Determination of Fatty Acid Composition by Gas Chromatography: II. Analysis with Use of Flame Ionization Detector[1]

S. WATANABE, S. NAKASATO, S. HAYANO, H. KUWAMURA, T. NAGAI, M. NEGISHI, Y. SASAMOTO, H. SEINO and S. SHIRAISHI, Japan Oil Chemists' Society
Gas Chromatography Committee, Yushikogyokaikan 7F, 3-13-11, Nihonbashi, Chuo-ku, Tokyo 103 Japan

ABSTRACT

In this study known mixtures of four or five fatty acid methyl esters were analyzed collaboratively by gas chromatography with flame ionization detectors. The experimental data was treated statistically to examine inter- and intralaboratory scattering. Moreover the effect of the use of correction factors was investigated. Even if only the specific analytical values that scattered a little were chosen, the averages of such values did not always approach the actual values. In some laboratories a sort of regularity was observed in the deviation of analytical values from real values throughout the analyses of four samples. The application of correction factors to the analytical values obtained by these laboratories resulted in a considerable decrease of interlaboratory scattering and deviation from the real values. When a constant amount of sample was injected, intralaboratory scattering was decreased, whereas interlaboratory scattering was not. Injection of large sample sizes caused deviation. From this collaborative study it was recommended that 0.5-1.0 μl of 20% solution be injected.

INTRODUCTION

The Gas Chromatography Committee organized in 1967 in the Japan Oil Chemists' Society has established a standard method for determination of the fatty acid composition of fats and oils by a gas chromatograph with a thermal conductivity detector (TCD) (1). However flame ionization detectors (FID) have recently become popular, and it was desirable to set a standard method employing an instrument with a FID. Thus collaborative studies were performed to obtain some practical data to serve for establishing the standard method. In these studies scattering of data and deviation of analytical values from real ones were examined. In addition, an investigation was made to determine whether application of correction factors would give a significant improvement on accuracy of analytical values. Accordingly, only known mixtures with known compositions were used as samples throughout all collaborative analyses. The collaborators were 34 industrial, independent, university and government laboratories involved in fat and oil chemistry.

COLLABORATIVE STUDY I

Procedure

In this study two samples, A and B, which have the compositions shown in Table I, were analyzed collaboratively. The purity of the methyl esters used was greater than 99.5%. The analyses were made according to the standard gas chromatographic method for the determination of fatty acid composition using a TCD. Since details were not specified, the collaborators were permitted to use the procedure they found best for their instrument.

Based on the results of the previous collaborative studies with TCD (2), the collaborators were requested to change attenuator range or chart speed in order to keep each peak height more than one-third of a full chart scale and each peak width more than 5 mm at half height. Of course, attenuator range and chart speed were previously checked to insure that they gave no error when changed. Collaborators were further required to analyze samples A and B three times in succession under the same operating conditions and to report the results of three determinations for each sample and the averages of three analytical values for each component. The percentage of each component was calculated from the ratio of each area to the sum of the areas under all of the component peaks. Peak areas were determined by multiplying the height by width at half height. The height and width were corrected for attenuation and chart speed, respectively.

Results and Discussion

The results of analyses of samples A and B are listed in Table I. The average for each component was in excellent agreement with the known value, but the coefficient of variation (CV) was fairly large, especially for methyl laurate, which was eluted first, and methyl elaidate, which was last, although their peak sizes were enlarged according to the regulations. The CV of analytical values of sample B were much larger than those of sample A. This might be due to the fact that sample B contained the components in wider range of composition.

The CV reveals the amount of scattering of analytical values from their average, but when the average is in good agreement with the known value the CV is regarded as a measure of deviation of analytical values from the actual value.

Many reports have been published on relative response of FID (3-6). However the investigation of relative response in collaborative analyses has not been reported. The effect of the use of correction factors was examined from the results of analyses of samples A and B, which were dissimilar in composition. The authors determined correction factors of each methyl ester for every laboratory from the analytical values of sample A, where the correction factor was defined as a ratio of known value to analytical value. Then the analytical values of sample B were corrected by these factors; the corrected data are shown in Table I. No regularity was observed between the carbon number of methyl esters and the correction factors. In the table the averages and the CV of the corrected data are listed with those of the uncorrected. In spite of the correction, no improvement was observed in the interlaboratory scattering or the scattering in the averages of three experimental values obtained by each collaborator. Intralaboratory scattering, scattering in three experimental values obtained by one laboratory, was very large in several laboratories, and it is useless for such laboratories to apply the correction factors to the averages of their data. Twelve

[1]Presented at the JOCS-AOCS Joint Meeting, Los Angeles, April 1972.

TABLE I

Analyses of Samples A and B (Collaborative Study I)

Lab.	Uncorrected values of A, %					Uncorrected values of B					Uncorrected values[a]				
	$C_{12:0}$	$C_{14:0}$	$C_{16:0}$	$C_{18:0}$	$C_{18:1}$[b]	$C_{12:0}$	$C_{14:0}$	$C_{16:0}$	$C_{18:0}$	$C_{18:1}$[b]	$C_{12:0}$	$C_{14:0}$	$C_{16:0}$	$C_{18:0}$	$C_{18:1}$[b]
1	19.6[c]	18.2	24.7	20.3	17.2	5.8	10.7	52.2	20.7	10.7	5.1	10.4	52.3	21.3	10.9
2	15.9	17.1	25.4	22.5	19.1	5.2	10.4	50.6	22.5	11.4	5.9	10.6	50.9	21.7	10.9
3	17.1	18.3	26.7	21.9	15.9	5.6	11.6	56.8	18.8	7.3	6.0	11.6	55.0	18.9	8.4
4	17.5	17.8	25.7	21.3	17.7	5.8	11.0	52.4	19.2	10.1	6.0	11.3	52.6	19.7	10.4
5	18.1	17.9	25.6	20.9	17.4	5.1	10.1	54.1	19.7	11.1	5.1	10.1	53.5	19.8	11.5
6	20.6	18.7	25.1	18.9	16.6	4.7	9.9	56.5	19.3	9.8	3.9	9.2	55.2	21.3	10.4
7	18.8	17.8	25.1	20.2	17.3	5.1	10.1	53.0	21.5	10.3	4.7	10.0	52.3	22.5	10.5
8	18.2	17.8	25.1	20.3	18.5	5.2	10.2	52.0	21.0	11.7	5.0	10.1	51.7	22.0	11.2
9	21.0	18.2	24.6	19.3	16.8	5.6	10.6	52.9	21.6	10.2	4.5	10.0	51.9	23.1	10.5
10	18.3	18.1	25.0	20.9	17.7	5.0	10.1	53.0	21.4	10.5	4.9	9.8	53.1	21.6	10.5
11	19.5	18.2	26.0	19.8	16.5	4.6	9.5	58.1	18.8	9.0	4.2	9.3	56.3	20.3	9.9
12	17.2	17.7	26.1	21.6	17.5	4.7	9.8	55.0	20.7	9.9	4.9	10.0	54.0	20.7	10.3
13	17.2	17.7	25.6	21.2	18.3	5.3	10.7	52.7	20.5	10.7	5.5	10.9	52.0	20.9	10.7
14	15.8	17.7	26.5	21.5	18.4	4.8	10.1	53.7	21.2	10.2	5.5	10.4	52.1	21.7	10.2
15	18.2	18.0	25.8	20.9	17.1	4.8	9.8	55.1	20.2	10.0	4.6	9.7	54.3	20.7	10.6
16	17.0	19.9	26.1	20.7	16.4	6.6	12.0	48.2	22.3	10.8	6.9	10.8	47.2	23.2	11.9
17	18.2	17.8	25.4	21.0	17.6	5.2	10.2	52.0	21.7	10.9	5.0	10.2	51.5	22.1	11.1
18	16.8	16.9	25.7	21.5	18.6	4.4	9.5	54.1	23.3	8.7	4.7	10.0	53.4	23.5	8.4
19	16.6	17.9	25.7	22.1	17.8	5.3	10.5	51.8	21.8	10.6	5.8	10.5	51.5	21.4	10.8
20	18.0	17.7	25.7	21.5	17.1	5.0	10.0	53.3	21.1	10.6	4.9	10.2	52.5	21.2	11.2
21	18.1	18.3	25.4	20.9	17.1	5.2	9.7	53.7	21.0	10.5	5.1	9.4	53.1	21.4	11.0
22	18.7	18.3	25.6	20.4	16.9	5.3	10.3	53.9	20.4	10.1	5.0	10.0	52.9	21.4	10.7
23	17.8	18.0	25.4	21.2	17.7	5.5	9.8	52.6	21.5	10.6	5.4	9.7	52.3	21.8	10.7
24	17.6	17.6	25.5	21.5	17.8	5.3	10.4	52.3	21.4	10.6	5.4	10.5	51.9	21.4	10.7
25	17.1	17.6	25.9	21.6	17.8	5.3	10.5	53.0	20.8	10.4	5.6	10.8	52.1	20.9	10.6
26	19.9	18.2	25.3	20.2	16.4	5.7	10.7	53.7	20.3	9.7	5.0	10.4	52.9	21.2	10.5
$\bar{x}$	18.0	18.0	25.6	20.9	17.4	5.2	10.3	53.3	20.9	10.3	5.2	10.2	52.6	21.4	10.6
CV	7.10	2.98	1.88	3.80	4.33	8.64	5.57	3.62	5.22	8.46	11.80	5.52	3.14	4.77	7.56
Known value	17.7	17.8	25.2	21.4	17.9	5.3	10.4	52.6	21.2	10.5					

[a]Corrected value = analytical value x correction factor x 100/Σ (analytical value x correction factor) where correction factor was obtained by dividing known value by analytical value of sample A.

[b]Methyl elaidate.

[c]Average of three measurements.

TABLE II

Averages and Coefficients of Variation of Uncorrected and Corrected Values by All and Selected Laboratories, and Averages of Intralaboratory Coefficients of Variation

% and CV	Group of lab.[a]	$C_{12:0}$	$C_{14:0}$	$C_{16:0}$	$C_{18:0}$	$C_{18:1}$[b]
		Known value, %				
		5.3	10.4	52.6	21.2	10.5
$\bar{x}$, %	Bo (n=26)	5.2	10.3	53.3	20.9	10.3
	Bo'	5.2	10.2	52.6	21.4	10.6
	B_1 (n=12)	5.2	10.3	53.0	21.2	10.5
	B_1'	5.1	10.2	52.3	21.6	10.7
CV[c]	Bo (n=26)	8.64	5.57	3.62	5.22	8.46
	Bo'	11.8	5.52	3.14	4.77	7.56
	B_1 (n=12)	5.44	2.82	2.43	3.20	5.52
	B_1'	5.40	2.56	2.35	3.05	5.08
Average of intralaboratory CV	Bo (n=26)	4.16	2.55	0.77	1.95	2.14
	B_1 (n=12)	2.93	1.19	0.46	1.01	1.36
	Bo - B_1 (n=14)	5.21	3.71	1.03	2.76	2.81

[a]Bo: all laboratories; B_1: selected laboratories (small intralaboratory scattering); Bo': Bo corrected by correction factors; B_1': B_1 corrected by correction factors.

[b]Methyl elaidate.

[c]Interlaboratory coefficient of variation.

laboratories were chosen in which intralaboratory scattering was so small as to be less than 2.2% for any four components of the five in the analyses of samples A and B. The uncorrected and corrected data of analyses of sample B by these twelve collaborators are summarized in Table II. No significant difference was observed among the four averages in the table—the averages of the data of all laboratories and selected 12 laboratories, and the averages of the corrected data of both. The interlaboratory CV of the corrected and uncorrected values of both groups of all and selected laboratories are also shown in the table. In addition, Table I shows that the averages of intralaboratory CV for the selected laboratories are considerably less than those of the others (Bo - B_1).

COLLABORATIVE STUDY II

Procedure

In the second experiment samples C and D, having similar compositions as shown in Table III, were analyzed. The specified operating conditions were similar to those of the first experiment. However the collaborators were requested to choose and report only the results of three analyses in which CV for four components out of five in each sample were less than 2.2%.

Results and Discussion

As shown in Table III, the averages agreed very closely with the known values for both samples. The CV for samples C and D were less than those for sample B, but not less than those for sample A. Accordingly, it was found that choosing the data for which scattering was small did not always cause a decrease of interlaboratory scattering. The analytical values of sample D were corrected by the correction factors, which were calculated from the analytical values of sample C; the results are summarized in Table III. Significant difference was not observed between the corrected and uncorrected data. An analysis of variance of the data of the first and second study was made. As a result, the laboratories were placed into three classes depending upon their deviation of analytical values from the known values. In the first class, deviation showed a sort of regularity for every analysis and intralaboratory scattering was small. In the second, the deviation was so small that the correction factors were almost unity for all components in the analyses of the four samples. The deviation in the last class was large and correction factors showed no regularity; therefore the use of the correction factors was not effective. The corrected data for the analyses of sample D by all laboratories were divided into two groups—one group of data obtained by the first and second classes described above, which showed the correction factors with regularity, and the other group of data obtained by the third class, which showed no regularity. Then averages and CV of the data obtained by these two groups were calculated; they are shown in Table III. The CV of D_2 were larger than those of D_1. However the CV of

TABLE III

Effect of Correction for Analysis of Sample D by Laboratories Showing Correction Factors with Regularity and No Regularity

Sample		Lab.[a]	n	Before correction, % $C_{12:0}$	$C_{14:0}$	$C_{16:0}$	$C_{18:0}$	$C_{18:1}$[b]	After correction, % $C_{12:0}$	$C_{14:0}$	$C_{16:0}$	$C_{18:0}$	$C_{18:1}$[b]
C[c]	$\bar{x}$	Co	18	14.6	34.2	9.9	27.1	14.2					
	Known value			14.5	33.9	9.7	27.4	14.5					
	CV	Co	18	6.1	2.9	2.8	3.7	3.9					
		Do	18	15.7	32.4	11.3	25.4	15.1	15.6	32.2	11.2	25.8	15.2
D	$\bar{x}$	D_1	7	15.4	32.1	11.3	25.8	15.3					
		D_2	11	16.1	32.7	11.3	25.0	14.9	15.9	32.1	11.0	25.6	15.0
	Known value			16.2	32.5	10.9	25.7	14.7					
		Do	18	3.8	2.7	3.4	2.3	3.9	3.1	1.7	3.4	1.0	3.2
	CV	D_1	7	3.7	2.5	3.8	2.2	3.8					
		D_2	11	3.9	3.0	3.0	2.8	4.0	2.9	1.4	2.8	0.6	2.8

[a]Co: all laboratories; Do: all laboratories; D_1: laboratories that showed correction factors with no regularity; D_2: laboratories that showed correction factors with regularity.

[b]Methyl elaidate.

[c]Sample C analyzed to obtain correction factors.

TABLE IV

Analyses of Sample E (Collaborative Study III)

CV	Laboratory	Sample size, μl	$C_{12:0}$	$C_{14:0}$	$C_{16:0}$	$C_{18:0}$
			Known value, %			
			8.8	22.4	48.3	20.5
	$\bar{x}$, %	0.5	8.6	22.0	49.0	20.4
	n=5	1.0	8.3	22.1	49.8	19.8
		5.0	8.0	21.9	50.6	19.6
		0.5	7.87	0.65	3.71	6.23
CV[a]	Total	1.0	6.33	1.29	2.97	5.51
		5.0	3.04	3.81	1.49	8.07
	Lab.					
		0.5	1.55	1.69	0.84	1.31
	1	1.0	2.14	0.91	1.05	1.99
		5.0	2.26	0.59	0.53	1.22
		0.5	1.33	0.40	0.21	0.24
	2	1.0	1.15	1.18	0.48	0.74
		5.0	1.37	1.04	0.41	0.36
		0.5	2.95	2.54	1.38	3.69
CV[b]	3	1.0	2.05	2.78	2.64	5.53
		5.0	1.49	2.57	2.29	4.26
		0.5	1.18	1.17	0.35	0.57
	4	1.0	1.77	1.38	0.74	0.88
		5.0	2.41	1.48	0.63	1.51
		0.5	2.00	1.13	0.95	1.22
	5	1.0	3.03	0.94	1.03	1.32
		5.0	1.00	1.27	0.78	0.71

[a]Interlaboratory scattering.
[b]Intralaboratory scattering.

corrected D_2 were considerably smaller—less than 3% for all components.

COLLABORATIVE STUDY III

Procedure

This study was carried out to examine the relationship between sample size and scattering or deviation. Sample E, having the composition shown in Table IV, was diluted to 20% solution with chloroform prior to analysis. Sample sizes to be injected were 0.5, 1.0 and 5.0 μl of this solution. The collaborators were requested to make five determinations for each sample size using the same packing material and a column of 2 m length under operating conditions identical to those of the first study.

Results and Discussion

Experimental data were treated statistically without use of correction factors. Variation in sample size from 0.5 to 5.0 μl caused no significant decrease in the CV, which means that there was no decrease in interlaboratory scattering; however intralaboratory scattering was considerably smaller, as shown in Table IV. Large sample sizes caused more deviation from the known value. From the results it is expected that intralaboratory scattering may be decreased by injecting a constant amount of sample, which should be in an optimum range according to the composition of a sample.

ACKNOWLEDGMENT

T. Tsuchiya, T. Asahara and T. Hashimoto gave helpful comments. Members of the Gas Chromatography Committee aided through discussions. Nippon Oils and Fats Co., Ltd. provided the pure methyl esters.

REFERENCES

1. JOCS Standard Method 2.4.21-71 (1971).
2. Watanabe, S., S. Hayano, T. Akiya, K. Mimura, S. Nakasato, H. Seino and Y. Usui, JAOCS 50:357 (1973).
3. Ackman, R.G., J. Gas Chromatogr. 2:173 (1964).
4. Ackman, R.G., Ibid. 6:497 (1968).
5. Ettre, L.S., J. Chromatogr. 8:525 (1962).
6. Ackman, R.G., and J.C. Sipos, Ibid. 16:298 (1964).

[Received February 9, 1973]