the ring is destroyed. In almost every such cleavage, this ring opening takes place at the nitrogen atom (13). A nitrile could also be formed via a piperidine intermediate in which the ring is more easily cleaved.

ACKNOWLEDGMENT

The authors are indebted to R. Whiteman and the late H. Wisneski for their assistance. This work was supported in part by University of Massachusetts Experiment Station Project No. 443.

REFERENCES

1. Nawar, W.W., J.R. Champagne, M.F. Dubravcic and P.R. LeTellier, J. Agric. Food Chem. 17:645 (1969).
2. Metcalfe, L.D., and A.A. Schmitz, Anal. Chem. 33:363 (1961).
3. Budzikiewicz, H., C. Djerassi, A.H. Jackson, G.W. Kenner, D.J. Newman and J.M. Wilson, J. Chem. Soc. 89:1949 (1964).
4. Duffield, A.M., H. Budzikiewicz, D.H. Williams and C. Djerassi, J. Am. Chem. Soc. 87:810 (1965).
5. Porter, Q.N., and J. Baldas, "Mass Spectrometry of Heterocyclic Compounds," Wiley Interscience, New York, NY, 1971, pp. 310, 331, 376–384.
6. Millar, I.I., and H.D. Springall, "The Organic Chemistry of Nitrogen," Clarendon Press, Oxford, 1966, pp. 619–637, 667–696.
7. Fischer, H., and E. Bartholomaus, S. Physiol. Chem. 77:185 (1912); Chem. Abs. 2068 (1912).
8. Spiteller, G., in "Advances in Heterocyclic Chemistry," Vol. 7, 1966, pp. 317–319.
9. Gilpin, J.A., Anal. Chem. 31:935 (1959).
10. Sims, R.J., and J.R. Fioriti, JAOCS 52:144 (1975).
11. Lien, Y.C., and W.W. Nawar, J. Food Sci. 39:911 (1974).
12. Gohlke, R.S., and F.W. McLafferty, Anal. Chem. 34:1281 (1962).
13. Mooher, H.S., in "Heterocyclic Compounds," Vol. 1, edited by R.C. Elderfield, John Wiley and Sons, Inc., New York, NY, 1950, pp. 397–616.

[Received July 1, 1980]

A Gas Chromatographic Method for the Assessment of Used Frying Oils: Comparison with Other Methods[1]

A.J. PARADIS and **W.W. NAWAR**, University of Massachusetts, Amherst, MA 01003

ABSTRACT

The application of a simple and rapid method, based on gas chromatographic measurement of dimer triglycerides, is proposed for evaluation of the quality of used frying oils. The technique involves complete conversion of the oil to its methyl esters followed by analysis on a short column packed with 3% JXR. Parameters are adjusted to provide a pattern in which the dimeric esters emerge as a doublet peak with a retention time of ca. 3 min, whereas all other monomeric esters elute with the solvent peak. The relative heights of the two components of the doublet peak appear to reverse as the frying time is increased. The test correlated well with changes in dielectric constant measurements, as well as with "altered triglycerides" in continuously heated corn oil.

INTRODUCTION

The food industry requires simple objective methods to assess frying oil quality during the progression of a frying operation. An obvious economic advantage is the ability to determine the appropriate point at which the frying oil is no longer suitable for use. On the other hand, regulatory authorities are interested in methodology which can be used to enforce proper usage of frying fats.

During frying, decomposition products, both volatile and nonvolatile, are formed. The higher molecular weight compounds are more reliable indicators of frying oil abuse in view of their steady formation and low volatility.

Guillaumin (1) reports procedures for GLC analysis of cyclic monomers in heated fats. The procedures call for conversion of the fat to its methyl esters which are then hydrogenated to prevent overlapping with the 18:2 fatty acid esters. Perkins et al. (2) used gel permeation chromatography to follow the development of polymeric triglycerides in corn oil used for potato frying. Aitzetmuller (3) used high pressure liquid chromatography to isolate oxy-triglyceride dimers from nonpolar dimers in used fats. These methods, however, are relatively expensive and/or time consuming.

Several methods of a simpler nature have been recently introduced. These include column chromatography (4) and dielectric constant measurement (5). In this work, a simple and rapid gas chromatographic method for triglyceride dimers is introduced. First, it was desired to evaluate the performance of this GLC method as a monitor of oil usage. For the purpose of comparison, the column method described by Billek et al. (6) and the dielectric constant method used by Fritsch et al. (5) were used.

EXPERIMENTAL PROCEDURES

Materials

"Sweet-Life" corn oil (Sweet Life Corp., Suffield, CT) was purchased at a local store. It was stored below 10 C and used as purchased without further treatment. Reference compounds were of the highest available purity from commercial sources. High purity dimer and trimer acids were obtained courtesy of Emery Industries, Cincinnati, OH. BCl_3/methanol (10% w/v) was purchased from Applied Science Laboratories, State College, PA. Silica Gel G 70–230 mesh (ASTM) "for Column Chromatography" was supplied by E. Merck Darmstadt, Germany. GLC columns were packed with pretested 3% JXR (a methyl silicone) on Gas Chrom Q 100/200 mesh as supplied by Applied Science Laboratories. Two forms of Silica Gel G. (with binder), "according to Stahl" were used for TLC. One form, for hand-coated preparative TLC plates, was purchased from Analabs Inc., North Haven, CT. The other form, supplied by E. Merck, contained a stronger binder and was precoated onto 20 x 20 cm glass plates. Amberlite IRA-400 ion exchange resin was purchased from Mallinckrodt Chemicals, St. Louis, MO.

Thin Layer Chromatography

The plates were activated by heating at 110 C for 1 hr. They were cooled to room temperature in a drying chamber con-

[1] Presented at the IFS/AOCS World Congress, New York, April 1980.

taining a dessicant. Five μl of sample solution (10% w/v $CHCl_3$) were transferred to the plate surface via a 10 ul pipetting syringe. The spotted plates were developed in a paper-lined (Whatman No. 1 Chromatography Sheets) TLC tank, previously equilibrated with 204 ml of petroleum ether/diethyl ether/acetic acid, (80:20:2, v/v/v). After development (1 hr) and evaporation of the residual solvent (15 min), the TLC plates were sprayed with concentrated sulfuric acid. The TLC spots were visualized by heating the plates on a hot plate for 15 min at 135 C.

Preparative Thin Layer Chromatography (PTLC)

All preparative TLC plates (20 x 20 cm glass) were hand-coated to a 500-μ thick layer of Silica Gel G "according to Stahl." These plates were not prewashed, but were heat-activated for 1 hr at 110 C. Samples (ca. 100 mg/ml $CHCl_3$) were applied in four 500-μl portions to the plate surface using a TLC sample streaker (Applied Science Laboratories). The plates were developed for 1 hr in the same tank as already described. The component bands were visualized by saturation with iodine vapor. The desired bands were scraped off the plate into centrifuge tubes (25 ml) mixed with 10 ml diethyl ether and separated from the silica by centrifugation at 10,000 rpm for ca. 10 min. The supernatant was removed and this separation procedure was repeated twice more. The supernatant was concentrated to ca. 5 ml under nitrogen gas for further analysis.

Liquid Chromatography of Frying Oils

Some modifications of the method described by Billek et al. (6) are given next. Glass chromatography columns were filled with 150 ml of the first solvent system, i.e., petroleum ether/diethyl ether (87:13, v/v). Silica gel was hydrated to 5% of its weight by stirring in distilled water (5 g/95 g silica) for 10 min. The silica gel was placed in a dry 250-ml separatory funnel fitted to the top of the column. The funnel stopcock was set open and the silica was lightly sprinkled through the column solvent. This facilitated multiple production of uniformly packed columns. A nitrogen blanket was not used during the removal of solvent from the column fractions.

Dielectric Constant Measurements

The Foodoil Sensor model NI-20 marketed by Northern Instruments Company, Lino Lakes, MN, was used. Procedures for calibration and analysis were the same as in the operation manual.

Gas Chromatography of Oil Components

A Hewlett-Packard F&M 810 Research Chromatograph, Palo Alto, CA, equipped with flame detection and oven temperature programming was used. The oven temperature was programmed from 230 to 330 C at 30 C/min. The upper limit oven temperature was held 1 min prior to recycling. Both injector and detector blocks were set at 330 C. Carrier gas (N_2) flow rate was set at 20 ml/min. The oven was fitted with a short stainless steel (40 cm x 3 mm) column packed with 3% JXR.

Preparation of Samples for Dimer Analysis by GLC

Samples of the oil were saponified and methylated according to Metcalfe and Schmitz (7) with minor modifications to achieve complete conversion of the dimeric material to methyl esters. Saponification was done for 15 min with 40 ml/g of 0.5 N NaOH in methanol, and methylation required 15 min with BCl_3/methanol. The esters were extracted in petroleum ether, freed of moisture over sodium sulfate, and dried under nitrogen gas. Under conditions of this method, diglycerides normally present in an abused oil and in partial methylation reactions would co-elute with dimer acid esters. Therefore, transesterification of oil glycerides was deemed essential. Dimer esters eluted within 3 min whereas all other monomer esters eluted with the solvent peak.

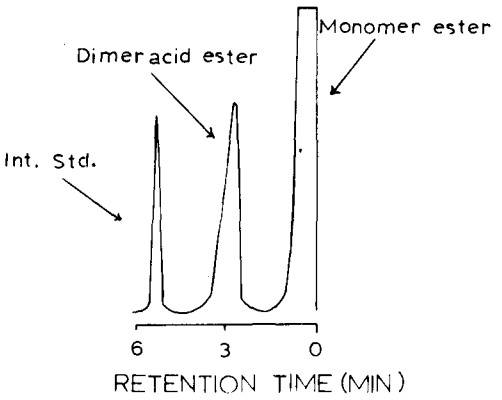

FIG. 1. GLC analysis of dimer acid ester (Dimer Acid 1010 courtesy Emery Industries). 3% JXR, 40 cm x 3 mm column temp. program 230-330 C at 30 C/min.

Quantitative GLC

Sample peaks were quantitated by comparison of their area to that of tripalmitin as the internal standard (Fig. 1). Peak areas were determined by triangulation.

RESULTS AND DISCUSSION

Correlation with Length of Use and Other Methods

To examine the correlation between GLC dimer ester values and heating time, 200 ml of a domestic pure corn oil was heated continuously, without agitation, at 185 C in a 500-ml round-bottomed flask fitted with a water condenser. Ten ml aliquots were taken during heating on a daily basis until the oil reached a level of 66% polar components (PC) according to the column method of Billek et al. (6). Detector response to dimer esters was found to be linear when amounts of the total esterified material in the range of 2 to 40 ug/ul solvent were injected. Tripalmitin was added (20 ug/ul) to the corn oil esters (ca. 60 ug/ul) as the internal standard.

Table I lists GLC values of the dimer esters expressed as the percentage of dimer present in the total esterified material. Values obtained for the same samples by the column and the dielectric constant methods are included. It can be seen that all values increased as the length of heating increased. Statistically, the GLC gave the highest correlation coefficient between the values obtained and hours of heating.

In the GLC analysis, the dimer esters appeared as a doublet peak with a retention time similar to that of the standard dimer ester (Fig. 2). The earlier eluting band of the doublet (peak #1, Fig. 2) was larger than the second (peak #2) for samples of oil heated up to 144 hr. Beyond that point, however, this trend was reversed and the second peak became

TABLE I

Effect of Continuously Heating Corn Oil at 185 C on Triglyceride Dimers, Polar Components (PC) and Dielectric Constant Readings

Hours at 185 C	GLC (% trig. dimer)	Column (% PC)	Dielectric constant (sensor reading)
0	0	0.8	0
48	1.2	16.9	1.8
120	3.2	27.8	3.7
216	6.1	65.8	Offscale (POS)

larger. At the end of heating (216 hr), this corn oil has a PC value of 66%.

To investigate the components responsible for the doublet peak, this highly abused sample (66% PC) was fractionated by column chromatography. Subfractions of the polar components were collected from this column via a fraction collector apparatus. When the methyl esters of the subfractions were analyzed by TLC, the least polar fractions gave only the first peak of the dimer doublet, whereas fractions of increasing polarity showed increasing amounts of the second peak (Fig. 3).

It should be understood that dimers of free acids were not found in any substantial amounts in these fats. What is being measured by this method are dimeric triglycerides after saponification and methylation. This was confirmed by isolating the acidic fraction of the heated oil as described by Hornstein et al. (8). The conversion of these acidic groups to methyl esters was simplified to a one-step reaction with methanolic boron trichloride (40 ml reagent/g starting oil). The fraction retained on the resin (acidic) did not yield dimer esters in any significant amount whereas methylation of the supernatant (nonacidic) produced large amounts of dimer esters.

The dimer esters of the abused oil were also collected as they eluted from the GLC column and were examined by TLC.

As can be seen in Figure 4, the major spot for the trapped GLC component (C) matches that of the dimer acid esters. For comparative purposes, a standard trimer acid ester (E) was also chromatographed on the same TLC plate. A weak spot in the abused corn oil esters had the same R_f value as the trimer ester. However, the material collected from the GLC column did not contain trimer esters on the same TLC plate. The methyl esters of fresh corn oil (A) showed no materials corresponding to dimer (D) or trimer acid esters (E). To further investigate the dimer triglyceride fraction, preparative TLC (PTLC) was used to fractionate the methyl esters of the total highly abused corn oil. About 200 mg of esters were applied as a narrow band onto a silica gel PTLC plate and developed in 200 ml of PE/Et_2O/HoAC (80:20:2, v/v/v). Iodine vapor visualization of the developed plate revealed three major bands, A, B and C as shown in Figure 5. The most intense band, band A, had the highest R_f value which matched that of authentic monomer esters (A in Fig. 4). Band B and the upper section of band C were eluted off the thin layer plate and gas chromatographed both separately and as a mixture on the short GLC column (Fig. 6). Band B (the less polar) gave rise to a major peak with a retention time equivalent to that of the first component of the doublet peak, whereas band C (the more polar) gave rise to a component represented by a peak with a longer retention time equivalent to the second member of the doublet. As expected, the mixture of B and C as well as the esters of the total fat gave rise to both components of the doublet peak. Thus, the doublet peak represents two dimer materials that differ in their degree of polarity. This agrees with the work of Billek and Heisz (9) who radioactively labeled a nonpolar dimer ester and found it to appear on TLC below monomer esters, but above polar dimer esters.

It is tempting to consider the reversal of the components of the doublet peak as a possible guide for determining more precisely the extent of oil degradation. However, such reversal was found to occur beyond the suggested 27% PC value of the column method.

Further work is obviously needed in order to provide a more detailed composition of the triglyceride dimer components produced in fats upon heating, and to establish the quantitative effects of frying parameters on the specific dimers formed.

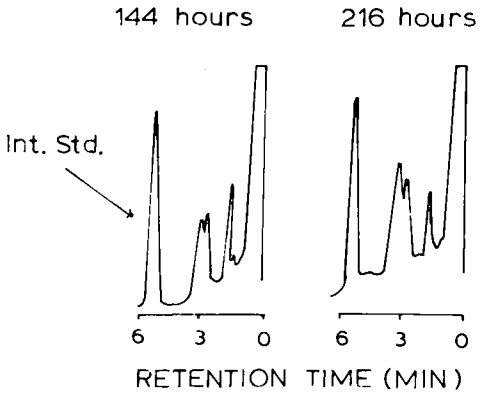

FIG. 2. GLC analysis of triglyceride dimer esters from corn oil continuously heated at 185 C. "1." and "2." are first and second eluting components of the dimer ester double peak.

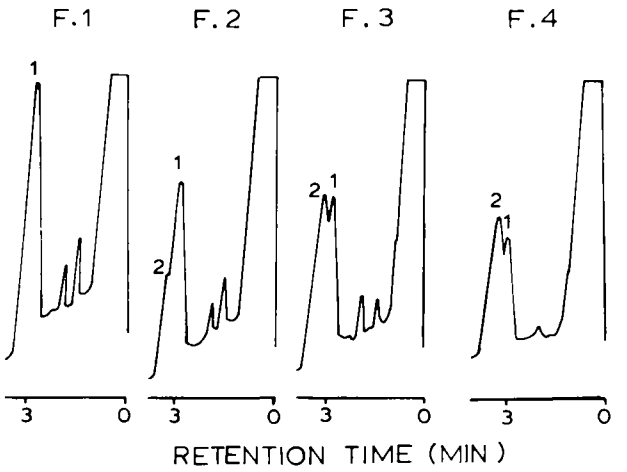

FIG. 3. GLC of dimer esters after fractionation of heated corn oil by column chromatography. F. 1: least polar. F. 4: most polar.

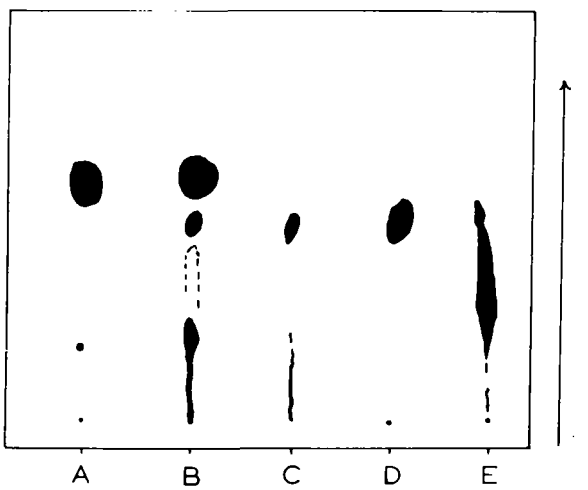

FIG. 4. TLC of heated corn oil (66% PC) dimer esters: A = esterified fresh corn oil; B = esterified heated corn oil; C = dimer ester component trapped from GLC; D = dimer ester standard; E = trimer ester standard. Solvent, petroleum ether/diethyl ether/acetic acid, 80:20:2 (v/v/v).

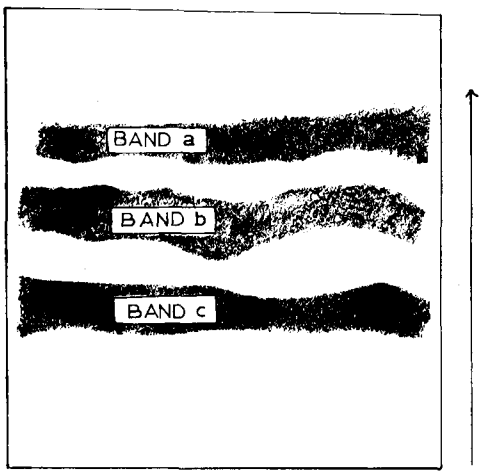

FIG. 5. Preparative TLC of esterified heated corn oil (66% PC). Band a: monomer esters. Bands b and c: dimer esters. Solvent: petroleum ether/diethyl ether/acetic acid, 80:20 2 (v/v/v).

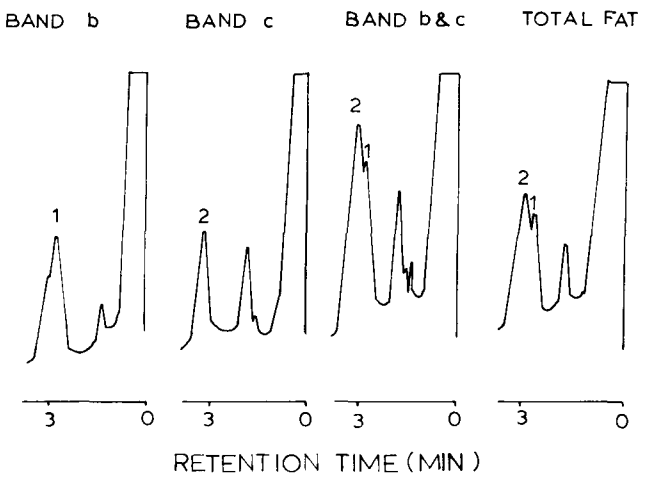

FIG. 6. GLC of bands isolated from preparative plate of Fig. 5.

ACKNOWLEDGMENTS

The authors thank R. Whiteman and F. Gouin for their assistance. This work was supported in part by Univ. of Mass. Experiment Station Project No. 443, and grants from KFC Corporation, Louisville, KY, and the U.S. Army.

REFERENCES

1. Guillaumin, G., Rev. Fr. Corps Gras 24:211 (1977).
2. Perkins, E., R. Taubold and A. Hsieh, JAOCS 50:223 (1973).
3. Aitzetmuller, K., Paper presented at the 3rd Int. Symp. on Metal-Catalyzed Lipid Oxidation, Institute des Corps Gras, Paris, September 27-30, 1973.
4. Guhr, G., and J. Waibel, Fette Seifin Anstrichm. 81:511 (1979).
5. Fritsch, C.W., D.C. Egberg and J.S. Magnuson, JAOCS 56:546 (1979).
6. Billek, G., G. Guhr and J. Waibel, Ibid. 55:728 (1978).
7. Metcalfe, L.D., and A.A. Schmitz. Anal. Chem. 33:363 (1961).
8. Hornstein, I., J. Alford, A. Elliott and P. Crowe. Anal. Chem. 32:540 (1960).
9. Billek, G., and O. Heisz, Fette Seifen Anstrichm. 71:189 (1969).

[Received July 18, 1980]

High Performance Liquid Chromatography of Triglycerides: Controlling Selectivity with Reverse Phase Columns[1]

R.D. PLATTNER, Northern Regional Research Center, Agricultural Research, Science and Education Administration, U.S. Department of Agriculture, Peoria, IL 61604

ABSTRACT

Rapid separations of triglycerides by chain length and degree of unsaturation have been made by high performance liquid chromatography (HPLC) using reverse phase columns. Several different bonded columns were evaluated for use in reverse phase HPLC of triglycerides, and differences in selectivity are discussed. Selectivity was also modified by adding silver ion to the solvent, which produces marked changes in selectivity of solute triglycerides.

INTRODUCTION

High performance liquid chromatography (HPLC) is a relatively new technique for the analysis of triglycerides. Separations based on chain length and degree of unsaturation have been reported for triglycerides using reverse phase (1-7) and conventional silica HPLC systems (8). Triglycerides occur naturally in complex mixtures containing many similar components, so that useful HPLC separations are difficult. The components may differ by as little as one double bond or a few carbon atoms or even differ only in the positions of the acyl groups on the glycerol molecule. An analysis of the two most important factors that control the separations, i.e., the column and the eluting solvent system, has not been reported. During the course of our work with HPLC of lipid mixtures, we have used various types of HPLC columns and solvent systems. Control of these parameters can greatly affect the separations.

The first choice that must be made in developing an HPLC separation is that of the column. Reverse phase columns are generally considered to be best for separating a homologous series of components. Fats and oils contain triglycerides made up of a complex mixture of homologous series of chain lengths and a homologous series of degrees of unsaturations, so that reverse phase HPLC would appear to be the procedure of choice. Despite the surprising fact that separations of chain length among triglycerides could be made on silica columns (8), the most efficient separations have indeed been obtained with the reverse phase (RP) columns. Because it is important that columns give reproducible results, both packing material and packing

[1] Presented at the AOCS meeting in San Francisco, May 1979.