油滴の微細化がO/Wエマルション系での脂質酸化に及ぼす影響

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Effect of Reducing Oil Droplet Size on Lipid Oxidation in an Oil-in-water Emulsion

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Lipid oxidation processes were simulated for O/W emulsions with different oil-droplet sizes based on autocatalytic type rate expression in order to examine the effect of oil-droplet size on oxidation. Unoxidized lipid molecules were converted to oxidized ones momentarily or gradually, and the conversion occurred evenly or randomly in the droplets. When the oxidized molecules were formed momentarily, the droplet size scarcely affected the oxidation process, except for when the number of oxidized molecules was equal to or smaller than the number of oil droplets. In contrast, the oil droplet size significantly affected the oxidation process when the oxidized molecules were gradually and randomly formed at regular intervals. The oxidation was more retarded for the emulsions with smaller oil droplets. Because the latter case would be closer to a real system, the reduction in the oil droplet size in O/W emulsions is expected to increase the stability of lipids to protect against oxidation.

1. Introduction

Lipid containing unsaturated fatty acids is prone to autoxidization in both bulk and dispersed systems, and some products of lipid oxidation exhibit undesirable health effects [1]. In an oil-in-water (O/W) emulsion containing unsaturated fatty acids, the fatty acids are oxidized using the oxygen supplied from the aqueous phase through the oil-water interface. Because an insufficient amount of oxygen is supplied through the interface for a large oil droplet on a small specific surface area, the mass transfer of oxygen through the interface seems to decelerate lipid oxidation.

Much attention has recently been paid to nano-emulsions that contain very small oil droplets. When the oil droplet size is sufficiently small, the lipid oxidation itself would be a rate-limiting step, and the oxidation rate would not depend on the oil droplet size. However, the dependence of the lipid oxidation on oil droplet size is complex. Gohtani *et al.* [2], Lethuaut *et al.* [3], Lee *et al.* [4], and Kuhn and Cunha [5] reported that lipid oxidation was accelerated by reducing oil droplet size, whereas Kanaya *et al.* [6], Ries *et al.* [7], O'Dwyer *et al.* [8], and Hguyen *et al.* [9] demonstrated retardation of lipid oxidation for smaller oil droplets. We also showed that there is a smaller rate constant for autoxidation of methyl linoleate for smaller oil droplets and proposed a model for explaining the dependence of the rate constant on oil droplet size [10]. Other studies previously reported no significant effect of oil droplet size on the lipid oxidation rate [11-15]. The difference in the dependence of autoxidation rate of lipid on the oil droplet size could be ascribed to certain factors, such as the kind of lipid used, the oil droplet size range, and the temperature tested, although the reason for the difference has not been specified.

It is not experimentally easy to examine the effect of oil droplet size on lipid oxidation for a wide range of oil droplet sizes under constant conditions other than oil droplet size. Therefore, the effect was examined by computational simulation under ideal conditions where factors other than droplet size do not need to be considered. Lipid oxidation is a complicated process consisting of initiation, propagation, and termination steps, and the initiation step is triggered by the conversion of unoxidized

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lipid molecule to oxidized one *via* radical generation. The autocatalytic type rate equation can express the entire process of lipid oxidation [16, 17]. The formation of oxidized molecules is reflected in the initial condition for the rate equation. The rate equation was applied to examine the effect of droplet size on lipid oxidation process for two ideal cases where unoxidized molecules are converted to oxidized ones momentarily at the beginning of storage or gradually converted at regular intervals.

2. Theoretical Considerations

2.1 Lipid oxidation kinetics

The autocatalytic type rate equation is expressed by Eq. (1).

$$\frac{dC}{dt} = -kC(C_t - C) \tag{1}$$

where C is the concentration of the unoxidized lipid, C_t is the initial concentration of unoxidized lipid, t is the time, and k is the rate constant. Eq. (1) indicates that the lipid oxidation rate is proportional to the product of concentrations of unoxidized and oxidized lipids, and that conversion of an unoxidized lipid molecule to oxidized one in a closed system triggers the oxidation of all the lipid molecules in the system. Eq. (1) can be converted to the dimensionless form in Eq. (2).

$$\frac{dY}{d\theta} = -Y(1-Y) \tag{2}$$

where Y ($=C/C_t$) is the fraction of unoxidized lipid, and θ ($=kC_t t$) is the dimensionless time. Let us consider an oil droplet containing N lipid molecules. When n unoxidized lipid molecules are converted to oxidized ones at time $\theta=0$, the initial condition of Eq. (2) is given by

$$Y = Y_0 = (N-n)/N \tag{3}$$

When the oil droplet is divided into m small droplets, which are hereafter called sub-droplets in order to distinguish with the original droplet, and n_i unoxidized molecules are converted to oxidized ones in the i-th sub-droplet, the initial value of unoxidized lipid fraction for the sub-droplet is calculated by

$$Y_{i,0} = \frac{N/m - n_i}{N/m} \tag{4}$$

By solving Eq. (2) under the condition of Eq. (4), the fraction of unoxidized lipid for the i-th sub-droplet, Y_i, at any dimensionless time θ is expressed as follows:

$$Y_i = \frac{1}{1+\exp\left(\theta + \ln\dfrac{1-Y_{i,0}}{Y_{i,0}}\right)} \tag{5}$$

2.2 Momentarily triggering oxidation model

An oil droplet consisting of N lipid molecules was divided into m sub-droplets of the same volume, and n unoxidized molecules were converted to oxidized ones momentarily at a given moment, $\theta=0$. Two extreme cases were considered: in the first case, the conversion occurred evenly in the m sub-droplets, and in another one, the conversion did randomly in the sub-droplets. The oxidation process of lipid in the sub-droplets for both the cases was calculated by Eq. (5), and the fractions of unoxidized lipid molecules were averaged over all of the sub-droplets.

2.3 Gradually triggering oxidation model

An oil droplet consisting of N lipid molecules was also divided into m sub-droplets of the same volume. n unoxidized molecules were randomly converted to oxidized ones in the m sub-droplets one-by-one at regular intervals of $\Delta\theta=1$. The dimensionless time when the conversion occurred in the i-th sub-droplet was designated as $\theta_{s,i}$, and the oxidation process of the lipid in the sub-droplet at $\theta \geq \theta_{s,i}$ was calculated by

$$Y_i = \frac{1}{1+\exp\left((\theta-\theta_{s,i}) + \ln\dfrac{1-Y_{i,0}}{Y_{i,0}}\right)} \tag{6}$$

The $Y_{i,0}$ for Eq. (6) is given by

$$Y_{i,0} = \frac{N/m - 1}{N/m} \tag{7}$$

When the conversion of another unoxidized molecule to oxidized one occurred in the sub-droplet during the oxidation process, its effect on the Y value was ignored because the large number of oxidized molecules had been formed in the sub-droplet by the time.

3. Results and Discussion

Let us consider an oil droplet that is 10 μm in diameter. When we assume that the oil droplet consists of methyl linoleate, the droplet would be estimated to contain *ca.* 10^{12} molecules from its density (*ca.* 900 kg/m^3) and molecular mass (*ca.* 0.3 kg/mol). The droplet was divided into 1 to 10^5 sub-droplets of the same size, and the oxidation processes of methyl linoleate in the sub-droplets were calculated when 10^3 or 10^5 unoxidized lipid molecules were converted to oxidized ones momentarily or gradually to trigger the oxidation of lipid in the sub-droplet.

3.1 Oxidation processes by momentary appearance of oxidized lipid

A droplet containing 10^{12} lipid molecules were divided into 10^3 sub-droplets of the same size, and each sub-droplet contained 10^9 molecules. Curves a and b in Fig. 1 are the lipid oxidation processes when the conversion of 10^5 unoxidized molecules to oxidized ones occurred evenly and randomly, respectively, in the sub-droplets at $\theta=0$. When the conversion occurred randomly, the fraction of unoxidized lipid, Y, at any time θ was different from sub-droplet by sub-droplet. The Y values were averaged over all of the sub-droplets. Curve b in Fig. 1 was drawn based on the averaged Y values. The standard deviation was also calculated, and it was very small and within the line width in the figure. Because the number of oxidized molecules was much larger than that of sub-droplets, the oxidation processes were almost the same in both cases where the conversion occurred evenly and randomly.

When the conversion of 10^3 unoxidized molecules to oxidized one occurred evenly in 10^3 sub-droplets (1 oxidized molecule per sub-droplet), the oxidation was retarded, as shown by curve c, compared with that for the case where 10^5 unoxidized molecules were converted to the oxidized ones in a sub-droplet (curve a). The retarded oxidation, as determined by the elongation of induction period, was ascribed to a larger Y_0 value at 1 oxidized molecule per sub-droplet than at 10^2 oxidized molecules per sub-droplet. The Y_0 values were nine nines and seven nines for the former and latter cases, respectively.

When the conversion of 10^3 unoxidized molecules to oxidized ones occurred randomly for 10^3 sub-droplets at $\theta=0$ (curve d), the length of the induction period was the same as that in the case of even conversion of unoxidized molecules to oxidized ones. However, because no oxidized lipid molecules were formed in some sub-droplets, the lipid in the sub-droplets was not oxidized. Therefore, the oxidation rate was slower, and the fraction of unoxidized lipid leveled off at $Y=0.369$, which can also be stochastically calculated as $[(10^3-1)/10^3]10^3$. This result suggests that reduction of oil droplet size in O/W emulsion retards lipid oxidation when a specific number of unoxidized lipid molecules are randomly converted to oxidized ones, because the probability that the sub-droplets have no oxidized molecules increases with a decrease in the oil droplet size.

Because the above results suggest that the number of oxidized molecules formed in a sub-droplet played an important role in lipid oxidation, the effect of the averaged number of oxidized molecules in a sub-droplet on lipid oxidation was examined. 10^{12} lipid molecules were divided into 1, 10, 10^2, 10^3, 10^4, or 10^5 sub-droplets, and the oxidation processes were calculated for the random formation of 10^5 oxidized molecules (Fig. 2). There was no significant difference among the oxidation processes for 1, 10, 10^2, 10^3, and 10^4 sub-droplets because the averaged number of oxidized molecules per sub-droplet was large. However, the oxidation was retarded and leveled off at 10^5 sub-droplets because the number of oxidized

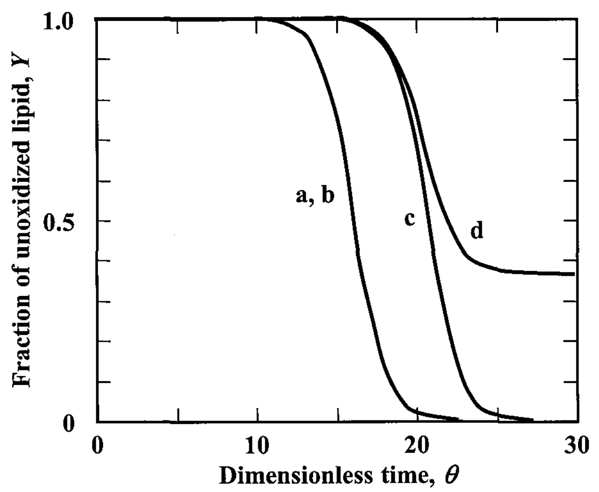

Fig. 1 Oxidation processes of lipids in 10^3 sub-droplets. Each sub-droplet contained 10^9 lipid molecules. The conversion of (a, b) 10^5 or (c, d) 10^3 unoxidized lipid molecules to oxidized ones occurred momentarily at $\theta=0$ (a, c) evenly or (b, d) randomly for the sub-droplets.

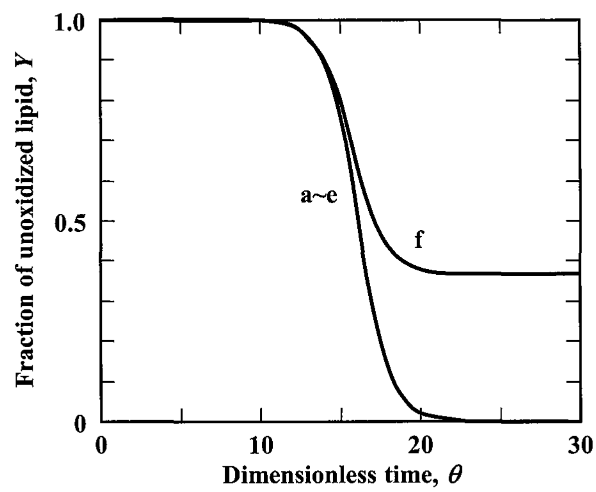

Fig. 2 Oxidation processes of lipids with momentary and random appearance of 10^5 oxidized lipid molecules. 10^{12} lipid molecules were divided into (a) 1, (b) 10, (c) 10^2, (d) 10^3, (e) 10^4, or (f) 10^5 sub-droplets.

molecules was the same as that of sub-droplets, and no oxidized molecules appeared in some sub-droplets.

3.2 Oxidation processes by gradual appearance of oxidized lipid

10^{12} lipid molecules were divided into 1, 10, 10^2, 10^3, 10^4, or 10^5 sub-droplets. The larger number of sub-droplets indicates that the oil droplet size is smaller. 10^5 oxidized molecules appeared one-by-one at intervals of $\Delta\theta=1$ in any sub-droplet, and the oxidation processes were calculated as shown in Fig. 3. When all of the lipid molecules existed in an original droplet (curve a), the lipid molecules underwent oxidation rapidly once an oxidized lipid molecule appeared. As the number of sub-droplets became larger, the lipid oxidation was more retarded (curves b to f) (Fig. 3). When the oxidized molecule formed was isolated in a sub-droplet, the lipid molecules in the sub-droplet oxidized according to Eq. (5), but the molecules in other sub-droplets were not affected by the oxidized molecule. This is why lipid oxidation is more retarded when the molecules are divided into more sub-droplets. These results indicate that reduction of oil droplet size in O/W emulsion effectively retards lipid oxidation.

Because appearance of oxidized lipid molecules in sub-droplets is probabilistic, every sub-droplet exhibits a different oxidation process. That is, the amount of time it takes to reach a specific fraction of unoxidized lipid depends on the sub-droplets. For the case where 10^{12} lipid molecules were divided into 10^2 sub-droplets, Fig. 4 shows the distribution of dimensionless time when the fraction of unoxidized lipid reached 0.75, 0.5, or 0.25. The distribution became broader at a lower fraction of unoxidized lipid. This result suggests the possibility that the experimentally observed fraction measurement depends on the sample size or volume.

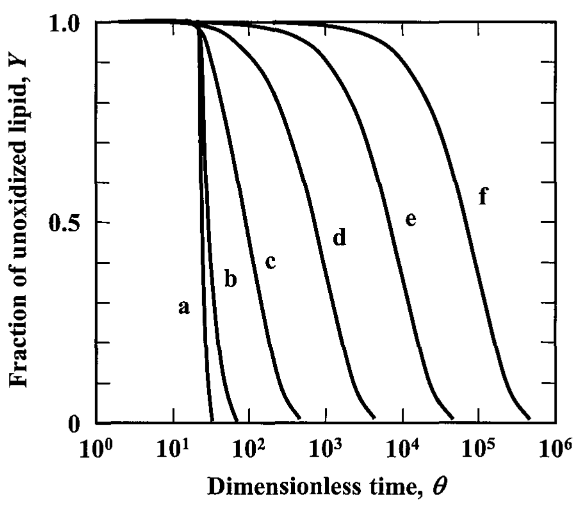

Fig. 3 Effect of droplet size on the oxidation of 10^{12} lipid molecules, which were divided into (a) 1, (b) 10, (c) 10^2, (d) 10^3, (e) 10^4, or (f) 10^5 sub-droplets. 10^5 oxidized lipid molecules gradually appeared at regular intervals of $\Delta\theta=1$.

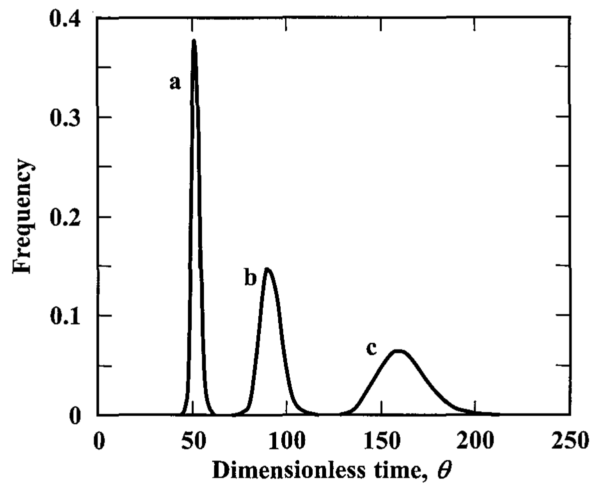

Fig. 4 Distribution of dimensionless time taken to reach the unoxidized lipid fractions of (a) 0.75, (b) 0.5, and (c) 0.25. 10^{12} lipid molecules were divided into 10^2 sub-droplets.

4. Conclusions

The effect of oil droplet size in O/W emulsions on lipid oxidation was simulated for two cases where the oxidized lipid molecules appeared momentarily at a given moment ($\theta=0$) and gradually at regular intervals of $\Delta\theta=1$. In the former case, there was no significant recognized effect of reduction of oil droplet size on retardation of lipid oxidation. However, in the latter case, lipid oxidation can be significantly retarded by the reduction of oil droplet size. The smaller oil droplet has a more remarkable effect on retardation. Therefore, simulation in this study indicated the possibility that lipid is more stable against oxidation in nano-emulsion than micro-emulsion.

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◇◇◇ 和文要約 ◇◇◇

油滴の微細化がO/Wエマルション系での脂質酸化に及ぼす影響

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O/Wエマルション系における脂質酸化に及ぼす油滴径の影響は，次のように考えるのが一般的であろう．油滴径が大きいと比表面積が小さく，油水界面での酸素の物質移動の影響により酸化が遅延される．一方，油滴が微細化すると，油水界面を通して十分量の酸素が供給されるので，反応自体が律速となり，脂質の酸化速度は油滴径に依存しない．しかし，既往の論文では，油滴を微細化すると酸化が促進されるとするもの [1-5] と，逆に遅延されるとするもの [6-10] がある．また，油滴径は脂質の酸化に顕著な影響を及ぼさないという報告もある [11-15]．このように結果が大きく異なる理由として，使用されている脂質の種類，油滴の大きさの範囲，酸化過程を測定する温度などが異なることに加えて，広い油滴径の範囲にわたり，脂質の状態がまったく同じO/Wエマルションを調製することが，実験的には困難であることがあげられる．そこで本研究では，コンピュータ・シミュレーションにより，O/Wエマルション系における脂質酸化に及ぼす油滴径の影響について検討した．すなわち，N分子の脂質をm個の油滴に分割し，ある瞬間に（無次元時間0で）n分子のラジカルがそれぞれの油滴に均等またはランダムに発生する場合を想定し，発生したラジカルの影響はその油滴内に留まると仮定して，全体の平均的な酸化過程を計算した．また，一定の時間間隔で1個ずつラジカルがランダムに発生する場合の酸化過程についても計算した．脂質の分子数Nは一定としたので，油滴の数mが大きいほど，油滴が微細化されたことに相当する．

10^3個の油滴に対して，初期（無次元時間0）に10^5または10^3分子のラジカルを均等またはランダムに発生させて，脂質の未酸化率の変化を計算した（Fig. 1）．発生するラジカル数が油滴の数より大きいとき（$n>m$）には，ラジカルの発生が均等でもランダムでも脂質の酸化過程はほぼ同じであった．一方，ラジカルの発生数が油滴数と同じ10^3分子で，均等に発生する場合には，発生数が10^5分子の場合に比べて，酸化誘導期は遅延されるが，酸化速度には大きな差異は認められなかった．しかし，ラジカルの発生がランダムなときには，酸化速度がやや遅くなり，脂質の酸化はある未酸化率で停止した．その未酸化率は確率的に計算できる．

次に，初期に10^5分子のラジカルが，1，10，10^2，10^3，10^4または10^5個の油滴に対してランダムに発生する場合の脂質の酸化過程を計算した（Fig. 2）．油滴数が発生するラジカルの数より少ない1〜10^4個の場合には，油滴の大きさに関わらずほぼ同じ酸化過程を示したが，10^5個の場合には，Fig. 1と同様であるが，一定の未酸化率で脂質の酸化が停止した．

上記では，10^5分子のラジカルが初期に一斉に発生したが，同数のラジカルが一定の時間間隔で1個ずつランダムに発生する場合の酸化過程を計算すると（Fig. 3），油滴数が多い，すなわち，油滴が微細化するほど，酸化の誘導期が延長され，酸化速度も遅くなった．また，未酸化率が0.75，0.5または0.25に到達する時間の分布は，当然ながら，未酸化率が低くなるほど分散が大きくなった．

ラジカルは油滴に対して均等かつ一斉に発生するのではなく，徐々にかつランダムに発生すると考えるのが妥当であろう．したがって，Fig. 3に示すように，油滴を微細化すると，発生したラジカルの影響が及ぶ範囲が狭くなるので，脂質全体の酸化は遅延される．また，ここで示した効果のほかに，油滴を微細化すると，油滴の表面を覆っている乳化剤の疎水基により脂質が希釈される効果が顕在化して酸化速度が小さくなると予測するモデルを提出している [10]．これらの2つの効果により，乳化直後の脂質の状態がまったく同じであれば，ナノエマルション系ではマイクロエマルション系よりも酸化が遅延されることが期待される．

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