The role of tocopherol on photooxidation reactions palm oil emulsion in water

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Abstract. Lipid oxidation is one of the main deteriorative reactions that takes place during preparation and storage of many food products. This can make it unacceptable for human consumption. In food emulsion, oxidation reaction can be occurred by either diradical triplet oxygen or nonradical singlet oxygen. The singlet oxygen can be formed from triplet oxygen by photooxidation. The purpose of this research was to study the effect of singlet oxygen quencher such as tocopherol on lipid oxidation rate in the erythrosine-sensitized photooxidation of oil-in-water emulsion. Palm oil-in-water emulsion, containing erythrosine 100 ppm and tocopherol 0, 25, 50, 75 and 100 ppm, were prepared with polyoxyethylene sorbitan monolaurate (Tween 20). The mixtures were stored under 4,000 lux fluorescent light for 10 h and peroxide values were measured at 2 h interval. Erythrosine greatly increased the photooxidation of palm oil-in-water emulsion, as expected. Lipid oxidation rates, as determined by the formation of lipid hydroperoxides in palm oil-in-water emulsion, decreased with increasing tocopherol concentration. At pH 3, the peroxide value was higher than at pH 7, i.e., from 8.03 to 6.08 respectively. The results indicate that tocopherol is an effectively singlet oxygen quencher in palm oil-in-water emulsion.

Keywords: photooxidation, tocopherol, palm oil in water emulsions

1. Introduction
Lipid oxidation has been studied extensively in large amounts of fats and oils. So far there has been a fairly good understanding of the mechanisms and factors that affect the oxidation in the system. On the other hand, lipid oxidation is still not well understood in the oil-in-oil emulsion system. Whereas a large amount of food is in the form of emulsions [1].

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 [2].

In food emulsion, oxidation reaction can be occurred by either diradical triplet oxygen or nonradical singlet oxygen. The singlet oxygen can be formed from triplet oxygen by photooxidation reaction. Triplet oxygen oxidation (autoxidation) does not fully explain the initiation step of lipid oxidation [3]. [4, 5] 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 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. 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 [6,7].

To reduce the undesirable singlet oxygen oxidation in lipid foods, tocopherol may act as antioxidant by singlet oxygen quencher [8, 9, 10]. Therefore, the objectives of this study was to investigate (1) the effects of erythrosine on the photooxidative stability of palm oil emulsion in water; (2) the effects of tocopherol as singlet oxygen quencher and pH on the erythrosine sensitized photooxidation in palm oil emulsion in water.

2. Materials and methods

2.1. Materials

Refined, bleached and deodorized palm oils was obtained from Bina Karya Prima Inc (bkpkjt.com). Silicic acid, celite, activated charcoal, quercetin, polyoxyethylene (100) stearyl ether (Brij 700), α-tocopherol and β-carotene was purchased from Aldrich Chemical Co. Erythrosine was obtained from Inti, Yogyakarta. Hexane, chloroform, acetic acid glacial, potassium iodide, polyoxyethylene (20) sorbitan monolaurate (Tween 20) was purchased from Sigma Chemicals Co.

2.2. 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 [9]. The oil passed through the column was purified palm oil. It was colorless and contained low peroxide, free fatty acids, tocopherols or carotenoids.

2.3. Chemical Analysis of Purified palm Oil

Tocopherols were determined by the high pressure liquid chromatography of [2], and carotenoids were determined by the spectrometric method of [17]. Peroxide value, and free fatty acids were determined by AOCs methods [19].

2.4. 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 [9,16] with modifications by [20]. 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 [19]

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

To study the effects of pH on erythrosine sensitized photooxidation in palm oil-in-water emulsion, emulsions were prepared as described by [9,16] with modifications by [20]. 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 [19]

2.6. Effects of Tocopherol on Erythrosine Sensitized Photooxidation of Palm Oil-in-Water Emulsion

To study the effects of tocopherol on the photosensitized oxidation in palm oil-in-water emulsion, emulsions were prepared as described by [9,16] with modifications by [20]. 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 [19].
3. Results and Discussion

3.1. 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 did not contain detectable concentrations of conjugated dienes.

3.2. Effects of Erythrosine as Photosensitiser in Palm Oil Emulsion in Water
The effects of 0, 50, 100, 150 and 200 ppm erythrosine on the peroxide values of palm oil-in-water emulsion are shown in Table 1 and Figure 1.

| Treatment | Storage time (h) |
|-----------|------------------|
|           | 0    | 2    | 4    | 6    | 8    |
| Control (E 0) | 0.00 | 0.98 | 1.19 | 1.34 | 1.38 |
| E 50      | 0.00 | 3.38 | 6.59 | 9.03 | 11.95|
| E 100     | 0.00 | 7.57 | 11.89| 15.39| 19.44|
| E 150     | 0.00 | 8.16 | 13.28| 16.74| 20.35|
| E 200     | 0.00 | 8.54 | 13.21| 15.69| 20.57|

Each value is expressed of three replicates.

Table 1. The Effects of erythrosine (E) on the peroxide values (meq/kg of sample) of palm oil emulsion in water that stabilized Tween 20 under fluorescent light.

50 ppm, 100 ppm, 150 ppm, and 200 ppm erythrosine concentrations increased the peroxide value by 1.38 to 11.95, 19.44, 20.35 and 20.37 meq/kg oil in palm oil emulsion in water stabilized Tween 20, 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 significantly different (P>0.05) in emulsion stabilized Tween 20.

Figure 1. Effects of erythrosine on the peroxide values of palm oil emulsion in water that stabilized Tween 20 under fluorescent light during 8 h.
3.3. Effects of pH on the Peroxide Values in Palm Oil Emulsion in Water

The role of pH has also been demonstrated in this research on the photooxidation of palm oil emulsion in water stabilized Tween 20 containing erythrosine 100 ppm under fluorescent light. Measurements were made at pH 3, pH 4, pH 5, pH 6 and pH 7 presented in Table 2 and Figure 2.

**Table 2.** Effect of pH on the peroxide values (PV, meq/ kg of sample) of palm oil emulsion in water containing erythrosine 100 ppm under fluorescent light: time curve (hour, h).

| Treatment | pH 3 | pH 4 | pH 5 | pH 6 | pH 7 |
|-----------|------|------|------|------|------|
| 2 h       | 5.94 | 5.89 | 5.28 | 4.67 | 4.14 |
| 4 h       | 8.03 | 7.69 | 7.2  | 6.79 | 6.08 |

Each value is expressed of three replicates.

![Figure 2](image_url)

**Figure 2.** Effect of pH on the peroxide values (PV) of palm oil emulsion in water containing erythrosine 100 ppm under fluorescent light: time curve.

Figure 2 shows the amount of hydroperoxides formed increased with decreasing pH between 2 and 4 hours. In both 2 h and 4 h, the amount of hydroperoxides formed in palm oil emulsion 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 [5, 12]. 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 [4,5,7, 13]. If endogenous transition metals are active prooxidant in the emulsion, the authors would expect that lipid oxidation rates will depend on surfactant type.

3.4. Effect of Tocopherol on the Photosensitized Oxidation in Palm Oil Emulsion in Water

Erythrosine was extremely effective as a photosensitizer to accelerate the oxidation in palm oil emulsion in water 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 [17,23, 24].
Ability of 0, 25, 50, 75 and 100 ppm tocopherol as singlet oxygen quencher on the photosensitizing effect of erythrosine in palm oil emulsion in water during 10-h storage under 4,000 lux fluorescent light are shown in Table 3; Figure 3 and 4.

Table 3. The role of tocopherol (T) and quercetin (Q) 100 ppm on the peroxide values in palm oil emulsion in water during storage under fluorescent light at pH 3 and pH 7

| Treatment | 0 h | 2 h | 4 h | 6 h | 8 h | 10 h |
|-----------|-----|-----|-----|-----|-----|------|
|           | pH3 | pH7 | pH3 | pH7 | pH3 | pH7  |
| T 0       | 5.87| 3.82| 9.26| 5.78| 11.51| 8.15 |
| T 25      | 5.22| 3.36| 7.03| 4.76| 10.15| 6.28 |
| T 50      | 4.42| 3.25| 6.61| 4.23| 9.92 | 5.76 |
| T 75      | 3.76| 2.63| 5.22| 3.37| 8.01 | 4.72 |
| T 100     | 2.56| 1.97| 3.68| 2.68| 6.72 | 4.19 |
| Q 100     | 2.03| 1.12| 2.82| 1.89| 4.59 | 3.46 |

Each value is expressed of three replicates

Figure 3. The role of tocopherol and quercetin 100 ppm on the peroxide values on the peroxide values palm oil emulsion in water during storage under fluorescent light at pH 3

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 [14,20]. 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 [15]. 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 [6,20,23].

Minor natural food component such as tocopherols, carotenoids, and ascorbic acid can act as effective singlet oxygen quencher [24,25]. Tocopherols is act as antioxidants by scavenging radicals that include superoxide anion, lipid peroxide radicals and hydroxyl radicals. Other mechanisms of action of tocopherols include singlet oxygen quencher [26].
Figure 4. The role of tocopherol (T) and quercetin (Q) 100 ppm on the peroxide values in palm oil emulsion in water during storage under fluorescent light at pH 7.

Tocopherols was extremely effective at minimizing erythrosine-sensitized photooxidation in palm oil emulsion in water. The peroxide values of erythrosine-sensitized photooxidation in palm oil emulsion in water with 0, 25, 50, 75, and 100 ppm tocopherols after 10-h storage under fluorescent light at pH 3 and pH 7 were decrease. 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 emulsion in water 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 is less effective than quercetin 100 ppm in emulsion system. Like chain-breaking antioxidant, singlet oxygen quencher differs 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 [27].

4. Conclusion

Erythrosine effectively act as a photosensitizer to accelerate the photooxidation in palm oil emulsion in water under the light storage. Therefore, erythrosine can produce singlet oxygen from triplet oxygen under the light exposure. Singlet oxygen formed by photosensitizers can increase hydroperoxide formation in palm oil emulsion in water. Tocopherols was extremely effective at minimizing erythrosine-sensitized photooxidation in palm oil emulsion in water. That is, tocopherols can act as a singlet oxygen quencher, also had a stronger antioxidative activity in photosensitized oxidation (antiphotooxidative activity). In the emulsions stabilized by Tween 20, the rate of lipid oxidation was faster at pH 3 than at pH 7. This research suggests that the tocopherols could be an important determinant in the oxidative stability of palm oil emulsion in water.
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