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Antiphotooxidative Effect of Ascorbic Acid Microemulsion in Virgin Coconut Oil

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Abstract: This study was intended to determine the effectiveness of ascorbic acid microemulsion for inhibiting photooxidation of virgin coconut oil (VCO). The ascorbic acid microemulsion was prepared by mixing ascorbic acid, deionized water, surfactant mixture, and VCO as continuous phase. Ascorbic acid microemulsion at 50, 100, 150, 200, or 250 ppm was dispersed into VCO. The same level of ascorbyl palmitate, TBHQ (tertiary butylhydroquinone), and BHA (butylated hydroxyanisole) were added into VCO and used for comparison. All of these samples were subsequently subjected to photooxidation under fluorescent light exposure (4,000 lux) for up to 8 hours at room temperature ($30 \pm 1^\circ\text{C}$). Peroxide values and *p*-anisidine values of photooxidized samples were measured at 1 hour interval. The result indicated that at the level of 250 ppm, ascorbic acid which was included into the microemulsion system effectively inhibited photooxidation of VCO in comparison with the other antioxidants. This study confirmed that a highly hydrophilic singlet oxygen quencher (SOQ) such as ascorbic acid can be successfully incorporated into the microemulsion system and the addition of ascorbic acid microemulsion effectively inhibited photooxidation of VCO during storage under fluorescent light.

Key words: Ascorbic acid, microemulsion, photooxidation, virgin coconut oil, singlet oxygen quencher.

1. Introduction

Virgin coconut oil (VCO) is susceptible to quality deterioration due to photooxidation. Photooxidation is initiated by a light reaction in the presence of a sensitizer [1]. Chlorophyll and its derivatives are common sensitizers that act as promoters of photooxidation in vegetable oils [2]. According to Anwar et al. [3], photooxidation during food processing and preservation has been demonstrated to display highly detrimental effects on the quality of foods due to an accelerated rate reaction. The reaction rate of photooxidation, also known as singlet oxygen oxidation, is at least 1,000-1,500 times faster than that of autooxidation [4]. This reaction can be inhibited by

addition of a singlet oxygen quencher (SOQ).

Naturally present SOQ in the VCO were not effective for inhibiting photooxidation [5]. Ascorbic acid was reported as an effective SOQ in aqueous solutions [6-8]. However, due to its very poor solubility in non-aqueous media, ascorbic acid cannot be easily dispersed in hydrophobic medium such as VCO. Water-in-oil (w/o) microemulsion system may be used to overcome this problem. On the other hand, synthetic antioxidants, especially TBHQ, have a strong singlet oxygen quenching ability [9].

Because of its several advantages such as transparent, thermodynamically stable, low viscosity, easy formulation (low interfacial tension and almost spontaneous formation) and high surface area (high solubilisation capacity), the use of water-in-oil (w/o) microemulsion to include ascorbic acid may be

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compatible in the VCO system. The objectives of this study were: (1) to determine the photooxidative stability of ascorbic acid microemulsion; and (2) to evaluate the antiphotooxidative effect of ascorbic acid microemulsion in VCO compared with that of common synthetic antioxidants.

2. Materials and Methods

2.1 Preparation of Ascorbic Acid Microemulsion

Referring to our previous study [10], the ascorbic acid microemulsion was prepared by dissolving ascorbic acid (1% w/w of microemulsion formula) into the deionized water. This solution was subsequently added with surfactant mixtures which consist of 16.6% Tween 20 (polyoxyethylene sorbitan monolaurate), 15.0% Span 20 (sorbitan monolaurate), and 68.4% Span 80 (sorbitan monooleate) and mixed on a hot-plate stirrer and then the dehydrated VCO was added dropwise while stirring. The formation of ascorbic acid microemulsion was characterized by the presence of 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 20 mL ascorbic acid microemulsions were placed in 30 mL transparent serum bottles with rubber caps. Another set of microemulsion without ascorbic acid was also prepared and used for comparison. Photooxidation was performed under accelerated condition using fluorescent lights with intensity of approximately 4000 lux and samples were exposed to that light for up to 5 hours at room temperature ($30 \pm 1^\circ\text{C}$). Peroxide values (PV) of the samples were measured according to the method proposed by the AOCS Official Method [11].

2.3 Photooxidation Experiments

To evaluate the antiphotooxidative effect of ascorbic acid microemulsion in VCO, a portion of 20

mL VCO samples containing ascorbic acid microemulsion were placed in 30 mL transparent serum bottles with rubber caps. The levels of ascorbic acid in VCO samples were adjusted to 50, 100, 150, 200, or 250 ppm. The same levels of ascorbyl palmitate (AP), TBHQ, or BHA were mixed into the VCO and used for comparison. All of these samples were subsequently subjected to photooxidation under accelerated condition using fluorescent light exposure (4,000 lux) for 40 to 8 hours at room temperature ($30 \pm 1^\circ\text{C}$). The peroxide values (PV) and *p*-anisidine values (*p*-AnV) of photooxidized VCO samples were measured at 1 hour interval according to the AOCS Official Method [11]. They were used to calculate the total oxidation (TOTOX) values of the samples.

3. Results and Discussion

3.1 Photooxidative Stability of Ascorbic Acid Microemulsion

Photooxidation is a reaction that initiates quality deterioration in fatty products which is indicated by the formation of peroxide compounds. Intensified light exposure in photooxidation induces an increased rate of peroxide formation in the oil [12]. The PV is a measure of the concentration of peroxide and hydroperoxide formed in the initial stage of lipid oxidation. The accumulation of peroxides reflects its oxidative level and thus its tendency to become rancid [12, 13]. The PV changes of microemulsion which were exposed to fluorescent light were shown in Table 1.

Table 1 shows that the microemulsion without ascorbic acid after being exposed to light had a significantly ($P < 0.05$) higher PV than that of the samples before light exposure. The PV became zero if the microemulsion was loaded with ascorbic acid. These results indicated that ascorbic acid which was incorporated in the water-in-VCO microemulsion system could play as SOQ and inhibit the photooxidation reaction. As reported by Bodannes and Chan [6], ascorbate can scavenge singlet oxygen, superoxide and peroxide. In comparison with the said

Table 1 Photooxidative stability of ascorbic acid microemulsion.

Treatment	Peroxide value (meq/kg)*	
	Microemulsion without ascorbic acid	Ascorbic acid microemulsion
Before light exposure	0.098 ± 0.009 ^b	0 ^a
After light exposure	1.298 ± 0.024 ^c	0 ^a

*: average 33 six replicates.

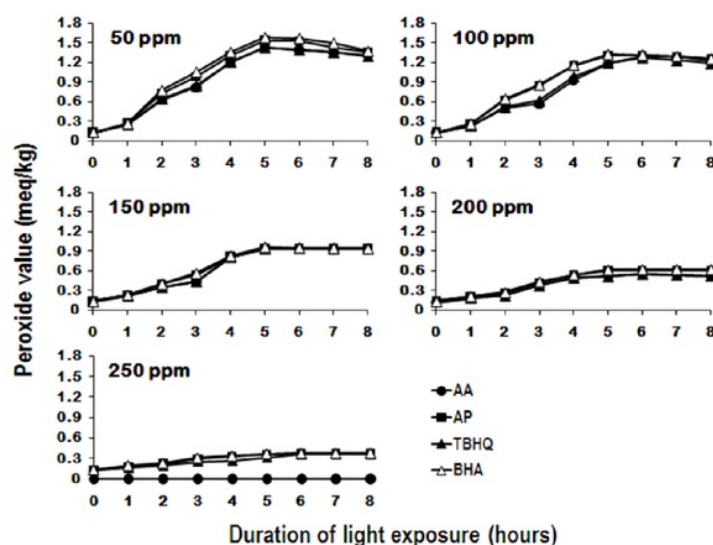
Different letters in the same column or row indicate significant difference ($P < 0.05$).

Fig. 1 Peroxide value of light exposed VCO containing various levels of ascorbic acid microemulsion (AA, ●), ascorbyl palmitate (AP, ■), TBHQ (▲), or BHA (Δ).

study, this experiment resulted in a similar trend which was indicated by the zero values of PV in ascorbic acid microemulsion, even after they were exposed to fluorescent light. This result indicated that the ascorbic acid microemulsion was very stable and resistant to photooxidation reaction.

3.2 Effect of Ascorbic Acid Microemulsion on Photooxidized VCO

The effect of ascorbic acid microemulsion and the other antioxidants on photooxidized VCO was evaluated by measuring PV, *p*-AnV, and TOTOX values. The PV of light exposed VCO samples containing various levels of ascorbic acid microemulsion, AP, TBHQ, or BHA was shown in Fig. 1.

Fig. 1 shows that the light exposed VCO samples containing ascorbic acid microemulsion have a

significantly ($P < 0.01$) lower PV than that of VCO containing AP, TBHQ, or BHA at all levels of antioxidants. They indicated that the ascorbic acid microemulsion was more effective in inhibiting photooxidation of VCO in comparison with the other antioxidants. Even ascorbic acid addition at the level of 250 ppm, the PV of light exposed VCO containing ascorbic acid microemulsion became zero, very significantly ($P < 0.01$) different from the VCO containing the other antioxidants. It was indicated that at the level of 250 ppm, ascorbic acid microemulsion could effectively prevent photooxidation reaction.

Fig. 1 also shows that the PV slightly decreased or was relatively constant after 5 hours of light exposure in all of the treatments. In this condition, hydroperoxides will have decomposed into secondary oxidation products. If the rate of hydroperoxide decomposition

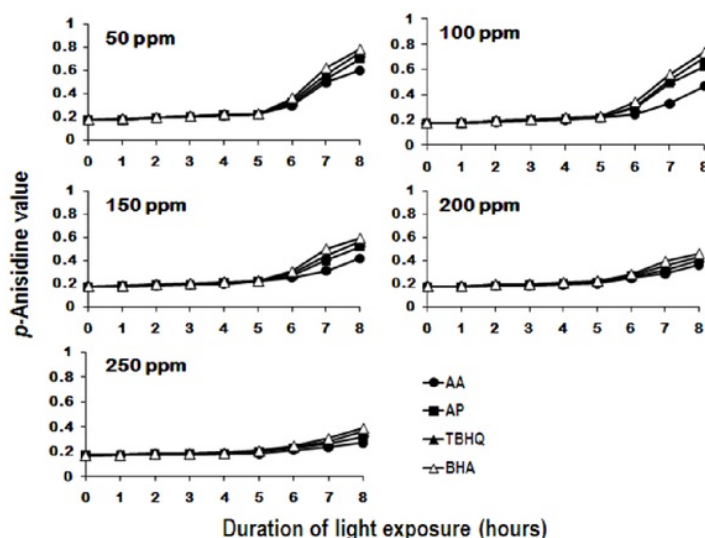


Fig. 2 *p*-Anisidine value of light exposed VCO containing various levels of ascorbic acid microemulsion (AA, ●), ascorbyl palmitate (AP, ■), TBHQ (▲), or BHA (△).

is greater than that of the hydroperoxide formation in the oil, it could have very low hydroperoxide content. Therefore, it was considered to determine the secondary oxidation products.

The *p*-AnV method measures the content of aldehydes (principally 2-alkenals and 2,4-alkadienals) generated during the decomposition of hydroperoxides. A higher level of *p*-AnV revealed a higher extent of the secondary oxidation products forming in light exposed oils which might be due, in part, to the enhanced rate of oxidative deterioration [14]. The *p*-AnV in light exposed VCO samples containing various levels of ascorbic acid microemulsion, AP, TBHQ, or BHA was shown in Fig. 2.

Fig. 2 shows that the *p*-AnV of light exposed VCO samples containing ascorbic acid microemulsion was significantly ($P < 0.01$) lower than that of VCO containing AP, TBHQ, or BHA at all levels of antioxidants. It was indicated that ascorbic acid microemulsion system was more capable for inhibiting the formation of secondary oxidation product as compared with the other antioxidants. Ascorbic acid could act as peroxide scavenger [6, 15],

so that it could prevent the development of secondary oxidation products.

Fig. 2 also shows that the *p*-AnV was relatively constant until 5 hours of light exposure in all of the treatments. This result clearly indicated the lag stage needed for the formation of the secondary oxidation product of lipid hydroperoxides. Within this period, the light exposed VCO samples produced hydroperoxides as shown in Fig. 1. According to Osborn and Akoh [16], the hydroperoxides must first be present before they can decompose into the secondary oxidation products.

The *p*-AnV increased after 5 hours of light exposure in all of the treatments. It was indicated that intensified light exposure in VCO leads to the accumulation of secondary oxidation products which was revealed in the increasing *p*-AnV. The lower *p*-AnV in VCO samples containing ascorbic acid microemulsion than that of VCO containing AP, TBHQ, or BHA indicated that ascorbic acid microemulsion effectively inhibited the photooxidation reaction and prevented the accumulation of hydroperoxides that could decompose to be the secondary oxidation products. The antiphotooxidative

effect of ascorbic acid microemulsion could also be evaluated by its TOTOX value.

The TOTOX value is a measure of the total oxidation, including primary and secondary oxidation products. It was expressed as $2\text{ PV} + p\text{-AnV}$. The PV and $p\text{-AnV}$ reflect the oxidation level at the early and later stages of oxidation reaction, respectively. During lipid oxidation, it is often observed that PV first rises and then falls as hydroperoxides decomposed. The TOTOX value measures both hydroperoxides and their breakdown products, and provides better estimation of the progressive oxidative deterioration of fats and oils [17]. The TOTOX values of light exposed VCO samples containing various levels of ascorbic acid microemulsion, AP, TBHQ, or BHA was shown in Fig. 3.

Fig. 3 had a similar trend to Fig. 1. Light exposed VCO samples containing ascorbic acid microemulsion showed a very significantly ($P < 0.01$) lower TOTOX value than that of VCO containing AP, TBHQ, or BHA at all levels of antioxidants. Intensified light exposure with relatively high intensity (4,000 lux) was very effective to initiate photooxidation [18]. The light

energy from that light exposure was absorbed by chlorophyll contained in VCO and it will become excited chlorophyll. According to Rawls and Van Santen [19], the excited chlorophyll transferred the energy to triplet oxygen to form the more reactive singlet oxygen. This singlet oxygen subsequently reacted with unsaturated fatty acid producing alkyl radical (L^{\bullet}). The alkyl radical reacted with oxygen molecules resulting in the formation of peroxy radical (LOO^{\bullet}), which had a higher energy than that of the alkyl radical. Thus, the peroxy radical could abstract hydrogen from another unsaturated fatty acid and produced a lipid hydroperoxide ($LOOH$) and a new alkyl radical [20].

Intensified light exposure in the VCO caused the decomposition of primary oxidation products, i.e. hydroperoxides, generating alkoxy (LO^{\bullet}) and hydroxyl radical ($\bullet OH$). The alkoxy radical, which was more energetic than either the alkyl or peroxy radical, could enter into a number of different reaction pathways known as β -scission reaction generating secondary oxidation products such as aldehydes, ketones, alcohols, and short-chain hydrocarbons [2].

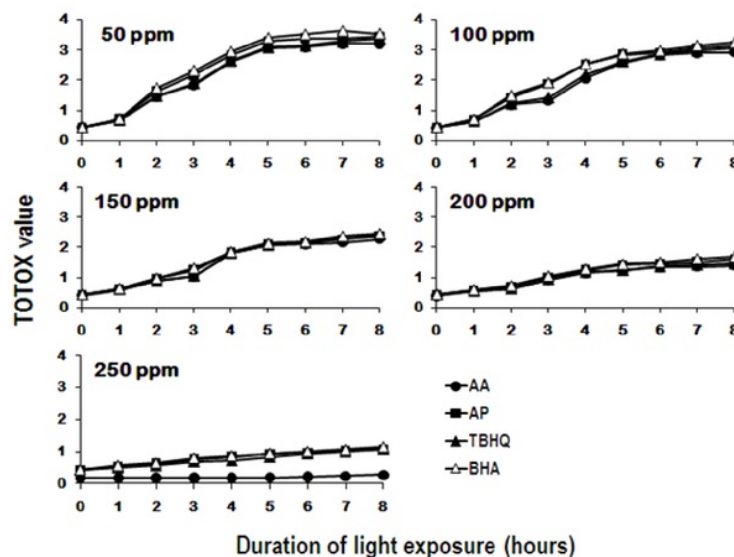


Fig. 3 TOTOX value of light exposed VCO containing various levels of ascorbic acid microemulsion (AA, ●), ascorbyl palmitate (AP, ■), TBHQ (▲), or BHA (△).

The hydroperoxides which were produced during light exposure of VCO samples had more polar characteristics than the triacylglycerols composed of VCO. They were able to diffuse to the more polar zone and interact with the compounds present in the aqueous phase [21]. The more polar zone in this study was the interface layer of microemulsions which contained ascorbic acid in their microdroplets. For this reason, the hydroperoxides were quenched more easily by the ascorbic acid than by the lipophilic antioxidants (TBHQ or BHA) or the oil-soluble ester antioxidant (AP). Moreover, ascorbic acid could also act as SOQ and prevent the alkyl radical formation that initiated the oxidation chain reactions. It resulted in a lower TOTOX value of VCO containing ascorbic acid microemulsion compared with those of synthetic antioxidants.

4. Conclusion

Ascorbic acid, which was incorporated into water-in-oil microemulsion system, was more effective antioxidant than other lipophilic antioxidants in inhibition of photooxidation reaction of VCO. Anti-photooxidation capability of ascorbic acid microemulsion was the highest, and followed by TBHQ, ascorbyl palmitate, and BHA. It would be of great interest for the future study to determine synergistic effect of the ascorbic acid microemulsion and lipophilic antioxidants such as α -tocopherol and β -carotene which are naturally present in VCO.

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