day of operation in which the coalescer was overloaded for about one day due to low operating temperature coincident with very high fat concentrations in the acid water feed.

After the 15 days of continuous operation, the coalescer was subjected to a regeneration cycle. This was done in part because of very gradually increased fat levels in the acid water effluent (but not yet exceeding 100 ppm), and in part to gain more experience with the regeneration procedure and possible effects on pressure drop and structural integrity of the coalescer after a long operating cycle.

In Figure 3, the fat concentrations achieved after regeneration in a further 15 days of operation are shown. The concentrations achieved were generally below 50 ppm, except for a period of relatively poor performance at 150-200 ppm between the third and the fourth day after regeneration. At the end of the 15-day period, another regeneration procedure was done to test bed-integrity. The run was then terminated.

These tests show that continuous operation of at least two weeks, and possibly even of one month, can be expected. Also, it is possible that a fiberglass coalescer as used in these tests can be regenerated repeatedly without adverse effects on pressure drop or structural integrity.

REFERENCES

1. Standard Methods for the Examination of Water and Waste-Water, 14th edn., American Health Association, Washington, DC, 1975, Method 502C.
2. Todd, D.B., and J.E. Morren, JAOCS 42:172A (1965).
3. Crauer, L.S., JAOCS 42:661 (1965).
4. Becher, P., Emulsions: Theory and Practice, Chapter 9, Demulsification, ACS Monograph no. 162, Reinhold Publishing, New York, 1965.
5. Spielman, L.A., and Y.-P. Su, Ind. Eng. Chem. Fundam. 16:272 (1977).

[Received November 10, 1982]

Quantitation of Estolide Triglycerides in Sapium Seeds by High Performance Liquid Chromatography with Infrared Detection

KATHLEEN PAYNE-WAHL and **ROBERT KLEIMAN**, Northern Regional Research Center, Agricultural Research Service, US Department of Agriculture, Peoria, IL 61604

ABSTRACT

The kernel oil of Chinese tallow (*Sapium sebiferum*) seed contains tetraester triglycerides composed of *trans*-2,*cis*-4-decadienoic acid joined in an estolide linkage to 8-hydroxy-5,6-octadienoic acid. A rapid (< 10 min) method of separation and quantitation of the triglyceride and estolide triglyceride fractions of the oil has been developed using high performance liquid chromatography with infrared detection.

The Chinese tallow tree, *Sapium sebiferum*, a rapidly growing 30-40 ft deciduous tree that grows well in the southern coastal United States, is being examined as a potential crop for marginal land (1). It has virtually no natural enemies, is tolerant of wetlands and salt marshes, and annually produces large quantities of white seeds approximately the size of a pea (1). The seeds are covered with a vegetable tallow (12-35 wt % per seed) composed of triglycerides containing saturated and monounsaturated fatty acids. The seed kernel contains a liquid oil (13-32 wt % per kernel), Stillingia oil, which is unusual in that it contains tetraester triglycerides composed of an allenic hydroxy acid (8-hydroxy-5,6-octadienoic acid) joined in an estolide linkage to *trans*-2, *cis*-4-decadienoic acid (2, 3). To facilitate measurement of the intact estolide triglycerides for plant breeding and further study, we have developed a rapid method of separation and quantitation of triglycerides and estolide triglycerides by high performance liquid chromatography (HPLC) with infrared (IR) detection.

MATERIALS AND METHODS

Seeds of *S. sebiferum* were provided by Simco of Texas, Inc. (Houston, TX). Whole seeds were refluxed for 6 hr with hexane to remove the vegetable tallow from the outer seed coat. The seeds were then ground and extracted with petroleum ether using a Butt apparatus to obtain the kernel oil.

The kernel oil was analyzed on a high performance liquid chromatographic system consisting of an M-6000A pump (Waters Assoc., Milford, MA), a column (25 cm x 4.6 mm) of Partisil PXS 10/25 PAC (Whatman, Clifton, NJ) and an IR detector for liquid chromatography (Du Pont, Wilmington, DE) set at 1750 cm^{-1} and 0.1 A full scale. Samples were eluted within 10 min with 10% tetrahydrofuran in hexane at a rate of 2 mL/min. A typical injection contained 2-3 mg of oil in 10 μL of eluting solvent. A laboratory-wide computer system (4, 5) was used to determine peak areas.

To obtain purified estolide for standards, 1 g of kernel oil was placed on a dry column (1 cm [id] × 21 cm) of 60/200 mesh Hi-Flosil column support (Anspec. Co., Inc., Ann Arbor, MI) and eluted successively with 250 mL each of 3%, 10% and 20% diethyl ether in hexane at ~5 mL/min. During the chromatography, 50-mL fractions were collected and progress was monitored by HPLC. The oil was separated into two components by column chromatography

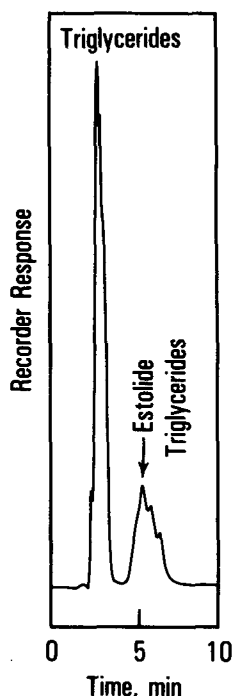

FIG. 1. HPLC chromatogram of *Sapium sebiferum* seed kernel oil on Partisil PXS 10/25 PAC column eluted with 10% tetrahydrofuran in hexane at 2 mL/min.

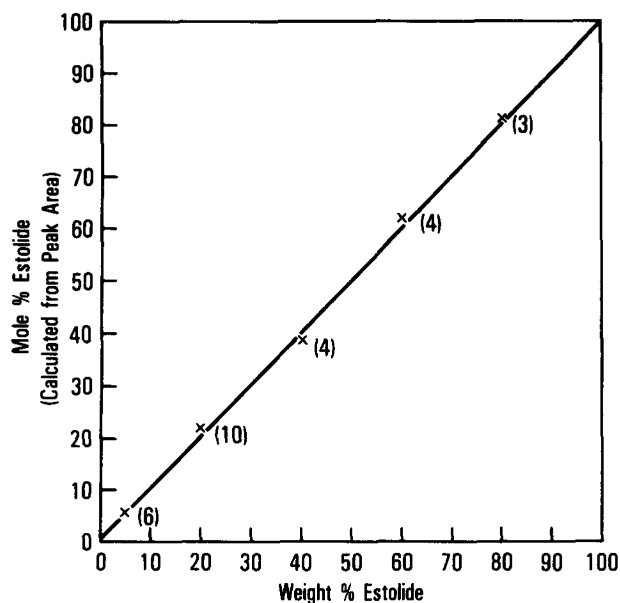

FIG. 2. Weight % estolide vs mole % estolide as calculated from peak areas. Each point represents an average of the number of trials indicated in parentheses.

and the infrared spectra of each was obtained neat on NaCl disks. Thin layer chromatography (TLC) was performed on silica gel by double development with 80:20 hexane/ether.

RESULTS AND DISCUSSION

Baseline separation between triglycerides and estolide triglycerides was achieved by HPLC of the intact kernel oil (Fig. 1). To obtain purified material for quantitation standards, the oil was separated into two components by column chromatography. Fractions 1-5 each contained a single component with HPLC retention time (RT) of 3.0 min, the same as known triglyceride standards. HPLC separated fraction 6 into two peaks with RT = 3.0 and 5.5 min, respectively. Fractions 7-11 contained only the later eluting component with the major portion of the material concentrated in fractions 7-9. Combining fractions 7-11 yielded 0.2600 g of material.

The IR spectrum of the combined fractions 1-5 was typical of normal triglycerides. The IR spectrum of the combined fractions 7-11 was consistent with the published spectrum of the estolide fragment of the oil (3). Bands were present at 1950 cm^{-1} characteristic of an allene (6) and at 1620 cm^{-1}, 1590 cm^{-1} and 990 cm^{-1}, indicative of *trans* unsaturation within a conjugated system (7).

The determine the linearity of the response under HPLC conditions, standard mixtures of the isolated triglycerides (TG) and estolides (EST) were prepared in concentrations of ca. 95:5, 80:20, 60:40, 40:60, and 20:80 w/w. To allow for the difference in the IR detector response of triglycerides (3 carbonyl groups) and estolides (4 carbonyl groups), the following calculation was used to convert peak area to mole %:

$$\text{mole \% estolide} = \frac{3/4 \text{ EST area}}{3/4 \text{ EST area} + \text{TG area}} \times 100$$

A linear response of mole % estolide was obtained throughout the range of standard mixtures (Fig. 2). A standard deviation of ± 1.4 on the 20% estolide mixture was obtained from 10 trials over several days.

Seventy samples of *S. sebiferum* seeds from different trees were analyzed by this method. The samples were also examined by TLC and no free acids were found. Free fatty acids have HPLC retention times similar to the *S. sebiferum* estolides and their presence in the oils would require removal before analyses. The estolide content of the 70 samples varied from 18 to 28 mole % of the oil with 90% of the samples containing between 22 and 27 mole % estolide.

Seeds of *Sapium japonicum*, a deciduous tree commonly found at the base of mountains in Japan, were also analyzed. The leaves of *S. japonicum* contain an antifungal constituent identified as methyl 8-hydroxy-5,6-octadienoate (8). The seeds have no vegetable tallow coat and contain 70.9% oil. TLC and HPLC of *S. japonicum* oil were the same as *S. sebiferum* oil. The IR spectrum of the oil contained the same significant bands indicating allenic and *trans*-conjugated unsaturation as the IR of the *S. sebiferum* estolide fraction. The estolide of *S. japonicum* was 24.2 mole % of the oil by HPLC.

REFERENCES

1. National Science Foundation, The Chinese Tallow Tree as a Source of Petroleum Substitutes, Simco, Inc. (Weston, CT), Phase I Final Report, PFR 8010567, Sect. III, 1981.
2. Christie, W.W., Biochim. Biophys. Acta 187:1 (1969).
3. Sprecher, H.W., R. Maier, M. Barber and R.T. Holman, Biochem. 4:1856 (1965).
4. Butterfield, R.O., W.K. Rohwedder, E.D. Bitner, J.O. Ernst, D.J. Wolf and H.J. Dutton, Prog. Lipid Res. 17:93 (1978).
5. Payne-Wahl, K., G.F. Spencer, R.D. Plattner and R.O. Butterfield, J. Chromatogr. 209:61 (1981).
6. Wotiz, J., and W.D. Celmer, J. Am. Chem. Soc. 74:1860 (1952).
7. Nakaneshi, K., Infrared Absorption Spectroscopy Practical, Holden-Day, Inc., San Francisco, CA, 1962, p. 24.
8. Ohigashi, H., K. Kawazu, H. Egawa and T. Mitsui, Agric. Biol. Chem. 36:1399 (1972).

[Received November 8, 1982]