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DETERMINATION OF THE CONTENT OF HYDROXY GROUPS IN COTTONSEED OIL

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UDC 547.915:665.31

IR spectroscopy has been used to determine the content of hydroxy groups in cottonseed oil in relation to the periods of ripening. It has been found that the largest amount of hydroxy groups is present in the oil of the early stages of ripeness.

The oil of ripe cottonseeds contains a number of compounds with hydroxy groups, and observations have shown that their amount decreases as the seeds ripen. In view of this, the necessity arose for having available rapid and fairly accurate methods for the quantitative estimation of oxygen-containing compounds in the oil.

The present paper describes a method for determining hydroxy groups in the oil of cottonseeds during their ripening by IR spectroscopy.

The use of IR spectroscopy for the quantitative determination of hydroxy groups, ketones, and ether/ester groups in oxidized fatty esters and similar compounds has been reported previously [1, 2]. The method is based on finding the intensity of the characteristic absorption of the spectrum at the wavelength of the absorption maximum. It can be performed rapidly, requires only small amounts of sample (about 20 mg) and gives results agreeing well with lengthier chemical methods requiring considerably larger amounts of material. It is desirable to use the IR-spectroscopic method for the quantitative estimation of hydroxyl groups in cottonseed oils in relation to the vegetation periods when it is difficult to obtain the

Institute of the Chemistry of Plant Substances, Academy of Sciences of the Uzbek SSR, Tashkent. Translated from Khimiya Prirodnykh Soedinenii, No. 3, pp. 300-303, May-June, 1979. Original article submitted January 26, 1979.

0009-3130/79/1503-0259$07.50

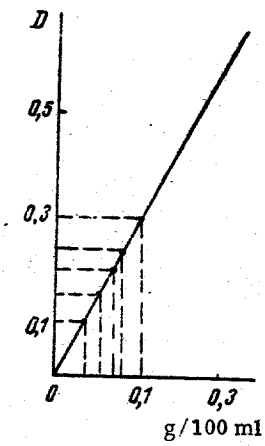

Fig. 1. Dependence of the optical density (D) in methyl ricinoleate on the product of the concentration and the layer thickness (c·ℓ): K_{ric} = 0.20; ℓ = 0.9 cm.

amount of oil necessary for chemical analysis. However, the content of hydroxy groups in the oil investigated proved to be below the resolving capacity of IR spectroscopy. To increase their concentration it was necessary first to separate the materials to be investigated into narrower fractions.

The samples for the analyses were prepared in such a way as to exclude errors connected with the association of the molecules. For this purpose, solutions of the samples were diluted until the absorption characteristic for associated hydroxy groups (broad band in the 3400-3500 cm^{-1} region) disappeared. Dilute solutions of the samples gave a narrow absorption band at about 3623 cm^{-1}, which corresponds to the vibrations of free hydroxy groups.

The performance of the analyses of solutions of the samples was preceded by the determination of the absorption coefficient of the pure substance at the characteristic frequency selected for analysis. The intensity of this absorption band was used to determine the percentage of hydroxy groups from the formula:

$$\%OH = \frac{K_{spec} \cdot 100}{K_{st}}, \quad (1)$$

where K_{spec} is the absorption coefficient of the specimen, and K_{st} is the absorption coefficient of a standard.

The absorption coefficient (K) was determined from a relation based on the Lambert–Beer law

$$K = \frac{D}{c \cdot l}, \quad (2)$$

where D is the optical density; c, the concentration of the specimen, g/liter of solution; and ℓ, the cell thickness, cm.

Methyl ricinoleate with its strongest absorption band in the region of about 3623 cm^{-1} was selected as standard. The graph, which reflects the dependence of the optical density of methyl ricinoleate on the concentration and the layer thickness, shows the absence of intermolecular associates. The mean absorption coefficient of methyl ricinoleate is 0.2.

The content of hydroxy groups in the oil was calculated on the basis of the quantitative characteristics of the IR spectrum of fractions enriched with hydroxy products, the gravimetric proportion of these fractions, and the total amount of fatty acids. Let us show the calculation of the amount of hydroxy groups in the oil on the basis of experiment 2. A sample of oil weighing 1.0410 g was taken for the determination and the fatty acids were isolated, their amount being 0.8757 g or 84.12%, and these were then separated by preparative TLC on silica gel and a fraction enriched with hydroxy products was isolated — it weighed

0.0196 g, i.e., 6.18% of the total fatty acids. This fraction was methylated, and it was subjected to spectrometry in the form of the methyl esters (0.0072 g). The amount of hydroxyls in it, according to the IR spectrum, was 72.5%. Then the percentage of hydroxy groups with respect to the fatty acids is

$$\frac{72{,}5 \cdot 6.18}{100} = 4{,}5\%,$$

and with respect to the oil

$$\frac{4{,}5 \cdot 84{,}12}{100} = 3{,}87\%.$$

Below we give the results of experiments reflecting the dynamics of the change in the amount of hydroxy groups in the oil as the seeds ripen:

Exp. No.	Ripeness of the seeds, days	Yield of fatty acids from oil, %*	Yield of fraction enriched with hydroxy products, %†	Amt. of OH groups according to IRS, %‡	Amt. of OH groups, % on fatty acids	Amt. of OH groups in the oil, %
1	10—20	64,1	12,4	145	18,0	11,5
2	30—40	84,1	6,2	72.5	4,5	3,8
3	60—70	94,6	3.3	65,0	3,1	2,0

Thus, as the seeds of the cotton plant ripen the amount of hydroxy groups in the oil decreases. The relatively high amount of OH groups in the oil of seeds 10- to 20-days ripe is due, according to preliminary results, to the fact that in addition to hydroxytriglycerides they contain an appreciable amount of such hydroxyl-containing compounds as high-molecular-weight alcohols, sterols, etc., this amount decreasing sharply as ripening proceeds.

EXPERIMENTAL

The IR spectra were recorded on a UR-20 instrument. Dismountable cells with sodium chloride windows were used. The solvent was carbon tetrachloride. The concentrations of the solutions of the samples at which the absorption characteristic for associated hydroxyls ceased were 0.7-1.4 mg/ml. The thickness of the layer of absorbing solution was 0.91 cm.

Preparation of the Samples. The oil (0.5-1 g) was extracted from freshly collected cottonseeds as they ripened by chloroform–methanol extraction according to Folch [3] and the fatty acids were isolated from them by exhaustive extraction with diethyl ether (after the separation of the unsaponfiables with petroleum ether). Then a fraction enriched with hydroxy compounds was isolated by preparative TLC on silica gel (from 0.2-0.5 g of fatty acids) [4] and were spectrophotometered in the form of their methyl esters (8-16 mg).

Preparation of Methyl Ricinoleate. The total fatty acids were extracted from castor oil by hot saponification and (after the removal of the unsaponifiables) they were converted into their methyl esters, and then pure methyl ricinoleate was isolated by descending column chromatography [5]. The purity was checked by thin-layer chromatography on Silufol plates in the solvent system hexane–diethyl ether (8:2).

SUMMARY

It has been established by IR spectroscopy that the amount of hydroxy groups in the seed oil decreases as cottonseeds ripen from 11.5% in 10-day seeds to 2.0% in ripe seeds.

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*In experiments 1 and 2, the yields of fatty acids were determined as percentages of the total lipids, since in the early stage of ripening of the seeds triglycerides proper are present in extremely small amounts.

†With respect to the fatty acids.

‡In the fraction enriched with hydroxy compounds.