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Separation and Determination of Mono-, Di-, and Triglycerides in Monoglyceride Concentrates

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MONOGLYCERIDE CONCENTRATES are the most commonly used emulsifier in shortenings. They contain varying amounts of tri-, di-, and monoglyceride and free glycerine, depending on their method of preparation and subsequent concentration treatment. The α-monoglyceride content of the monoglyceride concentrate can be determined by periodate oxidation methods (8), but no direct chemical procedure exists for determining total monoglyceride, diglyceride, and triglyceride in the presence of each other. The glycerides may be separated from each other by batchwise (6) and countercurrent extractions (4), by separation of their urea adducts (1), or by micromolecular distillation (9). Some of these methods require expensive equipment and considerable time for each determination, and others lack the desired accuracy.

Chromatographic methods appear to be the best approach to the separation and determination of the glycerides. For very small amounts of glycerides, paper chromatographic separations have proved very useful for semiquantitative work (3, 7). Quantitative results have been obtained when column chromatographic methods are used. Displacement chromatographic methods (5) have been applied to model mixtures of glycerides. Good results have been reported when a reverse phase partition chromatographic procedure (11) is used. The latter method is difficult to use because precise preparation of the siliconed kieselguhr absorbent and rigid conditions of temperature, sample size, and solvent flow through the column must be maintained.

Borgström (2) reported a silicic acid chromatographic procedure for separating mono-, di-, and triglycerides formed during the hydrolysis of triglyceride by pancreatic lipase. The present method offers several advantages over that of Borgström. When silica gel rather than silicic acid is used, larger amounts of mixed glyceride can be separated with small volumes of solvent. In addition, alcohol has been eliminated from all the solvent mixtures since its presence tends to cause isomerization of the monoglycerides. Since the present method was submitted for publication, a silica gel column chromatographic method for the separation of mono-, di-, triglycerides and mineral oil was published (10).

The present investigation has shown that adsorption chromatography affords a simple method for separating quantitatively mixtures of tri-, di-, and monoglyceride. The glycerides are adsorbed on a silica gel column. The number of hydroxyl groups in the glyceride affect the tenacity of adsorption. The chain length, position, and unsaturation of the fatty acid group have a minor effect on the adsorption. Thus monoglyceride having two hydroxyl groups is the most strongly adsorbed, followed by diglyceride, then by triglyceride, the least strongly adsorbed. The triglyceride can be readily eluted with benzene and diglyceride with 10% ethyl ether in benzene. Monoglyceride is eluted with ethyl ether. By using 200 ml. of these solvents, good separations between the tri-, di-, and monoglyceride fractions were obtained. An elution curve for a typical commercial product is given in Figure 1. The diglyceride and monoglyceride are adsorbed at the top of the column and remain in the upper third of the adsorbent during the elution of the triglyceride. Monoglyceride moves to the middle third of the column during the elution of diglyceride.

Apparatus and Reagents. Chromatographic columns (Figure 2): available from H. S. Martin Company.

Separatory Funnel: 250 ml., with 19/22 joint sealed on.

Solvents: Petroleum ether, benzene, chloroform, and ethyl ether—reagent grade.

Silica Gel: Davidson Grade 923, 100–200 mesh, adjusted to contain 5% water. Mixing in a Patterson-Kelly Twin Shell Blender gives a homogeneous mixture.

Sample Preparation. Accurately weigh a 1.0-g. sample into a 50-ml. beaker. Add 15 ml. of chloroform to dissolve the sample. The sample may be

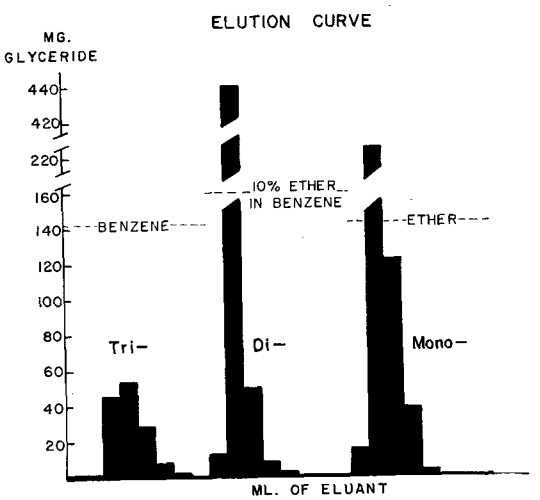

FIG. 1. Elution curve of a commercial product.

warmed a few minutes on the steam plate if necessary to bring about dissolution. It should be cooled to room temperature before adding to the column.

Column Preparation. Assemble the apparatus as shown in Figure 2 except that the dropping funnel is not attached. Cover 30 g. of hydrated silica gel contained in a 150-ml. beaker with petroleum ether. Stir the mixture with a glass rod to expel air bubbles. Add the slurry to the column. Rinse all of the silica gel into the column with additional increments of petroleum ether and wash down the side of the column with the same solvent. When the solvent level falls to 2 cm. above the top of the silica gel, add the sample slowly so that channels are not formed. Rinse the beaker with 5 ml. of choloroform, and add to the column when the level falls to 2 cm. above the adsorbent. The columns should never be allowed to become dry on top. The stopcock at the bottom of the column is adjusted so that 1.5–2 ml. of effluent are collected per minute. This rate is maintained throughout the procedure.

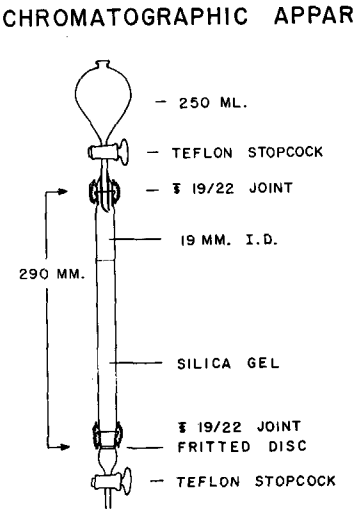

FIG. 2. Chromatographic apparatus.

Elution of Tri-, Di-, and Monoglyceride. Attach the dropping funnel to the column, and add 200 ml. of benzene. Begin collecting the effluent in a 250-ml. tared Soxhlet. After all the benzene has been added and the solvent head is at the 2-cm. level above the adsorbent, add 200 ml. of 10% ethyl ether in benzene (V/V). Change receivers to a tared 250-ml. Soxhlet for collecting diglyceride. When all of the diglyceride eluant has been added and the solvent is at the 2-cm. level above the silica, add 200 ml. of ethyl ether for elution of monoglyceride. When the ether is added, the column may go dry if the pressure in the column is not released. This is done by lifting the connection at the top of the column and allowing the solvent to flow in from the funnel. Change receivers and collect the monoglyceride in a tared 250-ml. Soxhlet.

Gravimetric Determination of the Separated Glycerides. Evaporate the eluates on a steam bath under a stream of nitrogen or clean, dry air. Allow the Soxhlets to stand on the table top for 15 min. before weighing. Put the sample under the nitrogen jets for 5 min., and weigh again after 15 min. If the two weights do not agree within 0.002 g., repeat the 5-min. evaporation procedure. Subtract the tare weight from the total weight. Calculate the percentage of glyceride as follows:

$$\% \text{ triglyceride} = \frac{\text{grams triglyceride} \times 100}{\text{sample weight}}$$

$$\% \text{ diglyceride} = \frac{\text{grams diglyceride} \times 100}{\text{sample weight}}$$

$$\% \text{ monoglyceride} = \frac{\text{grams monoglyceride} \times 100}{\text{sample weight}}$$

Results and Discussion

A simulated monoglyceride concentrate was made up by mixing known weights of tripalmitin, distearin, monopalmitin, and glycerine. The results are summarized in Table I.

TABLE I
Mono-, Di-, and Triglyceride in Known Mixtures

Component	Calculated %	Wt. % found	Average	Averge deviation from calculated value
Tripalmitin	15.1	14.4, 14.7, 14.9	14.7	0.4
Distearin	45.1	45.2, 45.2, 45.5	45.3	0.2
Monopalmitin	37.8	37.5, 37.4, 36.7	37.2	0.6
Glycerine	2.0	....	....	....

Infrared spectra of the individual glycerides before and after separation on the silica gel were identical.

To test the reproducibility of the method, commercial monoglyceride concentrates were chromatographically separated. The data obtained for these samples together with the glyceride distribution of some concentrates containing different levels of mono-, di-, and triglyceride are included in Table II.

TABLE II
Mono-, Di-, and Triglyceride in Production Samples

	1	2	3	4	5	6
Triglyceride % found	12.4, 13.0 12.4, 13.0	12.8, 13.0 12.6, 12.9	2.8 3.7	18.2	4.4	11.6 11.7
Av.	12.7	12.8				
Av. deviation from mean	0.3	0.2				
Diglyceride % found	47.4, 47.3 47.4, 47.3	45.7, 45.5 46.3, 45.6	28.9 28.8	41.8	34.6	43.3 43.3
Av.	47.4	45.8				
Av. deviation from mean	0.1	0.3				
Monoglyceride % found	35.0, 36.1 35.2, 35.1	38.3, 37.5 37.6	52.2 52.8	36.9	56.4	42.1 42.0
Av.	35.4	37.8				
Av. deviation from mean	0.4	0.3				

The reproducibility of the method is good. Although the commercial samples contain variations in fatty acid chain length and degree of unsaturation, the separation between the mono-, di-, and triglyceride fractions was sharp. No detectable differences were obtained when various lots of adsorbent and solvent were used. The total glyceride content of the monoglyceride concentrates determined ranged from 85–97%; the difference was the amount of free glycerine present in each receipt. Free glycerine remains strongly adsorbed on the column.

The method can be used as a routine procedure. Eight hours of elapsed time are required to complete a single analysis, and at least six determinations can

be run simultaneously by one analyst. Sufficient quantities of the separated glycerides are obtained so that characterization by chemical analysis can be made. The method is applicable to mixtures containing mono-, di-, and triglycerides and glycerine.

Summary

Monoglyceride concentrates are quantitatively separated into mono-, di-, and triglyceride components on silica gel columns by an adsorption chromatographic technique. The separated glycerides are determined gravimetrically. The adsorption on silica gel is dependent on the number of hydroxyl groups in the molecule, and the influence of unsaturation and chain length is minimized. Combinations of benzene and ethyl ether are used for elution, thus preventing isomerization, which frequently results when polar solvents such as alcohols are used.

The procedure for the chromatographic separation is simple and straightforward. The equipment used is easily obtainable. The silica gel adsorbent requires only adjustment of its water content before use.

Mixtures of both saturated and unsaturated glycerides and those having different fatty acid chain length have been separated. Known mixtures have also been analyzed. For a known mixture containing 15% tripalmitin, 45% distearin, and 38% monopalmitin the average deviations from the calculated values were 0.4%, 0.2%, and 0.6%, respectively.

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An Instrument for Measuring the Hardness of Fats and Waxes[1,2]

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APPARENTLY a really satisfactory method of measuring the hardness of fats has heretofore not been described in the literature. The needle and cone penetrometers, together with their various modifications, are suitable for measuring the consistency of plastic, semi-solid fats but are not suitable for measuring the hardness of solid or substantially solid fats. Instruments which do measure hardness are commercially available but leave something to be desired when used on products like cocoa butter and confectionery fats.

The Shore Durometer is currently in wide use to measure the hardness of wax-like products. It consists essentially of a frustoconical indentor, a spring to force the indentor into the test sample, and a scale-and-pointer arrangement to indicate the depth of the indentation. Among the undesirable features of this instrument are the variation of load with depth of indentation, the use of a fatigue-susceptible spring to supply the load, an arbitrary scale (0–100), and a limited range. The latter results in readings of 100 for different samples of quite hard materials, which obviously do not have the same hardness.

A survey of other instruments and procedures which might be used with hard fats and waxes indicated that a modified Brinell hardness test would be most likely to meet all requirements. The Brinell test (A.S.T.M. Method E 10-54 T) has been used for many years to determine the hardness of metallic materials. Confectionery fats, which may consist of 75% or more of hard crystals at room temperature, and completely solid fats and waxes have a number of properties in common with metal crystals.

Tests of the Brinell types consist of pressing a perfectly round ball onto the test surface until there is obtained an impression, the diameter of which is equal to a fraction of that of the steel ball. Sometimes the impression is so slight that it is barely visible to the naked eye. Under such slight deformation of the test surface the results tend to be removed from the realm of empiricism and placed on a more or less fundamental basis. The hardness units tend to be related to well-defined physical properties. It has been demonstrated (2), both theoretically and experimentally, that when a ball of given diameter under a given force penetrates an elastic, isotropic material, the depth of indentation is determined solely by the elastic modulus of the material, provided the strain is small. While solid fats and waxes are not elastic, isotropic materials, they would be expected to behave to some degree like such materials.

There appears to be no published evaluation of the adaptation of the Brinell test to determining the hardness of solid or substantially solid fats. Ravich and Volnova (1) used one adaptation of the test to measure the hardness of mixtures of tripalmitin and tristearin but did not report all of the conditions under which their tests were made. Also they were not concerned with the effect of operating procedures on the hardness values. Von Rosenberg (2) described a test procedure for determining the hardness of waxes which embodied some of the principles of the Brinell test. He devised an instrument for forcing a plunger, which measured 5 mm. in diameter and had been ground to a hemispherical point, into the test sample; the force was such that the depth of penetration was usually less than 0.5 mm. With the load on the plunger the penetration was measured in 0.001-mm. units. Then the load was removed, and the penetration was again measured. From these values the elastic and permanent deformations were calculated, and the latter was then used to calculate a hardness index.

The present report will be concerned with the

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