Absolute Neutral Oil Content of Vegetable Oils by Radiochemical Technique

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Abstract

Laboratory refining loss, determined by the chromatographic method, is checked by a radiochemical procedure. Neutral triglycerides are labeled with C^{14}-tripalmitate and passed through an alumina column to find out if the alumina retains a percentage of them.

Cottonseed oil is also labeled and, by applying the calculations of the isotope dilution analysis, the absolute neutral oil content and therefore the theoretical refining loss are determined.

The theoretical and the chromatographic refining losses are compared, and a relation between them is proposed for cottonseed oils.

Introduction

THE REFINING EFFICIENCY of a vegetable oil is given by the ratio $N/N + N_1$, where N is the refinery yield of neutral triglycerides calculated as the weight percentage of the crude oil and $N + N_1$ the actual neutral oil, i.e., the actual percentage of neutral triglycerides in the crude oil as determined by analysis. To check refining efficiency, both N and $N + N_1$ are required. From these data the amount of neutral oil which is lost (N_1) through saponification and emulsification may be determined.

The refinery yield of neutral oil may be determined by various methods (1–3), but the actual percentage of neutral triglycerides is usually determined by one of the three generally recognized methods: acetone insoluble (4–9), Wesson (10–12), and chromatographic (13–20).

Since the actual results of any of these three methods differ, attempts have been made to establish a correlation between them. Purdum and Werber (8) determined the refining loss of many samples of cottonseed and soybean oil by the Wesson and acetone-insoluble methods and found relationships between them and the cup-test (3); Marcopoulos (21), in examining all three methods for pistacia seed oils, found relations between them and between the refining loss according to the chromatographic method and the FFA content.

However the problem of the precision of these methods still exists, and a scientific approach in determining their accuracy for the determination of neutral oil content would be interesting. Since the results of the three methods have already been correlated, the determination of the error in one of them could mean the simultaneous evaluation of the other two. Also, since the difficulty in any classical method is found in the absolute isolation of the whole amount of the neutral triglycerides content of the oil, the application of a technique by which this can be determined without complete isolation should be helpful.

This work concerns control of the chromatographic method by a radiochemical technique and determination of the absolute neutral oil content in a variety of Greek cottonseed oils. Also, the chromatographic refining loss is compared with the one determined by the radiochemical technique.

Experimental Section

Materials and Equipment

Chromatographic Columns: constructed according to the official AOCS method (19).

Alumina: Grade F 20, Mesh 80–200, from Aluminum Company of America, Chicago, Ill. Before use it was treated according to the official AOCS method (19).

Solvent: mixture of 975 ml of ether with 25 ml of methanol (Solution E-M) (19).

Labeled Triglycerides: glyceryl-1-C^{14}-tripalmitate was obtained from the Radiochemical Center, Amersham, Buckinghamshire, England.

C^{14}-Tripalmitate Carrier-Free Solution (Solution A): Prepared by dissolving 0.02 g of glyceryl-1-C^{14}-tripalmitate in 200 ml of E-M solution (total activity 1 μC).

Counting Techniques

The liquid scintillation counting technique was chosen as the more convenient for the low activities of β-radioactive C^{14}. As liquid scintillator, a solution of 4 g of 2,5 biphenyloxasol and 0.2 g 1,4 bi-5(2-phenyl)oxazolyl benzene per liter of toluene was used (Solution Sc).

Active samples were measured in small glass tubes (2×5 cm) 15 min after restoration in the counter's refrigerator (Packard Tricarb Liquid Scintillation Counting System, Model 314 EX).

Measurements were taken under conditions of HV 1050, amplification 1000, window 100–700 V. The used amount of radioactive tripalmitate was precalculated each time in order to give a count rate of the order of 10,000 c/3 min in the measured active samples. Under these conditions the efficiency of the counting system was found to be 66%. The repeatability of the measurements was checked by measuring the same sample repeatedly. The relative standard deviation of the values was found to be less than 1%.

Check of Chromatographic Method

Procedure

The radiochemical check of the chromatographic method was carried out by comparing the activity of a given amount of C^{14}-tripalmitate before and after passing through the alumina column. Since FFA, moisture, phosphatides, and volatile matter are kept in the alumina, the check of the chromatographic method concerns the possible amount of neutral triglycerides also retained in the alumina. For this purpose, neutral triglycerides were labeled by C^{14}-tripalmitate, and the chromatographic loss was checked as follows.

Neutral triglycerides from various cottonseed oils were isolated by chromatography. Then 2.5 g were dissolved with 20 ml of Solution A (containing 0.002 g of C^{14}-tripalmitate of total activity 0.1 μc). The solution was well homogenized and placed on an

alumina column. The percolate with the washes (Solution E-M) was collected in a volumetric flask of 200 ml, which was then filled with Solution E-M. Next, 5.0 ml (0.00005 g C^{14}-tripalmitate, 0.0025 μc) were taken and mixed in a glass tube with 5.0 ml Sc; the activity was measured.

A simultaneous blank determination was also carried out. First, 2.5 g of neutral triglycerides were dissolved with 20 ml of solution A in a volumetric flask of 200 ml, which was then filled with Solution E-M; 5.0 ml were taken, mixed with 5.0 ml of Sc in a glass tube, and counted. Results were compared.

The same procedure was followed without the amount of neutral triglycerides i.e., with C^{14}-tripalmitate as the only triglycerides present. Owing to the relatively small amount of carrier (specific activity 50 $\mu C/g$), the C^{14}-tripalmitate could be considered as "carrier-free."

Results

The control of the chromatographic method with triglycerides labeled with C^{14}-tripalmitate was carried out for triglycerides from three different cottonseed oils and was repeated five times for each sample. In Table I the count rates for each sample-blank pair were written. The comparison of the mean values showed that the difference between sample- and blank-measured activity did not exceed the 1% found as the limit of counting error. Consequently, if a difference between sample and blank count-rate existed, it was negligible and it might therefore be concluded that the whole quantity of neutral triglycerides was passed through the column.

In Table II the results from the treatment of carrier-free C^{14}-tripalmitate are shown. The procedure was followed for three different activities, and it was repeated five times for each one. The results show that the mean values of the count rates of each sample-blank pair are absolutely comparable and that the difference between them does not exceed the 1%.

In both cases, with or without carrier, the C^{14}-tripalmitate has been passed thoroughly through the column. Therefore, from this point of view, no error has been observed in the chromatographic method.

Determination of Refining Loss

The case may be different with a vegetable oil which may be considered as a mixture. By applying the chromatographic method for the determination of the refining loss, there is no way to check whether the neutral triglycerides have been thoroughly separated from the other substances or whether a small percentage has been retained in the column. This may be checked by radiochemical procedures, especially by the known "isotope dilution analysis."

A given amount of oil, containing the unknown weight of neutral triglycerides W_o, is mixed with the known weight W_1 of pure, labeled triglycerides, which has total radioactivity R. The specific activity S_1 of W_1 may be found from the relation,

$$S_1 = \frac{R}{W_1} \qquad [1]$$

The total amount of triglycerides in the mixture is now $W_o + W_1$ with the same total activity R but with the specific activity S_2 given from the relation,

$$S_2 = \frac{R}{W_o + W_1} = \frac{R'}{p} \qquad [2]$$

where R′ is the total activity of a fraction p of the quantity $W_o + W_1$.

It seems clear that, for the determination of S_2, it is not necessary to separate the total amount of $W_o + W_1$ but just a fraction p, no matter what percentage of $W_o + W_1$ has been retained in the column.

By comparing (1) and (2), the quantity W_o may be calculated without absolute separation:

$$W_o = \frac{W_1(S_1 - S_2)}{S_2} \qquad [3]$$

TABLE I

Radiochemical Control of the Chromatographic Method by Neutral Triglycerides[a] Labeled with C^{14}-Tripalmitate

Sample	1		2		3	
Specimen	Blank c/3m	Sample c/3m	Blank c/3m	Sample c/3m	Blank c/3m	Sample c/3m
1	11017	10924	10498	10318	11151	11108
2	11168	10963	10386	10426	11226	10992
3	11059	10982	10392	10404	11314	11154
4	11100	11005	10405	10502	11285	11186
5	11102	11018	10304	10328	11250	11224
Mean values	11089	10978	10397	10395	11245	11132

[a] Total weight of triglycerides taken: 2.5 g inactive triglycerides + 0.002 g C^{14}-tripalmitate (activity 0.1 μC).

TABLE II

Radiochemical Control of the Chromatographic Method by Carrier-Free C^{14}-Tripalmitate

Total weight	0.002 g		0.01 g		0.0004 g	
Radioactivity	0.1 μC		0.5 μC		0.02 μC	
Specimen	Blank c/3m	Sample c/3m	Blank c/3m	Sample c/3m	Blank c/3m	Sample c/3m
1	11002	10972	51039	50836	2232	2214
2	10953	10950	51026	51005	2200	2186
3	11028	10935	51805	50950	2216	2295
4	11056	11008	51560	51183	2228	2208
5	10980	10890	51762	51020	2195	2256
Mean values	11004	10951	51438	50998	2214	2231

Procedure

To take always the same amount of W_1, 0.02 g of C^{14}-tripalmitate was dissolved in a 200-ml volumetric flask with E-M solution, and the flask was filled (Solution A); 5 ml (containing 0.0005 g of C^{14}-tripalmitate) were taken each time.

The official AOCS method was followed. A given amount of oil was diluted in a small Erlenmeyer flask with 10 ml of E-M solution and 5 ml of Solution A. After the solution had been well homogenized, it was transferred to the alumina column. To take always a weight (p) of the same order and to avoid errors from the difference in density during the counting, a small modification was made. Instead of putting the percolate with the washes in a soxhlet flask, they were collected in a 200-ml volumetric flask, which was then filled with E-M solution. A 20-ml aliquot was taken and evaporated in a glass tube according to the official AOCS method. After drying, cooling, and weighing (wt p), 5 ml of Sc and 5 ml of E-M solution were added, the content was dissolved, and the activity R′ was measured. The specific activity S_2 was calculated.

Since preliminary experiments showed that the count rate of active samples varied with the density and the color of the solution, S_1 was calculated under the same conditions by determining blanks as follows. The same amount of oils was dissolved with 15 ml of E-M solution (no radioactive addition) and transferred to the column. The percolate and the washes

were collected in a 200-ml volumetric flask, 5 ml of Solution A were added, and the flask was filled with E-M solution. A 20-ml aliquot was taken, treated as above, and counted.

From the relation (3) W_o, the absolute neutral oil content can be calculated whether it is completely separated or not.

Results and Discussion

The refining loss and the neutral oil were determined by both the radiochemical procedure and the classical chromatographic one for 15 samples of Greek cottonseed oils with a great range in FFA content. For each sample the determination was carried out in triplicate, and mean values were calculated.

In Table III the results concerning the refining loss, as determined by both procedures, are tabulated. Oils are listed by increasing FFA content. These results show that the chromatographic refining loss in all cases is a little greater than the one calculated by the radiochemical procedure. Possibly this is attributable to an error in the chromatographic refining loss.

In the last column of Table III the percentage of the difference between the two methods is tabulated as calculated on the basis of radiochemical refining loss.

A plot of mean values of the refining loss, according to the two procedures, gives a straight line. The slope and the intercept of this line, calculated by the method of least squares, were found to be respectively $b = 1.06$ and $a = 0.16$. The sd of b and a were found to be $S_b = \pm 0.010$ and $S_a = \pm 0.20$.

The correlation coefficient of the straight line which was obtained was found to be $r = 0.99$, giving a good criterion of linearity. S_a is greater than a, raising doubts about the existence of an intercept. But the previous experiments, where neutral triglycerides passed completely from the column, showed that, where the theoretical refining loss equals zero, no chromatographic refining loss could be observed. Therefore a ought to be eliminated.

Consequently, for the average relationship between theoretical refining loss (determined by the radiochemical procedure) and chromatographic loss, the following expression may be proposed:

$$\text{Chromatographic refining loss} = 1.06\ (\text{theoretical refining loss}) \qquad [4]$$

The fact that the chromatographic refining loss exceeds the theoretical loss only when the oil contains FFA and other substances, but not neutral triglycerides, indicates that, during the separation, alumina retains a small percentage of neutral triglycerides in addition to FFA and the other matter. It will be worth while to find out whether this is attributable only to FFA or to another kind of mixed matter. This work has already been undertaken, and it will be reported soon.

TABLE III

Refining Loss of Greek Cottonseed Oils, Determined by the Chromatographic Method (C%) and Calculated by Radiochemical Procedure (R%)

Sample	% FFA	Refining Loss		
		R% Theoretical	C% Chromatographic	100 (C-R) / R
1	0.2	0.2 ± 0.04	0.2 ± 0.04	0.0
2	8.8	9.1 ± 0.10	9.2 ± 0.06	1.1
3	10.9	12.7 ± 0.26	13.8 ± 0.06	8.7
4	12.4	13.5 ± 0.04	14.8 ± 0.14	9.6
5	12.4	13.8 ± 0.15	15.0 ± 0.14	8.7
6	14.4	15.6 ± 0.24	16.6 ± 0.32	6.4
7	16.2	17.5 ± 0.32	19.1 ± 0.10	9.1
8	20.3	20.7 ± 0.17	22.1 ± 0.04	6.8
9	24.0	24.1 ± 0.27	25.4 ± 0.10	5.4
10	24.2	24.5 ± 0.63	26.6 ± 0.17	8.5
11	26.5	29.2 ± 0.04	30.5 ± 0.07	4.4
12	27.8	29.1 ± 0.55	30.8 ± 0.04	5.8
13	27.9	29.0 ± 0.13	30.6 ± 0.04	5.5
14	28.2	28.3 ± 0.60	30.5 ± 0.21	7.7
15	32.7	35.1 ± 0.79	37.4 ± 0.17	6.5

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