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Antioxidative Effect of Ascorbic Acid Solubilized via Reversed Micelle in Perilla Oil

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Abstract

Ascorbic acid was solubilized in perilla oil via reversed micelle using small amount of water and lecithin as surfactant. The effect of the solubilized ascorbic acid on the oxidative stability of perilla oil was investigated. The autoxidation of the oil was greatly retarded with the solubilized ascorbic acid compared to the synthetic antioxidants employed. However, the combination with δ-tocopherol did not show any significant synergism.

Key words: perilla oil, antioxidative effect, reversed micelle, ascorbic acid.

Introduction

During the last decade, interest in the physiological effect of 'n-3 family' of polyunsaturated fatty acids (n-3 PUFA) has been increased. The beneficial effects of PUFAs have been ascribed mainly to their ability to lower serum lipids and cholesterol levels.[1-3]

Besides fish oil containing large amount of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), perilla oil is also deserved to attract attention as a source of n-3 PUFA by its high content of α-linolenic acid. Perilla oil has been used as a dietary oil for a long time in Korea due to its mild and pleasant flavor. The oil is prepared from the seed of the plant *Perilla ocymoides* by pressing method, and its production amounted to about 6,529 M/T in 1987.[4,5] The oil contains as high as 60-70% of α-linolenic acid which can not be made by animals for themselves.[4,6] In a mammalian system, it has been demonstrated that dietary α-linolenic acid is converted to EPA and/or DHA through 'n-3 route', which are reported to be effective for the prevention of cardiovascular diseases.[2,7]

Generally, it is conceded that the principal route of deterioration and possible economic loss of oil is through rancidity resulting from the oxidation which takes place at the double bond sites in the triglyceride molecules. Therefore, the higher the degree of unsaturation, the more susceptable it is to oxidative deterioration.[8] This is true for perilla oil which contains abundant quantities of unsaturated fatty acids.

Antioxidants are one means of fending off oxidation; there are also vacuum packaging or packing under an inert gas to exclude oxygen and refrigeration/freezing which greatly reduce the rate of autoxidation. Among these various means, using antioxidant seems to be effective, easily applied and inexpensive. The antioxidants permitted in Korea by Ministry of Health and Social Affairs are butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), tert-butylhydroquinone (TBHQ), ascorbyl palmitate (AP), propyl gallate (PG), ethylenediaminetetraacetic acid (EDTA), EDTA-calcium, ascorbic acid, iso-ascorbic acid and tocopherol at this writing.

However, there is a tendency for the consumers to reject synthetic antioxidants due to their possible hazard.[9] In this respect, the application of ascorbic acid and tocopherol, which are naturally occurring antioxidants as well as nutrients, would be more favorable. However, ascorbic acid has a very low solubility in oils and thus is not usually employed as an antioxidant in this media.

To resolve this difficulty, an application of re-

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versed micelle to the oil system was developed in the previous study.[10] Reversed micelle was formed by lecithin possessing both hydrophilic and lipophilic properties, and water. This reversed micelle was able to solubilize ascorbic acid in oil.

In the present study, the effect of the ascorbic acid solubilized via reversed micelle on the oxidative stability of perilla oil was studied and compared to those of synthetic antioxidants. Synergism between solubilized ascorbic acid and δ-tocopherol was also investigated.

Materials and Methods

Preparation of perilla oil and antioxidants used

Perilla seed was purchased from a local market in Korea. Perilla oil was prepared by the traditional pressing method: the seeds were washed, air-dried and roasted for 5 min on a pan heated by direct fire and then the oil was extracted with a press.

The antioxidants containing BHA, BHT, AP, PG, δ-tocopherol and L-ascorbic acid were of laboratory grade obtained through Sigma Chemical Co. (U.S.A.). Lecithin (fluid blend of natural phospholipids and soybean oil) from Central Soya Co. (U.S.A.) was used as surfactant for the preparation of reversed micelle.

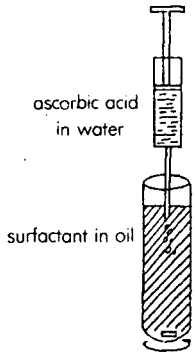

Fig. 1. The injection method to solubilize ascorbic acid in perilla oil via reversed micelle.

Table 1. Some chemical characteristics of perilla oil used

Peroxide value	2.4 ±0.2
Anisidine value	30.8 ±0.5
Acid value	2.42±0.06
Thiobarbituric acid value	1.03±0.08
Iodine value	189.4 ±0.5
Conjugated diene value	0.25±0.01

Analysis of the oil

The chemical characteristics of perilla oil used in the study are given in Table 1. AOCS Official Methods[11] were used for the determination of peroxide, acid, iodine and conjugated diene values. Anisidine value was obtained using IUPAC Method.[12] Thiobarbituric acid value was determined by the method described by Sidwell *et al.* [13]

The percentage fatty acid composition of the oil was determined according to the AOCS Official Method[11]: 6.35 palmitic; 2.41 stearic; 19.78 oleic; 12.92 linoleic; 58.25 α-linolenic. Trace of palmitoleic acid was detected.

Preparation of oil samples with antioxidants

Methods similar to those as described in the previous paper were used in the present study.[10] To solubilize ascorbic acid in perilla oil via reversed micelle, the so-called 'injection method'[14] was used as shown in Fig. 1. The final lecithin, ascorbic acid and water concentrations in perilla oil were 0.3% (w/w), 0.02% (w/w) and 0.05% (w/w), respectively.

Each antioxidants excluding ascorbic acid were added directly with a magnetic stirrer under an inert gas at a permitted level: BHA and BHT at 0.02% (w/w); AP and PG at 0.01% (w/w); δ-tocopherol at 0.2% (w/w). For the evaluation of synergistic effect, δ-tocopherol was added directly to the oil containing reversed micelle-solubilized ascorbic acid.

Measurement of antioxidant activity

To assess the effect of each antioxidants on the

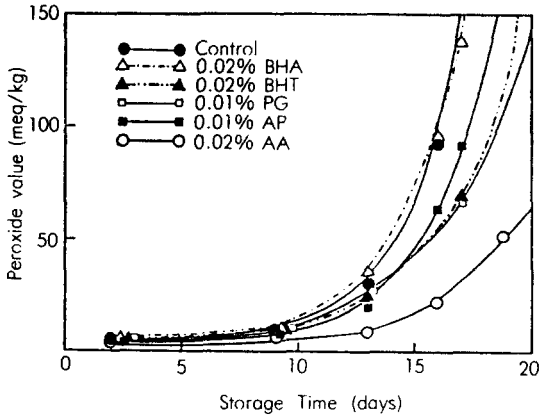

Fig. 2. Effect of butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate (PG), ascorbyl palmitate (AP) and ascorbic acid (AA) on the oxidation of perilla oil stored at 60°C.

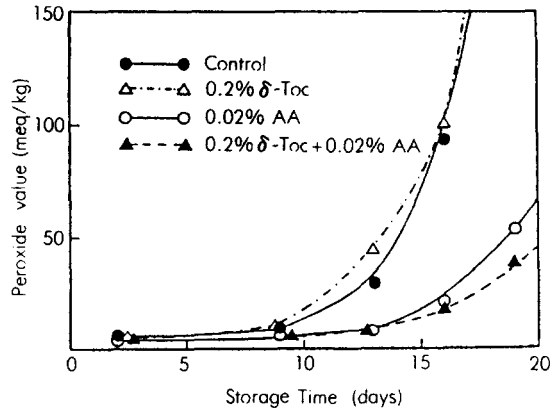

Fig. 3. Combined effect of δ-tocopherol (δ-Toc) with ascorbic acid (AA) on the oxidation of perilla oil stored at 60°C.

oxidative stability of perilla oil, 5g of oil samples containing each antioxidants was put in a Petri dish (87mm × 15mm) and placed in a dark oven at 60°C throughout the storage period. Control with no antioxidant was placed in the same condition.

The stability was determined by peroxide value (POV) measured with the AOCS Method Cd 8-53.[11]

Results and Discussion

The effects of some synthetic antioxidants and ascorbic acid on the oxidative stability of perilla oil are shown in Fig. 2. This result showed that all the antioxidants except BHA tested had an improving effect on the stability of the oil. After 9 days, the effect was found to be profound defending on the types of antioxidants except BHA. BHA did exhibit a slight negative effect on the oxidative stability of the oil. The curves also showed that the effect of the ascorbic acid solubilized via reversed micelle was superior to any other synthetic antioxidants. It is suggested that ascorbic acid seemed to act as a primary antioxidant itself by stopping the free radical chain reaction. The antioxidative effect of lecithin alone appeared negligible (data not shown here).

The combined effect of ascorbic acid with δ-tocopherol on the oxidation of perilla oil is shown in Fig. 3. During the storage, it is observed that δ-tocopherol added at a level of 0.2% (w/w) failed to show any synergistic activity. The induction periods (days needed to reach 40 meq/kg POV)[15] of samples containing δ-tocopherol alone, ascorbic acid alone or in combination were 12.5, 17.8 and 19.4, respectively. This result showed that the induction period for the oil containing both ascorbic acid and δ-tocopherol was not extended to the sum of the induction period observed when either δ-tocopherol or ascorbic acid was used alone. It seemed that the combination of δ-tocopherol with ascorbic acid did not provide any synergistic effect in perilla oil even though the induction period was slightly prolonged.

Another experiment shows that the antioxidative effect of the solubilized ascorbic acid proved to be more remarkable in fish oil than this result for perilla oil.[16]

In conclusion, the ascorbic acid solubilized via reversed micelle has an effective primary antioxidant activity in perilla oil. However, the combination with δ-tocopherol did not show any significant synergism. Based on this finding it is recommended that ascorbic acid be added to perilla oil via reversed micelle for improving the storage stability of the oil.

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역미설계를 이용한 들깨기름의 산화안정성 향상에 관한 연구

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역미설계를 이용하여 들깨기름에 아스코르브산을 용해하여 이의 산화방지 효과를 측정하였다. 계면활성제로서 레시틴과 소량의 물을 사용하여 역미셀을 제조하였으며 이렇게 용해된 아스코르브산을 첨가함으로써 여러 종류의 합성 산화방지제에 비하여 들깨기름의 산화를 현저히 억제할 수 있었다. 한편, 아스코르브산과 델타-토코페롤과의 상승효과는 나타나지 않았다.