ANTIOXIDANT ACTIVITIES OF PALM VITAMIN E WITH SPECIAL REFERENCE TO TOCOTRIENOLS

Keywords: Elaeis; Antioxidant activities; Palm Vitamin E; Tocopherols; Tocotrienols

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The antioxidant activities of palm vitamin E and tocotrienols (T3) from Elaeis guineensis *were investigated in model systems using distilled palm methyl ester (DME) and vitamin E-free RBD palm olein respectively. Oxidative stability was measured by the Rancimat method. Addition of 500 ppm vitamin E concentrate, which consisted of α-tocopherol (21.9%), α-tocotrienol (31.1%), γ-tocotrienol (37.7%) and δ-tocotrienol (9.3%) to DME was found to increase the oxidative stability of the substrate by a factor of about 2.6. Vitamin E-free RBD palm olein was found to be relatively unstable. Addition of 200-2000 ppm α-tocotrienol, γ-tocotrienol or δ-tocotrienol individually to vitamin E-free RBD palm olein showed that these compounds were effective antioxidants and that the activity increased with increasing concentration. At 200 ppm, α-tocotrienol improved the stability of the substrate by a factor of about 6.3. The order of antioxidant activities of tocotrienols was found to be γ-T3 ≥ δ-T3 > α-T3 : γ-T3 had about twice the activity of α–T3.*

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INTRODUCTION

The reaction of unsaturated fatty acids with oxygen is mainly responsible for the alteration in organoleptic properties and nutritive value of oils and fats during oxidative degradation. Since oils and fats are susceptible to this process, studies related to the stability of oils and fats towards oxidation are of considerable significance to the related industries. The process of oxidation has been found to proceed via a chain reaction mechanism and it is inhibited by antioxidants such as vitamin E and tertiary butylhydroquinone (TBHQ). Most vegetable oils contain naturally-occurring vitamin E in small amounts. Unlike other vegetable oils, which contain tocopherols as the major components of their vitamin E, palm oil has been found to contain about 800 ppm of vitamin E consisting of α – tocopherol (20%), α–tocotrienol (22%), γ-tocotrienol (46%) and δ–tocotrienol (12%); thus the major components are tocotrienols (Hashimoto *et al.*, 1980; Ab Gapor *et al.*, 1981). The antioxidant activities of tocopherols have been more widely studied then those of tocotrienols (Swern, D, 1964). However, Seher *et al.*(1973) reported that tocotrienols were more active than the corresponding tocopherols in lard. Using methyl linoleate as the substrate, Yamaoka *et al.*, (1985) found that tocotrienols had antioxidant activities superior or equal to those of the corresponding tocopherols. The purpose of the present study was to evaluate the antioxidant activities of palm vitamin E and α–, γ- and δ–tocotrienols in a model system using purified palm methyl esters and vitamin E-free RBD palm olein as the substrates.

MATERIALS AND METHODS

Materials

Palm fatty acid distillate (PFAD) and RBD palm olein were gifts from Pandamaran Oil Products, Malaysia. Silica gel for column chromatography, silica gel for thin layer chromatography, and aluminium oxide 90 active for column chromatography were from Merck. All other chemicals and solvents used were of analytical grade. The Rancimat 617 apparatus was from Metrohm, Switzerland.

Preparation of Palm Vitamin E Concentrate

Palm vitamin E concentrate was obtained from PFAD. The process involved the conversion of PFAD into methyl esters by the usual esterification process (Christie, 1982) and subsequently the distillation of the esters under high vacuum leaving a residue which was rich in vitamin E. The residue was then subjected to silica gel column chromatography and thin layer chromatography. The final vitamin E concentrate was found to be 66.5% pure, consisting of α–tocopherol (21.9%), α–tocotrienol (31.1%), γ-tocotrienol (37.7%) and δ–tocotrienol (9.3%).

Preparation of Individual Palm Vitamin E Components

Palm vitamin E concentrate as prepared above was fractionated into its individual components by thin layer chromatography according to Meijboom and Jongenotter (1979). The individual components were further chromatographed repeatedly until a purity of about 90% was obtained.

Analysis of Vitamin E

Vitamin E in various samples was analysed by high performance liquid chromatography using a fluorescence detector (Ab Gapor *et al.*, 1981).

Preparation of Vitamin E-free Palm Oil Methyl Esters

Purified palm methyl esters were obtained by the process of transesterification of RBD palm oil, followed by distillation under vacuum in the usual manner. The vitamin E content of the distilled methyl esters was confirmed to be nil before the material was used for the oxidation tests.

Preparation of Vitamin E-free RBD Palm Olein

The naturally-occurring vitamin E in RBD palm olein could be stripped from the oil by column chromatography on alumina. RBD palm olein (100g) was dissolved in the minimum amount of hexane and then introduced into a column (2.7 cm internal diameter) con-

TABLE 1.
EFFECT OF PALM VITAMIN E CONCENTRATE ON DISTILLED PALM METHYL ESTERS (DME)

Concentration of Vitamin E in DME sample (ppm)	Induction Period (hr)
0	16
500	57
1000	58
1500	57

taining alumina (100 g). The oil was then eluted with 80 ml hexane and recovered from the eluate by evaporation of the solvent under vacuum. The oil was checked for its vitamin E content; it was found that a single pass through such a column was sufficient to free the oil from all its initial vitamin E.

Preparation of Known Concentration of Vitamin E in Palm Methyl Esters

Palm vitamin E concentrate was added to distilled palm methyl esters to give samples containing 500, 1000 and 1500 ppm total vitamin E.

Preparation of Known Concentration of Palm Tocotrienols in RBD Palm Olein

Known concentrations of α-tocotrienol,γ-tocotrienol and δ-tocotrienol (200, 400, 600, 1000, 1500 and 2000 ppm) were prepared by dissolving the individual tocotrienol in vitamin E-free RBD palm olein.

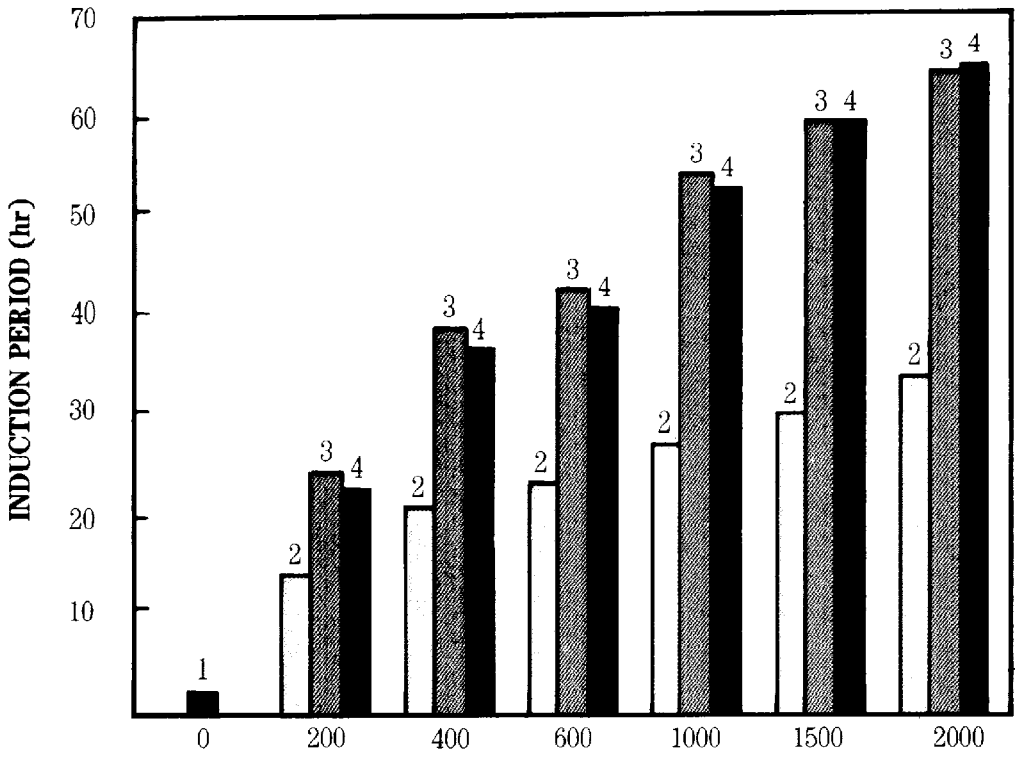
Measurement of Oxidative Stability

A 2g of each vitamin E-free sample (control) and samples with added vitamin E concentrate and tocotrienols were subjected to the Rancimat test at 100°C and the results were expressed in terms of induction period (IP) in hours (Loliger, 1983). Each sample was tested in duplicate or triplicate.

RESULTS AND DISCUSSION

The effect of adding palm vitamin E concentrate, which was a mixture of α-tocopherol (21.9%), α-tocotrienol (31.1%), γ-tocotrienol (37.7%) and δ–tocotrienol (9.3%) at 500, 1000 and 1500 ppm to purified palm methyl esters can be seen in Table 1. The stabilization of palm methyl esters by the addition of palm vitamin E suggests that it is an antioxidant, as expected. The present data show that the addition of palm vitamin E at concentrations higher than 500 ppm gave no further improvement in the stabilization of palm methyl esters, indicating that the optimum level of stabilization for the model had been reached at 500 ppm or less of palm vitamin E.

The results of adding individual α–, γ- and δ–tocotrienols at 200, 400, 600, 1000, 1500 and 2000 ppm to vitamin E-free palm olein as measured by the Rancimat test are given in *Figure 1*. It was noted that the Vitamin E-free RBD palm olein had a relatively low stability (IP *ca.* 2 hr) compared with fresh RBD palm olein (IP *ca.* 48 hr), suggesting that vitamin E-



1. Vitamin E- free RBD Palm Olein (Substrate) - Control
2. Substrate + α - tocotrienol
3. Substrate + γ - tocotrienol
4. Substrate + δ – tocotrienol

Figure 1.
Antioxidant activities of Tocotrienols in vitamin E-free RBD Palm Olein

free olein was highly susceptible to oxidation. Addition of tocotrienols to vitamin E-free RBD palm olein improved its oxidative stability significantly and the antioxidant effect was found to increase with increasing concentration of tocotrienols. The induction period of the substrate increased by factors of about 6.3 and 17.1 at 200 ppm and 2000 ppm α–tocotrienol respectively. Thus the phenomenon of an antioxidant showing pro-oxidant activity over a certain concentration range as can happen in certain systems (Swern, 1964; Cillard, *et al.*, 1980), was not observed in the present case.

As regards the relative activity of tocotrienol homologues: γ-tocotrienol had about twice the antioxidant activity of α – tocotrienol. The present results seem to suggest that δ–tocotrienol had slightly less or equal antioxidant activity as compared to γ-tocotrienol; Seher *et al.* (1973) found δ – tocotrienol to be more active than γ-tocotrienol in a lard model. This anomaly is currently under investigation taking into account that, apart from experimental error, other factors such as antioxidant concentration, substrate and temperature are also known to influence the activity of an antioxidant (Lea, 1960).

Various other aspects of the antioxidant activities of palm vitamin E and tocotrienols are currently under study. These include the stabilization of palm oil products by these compounds and the relative antioxidant activities of tocotrienols as compared to tocopherols. On the latter aspect, preliminary results seem to suggest that tocopherols have slightly bet-

ter or equal activity by comparison with the corresponding tocotrienols.

CONCLUSION

The presence of vitamin E in RBD palm olein was shown to be extremely important by the fact that vitamin E-free olein had very low oxidative stability.

Palm vitamin E was found to be an effective antioxidant in a model using purified palm methyl ester. Addition of the major components of palm vitamin E, *i.e.* tocotrienols, improved the oxidative stability of vitamin E-free RBD palm olein significantly. No evidence of pro-oxidant activity of tocotrienols up to a concentration of 2000 ppm in the oil model was observed. γ- and δ – tocotrienols were found to be markedly superior to α–tocotrienol.

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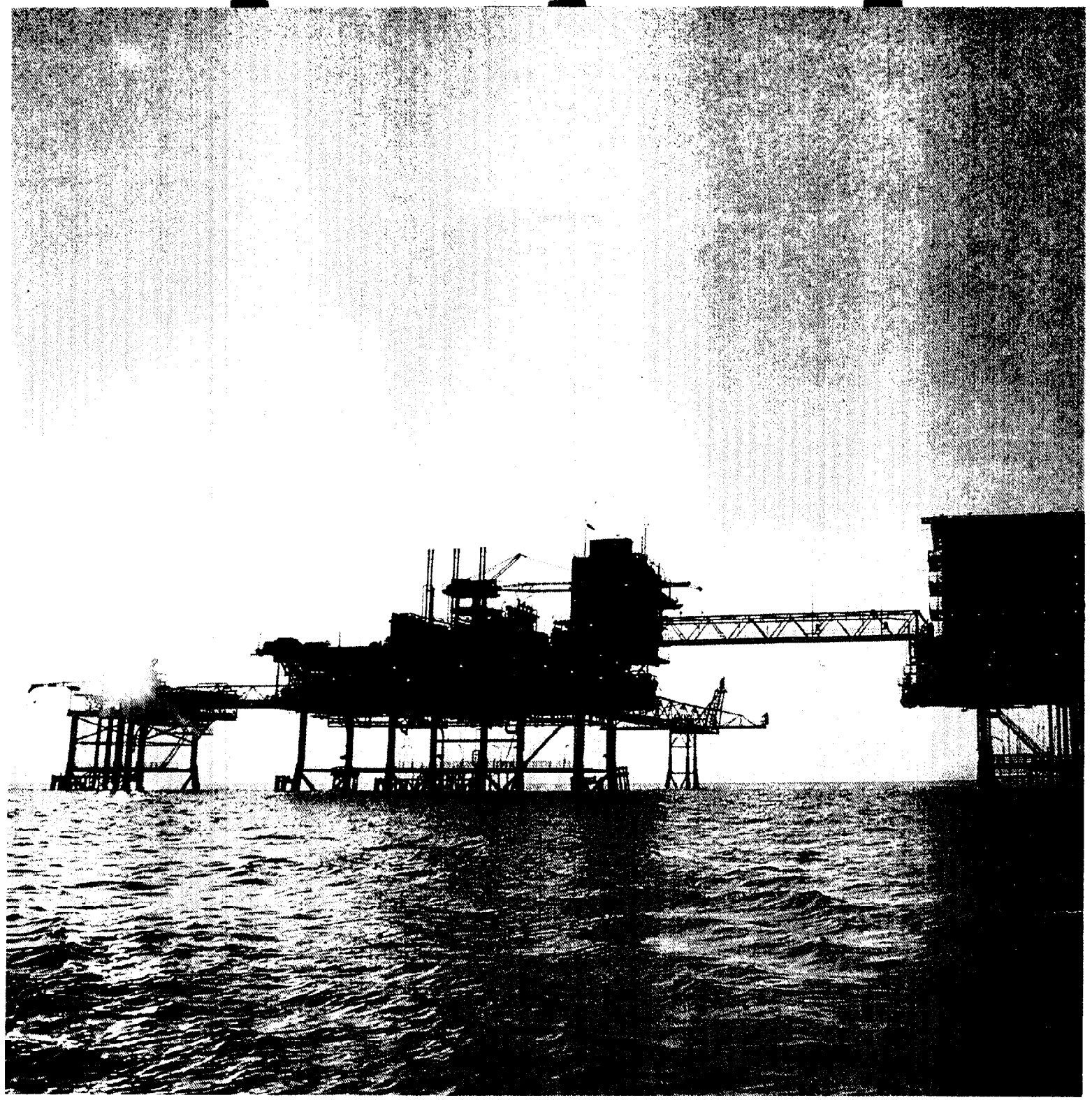

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