that detector response for these materials is not proportional to the *number* of active carbon atoms (n), but to the *relative weight percents* of these active carbons in the molecules.

On the basis of detector response proportional to the relative weight percents of n carbon atoms for the methyl esters of saturated fatty acids of chain length n, taking stearate as 1.00, the corrections to wt percent composition for methyl laurate, myristate, and palmitate are respectively 1.077, 1.044 and 1.019. The application of these factors, rounded off to 1.08, 1.04 and 1.02, (Table I) gives a significant improvement in correlation of wt percent composition and corrected area response over that for area response alone. Although not statistically significant, the average correction factors calculated from the experimental data are respectively 1.077,1.049 and 1.022 for laurate, myristate and palmitate relative to stearate as 1.00. The average deviations, respectively of 0.013, 0.007 and 0.011, illustrate however that the application of these correction factors to single analyses where the experimental errors are of the same order of magnitude may be of limited value.

The influence of unsaturation is less marked, and correction factors have only a small effect on calculated iodine values. Appropriate correction factors in the C_{18} chain lengths, relative to stearate as 1.00, are respectively 0.993, 0.987 and 0.980 for methyl oleate, linoleate and linolenate. In analyses of linseed oil methyl esters the application of correction factors to all significant components reduced the calculated I.V. to 182.3 from the value of 183.3 calculated from the uncorrected areas (I.V. Found, 181.9). Similarly in an analysis of a complex methyl ester mixture from a marine lipid, with acids ranging from myristic to docosahexaenoic, the reduction in calculated I.V. with correction factors was only from 244–238 (I.V. Found, 240). Although in the latter analyses agreement is seldom as close, the correction is normally an improvement.

The theoretical correction factors should be independent of most normal operating variables (cf. 8) excepting detector overload. In practice, in addition to recorder-integrator errors (9) in determining peak areas, numerous other sources of error may occur, such as transesterification with polyester phases (10) or temporary adsorption in the column (11), or systematic errors arising from injection procedure, tailing or partial overlap of certain peaks etc. The application of the theoretical response factors is necessary only when it may be shown that these other sources of error are small. In some cases they could be incorporated into general correction factors including other systematic errors, and in routine analyses of typical samples could be applied arithmetically to the percent area responses.

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Glass Paper Chromatography and Elatography of Triglycerides[1,2]

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Abstract

A degree of reverse phase separation of triglycerides can be obtained with ascending chromatography on uncoated glass paper using a pyridine: water solvent system. The same R_f values result when silica gel coated paper is used. To extend the usefulness of this separation, the glyceryl esters are converted to methyl esters in situ using sodium methoxide. This permits, on silica gel coated paper, glyceryl ester separation in one dimension, elatography to the methyl esters, and methyl ester separation in the second dimension. In mixtures of lipids such as serum extracts, the lipid groups may be separated in one dimension and the fatty acid methyl esters in the second dimension.

Introduction

THE COMPLEX METHODS of separation of triglycerides have made analysis of these compounds difficult. This paper describes a chromatographic micro-method of partial separation of triglycerides and a method of producing methyl esters of fatty acids from triglycerides and other lipids on a chromatogram.

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[2] Supported in part by Public Health Service Research Grants HE-06774 and HE-07116 from the National Heart Institute.

Experimental Procedures and Data

Glass fiber filter paper (Schleicher & Shuell No. 29) cut to 23 x 23 cm size, was coated with silica gel by a previously described method (1). Reagent grade

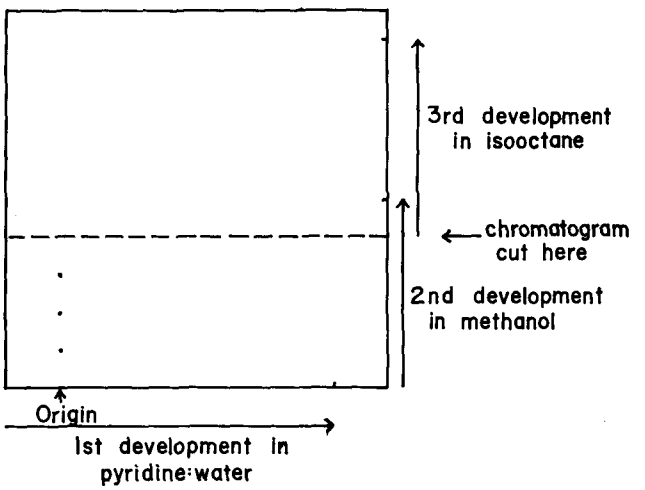

FIG. 1. Schematic drawing of the complete two-dimensional procedure. Elatography is done between the first and second development.

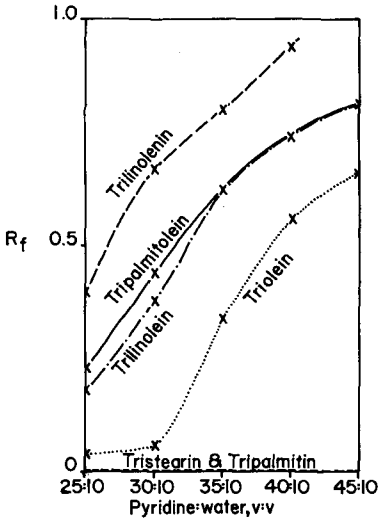

FIG. 2. R_f values obtained with triglyceride standards.

pyridine, isopropyl acetate and methanol were distilled before use. Isooctane (2, 2, 4 trimethyl pentane, Phillips Petroleum Co., 99.4% pure) was prepared for use by passage through an alumina column (Woelm, neutral, activity Grade I). A sodium methoxide solution was prepared by adding approximately 0.25 c.cm of reagent grade metallic sodium to 100 ml absolute methanol. Surface-cleaning of the sodium was accomplished by placing it briefly in a separate container of methanol. Triglyceride standards were obtained from The Hormel Institute. The natural triglycerides were commercial products or from human sources. The commercial triglycerides were dissolved in ether and diluted to a concentration of 5 μg/10 μ liter. Serum samples were prepared by making a Bloor's extract of 1 ml serum and diluting this to 10 ml. Human depot fat was extracted similarly after subcutaneous fatty tissue had been ground in an all-glass tissue grinder. All chromatography was ascending and done in rectangular glass tanks, 10 x 10 x 3 in. Drying of chromatograms was done in a vacuum oven at 50–55C with nitrogen flowing through continuously. The spots were located by sulfuric acid char (2).

Figure 1 illustrates the two dimensional technique used for this procedure. For the first dimensional separation, three separate 10 μl samples were spotted at the origin using only one-half of the paper. For triglyceride separation, varying ratios of pyridine: water were used as the developing fluid, with 40:10, v:v, as the best ratio for common dietary and human triglycerides. To separate lipid mixtures into the component groups, the developing fluid was isooctane: isopropyl acetate, 100:1, v:v or a similar developer as previously described (1). After the lipid separation, the chromatogram was dried in the vacuum oven for one hr when pyridine:water was used, or for 20 min after volatile solvents. Next, the unused half of the chromatogram was covered with glass while the area with the separated lipids was sprayed with sodium methoxide:methanol until moist. The chromatogram was immediately suspended in an anhydrous methanol vapor at 60C for 2 hr. After drying, a methanol sweep was made in the second dimension just far enough to move all lipids to a common origin on the unused half of the chromatogram. The used half is cut off and discarded. With mixed lipids a single sample was satisfactory and the methanol sweep was eliminated. The chromatogram, after vacuum drying for 1 hr, was developed in the second dimension in isooctane to separate the methyl esters.

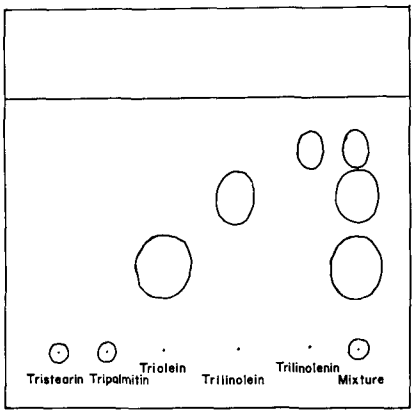

FIG. 3. Chromatogram of triglyceride standards in pyridine: water, 40:10, v:v.

Pyridine:water development gave a reverse phase separation of triglycerides (Fig. 2). The separation of triglycerides was complete when there was a difference of three double bonds. Saturated triglycerides did not move except with pyridine:water ratios of 80:10, v:v, or higher and then streaking resulted. All unsaturated triglycerides gave a 25-mm spot at an R_f of 0.3 and a 12-mm spot at an R_f of 0.8 (Fig. 3). The separation of triglycerides was identical on uncoated glass paper.

With triglyceride separation, elatography and methyl ester separation, the differences in the major triglycerides of natural mixed triglycerides could be estimated. The differences between Crisco, Mazola oil, peanut oil and human triglycerides were quite apparent.

Lipid group separation, elatography, and methyl ester separation of serum lipids permitted identification of the fatty acids of the groups on a single chromatogram (Fig. 4).

Discussion

The partial separation of triglycerides in pyridine: water may be a solubility phenomenon similar to that described by Arnold et al. (3). This separation is

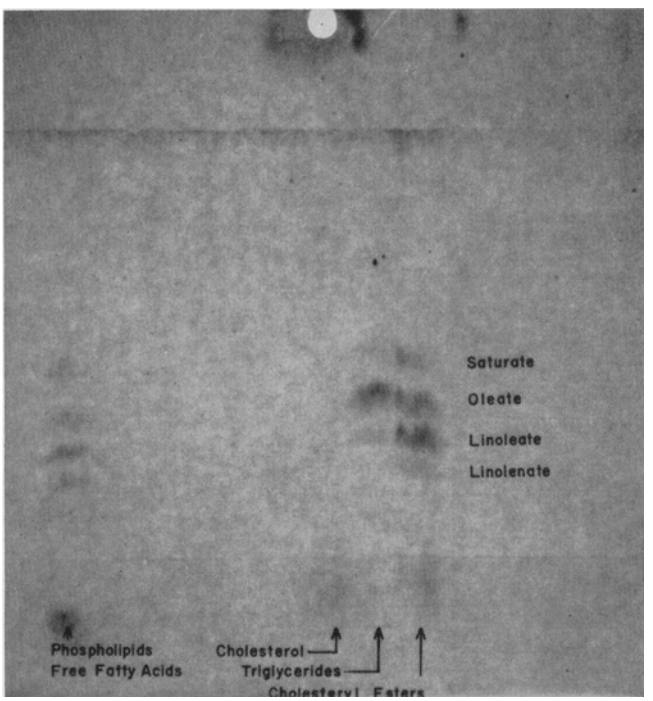

FIG. 4. Two-dimensional chromatogram of serum lipids.

limited both by degree of resolution and by the diffuseness of the spots, especially at low R_f values. With natural mixed triglycerides there is much overlapping and the separations achieved do not become apparent until the second dimensional separation of the fatty acids is done. However, since any given fatty acid can be present in several different triglycerides the methyl ester appears as a streak elongated in the first dimension. The major triglycerides can be estimated from the relative density of the fatty acids at any given first dimension R_f zone. The analysis of triglycerides presented here is less exact than that of Privett and Blank (4); however, it is a simpler procedure in that it is all done on a single chromatogram.

Incomplete transesterification of fatty acids may be caused by a high relative humidity, drying of the chromatogram in the methanol vapor, or excess sodium methoxide. Cholesteryl esters incompletely transesterify. Spreading or unwanted migration can be caused by too heavy spraying or condensation of the methanol vapor.

The total length of time to complete a two dimensional chromatogram is 7 hr for the triglycerides and 4 hr for the mixed lipids.

The transesterification by elatography has been very useful in our hands, particularly with mixed lipids. It is also useful with column chromatography for quickly determining the fatty acids in fractions; in which case different fractions are spotted on a single chromatogram, transesterified, and the methyl esters separated.

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Catalysts for Selective Hydrogenation of Soybean Oil.[1] I. An Experimental Method for Evaluating Selectivity

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Abstract

The undesirable flavor reversion properties of soybean oils may be counteracted by selective hydrogenation of the linolenate components. Screening of catalysts for this purpose was accomplished by a standardized laboratory hydrogenation of a refined, bleached soybean oil under atmospheric pressure. A mathematical derivation utilizes analytical chromatographic data to determine linolenate/linoleate reaction rates as a selectivity index S_L for a given catalyst.

Introduction

THE VALUE of soybean oil as an edible product has long been recognized. However, the formation of flavors is an undesirable aspect which has received considerable attention. It is rather generally accepted that the linolenic acid constituents are a primary precursor of flavors (1). Thus, if linolenic constituents can be selectively hydrogenated, improved soybean oil could find expanded use both as a cooking and a salad oil. A major problem is to evaluate whether present catalysts are sufficiently selective to accomplish the specified hydrogenation and if not, to develop heterogeneous catalysts which can achieve the desired goals.

In order to obtain such information, the conventional approach would involve periodic sampling of an experimental reaction, plotting concn of components against time or percentage converted and then empirically adjusting constants for specific reaction rate (2). This approach would not be useful for a screening program where many catalysts would require evaluation. Dutton (3) has developed a procedure based on the kinetic equations for consecutive first order reactions which determines the ratio of reaction rate constants of linolenate and linoleate carbon-carbon double bond hydrogenation. The method requires that the test mixture be comprised of equal amounts of linolenate and linoleate components, either triglycerides or monoesters. Then a single experimental hydrogenation (with 0.5 mole hydrogen/mole mixture) and analytical determination of the triene, diene, and monene components provides all the information needed to give the ratio of the linolenate/linoleate reaction rates.

While Dutton's method is precise and useful, it does not evaluate catalyst selectivity for the oil product of immediate interest, i.e., soybean oil. Therefore, it was deemed more direct to use a typical soybean oil in a standard hydrogenation experiment and employ the analytical data to provide similar kinetic information for evaluating a wide variety of catalysts.

Criteria for Selectivity

The maximum selectivity would occur if hydrogenation would reduce only the linolenic component of soybean oil without appreciably changing any other constituent. This result would provide an equivalent increase in linoleic concn, but no change in oleic or stearic concn. Thus, a cursory examination of a hydrogenated product by gas chromatography would reveal many catalysts which had little or no selectivity. Such an approach does not take full advantage of the available data nor does it permit relative ratings where differences are small.

Assume that the rate of hydrogenation of each component is given by a relation

$$-dA/dt = k' A(H_2) F(C) \qquad [1]$$

where A is Ln, Lo, O (linolenic, linoleic, oleic) concn
H_2 is hydrogen concn
F(C) is a function of catalyst concn.

If all experiments are performed at constant pres-

[1] Presented at the AOCS Meeting, Toronto, 1962.