was won by: W. N. Kesler, Woodson-Tenent Laboratories, Little Rock, Ark., with a rating of 48.06; T. J. Potts, Ralston Purina Co., St. Louis, Mo., was second with a rating of 39.73.

Last year, the Smalley Cup, awarded annually for combined proficiency in the determination of moisture, oil and nitrogen in meal, was retired by H. L. Hutton of the Woodson-Tenent Laboratory in Clarksdale, Miss. A new cup was graciously donated by Mr. Tenent to be awarded this year and was won by Biffle Owen, Planters Mfg. Co., Clarksdale, Miss., with a rating of 66.89. This gives Mr. Owen permanent possession of the new cup, having won two legs in 1956–57 and 1961–62. H. L. Hutton was second with a rating of 57.75.

The following chemists, while not winning certificates, did outstanding work in the past season's program.

Meal

W. D. Simpson, Woodson-Tenent Laboratories, Wilson, Ark.

Cottonseed

R. C. Pope, Pope Testing Laboratories, Dallas, Tex.

Soybean

W. D. Simpson, Woodson-Tenent Laboratories, Wilson, Ark.

Peanut

Stephen Prevost, Law and Co., Wilmington, N. C.

Vegetable Oil

R. C. Pope, Pope Testing Laboratories, Dallas, Tex. and O. S. Simpson, The Procter & Gamble Co., Dallas, Tex.

Tallow and Grease

J. W. Thomas, Southern Testing Labs., Westwego, La., and E. Nesom, Swift and Co., Chicago, Ill.

Glycerine

S. B. Stearn, The Procter & Gamble Co., Port Ivory, N. Y.

Drying Oil

W. A. Moe, Spencer Kellogg Co., Minneapolis, Minn., and V. Bloomquist, Minnesota Linseed Oil Co., Minneapolis, Minn.

Edible Fat

S. D. Jones, Hunt Foods, Gretna, La., and L. J. Brown, Canada Packers, Ltd., Edmonton, Alberta, Can.

M. J. ANDERA
L. V. ANDERSON
T. J. BALDWIN
R. T. DOUGHTIE, JR.
K. H. FINK
W. H. KOESTER
W. J. MILLER, Chairman

• *Letters to the Editor*

A Simple Method of Calibrating a GLC Column for Quantitative Fatty Acid Analysis

RELATIVE chromatogram peak area is a function of molecular weight and molecular structure. Relative molar response values have been calculated for application to methyl esters of even carbon number fatty acids from C_2 through C_{22}, and for methyl esters of the cis unsaturated series of C_{18} fatty acids (1,2,3).

Many workers are interested in obtaining quantitative analyses of mixtures of methyl esters of fatty acids in animal tissues that range from dodecanoic acid to docosahexaenoic acid as common and experimentally induced constituents. It has been observed in our laboratory that the correction factors required in such analyses are very large and are probably caused by detector response to differences in molecular weight and structure as well as reaction of the sample with the liquid phase of some columns. To meet the needs of routine quantitative analyses of samples containing large numbers of long chain polyunsaturated fatty acids the following technique was developed for routine laboratory usage.

A mixture of known composition containing methyl octadecanate, methyl eicosenoate, methyl docosanoate, methyl eicosatetraenoate, methyl eicosapentaenoate, and methyl docosahexaenoate is analyzed 5 times and the correction factors calculated by using methylstearate as a base of one. It is apparent that there is almost a curvilinear relationship between retention time and correction factor. Assuming the relationship is logarithmic, regression analysis is used to obtain the equation for the line of best fit for the points obtained (Fig. 1). This equation can then be used for calculating correction factors to be used with all other chromatograms obtained with this column under the same experimental conditions. Under our experi-

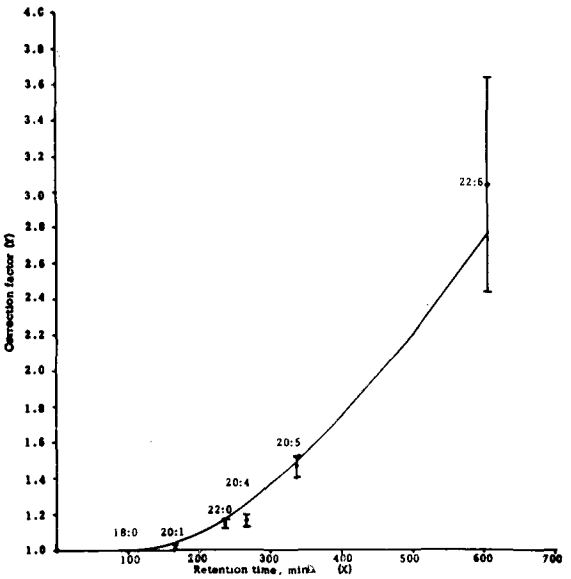

FIG. 1. Correction factor as a function of retention time. Mean correction factors and standard errors of mean. Numbers by means identify fatty acids—first number indicates length of carbon chain, second denotes number of double bonds. Equation from regression analysis: Log $Y = 0.001007X - 0.161$. Instrument: Perkin Elmer 154C, ¼" × 84" Cu tubing with 30% degs on acid washed chromosorb W, He at 55 psi and 50 ml/min flow rate, isothermal at 220C.

TABLE I
Application of Correction Factors to a Fraction of Menhaden Oil Methyl Esters

Sample	Fatty acid—no. carbons : double bonds													
	14:0	16:0	16:1	18:0	18:1	18:2	18:3	18:4	20:0	20:4	20:5	22:0	22:5	22:6
	%	%	%	%	%	%	%	%	%	%	%	%	%	%
Fraction of menhaden oil (M.O.) methyl esters—uncorrected	0.4	5.3	3.2	5.5	12.8	3.4	5.0	4.9	....	0.9	22.3	....	1.8	34.4
Fraction of M.O. methyl esters—corrected	0.3	3.7	2.2	3.6	8.5	2.3	3.4	3.7	....	0.8	21.5	....	2.2	47.7
Sum of acids of a carbon length M.O. methyl esters—uncorrected	0.4	8.5	....	31.6	....	....	....	....	23.2	....	....	36.2	....	....
Sum of acids of a carbon length M.O. methyl esters—corrected	0.3	5.9	....	21.5	....	....	....	....	22.3	....	....	49.9	....	....
Hydrogenated M.O. methyl esters	....	6.3	....	19.2	....	....	....	....	28.1	....	....	46.4	....	....

mental conditions it has been found that the column has a useful life of approximately 300 hr and that any standardization conducted during this time will give the same correction factors for the various retention times. Therefore, the standardization method outlined need only be carried out once for a column. It should be stressed that this curve and the correction factors for various retention times are not applicable to any other instrument or, in our experience, with another column in this same instrument. However, as a column is used, the retention time usually decreases and, therefore, the correction factor that must be applied from day to day decreases. The use of the relationship between retention time and correction factor makes it possible to eliminate the determining of correction factors each day.

Application of correction factors to an analysis of a fraction from menhaden oil is shown in Table I. The analysis of the same sample completely hydrogenated is also given. Only very small correction factors were required for 22:0 for the hydrogenated sample since it had a retention of 158 spaces. When correction factors were not applied, abnormally high amounts of 16 and 18 carbon fatty acids would appear to be present while the 20 and 22 carbon acids were underestimated. Note the good agreement in the quantities of 22 carbon atom fatty acid in the hydrogenated and corrected sample (46.4 vs. 49.9).

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Errors in the Azelaoglyceride Technique

IN A COMMUNICATION from this laboratory (1), it was pointed out that there are two sources of error in Kartha's method for the determination of glyceride composition (2) *viz.*, formation of incompletely oxidized glycerides containing *presumably* acetylated α-ketols (e.g. $GS_2S°$) and liberation of saturated acids by hydrolysis of azelaoglycerides. In several recent communications (3,4,5), Kartha contends that these observations are based on artifacts of procedures used by us.

While it is true that the presence of ketols was not confirmed by specific keto group reactions, it is certain that some neutral products are formed during the oxidation, which influence the yield, A.V. and S.V. of the insoluble azelaoglycerides (IAG). It is regretted that Kartha has cited only one example in support of his contention. Even so, the data and reasoning presented by him are entirely misleading. In the particular example cited (Tables IV & VI, expt. 6, ref. 1), about 12% of $GS_2S°$ were obtained; and a careful reader will observe that the yield of IAG in this case is 91.3% (theoretical 92.6%, assuming formation of $GS_2S°$) and not 89.3%, as wrongly cited by Kartha. He has also overlooked a number of other points. The yield and S.V. of IAG from GS_2U are not enough proof for the absence of $GS_2S°$, as these are based on the net effect of incomplete oxidation, which increases both of these and hydrolysis, which decreases the same. In the absence of $GS_2S°$ the yield should have been 89.9, however, the experimental value of 91.3 clearly shows that it is in between the values cited above.

As further proof of the absence of $GS_2S°$, Kartha states that the S content of a fat obtained from the azelaoglycerides is the same as that obtained by the oxidation of its mixed fatty acids. However, in the latter there is no saponification with alcoholic KOH prior to its estimation by Bertram separation (6). This agreement is based entirely on the yield of S. A closer examination would show that an increase in yield, due to incomplete oxidation of oleic acid, would be compensated by the loss of stearic acid due to secondary oxidation, as is unequivocally proved (7), but not referred to by Kartha in any of his communications (3,4,5). Furthermore, a sample of methyl oleate containing 2% of methyl stearate, on Kartha's oxidation, carbonate washing and chromatographic adsorption gave 1.6% of neutral products (S° of S.V. 343) after accounting for stearate and unoxidized oleate (8).

Kartha also calculated the S.V. of the scission products lost in Bertram separation by assuming that the α-ketol (from oleins) on saponification with alcoholic KOH yields *quantitatively* azelaic and nonoic acids. However, this was shown to be incorrect (9). On the basis of this calculation, he deduces that the neutral products eluted from the alumina column, contain distearononoin and stearodinonoin formed by ester interchange. Replacement of a half-esterified dicarboxylic acid (azelaic) in azelaoglycerides with a free