Analysis of Linoleate and Linolenate by Isomerization with Potassium *Tertiary* Butoxide in Mixtures of *Tertiary* Butanol and Ethylene Glycol Dimethyl Ether

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Abstract

A simple spectrophotometric procedure is described for determining the content of linoleic and linolenic acid in natural oils. Isomerization is accomplished at 70C with potassium *tertiary* butoxide (0.57 M) in 110 min. The absorptivities of linoleic acid ($k_{233} = 86.9$) and linolenic acid ($k_{233} = 66.9$ and $k_{268} = 46.9$) attain a maximum and remain constant thereafter.

Introduction

POLYUNSATURATED FATTY ACIDS can be determined by alkali isomerization, followed by spectrophotometric determination of the amount of conjugated material formed. The official AOCS method (1) has several disadvantages (2) however.

Isomerization catalyzed by potassium *tertiary* butoxide has been proposed, *e.g.*, for 20 hours at 60C (2) and for two hours at 140C (3). These methods also suffer from the disadvantage that the absorptivities of linolenic acid at 233 and 268 mμ reach a maximum and then decrease again before the absorptivity of linoleic acid has attained its maximum value.

It is known that olefin isomerizations catalyzed by potassium *tertiary* butoxide proceed much faster in ethylene glycol dimethyl ether than in *tertiary* butanol (4). This can be used to improve the analytical procedure for the determination of polyunsaturated fatty acids. Practical considerations and the results of a kinetic investigation of the potassium *tertiary* butoxide catalyzed isomerization of methyl linoleate (5) led to the selection of optimum conditions for the analysis.

Experimental Procedure

A 1.1-M solution of potassium *tertiary* butoxide is prepared by dissolving potassium in dry *tertiary* butanol. An equal volume of ethylene glycol dimethyl ether is added. The base concentration is then about 0.57 M.

About 100 mg of an oil are weighed accurately in a 1-ml glass beaker. The contents of the beaker are added to 10 ml of the isomerizing reagent in a 60-ml, long-necked reagent bottle. After 110 min at 70C the reaction is stopped by dilution with 20 ml of methanol and addition of 10 ml of 6 M HCl [to avoid the so-called glycerol effect (2)]. The mixture is transferred to a 100-ml volumetric flask and diluted with methanol to 100 ml; 110 min are the minimum time required. The same results are obtained when the reaction time is prolonged.

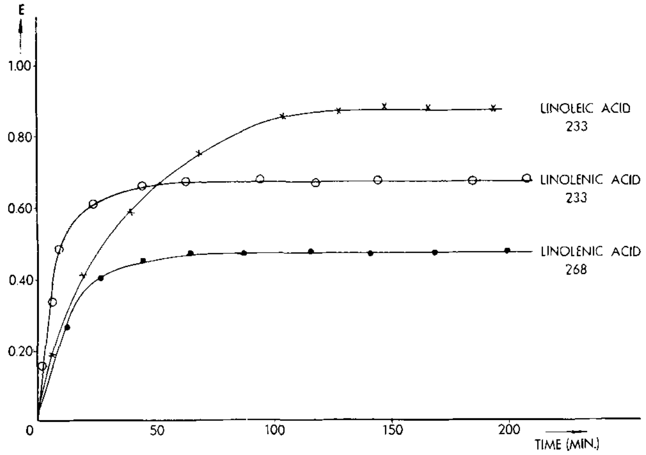

FIG. 1. Isomerization of pure methyl linoleate and linolenate by potassium *tertiary* butoxide in *tertiary* butanol/ethylene glycol dimethyl ether at 70C.

After isomerization, absorptivities at 233 and 268 mμ were measured with a Beckman DU spectrophotometer. The absorptivities of methyl linoleate and methyl linolenate were calculated after isomerization of the pure esters. The values calculated for the acids are linoleic acid $k_{233} = 86.9$, linolenic acid $k_{233} = 66.9$, and $k_{268} = 46.9$.

The amount of linoleate and linolenate in a natural oil can be calculated as follows:

$$\%\ \text{linoleic acid} = 1.151\, a_{233} - 1.493\, a_{268}$$

$$\%\ \text{linolenic acid} = 2.132\, a_{268}$$

Suitable corrections for preformed conjugation should be made.

Results and Discussion

Four natural oils were analyzed according to the AOCS method (1), the two methods using *tertiary* butanol as solvent (2,3), and the method proposed in this paper. Results are given in Table I.

The proposed method has distinct advantages over the three older methods. It gives reproducible results under mild conditions, and absorptivities at 233 and 268 mμ remain constant for a considerable time.

A longer reaction time, changes in temperature (between 50C and 75C), and changes in base concentration (between 0.15 and 0.80M) have no influence on the absorptivities (5).

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TABLE I
Analysis of Fatty Oils by Four Different Methods

Method	Reaction time	Temperature	Cottonseed		Soybean		Rapeseed		Sunflower	
			A	B	A	B	A	B	A	B
KOH AOCS (1)	25 min	180C	50.2	0	50.2	6.0	13.6	7.9	56.8	0
K-t-butoxide (2)	1200 min	60C	48.2	1.2	51.0	6.4	12.7	6.7	54.7	1.8
K-t-butoxide (3)	120 min	140C	50.5	2.2	50.1	9.0	13.8	8.9	57.9	2.3
This investigation	110 min	70C	52.4	1.1	50.5	8.7	13.5	9.1	57.0	1.1

[a] The measurements were made in triplicate. Dilutions were made so that absorbances were between 0.7 and 1.0. Absorptivities did not vary more than 2% between the three runs. A = % conjugatable diene, B = % conjugatable triene.

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