

Singlet Oxygen Quenching Effect of Quercetin in Erythrosine-Sensitized Photooxidation of Oil-in-Water Emulsion

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ABSTRACT

Oxidation reaction can be initiated by either diradical triplet oxygen or nonradical singlet oxygen. The singlet oxygen can be formed in foods from triplet oxygen by photosensitized reaction. This research was intended to study the quenching effect of quercetin on lipid oxidation rate in the erythrosine-sensitized photooxidation of oil-in-water emulsion. Palm oil-in-water emulsion, containing erythrosine 100 ppm and quercetin 0, 25, 50, 75 and 100 ppm, were prepared with polyoxyethylene 100 stearyl ether (Brij 700) or polyoxyethylene sorbitan monolaurate (Tween 20). Structurally Brij 700 has 5 times longer polyoxyethylene groups than Tween 20. The mixture were stored under 4000 lux fluorescent light for 10 h and peroxide values were measured at 2 h interval. Erythrosine effectively sensitized the photooxidation of palm oil-in-water emulsion, as expected. Lipid oxidation rates, as determined by the formation of lipid hydroperoxides and headspace oxygen, in palm oil-in-water emulsions containing erythrosine decreased with increasing quercetin concentration. At pH 3, the peroxide value was higher than at pH 7. Brij 700 decreased production of lipid hydroperoxides from palm oil-in-water-emulsions compared to emulsions

stabilized by Tween 20. The results indicate that quercetin is an effective singlet oxygen quencher in palm oil-in-water emulsion and the surfactant headgroup size could be an important determinant in the oxidative stability of food emulsions.

Keywords: Quercetin, photooxidation, singlet oxygen quencher, oil-in-water emulsion

INTRODUCTION

Lipid oxidation is one of the main deteriorative reactions that takes place during preparation and storage of many food products, and it can make them unacceptable for human consumption. Therefore, lipid oxidation has been extensively studied in bulk fats and oils, and there is fairly good understanding of mechanisms and the factors that affect oxidation in such systems. On the other hand, lipid oxidation is still not well understood in systems in which the fat is dispersed as emulsion droplets, although a large number of food exist partially or entirely in the form of emulsions (Frankel, 1998; Ponginebbi *et al.* 1999; Kubouchi *et al.* 2002).

Food emulsion are complex, multicomponent, heterogeneous systems in which different molecular species interact with each other. In many foods, lipids exist as emulsifier-stabilized dispersions. These emulsions can be considered to contain three regions,

i.e., the interior of a droplet, the continuous phase, and the interfacial membrane. The interfacial membrane consists of a narrow region is potentially very important in lipid oxidation since it represents the region where lipid – and water – soluble components interact and it is where surface – active compounds such as lipid peroxides and chain breaking antioxidants concentrate (McClements and Decker, 2000). Recent studies has shown that the oxidation of oil-in-water emulsions involves the decomposition of lipid peroxides by transision metals, with iron being the most important prooxidant metal (Decker and McClements, 2001). The interaction of continuous phase iron with lipid-containing emulsion droplets is influenced by the properties of the emulsion droplets interfacial membrane. Oil-in-water emulsions prepared with anionic, cationic, and nonionic surfactants have various susceptibilities to oxidation. Lipid peroxide are more rapidly broken down by Fe^{+2} in anionic emulsion droplets prepared with sodium dodecyl sulfate (SDS) than in emulsion stabilized with nonionic (polyoxyethylene 100 lauryl ether; Brij 700 or Tween 20) or cationic (dodecyltrimethylammonium bromide; DTAB) surfactants (Mei *et al.* 1998; Mancuso *et al.* 1999).

This increased reactivity of iron is believed to be because of the electrostatic attraction between the positively charged metal and the negatively charged emulsion droplet membrane. Although several studies have shown that emulsion droplet charge is an important factor in the oxidative stability of emulsified oil, very little is known about how other emulsion droplet interfacial membrane properties impact oxidation rates. The characteristics of surfactant polar headgroups, we would have expected can be an important factors in the oxidative stability of oil-in-water emulsions.

On the other hand, triplet oxygen oxidation (autoxidation) does not fully explain the initiation step of lipid oxidation (Lee *et al.*, 1997). Rawls and VanSanten (1970) suggested that singlet oxygen is involved in the initiation of triplet oxygen lipid oxidation because singlet oxidation can directly react with double bonds without the formation of free radicals. Singlet oxygen oxidation can be very rapid in foods due to the low activation energy required for the chemical reaction.

Singlet oxygen is produced by photosensitizers in the presence of light and triplet oxygen. Photosensitizers such as chlorophyll, riboflavin, and synthetic colorants in foods can absorb energy from light and transfer it to triplet oxygen to form singlet oxygen. The photosensitizer absorbs the ultraviolet or visible radiation energy rapidly and becomes an unstable, excited, singlet state molecule (1sen). The excited singlet photosensitizer loses its energy by internal conversion, emission of light, or intersystem crossing.

Synthetic food colorants, like erythrosine, which have been used to improve the appearance of foods, may act as photosensitizers due to the highly conjugated double bonds. Photosensitizing synthetic colorants affect the lipid oxidation and the safety of foods. Erythrosine or FD&C Red No.3 has been reported to be a photosentizer leading to the oxidation of pork product, methyl linoleate, and cholesterol (Chung, *et al.*, 1997).

To reduce the undesirable singlet oxygen oxidation in lipid foods, quercetin may act as antioxidant by singlet oxygen quencher (Takahama, 1984; Nakagawa *et al.* 2000). However, studies on the lipid oxidation by singlet oxygen in palm oil-in-water emulsion have been limited. Therefore, the objectives of this study was to investigate (1) the effects of erythrosine and two different surfactants that varied in hydrophilic headgroup size, namely, polyoxyethylene 100 stearyl ether (Brij 700) or polyoxyethylene sorbitan monolaurate (Tween 20) on the photooxidative stability of palm oil-in-water emulsions. Structurally Brij 700 containing 5 times longer polyoxyethylene groups than Tween 20 and (2) the effects of quercetin as singlet oxygen quencher and pH on the erythrosine sensitized photooxidation in palm oil-in-water emulsions.

MATERIALS AND METHODS

Materials

Refined, bleached and deodorized palm oils was obtained from PT Astra Agro Lestari, Medan, North Sumatera. Silicic acid, celite, activated charcoal, quercetin, polyoxyethylene (100) stearyl ether (Brij 700), a-tocopherol and b-carotene was purchased

from Aldrich Chemical Co. Erythrosine was obtained from from Inti, Yogyakarta. Hexane, chloroform, acetic acid glacial, potassium iodide, polyoxyethylene (20) sorbitan monolaurate (Tween 20) was purchased from Sigma Chemicals Co.

Preparation of Purified Palm Oil

To prepare purified palm oil, it was passed through a chromatographic column (60 cm x 4 cm) packed with a series of activated silicic acid, 2:1 mixture of activated charcoal and celite, 2:1 mixture of powder sugar and celite, and activated silicic acid as described by Lee and Min (1988). The oil passed through the column was purified palm oil. It was colorless and contained low peroxide, free fatty acids, tocopherols or carotenoids.

Chemical Analysis of Purified palm Oil

Tocopherols were determined by the high pressure liquid chromatography of Carpenter (1979), and carotenoids were determined by the spectrometric method of Proctor and Snyder (1987). Peroxide value, and free fatty acids were determined by AOCS (1980) methods (Shahidi and Wanasundara, 2002).

Effects of Erythrosine on the Photooxidation in Palm Oil-in-Water Emulsion

To study the effects of erythrosine on the photosensitized oxidation in palm oil-in-water emulsion, emulsions were prepared as described by Ponginebbi *et al.* (1999) with modifications. Purified palm oil (10 g) was weighed into a 250 mL beaker glass. Then, exactly 90 mL of acetate buffer at pH 3 containing 0, 50, 100, 150, and 200 ppm (wt/vol) erythrosine and 1% Brij 700 or Tween 20 were added to the flask. The mixture was subsequently mixed gently with magnetic stirrer under nitrogen for 15 min and then emulsified by means of a blender (waring commercial blender) for 15 min at 4°C. Fifteen mL of the prepared palm oil-in-water emulsion was transferred into 25 mL serum bottles in duplicate. The bottles were sealed airtight with rubber septa and aluminium caps and place in a light storage box described in detail by Lee and Min (1988). The light sources, four Sylvania 15 watt cool white fluorescent lamps, were placed on the bottom of wooden box. The light intensity at the

sample level was 4,000 lux. The degree of oxidation palm oil-in-water emulsion was determined by measuring peroxide value every two hour for 8 h by using the AOCS method (Shahidi and Wanasundara, 2002).

Effects of pH on Erythrosine Sensitized Photooxidation in Palm Oil-in-Water Emulsion

Emulsions were prepared as described by Ponginebbi *et al.* (1999) with modifications. Purified palm oil (10 g) was weighed into a 250 mL beaker glass. Then, exactly 90 mL of acetate buffer at pH 3 and pH 4 or of phosphate buffer at pH 5; pH 6 and pH 7 containing 100 ppm (wt/vol) erythrosine and 1% Brij 700 or Tween 20 were added to the flask. The mixture was subsequently mixed gently with magnetic stirrer under nitrogen for 15 min and then emulsified by means of a blender (Waring commercial blender) for 15 min at 4°C. Fifteen mL of the prepared palm oil-in-water emulsion was transferred into 25 mL serum bottles in duplicate. The bottles were sealed airtight with rubber septa and aluminium caps and place in a light storage box described in detail by Lee and Min (1988). The light sources, four Sylvania 15 watt cool white fluorescent lamps, were placed on the bottom of wooden box. The light intensity at the sample level was 4,000 lux. The degree of oxidation palm oil-in-water emulsion was determined by measuring peroxide value every two hour for 10 h by using the AOCS method (Shahidi and Wanasundara, 2002).

Effects of Quercetin on Erythrosine Sensitized Photooxidation of Palm Oil-in-Water Emulsion

To study the effects of quercetin on the photosensitized oxidation in palm oil-in-water emulsion, emulsions were prepared as described by Ponginebbi *et al.* (1999) with modifications. Purified palm oil (10 g) was weighed into a 250 mL beaker glass. Then, exactly 90 mL of acetate buffer at pH 3 and of phosphate buffer at pH 7 containing 100 ppm (wt/vol) erythrosine and 1% Brij 700 or Tween 20 were added to the flask. The mixture was subsequently mixed gently with magnetic stirrer under nitrogen for 15 min and then emulsified by

means of a blender (waring commercial blender) for 15 min at 4°C. Emulsion were added 0, 25, 50, 75 and 100 ppm quercetin. Samples containing 100 ppm (wt/vol) a-tocopherol were used as a positive control in the system. Fifteen mL of the prepared palm oil-in-water emulsion was transferred into 25 mL serum bottles in duplicate. The bottles were sealed airtight with rubber septa and aluminium caps and place in a light storage box described in detail by Lee and Min (1988). The light sources, four Sylvania 15 watt cool white fluorescent lamps, were placed on the bottom of wooden box. The light intensity at the sample level was 4,000 lux. The degree of oxidation palm oil-in-water emulsion was determined by measuring peroxide value every two hour for 10 h by using the AOCS method (Shahidi and Wanasundara, 2002).

Droplet Size Measurement

The droplet size distribution was measured as described by Zhang and Proctor (1997) with modifications. The emulsion samples were smeared on superfrost microscope slide and droplet size observed with using objective micrometer and ocular micrometer. Then, microscope was connected with camera PCI TVM.

Determination of Emulsion Stability

The method of Tornberg and Hermanssons (1977) and Aiko *et al.* (1984) were modified for use in this study. Emulsion stability was determined on the basis of the percentage change of fat in the aqueous phase after low speed centrifugation. Thirty (30 g) of the emulsion were placed into centrifuge tube. The samples were centrifuged at 200 rpm for 15 min. after centrifugation of the emulsion, 5 ml of the lower phase was carefully removed with a syringe for fat determination by the Mojonnier method (IDF 16C 1987). The following equation was used to calculate emulsion stability.:

$$\text{Emulsion Stability} = \frac{\% \text{ fat in the lower phase}}{\% \text{ fat in the original emulsion}} \times 100$$

Determination of Oxygen in Headspace

The emulsion samples was transferred into 25 mL serum bottles was connected with oxygen meter equipped and instalated with Logger Pro 3 program. The decreasing of oxygen in the headspace observed for 5 h under fluorescent lamp.

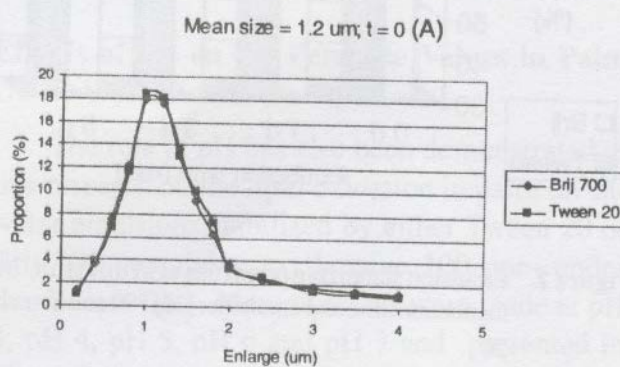
RESULTS AND DISCUSSION

Purified Palm Oil

The purified palm oil obtained by column chromatography was colorless and contained peroxide value 0.73 meq/kg oil, free fatty acids 0.08%, tocopherols 7.67 ppm or carotenoids 4.21 ppm, and dit not contain detectable concentrations of conjugated dienes.

Droplet Size Distribution of O/W Emulsion

Droplet size distributions were measured periodically and obtain mean emulsion droplet diameters 1.2 – 1.3 uM. Droplet size and distribution were quite stable for at least a 5-d storage at 40°C (Fig. 1). A small increase in the mean droplet size (from 1.2 to 1.3 uM) during 5 d incubation was due to the presence of a few particle above 2 uM diameter.



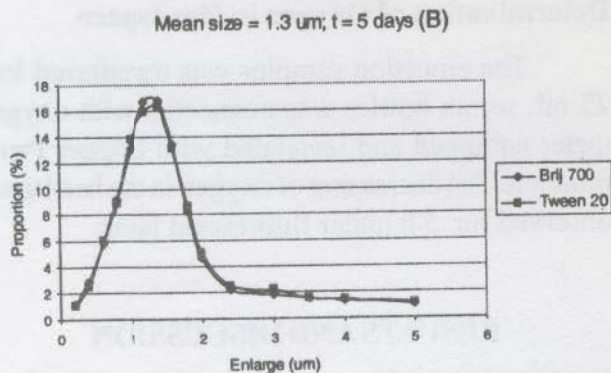


Figure 1. Droplet size distribution of oil-in-water (o/w) emulsions at t = 0 d (A) and 5 d (B) of aging

Duncan's multiple range tests showed that the mean droplet diameters of Tween 20 and Brij 700-stabilized emulsion were not significantly different ($P > 0.05$) both 0 d and 5 d aging, indicating that coalescence or Oswald ripening did not occur. The emulsion stability, expressed on the basis of the percentage change of fat in the aqueous phase after low speed centrifugation showed did not significantly different during 5 d aging (Fig. 2).

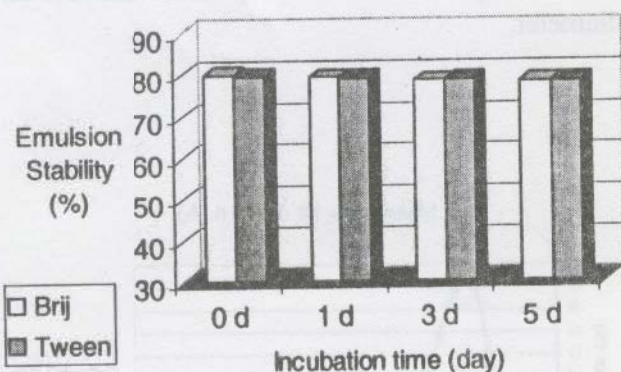


Figure 2. Emulsion stabilizing properties as a function of incubation time and emulsifier Brij 700 and Tween 20.

Emulsions are thermodynamically unstable system because of the positive free energy needed to increase the surface area between the oil and water phases and because oil and water have different densities. For this reason, emulsion tend

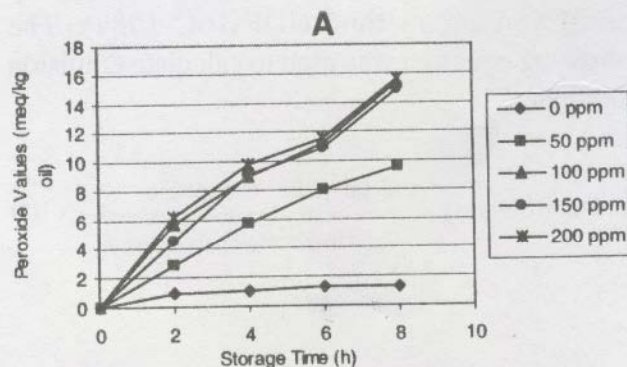
to separate into a system that consists of a layer of oil (lower density) on top of a layer of water (higher density) so as to minimize the contact area between oil and water. To form emulsions that are kinetically stable for a reasonable period of time (a few weeks, months or years), chemical substances known as emulsifiers must be added prior to homogenization.

An efficient emulsifier produces an emulsion in which there is no visible separation of the oil and water phase over time (McClements, 1999). Phase separation may not become visible to the human eye for a long time, even though some emulsion breakdown has occurred. Consequently, it is important to have analytical tests which can be used to detect the initial stages of emulsion breakdown, so that their long-term stability can be predicted.

One widely used test is to centrifuge an emulsion at a given speed and time and observe the amount of creaming and/or oil separation which occurs (Tornberg and Hermansson, 1977; Aoki *et al.* 1984; Srinivasan *et al.* 2001). This test can be used to predict the stability of an emulsion to creaming using relatively low centrifuge speeds or to coalescence by using speeds which are high enough to rupture the interfacial membranes. The greater the degree of creaming or oil separation that occurs, the greater the instability of an emulsion and the less efficient the emulsifier.

Photosensitizing Effects of Erythrosine in Palm Oil-in-Water Emulsion

The effects of 0, 50, 100, 150 and 200 ppm erythrosine on the peroxide values of palm oil-in-water emulsion are shown in Figure 3.



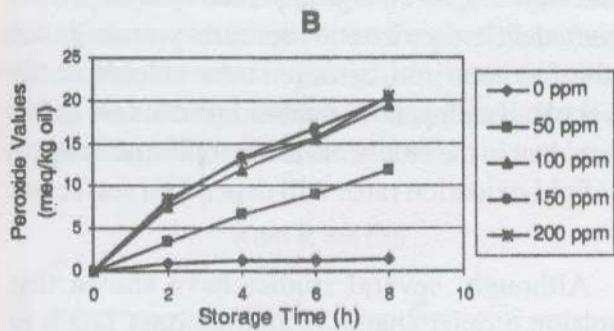


Figure 3. Effects of erythrosine on the peroxide values of palm oil-in-water emulsion which stabilized Brij 700 (A) and Tween 20 (B) under fluorescent light during 8 h.

The concentration effects of erythrosine increased from 0 to 50, 100, 150 and 200 ppm, the peroxide value increased by 1.29 to 9.59, 15.32, 14.93 and 15.56 meq/kg oil in palm oil-in-water emulsion which stabilized Brij 700, respectively during 8 h under fluorescent light. Meanwhile, in palm oil-in-water emulsion which stabilized Tween 20, the concentration effects of erythrosine increased from 0 to 50, 100, 150 and 200 ppm, the peroxide value increased by 1.38 to 11.95, 19.44, 20.35 and 20.37 meq/kg oil respectively during 8 h under fluorescent light. However, Duncan's multiple range tests showed that the peroxide value of samples of 100, 150, and 200 ppm erythrosine were not significant different ($P > 0.05$) both in emulsion stabilized Tween 20 and Brij 700.

Photosensitizers can produce singlet oxygen from triplet oxygen only under fluorescent light exposure. Singlet oxygen formed by photosensitizers can accelerate the oxidation of lipid and the headspace oxygen content decrease. The schematic diagram for the formation of oxidized products (AO_2) via singlet-oxygen oxidation is as follows (Foote, 1979):

Figure 4 shows the development of singlet oxygen and its subsequent reaction with compound (A) to form the oxidized product (AO_2). At every stage in this reaction, there is at least 3 alternate routes, which, if taken, would minimize the oxidation of the compound (A). The 1st step represents when a sensitizer (Sen), such as erythrosine, in oil absorbs light energy, it becomes an excited singlet sensitizer

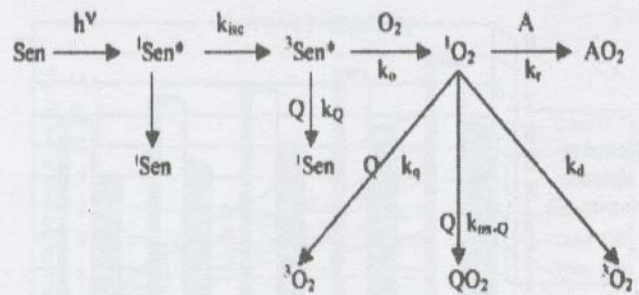


Figure 4. Formation of singlet oxygen and its reaction with substrate A to produce the oxidized product AO_2 . The formation of AO_2 can be prevented the reaction of 3Sen or 1O_2 with a quenching agent. (Min and Boff, 2002)

(1Sen). The return of the excited singlet sensitizer (1Sen) to ground state (1Sen) without intersystem crossing (isc) to form the excited triplet sensitizer (3Sen). The 2nd represents reaction with a quenching agent (Q) at a rate represented as k_Q , returning the excited triplet sensitizer (3Sen) to ground state (1Sen) prior to reaction with triplet oxygen. The excited triplet sensitizer (3Sen) may react with triplet oxygen (3O_2) to form singlet oxygen (1O_2). Following its creation, there are 3 fates for singlet oxygen in foods: (1) it may naturally decay to the ground state; (2) it may react with a singlet-state compound (A) forming the oxidized product AO_2 ; and (3) it may be destroyed by a quenching agent by either combining with the quencher.

Effects of pH on the Peroxide Values in Palm Oil-in-Water Emulsion

The role of pH has also been demonstrated in this research on the lipid oxidation in palm oil-in-water emulsions stabilized by either Tween 20 or Brij 700 containing erythrosine 100 ppm under fluorescent light. Measurements were made at pH 3, pH 4, pH 5, pH 6 and pH 7 and presented in Figure 5.

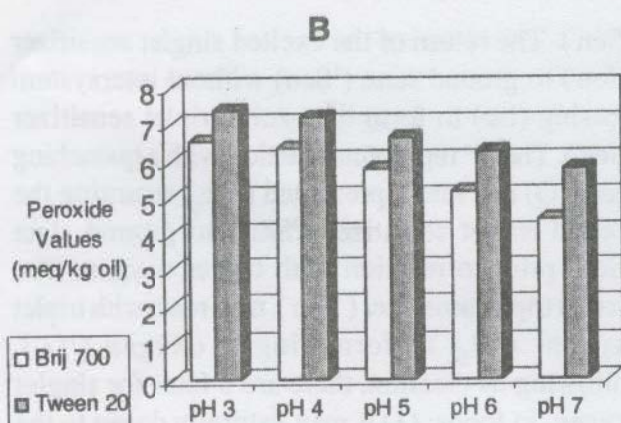
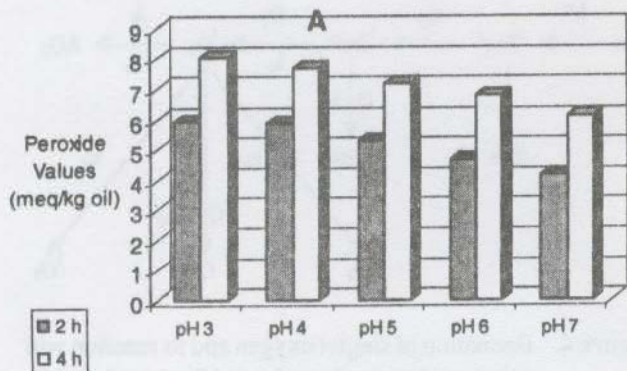


Figure 5. Effect of pH on peroxide values of palm oil-in-water emulsion containing erythrosine 100 ppm under fluorescent light: time curve (A); stabilized by either Tween 20 or Brij 700 (B)

Figure 5A shows the amount of hydroperoxides formed increased with decreasing pH between 2 and 4 hours. In both day 2 and day 4, the amount of hydroperoxides formed in palm oil emulsions increased in the following order: pH 3 > pH 4 > pH 5 > pH 6 > pH 7. In the emulsions stabilized by the nonionik surfactant, the rate of lipid oxidation was faster at pH 3 than at pH 7 (Donnelly *et al.* 1998; McClements and Decker, 2000). As the electrical charge of the droplets stabilized by nonionic

surfactants did not change appreciably with pH, the observed difference in oxidation rates was attributed to the fact that iron is more water soluble at the lower pH. If endogenous transition metals are active prooxidant in the emulsion, the authors would expect that lipid oxidation rates will depend on surfactant type.

Although several studies have shown that emulsion droplet charge is an important factor in the oxidative stability of emulsified oil, very little is known about how other emulsion droplet interfacial membrane properties impact oxidation rates. This research was to use two different nonionik surfactants that varied in hydrophilic headgroup size to evaluate their impact on the stability of lipid peroxides in palm oil-in-water emulsion. This was accomplished by preparing emulsions with polyoxyethylene 100 stearyl ether (Brij 700) or polyoxyethylene sorbitan monolaurate (Tween 20). Structurally Brij 700 containing 5 times longer polyoxyethylene groups than Tween 20. For both surfactants, peroxide values increased ($P < 0.05$) in the absence of added Fe^{+2} (Figure 5B), with hydroperoxide formation being lower in Brij 700-stabilized than in the Tween 20-stabilized emulsions ($P < 0.05$). In both surfactant system, Fe^{+2} significantly increased hydroperoxide formation, with Brij 700-stabilized emulsions again having less peroxide values formation than Tween 20-stabilized emulsions. This is, the characteristics of surfactant polar headgroups can be important factor in the oxidative stability of oil-in-water emulsion. This research suggests that the surfactant headgroup size could be an important determinant in the stability of lipid peroxide to oxidize fatty acid.

Effect of Quercetin on the Photosensitized Oxidation in Palm Oil-in-Water Emulsions

Erythrosine was extremely effective as a photosensitizer to accelerate the oxidation in palm oil-in-water emulsions under fluorescent light. This result agrees with previous reports on the photosensitizing effect of erythrosine on the oxidation soybean oil in acetone model system under the light storage (Yang *et al.* 2002).

Ability of 0, 25, 50, 75 and 100 ppm quercetin as singlet oxygen quencher on the photosensitizing effect of erythrosine in palm oil-in-water emulsion during 10-h storage under 4.000 lux fluorescent light are shown in Figure 6 and Figure 7.

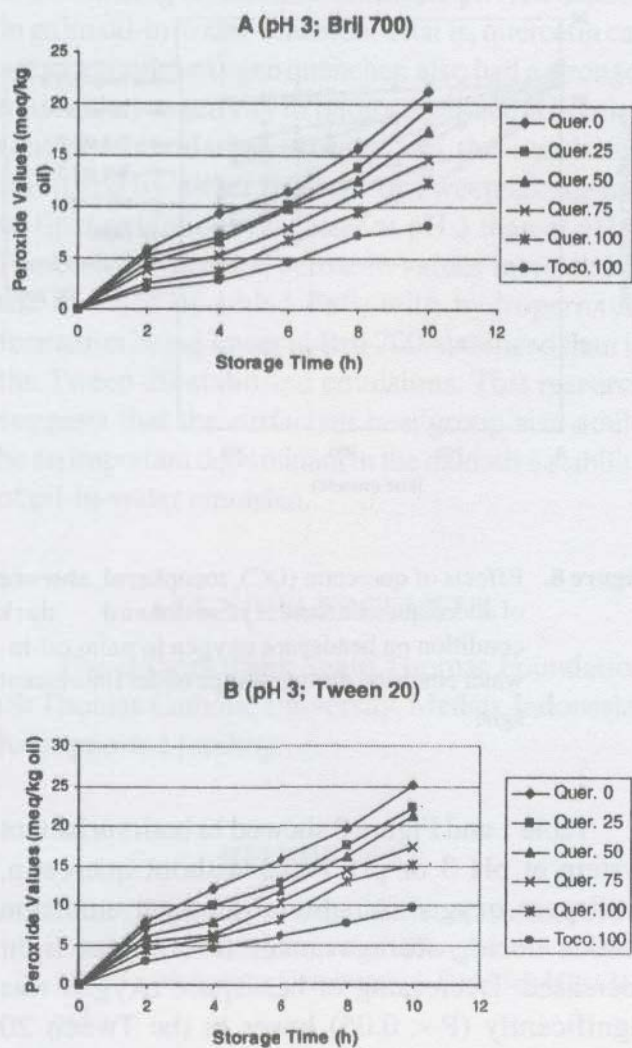


Figure 6. Ability of low concentration of quercetin and tocopherol 100 ppm as singlet oxygen quencher on the photosensitizing effect of erythrosine in palm oil-in-water emulsion during storage under fluorescent light at pH 3: stabilized by Brij 700 (A), and stabilized by Tween 20 (B)

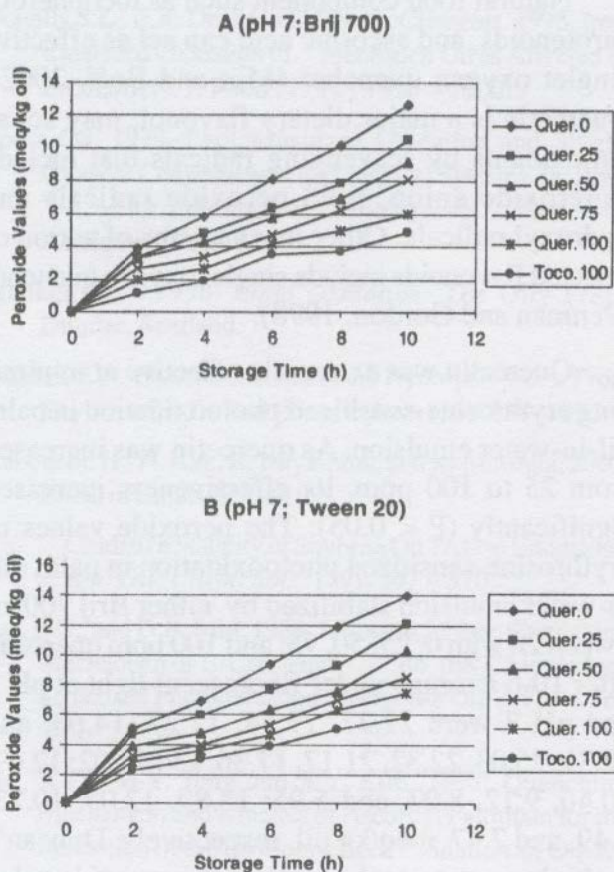


Figure 7. Ability of low concentration of quercetin and tocopherol 100 ppm as singlet oxygen quencher on the photosensitizing effect of erythrosine in palm oil-in-water emulsion during storage under fluorescent light at pH 7: stabilized by Brij 700 (A), and stabilized by Tween 20 (B)

The light-induced oxidation of lipids in foods and foodstuffs is not only due to absorption by chromophoric groups present in lipids but can also be a consequence of photosensitized oxidation. Light absorption, either by naturally occurring pigments or synthetic food additives, are particularly relevant in food products that are displayed in transparent containers under illuminated condition (Pan *et al.* 2005). Preliminary studies showed that the peroxide values of purified palm oil in methylene chloride containing no erythrosine did not change during 5 hr of storage under light and the peroxide values of the oils with and without erythrosine after 5 hr of storage in the dark were not detectable (Sibuea, *et al.* 2005).

Natural food component such as tocopherols, carotenoids, and ascorbic acid can act as effective singlet oxygen quencher (Min and Boff. 2002). Quercetin is a major dietary flavonol, may act as antioxidants by scavenging radicals that include superoxide anion, lipid peroxide radicals and hydroxyl radicals. Other mechanisms of action of selected flavonoids include singlet oxygen quencher (Penman and Gordon, 1998).

Quercetin was extremely effective at minimizing erythrosine-sensitized photooxidation in palm oil-in-water emulsion. As quercetin was increased from 25 to 100 ppm, its effectiveness increased significantly ($P < 0.05$). The peroxide values of erythrosine-sensitized photooxidation in palm oil-in-water emulsion stabilized by either Brij 700 or Tween 20 with 0, 25, 50, 75, and 100 ppm quercetin after 10-h storage under fluorescent light at pH 3 and pH 7 were 21.27, 19.54, 17.27, 14.63, and 12.31; 25.03, 22.32, 21.17, 17.36, and 15.02; 12.67, 10.46, 9.12, 8.07, and 5.95; 13.89, 12.05, 10.23, 8.49, and 7.47 meq/kg oil, respectively. Duncan's multiple range tests showed that the peroxide value of samples treated with quercetin were significantly lower than the control (no quercetin added) after 10-h storage under fluorescent light ($P < 0.05$).

As expected, addition of tocopherol 100 ppm to the palm oil-in-water emulsion stabilized by either Brij 700 or Tween 20 resulted in a dramatic decrease peroxide values formation at both pHs with oxidation proceeding faster at pH 3 than pH 7. These results showed, tocopherol 100 ppm was more effective than quercetin 100 ppm in emulsion system. Like chain-breaking antioxidant, singlet oxygen quencher differ in their effectiveness in inhibiting lipid oxidation, partly because of their chemical properties, but also because of their physical location within a system. Antioxidants that are effective at retarding lipid oxidation in bulk oils may not be as effective in emulsions. For example, hydrophilic antioxidants are less effective in oil-in-water emulsion than lipophilic antioxidants, whereas lipophilic antioxidants are less effective in bulk oils than hydrophilic antioxidants. (Frankel, 1999; McClements and Decker, 2000).

For both surfactants, the quantitative effects of quercetin, tocopherol, absence of added quercetin and erythrosine and dark condition on headspace oxygen in palm oil-in-water emulsion during storage under fluorescent light are presented in Fig.8.

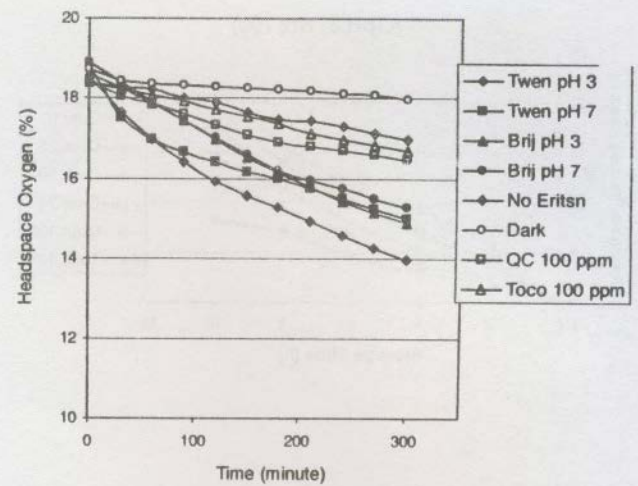


Figure 8. Effects of quercetin (QC), tocopherol, absence of added quercetin and erythrosine and dark condition on headspace oxygen in palm oil-in-water emulsion during storage under fluorescent light.

Table 1 and Figure 8 showed in both surfactant system at pH 3 or pH 7 and without quercetin, headspace oxygen in palm oil-in-water emulsion bottles during storage under fluorescent light decreased. Decreasing of headspace oxygen was significantly ($P < 0.05$) lower in the Tween 20 stabilized than in the Brij 700. However, the headspace oxygen content of sample of dark condition had significantly higher than the emulsion added quercetin, tocopherol and in the absence of added erythrosine during storage under fluorescent light.

CONCLUSION

Erythrosine effectively act as a photosensitizer to accelerate the oxidation in palm oil-in-water under the light storage. Therefore, erythrosine can produce singlet oxygen from triplet oxygen under the light exposure. Singlet oxygen formed by photosensitizers

- Penman, A.R., and M.H. Gordon. 1998. Antioxidant Properties of Myricetin and Quercetin in Oil and Emulsions. *J. Am. Oil. Chem. Soc.* (75): 169 – 180.
- Ponginebbi, L., W.W. Nawar., and P. Chinachotti. 1999. Oxidation of Linoleic Acid in Emulsions: Effect of Substrate, Emulsifier, and Sugar Concentration. *J. Am. Oil. Chem. Soc.* (76): 131 – 138.
- Proctor A. and H. Snyder. 1987. Adsorption of Lutein From Soybean Oil on Silicic Acid Isotherm. *J. Am. Oil. Chem. Soc.* (64): 1163 - 1167.
- Rawls, H.R, and P.J. VanSanten. 1970. A Possible Role for Singlet Oxygen in the Initiation of Fatty Acid Autooxidation. *J. Am. Oil. Chem. Soc.* (47): 121 – 125.
- Shahidi, F., U.N. Wanasundara. 2002. Methods for Measuring Oxidative Rancidity in Fat and Oils. . In: Akoh C.C., and Min, D.B. Editor: *Food Lipids: Chemistry, Nutrition, and Biotechnology*. Marcel Dekker, Inc. New York, Basel.
- Sibuea, P., S. Raharjo., U. Santoso., Z. Noor., M. Astuti And Suparmo. 2005. Quenching Mecanism and Kinetics of Quercetin for Reduction of the Photosensitized Oxidation of Palm Oil and Linoleic Acid. Accepted for Publication in *Indonesian Food and Nutrition Progress*. UGM Yogyakarta-Indonesia.
- Srinivasan, M., H. Singh, and P.A. Munro. 2001. Creaming Stability of Oil-in-Water Emulsions Formed with Sodium and Calsium Caseinates. *J. Food. Sci.* (66): 441- 446.
- Takahama, U. 1984. Hydrogen Peroxide dependent Oxidation of Quercetin by Intact Spinach Chloroplasts. *Plant Physiol.* (74): 852-857
- Tornberg, E. and A.M. Hermansson. 1977. Function and Characterization of Protein Stabilized Emulsion: Effect of Processing. *J. Food. Sci.* (42): 468 - 472.
- Yang, W.T., L.H. Lee., and D.B. Min. 2002. Quenching Mechanisms and Kinetic of A- Tokoferol and B- Carotene on the Photosensitizing Effect of Synthetic Food Colorant FD&C red No.3. *J. Food. Sci.* (67): 507 – 510.
- Zang, F. and A. Proctor. 1997. Rheology and stability of Phospholipid-Stabilized Emulsion. *J. Am. Oil. Chem. Soc.* (74): 869 - 874.

can increase hydroperoxide formation in palm oil-in-water emulsion. These results showed there are positive correlation between the headspace oxygen content and peroxide value of oxidized palm oil-in-water emulsion. Quercetin was extremely effective at minimizing erythrosine-sensitized photooxidation in palm oil-in-water emulsion. That is, quercetin can act as a singlet oxygen quencher, also had a stronger antioxidative activity in photosensitized oxidation (antiphotooxidative activity). In the emulsions stabilized by either Brij 700 or Tween 20, the rate of lipid oxidation was faster at pH 3 than at pH 7. For both surfactants, peroxide values increased in the absence of added Fe⁺², with hydroperoxide formation being lower in Brij 700-stabilized than in the Tween 20-stabilized emulsions. This research suggests that the surfactant headgroup size could be an important determinant in the oxidative stability of oil-in-water emulsion.

ACKNOWLEDGEMENT

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REFERENCES

- Aoki, H., Y. Shirase., J. Kato, and Y. Watanabe. 1984. Emulsion Stabilizing Properties of Soy Protein Isolates Mixed with Sodium Caseinates. *J. Food Sci.* (49): 212 -216.
- Carpenter, A.P. Jr. 1979. Determination of Tocopherol in Vegetable Oil. *J. Am. Oil Chem. Soc.* (56): 668 – 673
- Chung, M.G., J.Kim, and H.S. Shin. 1997. Effects of Erythrosine on the Cholesterol Stability in an Aqueous Model System. *Korean J. Food Sci. Tech.* 28 (4): 197
- Decker, E.A and D.J. McClements. 2001. Transition Metal and Hydroperoxide Interaction: An Important Determinant in the Oxidative Stability of Lipid Dispersions. *Inform* (12): 251 - 255.
- Decker, E.A. 2002. Antioxidant Mechanisms. In: Akoh C.C., and D.B. Min. Editor: *Food Lipids: Chemistry, Nutrition, and Biotechnology*. Marcel Dekker, Inc. New York, Basel.
- Donnelly, J.L., E.A. Decker and D.J. McClements. 1998. Iron-Catalyzed Oxidation of Menhaden Oil as Affected by Emulsifiers. *J. Food Sci.* (63): 987 – 1000.
- Foot CS. 1979. Photosensitized Oxidation and Singlet Oxygen: Consequences in Biological Systems. In: Pryor WA, editor. *Free Radicals in Biology*. Vol. 2. New York: Academic Press. p 85.
- Frankel, E.N. 1998. *Lipid Oxidation*. The Oily Press. Dundee, Scotland.
- Frankel, E.N. 1999. Antioxidants and Hydroperoxides: From Soybean Oil To Red Wine. *Inform* (10): 889 – 896
- Kubouchi, H., H. Kai., K. Miyashita., and K. Matsuda. 2002. Effect of Emulsifiers on the Oxidative Stability of Soybean Oil TAG in Emulsions. *J. Am. Oil Chem. Soc.* (79): 567 – 570.
- Lee, E.C., and D.B. Min. 1988. A Research Note: Quenching Mechanism of B-Carotene on the Chlorophyll Sensitized Photooxidation of Soybean Oil. *J. Food Sci.* (53)
- Lee, K.H., M.Y. Jung and S.Y. Kim. 1997. Quenching Mechanism and Kinetics of Ascorbyl Palmitate for the Reduction of the Photosensitized Oxidation of Oil. *J. Am. Oil Chem. Soc.* (74): 1053 - 1057.
- McClements, D.J. 1999. *Food Emulsions: Principles, Practice and Techniques*. CRC Press. Boca Raton, London, New York Washington, D.C.
- McClements, D.J., and E.A. Decker. 2000. Lipid Oxidation in Oil-in-Water Emulsions: Impact of Molecular Environment on Chemical Reactions in Heterogeneous Food Systems. *J. Food Sci.* (65): 1270 – 1282.
- Mei, L.Y., D.J. McClements., J.N. Wu and E.A. Decker. 1998. Iron-Catalyzed Lipid Oxidation in Emulsion as Affected by Surfactant, pH and NaCl. *Food Chem.* (61): 307 – 312.
- Min, D.B and J.M. Boff. 2002. Chemistry and Reaction of Singlet Oxygen in Foods. *Food Sci. and Food Safety.* (1): 58 – 72.
- Nakagawa, K., M. Kawagoe., M. Yoshimura., H. Arata., T. Minamilanwa., M. Nakamura and A. Matsumoto. 2000. Differential Effect of Flavonoid Quercetin on Oxidative Damages Induced by Hydrophilic and Lipophilic Radical Generators in Hepatic Lysosomal Fractions of Mice. *J. of Health Sci.* (46): 509 - 512
- Pan, X., H. Ushio, and T. Ohshima. 2005. Effects of Molecular Configurations of Food Colorants on Their Efficacies as Photosensitizers in Lipid Oxidation. *Food Chem.* (92): 37 – 44.