

many of his observations would not be valid. To test this possibility olive oil was oxidized with potassium permanganate with and without the addition of acetic acid. The fatty acids were recovered and subjected to the Bertram procedure (21). No significant difference in the saturated acids determined by the two oxidation methods could be observed. Thus while Kartha's method is obviously of limited accuracy, it has not been shown that his conclusions are necessarily untrue.

Summary

Various procedures for the permanganate oxidation of fats have been tried on purified model fatty acid esters. It was found that all the procedures tested except Kartha's procedure caused considerable loss of ester groups. Kartha's procedure, on the contrary, caused ester groups to be synthesized during the oxidation. It was concluded that none of the oxidation procedures that were tried is a very reliable method for the study of glyceride structure in fats. However the limitations which were discovered in Kartha's procedure could not be shown to invalidate Kartha's hypothesis of glyceride structure.

REFERENCES

- Hilditch, T. P., and Lea, C. H., *J. Chem. Soc.*, 1927, 3106.
- Kartha, A. R. S., *J. Am. Oil Chemists' Soc.*, 30, 280 (1953).
- Hilditch, T. P., "The Chemical Constitution of Natural Fats," p. 405, John Wiley and Sons Inc., New York, 1940.
- Hilditch, T. P., *J. Am. Oil Chemists' Soc.*, 31, 433 (1954).
- Vander Wal, R. J., *J. Am. Oil Chemists' Soc.*, 32, 240 (1955).
- Hilditch, T. P., *J. Am. Oil Chemists' Soc.*, 32, 241 (1954).
- Hammond, E. G., and Lundberg, W. O., *J. Am. Oil Chemists' Soc.*, 31, 427 (1954).
- Clarkson, C. E., and Malkin, T., *J. Chem. Soc.*, 1934, 666.
- Swern, Daniel, and Parker, W. E., *J. Am. Oil Chemists' Soc.*, 29, 431 (1952).
- Hammond, E. G., and Lundberg, W. O., *J. Am. Oil Chemists' Soc.*, 30, 433 (1953).
- Deuel, H. J., "Lipids," vol. 1, p. 120, Interscience Publishers Inc., New York, 1951.
- Chandlee, P., Eshelman, L., Pauls, J. F., Hammond, E. G., and Bird, E. W., *J. Dairy Sci.*, in press.
- Hunter, L., and Hyde F., *Analyst*, 58, 523 (1933).
- Keppeler, J. G., *Rec. trav. chim.*, 76, 49 (1957).
- Kalbe, H., *Hoppe-Zyler Zeit. physiol. chem.*, 397, 19 (1954).
- Armstrong, E. F., and Hilditch, T. P., *J. Soc. Chem. Ind.*, 44, 43T (1952).
- Knight, H. B., Koos, R. E., and Swern, Daniel, *J. Am. Chem. Soc.*, 75, 6212 (1953).
- Waters, W. A., in "Organic Chemistry an Advanced Treatise," Gilman, H., editor, vol. IV, p. 1,210, John Wiley and Sons Inc., New York, 1953.
- Yakubov, M. K., *Masloboino-Zhirovaya Prom.*, 21, 14 (1956).
- Steger, A., and van Loon, J., *Rec. trav. chim.*, 52, 593 (1933).
- Markley, K. S., "Fatty Acids," p. 402, Interscience Publishers Inc., New York, 1947.

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Correlation of Chromatographic Absolute Loss Determinations with the A. O. C. S. Cup Refining Method in Soybean Oil

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AMONG THREE METHODS (chromatographic, acetone-insoluble, and Wesson methods) available for determining refining efficiency, the chromatographic method is more and more the focus of interest because of its simplicity. While the acetone-insoluble determinations and the Wesson method are time-consuming and elaborate and require extreme skill on the part of the analyst, the chromatographic method can be run rapidly with relatively little experience. A series of two or more columns can be handled simultaneously, and a number of samples can be put through the adsorbent in succession.

The method is based on the relative difference in affinity of neutral oil and so-called minor constituents of crude oil toward the alumina adsorbent. Free fatty acids, phosphatides, moisture, volatile materials, certain pigments and impurities, such as meal and linters, having more affinity than neutral oil, are adsorbed by the column and cannot be eluted with ether. Neutral oil, on the other hand, can be recovered quantitatively with ether.

The technique was first developed by the International Chemical Union and published by Handshumaker and Linteris (1). The brief history of the method and its significance, compared to other methods for neutral oil determinations and the A.O.C.S. Cup Refining method, were presented at the A.O.C.S. Short Course in 1955 (2).

Previous studies in this laboratory and others have shown lack of correlation between the phosphorus and free fatty acid (FFA) content of degummed soybean oil and the A.O.C.S. Cup Loss method. It is also known that some degummed oils cannot be fairly val-

ued for trading by means of the A.O.C.S. Cup Loss method because the increased loss incurred in degumming is not always compensated by additional oil premium.

Since the Chromatographic Loss method is a simple and accurate index for unavoidable losses resulting from the nonneutral oil fraction of the oils, it offered good opportunity to study the behavior of the A.O.C.S. Cup Loss method.

Experimental

The method used in our laboratory is similar to that used by the F.A.C. subcommittee for total neutral oil for collaborative work in 1956 and is called the "1954 Modified Chromatographic Method."

The 20 x 400-mm. chromatographic tubes with coarse fritted discs were ordered from Corning Catalogue L. P. 34, No. 38, 450. Activated alumina, Grade F-20, Mesh 80-200, was obtained from the Aluminum Ore Company, East St. Louis, Ill.

Instead of the ether-methanol solution however, one lot of U.S.P. ether was used and was found quite satisfactory for our study. Nevertheless it should be pointed out that, in the case of reproducing results between laboratories, U.S.P. ether is unsatisfactory because of its varying alcohol content. Hence the ether methanol solution was adopted by the F.A.C. subcommittee on neutral oil, which developed the chromatographic method referred to in this paper.

Two g. of sample were used for crude soybean oils, and four g. were used for degummed oils.

Special precaution was taken in pouring the dissolved oil-ether solution into the tube. A plastic

wash-bottle proved to be very valuable in preventing creeping of the ether over the lip of the beaker.

The evaporation of the ether eluate is the most critical step in the process. Unless sufficient draft is provided over the steam bath, creeping occurs. It was found in our laboratory that lowering the lid of the hood to about three inches above the bench level will provide the desired conditions. The 250-ml. flasks were also fitted with an adapter in order to prevent further creeping losses.

The final drying step was carried out in forced air oven at 105°C. for one hour. Comparative results did not show any difference between drying in the forced air oven and the vacuum oven.

Each change of 20 g. of alumina was found to be good for three samples. With certain types of crude oils however the absorbent gives occasionally inaccurate results the third time.

Accuracy, Reproducibility, Time

There were 171 analyses run in duplicate. The samples which represented degummed and nondegummed oils from four different plants were analyzed over the period of several weeks by the same analyst.

The arithmetic mean of variations between duplicate samples was .052% with a standard deviation of .052.

From the 171 samples analyzed, 151 or 88.5% of the total analyzed within .1% between duplicates (94.5% between .15%). The standard deviation computed from samples, which analyzed within .1%, was .028. These figures include the period when the analyst, relatively inexperienced, was running .07% differences in duplicates. At present the same analyst is averaging .03% difference between duplicate samples.

These figures reveal a greater accuracy for duplicate analyses than could be expected from the Wesson method, and one experienced analyst can run approximately 24 samples in duplicate a day. Thus, for efficiency, the chromatographic refining loss method has great merits.

Correlation with Cup Loss

Simultaneous A.O.C.S. cup refining determinations were run for each chromatographic analysis to see whether the former can be "predicted" by the latter. At first the following approach was tried to express the relationship between the two methods.

The correlation was expressed by the ratio of cup loss/chromatographic loss from now on referred to in the text as R (Figure 1).

It was found that R gave good correlation between the two methods when chromatographic losses exceeded 1.0%. The arithmetic mean of R was found to be 1.56 for samples with more than 1.0% chromatographic loss with a standard deviation of .13 ($\pm 8.5\%$) from the mean. Only 14 (16%) of 88 samples were off more than $\pm 10\%$ from the arithmetic mean. Only 10% were off more than $\pm 12.5\%$, 7% more than $\pm 15\%$, and 3% more than $\pm 20\%$. If we assume that there is an accepted variation of $\pm .3\%$ between laboratories for the cup loss determinations, this variation alone can explain most of the discrepancy.

On the other hand, when chromatographic losses are less than 1.0%, the accuracy of prediction, by using a simple conversion factor (R), is lowered because of the larger effect by percentage of the accepted variations in the cup loss determinations. Occasional

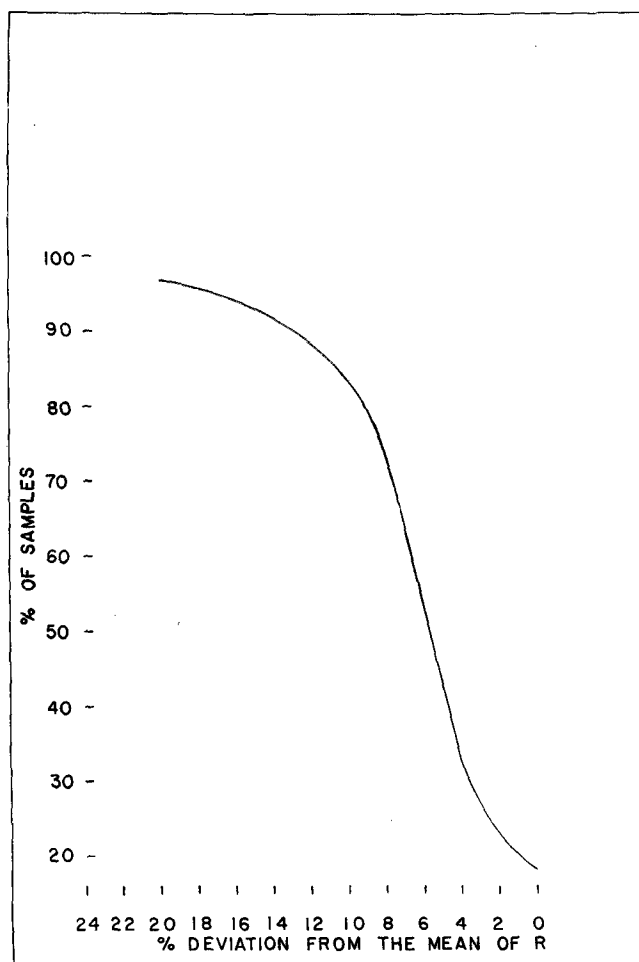


Fig. 1. Standard deviation from the mean of $R = \text{cup loss} \div \text{chromatographic loss}$ for samples analyzed over 1% chromatographic loss (expressed on a percentage scale).

difficulties encountered in separating "sloppy" soapstocks at low levels of cup refining losses further lower the reliability of the Cup Refining method.

The graphical representation of this correlation is seen in Figure 2, where R is plotted against chromatographic losses.

Class intervals of the .1% chromatographic losses were used as points in the construction of the curve. At least four duplicate determinations were made within a class interval for each point. At points where there was an insufficient number of analyses available, the curve is drawn with a dotted line. Each .1% represents the average value of samples found to be .1% below and above the indicated value (example: 3.3 [3.2-3.4]).

It is apparent that a relationship very similar to an exponential function exists between the chromatographic loss determination and the ratio (R) of the cup loss and chromatographic determinations. At higher levels this ratio is rather constant; at the neighborhood of 1% however R increases rapidly.

The standard deviation (S) of R values from the mean, computed for each .1% chromatographic loss, shows a similar pattern. It runs between .1-.15 until it reaches approximately 1.2% chromatographic loss, at which point it increases suddenly, up to .58 for losses lower than 1%.

The class intervals, the number of samples analyzed

TABLE I
Class Intervals of Correlation and Accuracy of Correlation

Chromatographic loss %	No. of samples	R		Cup loss		(S) of R
		(1)	(2)	(1)	(3)	
3.3 (3.2-3.4)	9	1.50	1.52	4.97	5.00	.11
3.2 (3.1-3.3)	20	1.49	1.53	4.80	4.90	.11
3.1 (3.0-3.2)	26	1.50	1.53	4.66	4.75	.11
3.0 (2.9-3.1)	21	1.54	1.53	4.65	4.60	.11
2.9 (2.8-3.0)	13	1.58	1.54	4.58	4.45	.11
2.8 (2.7-2.9)	12	1.57	1.54	4.38	4.30	.11
2.7 (2.6-2.8)	9	1.57	1.54	4.29	4.15	.11
1.6 (1.5-1.7)	4	1.65	1.57	2.67	2.50	.13
1.5 (1.4-1.6)	5	1.60	1.58	2.47	2.40	.14
1.4 (1.3-1.5)	6	1.58	1.58	2.20	2.20	.15
1.3 (1.2-1.4)	8	1.58	1.59	2.03	2.05	.15
1.2 (1.1-1.3)	8	1.60	1.64	1.90	1.95	.18
1.1 (1.0-1.2)	12	1.69	1.70	1.80	1.85	.19
1.0 (.9-1.1)	23	1.88	1.80	1.81	1.80	.23
.9 (.8-1.0)	36	2.02	1.97	1.81	1.80	.33
.8 (.7-.9)	28	2.15	2.24	1.78	1.80	.42
.7 (.6-.8)	26	2.68	2.58	1.79	1.81	.48
.6 (.5-.7)	23	2.96	3.12	1.85	1.87	.58
.5 (.4-.6)	7	3.80	3.82	1.93	1.91	.58
.4 (.3-.5)	4	4.09				

(1) Average values found for each class interval.
 (2) Results taken from the curve in Figure 2.
 (3) Results taken from the curve in Figure 3.

in each interval, the means and values for the standard deviations (S) for R are summarized in Table I, which also gives the values for cup losses as they can be read on the curve shown in Figure 3. The slight discrepancy between the actual values found for each class interval and the values shown in Figure 2 illustrates the extent of scattering along the curve.

In Figure 3 cup refining losses are plotted against chromatographic losses. This curve gives an approximate idea of the practical aspects of the correlation

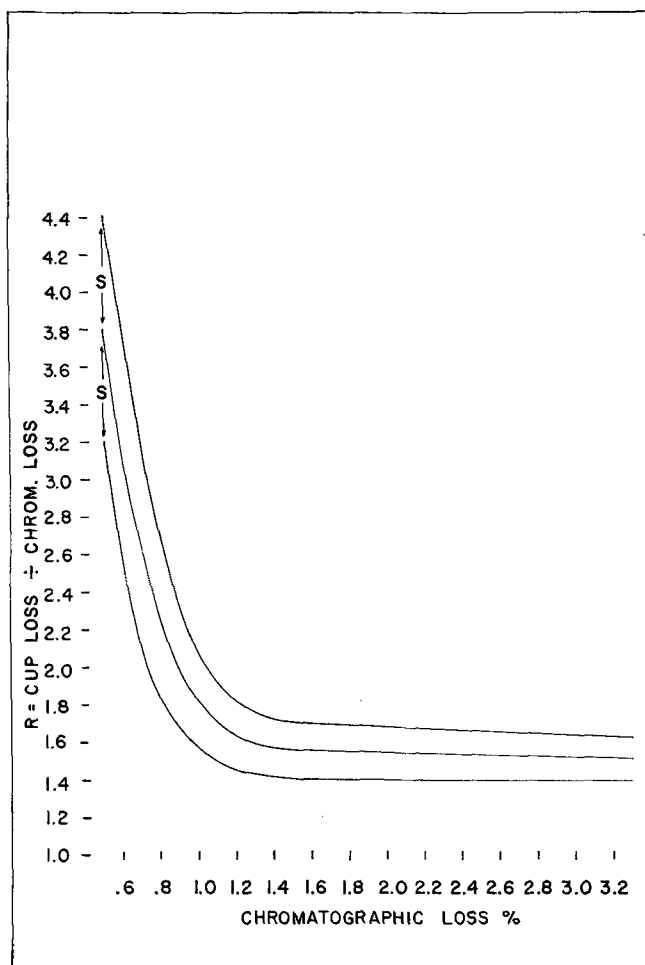


FIG. 2. Correlation between chromatographic loss determinations and the ratio of cup loss/chromatographic loss.

between the two methods. It shows to what extent cup losses can be predicted when the chromatographic losses are known. The standard deviation of cup losses from the mean at the corresponding chromatographic losses are shown at the one Sigma level. It can be seen, for example, that there is a corresponding decrease in cup loss for a decrease in chromatographic loss up to about the 1.0% level. Further decreases in chromatographic losses, as a rule, are not paralleled by cup losses. A slight increase is even possible with decreasing chromatographic losses under 1.0%. This indicates that the degumming of crude soybean oils below 1.0% absolute loss is not desirable from the point of view of the processor since cup loss determinations will not reflect the increased quality beyond that point.

TABLE II
The Equation of Regression Lines Relating Chromatographic Losses to A.O.C.S. Cup Refining Losses

Nondegummed oils	
Y = .680 + 1.308X (Y = A.O.C.S. Cup Refining Loss)	
Degummed oils	
Y = 2.335 - 1.429X + .930X ² (Y = A.O.C.S. Cup Refining Loss)	
Derivative or minimum of the curve for degummed oil.....	.77
Standard error of estimate	
Sy: nondegummed oils279
Sy ₁₂ : degummed oils252
Correlation coefficient (r), nondegummed oils.....	.751
Correlation coefficient (R), degummed oils.....	.642

Note also that, in spite of the large deviation of R from the mean below 1.2% absolute loss, the standard deviation of cup loss percentages from their mean is a fairly constant value ($\pm 3-4\%$). This deviation, as it was mentioned before, represents naturally a larger deviation by percentage, for example, for samples at the 2.0% level than for samples at the 4.0% level.

Another example: at .5% chromatographic loss the standard deviation (S) of R from the mean is .58. At this point the exact value for R is 3.82, which at a .58 standard deviation (S) would correspond to a range of 3.24-4.4, or in terms of predicted cup losses, to a range of 1.62-2.20%, which is equivalent to $\pm 3\%$. At 3% chromatographic loss, R being equal to 1.53 with a standard deviation of only .11, would represent a range of 1.42-1.64 or the equivalent, in terms of cup losses, of 4.25-4.92%—again a standard deviation (S) of approximately $\pm 3\%$, the same as in the case of a .5% chromatographic loss.

We have arrived at two equations which describe the foregoing observations in a more general way. One is for nondegummed oils and expresses the linear pattern of correlation. The other is for degummed oils and reflects a curvilinear (parabolic) pattern of correlation.

The equation of the regression line relating chromatographic losses to A.O.C.S. cup refining losses in case of nondegummed oils is as follows:

$$Y = .680 + 1.380X \text{ (Y = A.O.C.S. Cup Refining Loss)}$$

The equation of the curvilinear of multiple regression line for degummed oils is:

$$Y = 2.335 - 1.429X + .930X^2 \text{ (Y = A.O.C.S. Cup Refining Loss)}$$

The derivative or minimum of the curve for degummed oils was calculated to be .77. Standard error of estimate (Sy) for nondegummed oils was .279 and (Sy₁₂) for degummed oils was .252.

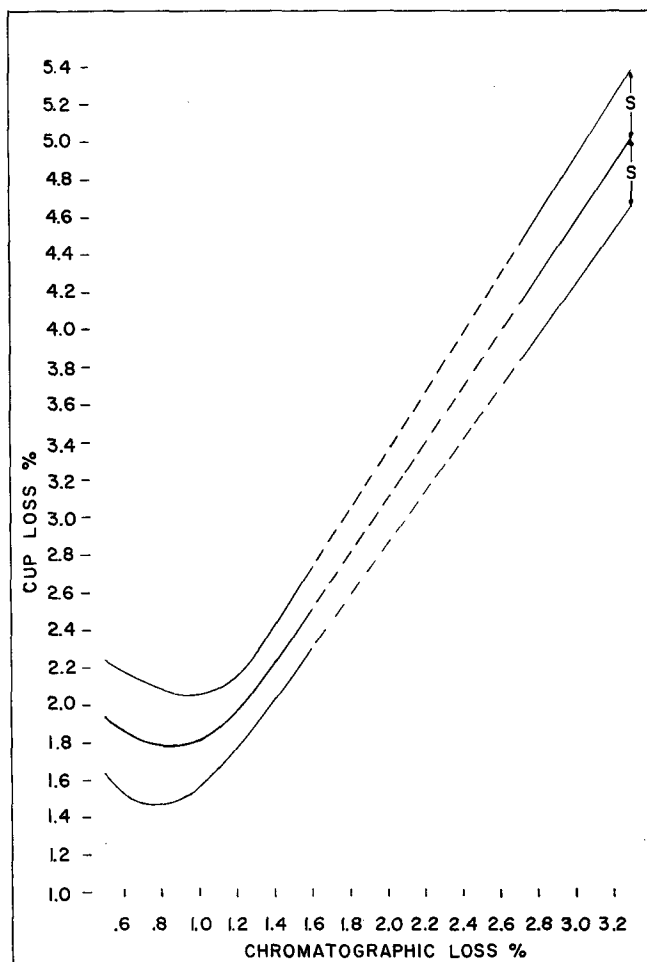


Fig. 3. Correlation of chromatographic losses with cup losses.

The correlation coefficient (r) for nondegummed oils was .751, and the multiple correlation coefficient (R) for degummed oils was .642.

These data show, as in the case of the curve constructed by values obtained from class intervals, that, although there are only slight differences from the standard errors of estimate between degummed and nondegummed oils, the correlation coefficients (r , R) indicate a definitely better correlation for the nondegummed samples than for the degummed samples. Nevertheless, as perfect correlation exists when r or $R = 1$, the correlation coefficient value of .642 and .751 are significant and considered to be good.

It should be pointed out that, at least in the types of oils analyzed in this investigation, the shape of the multiple regression curve for degummed oil is very shallow. The minimum point at .77% chromatographic loss is therefore just a theoretical and not a sharply defined point. In view of this and the $\pm .3\%$ standard deviation of cup losses from the mean, the following should be said in case the chromatographic losses are below or in the neighborhood of 1.0%: although it is possible to predict the approximate value of cup losses within the range indicated by the standard error of estimate (S_y , $S_{y_{12}}$), it is not possible to predict the trend which will be followed by one variable when the other variable is changing in one direction.

Summary

It can be concluded, on the basis of our experiments, that chromatographic loss determinations can be well correlated with A.O.C.S. Cup Refining determinations. This correlation is good for samples above 1% chromatographic losses and somewhat less accurate for samples below 1.0%.

Most of the discrepancy in correlation is inherent in the Cup Loss method because it is less precise in general than the chromatographic determination.

On the other hand, the chromatographic loss is an accurate, easily obtainable index for unavoidable losses resulting from the nonneutral oil fraction of the oils. In the majority of cases the degumming of the oil to levels below 1% chromatographic loss is not indicated by a parallel decrease in cup losses. Thus, in these cases, as long as premiums are paid on the basis of the A.O.C.S. Cup Loss method, the processor is placed in a very disadvantageous position.

It should be pointed out that all our determinations were made on the oils from the 1956 crop during the summer months. The possibility that different crop years will result in different correlation curves might exist, and we are planning to do further work in the future.

It is hoped that by presenting statistically significant figures on this subject, enough interest will be stimulated for more collaborative work, which might result in a review of the industry's present method for the determination of refining losses in soybean oil.

REFERENCES

1. Linteris, J. L., and Handschumaker, E., *J. Am. Oil Chemists' Soc.*, **27**, 260-264 (1950).
2. James, E. M., *J. Am. Oil Chemists' Soc.*, **32**, 581-587 (1955).

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The Synthesis of 2-Heptenal

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DURING A STUDY on the reversion of soybean oil undertaken some years ago it became necessary to prepare some pure 2-heptenal. Several methods for preparing this α - β unsaturated aldehyde are recorded in the literature. Delaby and Guillot-Allégre (3) and Martin, Schepartz, and Daubert (6) have described the chromic acid oxidation of the corresponding unsaturated alcohol. More recently Bedoukian (1) reported the preparation of a series of α - β unsat-

urated aldehydes by bromination of the appropriate enol acetate, followed by the debromination of the resulting dimethyl bromoacetal.

The yields of 2-heptenal obtained by previous workers were low. Delaby and Guillot-Allégre (3) do not record all of their yields; however, from the data given, the over-all yield of 2-heptenal appears to be about 7-10%. Several attempts to prepare pure 2-heptenal according to the method of Martin *et al.* (6)