SHORT COMMUNICATION

Fatty Acid Distribution of Fats, Oils and Soaps by High-Performance Liquid Chromatography Without Derivatization

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A high-performance liquid chromatography (HPLC) procedure without derivatization was developed for quantitating fatty acid components of various soap-related fats and oils, as well as for the direct quantitation of fatty acids from soap. The fatty acids are detected by refractive index after isocratic reverse-phase chromatography. The method has been developed with radial compression and stainless-steel column technology. The triglycerides are saponified and acid-hydrolyzed into fatty acids, and they are dissolved in a solvent and injected. The soaps are dissolved in methanol and injected into the HPLC, where they are acid-hydrolyzed directly on the column by an acid-modified mobile phase. The total run time after injection is approximately 20 min, with quantitation performed on an NEC Powermate® computer driven by PE/Nelson Analytical Software. The typical carbon chains analyzed are from C6 to C20.

KEY WORDS: Animal fat, fatty acid, HPLC, radial compression system, soap, vegetable oil.

High-performance liquid chromatography (HPLC) has become an important analytical technique for separating and quantitating compounds in biomedical, pharmaceutical, industrial and environmental operations (1,2). Various HPLC methods have been described to separate fatty acids with ultraviolet (UV) detection and derivatization into phenacyl esters (3). Marini (4), in particular, presents an excellent paper on lipid HPLC chromatography with various detector systems after derivatization.

The fatty acid distribution of fats and oils is important to the user because important physical and chemical properties of the material can be ascertained. For the soap maker, the performance characteristics of a finished product are affected by its fatty acid distribution. Most quality household soaps are alkali-metal salts of blends of various fats and oils, typically beef tallow and coconut oil. For beef tallow, the predominant fatty acid is the monounsaturated C18:1, oleic acid, followed by the saturated C16:0 and C18:0 acids, palmitic and stearic acids. On the other hand, coconut oil is comprised of predominantly shorter-chain saturated C12:0 and C14:0 acids, lauric and myristic acids (Table 1). An all-tallow soap would essentially produce a slow-foaming, tight-bubbled lather, whereas coconut soap will produce a quick-foaming, large-bubbled lather. The tallow-based lather would be somewhat more stable than that of coconut. Generally, the amount and type of lather or foaming are related to the solubility of each fatty acid soap present. For example, the solubility of a soap will decrease as the chainlength increases, but it will increase as the degree of unsaturation increases. By blending various amounts of beef tallow and coconut oil, the soap maker can achieve the desired performance and processing characteristics. Palm oil and palm kernel oil are often used as substitutes for tallow and coconut oil, respectively. The fatty acid distribution will also allow the analyst to generate other information, such as acid values and iodine values, by mathematically applying appropriate constants to the distribution.

TABLE 1

Typical Fatty Acid Distributions of Tallow and Coconut Oil[a]

Carbon number	Edible tallow (%)	Edible coconut oil (%)	HPLC elution time (min)[b]
C8	0.00	5.60	4.3
C10	0.00	5.80	5.2
C12	0.00	46.20	6.5
C14:1	0.70	0.00	7.1
C14	2.90	18.60	8.5
C18:3	0.33	0.00	9.3
C16:1	2.17	0.00	9.8
C15	0.50	0.00	10.5
C18:2	1.70	1.90	10.7
C16	25.50	10.30	11.4
C18:1	46.00	8.20	12.6
C18	19.20	3.10	15.7
C20	trace	0.00	21.8

[a]The tables were generated from an internal database (Original Bradford Soap Works, West Warwick RI).
[b]Conditions were described in the Experimental Procedures section for the Radial Compression Separation System. HPLC, high-performance liquid chromatograhy.

EXPERIMENTAL PROCEDURES

Materials. All fatty acid standards were obtained from Aldrich Chemical Co., Inc. (Milwaukee, WI). Commercial soap bases produced at Original Bradford Soap Works (West Warwick, RI) were used throughout the study. The various fats and oils were obtained from commercial domestic (U.S.) suppliers, and all solvents were HPLC-grade and were filtered through a 0.45μ Millipore (Milford, MA) filter before use.

HPLC apparatus. The apparatus consisted of a constant-delivery pump (Model 6000A; Waters Associates, Milford, MA) fitted with a septumless injector (Model U6K; Waters) having a 2-mL loop. The separations were obtained with a Waters fatty acid stainless-steel column (3.9 mm i.d. × 30 cm) and a radial compression separation system (RCSS) consisting of two Radial Pak 8 mm, 4μ Phenyl columns, 8 mm × 100 mm and used in conjunction with two radial compression modules (Model RCM-100; Waters) connected in series. A refractive index detector (Model 401; Waters) was used to detect the fatty acids.

Sample preparation. Free fatty acids (FFA) were prepared from the fats and oils according to AOCS Official Method Cc 12-59 (5). Tallow and stearic fatty acids are

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dissolved in tetrahydrofuran and methanol (50:50, vol/vol), at the ratio of 0.8 g sample to 20 g solvent. All other fatty acids were dissolved in methanol at the same w/w ratio. All soap samples were dissolved in methanol at the ratio of 1 g sample to 20 g solvent. All solutions were filtered through a 0.45μ Acrodisk CR (Gelman Inc., Ann Harbor, MI) before injection.

HPLC operating conditions. For the separation in the stainless-steel column, the mobile phase consisted of 45% acetonitrile, 20% tetrahydrofuran, 34.5% water and 0.5% glacial acetic acid with a flow rate of 1.1 mL/min. The sample size for analysis was 25 μL for the fatty acid standard solutions. The RCSS uses the same mobile phase as the stainless-steel column with a flow rate of 2.0 mL/min and a sample size of 30 μL.

Data acquisition. The data were acquired *via* PE/Nelson Analytical Model 2600 Chromatography Software (3000 Series Chromatography Data System; Nelson Analytical Inc., Cupertino, CA) in a NEC Powermate® computer. Raw data were transferred to Microsoft Excel® for presentation purposes.

RESULTS AND DISCUSSION

Figure 1 demonstrates the separation of the twelve fatty acid standards obtained with the RCSS. The FFA profiles for a typical soap base, consisting of 85% beef tallow and 15% edible coconut oil, are shown in Figure 1 (panel C). By following the dotted tie-lines from the standards from the top down, it is possible to identify the peaks in the soap chromatograms. The elution order is based on total carbon number, from lower to higher, and on unsaturation before saturation. Fatty acids from carbon numbers C15, C16:1 and C18:3 co-elute from the stainless-steel column but are better resolved with the higher efficiency of the RCSS columns. These fatty acids, however, do not represent a significant amount in the fats and oils used in soapmaking, typically less than 3% collectively. The normal-chain saturated acids are fairly straightforward in elution order. However, the unsaturated acids show the effects of a functional group, unsaturation and alkene geometry on elution order. Although ricinoleic acid, C18:1-OH, contains 18 carbons, the hydroxyl group produces an elution time

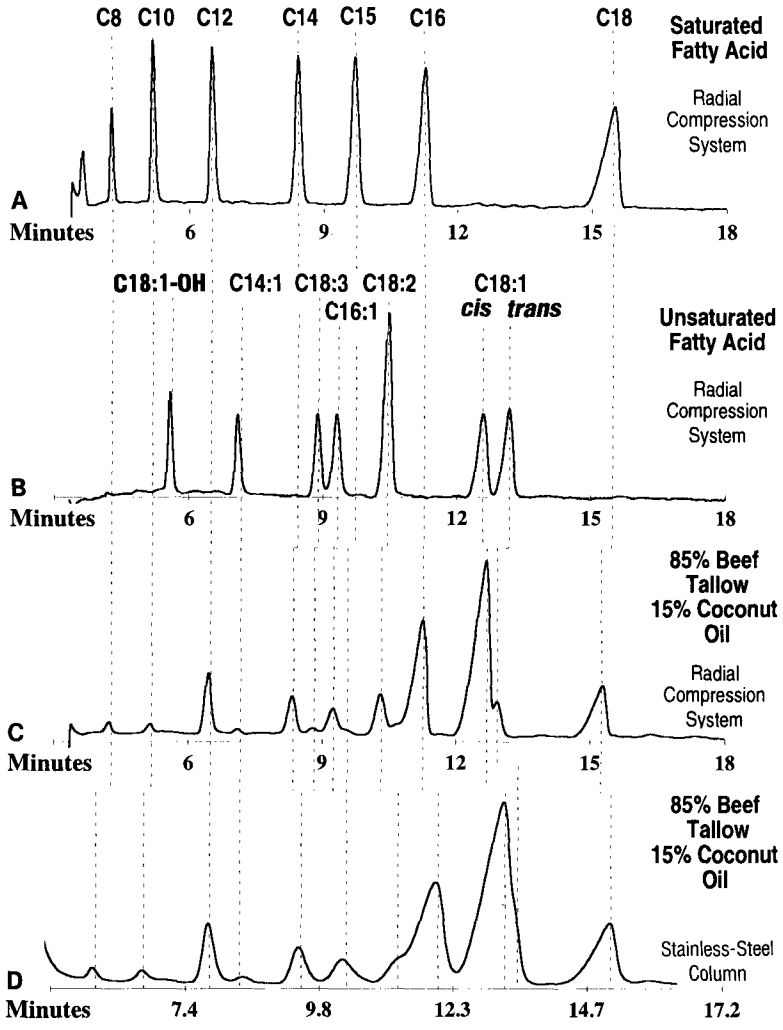

FIG. 1. Fatty acid standards separated with the radial compression system and resulting fatty acids from commercial soap base separated on a radial compression system vs. stainless-steel column.

TABLE 2

Comparative Analysis by High-Performance Liquid Chromatography (HPLC) and Gas Chromatography (GC) of Fatty Acids Obtained from Commercially Available Soap Bars

	Product											
	1		2		3		4		5		6	
Carbon number	HPLC	GC[a]	HPLC	GC	HPLC	GC	HPLC	GC	HPLC	GC	HPLC	GC
C8	1.08	0.88	2.32	1.73	0.81	0.66	1.15	0.96	1.99	1.60	1.39	1.12
C10	1.02	0.96	2.09	1.84	1.00	0.97	1.20	1.09	2.07	1.84	2.07	1.88
C12	7.80	8.23	17.32	17.56	14.62	14.65	9.85	9.93	25.04	24.48	16.11	16.14
C14:1	0.48	0.49	0.27	0.29	0.40	0.40	0.51	0.58	0.00	0.32	0.21	0.23
C14	5.38	5.48	8.52	8.86	6.92	7.16	6.31	6.75	10.17	10.27	7.54	8.07
C18:3	0.17	0.16	0.13	0.13	0.12	0.13	0.24	0.15	—	—	0.14	0.15
C16:1	1.14	1.85	0.88	1.45	0.78	2.15	1.58	2.22	0.50	1.36	0.94	1.24
C15	0.26	0.29	0.20	0.31	0.18	0.29	0.36	0.41	0.11	0.22	0.22	0.26
C18:2	2.88	3.47	2.46	2.07	1.55	2.52	2.40	2.19	0.92	1.56	2.85	2.70
C16	29.18	29.40	20.74	19.30	24.56	23.76	23.40	22.91	17.77	15.53	28.74	29.05
C18:1*cis*	32.23	32.36	27.51	30.04	23.66	32.13	33.47	33.25	18.79	29.66	27.19	25.44
C18:1 *trans*	4.43	—	3.43	—	12.89	—	3.74	—	11.04	—	1.61	—
C18	13.92	13.94	14.12	14.07	12.52	13.18	15.77	16.80	11.60	11.11	10.65	11.17

[a]GC analysis performed in accordance with Reference 6.

of 5.5 min, where the lower-chain saturated acids normally elute (Fig. 1).

Oleic and elaidic acid are monounsaturated C18:1 isomers that are difficult to separate because their elution times are close to each other. Oleic acid is a liquid, whereas elaidic acid is a solid at room temperature. Despite this difference, the dual RCSS is able to separate these isomers, whereas in the stainless-steel system, elaidic acid appears as a shoulder on the oleic acid peak.

The FFA profile of edible beef tallow, refined bleached and deodorized (RBD) palm oil, edible coconut oil and RBD palm kernel oil, as well as their soaps, also can be determined by this procedure.

The soaps, however, must be acid-hydrolyzed to FFA prior to analyses by HPLC. This can be achieved *in situ* by modifying the mobile phase with an acid, such as glacial acetic, thus allowing the direct injection of an alcoholic soap solution into the HPLC system. The soap is hydrolyzed immediately after injection, for subsequent separation on the column. As shown in Figure 1, the RCSS system is more efficient in separating the *cis* and *trans* isomers of C18:1 than the stainless-steel system and is better at resolving minor components.

Table 2 shows excellent correlation between this procedure and standard gas chromatographic (GC) methods. Fatty acids were obtained from leading bar soaps currently on the market. Methyl esters of the fatty acids were used in the GC analysis.

We currently use the RCSS technique for quality control of incoming raw materials, soap bases and finished soap products, as well as for research and development projects and have developed an extensive database in support of manufacturing operations.

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