moles per kilo. The maximum value for sample B was 212 after 16 hrs. while the maximum for samples C, D, and E was 242, 286, and 325 after 12 hrs. Sample A showed a continuous increase in carbonyl and did not exhibit a maximum concentration in carbonyl on continued treatment as found in samples B to E.

## Discussion

It was shown that the thermal oxidation of corn oil at 200°C. caused a decrease in iodine value and increases in refractive indices and viscosity. The linoleic acid content of the oil decreased from 53% to 30% during a 24-hr. treatment. However during the treatment the monounsaturated acid content of the oil increased from 26% to 39.9%, suggesting that only one double bond of the linoleic acid was involved in part of the reactions.

Since only one oxygen containing functional group, *e.g.*, carbonyl, was produced in any substantial amount, it was possible that this group played some part in secondary reactions. It is doubtful that the many changes which took place during the twelfth to sixteenth hour of heating were mere coincidence. Increased aeration during heating appeared to produce a more pronounced change but did not appear to change the type of reaction. Even samples which had not been aerated were found to decrease 10% in iodine value.

In the sample aerated at 2,400 ml. per minute per kilo the decrease in iodine value was greatest during the first 14 hours. and was least during the final ten hrs. In addition, the rise and decline of the carbonyl value paralleled that of samples B through D. It appears that the changes produced during the first 12 to 16 hrs. differ from those produced later. It has been suggested that the initial decrease in iodine value might be caused by the formation of cyclic monomers (12). However the presence of air or oxygen might give rise to other reactions which could also give a decrease in iodine value. Previous studies (3, 8) have been carried out in the absence of air or oxygen. The changes noted in the present study were carried out in the presence of oxygen and therefore could differ from those in which the production of cyclic monomeric material has been reported.

Initially the oil was attacked by the oxygen to produce carbonyl groups. Part of this attack was at the double bonds, but some changes must have occurred at methylene groups since the presence of $\alpha,\beta$-unsaturated carbonyl groups was indicated (6). Other reactions involving the double bonds were also taking place, including conjugation. Some of the products of this initial period are easily polymerized, and when these have built up to a certain concentration, after 12 to 16 hrs. of treatment, polymerization increases, producing the final products.

## Summary

The thermal oxidation of corn oil proceeds in two steps, an initial period of 12 to 16 hrs., characterized by a decrease in iodine value and a rapid increase in carbonyl value, and a second phase in which a slower decrease in iodine value, a slight decrease in carbonyl value, and a rapid increase in viscosity occurred. Increasing the rate of aeration caused greater magnitude in changes but did not alter the over-all two-phase reaction.

REFERENCES

1. Brice, B. A., Swain, M. L., Schaeffer, B. B., and Ault, W. C., J. Am. Oil Chemists' Soc., *29*, 279 (1952).
2. Chang, S. S., and Kummerow, F. A., J. Am. Oil Chemists' Soc., *28*, 285 (1951).
3. Crampton, E. W., Common, R. H., Farmer, F. A., Wells, H. F., and Crawford, D., J. Nutrition, *44*, 333 (1951).
4. Fugger, J., Cannon, J. A., Zilch, K. T., and Dutton, H. J., J. Am. Oil Chemists' Soc., *28*, 285 (1951).
5. Johnson, O. C., Sakuragi, T., and Kummerow, F. A., J. Am. Oil Chemists' Soc., *33*, 433 (1956).
6. King, G., J. Chem. Soc., 1980 (1951).
7. Lappin, G. R., and Clark, L. C., Anal. Chem., *23*, 541 (1951).
8. Mehta, T. H., and Sharma, S. A., J. Am. Oil Chemists' Soc., *33*, 38 (1956).
9. "Official and Tentative Methods" of the American Oil Chemists' Society, Chicago, Ill., 1946.
10. Paschke, R. F., Jackson, J. E., and Wheeler, D. H., Ind. Eng. Chem., *44*, 1113 (1952).
11. Paschke, R. F., and Wheeler, D. H., Oil and Soap, *21*, 52 (1942).
12. Paschke, R. F., and Wheeler, D. H., J. Am. Oil Chemists' Soc., *32*, 473 (1955).
13. Powers, P. O., Ind. Eng. Chem., *41*, 304 (1949).
14. Wheeler, D. H., Oil and Soap, *9*, 89 (1932).

[Received November 7, 1956]

# The Analysis of Nitrogen in the Smalley Oilseed Meal Series, 1955-56

C. H. PERRIN, Canada Packers Ltd., Toronto, Canada

CANADA PACKERS' RESEARCH LABORATORY entered the Smalley Oilseed Meal Series in August, 1955. The nitrogen analyses were performed by the rapid Kjeldahl procedure (4) developed by this laboratory because this is the method in routine use by our control laboratories. This method requires a digestion time of about 15 min. on 1-g. samples, and the temperature of the digestion and concentration of mercury are considerably higher than that obtained in the official A.O.C.S. Method (2). From the beginning it was apparent that our nitrogen analyses were consistently higher than the Smalley median value used in scoring the series, and our problem was to establish whether these higher nitrogen analyses were true or false.

In order to increase the accuracy of our work the following precautions were taken.

1. Weight burette technique was used in all titrations, including standardization of solutions.

2. The accuracy of our Gram-atic balance was checked against one-piece weights bearing a certificate from the National Physical Laboratory, England.

3. The sulfuric acid used for titrating the ammonia was standardized against sodium carbonate, mercuric oxide, and sodium hydroxide solution which had been standardized (by weight) against acid potassium phthalate obtained from the U. S. National Bureau of Standards.

4. The reagent blanks were measured both by the usual digestion with sugar and directly by the use of Nessler reagent. They were found to be insignificant.

5. To assure complete recovery of ammonia a new solid glass still was designed. A glass leg was blown onto the end of the trap. Inside the leg is a long, glass, cold finger which eliminates condensation on the outside surface.

The end of the condenser dips directly into the receiving acid. At the end of each distillation the cooling water is turned off. In approximately 30 seconds steam issues from the end of the condenser, eliminating the retention of traces

of ammonia. Thus all rubber connections are eliminated except for the stopper.

6. To reduce alkali carryover to the lowest practicable level no zinc was used to prevent bumping. Also the excess alkali was held to a minimum.

7. Frequent checks were made to prove that the samples had not dried out prior to the nitrogen analyses. In many cases the residue from the moisture analysis (representing a 5-g. sample) was transferred to the 800-ml. Kjeldahl flask and digested.

Table I illustrates our consistent tendency to find a higher percentage of nitrogen than the median values used in scoring the Smalley Series.

TABLE I

Nitrogen Analysis of 1955–56 Smalley Oilseed Meal Samples

| Sample No. | Smalley median | Our average | Difference |
|---|---|---|---|
| | % | % | % |
| 1 | 6.50 | 6.54 | 0.04 |
| 2 | 7.40 | 7.47 | 0.07 |
| 3 | 7.22 | 7.27 | 0.05 |
| 4 | 7.20 | 7.25 | 0.05 |
| 5 | 8.27 | 8.27 | 0.00 |
| 6 | 7.21 | 7.24 | 0.03 |
| 7 | 7.25 | 7.31 | 0.06 |
| 8 | 7.35 | 7.40 | 0.05 |
| 9 | 7.46 | 7.52 | 0.06 |
| 10 | 7.50 | 7.55 | 0.05 |
| 11 | 7.34 | 7.40 | 0.06 |
| 12 | 7.08 | 7.13 | 0.05 |
| 13 | 9.65 | 9.73 | 0.08 |
| 14 | 7.66 | 7.70 | 0.04 |
| 15 | 7.73 | 7.78 | 0.05 |
| Mean | ..... | ..... | 0.05 |

Experiments will now be described which support the conclusion that the Seedmeal Check Samples contain more nitrogen than the median values indicate.

*Experiment I.* Sample: Smalley No. 11, 1956. Median value = 7.34% N. A 1.4-g. sample was digested exactly as instructed in Ba 4-38 of the A.O.C.S. Official Methods. The distillation was performed, using our improved glass condenser, and the ammonia was received in boric acid. The indicated percentage of nitrogen was 7.39. The titrated contents of the receiver were placed in a Kjeldahl flask with a slight excess of U.S.P. grade sodium hydroxide, and the distillation was repeated. Again the indicated percentage of 7.39 was obtained. The titrated contents of this second receiver were transferred to another Kjeldahl flask, and, using our improved glass condenser, the ammonia was received in an evaporating dish containing excess chloroplatinic acid. The weight of the ammonium chloroplatinate corresponded to 7.39% nitrogen in the sample. It was confirmed that the ammonium chloroplatinate was completely soluble in hot water. A control experiment on pure dried ammonium sulfate yielded the theoretical weight of ammonium chloroplatinate.

Chloroplatinic acid differentiates between ammonium and sodium (the only other possible alkali present).

*Experiment II.* Several Kjeldahl distillations were performed using distilled water in the receiver. The ammonia was eliminated by boiling down the contents of the receiver to dryness. No visible residue of fixed alkali could be detected. On adding distilled water and indicator, no addition of standard acid was necessary to bring to neutrality.

*Experiment III.* The active purity of the Mallinckrodt "Primary Standard" sodium carbonate used to standardize our sulfuric acid was determined. (The Mallinckrodt assay of this substance was 99.95% to 100.05% $Na_2CO_3$.) By boiling it in solution with a slight excess of acid potassium phthalate and back-titrating, assays of 99.98% and 99.98% $Na_2CO_3$ were obtained. When the same sodium carbonate was titrated with a standard solution prepared by weight from constant boiling point hydrochloric acid, the assay was 99.94% $Na_2CO_3$.

*Experiment IV.* Fisher Reagent ammonium sulfate was boiled with a small excess weight of sodium hydroxide solution which had been standardized by weight against N.B.S. acid potassium phthalate in the absence of carbon dioxide. The solution was back-titrated with standard acid used in the Kjeldahl work. The indicated percentage nitrogen was 21.18.

*Experiment V.* One gram of dried ammonium sulfate was boiled in a solution containing excess, dried, sodium carbonate, and the residual alkali was titrated with our standard acid. The calculated recovery of ammonium sulfate was 0.9998 g. It is probable that the sodium carbonate and the ammonium sulfate were about equally pure.

*Experiment VI.* Standard hydrochloric acid was made up by weight from a constant boiling point concentrate. Its calculated strength checked closely with its estimated strength, using sodium carbonate as a primary standard. When this standard hydrochloric acid was used in the analysis of check sample No. 14, the indicated percentage nitrogen was 7.70, agreeing perfectly with our original analysis (Table I).

It was frequently observed that we enjoyed greater precision when analyzing pure chemicals than when analyzing Smalley check samples. Experiments VII, VIII, and IX indicate that significant errors derived from failure of the seedmeal samples to remain homogeneous during necessary handling.

*Experiment VII.* In the case of a few of the check samples, parallel determinations were run on 0.1-, 0.3-, 1.0-, 2.0-, and 5.0-g. samples. The weights of the reagents were constant except for the sulfuric acid, which was carefully adjusted to maintain a digestion temperature of approximately 370°C. There was no significant difference between the average analyses obtained, proving the absence of a serious proportional error. However the precision of the analyses noticeably increased with the sample size.

*Experiment VIII.* Check sample No. 8 was divided in half. One-half was analyzed eight times, and the average deviation from the mean was 0.021% units of nitrogen. That fraction of the other half passing a 60-mesh screen was analyzed six times, and the average deviation from the mean was reduced to 0.0042% units of nitrogen.

*Experiment IX.* Another check sample was separated into several screen fractions. The portion retained on 35-mesh had a nitrogen content of 4.68%. The fraction retained on an 80-mesh screen analyzed 6.91% N. The fraction passing 100-mesh analyzed 8.07%. When the same sample was poured out of the bottle into a small conical pile, a portion taken from the bottom of the pile analyzed 6.17% N. A portion taken from the top of the pile had a different appearance and an analysis of 6.68% N.

*Experiment X.* Table II presents our analyses of two samples of ammonium sulfate and a single large crystal of monoammonium phosphate received from the U. S. National Bureau of Standards. The crystal was prepared for piezoelectric work, and it is believed that it closely approaches theoretical composi-

TABLE II
Nitrogen Analyses of Pure Ammonium Salts

| Sample | Theoretical % N | % N found by Kjeldahl method | % N found formaldehyde titration A.O.A.C. 2.26 (1) | pH of 5% solution |
|---|---|---|---|---|
| Fisher reagent ammonium sulfate | 21.20 | 21.18 | 21.19 | 5.2 |
| A.O.C.S. standard ammonium sulfate | 21.20 | 21.17 | 21.18 | 5.5 |
| N.B.S. monoammonium phosphate | 12.18 | 12.17 | ..... | ..... |

tion. The A.O.C.S. standard ammonium sulfate is marked "lot No. 42848, 25.70% $NH_3$." This corresponds to 21.14% of nitrogen. A 5% solution of pure ammonium sulfate should have a pH of 5.2 to 5.3 at room temperature.

Table III presents our analyses of a number of reference chemicals. These analyses were obtained over a period of several months (during our participation in the Smalley Series), and the results were collected and compiled.

TABLE III
Nitrogen Analyses of Reference Chemicals

| Substance | Theory | No. of analyses | Mean | Average deviation |
|---|---|---|---|---|
| Ammonium sulfate reagent | 21.20 | 8 | 21.18 | 0.009 |
| Ammonium oxalate reagent | 19.71 | 4 | 19.69 | 0.007 |
| l-Cystine reagent | 11.66 | 3 | 11.59 | 0.000 |
| Acetanilide reagent | 10.36 | 8 | 10.31 | 0.005 |
| Benzidine reagent | 15.21 | 4 | 15.09 | 0.008 |
| U.S.P. nicotinic acid | 11.38 | 3 | 11.37 | 0.007 |

NOTE: Identical nitrogen analyses were obtained on three specimens of acetanilide. These were sample 141 from the U. S. National Bureau of Standards, the micro-analytical standard of the British Drug Houses, and an Eastman Kodak sample.

## Discussion

Our rapid Kjeldahl method (4) has been modified to use sample weights up to 5 g., new one-piece distilling apparatus, and weight burette technique in the titrations. In the distillation step, zinc was eliminated and a minimum of excess alkali was used. These improvements, along with other refinements described above, resulted in improved precision.

This special investigation (which included analyses of reference chemicals, precipitation of ammonium chloroplatinate, demonstration that the alkali isolated was completely volatile, intercomparisons of standards, and standardized solutions and other checks) proves that the Smalley Oilseed Meal Samples contain about 0.05% units more of nitrogen than the average analyses of the participating laboratories which used the official A.O.C.S. procedure (2). Had these laboratories used the latest modification of the A.O.A.C. procedure (1), it is likely that somewhat higher values would have been obtained. We believe that the A.O.A.C. procedure is superior to the A.O.C.S. procedure because the digestion temperature is higher, the digestion heating intensity is specified, and the acid used in measuring the ammonia is standardized directly and then checked against the standardized sodium hydroxide solution.

The report of R. C. Berry (3) compared high and low digestion temperatures on cottonseed meal, meat scrap, and fish meal. The increase in protein recovery was 0.937, 0.57, and 0.713% units, respectively. It is not surprising therefore that we should obtain slightly higher nitrogen values in using our very intense digestion conditions, wherein complete mineralization of the nitrogen is obtained in about 15 min. on 1-g. samples. Insufficient sample was available to us to investigate the relative importance of the two factors likely responsible for our higher nitrogen recoveries. These are a more intense digestion and a one-piece distilling apparatus, which is purged at the end of each distillation. Insufficient sample also made it impossible for us adequately to estimate the importance of the errors resulting from segregation of the particles during the filling of the containers and during the weighing out for analysis. It is felt that better agreement among laboratories and better precision within laboratories would result if samples could be issued which have a lesser tendency to become heterogeneous on handling.

## Acknowledgment

Edward Wichers of the U. S. National Bureau of Standards gave helpful advice on reference standards and kindly donated the pure single crystal of monoammonium phosphate. The author is also grateful to Phillip Ferguson of Canada Packers' Research Laboratories for performing many of the analyses presented.

REFERENCES

1. Association of Official Agricultural Chemists, Methods of Analysis.
2. American Oil Chemists' Society, Official and Tentative Methods, Ba 4-38.
3. R. C. Berry, J. Assoc. Offic. Agr. Chemists, *31*, p. 617.
4. C. H. Perrin, Analytical Chem., *25*, p. 968.

[Received January 7, 1957]

# Separation of the Oxidation Products of Fatty Acids by Means of Gas-Liquid Partition Chromatography[1]

JANINA NOWAKOWSKA, E. H. MELVIN, and RICHARD WIEBE, Northern Utilization Research and Development Division,[2] Peoria, Illinois

A. T. JAMES AND A. J. P. MARTIN (2) have shown that gas-liquid partition chromatography can be successfully used for the analysis of natural fats and applied the method to goat's milk, olive oil, and fatty extracts from bacterial culture. The work reported here is a preliminary study of the oxidation products of soybean-oil fatty acids by means of gas-liquid chromatography.

## Apparatus and Procedure

A stainless steel column, 6 ft. 3 in. long, and 8-mm. I.D., was packed with Celite [3] impregnated with D.C.

[1] Presented at the fall meeting, American Oil Chemists' Society, Chicago, Ill., September 24–26, 1956.
[2] One of the divisions of the Agricultural Research Service, U. S. Department of Agriculture.
[3] The mention of firm names or trade products does not imply that they are endorsed or recommended by the U. S. Department of Agriculture over other firms or similar products not mentioned.