Effect of Droplet Size on Lipid Oxidation Rates of Oil-in-Water Emulsions Stabilized by Protein

Laurent Lethuaut, François Métro, and Claude Genot*

Laboratoire d'Etude des Interactions des Molécules Alimentaires, INRA, F-44316 Nantes cedex 3 France

ABSTRACT: In emulsions lipid oxidation is mainly influenced by the properties of the interface. The aim of this work was to investigate the effects of droplet size and interfacial area on lipid oxidation in protein-stabilized emulsions. Emulsions, made of stripped sunflower oil (30% vol/vol) and stabilized by BSA were characterized by surface area values equal to 0.7, 5.1, and 16.3 $m^2 \cdot cm^{-3}$ oil. The kinetics of O_2 consumption and conjugated diene (CD) formation, performed on emulsions and nonemulsified controls, showed that emulsification prompted oxidation at an early stage. On condition that oxygen concentration was not limiting, the rates of O_2 consumption and CD formation were higher when the interfacial area was larger. Protein adsorbed at the interface probably restrained this pro-oxidant effect. Once most of the O_2 in the system was consumed (6–8 h), CD remained steady at a level depending directly on the ratio between oxidizable substrate and total amount of oxygen. At this stage of aging, the amounts of primary oxidation products were similar whatever the droplet size of the emulsion. Hexanal and pentane could be detected in the headspace of emulsions only at this stage. They were subsequently produced at rates not depending on oil droplet size and interfacial area.

Paper no. J10026 in *JAOCS 79*, 425–430 (May 2002).

KEY WORDS: Conjugated dienes, droplet size, interfacial area, lipid oxidation in emulsions, oxygen, protein, volatile compounds.

Many foods are oil-in-water emulsions stabilized by protein and lipid surfactants in a protein or polysaccharide matrix. In these systems, oxidation of unsaturated FA leads to the formation of volatile compounds, which are major contributors to the aroma or to undesirable off-odors and off-flavors (1). Oxidation in bulk and emulsified oils is influenced by several common factors, but additional factors are important in emulsions (2). These include the structure of the emulsions and the physicochemical properties of the aqueous phase and of the droplet membrane.

The size of the oil droplets is often claimed as an important factor. On one hand, small droplet size signifies a large surface area, implying a high potential of contact between diffusing oxygen, water-soluble free radicals and antioxidants, and the interface. It also implies a high ratio of oxidizable FA located near the interface to FA embedded in the hydrophobic core of the droplets (3). Decreasing the size of the oil droplets is therefore expected to favor development of oxidation. On the other hand, when the droplet size decreases, the number of lipid molecules per droplet diminishes and the amount of surface-active compounds adsorbed at the interface is increased. This could limit initiation and propagation, as surface-active compounds may act as a barrier to the penetration and diffusion of pro-oxidants or even interfere with oxidation. For instance, homogenization is reported to protect milk fat from oxidation catalyzed by metal complexes (4) because casein, which adsorbs to the droplet surface, is an efficient antioxidant protein in milk.

According to these contrasting views, data concerning the influence of the size distribution of oil droplets and the interfacial area on lipid oxidation in emulsions are scarce and somewhat confusing. Shimada *et al.* (5) found no effect of droplet size of soybean oil emulsions, whereas Gohtani *et al.* (6) observed that large droplet size (6.4 μm) DHA-in-water emulsions were less oxidizable than 3.4-μm oil droplet size emulsions. Jacobsen *et al.* (7) found that addition of propyl gallate to fish-oil-enriched mayonnaise both increased the mean droplet diameter and prompted oxidation and flavor deterioration. On the contrary, in the initial stage of storage, an increase in the size of the oil droplets was positively correlated with decreased oxidation in the mayonnaises (8). Rampon *et al.* (9,10) also observed that the smallest droplet size of protein-stabilized emulsions favored the formation of fluorescent pigments resulting from reactions between oxidizing lipids and proteins.

The objective of this work was to evaluate the influence of the interfacial area on the development of lipid oxidation in protein-stabilized oil-in-water emulsions with special emphasis on the formation of primary products of oxidation.

MATERIALS AND METHODS

Experimental design. As lipid oxidation is a train of chain reactions, it was characterized with methods making it possible simultaneously to evaluate the oxygen consumption, the formation of primary products of oxidation (conjugated dienes: CD), and the development of selected volatile products (pentane and

*To whom correspondence should be addressed at Laboratoire d'Etude des Interactions des Molécules Alimentaires, INRA, BP 71627, F-44316 Nantes cedex 3 France. E-mail: genot@nantes.inra.fr

hexanal). The physical stability of the emulsion was also monitored. The kinetics were measured in closed vessels at time intervals selected with the purpose of evaluating the early stage of oxidation development as well as the latest stages.

In the first experimental step, three large (LE), medium (ME), and small (SE) droplet size emulsions were prepared independently and stored in sealed vials at 47°C for 24 h. For each emulsion, at set time intervals (30 min for CD and volatiles measurements and 1 or 2 h for oxygen), analyses were made on the sample contained in one vial. Two oil–water and oil controls, made of unemulsified stripped oil plus water and stripped oil only, were also prepared, stored at 47°C, and sampled as just described for 28 to 32 h.

As a second experimental step, only medium and small droplet emulsions were prepared and stored at 47°C to evaluate volatile development for a longer period of storage (4 d). Three vials were analyzed every day for volatile development ($n = 3$).

Materials and chemicals. Powdered BSA (ref. 103703; 95–98% purity) was obtained from ICN Biochemicals Inc. (Orsay, France). Sodium azide for synthesis and hydrochloric acid (1.09970. Titrisol) were purchased from Merck (Pessac, France). SDS (L-5750) was obtained from Sigma Chemical Co. (St. Quentin Fallavier, France). Pentane and isopropanol of analytical quality came from Carlo Erba (Val de Reuil, France). Ultrapure water (18 MΩ) was prepared with Milli Q system (St. Quentin Yuelines, France) and used for all preparations. Commercial sunflower oil was stripped of tocopherols and minor compounds using adsorption chromatography on an alumina column (alumina N, Akt I, ref. 020287; ICN) (11). Pentane used as eluting solvent was evaporated from stripped oil under vacuum and flushed with nitrogen until no trace could be detected by headspace GC. Stripped sunflower oil contained less than 0.4 $mg \cdot kg^{-1}$ tocopherol. CD, hydroperoxides, and FFA were within the detection limits of the methods. The oil was mainly composed of TAG (99.7 g/100 g) and contained small amounts of MAG and DAG (0.17 and 0.12 g/100 g, respectively). Its FA composition (g/100 g total FA) was as follows: 16:0, 6.3; 18:0, 4.8; 20:0, 0.3; 22:0, 0.7; 16:1, 0.1; 18:1, 21.9; 20:1, 0.1; 18:2, 65.6; 18.3, 0.2.

Preparation and storage of emulsions. To obtain LE, 105 mL of 20 $g \cdot L^{-1}$ BSA solution (pH 4.3; 0.4 $g \cdot L^{-1}$ NaN_3) and 45 mL of stripped oil were emulsified for 2 min at 23,000 rpm with a rotor-stator homogenizer (Polytron PT 3000; Kinematica, Littau, Switzerland) fitted with a 12-mm diameter head. (NaN_3 was included to prohibit the development of bacteria.) To reduce the size of the oil droplets, the coarse emulsion was circulated for 12 min through a one-valve (15 bar) or a two-valve (first and second valves set at 200 and 40 bar, respectively) high-pressure homogenizer (A0812W-A-CD; Stansted Fluid Power, Stansted, United Kingdom). Aliquots (3.4 mL) of emulsions were distributed in 22.4-mL headspace vials (ref. 5182-0837; Hewlett-Packard), sealed with polytetrafluoroethylene/silicon septa and aluminum crimp seals. Vials containing either 1 mL of stripped sunflower oil and 2.4 mL of ultrapure water (0.4 $g \cdot L^{-1}$ NaN_3) or 3.4 mL stripped oil were used as a control. The vials were rotated at 20 rpm with a test-tube rotator oriented at 30° vs. vertical position (Labinco B.V., Ac Breda, The Netherlands) and kept in the dark at 47 ± 2°C.

Characterization of droplet size distribution of emulsions. Droplet size was determined with a Mastersizer 3600 (Malvern Instruments, Malvern, United Kingdom) fitted with a 45-mm lens and a liquid sampler MS 15. Prior to the measurements, the emulsions were diluted in 5 vol of pure water or 10 $g \cdot L^{-1}$ SDS solution. Samples were then diluted in the sample cell containing water until 15 to 20% of the incident light was absorbed. Measurements were performed in triplicate on each emulsion. Particle sizes were reported as specific surface or interfacial area: ($m^2 \cdot cm^{-3}$ oil); volume mean diameter: D[4,3] (μm) = $\Sigma d^4/\Sigma d^3$; surface mean diameter: D[3,2] (μm) = $\Sigma d^3/\Sigma d^2$ [where d is the diameter (μm) of a droplet]; and the diameter of oil droplet corresponding to the 10th 50th, and 90th percentiles of droplet volume (v) distribution: D[v, 0.1]; D[v, 0.5]; D[v, 0.9] (μm).

Oxygen consumption. Oxygen concentration in the headspace of emulsions and controls was evaluated with a gas chromatograph paired with a mass spectrometer (NERMAG R 10-10 C) used as a gas analyzer. Forty microliters of the headspace was injected with a gas-tight syringe into the injector (split rate, 10 $mL \cdot min^{-1}$; injector temperature, 120°C) connected to an inert column (0.32 mm i.d.). The carrier gas was helium (flow rate: 1 $mL \cdot min^{-1}$). In these conditions, permanent gases were co-eluted. Molecules were ionized at 70 eV electronic impact and quantified using their specific ions. The ionic flux corresponding to 32 mass units (mass of oxygen) was recorded and integrated with SIDAR (Nermag, France) software. To take into account the time-drift of the signal, the mean value ($n = 3$) obtained with the sample was compared to the mean signal of the same volume of surrounding air injected in triplicate before and after the sample. Initial amounts of oxygen in the vials containing the emulsions were calculated using a volume balance of constituents, 219.46 $mg \cdot g^{-1}$ for oxygen concentration in ambient air (12), 9.06 $mg \cdot L^{-1}$ for oxygen solubility in air-saturated water [20°C, 760 mm Hg (13)] and a solubility of oxygen in oil 4.4 times higher than in water (14). The initial quantity of oxygen in the vials was thus approximately 187.8 μmol, of which only 1.8 μmol was dissolved in the liquid phase. This total value allowed results to be expressed in mmol of oxygen consumed per kg of oil (mmol $O_2 \cdot kg^{-1}$). The initial rate of oxygen consumption (V_{i,O_2}) was calculated in the linear part of the curve [consumed O_2 (mmol $O_2 \cdot kg^{-1}$) vs. time (h)] by step-by-step linear regression.

CD. CD were measured using the UV absorbance at 233 nm. Aliquots of emulsions and controls were diluted in isopropanol to obtain lipid concentrations of 250 $mg \cdot L^{-1}$. The solutions were centrifuged for 4 min at 5,600 × g (Bioblock, TH12). The absorbance spectra of the supernatants were recorded between 210 and 310 nm (data interval, 1 nm; scan speed, 240 $nm \cdot min^{-1}$; band path, 2 nm) with a double beam UV-Vis spectrophotometer (PerkinElmer Lambda 12). The reference cell contained isopropanol for the controls and 6.4

μL ultrapure water in 10 mL isopropanol for emulsions. Results were means of three determinations. They were expressed in millimoles CD per kilogram of oil (mmol CD·kg^{-1} oil) using 27,000 M^{-1}·cm^{-1} as the molar extinction coefficient of CD at 233 nm (15). The initial rate of CD formation ($V_{i,\,CD}$) was calculated by linear regression in the linear part of the curve [mmol CD·kg^{-1} vs. time (h)].

Volatile compounds. The presence of pentane and hexanal is often used as a marker of lipid oxidation in vegetable oils (16,17). Their development in the sunflower oil emulsions was followed by manual static headspace/GC (HS-GC) analysis. The samples were equilibrated at 50°C for 15 min, and 500 μL of each headspace was injected with a gas-tight syringe into the injector (splitless mode, injector temperature: 250°C) of a gas chromatograph (HP 5890, series II; Hewlett-Packard). The GC was fitted with a DB5 column (J&W Scientific, Folsom, CA) (length, 30 m; i.d., 0.32 mm; film thickness, 1 μm). The initial and final oven temperatures were, respectively, 40 and 140°C, with a rate of increase of 5°C·min^{-1}. The flow rate of hydrogen carrier gas was 2.3 mL·min^{-1}. The temperature of the FID was set at 250°C. Pentane and hexanal were identified by comparison of their retention times with that of authentic reference pentane (1.8 min) and hexanal (6.2 min) and by GC–MS. The peak area was integrated (APEX Software, SRA Instruments, France) and expressed in mV·s.

Statistics analyses. Means and SD were calculated for each experimental series (n = 3 emulsions or n = 2 for controls). Linear regression was used to calculate, emulsion by emulsion, the initial rates of CD formation and O_2 consumption using Excel software (Microsoft Product, Ltd.). The influence of droplet size on initial oxidation rate was tested with one-way ANOVA, and Neuman–Keuls tests were performed at different probability levels. The statistical Statgraphics software package (Statistical Graphics Corp., Englewood Cliffs, NJ) was used.

RESULTS AND DISCUSSION

Droplet size distribution of emulsions. The rotor stator and low-pressure and high-pressure valve homogenizers gave, with good reproducibility, emulsions characterized by different droplet size distributions (Fig. 1). The coarse emulsions (LE) prepared with the rotor-stator homogenizer were multimodal and mainly composed of oil droplets ranging between 10 and 80 μm in diameter. This population accounted for around 90% of the volume distribution. A minor population, with droplet diameters between 0.5 and 5 μm, constituted 10% of the volume distribution. D[4,3] of the large droplet size emulsions was 27.0 ± 6.9 μm (n = 3), and their total droplet interfacial area was 0.7 ± 0.2 m^2·cm^{-3} oil (Table 1).

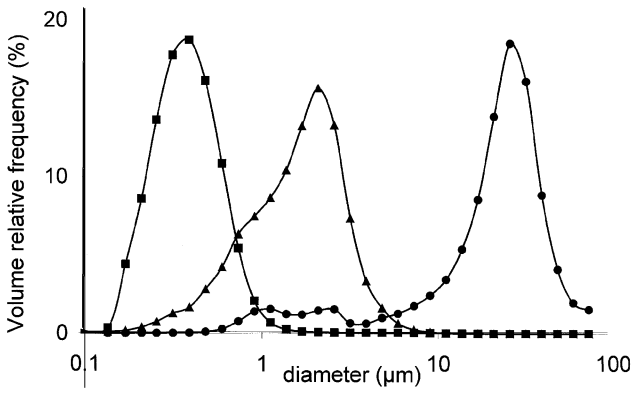

FIG. 1. Distribution of droplet size of emulsions composed of stripped sunflower oil and BSA solution (20 g·L^{-1}), obtained by rotor-stator homogenizer (●) and valve homogenizers set at 15 (▲) or 200 (■) bar.

Emulsions made with the high-pressure homogenizers were roughly monomodal (Fig. 1). A pressure of 15 bar gave emulsions characterized by D[4,3] = 1.9 ± 0.1 μm and interfacial area = 5.1 ± 0.1 m^2·cm^{-3} oil (ME). Emulsions prepared with a pressure of 200 bar (SE) were characterized by the smallest D[4,3] (0.5 ± 0.03 μm) and the largest interfacial areas (16.3 ± 1.1 m^2·cm^{-3} oil).

Droplet size distributions of emulsions were similar, with or without SDS (Table 1), showing that fresh emulsions did not aggregate.

Droplet sizes of LE were stable for at least 48 h at 47°C. Creaming was avoided as the test-tube rotator regularly turned the vials upside down. Coalescence was detected in ME between days 10 and 15 of storage and in SE from day 8, followed by phase separation at day 10 (9).

Lipid oxidation in freshly prepared emulsions. Emulsification resulted in minimal lipid oxidation in freshly prepared emulsions (time = 0) as emphasized by the second derivative of UV spectra (not shown). These derivative spectra showed very small peaks with maxima located at 233 and 242 nm, demonstrating the presence of very small quantities of *trans-trans* and

TABLE 1
Characteristics of Droplet Size of Emulsions Prepared with Different Emulsification Procedures[a]

Emulsification process	Code	SDS	Specific surface (m^2·cm^{-3})	Volume mean diameter D[4,3] μm	Surface mean diameter D[3,2] μm	Diameter at 10% D[v,0.1] μm	Diameter at 50% D[v,0.5] μm	Diameter at 90% D[v,0.9] μm
Rotor-stator	LE	–	0.7 ± 0.2	27.5 ± 7.8	8.5 ± 1.8	4.9 ± 2.8	25.4 ± 5.5	44.0 ± 7.9
		+	0.7 ± 0.2	27.0 ± 6.9	8.1 ± 1.7	4.3 ± 2.7	24.7 ± 4.8	42.4 ± 6.9
Low pressure	ME	–	5.0 ± 0.2	1.9 ± 0.1	1.2 ± 0.0	0.6 ± 0.0	1.7 ± 0.1	3.2 ± 0.2
15 bar		+	5.1 ± 0.1	1.9 ± 0.1	1.2 ± 0.0	0.6 ± 0.0	1.7 ± 0.1	3.3 ± 0.3
High pressure	SE	–	15.3 ± 1.5	0.5 ± 0.06	0.4 ± 0.0	0.2 ± 0.0	0.5 ± 0.1	0.7 ± 0.1
200 bar		+	16.3 ± 1.2	0.5 ± 0.03	0.4 ± 0.0	0.2 ± 0.0	0.4 ± 0.0	0.9 ± 0.1

[a]Measurements were carried out without (–) or with (+) SDS. Data represent mean ± SD (n = 3).

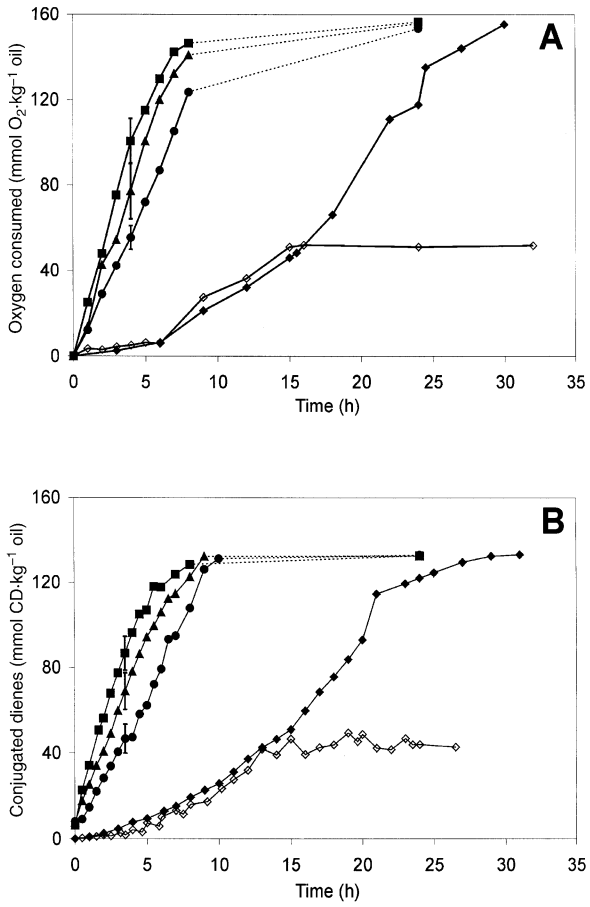

FIG. 2. Effect of emulsification and droplet size on oxygen consumption (A) and the conjugated diene (CD) formation (B) in controls [3.4 mL stripped sunflower oil (◇) or 1.4 mL stripped sunflower oil + 2 mL ultrapure water (◆)] and in emulsions (3.4 mL) characterized by large (●), medium (▲), and small (■) droplet size. The temperature of storage was 47°C. Error bars represent mean ± SD for large, medium, and small droplet size emulsions (emulsions: $n = 3$; controls: $n = 2$).

cis-trans CD (15). CD concentrations of freshly prepared emulsions varied between 0 and 10 mmol CD·kg^{-1} oil. No effect of the emulsification procedure used to prepare the emulsions was observed ($P > 0.05$).

O_2 uptake and CD formation during aging of controls and emulsions. Measurements of oxygen consumption and CD formation provided very similar pictures of oxidation development during aging (Fig. 2). Both methods evidence sizable differences in oxidation kinetics in controls and in emulsions.

In controls, O_2 consumed and amounts of CD formed increased slowly for about 6 h, underscoring something akin to a lag phase. They subsequently rose sharply for several hours to level off when oxygen detected in the headspace reached about 4.5% of the quantity of oxygen initially present. In emulsions, lipid oxidation kinetics were very different from those observed with controls. No lag phase was observed. A fast and linear phase appeared immediately over the first 6 to 9 h of storage, until the O_2 concentration in the headspace and CD amounts reached about the same values as measured in the controls. Thus, the plateau appeared earlier than in the controls, but the final levels of consumed O_2 and CD formed were similar, reflecting the similar initial oil and oxygen quantities in oil–water controls and in emulsions. This value, which remained stable for longer periods of storage, corresponds to about 155 mmol O_2 consumed kg^{-1} oil in oil–water controls and emulsions and to 55 mmol O_2·kg^{-1} in oil controls. At the same time, about 135 mmol CD·kg^{-1} of oil was measured in oil–water controls and emulsions and 45 mmol CD·kg^{-1} in oil controls. CD continued to increase slightly for longer periods of storage as did hydroperoxides (9). Residual oxygen, continually detected in the headspace even after long-term storage, probably reveals a limit in our sampling procedure. The limit could be due to pollution by the ambient air in the gas syringe when the pressure in the vials is below that of the atmosphere. However, as the limit value for oxygen uptake and the steady-state phase of CD formation were reached simultaneously, the limiting factor for oxidation development at this stage of aging was very likely oxygen concentration. Accordingly, initial quantities of oxygen in the vials were roughly the same in all samples, but lipid amounts were three times higher and oxidation (mmol O_2·kg^{-1} oil or mmol CD·kg^{-1}) was three times lower in oil controls than in oil–water controls and emulsions. To confirm this hypothesis, several vials were opened when oxygen consumption had steadied, then sealed and stored again at 47°C. After the air renewal, oxygen consumption and CD production resumed, followed by a second leveling-off.

After 1 d of storage, about 135 mmol CD kg^{-1} of oil was measured in oil–water controls and in emulsions and 45 mmol CD kg^{-1} in oil controls. These values were close to those for oxygen uptake. Apart from bias due to approximations used for calculation, the difference in O_2 uptake and calculated CD can result from three phenomena. First, although it is less sensitive to oxidation than linoleic acid, the oleic acid present in sunflower oil (22% of total FA) can participate in O_2 uptake but not in CD formation. Indeed, oleic acid transforms into primary products of oxidation that do not absorb UV light because they do not possess the CD structure. Differences observed between CD value and hydroperoxides measured by xylenol orange method (results not shown) provide arguments in favor of this explanation. Second, oxygen could be involved in reactions other than CD formation such as oxidation of the protein at the interface induced by lipid free radicals (9,18,19). Finally, one may note that CD value is the result of both CD produced and CD converted into secondary products of oxidation.

Effect of droplet size, or of interfacial area, on O_2 consumption and CD production in emulsions. The immediate development of oxidation in emulsions compared to non-emulsified systems (Fig. 2) has to be related to oil emulsification. It can be attributed to the related increase in the interface area, but other factors also could be involved. Figure 2 also shows that oxidation developed faster in SE than in ME and LE. This difference is illustrated by plotting V_{i,O_2} and $V_{i,CD}$ vs. interfacial area (Fig. 3). Rates of oxygen consumption

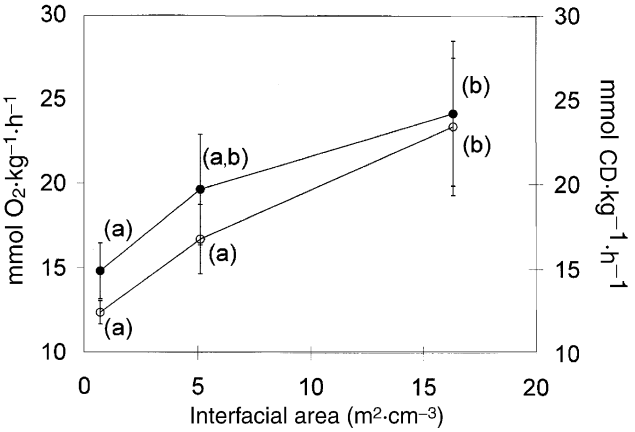

FIG. 3. Comparison of V_{i,O_2} (●) and $V_{i,CD}$ (○) as a function of the interfacial area of emulsions. Symbols with different lowercase roman letters correspond to mean values that differ at $P = 0.05$. Error bars represent SD of the three replicates.

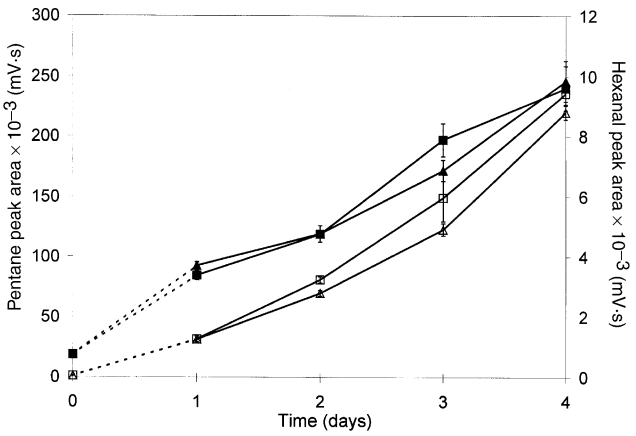

FIG. 4. Release of pentane and hexanal in the headspace of vials containing medium or small droplet size emulsions (ME and SE), stored at 47°C ($n = 3$). Closed symbols correspond to pentane release in ME (▲) and SE (■). Open symbols correspond to hexanal release in ME (△) and SE (□).

(V_{iO_2}) were 24.2 (±4.3), 19.6 (±3.3) and 14.8 (±1.6) mmol·kg^{-1}·h^{-1} for emulsions characterized by large (SE), medium (ME), and small interfacial areas (LE), respectively. Rates of CD formation were 23.5 (±4.3), 16.7 (±2.1), and 12.3 (±0.7) mmol CD·kg^{-1}·h^{-1}. Variance analysis evidenced a significant effect of emulsion type ($P = 0.0316$ for V_{i,O_2} and $P = 0.0064$ for $V_{i,CD}$). A multiple range test (Newman–Keuls) showed that V_{i,O_2} of emulsions characterized by the smallest (LE) and the largest (SE) interfacial areas were significantly different for a 0.05 threshold value. The same picture was drawn with $V_{i,CD}$. Higher threshold values (0.10 and 0.15 for CD and O_2, respectively) made it possible to distinguish ME from both SE and LE.

No correlation was found between the level of oxidation (CD) of freshly prepared emulsions and initial rates of oxidation ($P = 0.3097$). Thus, the enhancement of lipid oxidation in emulsions during storage is related to the increase in the interface area and not to the mechanical treatment and its possible pro-oxidant effect. As expected, the greater the interfacial area, the faster the oxidation of lipids in emulsions. Favored diffusion and access of oxygen and metal catalysts to the oil phase can be involved. However, in our experiment, the interfacial area increased by 7.3 (LE to ME) and then by 3.2 (ME to SE), but initial rates of O_2 uptake and CD production increased by about twofold, i.e., 1.3. This apparent discrepancy can be attributed to an antioxidant activity of the protein adsorbed at the interface. BSA has antioxidant properties in emulsion systems (20,21). Its activity is directly linked to the ability of its amino acid residues to react with lipid free radicals and hydroperoxides. Because the quantity of BSA directly in contact with the lipid phase (adsorbed BSA) increases with the interfacial area, the smaller the oil droplets are, the greater the antioxidant efficiency of the protein is. This agrees with previous results showing that tryptophanyl residues of the protein were damaged more seriously and faster when the droplet size of emulsions decreased (9,10). Formation of oxidized lipid-protein fluorescent product was also enhanced. As a consequence, the pro-oxidant effect linked to the smallest size of oil droplets is partially counteracted by the antioxidant activity of the adsorbed protein.

Release of volatile compounds in emulsions. At the same time that O_2 and CD measurements were made, analyses of volatile compounds released in the headspace of emulsions and controls were carried out. For all samples, the release of pentane and hexanal was undetectable for at least 8 h of storage. Amounts were too small to allow acceptable reproducibility before about 24 h of storage. Thus, release of volatiles was followed in ME and SE stored at 47°C for 4 d (Fig. 4). Peak areas of pentane and hexanal increased progressively with time with no influence of the droplet size. At the same time, CD amounts increased steadily at low and similar rates in SE and ME (not shown). This tallies with previous results (9) showing that droplet size did not influence hydroperoxides during long-term aging (up to 21 d) of emulsions kept in closed vessels. Therefore, at this stage of emulsion aging, the interfacial area is not yet the limiting factor for hydroperoxide formation and for volatile compound release. The limiting factor for hydroperoxide formation is oxygen concentration (22). The relative stability of CD amounts shows that at this stage of oxidation, the formation and decomposition of primary products of oxidation are nearly balanced and similar whatever the droplet size of the emulsions. The decomposition of primary products gives rise to volatile compounds that should transfer through the interface and the aqueous phase before being released into the headspace. BSA adsorbed at the interface does not modify the kinetics of release of volatile compounds (Brossard, C., personal communication), and total CD amounts are similar in ME and SE at this stage of aging. Thus, it should be assumed that, in the present experiment, hydroperoxide decomposition was not governed by the interfacial area but was at rates depending on hydroperoxide concentrations.

When oxygen is not the limiting factor for oxidation development, the formation of primary products of oxidation in

emulsions is clearly favored when the size of oil droplets decreases. The effect is partly offset because of the presence of protein at the interface that may act as antioxidant. When oxygen becomes the limiting factor for oxidation, the influence of droplet size and interfacial area is effective for the formation of oxidized lipid-protein fluorescent products (9,10) but is no longer valid when looking at formation and release of volatile secondary products of oxidation.

ACKNOWLEDGMENTS

We thank Benoît Bousseau for efficient oil purification, Nelly Fouquet for participating in CD measurements, Geneviève Llamas for droplet size determinations, Isabelle Bronnec for her help in static headspace analysis, and Nadine Mouhous-Riou and Vincent Rampon for their friendly and joyful participation in night-long kinetics. We are also grateful to Anne Meynier, Chantal Brossard, and Alain Riaublanc for their helpful advice. The funding of this work by the INRA and the Pays de Loire region (France) as part of the VANAM-II program "Interfaces, Emulsions et Mousses liquides" is greatly appreciated.

REFERENCES

1. Love, J., Contribution of Lipids to Desirable and Undesirable Flavors in Foods, in *Food Lipids and Health*, edited by R.E. McDonald and D.B. Min, Marcel Dekker, New York, 1996, pp. 287–314.
2. McClements, D.J., and E.A. Decker, Lipid Oxidation in Oil-in-Water Emulsions: Impact of Molecular Environment on Chemical Reactions in Heterogeneous Food Systems, *J. Food Sci. 65*:1270–1282 (2000).
3. Coupland, J.N., Z. Zhu, H. Wan, D.J. McClements, W.W. Nawar, and P. Chinachoti, Droplet Composition Affects the Rate of Oxidation of Emulsified Ethyl Linoleate, *J. Am. Oil Chem. Soc. 73*:795–801 (1996).
4. Hegenauer, J., P. Saltman, D. Ludwig, L. Ripley, and P. Bajo, Effects of Supplemental Iron and Copper on Lipid Oxidation in Milk. 1. Comparison of Metal Complexes in Emulsified and Homogenized Milk, *J. Agric. Food Chem. 27*:860–867 (1979).
5. Shimada, K., H. Okada, K. Matsuo, and S. Yoshioka, Involvement of Chelating Action and Viscosity in the Antioxidative Effect of Xanthan in an Oil/Water Emulsion, *Biosci. Biotechnol. Biochem. 60*:125–127 (1996).
6. Gohtani, S., M. Sirendi, N. Yamamoto, K. Kajikawa, and Y. Yamamo, Effect of Droplet Size on Oxidation of Docosahexaenoic Acid in Emulsion System, *J. Dispersion Sci. Technol. 20*:1319–1325 (1999).
7. Jacobsen, C., K. Hartvigsen, P. Lund, A.S. Meyer, J. Adler-Nissen, J. Holstborg, and G. Hølmer, Oxidation in Fish-Oil-Enriched Mayonnaise 1. Assessment of Propyl Gallate as an Antioxidant by Discriminant Partial Least Squares Regression Analysis, *Eur. Food Res. Technol. 210*:13–30 (1999).
8. Jacobsen, C., K. Hartvigsen, P. Lund, M.K. Thomsen, L.H. Skibsted, J. Adler-Nissen, G. Hølmer, and A.S. Meyer, Oxidation in Fish-Oil-Enriched Mayonnaise 3. Assessment of the Influence of the Emulsion Structure on Oxidation by Discriminant Partial Least Squares Regression Analysis, *Ibid.* 210:242–257 (2000).
9. Rampon, V., L. Lethuaut, N. Mouhous-Riou, and C. Genot, Interface Characterization and Ageing of Bovine Serum Albumin Stabilized Oil-in-Water Emulsion as Revealed by Front-Surface Fluorescence, *J. Agric. Food Chem. 49*:4046–4051 (2001).
10. Rampon, V., N. Mouhous-Riou, and C. Genot, Modification of Bovine Serum Albumin and Formation of Fluorescent Pigments in Oxidizing Emulsions as Revealed by Front-Surface Fluorescence, in *Recent Research Developments in Agricultural and Food Chemistry: Food Emulsions and Dispersions*, edited by M. Anton and S.G. Pandalaï, Res. Signpost, Kerala, India, in press.
11. Hamilton, R.J., C. Kalu, E. Prisk, F.B. Padley, and H. Pierce, Chemistry of Free Radicals in Lipids, *Food Chem. 60*:193–199 (1997).
12. Weast, R.C., *Handbook of Chemistry and Physics*, 61st edition, CRC Press, Boca Raton, 1980.
13. Hitchman, M.L., *Measurement of Dissolved Oxygen*, *Chemical Analysis Series*, edited by P.J. Elving and J.D. Winefordner, Wiley Interscience, New York, 1978, Vol. 49, 255 pp.
14. Gros, J.B., C. Achard, and C.G. Dussap, Solubilité de l'Oxygène dans les Milieux Alimentaires Liquides: Mesure et Prédiction, *Sci. Aliments. 12*:47–61 (1992).
15. Corongiu, F.P., and S. Banni, Detection of Conjugated Dienes by Second Derivative Ultraviolet Spectrophotometry, *Methods Enzymol. 233*:303–310 (1994).
16. Frankel, E.N., W.E. Neff, and E. Selke, Analysis of Autoxidized Fats by Gas Chromatography–Mass Spectrometry: VII. Volatile Thermal Decomposition Products of Pure Hydroperoxides from Autoxidized and Photosensitized Oxidized Methyl Oleate, Linoleate and Linolenate, *Lipids 16*:279–285 (1981).
17. Snyder, J.M., E.N. Frankel and E. Selke, Capillary Gas Chromatographic Analysis of Headspace Volatiles from Vegetable Oils, *J. Am. Oil Chem. Soc. 62*:1675–1679 (1985).
18. Davies, K.J.A., Protein Damage and Degradation by Oxygen Radicals. I. General Aspects, *J. Biol. Chem. 262*:9895–9901 (1987).
19. Leaver, J., A.J.R. Law, E.Y. Brechany, and C. McCrae, Chemical Changes in β-Lactoglobulin Structure During Ageing of Protein-Stabilized Emulsions, *Int. J. Food Sci. Technol. 34*:503–508 (1999).
20. Taylor, M.J., and T. Richardson, Antioxidant Activity of Cysteine and Protein Sulfhydryls in a Linoleate Emulsion Oxidized by Hemoglobin, *J. Food Sci. 45*:1223–1230 (1980).
21. Yukami, S., Autoxidation of Sodium Linoleate in the Protein Solution, *Agric. Biol. Chem. 36*:871–874 (1972).
22. Marcuse, R., and P.-O. Fredriksson, Fat Oxidation at Low Oxygen Pressure. I. Kinetic Studies on the Rate of Fat Oxidation in Emulsions, *J. Am. Oil Chem. Soc. 45*:400–407 (1968).

[Received July 9, 2001; accepted February 18, 2002]