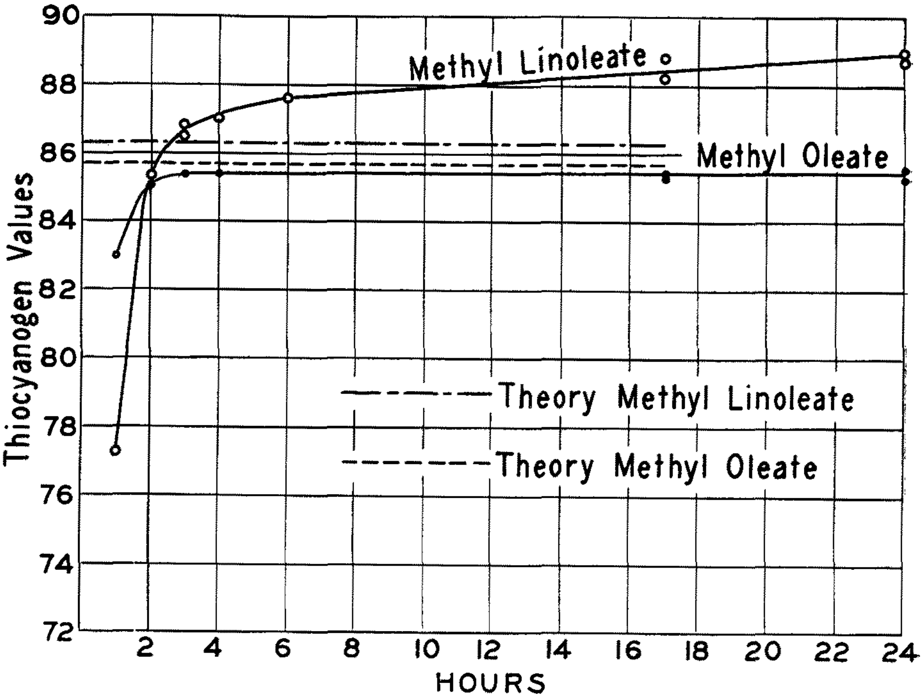

were used in the computations:

% methyl linoleate=1.159 × (iodine no. — SCN no.)

% methyl oleate=1.167 × (2 SCN no. — iodine no.)

The results of the analysis and the calculated compositions of the mixtures are given in Table IV.

For mixture A, which has only 10.6 percent of methyl linoleate, the calculated compositions show good agreement with the known, with the exception of that calculated from the Hanus iod. no. and 24-hour SCN no.

In the computed compositions of mixture B, which contained 51.0 percent of methyl linoleate, the discrepancies due to the 24-hour SCN. no., as well as to the Hanus iod. no., are quite evident. The composition calculated by using the 3-hour SCN. no. and Wijs iod. no. is practically identical with the known, and with that computed from the Wijs iodine number alone.

As a further indication of the reliability of the 3-hour absorption time, the percent of saturated esters in a distilled sample of methyl esters of 18 C atom acids of olive oil was computed from the Wijs iod. no. (98.5) and 3-hour SCN. no. (80.5). The calculated amount, 6.1 percent of saturated esters, agrees very well with 5.9 percent found by the Bertram procedure.

It should be noted that this investigation was limited to methyl esters. Until the study of thiocyanogen absorption is extended to include pure glycerides or mixtures of glycerides of known composition, the use of the 3-hour thiocyanogen absorption time directly on fats and oils may not be justified. Such an extension of the method is contemplated in future work.

Summary

Iodine numbers by the Hanus and Wijs methods and thiocyanogen numbers using various absorption periods, were determined on methyl oleate and methyl linoleate and on mixtures of these esters. It is concluded that iodine numbers by the Wijs method and three-hour thiocyanogen numbers are more satisfactory for methyl linoleate and for mixtures containing large amounts of this ester.

Small amounts of higher saturated acids were determined with a precision of about 0.1 unit-percent by means of the Bertram procedure. A purified specimen of methyl oleate was found to contain about 0.2 percent of saturated ester by this method.

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Variation in Free Fatty Acid Within a Seed Sample

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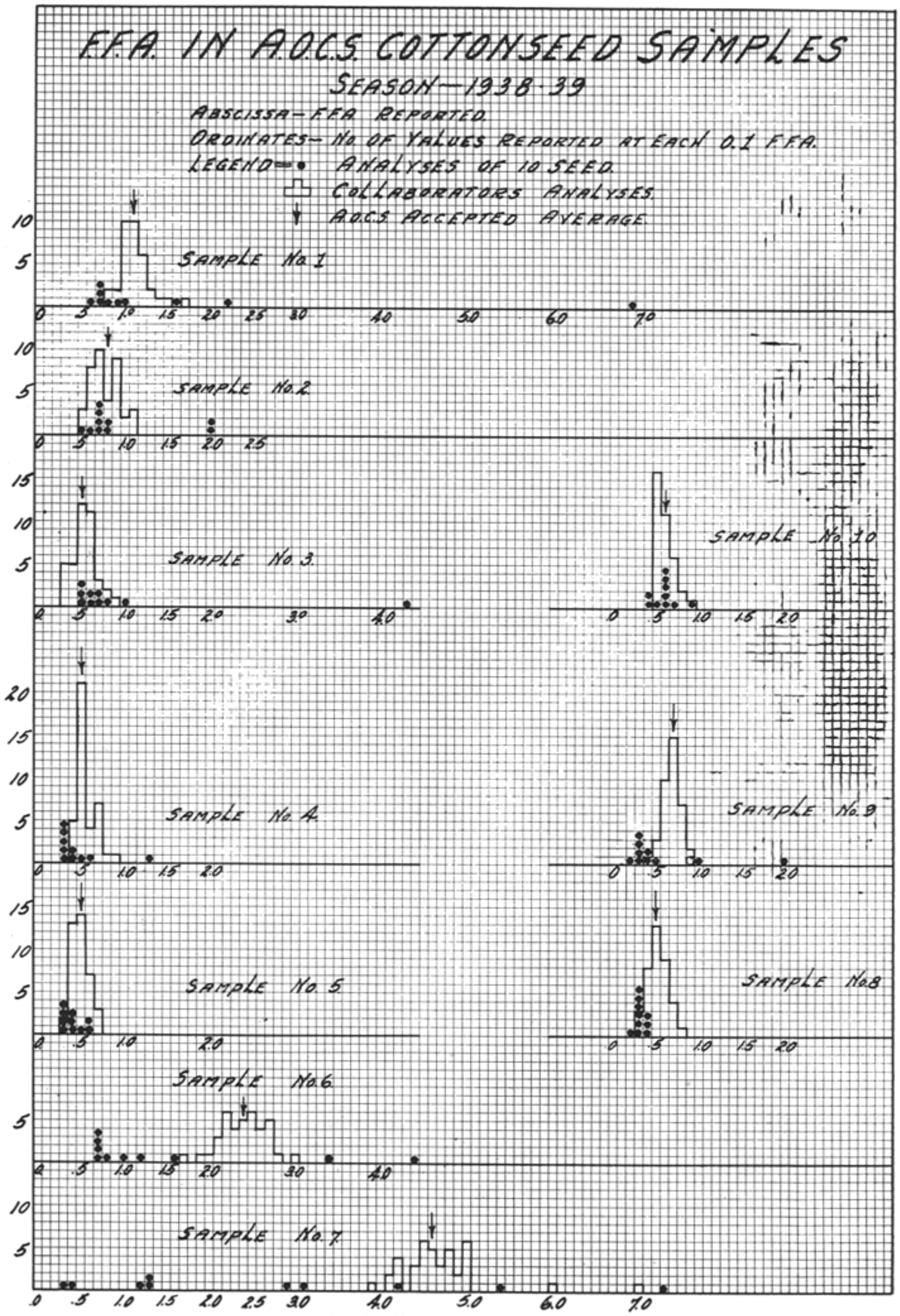
THE A. O. C. S. seed samples of the 1938-39 season have been analyzed by Edeler's method as described in Oil & Soap, Volume XV, pages 291-2. The F. F. A. was determined on ten samples of ten seed each. This was done on each of the ten A. O. C. S. samples. The analytical work was done by Mr. Reuben Lambert of the Procter & Gamble Service Laboratory.

The results were as follows:

Extraction No.	Seed No. 1		Seed No. 2		Seed No. 3		Seed No. 4	
	F.F.A.	Appearance	F.F.A.	Appearance	F.F.A.	Appearance	F.F.A.	Appearance
1	1.01	—	0.60	—	0.71	—	0.25	—
2	0.84	—	0.72	—	4.31	1 bad seed	0.27	—
3	6.87	—	0.68	—	0.65	—	0.42	—
4	2.15	—	0.82	—	0.61	—	0.61	1 br. spotted
5	0.67	—	0.67	—	0.46	—	0.31	—
6	0.74	—	0.54	—	0.51	—	1.33	1 bad seed
7	1.56	—	2.03	1 bad seed	0.53	—	0.26	—
8	0.89	—	0.71	—	0.61	—	0.34	—
9	0.70	—	0.77	—	0.76	—	0.36	—
10	0.59	—	1.98	—	0.98	—	0.48	1 translucent
Average	1.60		0.95		1.01		0.46	
Accepted Average by Seed Analysis Com.	1.1		0.8		0.5		0.5	

Extraction No.	Seed No. 5		Seed no. 6		Seed No. 7		Seed No. 8		Seed No. 9		Seed No. 10	
	F.F.A.	Appearance	F.F.A.	Appearance	F.F.A.	Appearance	F.F.A.	Appearance	F.F.A.	Appearance	F.F.A.	Appearance
1	0.38	—	0.67	O.K.	0.38	O.K.	0.27	O.K.	0.46	O.K.	0.58	O.K.
2	0.29	—	1.22	2 very dark	7.31	2 Brown	0.35	O.K.	0.97	1 lt. br.	0.62	O.K.
3	0.28	—	0.83	O.K.	1.16	1 Brown	0.34	O.K.	2.08	2 lt. br.	0.56	O.K.
4	0.50	—	4.42	1 Br.; 2 Tr.	4.21	1 Dr. Br.	0.32	O.K.	0.26	1 lt. br.	0.66	O.K.
5	0.34	1 dried up	0.69	O.K.	3.12	1 Br. Spot	0.35	O.K.	0.34	O.K.	0.86	O.K.
6	0.64	2 dried up	1.04	1 Sl. Br.	1.31	1 Br.; 1 lt. br.	0.23	O.K.	0.40	1 lt. br.	0.46	O.K.
7	0.34	—	0.69	1 Sl. Br.	1.25	1 Black	0.34	O.K.	0.43	O.K.	0.38	O.K.
8	0.42	—	1.61	O.K.	0.34	O.K.	0.34		0.30	O.K.	0.55	O.K.
9	0.41	—	0.68	O.K.	5.43	1 very dk.	0.36	O.K.	0.30	O.K.	0.35	O.K.
10	0.58	—	3.38	1 Br.; 1 Tr.	2.92	2 Br.	0.28	O.K.	0.20	O.K.	0.57	O.K.
Average	0.42		1.52		2.74		0.32		0.57		0.56	
Accepted Average by Seed Analysis Com.	0.5		2.4		4.6		0.5		0.7		0.6	



The comments about the appearance were made in advance of the analysis. In about half the cases the comments indicating unusual appearance were associated with high F. F. A.

The results on the ten seed samples and those of the A. O. C. S. collaborators are shown graphically in the appended diagram.

There seems to be a fairly close relationship between the scatter of the A.O.C.S. results and the scatter of the 10 seed results. In an effort to develop this mathematically, the standard (root-mean-square) deviations of the collaborators were computed. The standard deviation of the 10 seed values about the A. O. C. S. accepted averages were also computed. They are shown at bottom of this column.

For judging differences in order, the Spearman rank order correlation is used. This function produces a value of +1 for perfect agreement of order and —1 for completely opposite order.

The Spearman rank order correlation,

$$\text{rho} = 1 - \frac{6\,\Sigma D^2}{N\,(N^2-1)}$$

For the above differences, this is

$$1 - \frac{6\,(30.5)}{10\,(99)} = +\ .82$$

For practical purposes it is evident that the size of the A. O. C. S. scatter is predicted by the variation within the sample as shown by the 10 seed analysis. This is what one might expect and is not of any great importance if the amount of the scatter is not too large. To throw some light on this, the scatter of the *individual* seed values was computed from the A. O. C. S. values and from the 10 seed values.

Seed No.	1	2	3	4	5	6	7	8	9	10
AOCS Std. Dev.	.25	.16	.15	.11	.10	.26	.30	.08	.06	.10
10 Seed Std. Dev.	1.88	.562	1.21	.316	.135	1.52	2.87	.196	.55	0.143
Order AOCS	3	4	5	6	7½	2	1	9	10	7½
Order 10 seed	2	5	4	7	10	3	1	8	6	9
Differences in order	1	1	1	1	2½	1	0	1	4	1½

From statistical mathematics we know that the scatter of individual values is equal to the scatter of the means, times the square root of the number of individuals in the mean. Since the A. O. C. S. sample is 200 gms. we will assume 2000 seed to be represented. The A. O. C. S. values will be multiplied by $\sqrt{2000}$ and the 10 seed values by $\sqrt{10}$. The ten seed values should be given a further correction for the small size of the sample. This correction is $\sqrt{\frac{10}{9}}$

Tabulated, the results are shown below.

The calculation of the standard deviation of the individual seed from the standard deviation for groups of 10 is not too exact, since there are only ten samples for each large sample. There is undoubted significance, however, in the fact that the standard deviation of individual seed calculated from the collaborators is greater in every case than when calculated from samples of 10.

To the author, the relationships above indicate that the sampling of the sample itself is in need of attention. The collaborator may withdraw a portion of seed from the can without proper mixing. The hulling and separating may be carried out in such a way that the 40 gms. of meats are not truly representative of the entire meats in the seed.

It is recommended that the collaborators take special precautions in sampling the samples, both initially and after the hulling operation.

Seed No.	1	2	3	4	5	6	7	8	9	10
Std. Dev. AOCS	.25	.16	.15	.11	.10	.26	.30	.08	.06	.10
Std. Dev. individual seed (above x $\sqrt{2000}$)	11.2	7.2	6.71	3.82	4.47	11.62	13.41	3.58	2.68	4.47
Std. Dev. 10	1.88	.56	1.21	.316	.135	1.52	2.87	.196	.55	.143
Std. Dev. individual seed (above x $\sqrt{\frac{10}{9}}$ x $\sqrt{10}$)	6.26	1.87	4.03	1.05	.449	5.07	9.56	.66	1.83	.478

Note on the Detection of Myristic Acid in Alfalfa Seed Oil

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IN an earlier communication on the subject of the saturated fatty acid fraction of alfalfa seed oil[1], it was reported that indications had been found for the presence herein of myristic acid and that two of the others had been identified as palmitic and stearic. The latter pair, apparently because of the peculiar quantitative relationships existing between them in this case, had been regarded by others as a single entity, or margaric acid. Our conclusion in respect to myristic acid was in part based upon the fact that replicated fractional distillations of the methyl esters of the mixed saturated acids had yielded a fraction having a molecular weight which, in *a priori* grounds, suggested a two-component mixture consisting of 65 mol-pct. myristic ester and 35 mol-pct. palmitic. The molecular weight of the regenerated fatty acids had been found to be 238.3, or that of a mixture consisting of 64.6 mol-pct. of the former and 35.4 mol-pct. of the latter.

Because of the certainty of recovering, for characterization purposes, but very small quantities of material in view of the inevitable losses to be expected in attempts at affecting a separation of the constituents of this fraction, it was obviously desirable to approach the problem of establishing identity from the simpler physical rather than the more involved chemical angle. To that end, the solidification point of the mixture was determined.

The mixture was found to have a solidification point of 46.45° C. On referring this value to the binary acid solidification point curve for myristic-palmitic acids[2], it becomes apparent that a myristic acid content of either 64.3 or 80.5 mol-pct. is indicated. Inasmuch, however, as the former value, rather than the latter, is in substantial agreement with that obtained empirically by calculation, it follows that this fact lends confirmation to the conclusions as to the composition of the fraction in question.

This acid is only a minor component of the whole number comprising alfalfa seed oil. Fractional distillation of a 27-gram portion of the methyl esters of its saturated fatty acids yielded a small quantity containing approximately 0.9 gram of this acid, equivalent to *ca* 1.28 per cent of the oil. Its occurrence in alfalfa seed oil has not been previously reported.

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