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Fatty Acid Analysis on Short Glass Capillary Columns

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Analyses of fatty acid methyl esters (FAME) were compared on 100-, 10-, and 2-m glass capillary columns coated with SP2340. The accuracy and precision for the analysis of FAME standards were comparable for all three columns. When actual food samples were chromatographed, the 100-m column gave superior resolution of the many positional and geometric isomers in hydrogenated vegetable oils and ruminant animal fats; however, analysis times were 90–135 min/sample. The 10-m column was adequate for quantitation of major fatty acids, but some minor acids were not detected. Analysis time ranged from 5 to 30 min depending on the sample and the chromatographic conditions. Low resolution made the 2-m column undesirable, even though the major fatty acids could be separated in less than 3.5 min. Quantitative data from the analysis of peanut oil, rapeseed oil, shortening, cod liver oil, pork, beef, and beef liver samples on 100- and 10-m columns are compared, and the characteristics of the three columns are discussed.

In order to evaluate current dietary habits, plan and execute nutritional research, and implement current recommendations on dietary fats, nutritionists need detailed data on the amounts and kinds of fatty acids in foods consumed in the United States. Collection of such data would require quantitative analysis of a vast number of foods. The use of packed column gas chromatography (GC) for the quantitative analysis of fatty acids in foods presents problems. (1) Choice of an appropriate internal standard is difficult because complex foods, such as cheeseburgers, contain so many fatty acids. In the absence of an internal standard, fatty acids can be quantitated only as normalized weight percent rather than as the more useful absolute amounts. (2) Resolution of positional isomers of unsaturated fatty acids is inadequate. (3) Separation of the cis and trans isomers is incomplete so the percent trans fatty acids cannot be estimated. The high efficiency of long (60 and 100 m) glass capillary columns coated with SP2340 solved these problems (Slover & Lanza, 1979): (1) separation between peaks was adequate for insertion of the internal standard, methyl heneicosanoate; (2) many positional isomers, particularly the octadecenoate isomers, can be resolved; (3) separation of the geometric isomers was adequate for estimation of the amount of trans fatty acids. However, the analysis took 90–135 min, depending on the sample. Recently, Ettre & March (1974), Johansen (1977), and Rooney et al. (1978) described significant reductions in analysis time with short glass capillary columns. Unfortunately, these authors explored mainly the qualitative aspects of this technique. The purpose of our investigation was to study the use of short capillary columns for the quantitative analysis of fatty acids in foods and the compromises which have to be made between analysis speed and resolution.

MATERIALS AND METHODS

Gas Chromatography. A Hewlett-Packard Model 5840 gas chromatograph equipped with a flame ionization detector, a Hewlett-Packard Model 7671A automatic liquid sampler, and a J & W Scientific, Inc. (Orangevale, CA) glass capillary column splitter were used. All SP2340 glass capillary columns were purchased from Quadrex Corp. (New Haven, CT). SP2340 is a cyanosilicone phase which has chromatographic properties similar to those of polar polyesters. Helium was used as the carrier gas for all analyses. Chromatographic conditions appear in Table II.

Sample Preparation. *Extraction*: All foods were extracted with chloroform–methanol as described by Floch et al. (1957). *Methylation*: Fatty acids were methylated by a modification of the procedure of Metcalfe et al. (1966). For quantification, an internal standard, methyl heneicosanoate, was added to each sample.

Nomenclature. In this paper the designation used for fatty acid identity is the following: the number before the colon gives the number of carbon atoms in the chain; the

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Table I. Characteristics of 100-, 10- and 2-m SP2340 Capillary Columns[a]

length, m	100	10	2	2
$\bar{u}$, cm/s	25	30	11	30
k[b]	9.0	8.0	9.1	7.5
TZ[c] (18:1/19:1)	23	10	3.4	2.6
n[d] ($\times 10^3$)	210	32	4.8	2.7
N[e] ($\times 10^3$)	170	25	3.8	2.1
split ratio	1/100	1/260	1/300	1/300
sample size, μg/injection	23	11	11	11

[a] All calculations are for methyl stearate 18:0 chromatographed isothermally at 150 °C unless stated otherwise. [b] Partition ratio $k = t_R'/t_M$ where t_R' = corrected retention time and t_M = solvent hold-up time. [c] Trennzahl (TZ) or separation number for 18:1ω9/19:1ω9 is TZ = $(t_{R_2}' - t_{R_1}')/(w_{h_1} + w_{h_2}) - 1$, where t_{R_2}' = the corrected retention time for peak 2 of a homologous pair, t_{R_1}' = the corrected retention time for peak 1 of a homologous pair, w_{h_1} = the width at peak half-height for peak 1, and w_{h_2} = the width at peak half-height for peak 2. [d] Number of theoretical plates $n = 5.54\ (t_R/w_h)^2$ where w_h = the width at peak half-height. [e] Number of effective plates $N = 5.54\ (t_R'/w_h)^2$.

Table II. Comparison of Retention Times for Fatty Acid Methyl Esters on 100-, 10-, and 2-m SP2340 Capillary Columns

			retention time, min	
length, m	temp, °C	$\bar{u}$, cm/s	18:1ω9c	24:0
100	150-200 at 0.4 °C/min	25	49.0	109.6
10	150-190 at 1.0 °C/min	30	7.4	23.2
10	170	22	4.7	15.2
2	140-160 at 3 °C/min	30	2.3	7.1
2	140-160 3 °C/min	11	4.5	9.7

number immediately following the colon gives the number of double bonds. A number following a Greek omega (ω) as in 18:2ω6 is the number of carbon atoms after the double bond farthest from the carboxyl. The geometric configuration of double bonds may be indicated by cis or trans at the end of the number as in 18:2ω6ct.

RESULTS AND DISCUSSION

We tested three lengths of glass capillary columns (100-, 10-, and 2-m) coated with SP2340. The 100-m column was our standard analytical column, which we had used for some time. The 10-m column was chosen because it is a popular and commercially available length. The 2-m column was selected since it was the shortest glass capillary column that could fit conveniently in the HP5840 GC. Some characteristics of these columns appear in Table I. The partition ratio (k) for methyl stearate was similar for all three columns, indicating similar phase ratios between the columns. The separation number or Trennzahl (TZ), as described by Kaiser (1961), is the number of peaks with base-line separation which can be fitted between adjacent members of a homologous series. For example, the TZ for 18:1ω9/19:1ω9 for the 10-m column was 10. The TZ values were calculated for this pair since TZ values do vary for different compounds and we wanted to separate the 18:1 isomers. The 10-m column had only ~15% of the theoretical plates of the 100-m column but retained ~40% of the separating power for the octadecenoates. The 2-m column was very inefficient compared to the other capillary columns but still had more theoretical plates than many packed columns used for fatty acid analyses. The efficiency of the 2-m column could be increased by lowering the linear velocity, but it was still considerably less efficient than the 10-m column.

Retention times of a late eluting fatty acid, methyl lignocerate (24:0), and methyl oleate (18:1ω9c), the major fatty acid in most foods, were compared on all three columns (Table II). Considerable analysis time could be saved with the shorter columns. Analysis was most rapid with the 2-m column, but the resolution was the poorest. Lowering the linear flow velocity (Table I) improved the resolution but increased the retention time to about that of the 10-m column. Figure 1 shows the separation of fatty acid methyl ester (FAME) standards on the 2- and 10-m columns.

A FAME sample derived from triglyceride standards was chromatographed 6 times each on 2-, 10-, and 100-m SP2340 capillary columns. Neither the 10- nor the 2-m column separated the fatty acids with chain lengths shorter than laurate (12:0); otherwise, the accuracy and precision were similar for all three columns (Table III), suggesting that both 2- and 10-m columns could be used for quantitative analysis.

For analysis of foods on shorter columns, the sample must be diluted to avoid overloading, which causes the loss of many of the minor peaks. A sample concentration of about half of that used on the 100-m column worked well for both the 10- and 2-m columns but changed the lower limit of detection to peaks representing at least 0.1% of the total sample. In the analysis of beef fatty acids, this was not a serious limitation, since 11 fatty acids make up at least 95% of the total fatty acid concentration, and all

Table III. Accuracy and Precision of Fatty Acid Methyl Esters[a] Chromatographed on 100-, 10-, and 2-m SP2340 Capillary Columns

		100 m			10 m[b]			2 m[c]		
fatty acid	known amount	$\bar{x}$	SD	CV, %	$\bar{x}$	SD	CV, %	$\bar{x}$	SD	CV, %
1200	2.386	2.38	0.040	1.7	2.31	0.058	2.5	2.38	0.72	3.0
1400	2.264	2.24	0.028	1.3	2.22	0.040	1.8	2.27	0.25	1.1
1600	2.394	2.38	0.023	1.0	2.37	0.019	0.8	2.39	0.021	0.9
1800	2.684	2.67	0.013	0.5	2.70	0.024	0.9	2.67	0.014	0.5
1819	6.143	6.12	0.044	0.7	6.11	0.044	0.7	6.12	0.036	0.6
1826	2.289	2.28	0.010	0.4	2.29	0.002	0.1	2.27	0.014	0.6
2000	0.720	0.72	0.007	0.9	0.73	0.007	1.0	0.72	0.005	0.7
1833	1.035	1.04	0.011	1.0	1.05	0.013	1.2	1.03	0.009	0.9
2200	1.744	1.74	0.011	0.6	1.75[d]	0.011	0.6	1.75	0.005	0.3
2219	0.640	0.74	0.002	0.3	0.74	0.015	2.0	0.73	0.013	1.8
2400	0.328	0.33	0.005	1.5	0.33	0.006	1.9	0.33	0.006	1.7

[a] Fatty acid methyl esters derived from triglyceride standards, quantitated as milligrams of triglycerides (TG) per milliliter of solvent. [b] Sample chromatographed at 150 °C, 1 °C/min, to 190 °C; $N = 6$. [c] Sample chromatographed at 140 °C, 3 °C/min, to 165 °C; $N = 6$. [d] $N = 5$.

Table IV. Quantitative Comparison of Fatty Acids in Beef on Different-Length SP2340 Capillary Columns

fatty acid	raw,[a] g/100 g			cooked, g/100 g		
	100 m	10 m	2 m	100 m	10 m	2 m
14:0	1.66	1.68	1.60	1.61	1.51	1.41
14:1ω5c	0.52	0.55	*b*	0.47	0.45	*b*
16:0	9.19	9.36	8.64	9.16	8.91	8.21
16:1ω7c	1.68	1.59	1.83	1.61	1.53	1.72
17:0	0.51	0.54	0.57	0.52	0.56	0.56
17:1ω8c	0.45	0.40	0.53	0.44	0.48	0.50
18:0	3.33	3.43	3.19	3.50	3.55	3.24
18:1ω?t	1.61	1.55	*b*	1.64	1.50	*b*
18:1ω9c	12.12	12.39	13.84	12.35	12.30	13.45
18:1ω7c	0.57	0.57	0.17	0.58	0.62	*b*
18:2ω6	0.68	0.71	0.86	0.62	0.68	0.78
other	2.83	1.25	1.50	1.79	1.02	1.51
total fat[c]	36.57	36.57	36.57	37.06	37.06	37.06
all fatty acids (FA)	35.15	34.02	32.73	34.29	33.21	31.38
all known FA	34.41	33.65	31.63	33.57	32.90	31.17
saturated FA	15.68	15.50	14.18	15.31	14.95	13.51
cis monoene FA	15.71	15.81	16.37	15.29	14.94	15.84
trans monoene FA	1.61	1.55	*b*	1.69	1.59	*b*
total monoene FA	17.85	17.36	16.37	17.31	16.53	15.84
cis PUFA	0.81	0.79	1.08	0.74	0.81	1.03
trans PUFA	0	0	0	0.06	0.09	*b*
total PUFA	0.81	0.79	1.08	0.80	0.90	1.03
% trans FA	4.58	4.56	*b*	5.00	5.17	*b*
P/S	0.05	0.05	0.08	0.05	0.06	0.08
all FA/total fat	0.96	0.93	0.89	0.93	0.90	0.85

[a] Typical fatty acid analyses from one raw and one cooked beef sample quantitated as grams per 100 g dry weight. Average of two analyses per sample. [b] Not resolved from other components. [c] Total fat: as determined by Folch extraction. All FA: summation of amounts of all fatty acids. All known FA: summation of amounts of identified fatty acids. Saturated FA: summation of amounts of all saturated fatty acids. cis monoene FA: summation of amounts of all cis monoene fatty acids. trans monoene FA: summation of amounts of trans monoene fatty acids. Total monoene FA: summation of amounts of all monoene fatty acids. cis PUFA: amounts of cis polyunsaturated fatty acids. trans PUFA: amount of trans polyunsaturated fatty acids. Total PUFA: summation of amounts of cis and trans PUFA. Percent trans FA: trans (%) = [(trans monoene + trans PUFA)/all fatty acids] × 100. P/S = cis PUFA/all saturated fatty acids. All FA/total fat: all fatty acids/total folch extractable fat.

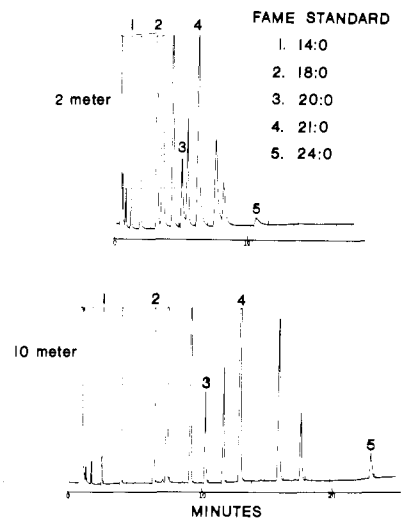

Figure 1. Fatty acid methyl ester standards chromatographed on 10- and 2-m SP2340 glass capillary columns. The 10-m column was temperature programmed from 150 to 190 °C at 1.0 °C/min, and the 2-m column was temperature programmed from 140 to 160 °C at 0.3 °C/min.

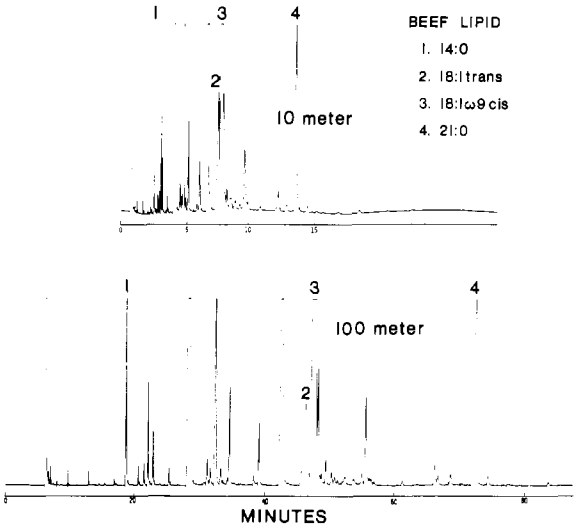

Figure 2. Fatty acid methyl esters derived from beef lipid chromatographed on 100- and 10-m SP2340 glass capillary columns. The 100-m column was temperature programmed from 150 to 200 °C at 0.4 °C/min, and the 10-m column was temperature programmed from 150 to 190 °C at 1.0 °C/min.

11 are present at concentrations greater than 1.0%. In Figure 2, separations of beef FAME on 100- and 10-m columns are compared.

The FAME from each of six different cuts of cooked and raw beef were chromatographed on 100-, 10-, and 2-m columns; data for one cooked and one raw sample appear in Table IV. The upper half of the table gives those 11 fatty acids that are present at more than 1% of the total fatty acids (normalized weight percent). The lower half of the table gives the summations of the individual fatty acids into specific classes and/or nutritionally relevant categories. Earlier work confirmed the accuracy of the 100-m column (Slover & Lanza, 1979). For all fatty acids present at less than 1%, the average relative difference was ~4% between the 100- and 10-m columns and ~11% between the 100- and 2-m columns. For the major fatty acids (14:0, 16:0, 18:0, and 18:1ω9c), the differences were 2 and 7% for the 10- and 2-m column, respectively. Beef

Table V. Comparison of Fatty Acids on a 100- and 10-m SP2340 Capillary Column

	g of TG/100 g[d]						g of TG/100 g[d]					
	corn oil		beef liver lipid		peanut oil		pork lipids		cod liver oil		rapeseed oil	
	100 m[a]	10 m[b]	100 m[a]	10 m[b]	100 m[a]	10 m[b]	100 m[a]	10 m[c]	100 m[a]	10 m[c]	100 m[a]	10 m[c]
14:0	0.02	0.02	0.07	0.07	0.06	0.03	0.18	0.09	4.00	3.93	0.04	0.04
16:0	12.08	11.54	1.10	1.11	10.53	9.16	2.01	1.95	13.34	13.07	2.59	2.44
16:1ω7c	0.05	0.09	0.08	0.11	0.04	0.04	0.29	0.28	10.76	11.09	0.11	0.13
18:0	2.04	1.99	0.99	1.01	2.52	2.29	0.98	0.98	2.78	2.82	1.05	0.96
18:1ω?t			0.04	0.03			0.02	0	0.41	0.40		
18:1ω9c	25.88	27.27	0.83	0.90	45.06	44.35	3.82	3.62	15.48	15.49	15.14	14.80
18:1ω7c	0.69		0.04		0.65		0.37	0.42	6.17	6.55	1.24	1.36
18:2ω6c	60.05	59.32	0.36		30.61	30.54	0.84	0.82	1.47	1.28	14.82	14.47
20:0	0.45	0.35	0.04		1.21	1.47	0.01	0	0.10	0.12	0.73	0.63
18:3ω3c	0.82	0.90			0.09		0.03	0.05	2.21	1.65	8.83	10.70
20:1ω9c	0.30				1.51	1.44	0.06	0.04	10.64	12.66	9.63	8.10
22:0	0.11		0.17	0.10	3.26	3.11			0.10	0	0.64	0.43
22:1ω9c					0.10	0.11			9.52	10.26	42.91	44.97
20:4ω6c			0.23	0.23			0.08	0.06	1.76	2.04		
24:0					1.41	1.96			10.08	9.30		
20:5ω3c			0.06	0.03								
22:6ω3c									8.80	7.92		
other FA	0.22	0.08	0.04	0.12	0.21	0.21	0.22	0.10	3.75	4.89	3.39	0.06
Summations of Fatty Acids												
total fat	100.00	100.00	6.15	6.15	100.00	100.00	9.41	9.41	100.00	100.00	100.00	100.00
all fatty acids (FA)	102.70	101.56	4.05	4.06	97.26	95.05	8.83	8.41	101.37	103.47	101.12	99.09
all known FA	102.70	101.56	4.05	4.02	97.23	94.84	8.69	8.37	98.00	102.02	89.03	99.06
saturated FA	14.82	13.96	2.20	2.27	19.11	18.18	3.16	3.06	20.27	20.98	5.08	4.51
cis monoene FA	26.92	27.36	0.94	1.27	47.43	45.97	4.51	4.36	52.57	56.05	70.29	71.96
trans monoene FA	0	0	0.04	0.03	0	0	0.02	0	0.41	0.40	0	0
total monoene FA	26.92	27.36	0.97	1.24	47.43	45.97	4.54	4.36	52.98	56.45	70.29	71.96
cis PUFA	60.87	60.22	0.82	0.71	30.70	30.54	0.96	0.88	24.32	32.19	15.34	15.95
trans PUFA	0	0	0	0	0	0	0	0	0.40	0.41	0	0
total PUFA	60.87	60.22	0.82	0.71	30.70	30.54	0.96	0.88	0.40	0.41	0	0
% trans FA	0.09	0	0.91	0.77	0	0	0.30	0.29	0.80	0.67	0	0
P/S	4.11	4.49	0.37	0.31	1.61	1.75	0.94	0.89	1.22	1.06	3.08	3.54

[a] Samples chromatographed at 150–200 °C at 0.4 °C/min. [b] Samples chromatographed at 150–190 °C at 1.0 °C/min. [c] Samples chromatographed isothermally at 170 °C. [d] Grams of fatty acids calculated as triglyceride (TG) per 100 g wet weight. Each sample was analyzed.

lipid samples chromatographed on the 10-m column produced ~30 peaks. Only minor fatty acids were missing: some short chain (<12:0), some branched chain, and a few unknown. On the 2-m column resolution was poor and only ~13 peaks appeared. Myristoleate (14:1ω5c) was not separated, the *trans*-octadecenoate isomers were not resolved, and *cis*-vaccenate (18:1ω7c) appeared only occasionally as a shoulder on the peak for oleate (18:1ω9c). The fatty acid summations listed in the lower half of Table IV show that results were similar for the 100- and 10-m columns. Most of the differences observed with the 2-m column were due to poor resolution which led to incorrect summations, loss of minor peaks, and no quantitation of the fatty acids with trans unsaturation. Some nutritionists now think that dietary fatty acids with trans unsaturation are undesirable and, therefore, their content in foods should be examined. Quantitation of trans isomers in various margarines with an SP2340 100-m capillary column gave good agreement with the established AOAC infrared procedure (Allen, 1969) and indicated that the technique was satisfactory for estimating the total trans fatty acid in foods (Slover & Lanza, 1977). In addition to the above problems with the 2-m column, its useful life was only about 1/10 that of the 10- and 100-m columns.

Data for six foods and/or oils obtained with the 10- and 100-m columns appear in Table V; the individual fatty acids appear in the first half and the summations in the second half. For nonhydrogenated corn oil, agreement was good between the 100- and 10-m columns, except that the positional isomers, 18:1ω7c and 18:1ω9c, were not well separated in the latter. For samples, such as beef liver, with long-chain PUFA (polyunsaturated fatty acids), chromatographic time could be reduced to one-fifth; for example, 22:5ω3 could be analyzed in 23 rather than 123 min. Peanut oil, another sample with late eluting fatty acids (22:0 and 24:0), could be chromatographed on the 10-m column in 15 instead of 110 min.

For pork lipid, cod liver oil, and rapeseed oil (Table V), the chromatographic conditions had to be altered in order to separate 18:3ω3 and 20:1ω9, since either one or both of these compounds were present in significant quantity. Fatty acid analysis of pork required only 11 min on the 10-m column, and the results differed from those with the 100-m column only in the lack of resolution between 18:1ω7 and 18:1ω9 and the loss of ~15 minor fatty acids usually present at concentrations of less than 0.1%. Even a 100-m column is probably inadequate to separate all the positional isomers of cod liver oil (Ackman and Eaton, 1978), but the major FAME can be well quantitated on the 10-m column. The positional isomers of 20:1 were separated on the 100-m but not on the 10-m column. For rapeseed oil in which erucic acid (22:1ω9c) often is monitored because it is thought to have harmful physiological effects (Beare-Rogers, 1979), a short analysis could be useful. The retention time of erucic acid was 90 min on the 100-m column and only 11 min on the 10-m column.

For the samples listed in Table V, the percent trans unsaturation was only 0–4% and was all from *trans*-octadecenoate isomers. When vegetable oils, which contain large amounts of linoleic acid (the 9,12-cis,cis isomer of the octadecadieneates), are hydrogenated, various trans isomers of linoleic acid also can be formed. Quantitation of linoleic acid is of considerable interest since it is the major essential fatty acid in most oils. Figure 3 shows that the

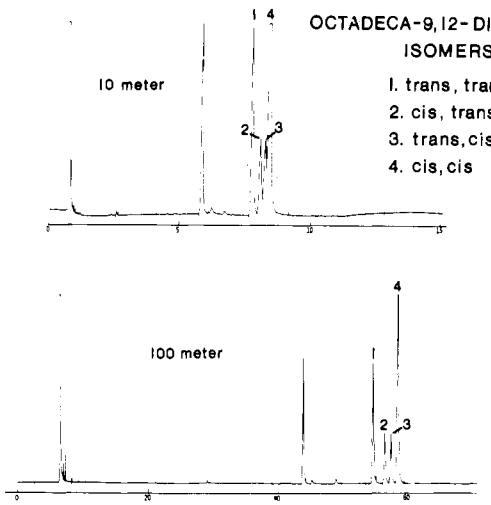

Figure 3. Methyl esters of the geometric isomers of 18:2ω6 chromatographed on 10-m and 100-m SP2340 glass capillary columns. The 10-m column was temperature programmed from 150 to 190 °C at 1.0 °C/min, and the 100-m column was temperature programmed from 150 to 200 °C at 0.4 °C/min.

100-m column completely separated the four geometric isomers of 18:2ω6cc, but the 10-m column did not completely separate the trans, cis, and cis,cis isomers.

CONCLUSION

Results were similar from the 10- and 100-m columns for the major fatty acids (greater than 1% normalized weight percent) and for the summation of individual fatty acids into various categories of interest to nutritionists, such as trans unsaturation, cis PUFA, and saturated fatty acids. The 10-m column could be useful for rapid analysis of large numbers of similar samples; however, the limitations of the 10-m as compared to the 100-m column for the separation and detection of minor fatty acids must be considered. The 2-m column was found to give rapid and reliable results for the analysis of standards or selective fatty acids, but it was inadequate for the quantitation of the complex mixtures of fatty acids found in most foods.

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Distribution and Amino Acid Composition of Protein Groups Located in Different Histological Parts of Maize Grain

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From normal and *opaque-2* maizes (*Zea mays*), the whole grain and its histological parts, germ, endosperm, and envelopes (combined tip cap, pericap, and probably aleurone layer), were characterized by their dry matter and nitrogen content. For germ and endosperm, nonprotein nitrogen, albumins, globulins, zein, and G_1-, G_2-, and G_3-glutelins were isolated; for envelopes only the first three fractions were extracted. Various protein fractions of similar solubility exhibit only slight differences in amino acid composition. Proteins, on basis of location in grain, can be classified as follows: (1) endosperm-specific proteins, consisting of zein and G_1- and G_2-glutelins and found virtually in endosperm only; (2) basic proteins, making up all other fractions and occurring in all tissues. The influence of extraction conditions on the isolation of protein fractions is discussed.

The maize caryopsis, connected to the rachis (cob) by the tip cap, is a complex physiological entity containing various histological elements with different genetic origins and with specific functions. Thus, the outermost tissues, which consist of the pericap and the seed outer layers with the aleurone layer excluded, are genetically identical with the maternal plant and have a protective function. The endosperm, the hereditary makeup of which is two-thirds maternal and one-third paternal, is a tissue filled with storage material used by the growing embryo during germination. The germ, which has equal inheritance from both parents, is composed of the scutellum, a storage element, and of the embryonic axis, which is at the origin of the new plant. Consequently, the proteins present in these various histological elements must be differentiated

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