Original paper

Kinetic Analysis of the Concentration of Conjugated Diene Structures in Glycerol Trilinoleate During Oxidation

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The concentration of conjugated diene structures produced by the oxidation of glycerol trilinoleate was measured spectrophotometrically. The test lipid was held at 5, 30, 40 and 50°C in air. The concentration was estimated using the molar absorption coefficient of conjugated methyl linoleate. The results showed that the concentration of conjugated diene structures was rapidly increased at higher temperatures. Rate constants were determined from the experimental results according to a chemical reaction model and its kinetic equations for the lipid oxidation process. Transient changes in the concentration of conjugated diene structures as calculated from the rate constant explained the experimental results well. The Arrhenius activation energy was 155 kJ/mol.

Keywords: lipid oxidation, kinetic analysis, glycerol trilinoleate, conjugated diene

Introduction

Food is typically a coarse mixture of heterogeneous components mainly comprised of water, carbohydrate, protein and lipid. Lipid oxidation is one of the major causes of deterioration in food, and the protection of lipid from oxidation is important in food manufacturing. Lipid oxidation in food has been investigated from various points of view because of the complicated environment of food components (Shima *et al.*, 2006, Park *et al.*, 2005, Nuchi *et al.*, 2001). For example, studies have focused on the oxidation of polyunsaturated fatty acids extracted from fish (Azuma *et al.*, 2009, Frankel *et al.*, 2002), encapsulated lipid within a spray dried powder (Fang *et al.*, 2005), a mixture effect with stable compounds (Ishido *et al.*, 2001), and the oxidation of small oil droplets dispersed in liquid as an emulsion (Adachi, 2015, Ma *et al.*, 2013).

Oxidation of lipid composed of polyunsaturated fatty acids occurs more easily than that made of monounsaturated fatty acids. There are several quantitative methods for detecting the initial process of lipid oxidation, i.e., decrement of the concentration of

unsaturated fatty acids, consumption of oxygen, increment of the concentration of peroxide and conjugated diene structures, and so on.

Polyunsaturated fatty acids in foods contain a 1,4-pentadiene structure in its hydrocarbon chain. This structure has a carbon atom at the bisallylic position, and the hydrogen atom at the carbon is highly reactive compared with the other hydrogen atoms in the hydrocarbon chain. This hydrogen atom is attracted to nucleophilic substrates, typically radical compounds. The polyunsaturated fatty acid from which a hydrogen atom is abstracted at the bisallylic carbon becomes a fatty acid radical, and this radical reacts with oxygen to produce fatty acid peroxide. This fatty acid peroxide abstracts a hydrogen atom, which is frequently at the bisallylic carbon of another polyunsaturated fatty acid. During this process, the two double bonds in the 1,4-pentadiene structure are converted to a conjugated diene structure, which is relatively thermodynamically stable. Because the conjugated diene has absorbance at 234 nm, the concentration of this structure can be quantitatively determined spectrophotometrically.

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Porter and co-workers thoroughly reviewed the kinetics and chemical reaction processes of lipid oxidation (Yin *et al.*, 2011). Maillard *et al.* reported rate constants for the peroxidation of free radicals, and the rate constants seemed to be sufficiently large compared to a diffusion-controlled process (Yin *et al.*, 2011, Maillard *et al.*, 1983). Compared with this rapid process, abstraction of a hydrogen atom at the bisallylic position carbon by a lipid peroxy radical is relatively slow. Therefore, this abstraction process is known as the rate-limiting process in the chain reaction of lipid autoxidation.

Niki and co-workers measured the extent of oxidation of polyunsaturated fatty acid methyl esters dissolved in acetonitrile, in which a radical generator was added as an initiator of oxidation (Yamamoto *et al.*, 1982). In their study, oxygen uptake, peroxide and conjugated diene formation were equivalent at the initial period of oxidation of methyl linoleate. Next, they calculated an equilibrium constant for the reaction in which lipid peroxide is produced by the addition of oxygen to lipid radical, and suggested that lipid peroxide is the major component at large oxygen concentrations such as in air.

In this study, the oxidation process of glyceryl trilinoleate was kinetically analyzed as the concentration of conjugated diene structures and the rate constant of oxidation was obtained at various temperatures.

Theoretical

The chemical reaction pathway of the oxidation process of unsaturated fatty acid residue in lipid is shown in Fig. 1. The variables t and C represent time and concentration, respectively. Subscripts CH, R, C·, O₂, COO· and COOH represent hydrocarbons at bisallylic positions, radicals intrinsic in the lipid, fatty acid radicals, oxygen, fatty acid peroxides and fatty acid hydroperoxides, respectively. Rate constants k_0 , k_1 and k_2 were for the reactions indicated in Fig. 1.

Reaction rates of the components are described below.

$$\left\{ \begin{array}{l} \frac{dC_{CH}}{dt} = -k_0 C_{CH} C_R - k_1 C_{CH} C_{COO\cdot} \quad \cdots \cdots \text{Eq. 1} \\ \frac{dC_{C\cdot}}{dt} = k_0 C_{CH} C_R + k_1 C_{CH} C_{COO\cdot} - k_2 C_{C\cdot} C_{O_2} \quad \cdots \cdots \text{Eq. 2} \\ \frac{dC_{COO\cdot}}{dt} = -k_1 C_{CH} C_{COO\cdot} + k_2 C_{C\cdot} C_{O_2} \quad \cdots \cdots \text{Eq. 3} \\ \frac{dC_{COOH}}{dt} = k_1 C_{CH} C_{COO\cdot} \quad \cdots \cdots \text{Eq. 4} \end{array} \right.$$

The steady state of the concentration of fatty acid radical was hypothesized because of its high reactivity (Eq. 5).

$$\frac{dC_{C\cdot}}{dt} = 0 \quad \cdots \cdots \text{Eq. 5}$$

According to Eqs. 2 and 3, Eq. 5 is transformed to below.

$$\frac{dC_{C\cdot}}{dt} = k_0 C_{CH} C_R - \frac{dC_{COO\cdot}}{dt} = 0 \quad \cdots \cdots \text{Eq. 6}$$

Fig. 1. Diagram of chemical reaction pathway of lipid oxidation.

therefore,

$$\frac{dC_{COO\cdot}}{dt} = k_0 C_{CH} C_R \quad \cdots \cdots \text{Eq. 7}$$

When Eq. 7 is integrated under $C_{COO\cdot} = 0$ at $t = 0$ as an initial condition, $C_{COO\cdot}$ is represented as Eq. 8 at time t , on the assumption that C_{CH} and C_R are constant.

$$C_{COO\cdot} = k_0 C_{CH} C_R t \quad \cdots \cdots \text{Eq. 8}$$

Time course of C_{COOH} was described as Eq. 9 from Eqs. 4 and 8.

$$\frac{dC_{COOH}}{dt} = k_1 C_{CH} k_0 C_{CH} C_R t \quad \cdots \cdots \text{Eq. 9}$$

C_{COOH} at time t is represented as Eq. 10, when Eq. 9 was integrated under $C_{COOH} = 0$ at $t = 0$ as an initial condition.

$$C_{COOH} = k_0 k_1 C_{CH}^2 C_R \left(\frac{1}{2} \right) t^2 \quad \cdots \cdots \text{Eq. 10}$$

Here $k_0 k_1 C_R$ was replaced by k as follows:

$$k = k_0 k_1 C_R \quad \cdots \cdots \text{Eq. 11}$$

therefore,

$$C_{COOH} = \frac{1}{2} k C_{CH}^2 t^2 \quad \cdots \cdots \text{Eq. 12}$$

Rate constant k is obtained from the second derivation of C_{COOH} with respect to t (Eq. 13).

$$\frac{d^2 C_{COOH}}{dt^2} = k C_{CH}^2 \quad \cdots \cdots \text{Eq. 13}$$

Materials and Methods

Materials Glyceryl trilinoleate was obtained from Sigma-Aldrich (St. Louis, Missouri, USA), with a purity of 98% (TLC) according to the supplier's information. Conjugated methyl linoleate was obtained from Sigma-Aldrich (St. Louis, Missouri, USA), and was a mixture of *cis*- and *trans*- isomers of 9,11- and 10,12-octadecadienoic acid methyl esters, with a purity of 99% (GC) according to the supplier's information. *n*-Hexane (GR grade) was obtained from Nacalai Tesque (Kyoto, Japan).

Measurement of specific gravity of glyceryl trilinoleate

Glyceryl trilinoleate used for the measurement of specific gravity was obtained from Tokyo Kasei Kogyo (Tokyo, Japan), with a purity of 95.8% (GC) according to the supplier's information. The test lipid was poured into a pycnometer, stored in a temperature-controlled water bath for an appropriate period, and then weighed using an analytical scale. Temperature equilibration and the weighing process was repeated using the same test lipid at ascending storage temperatures of 5, 30, 40 and 50°C. Oxidation during this procedure was assessed by measurement of the concentration of conjugated diene structures. Distilled water was used as a reference solution for specific gravity determinations at the same temperature.

Measurement of molar absorption coefficient of conjugated methyl linoleate An aliquot of conjugated methyl linoleate was weighed precisely in a test tube using an analytical scale, and diluted in *n*-hexane. Absorbance at 234 nm of the test solution was measured using a UV spectrophotometer (UVmini-1240; Shimadzu, Kyoto, Japan) with *n*-hexane as a reference solution. This process was repeated with varying concentrations of conjugated methyl linoleate.

Spectrophotometric measurement of transient changes in lipid oxidation Glyceryl trilinoleate (10 – 25 mg) was spotted on the bottom of each test tube (18 mm outer diameter and 165 mm in length) and weighed precisely using an analytical scale. The test tubes were placed in a temperature-controlled dry heat block at 30, 40 and 50°C or placed in a dry metal block located in a refrigerator at 5°C in air. The tubes were covered with aluminum foil.

At an appropriate interval, the test tube was removed and the test lipid was diluted with *n*-hexane to an appropriate concentration, and then poured into a quartz cell (1 cm in light path length). Absorbance at 234 nm was measured using a UV spectrophotometer, with *n*-hexane as a reference solution.

Results and Discussion

Specific gravity of glyceryl trilinoleate The specific gravity of glyceryl trilinoleate is shown in Fig. 2. The concentrations of conjugated diene structures in the test lipid before and after measurement were 0.088 mol/L and 0.11 mol/L, respectively. Specific gravity was used to convert the weight of glyceryl trilinoleate to a volume in the subsection 'Measurement of the lipid oxidation process'.

Molar absorption coefficient of conjugated methyl linoleate A molar absorption coefficient of 2.5×10^4 L/(mol cm) was obtained for conjugated methyl linoleate by spectrophotometric measurement at 234 nm (Fig. 3). This value was used to evaluate the concentration of conjugated diene structures in glyceryl trilinoleate. In a previous study, 2.8×10^4 L/(mol cm) was used as the absorption coefficient of conjugated diene structures at 230 – 236 nm (Yamamoto *et al.*, 1982).

Measurement of the lipid oxidation process Transient changes

Fig. 2. Specific gravity of glyceryl trilinoleate to distilled water at the same temperature.

Fig. 3. Absorbance of conjugated methyl linoleate dissolved in *n*-hexane at 234 nm.

in the concentration of conjugated diene structures in glyceryl trilinoleate stored at 5, 30, 40 and 50°C are shown in Fig. 4. The concentration of conjugated diene structures was represented as the amount of substance per liter (mol/L) in the test lipid, which was calculated using the specific gravity of the lipid at the same temperature. The solid curves in Fig. 4 represented calculated values of C_{COOH} using the rate constants k and Eq. 12, assuming that the majority of conjugated diene structures was held by fatty acid hydroperoxide at the initial period of lipid oxidation.

The rate constant k was obtained from the experimental results as follows. At first, the concentrations of conjugated diene structures C were plotted against time t on a linear scale graph, and an approximate curve was drawn for the data points. First derivations of C with respect to t were obtained by the graphical differential on the curve at various t . The first derivations were also plotted against t , and then an approximate straight line was drawn through the plotted points. Graphs in Fig. 5 show the plotted points and the approximate straight line for each temperature. Second

Fig. 4. Transient changes of the concentration of conjugated diene structure in glyceryl trilinoleate. Symbols $\circ$, $\triangle$, $\square$ and ∇ indicate 5, 30, 40 and 50°C, respectively. Solid curves represent calculated values using k and Eq. 12.

derivations of C_{COOH} with respect to t were obtained from the slope of the approximate lines in Fig. 5, and then k was calculated from Eq. 13 and C_{CH} , assuming that C_{CH} was nearly equal to the initial concentration of linoleate residue in glyceryl trilinoleate. C_{COOH} at $t = 0$ was assumed to be 0 in the theoretical section, but experimental results showed that some absorbance occurred at $t = 0$. Therefore, the initial value of the approximate curve was used to obtain the initial concentration of C_{COOH} on the calculated curve shown in Fig. 4. This absorbance might originate from the initial fatty acid hydroperoxide or glyceryl trilinoleate itself.

Part of the test lipid became insoluble in *n*-hexane after the period shown in Fig. 4 for 30, 40 and 50°C, and absorbance at 234 nm was decreased (data not shown). Measurement of lipid oxidation at 5°C was finished after about one week, and absorbance did not decrease within that period.

Arrhenius plot for the rate constant k The temperature dependency of k was analyzed using an Arrhenius plot (Fig. 6). All data points were in the neighborhood of an estimated straight line, and the Arrhenius activation energy was 155 kJ/mol, which was calculated from the slope of the line.

The Arrhenius activation energy obtained in this investigation may be used to estimate a rate constant of the oxidation of glyceryl trilinoleate at a temperature that was not investigated in this study, and a time course of lipid oxidation at the temperature may be estimated by this rate constant.

Discussion of the various oxidation rate constants Adachi *et al.* used the autocatalytic rate equation model to represent the entire process of lipid oxidation using the non-oxidized lipid fraction (Adachi *et al.*, 1995). The non-oxidized lipid fraction can be used as a quantitative index during the whole process of lipid oxidation, because this index is not affected by the development of various

kinds of secondary products generated in the later period of the lipid oxidation process. The non-oxidized fraction in our experiments was calculated as about 0.8 or higher. This suggested that the oxidation process investigated in this study was the initial period of the whole process, which can be evaluated using the non-oxidized lipid fraction.

The lipid oxidation model used in this study is a generally known process, provided that our model does not include the pathway to generate secondary oxidation products, such as the condensation reaction among fatty acid peroxides and so on. This hypothesis would be practical within the initial period, because of the low concentration of fatty acid peroxide, but might be inappropriate in the later period. If the secondary products retain the unchanged conjugated diene structure, the measured values would represent the sum of the concentration of conjugated diene structures included in fatty acid hydroperoxide and the secondary products.

Kinetic analysis of the termination reactions between fatty acid radicals and fatty acid peroxides, and within fatty acid radicals or within fatty acid peroxides, has been discussed by many researchers. Because the concentration of lipid peroxide was relatively large in the wide range of oxygen partial pressure, the reaction within lipid peroxides has been the main focus of attention (Yin *et al.*, 2011, Maillard *et al.*, 1983). Yamamoto *et al.* investigated the effects of oxygen concentration on the oxidation rate and ratio of the three types of termination reaction (Yamamoto *et al.*, 1982).

According to the model used in this study, k is represented by the multiple of k_0 , k_1 , and C_R . Increment of the concentration of fatty acid hydroperoxide, because of the progress of lipid oxidation, enhances the effect of k_1 . Other radical species would be produced

Fig. 5. Transient changes of the first derivations of the concentration of conjugated diene structure in glyceryl trilinoleate with respect to t . Figures **a**, **b**, **c** and **d** indicate 5, 30, 40 and 50°C, respectively.

Fig. 6. Arrhenius plot of k .

during lipid oxidation, such as fatty acid hydroxy- and alkoxy-radicals. In the model shown in this study, the effect of these radicals was included in k_1 . Therefore, k is an apparent rate constant that includes the effect of these reaction processes and the concentration of radical intrinsic in the lipid. In our study, the estimated curve calculated using k represented the experimental result well.

Conclusion

Glyceryl trilinoleate was stored at 5, 30, 40 and 50°C in air, and the concentration of conjugated diene structures generated during oxidation was measured using UV spectrophotometry. Transient changes in the concentration of conjugated diene structures were represented by the calculated curves using the estimated rate constants, which were defined by the kinetic equations for the chemical reaction model of the oxidation process. The dependence of k on temperature was represented as a straight line in the Arrhenius plot, and the Arrhenius activation energy was 155 kJ/mol.

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