

by introduction of sulfite ion, which makes active carbonyl groups unavailable for browning. In the absence of added pro-oxidative conditions this inhibition of active carbonyl-amine browning apparently favors reaction b), leading to the production of more intensely colored polymer. Because the rates of formation of brown color were not inhibited when active carbonyl-amine browning was inhibited, this reaction c) could not be the compulsory or dominant mechanism.

Antioxidants retard reactions b) and a) and may be expected to impede reaction c) to some extent, resulting in extremely slow development of color.

Exclusion of oxygen by substituting nitrogen for air produced an effect indistinguishable from the use of antioxidants.

Non-reactivity of an Amino Acid in Oxidizing Menhaden Oil Emulsion. A sensitive test for active carbonyl-amine condensation was the determination of the reactivity of an amino acid when in the actively oxidizing menhaden emulsion. Because of the ease of spectrophotometric analysis, phenylalanine was used as the test amino acid and phenylacetic acid was used as a control. The control, phenylacetic acid, similar in structure to phenylalanine but lacking an amino group, was used to correct for nonspecific losses of the amino acid in this reaction system.

After two hours the menhaden oil emulsion absorbed about 1.3 ml. of oxygen and 82% of the phenylalanine and 72% of the phenylacetic acid were recovered. There is no significant difference between the amino acid and the control, showing the absence of active carbonyl-amine condensation reaction during the initial oxidation of menhaden oil.

By these three types of studies: activation energy for the formation of brown color, inhibition analysis of the reaction mechanism leading to the formation of brown color, and measure of amino acid reaction during the oxidation, there is evidence against active carbonyl-amine browning as a dominant reaction mechanism for the formation of brown-colored oxypolymers. Because of the limitations in these methods it is not possible to exclude active carbonyl-amine browning as a minor reaction mechanism or to quantify any minor role that this reaction mechanism may play in the formation of these brown-colored oxypolymers.

Summary

The oxypolymerization of unsaturated fat in the presence of protein and amino acid was studied to determine whether the active carbonyl-amine-browning reaction is a dominant mechanism for the formation of brown-colored polymers. A highly reactive system of menhaden oil-aqueous egg albumin emulsion was studied in greatest detail. Three types of studies: activation energy of formation of brown color, inhibition analysis of the mechanism leading to formation of brown color, and measures of amino acid reaction in the oxidizing unsaturated fats, all gave evidence that active carbonyl-amine browning was not a dominant mechanism.

Acknowledgments

The authors gratefully acknowledge the assistance and many helpful suggestions of Harold S. Olcott, Department of Food Technology, University of California, Berkeley; W. Duane Brown, Fish and Wildlife Service, University of California, Davis; and Maurice E. Stansby, chief, Pacific Coast and Alaska Technological Research, Fish and Wildlife Service, Seattle.

REFERENCES

1. Bolland, J. L., Proc. Roy. Soc., *A186*, 218 (1946).
2. Casselman, W. G. B., J. Exptl. Med., *94*, 549 (1951).
3. Cordy, D. R., Cornell Vet., *44*, 310 (1954).
4. Dam, H., Experientia Suppl. I, "Symposium on Present Problems in Nutritional Research," *196* (1953).
5. Deane, D. L., in "Deterioration of Fats and Oils," Quartermaster Corps Manual 17-7, 1945, p. 13.
6. Fraenkel-Conrat, H., in Greenberg, D. M., "Amino Acids and Proteins," C. C. Thomas, Springfield, Ill., 1951, p. 547.
7. Garton, G. A., and Duncan, W. R. H., Biochem. J., *57*, 120 (1954).
8. Glavind, J., Hartmann, S., Clemmesen, J., Jessen, K. E., and Dam, H., Acta Pathol., *30*, 1 (1952).
9. Hartroft, W. S., J. Gerontology, *8*, 158 (1953).
10. Hartroft, W. S., Science, *113*, 673 (1951).
11. Hodge, J. E., J. Ag. Food Chem., *1*, 928 (1953).
12. Lea, C. H., and Hannan, R. S., Biochim. et Biophys. Acta, *3*, 313 (1949).
13. Maerz, A., and Paul, M. R., "A Dictionary of Color," McGraw-Hill Book Company, New York, 1930.
14. Mason, K. E., and Hartsough, G. R., J. Am. Vet. Med. Assoc., *69*, 72 (1951).
15. Mohammad, A., Fraenkel-Conrat, H., and Olcott, H. S., Arch. Biochem., *24*, 157 (1949).
16. Müller, R., Vitamine u. Hormone, *6*, 303 (1954).
17. Privett, O. S., and Nickell, C., J. Am. Oil Chemists' Soc., *33*, 156 (1956).
18. Robinson, K. L., and Coey, W. E., Nature, *168*, 997 (1951).
19. Stansby, M. E., Fishery, Leaflet, *429*, 24 (1956).
20. Tappel, A. L., Arch. Biochem. and Biophys., *44*, 378 (1953).
21. Tappel, A. L., Arch. Biochem. and Biophys., *54*, 266 (1955).
22. Wood, E. M., and Yasutake, B. A., Am. J. Pathol., *32*, 591 (1956).

[Received August 12, 1957]

Report of the Uniform Methods Committee 1956-57

AT A MEETING of the Uniform Methods Committee in the Netherland-Hilton hotel on September 30, 1957, the following proposed Methods and method revisions were discussed and the indicated actions were taken. The meeting was attended by five members of the Uniform Methods Committee; only Messrs. Houle and King were absent.

Spectroscopy Committee, R. T. O'Connor, chairman

Polyunsaturated Acids, Tentative Method Cd 7-48

- a) Several minor changes in equations under "Calculations" are recommended. In a number of cases these involve nomenclature only; in others small changes are made in the constants employed.
- b) The Spectroscopy Committee feels that this method, which has been held as "Tentative" for nearly 10 years, now has attained a rather permanent form within its defined scope. No further work on it is planned in the immediate future.

Its advancement from Tentative to Official status is recommended.

The Uniform Methods Committee concurs with these recommendations and has approved them for action by the Society.

Soap and Synthetic Detergent Analyses Committee, J. C. Harris, chairman

Determination of Copper Subcommittee, E. W. Blank, chairman

As a result of several years of study of the subject, a spectrophotometric method for trace amounts of "Copper in Soaps and Soap Products" is recommended for adoption as a Tentative Method. The Uniform Methods Committee approves this recommendation with the addition of the following sentence at the end of "Apparatus. A-1":

A photoelectric colorimeter may be used, provided it is equipped with a proper interference filter.

**Seed and Meal Analysis Committee,
T. H. Hopper, chairman**

**Urease Activity in Soybean Meal Subcommittee,
T. J. Potts, chairman**

A new method for "Urease Activity" in soybean meals, soybean flour, and mill feeds is recommended for adoption as "Tentative." If approved, it will be placed in Section Ba under "Oil Seed By-Products." This method, which is that of Caskey and Knapp, under various modifications has been in use for several years. It has been standardized and collaboratively tested by members of the soybean industry and the Association of Cereal Chemists, by whom it will be used. The Uniform Methods Committee approves its adoption with two minor additions as follows. At end of D-1 add this sentence: "Do not invert the tube during the process of mixing." At end of B-1 add this sentence: "The useful life of the buffer solution, prepared as described, is less than 90 days."

**Fat Analysis Committee
V. C. Mehlenbacher, chairman**

**Subcommittee on Continuous Flow Method of Sampling,
L. R. Brown, chairman**

At the 1955 Spring Meeting a proposed method of continuous flow sampling was rejected by the U.M.C. as being "too cumbersome." The chief objection was that, for each tank car loading or unloading, from 25 to 40 gallons of surplus oil are accumulated and the method contained no provision for its disposal, preferably by return to storage.

Since that time the method has been used by several large shippers and users of soybean oil, and a convenient means of disposal of the excess oil has been devised. The method has been found to be satisfactory in all respects so far as the drawing of an accurate sample is concerned. Accordingly a revised

method, with the incorporation of certain improvements has been submitted for approval.

The Uniform Methods Committee has studied the proposed revised procedure and has approved of its adoption as a Tentative Method to replace present "Petcock Method," C 1-47, D (a), but with the following minor changes and additions:

- a) In order to promote more rapid and complete drainage and to eliminate, in most cases, the need for the cleaning prescribed in D-5, the bottom of the drum should be replaced by an inverted cone bottom welded securely in place. Samples should be drawn off through the "draw-off line," presently shown in the drawing of the proposed Continuous Flow Sampler, but surplus oil to be returned to storage should be drained off through a connection to the apex of the conical bottom.
- b) The cone bottom should have an apex angle of approximately 120°, the two other angles with the horizontal being about 30° each. These angles should be shown in the revised drawing.
- c) Whenever a different type or kind of oil is to be sampled, the drum should be flushed thoroughly with the oil to be sampled. To accomplish this conveniently a one-inch line with a 45° swing joint should be installed to deliver oil from the loading line to the top center of the drum in such a way that by rotating the swing joint the oil may be used to flush down the sides of the drum into the cone bottom.

These changes should be worked out with the subcommittee on Continuous Flow Method of Sampling and must be reapproved by the subcommittee of the Fat Analysis Committee and by the Uniform Methods Committee.

With the above changes the Uniform Methods Committee approves adoption of this proposed replacement for C 1-47, D (a).

All of the above recommendations by the Uniform Methods Committee were approved by vote of the members present at the business session on October 2, 1957.

J. J. GANUCHEAU	R. J. HOULE
D. L. HENRY	R. R. KING
T. H. HOPPER	T. C. SMITH
J. T. R. ANDREWS, chairman	

An Improved Liquid-Liquid Extractor¹

R. P. A. SIMS² and G. A. ADAMS, Division of Applied Biology, National Research Council, Ottawa, Ontario

FOR STUDIES of thermal polymerization of vegetable oils and investigations of the viscosity of the resultant polymer, a method for fractionating heat-bodied oils became essential. The solvent fractionation method of Bernstein (1) was used, but removal of last traces of the high-boiling alcohols from the heavier fractions proved to be troublesome. Because of the marked influence of temperature on the solubility of thermally polymerized oils in acetone, extraction at progressively increased temperatures was tried as an alternative. This type of fractionation is possible in the apparatus to be described.

A number of liquid-liquid extractors having the advantages of thermostatic control (8), internal stirring (2, 3, 6), or all-glass construction (2, 4, 6) have been described. None however embodies all these features. An all-glass extractor was therefore constructed in which these and other advantages are incorporated.

In the present apparatus (Figure 1) stirring is effected by a Teflon-enclosed bar magnet, and channeling of the lighter liquid is further restricted by four baffles (2 cm. long and 1 cm. deep) set at approximately 30° from the vertical. To obviate the problem of alignment the central tube is sealed into the extractor wall. A 2.0-mm. hole at the bottom of the solvent delivery tube permits satisfactory solvent dispersal. The extraction area is surrounded by a water jacket, through which water from a constant-temperature bath is circulated.

The liquid return-line has been kept small and the vapor path large to direct the vapor to the condenser and thus avoid heating the condensate. To permit equalization of pressure in the event of a blocked, liquid return-line, a 2-mm. hole was made in the upper part of the solvent delivery tube near the seal.

The temperatures within the extractor, when acetone was the solvent, were measured one hour after the start of the experiment with the water jacket empty or containing water at various temperatures (Table

¹ Contribution from the Division of Applied Biology, National Research Laboratories, Ottawa, Canada. Issued as N.R.C. No. 0000.

² Present address: Chemistry Division, Science Service, Department of Agriculture, Ottawa, Canada.