Rapid Oleic/Linoleic Microanalytical Procedure for Peanuts

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A rapid microanalytical technique is described, whereby the oil from a portion of a peanut kernel was analyzed for the oleic/linoleic acid (methyl ester) ratio, with the remainder being planted for reproduction. Analysis of the methyl esters by gas–liquid chromatography required 2 to 4 min per sample, depending upon the equipment. The analytical techniques were evaluated for precision using 100 peanut varieties and the factors which influenced O/L ratios are reported. This procedure should assist in more rapid development of new peanut varieties that are demanded by the manufacturers of peanut products and other oilseed crops.

The peanut industry desires a Spanish-type peanut with a high oleic/linoleic (O/L) fatty acid ratio (less than 25% linoleic acid) for the production of peanut butter, peanut oil, and other roasted peanut products with a longer shelf-life. Because of possible health problems, the degree of unsaturation must not be decreased to an extremely low level.

Rosen and Altschul (1958) suggested that it might be possible to produce peanut oil with increased unsaturation by breeding. Significant changes have been induced in fatty acid composition of safflower (Knowles, 1965) by selective breeding practices. Previous work by Holley and Hammons (1968) and Mason and Matlock (1968) indicated that the genetic variation necessary to achieve the present goal of decreased unsaturation was present in peanuts.

In earlier work by Mason (1963), small composite samples of 10 to 20 peanuts were pressed and analyzed for O/L ratios. Jellum and Worthington (1966b) developed a rapid procedure for the analysis of fatty acid methyl esters and adapted the procedure for the analysis of individual corn kernels (Jellum and Worthington, 1966a). They reported an elution time of approximately 2.5 min for the methyl ester of linoleic acid. Putt *et al.* (1969) have made single seed analyses on a portion of single sunflower seed and saved the remainder for growing into a plant. Since only one or two drops of oil were necessary for the analysis, the amount of oil contained in a peanut is more than enough for this analysis. If a portion of the peanut kernel could be analyzed and the remaining portion planted, the development of the desired peanut variety would be more rapid. The technique described is quite similar to the half-seed technique reported by Downey and Harvey (1963) and used by Downey and Craig (1964) in the development of zero-erucic acid rapeseed.

MATERIALS AND METHODS

Apparatus. Glc analyses were performed on either a Perkin-Elmer Model 800 or a modified Barber-Colman Model 5000 gas chromatograph (Waller, 1967a); both were equipped with a flame ionization detector. Aluminum columns, 0.25 in. × 6 ft (or 4 ft), packed with 14.5% DEGS (Applied Science Laboratories) or stabilized DEGS (Analabs, Inc.) on Anakrom 100/110 mesh were used with helium as the carrier gas.

Selection of Sound Mature Kernels. Size alone was not sufficient for selection of sound mature kernels (SMK). Successful and highly reproducible chemical determinations of peanuts required a rigid and carefully controlled selection and classification of the kernel (Holley and Young, 1963; Newell, 1967; Pang, 1967; Young and Waller, 1969). Peanuts having dark-colored interior pericarp surfaces and very thin, faded pink-colored testa were classified as mature (M). In most varieties, the sound mature kernel was smooth with little or no wrinkling of the testa surface. Those having some white on the interior pericarp and pink testa were classified as high intermediate (HI). Those with some slight wrinkling of the skin in which the testa had not completely collapsed and the interior of the pericarp remained white were referred to as low intermediate (LI). Undersized, shriveled peanuts having white pericarp and thick testa were classified as immature (I).

Sample Preparation. The peanut kernel was held by the germ end, and a sharp scalpel was used to slice about one-third of the peanut from the opposite end. It was important to use at least one-fourth of the seed because of the variability of the oil within the peanut (Kartha, 1963) and it was best to remove and discard one-fourth of the seed in large seeded varieties before slicing a portion for analysis in order to obtain a representative sample.

Preparation of Methyl Esters for O/L Analysis. The procedure of Mason and Waller (1964) was slightly modified for the preparation of methyl esters. The sliced section of the peanut was placed in a 16 by 150 mm test tube, and the following reagents were added in order: 4 ml of sodium dried benzene; 0.1 ml of 2,2-dimethoxypropane redistilled from 76–70° (Dow Chemical Co.); and 0.5 ml of 0° anhydrous 2.8 *N* methanolic HCl. After shaking, the test tubes were covered and left overnight at room temperature (22–25°) to form the methyl esters. The reaction mixture was analyzed either by directly injecting 2–3 μl onto the glc column or by decanting, evaporating to near dryness on a hot water bath (80–85°), and injecting 50 nl of the concentrated mixture onto the glc column. The latter method gave slightly improved resolution with a longer column life.

Glc Analytical Conditions. Since operating parameters varied slightly from day to day, a standard peanut oil sample was used to adjust the equipment so that an accurate analysis could be obtained. The standard peanut oil sample was analyzed routinely for fatty acid composition to ensure that the proper O/L ratio was being used to standardize this study. The injection port was about 250° with an oven temperature of 235–240° and a helium flow rate of approximately 200 ml per min. The temperature of the hydrogen flame ionization detector on the Perkin-Elmer Model 800 was the same as the oven temperature. On instruments with small lines to the detector such as the modified Barber-Colman Model 5000, it was necessary to operate the detector temperature at 350°

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Table I. The Use of the Mass Spectrometer–Gas Chromatograph Multiple Ion Detector (MID) to Estimate the Contaminating Fatty Acids in the Oleic and Linoleic Acid glc Peaks when Measured by the Rapid O/L Procedure

Peanut sample number	MID			MID		
	Methyl oleate, M+	Methyl stearate, M+	Methyl stearate, %	Methyl linoleate, M+	Methyl linolenate, M+	Methyl linolenate %
Standard	296	298	1.10	294	292	1.60
3663	296	298	0.66	294	292	0.59
3718	296	298	0.65	294	292	0.80
3716	296	298	0.99	294	292	1.00
3691a	296	298	1.08	294	292	1.20
1092	296	298	1.61	294	292	0.89
Average			1.01			1.01

to avoid clogging. On the Perkin-Elmer Model 800, a 4:1 stream splitter was used since the flame was not to receive more than 50 ml per min of carrier gas. A typical analysis on the Perkin-Elmer Model 800 gas chromatograph with a stream splitter required slightly less than 2 min (Figure 1). A typical analysis using the Barber-Colman Model 5000 gas chromatograph required about 3.5 min.

RESULTS AND DISCUSSION

The purpose of this phase of research was to improve upon the method of Mason and Waller (1964), so that a much larger number of oil samples could be analyzed.

The major simplification of the method of Mason and Waller (1964) was elimination of the time-consuming step of hydraulically expelling the oil and the use of test tubes instead of expensive and more bulky flasks. Less reagents were used, and lastly neutralizing the methyl ester preparation before injection into the gas chromatograph was found to be unnecessary, providing the procedure described herein was followed. Covering the samples with a towel gave the same results as with stoppered tubes. Solid injection (Minini and Noryinberski, 1965) of samples was attempted but was very time consuming because of the time required to load and resume operation. Because there was no solvent present in this latter method, very good separation of oleic and linoleic acids was obtained and column life could be extended several-fold.

Portions of the peanut were analyzed, starting from the tip opposite the germ end; on the small-seeded type it was necessary to sample one-third to one-half of the seed to obtain accurate and reproducible results. Kartha (1963) fractionated peanut cotyledons and then combined similar portions to obtain enough sample for measuring the percent oil and iodine value at different locations on the cotyledon. According to Kartha (1963), the internal distribution varies, with the tip opposite the germ being the most unsaturated portion of the peanut kernel. Preliminary studies taking only one-fourth of the peanut kernel showed this to be true. The highest percentage of unsaturated fatty acids as found in the more immature peanuts, with the saturated fatty acids being the highest in a mature kernel (Pickett and Holley, 1960; Prasad and Diswas, 1956; Schenk, 1961). Since the tip opposite the germ is the furthest from the point where nutrients enter the seed, it might also be expected to be the most unsaturated portion of the kernel. In the same article, Kartha reported that the interior face in about the middle of the kernel had the lower iodine value. Thus, to take a representative sample it was necessary to sample sufficiently near the center of the kernel.

A new technique by Yermanos (1968) involved the immersion of oilseeds in liquid solvents, allowing the extraction of

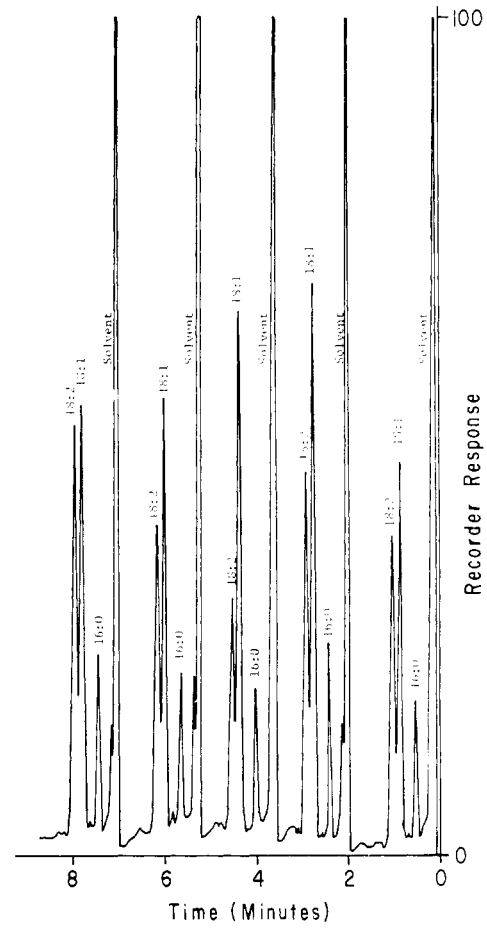

Figure 1. Gas–liquid chromatographic tracing of a series of five typical O/L analyses

enough oil for analytical purposes without destroying seed viability. This was attempted with peanuts, but it was difficult to obtain enough fat in the 48 hr and the ratio was somewhat lower, probably due to the extraction of the more unsaturated fatty acids present in the testa (Worthington, 1968).

After the reaction was completed, the samples were stable for approximately 30 hr, with some changes being observed by 48 hr. Thus no samples were kept for analysis more than 24 hr when prepared under the above conditions.

Figure 1 is a tracing of a glc chromatogram showing the excellent separation that was obtained with this technique. Base lines were drawn and peak heights only were measured for palmitic, oleic, and linoleic acids; the O/L ratio was calculated. The above three fatty acids accounted for approximately 90% of the total fatty acids. Oleic and linoleic in

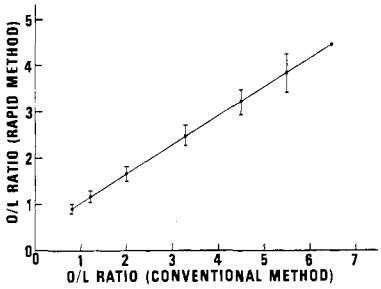

Figure 2. Calibration curve for O/L ratios

Table II. Range in O/L Ratios for Seed of Plants from F_3 Population (Perkins, Oklahoma, 1968) as Determined by the Rapid Microanalytical Technique

Cross	Generation	O/L Ratio range	Plants analyzed
P-939 × P-2	F_3	0.66–1.12	80
P-939 × P-6	F_3	0.61–1.23	74
P-190 × P-2	F_3	0.88–1.56	50
P-25 × P-2	F_3	0.88–2.42	48
P-960 × P-6	F_3	1.06–2.77	19
P-636 × P-6	F_3	0.91–1.32	49
P-962 × P-2	F_3	1.01–2.15	49
P-964 × P-2	F_3	0.98–2.95	50
P-15 × P-964	F_3	0.98–2.95	100

most varieties and strains comprised 80% of the total fatty acids.

To minimize glc errors, the largest peak should be at least 40% full scale deflection. Below this value the peak height error increased at a very fast rate due to changes in base line.

With the fast flow rates and high temperatures employed in this method, it was felt that a check on the purity of the oleic and linoleic methyl ester peaks was necessary. The technique of Sweeley *et al.* (1966) for the analysis of unresolved compounds in gas chromatographic effluents was utilized. This technique using a prototype of the LKB 9000 combination GC-MS (Waller, 1967b) employed a multiple ion detector so that a continuous recording of two values of *m/e*, separated by not more than 10% of the mass range, could be obtained. The results indicated the presence of trace amounts of methyl stearate (about 1%) under the methyl oleate peak and of methyl linolenate (also about 1%) under the methyl linoleate peak (Table I).

To test for accuracy, 100 samples of oils from a wide range of genetic material containing a range of O/L ratios from 1 to 6 were used. These oils had been analyzed earlier for their fatty acid content using the method of Worthington and Holley (1967). Ratios were calculated and compared with the values obtained by this rapid microanalytical method. Results were plotted and shown in Figure 2. Above a 1.8 O/L ratio, the rapid method gives low values; but corrected ratios for the conditions of this study were read directly from Figure 2. Each laboratory should prepare their own calibration curve.

A peanut oil of known composition was used as a standard in the rapid microanalytical technique when determining the O/L ratio on approximately 2250 samples for the plant breeders in 1969. A statistical treatment of these O/L values obtained from the standard during the entire study gave excellent reproducibility (1.125 ± 0.043), with a low standard deviation of the O/L ratios. Our results were in excellent agreement with an earlier study in which Mason and Matlock (1968) had reported a LSD value of 0.04, with differences greater than 0.05 being considered significant.

Data (Waller *et al.*, 1969) recorded in Table II illustrate the type of data now being derived by the peanut breeder using the rapid microanalytical technique. It should be noted that certain crosses give a narrow range of O/L ratios, *i.e.*, P-939 × P-2, whereas other crosses, *i.e.*, P-964 × P-2, showed a much wider range of O/L ratios.

LITERATURE CITED

Downey, R. K., Craig, B. M., *JAOCS* **41,** 475 (1964).
Downey, R. K., Harvey, B. L., *Can J. Plant Sci.* **43,** 271 (1963).
Holley, K. T., Hammons, R. O., University of Georgia Research Bulletin No. 32, 1968.
Holley, K. T., Young, C. T., *Proc. Peanut Improv. Working Group* 52 (1963).
Jellum, M. D., Worthington, R. E., *Crop Sci.* **6,** 251 (1966a).
Jellum, M. D., Worthington, R. E., *J. Amer. Oil Chem. Soc.* **43,** 661 (1966b).
Kartha, A. R. S., *J. Sci. Food Agr.* **14,** 515 (1963).
Knowles, P. F., *Econ. Bot.* **19,** 53 (1965).
Mason, M. E., "Procedures in Studying and Factors Influencing the Quality and Flavor of Roasted Peanuts," Ph.D. Dissertation, Oklahoma State University, 1963.
Mason, M. E., Matlock, R. S., "Progress Report VIII on Agronomics, Organoleptic, and Biochemical Study of Factors Responsible for the Flavor of Peanut Butter and Roasted Peanuts," Biochemistry Department, Oklahoma State University, 1968.
Mason, M. E., Waller, G. R., *Anal. Chem.* **36,** 583 (1964).
Minini, E., Noryinberski, J. K., *Biochem. J.* **95,** 1 (1965).
Newell, J. A., "Precursors of Typical and Atypical Roasted Peanut Flavor," Ph.D. Dissertation, Oklahoma State University, 1967.
Pang, L.-S., "The Influence of Maturity and Time of Harvesting Spanish Peanuts on Peanut Butter Quality," M.S. Thesis, Oklahoma State University, 1967.
Pickett, T. A., Holley, K. T., *Ga. Agr. Exp. Sta. Mimeogr. Ser.* no. 90 (1960).
Prasad, B. G., Diswas, B., *J. Oil Technol. Ass. India* **12,** 23 (1956).
Putt, E. D., Craig, B. M., Carson, R. B., *J. Amer. Oil Chem. Soc.* **46,** 126 (1969).
Rosen, G. D., Altschul, A. M., Ed., "Processed Plant Protein Foodstuffs," Academic Press, New York, N. Y., 1958, p 419.
Schenk, R. U., University of Georgia Technical Bulletin No. 22, 1961.
Sweeley, C. C., Elliott, W., Fries, I., Ryhage, R., *Anal. Chem.* **38,** 1549 (1966).
Waller, G. R., *Proc. Okla. Acad. Sci.* **47,** 265 (1967a).
Waller, G. R., *Proc. Okla. Acad. Sci.* **47,** 271 (1967b).
Waller, G. R., Odell, G., Matlock, R., Kirby, J., "Progress Report IX on Biochemical, Agronomic and Organoleptic Study of Factors Responsible for the Flavor of Peanut Butter and Roasted Peanuts," Biochemistry Department, Oklahoma State University, 1969.
Worthington, R. E., *Proc. 5th Natl. Peanut Res. Conf.* 87 (1968).
Worthington, R. E., Holley, K. T., *J. Amer. Oil Chem. Soc.* **44,** 515 (1967).
Yermanos, D. M., *Calif. Agr.* **22,** 15 (1968).
Young, C. T., Waller, G. R., American Chemical Society Southwest Regional Meeting, Tulsa, Okla., December 4–5, 1969, Abstract B10–11.

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