Food Sci. Technol. Res., **9** (4), 383–386, 2003

Note

Autoxidation of Fish Oil in Mayonnaise-Like O/W Type Emulsion

Jun-ichi TAKAI,[1] Yasushi ENDO,[1]* Masaaki OKUZAKI[2] and Kenshiro FUJIMOTO[1]

[1]*Graduate School of Agricultural Science, Tohoku University, 1-1 Tsutsumidori-Amamiyamachi, Aoba, Sendai 981-8555, Japan*
[2]*Research Laboratory, Tsukishima Food Industry Co., Ltd., 3-17-9 Higashi-Kasai, Edogawa, Tokyo 134-8520, Japan*

Received June 6, 2003; Accepted August 5, 2003

O/W type emulsions were prepared with salmon oil as lipid and soymilk or whole egg as an emulsifier at different pH, to know the effect of pH on the oxidation and flavor stability of mayonnaise-like emulsions. Emulsions were oxidized in the presence of hemoglobin as a catalyst at 20°C. Both emulsions prepared with soymilk and whole egg were stable at pH 4 to 6, when the oxidative stability of emulsions was estimated as the induction period. Volatile compounds produced during oxidation were quantified for emulsions at different pH. Total volatiles were highest at pH 4. Propanal was added as a typical volatile compound to emulsions at different pH. The quantity of propanal in the gas phase was highest at pH 4, while it was lowest in the liquid phase at pH 4. The inferior flavor stability of mayonnaise could be due to the high volatility of low molecular weight compounds at pH 4, but this is not true of the oxidative stability.

Keywords: emulsion, fish oil, flavor stability, lipid peroxidation, mayonnaise

Emulsion foods such as milk, margarine, dressing and mayonnaise are popular and are consumed all over the world because of the special texture and taste. Among these foods, mayonnaise used for salad and fried foods is a typical oil-in-water (O/W) type emulsion in which lipid is dispersed in water. It is very important to control the quality of mayonnaise, because it is often stored in a room exposed to sunlight and fluorescent light at normal temperature. In particular, lipid oxidation seriously deteriorates mayonnaise and may give it an unpleasant flavor and undesirable taste. Lipid oxidation in a complicated emulsion system such as mayonnaise, however, has not been understood completely in comparison with a bulk system, because it includes various components such as protein, carbohydrate and organic acids besides lipids. Mayonnaise generally consists of 70% oil (salad oil), 15% egg yolk, 10% vinegar, and 5% seasoning and spice. Especially, egg yolk contains phospholipids and proteins as emulsifiers, and tocopherols and irons which act as antioxidants and prooxidants, respectively.

Moreover, the mechanism of the lipid oxidation in the emulsion system is thought to be different from that in the bulk system. Emulsion foods consist of at least three phases: the oil phase, the water phase and the oil-water interface. The lipid oxidation may initiate in one of the three phases in the emulsion. Coupland and McClements (1996) reported that the transition metal and the water-soluble initiator produced the free radical in the water phase. On the other hand, Mei and his group (Mei *et al*., 1998a,b; Donnelly *et al*., 1998) reported that the iron ion acted as a prooxidant to catalyze oxidation on the surface of lipid particle. Today, it is thought that the lipid oxidation may initiate on the oil-water interface in an emulsion system.

The reason why the mayonnaise is labile to flavor deterioration has not been clarified, although it clearly has an undesirable flavor during storage in comparison with other foods. In this study, we prepared an O/W emulsion containing fish oil as a model of mayonnaise, because fish oil is more susceptible to oxidation than vegetable oil and is noted for its specific nutritional effect (Lees & Karel, 1980). We investigated the effect of pH on the oxidative and flavor stability of the mayonnaise-like O/W type emulsion, to learn the mechanism of flavor deterioration in mayonnaise.

Materials and Methods

Materials Refined salmon oil was provided from Tsukishima Foods Industry Co., Ltd. (Tokyo). The major fatty acids of salmon oil were 16 : 0 (14.8%), 18 : 1 (18.6%), 20 : 1 (9.1%), 20 : 5 (9.6%) and 22 : 6 (10.8%). Defatted soybeans were the product of Ajinomoto Co., Inc. (Tokyo) and eggs were purchased at a local market. Hemoglobin from bovine, propanal, 2,4-dinitrophenylhydrazine (2,4-DNPH) and other chemicals were purchased from Wako Pure Chemical Industries, Ltd. (Osaka).

Preparation of O/W type-emulsions The O/W type emulsions were prepared by mixing the salmon oil as a lipid and the soymilk or whole egg as a proteineous emulsifier at the ratio of 5 : 1 (w/w). Soymilk was prepared from the defatted soybeans according to the method of Yoshimoto and Sato (2001). The emulsions were adjusted at pH 2 to 8 by 25 mM phosphate buffer and 25 mM HCl. Consequently, the emulsions consisted of 70% buffer, 25% salmon oil and 5% emulsifier.

Oxidation of emulsions and determination of oxygen concentration Three milliliters of emulsions were put in a 5 ml-glass chamber of an oxygen monitor, and an oxygen electrode was inserted in it. The emulsion was kept at 20°C, and then 0.2 ml of hemoglobin solution (5 mg/ml) was added to start the oxidation. The oxygen consumed by the lipid oxidation in emulsion was measured by a YSI model 5300 biological oxygen mon-

*To whom correspondence should be addressed.
E-mail: endo@biochem.tohoku.ac.jp

itor (Yellow Springs Instrument Co., Inc., USA) (Lin *et al.*, 1993). The oxidative stability of emulsions was evaluated by the period when the oxygen concentration in the emulsion decreased to half of the initial concentration.

Determination of volatile compounds After 1.5 ml of the emulsions prepared with whole egg were put in a 6 ml-vial, the oxidation was started by addition of 0.1 ml hemoglobin solution (5 mg/ml) at 20°C. The volatile compounds in the headspace of the vial were periodically collected by the solid phase-micro extraction (SPME) method (Endo *et al.*, 1998). Polydimethylsiloxane-absorbed fiber (SUPELCO Co., Ltd.) was used for the SPME method. The collected volatile compounds were analyzed by gas chromatography (GC) equipped with a hydrogen flame ionization-detector including CPSil-88 capillary column (50 m× 0.25 mm, Chrompack Co., Ltd., Netherlands). The column temperature was programmed to hold at 50°C for 5 min and to rise to 200°C at 2.5°C/min. The injection and detection temperature was 250°C.

Determination of propanal in gas and liquid phases Five or ten milligrams of propanal were added to 1.5 ml of the emulsions prepared with whole egg at different pH in the 6 ml-vial. The vial was immediately sealed, and propanal in the head space (gas phase) was collected at 20°C for 1 h by the SPME method prior to GC analysis.

Propanal present in the emulsion (liquid phase) was measured by the method of Yukawa *et al.* (1993). Then, 0.2 ml emulsion, 0.8 ml ethanol and 1.0 ml acidic 2,4-DNPH solution were put into a test tube with a screw cap and heated at 50°C for 30 min; the mixture was cooled and then 5 ml of 10% KOH in 80% ethanol was added. After centrifugation at 1300 *g* for 20 min, 0.5 ml of the upper layer was collected and diluted with 4.5 ml ethanol. The absorption at 425 nm of the solution was measured with a spectrophotometer.

All experiments were repeated four times. Data were expressed as averages±standard deviation. Data were statistically analyzed by a one-way analysis of variance followed by Tukey's test.

Results and Discussion

The effect of pH on the lipid oxidation in emulsions with different emulsifier Figure 1 shows the effect of pH on the oxygen consumption in the emulsions prepared with soymilk as an emulsifier. The period when the oxygen concentration in the emulsion decreased to half of the initial was in the range of 26 to 42 min at pH 3 to 8, and was dependent on pH. It was longest at pH 6, whereas it was shortest for pH 8. As a result, the emulsion prepared with soymilk was stable for hemoglobin-catalyzed oxidation under acidic conditions.

The effect of pH on the oxygen consumption of the emulsions prepared with whole egg as an emulsifier is shown in Fig. 2. A similar observation was obtained for the emulsions prepared with whole egg. The period when the oxygen concentration in the emulsion decreased to half of the initial was 14 to 71 min at pH 2 to 8, and was dependent on pH. It was longest for pH 4 and 6, while it was shortest at pH 8. The emulsion prepared with whole egg was stable for hemoglobin-catalyzed oxidation under weakly acidic conditions, although it was very susceptible to oxidation at pH 2. Moreover, the emulsion prepared with whole egg was more stable than that with soymilk.

The oxidative stability of mayonnaise-like emulsions containing salmon oil depended on pH and emulsifiers. Different oxidative stability of emulsions seemed due to the variety of proteins in the emulsifiers. Miyashita and his group (Miyashita *et al.*, 1997; Kubouchi *et al.*, 2002) and Duh *et al.* (1999) reported that polyunsaturated fatty acids (PUFAs) such as eicosapentaenoic (20 : 5) and docosahexaenoic acids (22 : 6) which were unstable in the bulk system, were resistant to oxidation in emulsions, and that the oxidative stability of PUFAs depended on the variety of emulsifiers. They also found that the droplet size of lipid might be related to its oxidative stability. The protein structure of the soymilk and the egg contained in the emulsion might affect the size and form of lipid particles. Moreover, the electric charge that might affect the contact of iron in hemoglobin with lipid is different between soymilk and whole egg. Additionally, phospholipids might enhance the oxidative stability of emulsions as antioxidants, in emulsions prepared with whole egg.

On the other hand, the stability of emulsion under acidic conditions of pH 4 to 6 may be due to the electric charge of protein in emulsifiers. Yoshida and Niki (1992) and Fukuzawa and Fujii (1992) reported that the iron-catalyzed oxidation of lipids in the micelle system was promoted by the anionic surfactant of so-

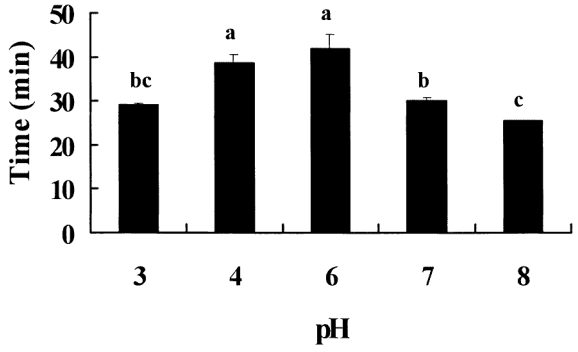

Fig. 1. The effect of pH on the hemoglobin-catalyzed oxidation of O/W type emulsions prepared with soybean milk as an emulsifier. The induction period was expressed as the period when the oxygen concentration in the emulsion decreased to half of the initial. Values with different superscript letters are significantly different ($p<0.05$).

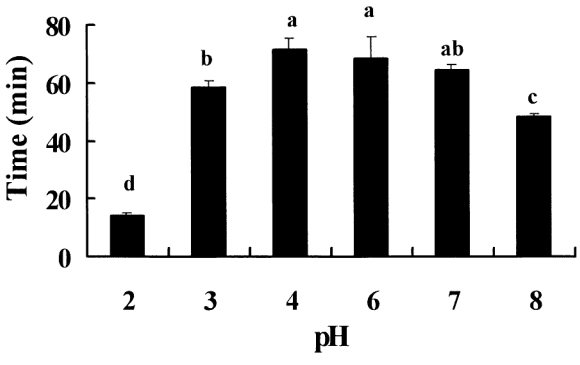

Fig. 2. The effect of pH on the hemoglobin-catalyzed oxidation of O/W type emulsions prepared with whole egg as an emulsifier. The induction period was expressed as the period when the oxygen concentration in the emulsion decreased to half of the initial. Values with different superscript letters are significantly different ($p<0.05$).

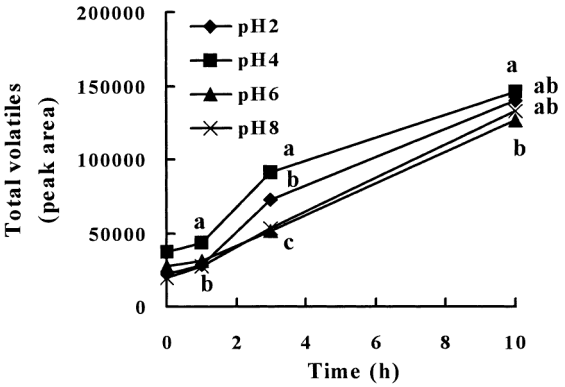

Fig. 3. The effect of pH on the total volatiles produced during oxidation of the emulsion prepared with whole egg as an emulsifier. Values in a vertical row with different superscript letters are significantly different ($p<0.05$).

dium dodecylsulfate and inhibited by the cationic surfactant such as tetradecyltrimethylammonium bromide. Mei *et al*. (1998b) reported that the iron was difficult to bring into lipid, when the interface of the lipid particle and water in emulsion had a positive charge. As the proteineous emulsifiers had positive charge due to the amino group at the acidic condition of pH 4 to 6, the electric charge could be positive at the interface of lipid and water. Therefore, since the iron in hemoglobin is a cation, it is usually unable to come into contact with lipid and catalyze the oxidation.

The high oxidizabiliy of emulsions under the extremely acidic condition of pH 2 might be due to the denatured proteins in emulsifiers and hemoglobin. The change of steric structure of emulsions might affect the size and form of lipid particle.

The effect of pH on the production of volatile compounds during oxidation O'Brien (1969) reported that heme compounds were very effective in decomposing lipid hydroperoxides at low pH. Therefore, we investigated the effect of pH on the volatile compounds produced from the decomposition of hydroperoxides during the oxidation of lipids in emulsion.

Three major peaks were observed at any pH, although several peaks were detected on the gas chromatogram. The major peaks were identified as propanal, 2-butenal and 2-pentenal, respectively by comparison with the retention time of authentic aldehydes. These aldehydes seemed to be derived from 20 : 5 and 22 : 6 of salmon oil. Most of volatile compounds were short-chain aldehydes with 4 to 7 carbon numbers in this experiment, although heptadienal, octanal and nonanone in addition to pentenal were reported to be off-flavor components of mayonnaise containing fish oil (Jacobsen *et al*., 1999).

Figure 3 shows total amounts of volatile compounds produced during oxidation of the emulsions prepared with whole egg as an emulsifier at different pH. Production of volatile compounds was increased with oxidation time, but their composition was not varied by pH. However, total amounts of volatile compounds depended on pH. Total volatiles were highest at pH 4 where the emulsion was most stable for oxidation, whereas they were lowest at pH 8 where it was most unstable. This observation was quite different from that obtained for oxygen consumption (Figs 1 and 2).

The effect of pH on the distribution of low molecular weight compounds in liquid and gas phases of emulsions The oxygen consumption should be proportional to amounts of volatile compounds, because the oxygen consumed during oxidation of an emulsion includes both production and decomposition of hydroperoxides. Low molecular weight compounds derived from the decomposition of hydroperoxides are major volatile compounds. To know whether this contradiction might be due to a different decomposition rate of hydroperoxides or volatility of low molecular weight compounds based on pH, we investigated the distribution of low molecular weight compounds in gas and liquid phases at different pH, using propanal as a typical volatile component. Propanal distributed in the liquid and gas phases was quantified after 5 mg or 10 mg of it was added to emulsions at pH 2 to 8 (Table 1). The quantity of propanal remaining in the liquid phase was somewhat varied by pH, when 5 mg propanal was added to emulsions at different pH. The amount of propanal in liquid phase was 283 μM (corresponding to 1.8 mg) at pH 4, and was lower than that (362 μM: 2.3 mg) at pH 8, when 10 mg propanal was added to the emulsions. The residual propanal was probably distributed in the headspace.

Table 1. Effects of pH on the distribution of propanal in liquid and gas phases of emulsions.

Propanal	pH			
	2	4	6	8
5 mg				
Liquid phase (μM)	329	324	358	347
Gas phase (peak area)	59300	75500	49100	40500
10 mg				
Liquid phase (μM)	301	283	325	362
Gas phase (peak area)	183600	213100	169000	121400

The quantity of propanal detected in the gas phase was markedly different by pH, although the addition level of propanal in the emulsions was the same at any pH (Table 1). The quantity of propanal in the gas phase was highest at pH 4, whereas it was lowest at pH 8. This result was very similar to that observed in Fig. 3. Especially, the quantity of propanal in the gas phase at pH 4 was 1.8 times higher than that at pH 8, when 10 mg propanal was added. This observation showed that the distribution of the volatile compounds was affected by pH, and it was consistent with the fact that the volatile compounds in the headspace of emulsions were most abundant at pH 4.

These results showed that propanal could easily migrate from liquid phase to gas phase at pH 4. The interaction of the carbonyl compounds (propanal) and proteineous emulsifier is probably weak under an acidic condition of pH 4, and might easily migrate from liquid phase to gas phase. Flavor stability of emulsions was inferior at pH 4, although their oxidative stability was superior at this pH. The inferior flavor stability of mayonnaise could be due to the higher volatility of low molecular weight compounds which are flavor components at pH 4 but this is not true of the oxidative stability.

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