# Formulation and Photooxidative Stability of Ascorbic Acid Microemulsion

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# Formulation and Photooxidative Stability of Ascorbic Acid Microemulsion

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## Abstract

The objective of this study was to determine the solubility of ascorbic acid in water-in-oil (w/o) microemulsion and to evaluate the stability of that ascorbic acid microemulsion against photooxidation. The solubility of ascorbic acid in w/o microemulsion was formulated by dissolving a predetermined amount of ascorbic acid (0.5, 0.75, 1.0, 1.5, 2.0, 3.0, 4.0, 5.0, or 10.0%) in deionized water. This solution was subsequently added dropwise with surfactant mixtures and dehydrated virgin coconut oil (VCO) until produced ascorbic acid microemulsion. The photooxidative stability of ascorbic acid microemulsion was evaluated by illuminating the samples with fluorescent light exposure or storage with and without light protection at room temperature (30 ± 1°C). The results indicated that at the level of 1.0% ascorbic acid gave the maximum solubility of ascorbic acid in w/o microemulsion. This microemulsion was highly stable against photooxidation and remains stable until 8 months. This study confirmed that the highly hydrophilic singlet oxygen quencher (SOQ) such as ascorbic acid can be successfully incorporated into w/o microemulsion and may suitable for inhibiting photooxidation of oil based food products.

*Keywords:* Ascorbic acid; microemulsion; singlet oxygen quencher; photooxidation.

## 1. Introduction

Ascorbic acid, recognized as vitamin C, is a natural compound which is present in many food products such as fruits, vegetables, and beverages. It is a colourless water-soluble vitamin and has many biochemical functions. Thus, it is frequently used as a functional additive to enhance nutrient quality or to increase oxidative stability. Ascorbic acid can act as natural antioxidant in foods due to its capability as electron or hydrogen donor to the free radical compounds in foods [1]. Ascorbic acid is well known an excellent electron donor because of the low standard 1-electron reduction potential (282 mV), the generation of relatively stable semi-dehydroascorbic acid and the easy conversion of dehydroascorbic acid to ascorbic acid. Due to the kinetic reaction speed of its electron or hydrogen atom transfer are rapid, resulting in ascorbic acid being an excellent antioxidant [2].

It was also reported that ascorbic acid can quench singlet oxygen [3] and excited triplet sensitizer [4, 5] effectively. Bodannes and Chan [6] and Jung et al. [7] reported that ascorbic acid is an effective singlet oxygen quencher (SOQ) that can be used to minimize photooxidation of water-soluble compounds in aqueous solutions. Unfortunately, due to its very poor solubility in non-aqueous media such as fats and oils, ascorbic acid can not be used in practice as antioxidant in this media. Therefore, ascorbic acid must be made less polar so that it will dissolve in fats and oils. Esterification of fatty acid to form a compound such as ascorbyl palmitate can over this difficulty. However, although these compound have more solubility than ascorbic acid, their solubility is still low compared with those of other fat-soluble antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) which were often incorporated in the oils. Considering the synthetic

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antioxidants such as BHA and BHT were decline in their usage probably because of the consumers' preference for natural antioxidants, that's why water-in-oil (w/o) microemulsion as ascorbic acid delivery system can be used to overcome this problem.

Microemulsion is defined as a system of water, oil, and amphiphile which is a single optically isotropic and thermodynamically stable liquid solution [8]. Normally, microemulsions are quartenary systems composed of a water phase, an oil phase, surfactant and co-surfactant [9]. Microemulsions have found numerous applications but the application in foods is limited by the types of surfactants which are used to facilitate microemulsion formation. Many surfactants are not permissible in foods; many more may only be added at low levels which are not enough to formulate microemulsions. We have successfully formulated w/o microemulsion using ternary food grade non-ionic surfactants [10]. This microemulsion may be suitable used as ascorbic acid delivery system and applied to prevent the photooxidation reaction in oil based food products. Szymula and Radzki [11] found that ascorbic acid can be solubilised in up to 60% in the microemulsion region of the SDS/pentanol/water system. However, ascorbic acid reportedly oxidized extremely fast in the presence of photosensitizers and light. According to Jung et al. [7], 1.2 x $10^{-4}$M ascorbic acid oxidized completely after a 12 minutes storage in the presence of 6.0 µg/mL riboflavin under 3300 lux fluorescent light. The objective of this study was to determine the solubility of ascorbic acid in w/o microemulsion and to evaluate the stability of that ascorbic acid microemulsion against photooxidation.

## 2. Materials and methods

### 2.1. *Formulation of ascorbic acid microemulsion*

Formulation of ascorbic acid microemulsion was conducted to determine the solubility of ascorbic acid in w/o microemulsion system. It was formulated by dissolving of a predetermined amount of ascorbic acid (0.5, 0.75, 1.0, 1.5, 2.0, 3.0, 4.0, 5.0, or 10.0%) in deionized water. This solution was subsequently added with surfactant mixtures which consist of 16.6% Tween 20, 15.0% Span 20, and 68.4% Span 80; mixed on a hot plate stirrer and then dehydrated virgin coconut oil (VCO) was added dropwise while stirring. The formation of ascorbic acid microemulsion was characterized by the presence of a clear and transparent solution without phase separation.

### 2.2. *Photooxidative stability test of ascorbic acid microemulsion*

To evaluate the photooxidative stability of ascorbic acid microemulsion, a portion of ascorbic acid microemulsion samples were placed in transparent serum bottles with rubber caps. Another set of w/o microemulsion without ascorbic acid was also prepared and used for comparison. This photooxidation was performed under accelerated condition using fluorescent light with intensity of approximately 4000 lux and samples were exposed to that light for up to 5 hours at room temperature (30 ± 1°C). Peroxide value (PV) of the samples was measured according to the method proposed by AOCS Official Method [12]. To evaluate the photooxidative stability of ascorbic acid microemulsion during storage, a portion of ascorbic acid microemulsion samples were placed in transparent serum bottles with rubber caps and another set of samples were protected from light by wrapping the transparent serum bottles with aluminum foil. All of samples were placed at room temperature (30 ± 1°C) for up to one year and the turbidity index of samples were measured according to the method proposed by Cho et al. [13] at one month interval.

## 3. Results and Discussion

### 3.1. *Solubility of ascorbic acid in w/o microemulsion*

In order to determine its solubility in w/o microemulsion, ascorbic acid at different levels (0.5, 0.75, 1.0, 1.5, 2.0, 3.0, 4.0, 5.0, or 10.0%) were loaded into the w/o microemulsion system. The resulting microemulsions were evaluated with regard to their appearance. The result indicated that incorporation of ascorbic acid at the level of higher than 1% (w/w) produced slightly turbid or cloudy solution with visible precipitations of ascorbic acid (Figure 1). According to Pakpayat et al. [14], incorporation of ascorbic acid in the formulated microemulsion leads to destabilization of the microemulsion structure and fail to obtain transparent isotropic systems as expected. They found that incorporation of ascorbic acid into the microemulsion system caused the displacement of the microemulsion zones to the lipophilic region. In this study, at the level of 1% ascorbic acid or higher give slightly cloudy solution with ascorbic acid precipitate in appearance. Thus, it indicated that the maximum solubilisation of ascorbic acid in the w/o microemulsions was 1%. They showed the maximum ability of w/o microemulsion in retaining the ascorbic acid without phase separation (Figure 1). This ascorbic acid microemulsion formula subsequently was used on photooxidation stability test.

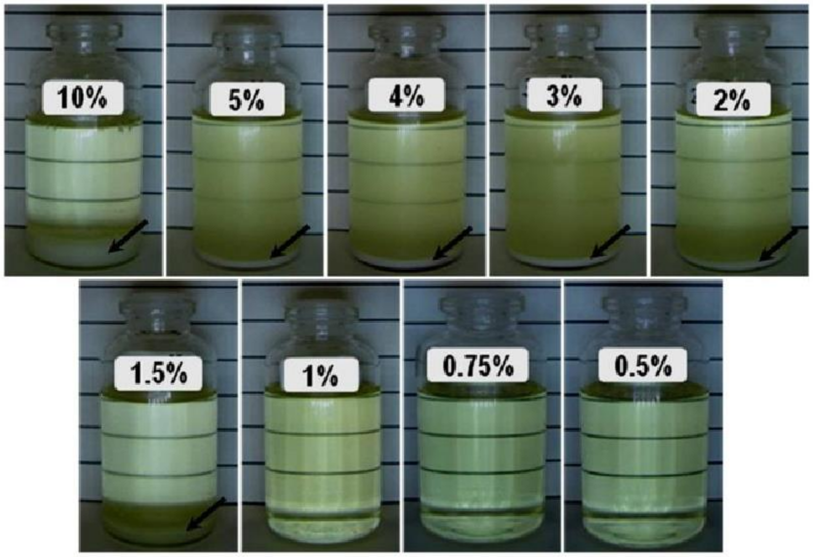

Figure 1. Ascorbic acid microemulsion photographs at different levels of ascorbic acid (arrows indicate ascorbic acid precipitates).

### 3.2. *Photooxidative stability of ascorbic acid microemulsion*

The photooxidative stability test of ascorbic acid microemulsions shows that after being exposed to light, the microemulsion without ascorbic acid had a significantly ($P < 0.05$) higher PV (1.298 ± 0.024 meq/kg) than that of the samples before light exposure (0.098 ± 0.009 meq/kg), whereas the PV became zero if the microemulsion was incorporated with ascorbic acid, both before and after light exposure. During photooxidative test which was performed under accelerated condition using fluorescent light with intensity of approximately 4000 lux for up to 5 hours, that intense light exposure induces an increased rate of peroxide formation. Photooxidation occurs when there is light, triplet oxygen, and photo-sensitizer [4]. Chlorophyll is common sensitizer that acts as promoters of photooxidation in vegetable oils [15]. Even at a very low level (0.098 mg/kg), chlorophyll which was naturally present in VCO that was used as oil phase could initiate a photooxidation reaction [16]. After absorption of energy from light, chlorophyll can transfer the energy to triplet oxygen ($^3O_2$) to form the more reactive singlet oxygen ($^1O_2$). Singlet oxygen is well known to react rapidly with C-C unsaturation to give hydroperoxides [4]. These hydroperoxides then can initiate a conventional free radical autooxidation to produce more hydroperoxide, so the PV increased rapidly after the samples were exposed to light. However, the hydroperoxides formation did not occur when there is ascorbic acid.

Although ascorbic acid is water soluble and have a limitation as antioxidants for fats and oils, this result indicated that ascorbic acid which was incorporated in the w/o microemulsion system could play as an effective SOQ and inhibit the photooxidation reaction. As reported that ascorbic acid could act as SOQ [3] or excited sensitizer quencher [4, 5], and superoxide or peroxide scavenger [6], so that it could prevent the development of oxidation process. In comparison with the said study, this experiment resulted in a similar trend which was indicated by the zero values of PV in ascorbic acid microemulsions, even after they were exposed to the fluorescent light. This result indicated that the ascorbic acid microemulsion resistant to the photooxidation reaction.

To discover the ascorbic acid microemulsion stability during storage, it has been evaluated by observing their appearance and measuring their turbidity index. The storage was conducted in two conditions; i.e. with and without light protection. The turbidity index of ascorbic acid microemulsion during storage was showed in Figure 2. The turbidity index is the most common way to measure the stability of microemulsion because it's proportional to the average particle diameter [13]. Thus the turbidity index changes can be used to derive changes in the particle resulting from either clustering or growth of the microemulsion droplets, and to obtain information on stability changes.

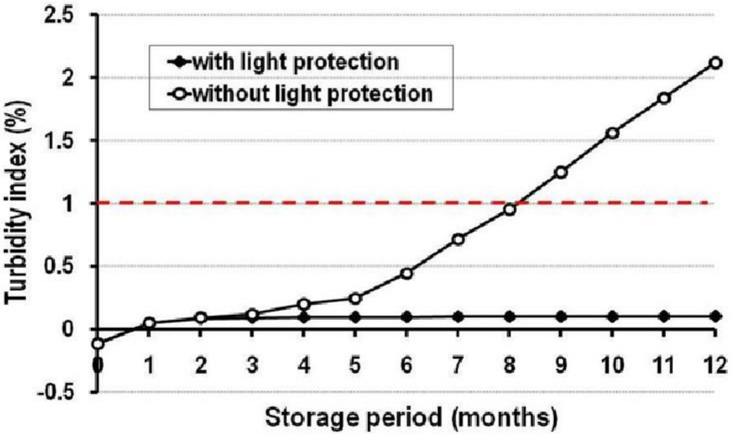

Figure 2. Turbidity index of ascorbic acid microemulsion during storage at room temperature (30 ± 1°C) with light protection (-♦-) and without light protection (-○-)

Figure 2 shows that ascorbic acid microemulsion which was stored with light protection has a relatively stable turbidity index. Until 12 months of storage, its turbidity index is still 0.1%, while the ascorbic acid microemulsion which was stored in transparent serum bottle with rubber cap but without light protection have increased turbidity index after 3 months of storage (although the value is still far below 1%). Increasing in turbidity index value occurs more evident starting in the 5th. Nevertheless, the turbidity index value was under 1% until 8 months of storage. At that moment, both ascorbic acid microemulsion with and without light protection showed no changes in appearance. They were still clear and transparent until 8 months. According to Cho et al. [13], the microemulsion with transparent appearance and a turbidity index value of less than 1% was defined as a stable microemulsion. Thus it can be stated that the ascorbic acid microemulsion remained stable for up to 8 months of storage period.

Visual observation indicated that the ascorbic acid microemulsion in transparent serum bottle with rubber cap but without light protection has already changed to yellow (light-brown) although it's still appears transparent. The longer it was stored; ascorbic acid microemulsion without light protection was browner and suffered to phase separation. Starting in 9 months of storage period, the ascorbic acid microemulsion appears increasingly brown (Figure 3). This suggests that the ascorbic acid incorporated in the microemulsion has undergone oxidation.

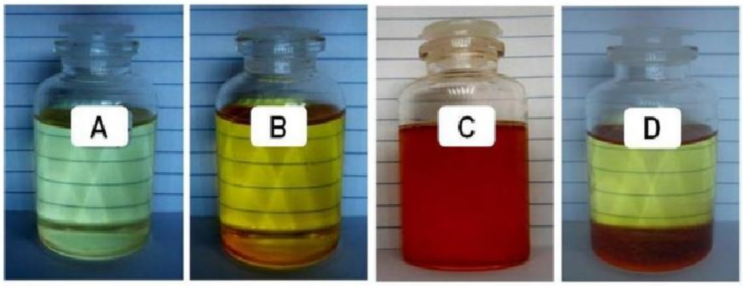

Figure 3. Ascorbic acid microemulsion without light protection which was stored for 1 month (A); 8 months (B); 9 months (C); 12 months (D)

This can be explained by photo-sensitising effect exerted by chlorophylls in VCO that was used as oil phase. Light energy from the day-light that illuminated in the room-storage could be absorbed by chlorophyll and transferred to triplet oxygen in the head-space of transparent serum bottles of the samples to produce singlet oxygen. This singlet oxygen tends to react with the unsaturated fatty acids of VCO causing the formation of hydroperoxides. That hydroperoxides had polar characteristics and they were able to diffuse to the more polar zone and interacted with the compounds present in the aqueous phase [17]. The more polar zone in this study was the interface layer of microemulsion which was contained of ascorbic acid in its microdroplets. Because of the reason, the hydroperoxides were quenched easily by ascorbic acid which was incorporated in the microemulsion system so that the ascorbic acid to become oxidized.

L-ascorbic acid is oxidized with loss of one electron to form a radical cation and then with loss of a second electron to form L-dehydroascorbic acid [18]. It can produce further oxidized brown compounds. This has led change in the appearance of ascorbic acid microemulsion to becomes brownish and undergo phase separation to form a brown precipitate if stored for long periods (more than 8 months) without light protection. Meanwhile the ascorbic acid microemulsion which was stored with light protection was still clear and transparent. Therefore, to prevent the deterioration of ascorbic acid microemulsion during storage, it should be done by using light barrier packaging material that can protect it from the light, for example using brown bottles or secondary packaging material such as sealed paper boxes.

## 4. Conclusions

Ascorbic acid can be successfully incorporated into the w/o microemulsion system which is formulated by using ternary food grade nonionic surfactants. At the level of 1% ascorbic acid give its maximum solubility. This ascorbic acid microemulsion is highly stable against photooxidation. Starting from 3 months of storage, ascorbic acid microemulsion which is stored without light protection has higher turbidity index value than that of stored with light protection. However, it defines as a stable microemulsion for up to 8 months of storage period, while if it's stored with light protection still stable until 12 months. Based on this study, although ascorbic acid is a hydrophilic antioxidant, if it's incorporated in the w/o microemulsion system, it can act effectively as an antiphotooxidative agent and may apply to prevent oil based products from photooxidation.

## Acknowledgements

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