Confectionery Fat Analysis with High Performance Liquid Chromatography

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ABSTRACT

Natural triglyceride mixtures have been analyzed by a variety of techniques. Earlier methods, such as crystallization, were unsuitable because they were very time-consuming, required large amounts of material and were not reproducible. Later advances in chromatography gave successful separations of triglycerides but required a combination of techniques to give suitable separations. High performance liquid chromatography (HPLC) with very efficient columns has been successfully applied to triglyceride separations on a routine basis. In the present work, triglycerides of cocoa butter as well as several replacement fats were separated by HPLC. The system consisted of a highly efficient column packed with octadecyl-bonded spherical silica (5 μ) and a mobile phase composed of acetone and acetonitrile. Peaks corresponding to eluted components were collected and their composition determined by gas chromatography (GC) of the corresponding methyl esters to allow unambiguous assignment of triglyceride structure to the components. A profile of the triglycerides present in such fats can be obtained within 30 min.

INTRODUCTION

Natural fats and oils are complex mixtures of triglycerides. This triglyceride makeup determines the physical nature of the fat or oil. Fat in foods contributes to mouth feel and texture. In confectionery coatings, fat also serves to suspend sugar and other solids. Cocoa butter is the desired fat for use in chocolate and confectionery coatings because of its physical properties. Cocoa butter is brittle below 20 C (68 F) and has a sharp complete melting point at ca. 35 C (95 F), which enhances its desirability. However, high cost precludes its use in many food products, and suitable replacements for it are desirable.

The main requirements for fats used in confectionery are: reasonably hard consistency, stability and a short melting range just below body temperature. If a fat is to be used in mixtures with cocoa butter, other factors are important, e.g., eutectic effects and crystal structure, and need to be considered. "It has been found that reduction in the number of glycerides in a mixture often leads to a product having desirable properties, particularly when a selection of glycerides, closely allied chemically, can be made" (1).

Glyceride selection increases the number of fats that are useful in confectionery. The original method of glyceride selection was to partially solidify a liquid fat under closely controlled conditions. The fat crystal and liquid fat were then separated by pressing into stearines, the higher melting fats and oleins, which are liquids. This technique is not selective enough for many applications, particularly for developing a replacement for cocoa butter. However, fats and oils can be modified in a variety of other ways including hydrogenation, random interestification, directed interestification and either fractional or controlled crystallization (2).

Hard brittle fats (stearines) with melting points close to that of cocoa butter can be produced from coconut and palm kernel oils. Such hard fats have a limited glyceride composition and are used readily as replacement fats in coatings. However, when these fats are mixed with cocoa butter, they produce eutectic mixtures with characteristics unsuitable for many applications. "To produce a satisfactory cocoa butter alternative fat free from eutectic effects when mixed with cocoa butter, a more specific selection of glycerides similar in chemical constitution to those present in cocoa butter itself is required" (1).

TRIGLYCERIDE ANALYSIS

Analysis of the complex mixtures of triglycerides present in natural fats has been carried out by many methods (2-7). Early analytical techniques included fractional (8) and gradient crystallization (9,10), as well as countercurrent distribution (11-16). These methods did not prove useful for routine analysis as they required large samples and long analysis times and were not highly reproducible.

In recent years, chromatography has received extensive use in triglyceride analysis. Argentation chromatography separates triglycerides based on the degree of unsaturation (16-18). Gas liquid chromatography (GLC) separates triglycerides based first on their boiling points and then according to their molecular weights (19-21). Reversed-phase chromatography separates triglycerides according to their equivalent carbon numbers and takes into account both the molecular weight and the degree of unsaturation (3-5,22,23). A combination of 2 or more of the above chromatographic techniques is necessary to obtain good fractionation of complex triglyceride mixtures. Once again, however, the time and effort required and the lack of reproducibility limit the routine use of these methods.

Cocoa butter is a relatively simple fat compared with most natural fats and oils. The major triglyceride compositional isomer present is POS (Table I) and the major fatty acids are oleic (O), stearic (S) and palmitic (P) acids (24-26).

The ability to quickly and easily analyze natural as well as synthetic fats is important to many areas of the food and allied industries. The development of high performance liquid chromatography (HPLC) has partially met this need and has been applied to many triglyceride investigations (27-30).

This study reports the application of HPLC to the analysis of confectionery fats developed to replace or substitute for cocoa butter in confectionery and chocolate products.

EXPERIMENTAL

The HPLC system used in the present work consisted of a Tracor-995 isochromatographic pump (Tracor Instruments, Austin, TX), a Rheodyne loop injector (Model 7120) with a 20 μL sample loop, a Waters R401 differential Refractometer (Waters Associates, Milford, MA) and a Hewlett-Packard 3385 A Electronic Integrator (Hewlett-Packard, Palo Alto, CA), which was used to obtain accurate retention times. The column used for the separations was a Supelcosil LC-18 column (octadecyl bonded [Sepelco Inc., Bellefonte, PA]), 25 cm long, 4.6 mm i.d. with a 5 μ particle size.

Sample size was between 5-10 μL of ca. 20% solutions of cocoa butter and substitutes in tetrahydrofuran. The mobile phase consisted of acetone/acetonitrile, 64.6:36.4 (v/v) at a flow rate of 2 mL/min. Acetone and tetrahydrofuran were of analytical reagent grade purchased from MCB Manufacturing Chemists, Inc. (Cincinnati, OH) and Mallinckrodt, Inc. (St. Louis, MO), respectively. Acetonitrile

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TABLE I

Fatty-Acid Composition of Major Peaks—Percentage as Methyl Esters

	Peak												
	I					II				III			
	14:0	16:0	18:0	18:1	18:2	16:0	18:0	18:1	18:2	16:0	18:0	18:1	18:2
Cocoa butter	–	58.3	5.9	29.6	6.2	38.5	26.5	34.9	–	1.1	59.9	38.8	–
Premium replacement	–	67.7	1.7	30.3	–	31.5	35.9	31.4	1.1	4.2	66.4	28.5	–
FVOSC	12.4	37.7	3.9	45.8	–	34.3	2.8	62.8	–	14.4	31.1	54.4	–
FVOP	–	64.2	1.8	33	–	28.5	36.1	34.3	1.1	3.2	66.9	29.8	–

Triglyceride moieties deduced from these data are indicated on Figures 1-4. The coding PPO indicates the mixture of triglycerides PPO, POP and OPP, not the individual isomer.

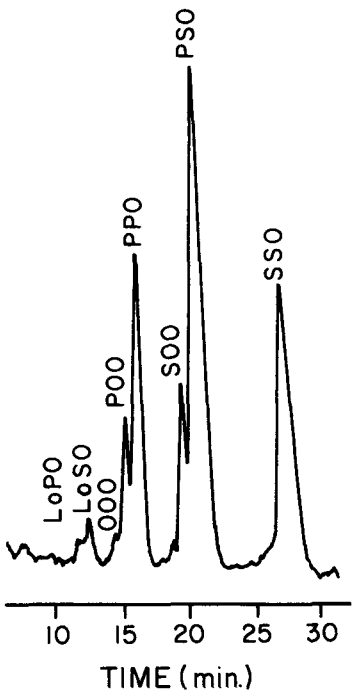

FIG. 1. Separation of cocoa-butter triglycerides. Column: 250 mm × 4.5 mm i.d. Supelco LC-18 (octadecyl bonded); mobile phase: acetone/acetonitrile (64.6:63.4, v/v); flow rate, 2.0 mL/min (P = palmitic acid, S = stearic acid, O = oleic acid, Lo = linoleic acid).

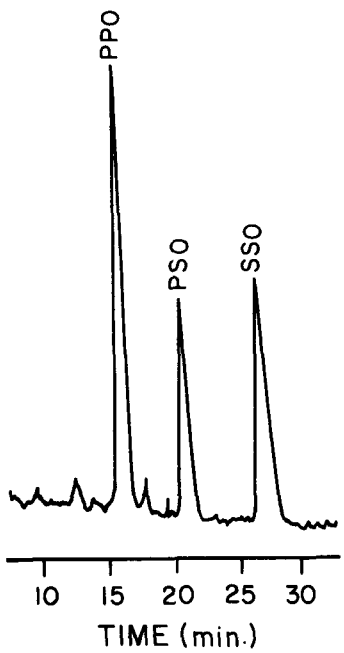

FIG. 2. Separation of premium-quality replacement triglycerides, conditions as in Figure 1.

was distilled in glass quality from Burdick and Jackson Laboratories, Inc. (Muskegon, MI).

Triglycerides peaks were collected from the HPLC effluent and analyzed as their methyl esters by GC. The methyl esters were prepared as described by Johnston (31). Analyses of the methyl esters were carried out on a 6 ft × 4 mm i.d. glass column packed with 10% SP-2330 on 100/120 Chromosorb WAW (Supelco Inc.) as previously described (29). The instrument used was a Hewlett-Packard Model 3385 gas chromatograph equipped with a flame ionization detector (FID [Hewlett-Packard]). Quantitation was accomplished by integration with a Hewlett-Packard model 18850A GC terminal (Hewlett-Packard) and comparison with standard mixtures. Triglyceride compositions of eluted peaks were determined by evaluation of the methyl ester composition of each eluted peak. The triglycerides abbreviated SSO, PSO, SOO, PPO and LoSO indicate the family of triglycerides composed of either palmitic (P), stearic (S), oleic (O) or linoleic (Lo) acids.

RESULTS AND DISCUSSION

Cocoa butter from 5 sources was analyzed by HPLC and all yield similar chromatograms; a typical cocoa-butter chromatogram is shown in Figure 1. Figures 2 and 3 show 2 replacement fats: a premium-quality replacement and fractionated vegetable oil (palm and others, FVOP), respectively, with glyceride compositions close to that of cocoa butter. Figure 4 illustrates the chromatogram of a substitute fat prepared from fractionated, hydrogenated vegetable oil (soy and cottonseed, FVOSC). Table I presents the fatty-acid composition data for the major peaks in the above-mentioned chromatograms.

The chromatograms shown in Figures 1-3 and the corresponding GC data from Table I indicate that the triglyceride compositions of cocoa butter, the premium-quality replacement and FVOP are very similar. The premium-quality replacement is used successfully in mixtures of all

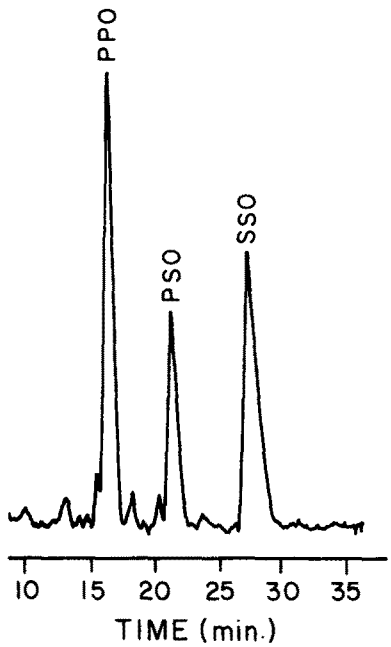

FIG. 3. Separation of fractionated vegetable-oil (palm and others) triglycerides; conditions as in Figure 1.

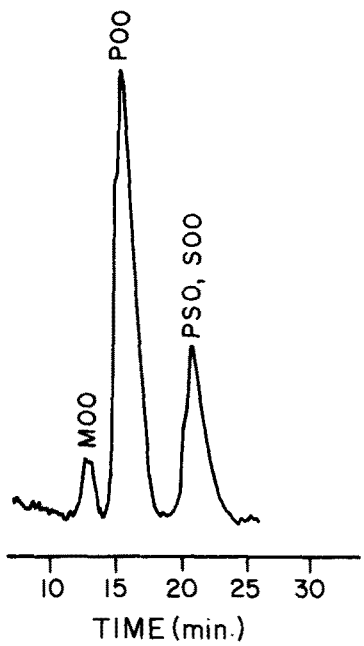

FIG. 4. Separation of fractionated vegetable-oil (soy and cottonseed) triglycerides; conditions as in Figure 1.

proportions with cocoa butter. FVOP has also been used in this way but with somewhat less success.

The chromatogram and fatty-acid composition of FVOSC show it to be dramatically different from the other samples. This fat does not mix well with cocoa butter. Even relatively small proportions result in eutectic mixtures. It is, however, used in other areas of chocolate confectionery.

Application of HPLC described here is a rapid method for the determination of the relative amounts of glycerides present in a fat. It can be used to monitor the modification of a fat as well as to provide an estimate of the compatibility of a fat as an extender or substitute for cocoa butter. This method also may be used to detect adulteration of fats and oils and is being applied to the detection of premium quality cocoa-butter replacements in cocoa butter.

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