Separation of Triglycerides by Column Chromatography on Silica Impregnated with Silver Nitrate

B. de VRIES, Unilever Research Laboratory, Vlaardingen, The Netherlands

Abstract

Silica gel impregnated with silver nitrate was used for the column-chromatographic separation of closely related triglycerides. Good results were obtained with 20–100 mg of the following glyceride mixtures:

1. Dipalmito-elaidin and dipalmito-olein.
2. Tristearin, dipalmito-olein, stearo-diolene and triolein.
3. Dipalmito-olein and dipalmito-linolein.

Six well-separated fractions were obtained by chromatography of palm oil and the total triglyceride composition of the oil was calculated from the composition of the fractions.

Introduction

At the moment the analysis of glyceride mixtures is being thoroughly investigated. Apart from the fatty acid components, which can be easily determined quantitatively by gas chromatography of the corresponding methyl esters, there is an increasing interest in the way in which the fatty acids are combined with glycerol to form glyceride molecules. The availability of more advanced techniques for the determination of the composition of triglyceride mixtures would indeed contribute considerably to our fundamental knowledge of interesting and highly important subjects, such as the metabolism of triglycerides in mammals and the relationship between the triglyceride structure and the consistency and rheology of dietary fats.

In earlier publications (1,2) a new chromatographic adsorbent was described for the separation of higher fatty acid methyl esters according to their degree of unsaturation or according to the configuration (cis or trans) of their double bonds. The adsorbent, which can also be applied to TLC (3,4,5), owes its high selectivity to the presence of a large amount of silver ions. The separation of mg amounts of closely related triglycerides using this type of adsorbent in column chromatography has been briefly communicated (1). The present report gives a detailed description of the results obtained so far.

Experimental

Adsorbent. The preparation of the chromatographic adsorbent (silica impregnated with silver nitrate) has already been described (2).

Solvents. The benzene used was of analytical grade; the diethyl ether distilled before use. The light petroleum was purified from aromates according to the method of Van der Ven et al. (6) and fractionated by distillation. The fraction with a boiling range of 40–60°C was used.

Triglycerides. The monoacid triglycerides tristearin (SSS) and triolein (OOO) were prepared by means of the reaction between glycerol and acid chloride (7). The asymmetric triglycerides, dipalmito-elaidin (PPE), dipalmito-olein (PPO) and stearo-diolene (SSO), were prepared from the monoglycerides (8) 1-mono-elaidin, 1-mono-olein and 1-monostearin respectively by acylation in the presence of pyridine and

1 Present at the AOCS Meeting, Toronto, 1962.
2 Present address: Unilever Research Laboratory, Duiven, The Netherlands.
chloroform (9).

A mixture of dipalmito-olein (PPO) and dipalmito-linolein (PPL) was obtained from cottonseed oil. A 100-g portion of the oil was crystallized from 100 ml acetone at -5°C. Separated crystals were collected and recrystallized from acetone. In order to remove oxidized triglycerides and mono- and diglycerides, 2 g crystals obtained were purified by column chromatography. A column (in diameter 25 mm) containing 30 g silicic acid (Mallinckrodt, 100 mesh, analytical grade) and 15 g Celite 535 was used according to the method of Quinlin et al. (10). A mixture of diethyl ether and light petroleum (5:95, v/v) was used as eluant. The fraction eluting between 150 and 250 ml was collected and contained 1.8 g oil. The fatty acid composition was (mole %):

- Palmitic acid 64.0%
- Oleic acid 10.8%
- Stearic acid 2.8%
- Linoleic acid 22.3%

This analytical result is in good agreement with a PPO/PPL ratio of 1:2. It is supposed that the oleoyl- and linoleyl-groups mainly occupy the β-positions in the triglyceride molecules (11).

**Palm Oil.** An amount of palm oil (2 g neutralized and bleached) was purified by chromatography over silicic acid (see above). The fatty acid composition was (mole %):

- Myristic acid 1.0%
- Oleic acid 38.6%
- Palmitic acid 44.4%
- Linoleic acid 9.8%
- Stearic acid 6.2%

**Column.** A chromatographic column (in diameter 11 mm, effective length 40 cm) provided with a cooling mantle and a Teflon-cook with a capillary attachment was used. A 250-ml reservoir for the eluant was placed on the column by a ground joint. The temp of the column was kept at 15°C.

**Elution.** Twenty to 100 mg triglyceride mixture was dissolved in 10 ml light petroleum or, if a considerable amount of saturated triglyceride was present, in a mixture of benzene and light petroleum (20:80 v/v) and subsequently applied to the column. The eluting solvents consisted of mixtures of benzene or diethyl ether and light petroleum. At the top of the column a pressure of 10-15 cm water was maintained while the flow-rate was adjusted at 30 ml/hr. During the chromatographic experiments, 10-ml fractions were collected and separately evaporated to dryness (50°C) in weighed small glass dishes by a stream of nitrogen. Subsequently, the glycerides were determined gravimetrically with an accuracy of ca. 0.2 mg. Fractions apparently belonging to one and the same chromatographic peak were combined and coded A, B etc. These fractions were converted into methyl esters and the fatty acid composition determined by means of GLC.

The gas-chromatographic analyses were carried out by a Pye instrument with gas density balance or with an Argon ionization detection. The column (length 120 cm) was packed with polyethylene glycol adipate on Celite (20% w/w). The determination