# An Investigation of the Basic Conditions for Tocopherol Determination in Vegetable Oils and Fats by Differential Pulse Polarography

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## ABSTRACT

The polarographic behavior of α-, γ-, and δ-tocopherols was studied according to the proposed official IUPAC method for tocopherol determination in vegetable oils and fats. Each of the tocopherols had a different polarographic response; however, the tocotrienols had the same half-wave potentials and probably also the same polarographic response as the corresponding tocopherols. Additives previously investigated plus several others were examined for possible interference. The results by polarography and a new high performance liquid chromatography (HPLC) method were compared. The analysis by t-test at 99% significance level showed no differences for the determinations of α-tocopherol, but the results for three of the γ-tocopherol results were less consistent under the same conditions. The results for the determination of δ-tocopherol were below the detection limit for polarography and could not be statistically evaluated. The polarographic method investigated was found to be uncomplicated and therefore suitable for routine work. However, when using the method, one has to take into account possible interference by additives and the limitations due to the lack of separation of β- from γ-tocopherol and/or the interference of tocotrienols with the corresponding tocopherol peaks. From this aspect the HPLC method gives better resolution.

## INTRODUCTION

At present there is a pressing need for a simple method for the determination of tocopherols in natural materials and industrial products.

Many methods for tocopherol determination have been published. One of them, for oils and fats, and now probably the most widely used, involves alkaline saponification, extraction of the unsaponifiables, separation of the tocopherols by thin layer chromatography (TLC), and their subsequent quantification by gas chromatography (1). As the tocopherols are very sensitive to oxygen in the presence of alkali, it is difficult to prevent losses. In general the method is complicated and time consuming.

Recently high performance liquid chromatography (HPLC) and pulse polarography have been investigated as to their applicability for the determination of tocopherols in oils and fats. It was shown that both techniques could offer simpler analytical procedures.

By HPLC it is possible to determine directly the tocopherols in a fat sample without preceding concentration of the tocopherols (2-4). One of the reported methods (4) has been developed in these laboratories. It has a high sensitivity and differentiates the tocopherols and the corresponding tocotrienols.

The earlier polarographic procedures used the electrochemical reduction of tocopherylquinones on the dropping mercury electrode for the tocopherol determination (5-9). To use the opposite reaction, the electrooxidation of tocopherols to tocopherylquinones, was possible after first developing special electrodes for positive potentials (10-12). One of these methods (10) was adapted for a direct tocopherol determination in oils and fats without preconcentration and is recommended to IUPAC as a standard method (13). By this method α-, (β+γ)-, and δ-tocopherol can be determined.

As the last-mentioned method (13) seemed to be suited for routine use, it was decided to investigate it further and compare the results by this method with those obtained by our HPLC method.

## EXPERIMENTAL PROCEDURES

### Materials

Ethanol for spectroscopy was purchased from Spritcentralen, Stockholm, toluene (Uvasol), petroleum spirit (analytical), and basic aluminum oxide (for column

TABLE I

Regression Lines (Amount x Peak Height) and Some Other Parameters for Tocopherols and Additives

| Substance | Regression line[a] r | k | b | Half-wave pot.[b] +mV | Peak-hgt[c] for 300 μg mm | Max. permitted conc.[d] ppm | mm[e] |
|---|---|---|---|---|---|---|---|
| α-tocopherol | 0.9977 | 0.365 | -0.866 | 517 | 109 | | |
| γ-tocopherol | 0.9999 | 0.460 | 0.280 | 618 | 138 | 200 | 36 |
| δ-tocopherol | 0.9999 | 0.215 | -2.829 | 705 | 62 | | |
| BHA | 0.9994 | 0.694 | -2.051 | 718 | 206 | 100 | 33 |
| PG | 0.9980 | 0.468 | -0.579 | 633 | 140 | 100 | 23 |
| Ascorbyl palmitate | 0.9967 | 0.073 | 1.982 | 632 | 24 | 500 | 20 |
| β-carotene | 0.9982 | 0.120 | 3.625 | 426 | 40 | 250 | 19 |
| BHT | 0.9979 | 0.192 | -3.021 | 1000 | 5 | 100 | 7 |

[a]Regression line equation: y=kx+b. r = coefficient of correlation; k = regression coefficient; b = y-intercept; x - μg substance in the polarographical cell; y = peak height in mm.
[b]Against saturated calomel electrode.
[c]0.5 ml tocopherol free soybean oil; 10 ml 0.25 N $H_2SO_4$ in toluene-ethanol 1:2 (v/v); 300 μg substance.
[d]Acc. to the Swedish food regulations (14).
[e]0.5 g oil sample; 10 ml 0.25 N $H_2SO_4$ in toluene-ethanol 1:2 (v/v).

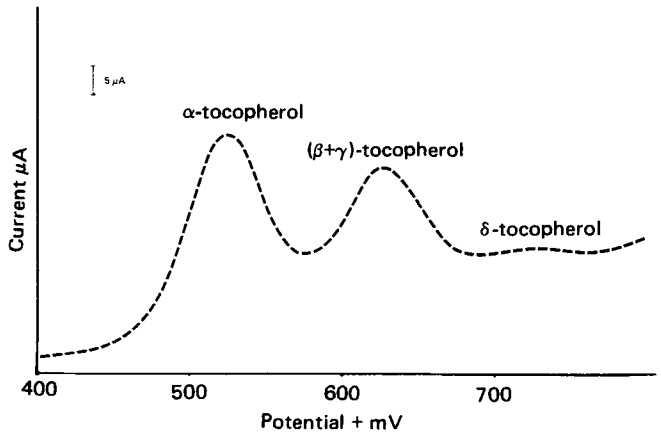


FIG. 1. A typical polarographic curve from the determination of tocopherols in ref. palm oil. (0.5 g sample; 50 μA current range).

chromatography) were purchased from E. Merck, (Darmstadt, Germany). α-Tocopherol was purchased from DPI Research (Rochester, NY) and from Sigma (St. Louis, MO), γ-tocopherol from Supelco (Bellefonte, PA) and δ-tocopherol from Fisher (Fairlawn, NJ). All the tocopherol standards were more than 97% pure according to the information from manufacturers. Butylated hydroxy anisole (BHA), butylated hydroxy toluene (BHT), and propyl gallate (PG) have been obtained from Naarden (Bussum, Holland). Ascorbyl palmitate and carotene (crystalline, and 20% in oil emulsion) were supplied by Roche (Basel, Switzerland).

Sulfuric acid 0.25N in ethanol-toluene 2:1 (v/v) was used as the base electrolyte and solvent.

### Apparatus

PAR model 174A Polarographic Analyser, Houston XY recorder Omnigraphic Model 2000-3-3, glassy carbon electrode, and Metrohm thermostated titration cell, were used.

### Procedure

Base electrolyte (10 ml) and an accurately weighed quantity of ca. 0.5 g oil sample were placed in the Metrohm cell and held at 25 C. The contents were rapidly blended by a magnetic stirrer, and thereafter the freshly polished glassy carbon electrode installed.

The current potential curve was obtained using the differential pulse mode in the potential range +300 to +1050 mV. Scan rate was 2 mV/sec, modulation amplitude 25 mV, and sensitivity 10-50 μA.

The peak heights were measured as the distance in mm between the top of each peak and the overall base line for all representative peaks.

## RESULTS AND DISCUSSION

### The Polarographic Responses

The linear regression lines (concentration: x-axis; peak height: y-axis) for the tocopherols and some additives are presented in Table I.

It is obvious that the regression lines (and consequently the polarographic responses) for the three studied tocopherols are not identical. The relation is as follows:

α-tocopherol:γ-tocopherol:δ-tocopherol 1:1.3:0.6

In view of these results it is not only diffusion, but also some other factor, which governs the height of a peak. However, no attempt has been made to explain this behavior.

TABLE II

Tocopherol Determination by HPLC and Polarography (Results in μg/g) and Reproducibility of the Polarographic Method[a]

| Oil sample | Number of determinations | | α-tocopherol | | | | γ-tocopherol | | | | δ-tocopherol | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | HPLC | Polarography | | | HPLC | Polarography | | | HPLC | Polarography | | |
| | HPLC | Polarography | $\bar{m}'$ | $\bar{m}$ | s | 100s/$\bar{m}$ | $\bar{m}'$ | $\bar{m}$ | s | 100s/$\bar{m}$ | $\bar{m}'$ | $\bar{m}$ | s | 100s/$\bar{m}$ |
| Sunflower 1 | 3 | 6 | 510 | 519 | 3.2 | 0.6 | 120 | 131 | 2.9 | 2.2 | 20 | <20 | | |
| Sunflower 2 | 2 | 6 | 507 | 507 | 10.7 | 2.1 | 108 | 111 | 2.3 | 2.1 | 3 | <20 | | |
| Sunflower 3 | 2 | 8 | 501 | 528 | 18.5 | 3.5 | 94 | 118 | 5.3 | 4.5 | --- | <20 | | |
| Sunflower 4 | 2 | 10 | 511 | 524 | 21.4 | 4.1 | 81 | 116 | 5.4 | 4.6 | --- | <20 | | |
| Cottonseed | 5 | 9 | 455 | 426 | 9.7 | 2.3 | 458 | 369 | 5.6 | 1.5 | tr | <20 | | |
| Ref. palm oil | 2 | 8 | 220 (130)[b] | 376 | 7.8 | 2.1 | tr (215)[b] | 192 | 2.2 | 1.2 | --- (22)[b] | 77 | 3.8 | 5.0 |

[a] $\bar{m}$: arithmetic mean; s: standard deviation.
[b] The values in parentheses are the corresponding tocotrienols.

A typical polarogram of tocopherols in ref. palm oil is shown in Figure 1.

### Interfering Substances

According to the Swedish food regulations (14), there are several permitted additives other than those already studied in the method (13). These are: citric acid and its salts and esters, ascorbic acid and its esters, β-carotene and some related dyestuffs and other additives without polarographic activity.

Ascorbic acid is not soluble in the polarographic base solution. Ascorbyl palmitate and β-carotene have peaks which lie in the actual potential area (Table I, col. 5). Ascorbyl palmitate fortunately is not detected by any method if added to vegetable oils.

The additives and tocopherols in their highest permitted concentrations (14) give peaks of about the same height. Only BHT has a noticeably lower peak.

### Model Trials with Interfering Substances

To examine the effect of the possible interfering substances, model mixtures were prepared of each tocopherol together with each of the additives. These mixtures were polarographed under standard conditions.

As expected, the peaks of γ-tocopherol and ascorbyl palmitate or propyl gallate do not separate at all; the same holds for the combination γ-tocopherol and BHA. The other combinations allow the evaluation of both peaks separately, but with an error of 0 to +25 rel.%. The combinations with δ-tocopherol are subject to the highest errors.

### Comparative Analyses

Six vegetable oils have been studied by two different methods: the newly developed HPLC method (4) and the polarographic method (13).

The results (Table II) show good agreement between the two methods, with only significant differences being shown by the t-test (99% sign. level) for γ-tocopherol in sunflower 3 and 4 and in cottonseed oil. It would appear that the values for ref. palm oil (which contains considerable quantities of tocotrienols besides tocopherols) are less satisfactory, but this sample is an exception which will be discussed in the next section.

### Behavior of Tocotrienols

According to evaluation of the HPLC chromatograms (assuming, that the fluorometric responses of the tocotrienols are the same as the tocopherols), the ref. palm oil contains approximately 130 μg/g α-tocotrienol, 215 μg/g γ-tocotrienol, and 22 μg/g δ-tocotrienol. It can be seen that the value for each "tocopherol" by the polarographic method is in fact a sum of the tocopherol and the corresponding tocotrienol, which are determined individually by the HPLC method.

These results could not be compared statistically because of the addition of random errors for the two determinations by HPLC. However, the results for α- and (β+γ)-"tocopherols" by polarography correspond well with the sum of values for tocopherols and tocotrienols by HPLC.

### High Melting Oils and Fats

Although the high melting oils and fats are difficult to dissolve in the recommended base solution, it is still possible to carry out the tocopherol determination. The molten fat is injected into the stirred base solution. The tocopherols dissolve before the fat solidifies and produces a finely dispersed suspension.

### Reproducibility and Detection Limits

The reproducibility of the method is max. ± 5 rel.% at 50-600 μg/g level for α-, (β+γ)-, and δ-tocopherol, as determined by repeated analysis of the six examined oils (Table II).

The detection limits have been estimated to 10 μg/g oil for α- and (β+γ)-tocopherols and to 20 μg/g oil for δ-tocopherol. The detection limit was defined as that tocopherol level whose detectable signal was at least double as high as the base line noise.

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