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Chromatographic Analysis of Mixtures of Mono-, Di-, And Tristearin Containing Mineral Oil

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THE ESTIMATING of the mono-, di-, and triglyceride contents of processed oils and fats by strictly chemical methods of analysis presents certain difficulties. Several chemical methods applicable to the analysis of glycerides have been reported in the literature (1, 2, 3) but are both time-consuming and tedious. Methods, other than chemical procedures, based on chromatographic resolution also have been applied to this problem. Börgstrom (4) reported the use of a chemical method followed by a chromatographic resolution, using silicic acid as the adsorbent to separate mixtures of glycerides. Hamilton and Holman (5) in their work utilized displacement chromatography for the separation of known mixtures of glycerides, using charcoal as the adsorbent. Later Diekert and Reiser (6) reported a partial separation of glyceride mixtures by use of paper chromatography utilizing silicic acid-impregnated glass fiber paper as the adsorbent. Other investigators (7, 8) have separated the glycerides from natural products by means of chromatographic procedures; however, in most instances, only qualitative separations were reported.

Kaufmann and Wolf (9) reported that silica gel adsorbs glyceryl stearates in the following order: mono-, di-, and tri-, the most polar component being adsorbed to the strongest degree. This sequence of adsorption suggests that it should be possible to elute, selectively, each component from a glyceride mixture in a relatively pure state with a proper solvent system. This would be achieved best by initially selecting a solvent system of low polarity subsequently followed by a gradual increase in polarity. As a result, the component that is least strongly adsorbed on the silica gel, *viz*, the triglyceride, would be eluted first while the component most strongly adsorbed, the monoglyceride, would be eluted last. This paper describes a method suitable for the quantitative separation and determination of mono-, di-, and tristearins in the presence and absence of mineral oil.

The procedure developed involves the separation of the component glyceride esters by elution chromatography with the subsequent determination of the separated components by gravimetric methods or saponification analysis.

Experimental

Apparatus. A 20-mm. borosilicate glass chromatographic column 45 cm. long was used. The column was packed by using a close fitting glass plunger. Aluminum moisture pans.

Reagents.

Ligroin (Skellysolve A,[2] boiling point range 40°C.–60°C.) purified by shaking with sulfuric acid, washing with water, then drying over calcium chloride and distilling

Isooctane[3] 99.5%—purified in the above manner for Skellysolve A

Mono-, di-, and triglycerol stearates, obtained by large scale chromatographic separation of a mixed glyceride sample

Isopropyl ether—purified by washing with ferrous sulfate solution to remove peroxides, washing with water, then drying over sodium sulfate and distilling

Anhydrous ethyl ether (reagent grade)

Absolute ethanol, aldehyde-free—distilled over magnesium

Benzene, thiophene-free, redistilled

Silica gel, Davison No. 922[4] (200 mesh), used as supplied from commercial source without further treatment

Packing the Column. Twenty-five grams of silica gel were packed incrementwise into the column, care was taken to obtain a uniform packing throughout the column. After filling the column, isooctane was allowed to percolate through the packing until the silica gel was completely wetted.

Preparation of the Sample Solution. One to two grams of the glyceride sample and 500 mg. of mineral oil were weighed separately. They were combined and dissolved in a 70% solution of benzene in isooctane with the application of gentle heat. The solution was transferred to a 100-ml. volumetric flask and filled to the mark.

Sample Addition and Development of the Column. A 10-ml. aliquot of the sample was pipetted onto the chromatographic column. The usual chromatographic precautions were taken while adding the sample and washing down following the addition. The eluant used to resolve the mixture had the following compositions:

Fraction	*Composition*
0– 6	16% isopropyl ether in isooctane
6–12	100% isopropyl ether
12–18	70% ethyl ether in isooctane
18–25	20% ethanol in isopropyl ether

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[3] Eastman Organic Chemicals, Distillation Products Industry, Rochester, New York.

[4] Davison Chemical Company, Baltimore, Md.

All percentages of the eluting solvents were computed on a volume basis.

Ten-ml. fractions from the column were collected and transferred to previously weighed aluminum moisture pans. The eluted solvent was allowed to evaporate spontaneously over a period of 24 hrs. Extreme caution was taken to prevent the entrance of any extraneous material during the evaporation. The residue in the pans was determined by reweighing. Blank determinations were made simultaneously.

Results and Discussion

The degree of separation obtained for a mixture consisting of mono-, di-, and tristearin and mineral oil is shown in Figure 1. The mineral oil was added to the glyceride mixture to determine whether or not a non-polar material might be differentially eluted from the silica gel. Results show that the mineral oil was eluted by the solvent of lowest polarity, indicating that relatively little adsorption of this component on the silica gel was obtained and that tristearin was actually polar enough to be absorbed.

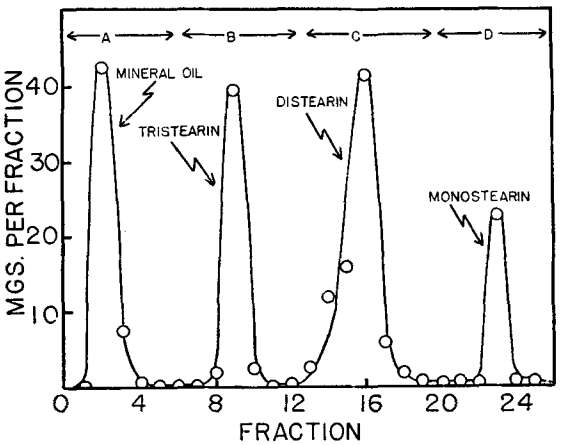

FIG. 1. Chromatogram of a glyceride mixture with mineral oil. The eluting solutions were:
A— 16% isopropyl ether in isooctane
B—100% isopropyl ether
C— 70% ethyl ether in isooctane
D— 20% absolute ethanol in isopropyl ether

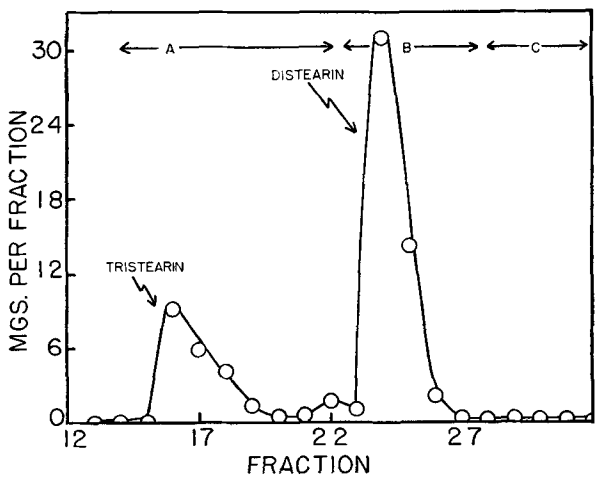

FIG. 2. Chromatogram of a glyceride mixture. The following eluants were used:
A— 30% ethyl ether in Skellysolve A
B— 60% ethyl ether in Skellysolve A
C—100% ethyl ether

It was found from this study that a solvent system containing various concentrations of ethyl ether in Skellysolve A had only sufficient polarity to elute the tri- and diglyceride and not the monoglyceride. An example of this resolution is illustrated in Figure 2. The monostearin which was more strongly adsorbed remained on the column until a more polar solvent system containing 20% ethanol in ethyl ether had been added. A combination of the two solvent systems gave a satisfactory separation of the mono-, di-, and tristearin. It had however one main disadvantage. The evaporation rate of the solvent from the aluminum pans was too rapid. As a result, the glyceride crept up the sides and eventually out of the pans, giving rise to a very serious source of error. This difficulty was avoided by using a solvent system containing different concentrations of isopropyl ether in isooctane followed by 15% ethanol in isopropyl ether. When pure isopropyl ether was used however, the polarity was insufficient to elute all of the distearin. Furthermore the 15% ethanol isopropyl ether system was of sufficient polarity that the monostearin and the distearin were not completely resolved. The results obtained by using these solvent systems are illustrated in Figure 3.

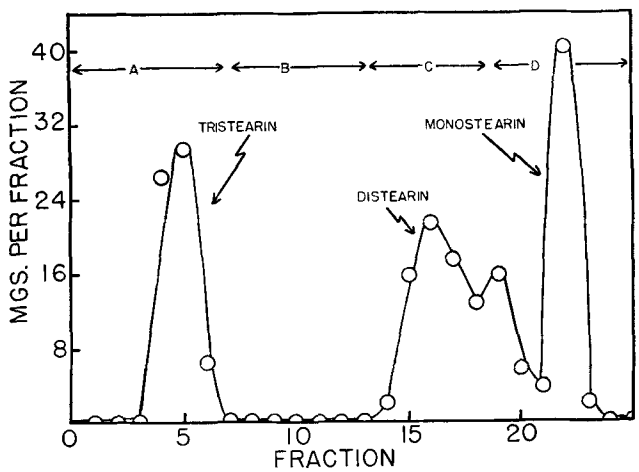

FIG. 3. Chromatogram of a glyceride mixture. The eluting solvents were:
A— 40% isopropyl ether in isooctane
B—100% isopropyl ether
C— 5% absolute ethanol in isopropyl ether
D— 15% absolute ethanol in isopropyl ether

Satisfactory separation of the glyceride mixture was obtained, using isopropyl ether followed by 70% ethyl ether in isooctane. This was then followed by 20% ethanol in isopropyl ether. The use of pure isopropyl ether resulted in the elution of the tristearin while the 70% solution of ethyl ether in isooctane eluted the distearin. The monostearin was recovered on the addition of 20% ethanol in isopropyl ether. For mixtures containing mineral oil a 16% solution of isopropyl ether in isooctane was sufficient to elute the mineral oil.

The solvent system developed from the preliminary studies was applied to several mixtures of glycerides, one being a commercial sample of tristearin. The chromatogram of the latter can be seen in Figure 4.

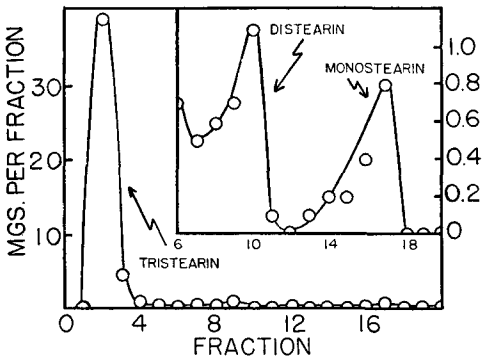

FIG. 4. Chromatogram of a commercially pure Tristearin sample. The eluting solvents were similar to solutions B, C, and D of Figure 1.

A summary of the analytical results are shown in Table I. The recovery appears to be quite satisfactory.

The synthetic mixtures used in this study were analyzed by a chemical method (10). The results of these analyses are shown in Table II. This gives a comparison of the accuracy of the two methods.

The above procedure was applied to two known mixtures of mono-, di-, and tristearin to test the reliability of the method. The results obtained are shown in Table III.

TABLE I

Recovery of Components of Glyceride Mixtures

Sample	Glyceride added	Mineral oil added	Mineral oil recovered	Recovered			Total
				mono-	di-	tri-	
	mg.	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	
1	150.0	51.9	51.4	24.5	80.0	43.6	199.5
2	100.0	50.3	49.7	35.7	50.5	12.6	148.5
3	100.2	50.0	49.8	15.9	53.1	31.2	150.0
Tristearin	50.5	.....	.....	1.5	3.1	45.7	50.3

TABLE II

Comparison of Chemical and Chromatographic Method of Analysis

Sample	Tristearin		Distearin		Monostearin	
	Chem.	Chrom.	Chem.	Chrom.	Chem.	Chrom.
	percentage		*percentage*		*percentage*	
1	29.00	29.07	55.80	53.50	15.20	16.20
2	11.10	12.60	51.20	50.50	37.70	35.70
3	.....	31.14	.....	52.99	15.80	15.87

TABLE III

Recovery of Components of Known Mixtures of Glyceryl Stearins

Mixture	Glyceride added	Mono-		Di-		Tri-		Total
		added	recov.	added	recov.	added	recov.	
	mg.	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
1	100.2	40.1	39.7	20.0	20.1	40.1	40.2	100.0
2	100.1	20.1	19.7	50.1	49.8	30.0	30.4	99.9

The precision and accuracy of the chromatographic method seems to be quite satisfactory for most purposes.

Summary

A procedure for the quantitative separation and determination of mono-, di-, and tristearin by elution chromatography is presented. The individual components are found to be absorbed on silica gel in the following order: mono-, di-, and triglycerides. By using a solvent system of suitable polarity, it is possible completely to separate individual components of a mixture from one another. Satisfactory separation of the stearin mixture is obtained by using 100% isopropyl ether, followed by a solution of 70% ethyl ether in isooctane. This latter solvent system is then followed by a solution of 20% ethanol in isopropyl ether. This chromatographic method compares favorably with the chemical method of analysis of glyceride mixtures.

Acknowledgment

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THE FOLLOWING TABLE gives the averages of the lint yield analyses obtained from three sets of samples sent out during the past year. All three samples were second-cut linters. No hull-fiber samples were sent out as very little of this material is sold at the present time.

Lab. No.	No. of tests	A Linter	B Linter	C Linter	Over-all average for the year
1	3	79.3	73.3	69.2	73.9
2	3	79.3	73.1	69.2	73.9
3	3	79.4	73.9	69.7	74.3
4	3	79.6	73.0	69.1	73.9
5	3	79.1	73.5	69.7	74.1
6	3	79.7	73.3	69.5	74.2
7	3	79.4	73.4	69.2	74.0
9	3	79.3	73.2	69.4	74.0
10	3	80.6	74.4	70.4	75.1
Average	....	79.5	73.5	69.5	74.2

As seen by the above results, very good checks were obtained showing that the laboratories are keeping their equipment in good shape. There were a few instances in which the laboratory would be off a little in one set but would get in line as soon as the check analyses were returned.

We believe that it is still a good idea to keep checking laboratories at least three times a year to keep all equipment in good shape. Therefore it is recommended that samples be sent out at least three times during the coming year to check on laboratories equipped to run cellulose yield analyses.

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