An Automated Method For Determining Tocopherol in Deodorized Soybean Oil

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Abstract

A method has been developed, utilizing the Technicon AutoAnalyzer, to determine the tocopherol content of deodorized oil. The chemistry of the Emmerie-Engel method, based upon the reduction of ferric chloride, is utilized in the development of this automated method. The tocopherol content of soybean oil is measured relative to a standard sample of d-α-tocopherol. The tocopherol content as measured by this method will include any other reducing substances present in the sample to be analyzed. The standard deviation, as derived from a pooling of the variances of four deodorized soybean oil samples is ± 0.0023% at the 0.1% level.

Introduction

In any wet chemical analysis where the amount of color development is measured after a specified reaction time, great care must be used to insure reproducible results. Since this type of timed-reaction analysis is so dependent upon the skill of the analyst, an automated procedure would be expected to obviate this difficulty.

In achieving reproducibility of analytical results, the manual procedure of the Emmerie-Engel method for tocopherol, as given by Mehlenbacher (1), was adapted to automation by using the Technicon AutoAnalyzer.

Experimental Procedures

Equipment

Technicon AutoAnalyzer (Technicon Corporation, Ardsley, New York) components: Sampler II, two-speed proportioning pump; colorimeter with 540 mμ filters and a 15 mm flow cell; heating bath with 80 foot coil providing a 10 min. time delay between points A, mixing of sample and reagents, and point B, measurement of color development, as shown in Figure 1.

Reagents

Solvent: 25:75 mixture (by volume) of ethanol and n-propanol; d-α-tocopherol (Distillation Products Industries of Eastman Kodak); α,α′-dipyridyl (Fisher

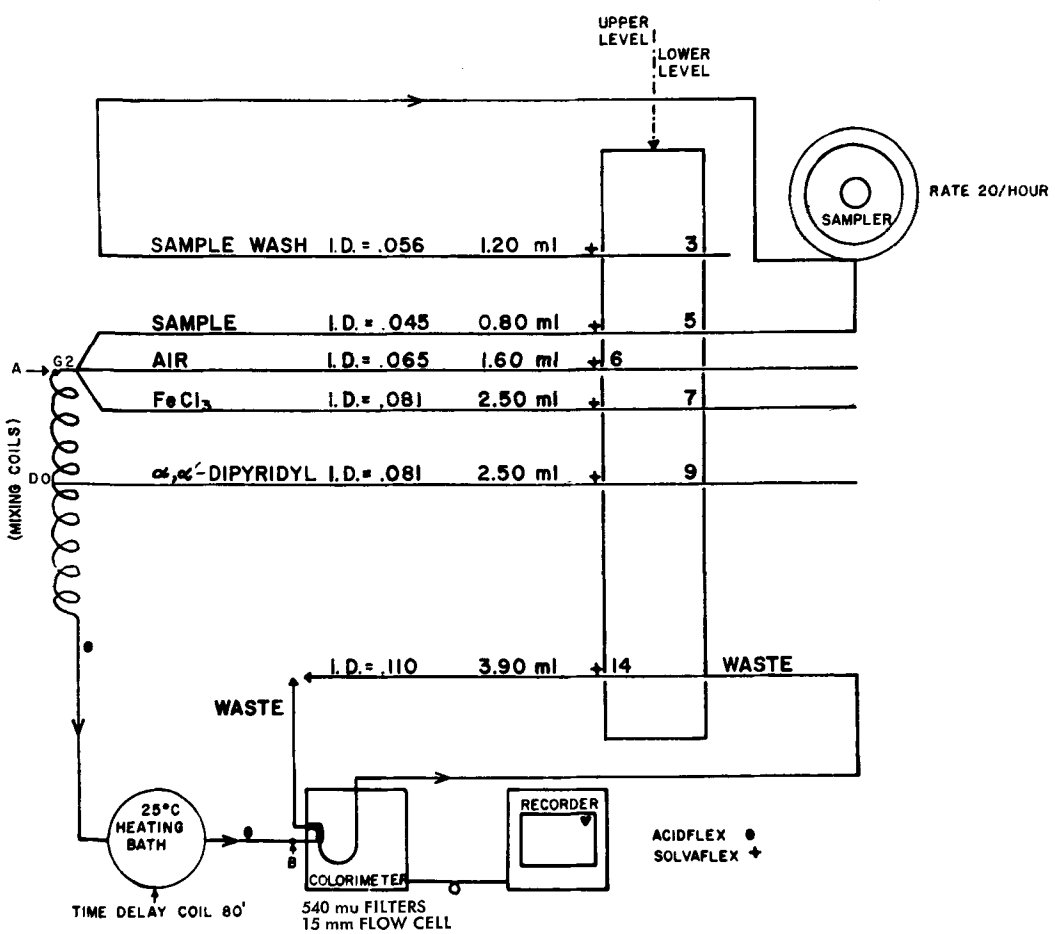

FIG. 1. Flow chart for AutoAnalyzer method of tocopherol determination using Technicon flow cell methodology.

TABLE I
Per Cent Reducing Material Expressed as d-α-Tocopherol in Deodorized Soybean Oil

Sample No.[a]	Arithmetic mean	Standard deviation
1	0.112	0.0015
2	0.114	0.0022
3	0.139	0.0022
4	0.106	0.0030

[a] Eight replicates of each sample were analyzed over a five day period.

Scientific Co.) 0.25 g/500 ml of solvent; and $FeCl_3 \cdot 6H_2O$ (Bakers Analyzed) 0.10 g/500 ml of solvent.

AutoAnalyzer Set-up

Arrange the AutoAnalyzer components as in Figure 1. Use Solvaflex (Technicon) tubing in the manifold to accommodate the organic solvents used and to help exclude light. Protect the mixing coils from light by wrapping in tape or housing in a closed cardboard box. Use black Acidflex (Technicon) tubing for transmission lines.

Preparation of Reference Standard

Transfer the 1 g sample of d-α-tocopherol to a 250 ml volumetric flask and dilute to volume with the solvent. Store this stock solution in the cold and dark. Prepare working standards from 0 to 40 ppm in increments of 5 or 10 ppm by dilution of the stock solution with the solvent.

Preparation of Samples

Weigh into a 25 ml volumetric 250–300 mg of oil and dilute to volume with the 25:75 mixture of ethanol and propanol solvent.

Determination of Tocopherol

Set up the AutoAnalyzer as shown in Figure 1 and pump reagents to establish a baseline. Set the recorder at zero absorbance when a steady baseline has been obtained. Place the standards on the sampler in increasing order of concentration and start the sampler. Read the absorbances of the standards and plot on graph paper to determine linearity (absorbance vs. concentration) and to determine if the function goes through zero absorbance.

Place the samples on the sampler with the appropriate concentration of standard placed after every fourth sample. The standard sample should have an absorbance close to that of the sample. The sample absorbance is then compared with the nearest standard absorbance and the per cent tocopherol obtained by a ratio and proportion calculation.

Results and Discussion

For the purpose of this work, the tocopherol percentages were calculated relative to a d-α-tocopherol standard without the use of the 0.91 correction factor (1) commonly employed to compensate for the differences in reaction rates of the various tocopherols with the Emmerie-Engel reagents. This direct approach was thought most likely to produce a uniformity of results as long as samples of similar origin were compared. The conditions of this automated method are quite close to the manual Emmerie-Engel method so that the usual 0.91 correction factor should be applicable.

The results obtained on four deodorized soybean oil samples are shown in Table I.

The four oil samples in Table I were held in the dark at 5 C for six months. The samples were reanalyzed and showed no change. Also, each of the four oil samples was spiked with a known amount of d-α-tocopherol. The average recovery for the four samples was 95.1%. Tocopherol results on several commercial products of deodorized soybean oil showed levels from 0.076% to 0.159% as determined by this method.

In conclusion, the simplicity and reliability of this automated method should produce greater uniformity of inter-laboratory results. This would be a way to achieve better process and quality control.

ACKNOWLEDGMENT

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REFERENCE

1. Mehlenbacher, V. C., "Analysis of Fats and Oils," Garrard Press, Champaign, Illinois, 1960, p. 586.

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