^{-1} as β-carotene, so for this reaction k_{inh} is 2.1 $\times$ 10^{-1} mM^{-1} s^{-1}. So the rate of oxidation in the olive oil used herein will be affected by the presence of endogenous antioxidants. In particular, the mechanism of peroxidation in homogeneous solutions follows the following scheme:

Initiation:

$$R - N = N - R \xrightarrow{k_d} 2eR^{\bullet} + N_2 + (1 - e)R - R \quad (4)$$

$$R^{\bullet} + O_2 \xrightarrow{\text{fast}} ROO^{\bullet} \quad (5)$$

$$ROO^{\bullet} + LH \xrightarrow{k_6} ROOH + L^{\bullet} \quad (6)$$

Propagation:

$$L^{\bullet} + O_2 \xrightarrow{k_0} LOO^{\bullet} \quad (7)$$

$$LOO^{\bullet} + LH \xrightarrow{k_p} LOOH + L^{\bullet} \quad (8)$$

Inhibition and termination:

$$LOO^{\bullet} + AH \xrightarrow{k_{inh}} LOOH + A^{\bullet} \quad (9)$$

$$LOO^{\bullet} + A^{\bullet} \xrightarrow{k_{ta}} LOOA \quad (10)$$

$$LOO^{\bullet} + LOO^{\bullet} \xrightarrow{k_t} \text{nonradical products} \quad (11)$$

where k_d, k_p, and k_t are rate constant for initiator decomposition, for chain propagation, and termination, respectively. LH is a molecule of lipid (olive oil triacylglycerol), L$^{\bullet}$ is a lipid derived radical, R$^{\bullet}$ is an initiator derived radical and so on. The rate of initiation can be derived as follows

$$R_i = 2k_d \times e[R - N = N - R] \quad (12)$$

The factor e is the cage parameter, that is, the fraction of initiator radicals that escape the solvent cage and depends on the medium and the concentration of antioxidants and initiator.[24] The "cage effect" causes radical molecules to recombine and slows down their diffusion through the solvent.

It is well-known that the R_i value can be determined by the inhibition period and or by the decay rate of an antioxidant. It is practical to calculate the R_i by using an antioxidant whose the number of radical trapped by each molecule, n, is known. α-tocopherol (TOH) has an n equal to 2 and so the R_i can be calculated from the inhibition period or from the decay rate of TOH as follows

$$-\frac{d[AH]}{dt} = R_i/n \quad (13)$$

TABLE 2: Rate of Initiation R_i, Cage Parameter e, Initial Rate of Vitamin E Degradation Calculated by Equations 12 and 13 at Different AMVN Concentrations

C_{AMVN}/mmol g^{-1} $\times 10^3$	R_i[a]/mmol s^{-1} $\times 10^8$	cage parameter e[a]	initial rate of vit E degradation[a]/ s^{-1} $\times 10^4$
8	2.5 ± 0.1	0.29 ± 0.02	2.0 ± 0.1
16	3.7 ± 0.2	0.22 ± 0.02	3.0 ± 0.2
32	6.2 ± 0.3	0.18 ± 0.02	5.0 ± 0.2

[a] Each value is expressed as a mean ± SD ($n = 3$).

In Table 2 are gathered the values of initial rates of vitamin E degradation at different AMVN concentration and the calculated values of R_i by measuring the rate of vitamin E degradation. Vitamin E shows a sharp peak at 310 nm in olive oil which is proportional to concentration. A calibration curve was made in olive oil for the calculation of the initial rate of degradation and R_i.[15]

In our system, the initial rate of oxidation is $R_i = k_i[ROO^{\bullet}]\cdot[LH]$ and being the concentration of lipid very high (homogeneous system) the reaction turns into a pseudofirst order kinetics. So we can assume that the initiation step depends only on the concentration of initiator-derived peroxyl radicals.

In a complex system such olive oil, the presence of antioxidant makes things more complicated. The kinetics of oxidation should take into account the action of endogenous antioxidants. To get the equation rate of oxidation in the presence of antioxidants, it should be assumed that during the inhibition period the rate of peroxyl radicals formed equals the rate of radicals trapped by antioxidant so

$$\frac{d[LOOH]}{dt} = R_{inh} = k_p[LOO^{\bullet}][LH] = \frac{R_i k_p[LH]}{[AH]2k_{inh}} \quad (14)$$

In whole olive oil with a radical initiator, LH will compete with AH to react with ROO$^{\bullet}$. The reactions below may affect the overall reaction

$$ROO^{\bullet} + AH \xrightarrow{k_{15}} ROOH + A^{\bullet} \quad (15)$$

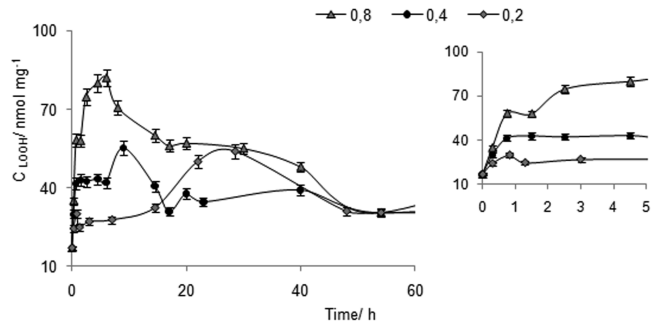


Figure 2. Concentration of hydroperoxides in nmol LOOH/mg of oil, measured by a fluorimetric method based on the DPPP probe, as a function of oxidation time at 40 °C for samples not emulsified containing different amounts of AMVN initiator. The inset shows in detail the oxidation behavior of samples at short times. The lines are a guide for the eye. Values are means of three repeated measurements ± SD.

$$ROO^{\bullet} + A^{\bullet} \xleftarrow{k_{16}} ROOA \qquad (16)$$

In our system, the rate constants for carotenoids at 37 °C, k_{15}, k_6, and k_p are 6.0×10^4, 3.0×10^3, and 1.4×10^1 M^{-1} s^{-1}, respectively (from ref 25), k_{inh} calculated is 2.1×10^2 M^{-1} s^{-1}. So the reaction between initiator derived peroxyl radicals and lipid or antioxidant is favorite and it is possible that generated $ROO^{\bullet}$ initiates new propagation steps and it is first trapped by AH. This may also explain the dependence of carotenoids degradation on the AMVN concentration.

Figure 2 shows the change in hydroperoxides concentration with storage time at 40 °C for whole olive oil containing different AMVN quantity.

As we can see from the inset in Figure 2, the initial rate of hydroperoxide formation depends on AMVN. The curves share a similar trend: a first period of fast increase in LOOH concentration followed by the achievement of a maximum at a time dependent on the initial concentration of AMVN. Furthermore, in samples with 0.2 and 0.4 wt % (8 and 16×10^{-3} mmol g^{-1}) AMVN, after less than one hour of oxidation a plateau is reached and the concentration of hydroperoxides stays almost constant up to 5 h of oxidation. This can mean that the oxygen becomes limiting when a first peak of hydroperoxides concentration is reached and the carotenoids present in olive oil become more effective because the eq 2 will be shifted to left.[22] In these conditions, endogenous carotenoids exert their maximum activity as showed by Kiokias et al.[22] In the sample containing 0.8 wt % AMVN (32×10^{-3} mmol g^{-1}), the quantity of radicals formed with time is high and antioxidants lose their activity soon. When the concentration of initiator increases the rate of oxygen consumption increases and the system reaches a maximum in hydroperoxides rapidly. After the maximum concentration in hydroperoxides is reached, a decreasing step is observed until the system goes to a stationary state in which the concentration of hydroperoxides stays almost constant unless the concentration of lipids changes significantly. Since the oil contains endogenous antioxidants, it can be assumed that the rate of oxidation will follow eq 14.

In heterogeneous systems, things may change when the kinetics of the oxidation reaction are considered. The next step is to create a wide interfacial area and to see whether the aqueous dispersed phase affects the oxidation kinetics.

Heterogeneous System. It has been shown that natural olive oils containing a little percentage of dispersed vegetation water are more resistant to the oxidative damage.[7] It is clear that the natural antioxidants of olive oil can partition between the water/oil phase and interface and in this way the oxidation pathway can be modified.[6] In our recent works, we showed that artificially veiled olive oils are less susceptible to UV-induced oxidation and that the antioxidant property of the dispersed phase can be enhanced by adding emulsifiers and hydrophilic antioxidants.[7,8,26] As far as the oxidation mechanism is concerned, some authors say that in a compartmentalized system the oxidation follows the same rate law of homogeneous systems,[27] but up to now the available data are mainly on liposome dispersion and on O/W emulsions but not much is known on water-in-oil emulsions.[28] Nevertheless, it is well-known that lipid oxidation can be strongly affected by the presence of a large interface in the better and in the worse. In this work, emulsions were prepared to simulate the natural cloudiness of veiled olive oils which form during the traditional olive oil extraction process.

Setting up Emulsion Formulation. A first step concerned the setting up of an emulsion formulation with low water content (from 0.5 up to 1% w/w) and kinetically stable for the experimental time course at 40 °C. From a first screening, the water content that gave the best stability was 0.7% with a water surfactant ratio 1:1 (w/w). Then, different emulsion formulations were prepared with 0.7% w/w of distilled water and 0.7% w/w of mixtures of Span 80 (Sorbitan Monoleate−HLB = 4.3) and Tween 80 (polioxyethylene sorbitan monoleate−HLB = 10) to achieve different HLB of the mixtures. In Table 3 are gathered the results of the macroscopic observations of the emulsions at different HLB after 24 h at room temperature. The emulsifiers used were all food grade and admitted for use in foodstuff according to the current EU Regulations.[30,31] It is worth noticing that emulsions that appeared stable after 24 h at 25 °C share a weak birifrangence. This indicates that the stability is due to the formation of a stable interface layer of liquid crystalline phases.[29] The emulsions that were stable for at least 24 h were subsequently tested through turbidimetric measurements according to Song et al.[13] Figure 3 shows the turbidity ratio R = absorbance 850 nm/absorbance 450 nm as a function of storage time at 40 °C. According to Song et al, the slope of the turbidity

TABLE 3: Macroscopic Characteristics of W/O Emulsions at Different HLB after 24 h Storage at 25 °C

HLB	appearance	birifrangence
4.3	turbid/sediment no creaming	no
5.2	turbid/sediment no creaming	yes
5.5	stable	yes
6.0	stable	yes
6.5	opalescent/sediment	no
7.0	opalescent/sediment	no
7.5	turbid/sediment	yes
8	opalescent/small sediment	no

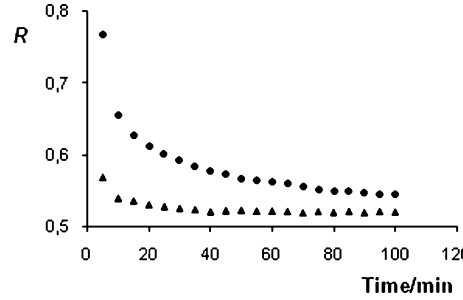


Figure 3. Turbidity ratio R as a function of storage time for W/Olive oil emulsion at different HLB of the Span 80/Tween 80 mixture. (•) HLB = 6.0; (▲) HLB = 5.5.

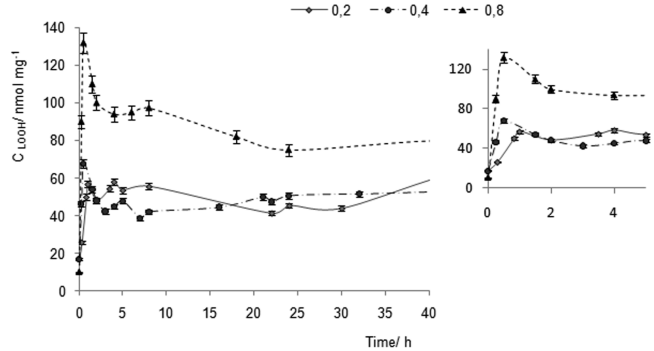


Figure 4. Concentration of hydroperoxides in nmol LOOH/mg of oil, measured by a fluorimetric method based on the DPPP probe, as a function of oxidation time at 40 °C for W/O emulsion samples with different amounts of AMVN. The inset shows in detail the oxidation behavior of samples at short times. The lines are a guide for the eye. Values are means of three repeated measurements ± SD.

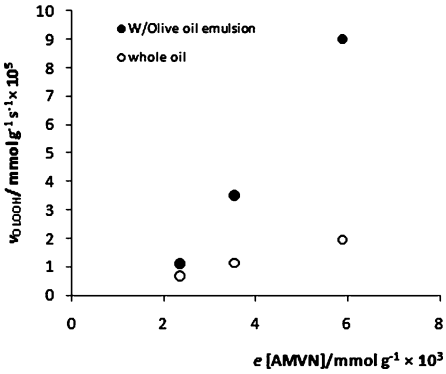


Figure 5. Initial rate of hydroperoxide formation as a function of $e \times$ AMVN concentration for emulsified and not emulsified samples.

ratio within the first 50 min from the preparation is well correlated to emulsion stability. In particular, lower slope of R versus time indicates higher stability.

So it is clear from Figure 3 that emulsion with HLB 5.5 is more stable than emulsion with HLB 6.0 because the slope of R versus time is lower. The conclusions drawn from turbidity measurements are in accordance with the visual tests of stability made up to 7 days at 25 °C.

Oxidation Experiments in W/O Emulsions. Then the optimum emulsion formulation to be used for oxidation studies was composed of 0.7 wt % emulsifiers mixture (HLB = 5.5) and 0.7 wt % distilled water. Figure 4 shows the change in hydroperoxides concentration as a function of oxidation time for W/Olive oil emulsions with different contents of AMVN. Data point out that a rapid increase of the concentration of hydroperoxides in W/Olive oil emulsion is followed by a decrease due to a decomposition of these compounds. Later on, the oxygen becomes available from the propagation reaction and because of the sampling procedure which requires to open the vial at different time intervals. After the maximum, the system enters in a stationary state in which the hydroperoxides formed are equal to those that decompose to secondary oxidation products. The trend observed is similar to that shown in Figure 2 but some differences may be easily noticed. A maximum of hydroperoxides concentration is reached for all samples but the time at which the maximum is achieved is lower for emulsions than for homogeneous olive oil systems. At a first sight, the rate of hydroperoxides formation is faster in emulsions than in whole olive oil. It can be easily calculated a rate of hydroperoxide formation from the curves in Figures 2 and 4 and a plot of this rate as a function of AMVN $\times$ e is shown in Figure 5. From Figure 5 it is clear that the dependence of the initial rate of oxidation on AMVN concentration is different for systems emulsified and not emulsified. In particular, both in homogeneous system and in W/O emulsion, the v_0 is linearly related to AMVN $\times$ e. So by taking into account eqs 12 and 14, the linear trend indicates that W/O emulsions and whole olive oil follow the same kinetic model of oxidation. Nevertheless, the oxidative behavior of emulsified samples differs markedly from that of whole oil at initiator concentration higher than 0.2 wt % (8×10^{-3} mmol g^{-1}). This indicates that AMVN works more efficiently in W/O emulsions where a large interphase area exists. We have previously calculated the rate of initiation of the oxidation reaction in homogeneous systems by applying the classical mechanism of peroxidation in oils. It can be argued that when the concentration of initiator increases the two oxidation pathways in homogeneous and heterogeneous systems separate because of a change in the cage parameter of the radical initiator and/or of an effect due to the presence of a W/O interphase. From the comparison of the linear trends in Figure 5, it can be argued that being the angular coefficient $k_p k_d$[LH]/k_{inh}[AH] (for eqs 12 and 14) and assuming [LH] constant, the difference between the two systems trends can arise from different [AH] values. In fact, in heterogeneous systems like W/O emulsions the local concentration of antioxidants may vary as a result of the partitioning of these molecules between the phases. In general, the higher angular coefficient of emulsion with respect to whole oil indicates a higher occurrence of oxidation-propagating events than antioxidant reactions. The presence of the interphase brings about the increase in the propagation reactions since initiator peroxyl radicals are more efficiently dispersed in the oil.

Effect of Emulsion Structure on Oxidation Kinetics. The effect of a water dispersed phase on the oxidation of olive oil can change in dependence on factors as concentration, size and size distribution, type and quantity of emulsifiers used, and presence of hydrosoluble antioxidants.[31] The knowledge of how all these factors can modulate the oxidative behavior of emulsions can be decisive for choosing the best way to protect complex food from oxidation. The structure of an emulsion can modulate strongly its properties. It is possible to correlate oxidation to structure by means of size data of the dispersed phase. Knowing the diameter of the droplets in a sample allows the calculation of the size distribution and of the moments of the distribution. Droplet size distributions of emulsions are best characterized by the moments

$$\mu_j = \sum_i f(x) x^j \tag{17}$$

where j is the moment order.

Once the jth moments are calculated, it is possible to derive other parameters that can be useful to study emulsions properties. When dealing with real emulsions with polydisperse droplets, it is useful to represent as one value the system by means of average diameters, as follows

$$\bar{D}_{nm} = \left(\frac{\mu_n}{\mu_m}\right)^{1/(n-m)} \tag{18}$$

So $\bar{D}_{10}$ is the number length diameter or mean, $\bar{D}_{32}$ is the volume to surface mean (or Sauter average), and so on. When the study of the reaction rate of a dispersed system is of concern, it is useful to use a mean diameter involving surface area.

Figure 6a shows the dependence of the initial rate of hydroperoxides formation on the number-surface mean diameter, $\bar{D}_{20}$, that is, the diameter of a spherical particle having a surface $(A_s/N) = \pi \bar{D}_{20}^2$, where N is the total number of dispersed particles, A_s is the surface area. This average surface mean diameter was calculated from the size distributions of emulsions with different amount of AMVN. The rate of oxidation decreases as the surfacemean diameter increases so indicating that the process of oxidation in emulsion is affected by the total surface area of dispersed phase, as expected (Figure 6a). As far as the size of droplets is concerned, the $\bar{D}_{20}$ decreases as the concentration of AMVN increases in emulsion (Figure 6b). This fact indicates that AMVN added to the oil phase prior to the agitation step with the water phase affects the structure of the emulsion causing a decrease in the mean droplets size. Obviously, this fact leads to an increase in the total surface area of emulsions as the AMVN concentration increases. These results show that the size of water droplets is a decisive factor in determining

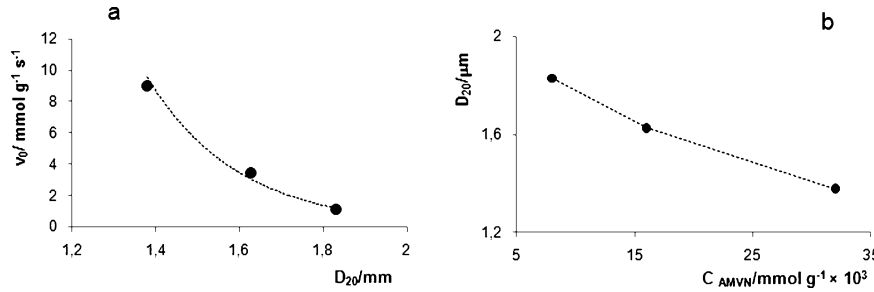


Figure 6. (a) Initial rate of hydroperoxides formation in W/O emulsion vs D_{20}. (b) Dependence of D_{20} on the concentration of AMVN in emulsion. The lines are a guide for the eye.

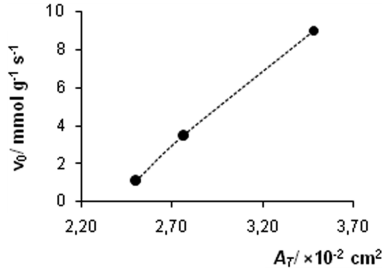


Figure 7. Initial rates of hydroperoxide formation as a function of emulsions total surface area of dispersed phase. The lines are a guide for the eye.

the rate of the oxidation reaction. In particular, these results seems to contradict what we have previously shown in experiments of oxidation in water emulsified olive oils.[32,33] In those works, the peroxide value, PV, decreased with increasing the surface area of the dispersed phase.

It must be pointed out, however, that the oil phase used herein is not a genuine olive oil but it is a commercial product with a constant composition according to the European Pharmacopoeia. The natural product is a complex system rich in minor compounds with powerful antioxidant action. Then, many factors can explain this discrepancy: the differences in the emulsion model especially regarding the oil phase, the presence of a mixture of emulsifiers to create a stable interfacial layer, and the method of oxidation which implies the use of an initiator. Up to now, we have considered the water dispersed phase of W/Olive oil emulsions as an antioxidant-like compound, whose effect on the oxidation reaction is dependent on its characteristics.[8,26] In general, antioxidants are not universal; they may protect in one environment but lack protection in another. In the same way, a water-dispersed phase in a vegetable oil may behave like an antioxidant in a system, but may trigger the reaction in another. In this context, if the initial reaction rates of the emulsion with different AMVN content are being compared, it is useful to make a comparison on the basis of the total surface area of dispersed phase A_T. In Figure 7 it is shown the dependence of the initial rate of hydroperoxides formation on the total surface area calculated from the size distribution data.

It is clear that the initial rate of hydroperoxide formation is dependent on the total surface area of dispersed phase. This finding is surely related to the presence of AMVN in the lipophilic phase prior to emulsion preparation. During the mixing step a quote of AMVN may be incorporated in the water−oil interface by the Tween 80/Span 80 mixture. Further, in the course of the oxidation reaction the peroxyl radicals generated in the bulk phase are expected to concentrate at the oil/water surface since they are more amphiphilic than the starting molecule.[34] In fact, Liang et al. demonstrated that the dipolar

TABLE 4: k_{clinh} in Emulsion and Whole Oil with and without AA

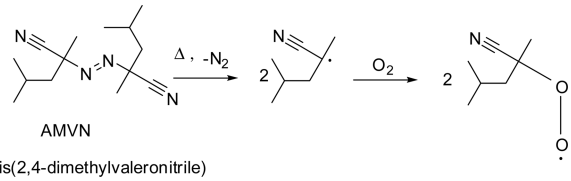
System	Calculated k_{clinh}[a]
whole olive oil with AMVN 8 × 10^{-3} mmol g^{-1}	273 ± 14
whole olive oil with AMVN 16 × 10^{-3} mmol g^{-1}	303 ± 15
whole olive oil with AMVN 32 × 10^{-3} mmol g^{-1}	311 ± 15
W/olive oil emulsion with AMVN 8 × 10^{-3} mmol g^{-1}	445 ± 20
W/olive oil emulsion with AMVN 16 × 10^{-3} mmol g^{-1}	935 ± 45
W/olive oil emulsion with AMVN 32 × 10^{-3} mmol g^{-1}	1440 ± 72
W/olive oil emulsion with AMVN 8 × 10^{-3} mmol g^{-1} + AA 1000 ppm	490 ± 24
W/olive oil emulsion with AMVN 8 × 10^{-3} mmol g^{-1} +AA 2100 ppm	114 ± 6
W/olive oil emulsion with AMVN 32 × 10^{-3} mmol g^{-1} + A A 2100 ppm	64 ± 3

[a] Each value is expressed as a mean ± SD (n = 3).

moment of the peroxyl radical generated by the lipophilic initiator AMVN is higher than the dipolar moment of the peroxyl radical generated by a hydrophilic initiator.[34] This observations may explain two facts. First, the decrease in droplet size with AMVN concentration in emulsion, keeping constant the other components, due to an interaction/adsorption at the interface; second, the increase in the initial rate of hydroperoxides formation with AMVN concentration in emulsion and finally, the higher values of these rates with respect to the respective homogeneous systems. These observations find confirmation in the fact that homogeneous and heterogeneous systems containing the lowest AMVN concentration (0.2 wt %, 8 mmol × 10^{-3} g^{-1}) show almost the same initial rate of oxidation (Figure 5). System behavior changes as the initiator concentration changes because of the presence of the dispersed phase which causes a redistribution of the components between the phases, interface included.

Antioxidant Effectiveness. In previous works on the oxidation of olive oil in emulsions, we have demonstrated that the water/oil interface plays a key role in determining the oxidative susceptibility of the oil, because of the partitioning of reaction products and/or antioxidants at the interphase or in the water phase.[32,33] These phenomena lead to positive or negative effects of the aqueous dispersions depending on the composition of the system.[7,8,26] In W/ Olive oil emulsions, the presence of ascorbic acid dispersion in oil protected the oil from oxidation.[8,35] The antioxidative reaction occurred through the mediation of the interface. Even in the case of AMVN-initiated emulsion oxidation, it is doubtless the important role of the interface in amplifying AMVN efficiency. If this hypothesis was correct, the addition of ascorbic acid (AA) in the water dispersed phase, that is, in the droplets, would lead to a decrease in the oxidation rate. In fact, recent works have demonstrated that ascorbic acid

SCHEME 1: Temperature-Induced Dissociation Reaction of AMVN



2,2'-azobis(2,4-dimethylvaleronitrile)

solutions dispersed in olive oil and in triolein have a strong antioxidant effect toward UV-promoted oxidation.[26,36] This effect was explained as the result of the interaction of ascorbate anion molecules with amphiphilic peroxyl radicals/hydroperoxides which adsorb on the W/O interfase. To make an easy comparison between emulsions with and without ascorbic acid, the kinetic chain length parameter $k_{clinh} = R_{inh}/R_i$ is introduced.[27] The k_{clinh} defines the number of chain propagations by each initiating radical in the presence of endogenous antioxidants, where R_{inh} is the oxidation rate in the presence of an antioxidant, and R_i is the initiation rate. We can see in Table 4 that the kinetic chain length increases with increasing AMVN content in emulsion while it remains almost constant with AMVN content in whole olive oil. Further, the kinetic chain length is always higher in emulsion than in whole oil thus indicating a higher number of chain propagating radicals available for the reaction. The hypothesis of a role of the W/O interface in promoting the effectiveness of the initiator derived radicals to promote and propagate the reaction can be confirmed by the k_{clinh} values of emulsions in the presence of droplets of ascorbic acid solutions. In the presence of 8×10^{-3} mmol g^{-1} AMVN, a 15 wt % (1000 ppm in emulsion) AA solution is not effective as antioxidant but it shows a pro-oxidant effect.

By increasing AA concentration in the water phase to 30 wt % (2100 ppm in emulsion), a strong antioxidant effect is evident both in emulsions with 8×10^{-3} and 32×10^{-3} mmol g^{-1}. It is worth noting that the presence of saturated aqueous solutions of AA dispersed in oil puts down the k_{clinh} with respect both to emulsions devoid of AA and whole oils. The dispersed phase behaves as an antioxidant not for itself but because it carries water-soluble antioxidants. In water-in-extravirgin olive oil emulsions and in natural cloudy olive oils, the antioxidants present in the aqueous phase are above all the endogenous polyphenols that partition spontaneously between the water and oil phase. When W/O emulsions were prepared with natural and genuine extravirgin olive oils, that is, those fresh olive fruit juices taken directly from the crusher, the characteristics of the water phase such as, size, polidispersity, concentration, were important because they affected the partitioning of antioxidants between the phases.[33,34] On the other hand, when a unnatural oil phase, such as commercially available triolein or olive oil used in this study, is used, the antioxidant activity of the dispersed phase is not evident unless a consistent quantity of antioxidant is added prior to the emulsification process.[26] An important evidence to point out is that the effectiveness of a certain amount of AA in emulsion increases when more radicals are present; in fact, if the initial concentration of initiator is 32×10^{-3} mmol g^{-1} the k_{clinh} is one-half the value calculated in the presence of 8×10^{-3} mmol g^{-1} of AMVN. It is doubtless there is mediation of the interface, since the total surface area of emulsions increases with increasing AMVN concentration. So AA effectiveness increases when the surface area increases because the surface is where the encounter between radicals and ascorbate anion occurs. The lowering of the k_{clinh} indicates that, as it is well-known, AA behaves like a chain breaking antioxidant because it interferes with the propagation reaction. A synergistic action of AA and endogenous antioxidants found in this oil is thought to be not the main mechanism on the basis of the antioxidant action observed at high concentration.[37] In fact, in recent works on W/extravirgin olive oil emulsion containing AA the antioxidant action of dispersed AA begins to be important at concentration higher than 100 ppm, while in triolein-based emulsions a concentration higher than 900 ppm is necessary to observe an antioxidant function.[8,26] It must be pointed out also the importance of the interface layer, whose composition and characteristics may affect the interaction between compounds in continuous and dispersed phase. When endogenous antioxidants were present, a minor quantity of AA was effective in protecting the oil from UV-promoted oxidation.[8] In the presence of a massive quantity of initiating radicals in the oil continuous phase, endogenous antioxidants are unarmed because they are deactivated by the great deal of radicals in the bulk; furthermore, the W/O interface works as a build-up zone for initiator derived radicals which in turns causes a higher rate of oxidation in emulsions than in whole oil. For the same reason, AA exerts a strong antioxidant function when it is dispersed as saturated solution, because more AA molecules are available to react with peroxyl radicals through the mediation of a large interface.

Conclusions

In this study we have used a model W/Olive oil emulsion to understand in-depth the role of the W/O interface in the oxidation of heterogeneous systems. The oxidation data of emulsions gathered as hydroperoxide concentration by a fluorimetric method are compared with that of a control consisting of whole olive oil (bulk oil). The oxidation reaction was carried out at 40 °C and at different AMVN concentration that forms radicals in the continuous phase. The oxidation rate is always higher for emulsions than whole oils. A classical mechanism of oxidation based on stationary state assumptions was hypothesized that takes into account also the presence of endogenous antioxidants. These compounds have been monitored with spectrophotometry UV−vis and it has been seen that their initial rate of degradation is directly related to AMVN concentration. The kinetic of the oxidation reaction in emulsion follows the same rate law of whole oil. The W/O interface seems to act as a build-up zone for radicals formed in the continuous phase so the radicals are more efficiently dispersed in the oil phase to promote oxidation. This fact explains the higher rates of oxidation in emulsion than in whole oils. When ascorbic acid solutions are dispersed in the oil, the rate of oxidation in emulsion falls down to values lower than whole oils at all AMVN concentrations. These results expressed in terms of kinetic chain length of the inhibited reaction k_{clinh}, show that the ascorbate anion reacts with radicals adsorbed on the W/O surface efficiently and the antioxidant activity is dependent on ascorbate concentration. This evidence points out the importance of using oils and fats in emulsion formulation of high quality, like extravirgin olive oils, free from radicals and prooxidants to increase the oxidative stability of food emulsions.

Abbreviations Used

AA, ascorbic acid; AMVN, 2,2′-azobis(2,4-dimethylvaleronitrile); DPPP, diphenyl-1-pyrenylphosphine; PV, peroxide value; TOH, α-tocopherol; UV, ultraviolet; UV−vis, ultraviolet-visible; W/O, water-in-oil; HLB, hydrophile−lipophile balance.

Acknowledgment. Support of this research from Consorzio per lo sviluppo dei sistemi a grande interfase (C.S.G.I.) is gratefully acknowledged.

References and Notes

(1) *Modern Aspects of Emulsion Science*; Binks, B. P., Ed.; The Royal Society of Chemistry: Cambridge 1998.
(2) *Food Emulsions*; Friberg, S. E., Larsson, K., Sjöblom, J., Eds.; Marcel Dekker, Inc.: New York, 2004.
(3) McClements, D. J. *Food Emulsions: Principles, Practice, and Techniques*; CRC Press: Boca Raton, FL, 2005.
(4) Frankel, E. N. *Lipid Oxidation*; The Oily Press: Dundee, Scotland, 1998.
(5) Halliwell, B. *Am. J. Med.* **1991**, *91*, 145–225.
(6) Porter, W. L. *Antioxidants: Chemical, Physiological, Nutritional and Toxicological Aspects*; Williams, G. M., Ed.; Princeton Scientific: Princeton, NY, 1993; pp 93−122.
(7) Ambrosone, L.; Mosca, M.; Ceglie, A. *Food Hydrocolloids* **2006**, *20*, 1080–1086.
(8) Mosca, M.; Ceglie, A.; Ambrosone, L. *Food Res. Int.* **2008**, *41*, 201–207.
(9) Shwarz, K.; Huang, S. W.; German, J. B.; Tiersch, B.; Hartmann, J.; Frankel, E. N. *J. Agr. Food Chem.* **2000**, *48*, 4874–4882.
(10) Dean, R. T.; Hunt, J. V.; Grant, A. J.; Yamamoto, Y.; Niki, E. *Free Radical Biol. Med.* **1991**, *11*, 161–168.
(11) Van Dick, S. M. O.; Verleyen, T.; Dooghe, W.; Teunckens, A.; Adams, C. A. *J. Agric. Food Chem.* **2005**, *53*, 887–892.
(12) Culbertson, S. M.; Porter, N. A. *J. Am. Chem. Soc.* **2000**, *122*, 4032–4038.
(13) Song, M. G.; Jho, S. H.; Kim, J. Y.; Kim, J. D. *J. Colloid Interface Sci.* **2000**, *230*, 213–215.
(14) Minguez-Mosquera, M. I.; Navarro, L. R.; Gandul-Rojas, B.; Sanchez-Gomez, A. H.; Garrido-Fernandez, J. *J. Am. Oil Chem. Soc.* **1991**, *68*, 332–336.
(15) Eitenmiller, R.; Lee, J. *Vitamin E food chemistry, composition, analysis*; Marcel Dekker, Inc.: New York, 2004; pp 8−10.
(16) Orr, C. In *Encyclopedia of Emulsion Technology*; Becher, P., Ed.; Marcel Dekker, Inc.: New York, 1983; Vol. 1, Chapter 6.
(17) Akasaka, K.; Takamura, T.; Ohrui, H.; Meguro, H.; Hashimoto, K. *Biosci. Biotech. Biochem.* **1996**, *60*, 1772–1775.
(18) Akasaka, K.; Sasaki, I.; Ohrui, H.; Meguro, H. *Biosci. Biotech. Biochem.* **1992**, *56*, 605–607.
(19) Okimoto, Y.; Warabi, E.; Wada, Y.; Niki, E.; Kodama, T.; Noguchi, N. *Free Radical Biol. Med.* **2003**, *35*, 576–585.
(20) Orlien, V.; Andersen, A. B.; Sinkko, T.; Skibsted, L. H. *Food Chem.* **2000**, *68*, 191–199.
(21) Niki, E. *Methods Enzymol.* **1990**, *186*, 100–108.
(22) Kiokias, S.; Oreopoulou, V. *Innovative Food Sci. Emerging Technol.* **2006**, *7*, 132–139.
(23) Woodall, A. A.; Lee, S. W. M.; Weesie, R. J.; Jackson, M. J.; Britton, G. *Biochim. Biophys. Acta* **1997**, *1336*, 33–42.
(24) Bowry, V. W.; Stocker, R. *J. Am. Chem. Soc.* **1993**, *115*, 6029–6044.
(25) Ozhogina, O. A.; Kasaikina, O. T. *Free Radical Biol. Med.* **1995**, *19*, 575–581.
(26) Mosca, M.; Ceglie, A.; Ambrosone, L. *J. Phys. Chem. B* **2008**, *112*, 4645–4651.
(27) Cubillos, M.; Lissi, E.; Abiun, E. *Chem. Phys. Lipids* **2000**, *104*, 49–56.
(28) Noguchi, N.; Yamashita, H.; Gotoh, N.; Yamamoto, Y.; Numano, R.; Niki, E. *Free Radical Biol. Med.* **1998**, *24*, 259–268.
(29) Mosca, M.; Murgia, S.; Ceglie, A.; Monduzzi, M.; Ambrosone, L. *J. Phys. Chem. B* **2006**, *110*, 25994–26000.
(30) Krog, N. J.; Sparso, F. V. In *Food emulsion*; Friberg, S. E., Larsson, K., Sjöblom, J., Eds.; Marcel Dekker: New York, 2004; Chapter 2.
(31) European Community, Regulation 95/2/EC, Official Journal of European Community, *L.61*, 1995.
(32) Walstra, P. In *Encyclopedia of Emulsion Technology*; Becher P., Ed.; Marcel Dekker: New York 1983; Vol. 1, pp 57−127.
(33) Ambrosone, L.; Cinelli, G.; Mosca, M.; Ceglie, A. *J. Am. Oil Chem. Soc.* **2006**, *83*, 165–170.
(34) Ambrosone, L.; Mosca, M.; Ceglie, A. *Food Hydr.* **2006**, *20*, 1080–1086.
(35) Liang, J.; Tian, Y. X.; Fu, L. M.; Wang, T. H.; Li, H. J.; Wang, P. *J. Agric. Food Chem.* **2008**, *56*, 10376–10383.
(36) Gallarate, M.; Carlotti, E.; Trotta, M.; Bovo, S. *Int. J. Farm.* **1999**, *188*, 233–241.
(37) Wayner, D. D. M.; Burton, G. W.; Ingold, K. U. *Biochim. Biophys. Acta* **1986**, *884*, 119–123.

JP911288E