Colorimetric Determination of Total Tocopherols in Palm Oil, Olein and Stearin

M.L. Wong, R.E. Timms and **E.M. Goh***
Kempas Edible Oil Sdn. Bhd., P.O. Box 75, 81707 Pasir Gudang, Johore, Malaysia

By using a preliminary heat-bleach at 250 C the Emmerie-Engel method has been adapted for the determination of total tocopherols (including tocotrienols) in crude as well as refined palm oil, olein and stearin. Total tocopherol contents found were: crude palm oil, 794 ppm (n=10); RBD palm oil, 563 ppm (n=13); RBD palm olein, 643 ppm (n=40); RBD palm stearin, 261 ppm (n=19), where n is the number of samples analyzed. During the detergent fractionation no tocopherols were lost, but the tocopherols were concentrated in the olein fraction.

The fate of the tocopherols during degumming, bleaching and steam refining/deodorizing of crude palm olein containing 978 ppm total tocopherol was studied. Over the whole refining process only 8% of the tocopherols were lost, 62% of the original tocopherols were retained in the RBD palm olein, while the remaining 30% were concentrated in the fatty acid distillate which contained 7,040 ppm tocopherol.

Tocopherols are important minor components of oils and fats because of their antioxidant properties. They are especially important in palm oil because of the uniquely long shipment and storage times which RBD (refined, bleached and deodorized) palm oil and its fractions undergo. Tocopherols are also important nutritionally because of their Vitamin E activity. Although oils such as soybean and corn oils contain somewhat higher levels of tocopherols (1), palm oil contains probably the highest ratio of tocopherols to polyunsaturated fatty acids found in any oil, which is believed to be important for the prevention of heart disease and cancer (2,3).

Tocopherols may be divided into two types: (i) tocopherols, with a saturated side chain, sometimes called tocols, and (ii) tocotrienols, with an unsaturated side chain (4). In this paper, where necessary to avoid confusion the designation "total tocopherol" is used to indicate both (saturated) tocopherols and (unsaturated) tocotrienols.

Palm oil contains unusually high levels of tocotrienols (5). Most other common vegetable oils have negligible contents of tocotrienols (1,6).

Total tocopherol contents of various oils and fats are given in "Bailey" (7), but this is old data not updated in the latest edition of this work. The value of 560 ppm given for palm oil is lower than recent results reviewed by Ab. Gapor et al. (5), which show a range of 600-1080 ppm with average values in the range 730-860 ppm.

Because of the desirability of preserving as much as possible of the natural tocopherols during processing of palm oil (PO), we wished to study any losses occurring during fractionation to give palm olein and stearin, and during subsequent physical refining to the RBD products. Ab. Gapor et al. (5) have published the only previous study of this problem. However, the results they reported are not consistent and appear to show unusually large losses or gains of total tocopherols. Their data for oils at different stages of refining show substantial absolute losses when mass balance calculations are made. There was also an unexpected rise in total tocopherol after bleaching, which was explained as due to regeneration of total tocopherols from esterified or dimerized tocopherols.

From preliminary studies at our refinery we considered these results anomalous and probably due to losses during sample handling and analysis or to incorrectly matched samples supplied by the refineries.

Accordingly, the objectives of the work reported in this paper were: to develop a method of total tocopherol analysis suitable for use in a Malaysian refinery for both crude and RBD palm oil, olein and stearin; to determine typical total tocopherol levels in the products from our refinery and to follow the fate of total tocopherols through the fractionation and refining processes.

Methods for tocopherol analysis have been reviewed by Parrish (4). The method of choice is now high performance liquid chromatography (HPLC), which is rapid, requires little sample pretreatment and can distinguish individual tocopherols and tocotrienols. However, HPLC is unsuitable for routine use in a Malaysian refinery because of the high capital and running costs of the equipment. Because we believe it is desirable for quality control (QC) laboratories in refineries to be able to determine tocopherols, we preferred a simpler and cheaper spectrophotometric method. Most refineries already have a spectrophotometer as standard laboratory equipment. The method we have adopted is the very widely used Emmerie-Engel method (4) in which tocopherols and tocotrienols reduce ferric ions to ferrous, which reacts to form a colored complex with dipyridyl. This procedure is sufficiently simple for factory control (8) and has been fully automated for analysis of RBD oils (9). Unfortunately, other reducing agents and colored pigments can interfere so that the method is often unsuitable for crude oils (10-12). However, using a simple pretreatment we were able to apply it successfully to the analysis of crude palm oil and fractions.

In the results reported below, tocopherol refers to total tocopherol as determined by the Emmerie-Engel method.

EXPERIMENTAL

Sample. All samples of palm oil, palm olein, palm stearin and fatty acid distillate were taken from road tankers, storage tanks or directly from the refinery at

*To whom correspondence should be addressed.

Kempas Edible Oil Sdn. Bhd., Pasir Gudang, Johore, Malaysia. Samples were stored at 5 C until analyzed.

Fractionation was by detergent fractionation (Alfa-Laval). A total amount of detergent solution equal to 55-60% of the weight of oil was used. The composition of the detergent solution was 0.45% sodium dodecyl sulfate and 1.75% magnesium sulfate. Refining was by physical refining comprising degumming (0.04-0.07% phosphoric acid at 85 C), bleaching (1-2% earth at 110 C), and steam refining/deodorization at 270 C (EMI).

Determination of tocopherol. Two hundred ± 10 mg of the oil sample are weighed accurately into a 10-ml volumetric flask. Five ml of toluene are added by pipette and the oil taken into solution. Three and one-half ml of 2,2'-bipyridine (0.07% w/v in 95% aqueous ethanol) and 0.5 ml of $FeCl_3$. $6H_2O$ (0.2% w/v in 95% aqueous ethanol) are added in that order. The solution is made up to 10 ml with 95% aqueous ethanol. After standing for one min the absorption at 520 nm is determined using as a reference a blank solution, prepared as above but omitting the oil (10). Solutions should be protected from strong light during color development.

The method was calibrated by preparing standards containing 0-240 μg of pure α-tocopherol in 10 ml of toluene and then analyzing as above.

The concentration of tocopherol in the sample was calculated as:

$$\text{Total tocopherol (ppm)} = (A - B) / M.W.$$

where A = absorption of sample in 10-mm cell
B = absorption of blank in 10-mm cell
M = gradient of absorbance vs weight graph for α-tocopherol calibration; was ca. 8.0×10^{-3} absorbance/μg α-tocopherol
W = weight of sample in g

A much smaller sample (~20 mg) of fatty acid distillate was taken for analysis because of the high level of tocopherol found.

The precision of the method was evaluated by analyzing RBD palm olein five times on each of two days with two independent calibrations. There was no significant difference between the days, and the standard deviation was 12.3 ppm with a mean of 685 ppm, giving 95% confidence limits of ± 29 ppm (± 4.2%) for a single determination and ± 20 ppm (± 3.0%) for the mean of duplicate determinations. Similar 95% confidence limits of ± 4.0% were calculated from the results of Honnold et al. (9).

The method was evaluated further by spiking a sample of RBD palm oil with 100, 200, 300 and 400 ppm of pure α-tocopherol. Samples were analyzed in triplicate on two separate days; the results are shown in Table 1. Provided that a smaller sample size was used for the 400 ppm addition, the observed increase in tocopherol content corresponded to the amount added within experimental error.

Analysis of crude palm oil and fractions. A sample of the crude oil (10-20 g) was heated in a round-bottomed flask under vacuum for 10 min at 250 C. The carotene was destroyed rapidly, and the color faded to a light yellow. The flask was cooled under vacuum and the oil analyzed as described above.

TABLE 1.

Analysis of RBD Palm Oil Spiked With Known Amounts of Pure Tocopherol

Tocopherol added (ppm)	Tocopherol analysis (ppm)		
	Day 1[a]	Day 2[a]	Mean
0	691	671	681
100	796	796	796
200	921	907	914
300	990	995	993
400	(1008)[b]	1079[c]	1079

[a]Each figure is the mean of triplicate analyses.
[b]Tocopherol level/absorbance too high.
[c]Half normal sample size used.

RESULTS AND DISCUSSION

Crude palm oil. To check the effectiveness of the heat-bleaching pretreatment at 250 C at destroying carotene, 750 ppm β-carotene was added to an RBD palm oil whose tocopherol content had been determined. Results given in Table 2 show the interfering effect of the added carotene. After heat-bleaching, the observed tocopherol level was not significantly different from that in the starting oil.

TABLE 2.

Analysis of RBD Palm Oil in the Presence of β-Carotene

Sample		Apparent tocopherols content (ppm)[a]
Code	Description	
A	RBD palm oil	535
B	A + 750 ppm carotene	944
C	B analyzed after heat-bleaching at 250 C for 10 min	516

[a]Each figure is the mean of duplicate analyses.

Crude palm oil does not contain any substances besides carotene which could significantly affect the tocopherol determination. Phosphorus levels are low (averaging 15 ppm). Any phospholipids present would be destroyed by the heat treatment, but recent work also has shown that most of the phosphorus is present as inorganic phosphates (13).

In Table 3 are given tocopherol and carotene (determined as β-carotene by absoprtion at 450 nm) contents of crude palm oils received from 10 major suppliers to our refinery. There was little variation among the suppliers, and the mean and range of the tocopherol contents are comparable with previous results (5).

There was no significant correlation between tocopherol and carotene contents (correlation coefficient = 0.426 for 8 degrees of freedom).

RBD palm oil, olein and stearin. Thirteen samples of RBD palm oil, 40 samples of RBD palm olein and 19 samples of RBD palm stearin were analyzed over a

TABLE 3.

Tocopherol and Carotene Contents of Crude Palm Oils From 10 Suppliers

Supplier[a]	Tocopherol[b] (ppm)	Carotene (ppm)
A	773	642
B	766	630
C	843	621
D	760	574
E	866	651
F	800	553
G	756	564
H	818	575
I	790	554
J	770	551
Mean	794.2	591.5
Range	756-866	551-642

[a]Each sample analyzed was a composite of one week's deliveries from the supplier.
[b]Each figure is the mean of duplicate analyses.

TABLE 4.

Tocopherol Contents (ppm) of Samples of RBD Palm Oil, Olein and Stearin Produced Over a 45-week Period

	Palm Oil	Palm Olein	Palm Stearin
No. of samples[a]	13	40	19
Weighted mean	563.2	643.4	261.2
Maximum	684	761	368
Minimum	482	528	135
Range	202	233	233

[a]Each sample analyzed was a composite of one week's production by the refinery.

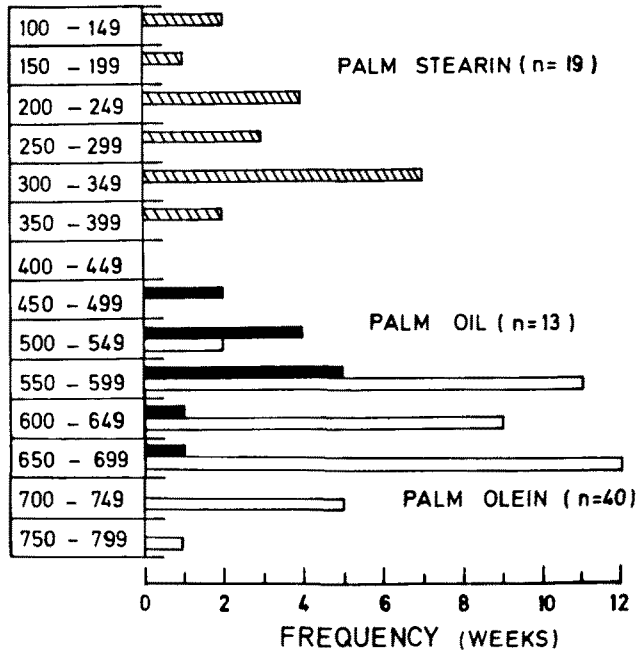

FIG. 1. Distribution of tocopherol contents (ppm) of RBD palm oil, olein and stearin samples produced over a 45-week period.

45-week period (Table 4). Each sample analyzed was a weighted composite of one week's production at the refinery. The results are presented as a histogram in Figure 1.

The results for RBD palm oil show lower levels and greater variability than for crude palm oil. The greater variability probably reflects the larger number of samples and longer time period. The results for RBD olein and stearin show even greater variation. This could be due to process variability and losses of tocopherol on storage, especially for stearin which may be stored for several weeks before refining. Clearly tocopherols are concentrated in the olein and depleted in the stearin. Assuming an average stearin yield of 20%, the tocopherol in the stearin and olein corresponds to a tocopherol content in the equivalent RBD palm oil of 567 ppm. This is close to the level found in RBD palm oil refined over the same period, suggesting that losses on fractionation and any subsequent storage cannot be very significant.

Fractionation of crude palm oil. The fate of tocopherols during fractionation of palm oil was studied by tracing a single batch of crude palm oil through the fractionation plant. Crude palm oil (FFA = 3.22%, IV = 50.8, Toc = 850 ppm) was fractionated to yield 77% of crude palm oil olein (FFA = 3.41%, IV = 56.4, Toc = 972 ppm) and 23% of crude palm stearin (FFA = 2.26%, IV = 31.9, Toc = 459 ppm), where FFA is free fatty acids, IV = iodine value and Toc = tocopherol.

By mass balance the tocopherol found in the stearin and olein was calculated to be equivalent to 854 ppm in the starting crude oil. Thus, confirming the tentative conclusions for RBD olein and stearin above, no absolute loss of tocopherols was found, merely a redistribution between olein and stearin.

The depletion of tocopherols in the stearin is accompanied by an enrichment in iron and phosphorus which accounts for the generally poor bleachability and oxidative stability of crude palm stearin.

Physical refining of palm olein. The reduction in tocopherols during refining, which is evident from the data in Tables 2 and 4, was studied in more detail by following the fate of tocopherol during the refining of crude palm olein. As far as possible, a single batch of olein was traced through the refinery. In a continuous refinery such as ours this is difficult, as no "batch" of oil can be isolated. However, by careful sampling it is possible to simulate passage of a batch of oil. Only between the bleacher and deodorizer, where the oil passes through a 100-T intermediate tank, is there some mixing of the "batch". This was allowed for by analyzing oil entering and leaving the tank. There was only a small difference of 13 ppm between these two analyses, which is not statistically significant. Over the whole sampling period the crude olein feed to the refinery was drawn from a single storage tank and remained almost constant.

Crude palm olein (FFA = 3.39%, Toc = 978 ppm) was degummed to give crude degummed palm olein (FFA = 3.49%, Toc = 944 ppm), bleached to give bleached and degummed olein (FFA = 3.52%, Toc = 920 ppm) and then transferred to the intermediate holding tank. Bleached and degummed olein (FFA = 3.47%, Toc = 907 ppm) was then fed from the intermediate tank to the deodorizer and physically refined (steam refining/deodorization) to yield 95.9% (of feed) of RBD palm olein (FFA = 0.08%, Toc = 636 ppm) and 4.1% of palm fatty acid distillate (FFA = 83.6%, Toc = 7040 ppm). Yields were calculated from the FFA analyses.

During degumming and bleaching there were small (2-4%) losses of tocopherol. During steam refining/deodorization there was a substantial reduction in tocopherol from 907-920 ppm in the feed degummed and bleached olein to 636 ppm in the RBD olein. By mass balance the tocopherol recovered in the RBD olein and the fatty acid distillate was calculated to be equivalent to 896 ppm in the feed oil. Thus there was no significant loss of tocopherols in the deodorizer, the apparent loss being caused entirely by a concentration of tocopherols in the fatty acid distillate. This result is in marked contrast to the results of Ab. Gapor et al., which were commented on earlier.

Over the whole refining process absolute losses were only 8%; 62% of the original tocopherol was retained in the RBD palm olein, while the remaining 30% was concentrated in the fatty acid distillate.

Loss of tocopherol during oxidation of palm oil. A sample of RBD palm oil was stored at 80 C for 10 days, until substantial oxidation had occurred. Tocopherol content and peroxide value (PV) were monitored to give the results shown in Figure 2. Loss of tocopherol was initially slow but proceeded rapidly when the PV rose above 10. The results show that the antioxidant effect of the tocopherols results in their destruction once oxidation is substantially under way.

However, we also found that peroxides themselves interfere with the tocopherol determination. In the presence of a PV of 50, addition of 500 ppm α-tocopherol increased the apparent tocopherol content by only 292 ppm. This suggests that the Emmerie-Engel method should not be used for highly oxidized oils (PV 10), and the results shown in Figure 2 can be considered to be only qualitatively correct.

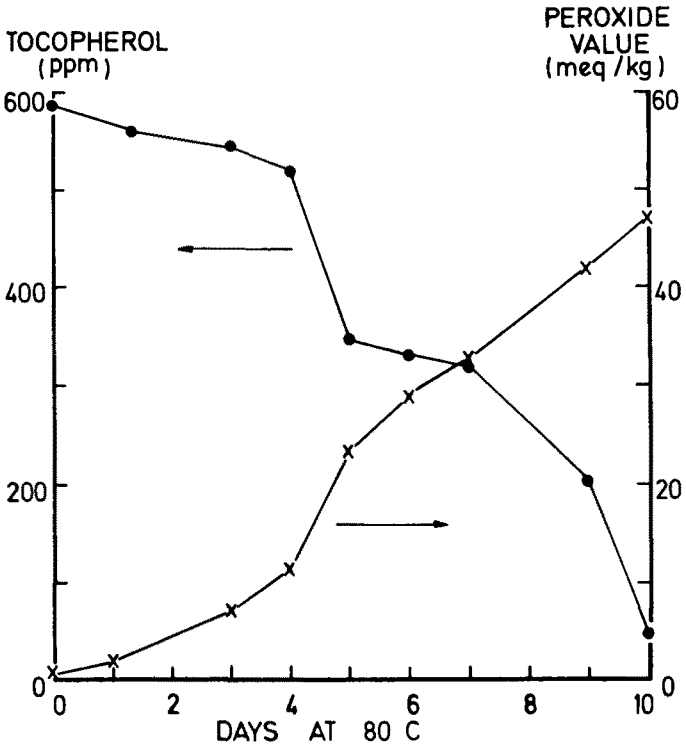

FIG. 2. Change of tocopherol content and peroxide value in RBD palm oil stored at 80 C.

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