principal causes of dispute regarding the color grading of cottonseed oils. The Priest-Gibson (N″) scale may now be considered as thoroughly established in America."

Having established the N″ scale, which is definitely linked to the use of a 35-yellow type, the Bureau of Standards and the Electrical Testing Laboratories set up identical procedures for checking glasses submitted to them for standardization. The standardization procedure used is taken as quoted from "Research Paper RP 653," p. 274: ". . . the glass to be tested is compared directly under artificial sunlight illumination with the standard glass or combination of standard glasses. The central portions of each are viewed in juxtaposition in the Martens photometer through the 35Y glass placed over the ocular. A standard glass or combination of standard glasses is selected, giving the best possible match in chromaticity. Since the smallest intervals between the standard glasses in use are differences of approximately 0.1 of an N″ unit, estimates between the tenths are made whenever differences of this small magnitude can be detected. When a combination of two or more standard glasses is necessary to secure the match, the glass being tested is combined with a corresponding number of clear glasses so that the light illuminating one-half of the photometric field passes through as many reflecting surfaces as that illuminating the other half. Three glasses are the maximum number ever used in one-half of the photometric field. By thus limiting the number of standard glasses, errors due to the accumulation of small errors in the standards are prevented. Because of this limitation and the restricted number of standard glasses available, it is frequently necessary, in order to obtain the N″ value for the glass tested, to combine a standard glass with the glass being tested and subtract its N″ value from that of the standard glasses in the other half of the photometric field.

"After the first decision the glass being tested and the standards are interchanged in position and a second decision is made. An average of these two readings is recorded as one determination. The final grade of each glass tested is based upon at least two separate determinations embodying two different combinations of standards. To increase the reliability of the average grade, a third or fourth combination of standard glasses is occasionally used, especially when the glass tested is of nominal grade greater than 10.0 Lovibond units."

The Electrical Testing Laboratories make comparisons against Bureau of Standards glasses set No. 74170, which was calibrated by the Bureau. Since procedures for the standardization of Lovibond glasses were established in 1934 and 1935, many such sets have been standardized for industrial firms. It is expected that numerous other sets have been calibrated visually against these standards. Accordingly it is expected that the Color Committee will recommend that A.O.C.S. Official Method Cc 13b-45 be amended to read as follows:

3. Color Glasses
   Color glasses calibrated to conform to National Bureau of Standards N″ Scale. Glasses may be calibrated by the Electrical Testing Laboratories or may be calibrated by comparison against a standard set calibrated by the Electrical Testing Laboratories or National Bureau of Standards.

The proposed change in the A.O.C.S. Method, if adopted, will not change anything now being done but will bring the method into accord with the facts. Many laboratories now using uncalibrated glasses will be enabled to obtain proper calibration, and a greater uniformity of results between laboratories should be achieved.

After reviewing the work completed by the Bureau of Standards on the calibration of Lovibond glasses and the literature concerning the standardization of new glasses, the committee feels that no additional changes in the method of calibration should be made.

R. C. STILLMAN, chairman
Color Committee

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Brown-Colored Oxypolymers of Unsaturated Fats[1]

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BROWN OR YELLOW lipide polymers or copolymers have been characteristically associated with both pathological *in vivo* oxidation of unsaturated fats and the advanced stages of oxidative fat rancidity in food products. In the yellow fat disease of vitamin E-deficient mink (14), rats (16), cats (3), chickens (4), pigs (7, 18), and fish (22) these yellow-brown polymers are formed in the adipose tissue. Similar yellow-brown polymers have been found in human atherosclerosis (9), where they appear related to pathological, unsaturated lipide oxidation (8). These polymers are similar to those named "ceroid," which are associated with choline deficiency (2). In the rusting of fish (19) and the yellowing of bacon (5) during refrigerated and frozen storage the color development follows unsaturated fat oxidation and is associated with rancidity.

These naturally occurring oxypolymerizations of unsaturated lipides proceed in the presence of proteins. Furthermore it has been demonstrated that proteins, especially hemoglobin, accelerate polymer formation (2, 10, 21). Also these proteins either copolymerize with the oxypolymerizing lipide or become occluded in it. If the yellow-brown polymers are, in fact, copolymers containing protein, then the mechanism is probably active carbonyl-amine browning (11, 21). By the active carbonyl-amine browning mechanism the carbonyls formed by decomposition of lipide peroxides could react with the amino groups of

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the proteins, and brown-colored copolymers would be the expected product.

It was the purpose of this study to determine if active carbonyl-amine browning is a dominant mechanism in the formation of brown oxypolymers similar to those found in nature.

Experimental

The formation of brown polymers was studied in emulsion systems similar to those studied previously (21) and consisting of 3 ml. of unsaturated lipide and 10 ml. of aqueous dispersion of protein.

The latter was prepared by dispersing one gram of protein or acetylated protein in 10 ml. of 0.1 M phosphate buffer of pH 7.0. There were two exceptions to this procedure: a) in the case of the sulfite emulsion and its control 0.2 ml. of polyoxyethylene lauryl alcohol (Brij 30, Atlas Powder Company) was added to each 10-ml. portion of buffer to compensate for emulsion instability in the presence of sulfite, b) in the case of sodium alginate emulsions the one gram of protein was replaced by 0.2 g. of Kelcosol (Kelco Company, San Diego, Calif.) per 10 ml. of buffer. After incorporation of 3 ml. of menhaden oil the mixture was passed three times through a hand-operated homogenizer; the single exception to this occurred in the case of the egg albumin-menhaden oil emulsion used for studying the temperature dependence of the rate of brown-color development when 0.3 ml. of oil was used in place of the customary 3 ml. Antioxidants and pro-oxidants were introduced into the emulsions by means of the buffer or the oil as their solubilities indicated.

Hemoglobin was prepared from fresh cattle blood. Defibrination was followed by three washings with saline. After laking with distilled water, stromata were removed and the solution was lyophilized.

Powdered egg albumin was purchased from the Nutritional Biochemicals Corporation. Acetylation of this material was carried out according to the procedure of Fraenkel-Conrat (6).

The menhaden oil and herring oil (Fishery Technological Laboratory, Seattle, Wash.) had saponification equivalents of 190 and 185 and iodine numbers of 187 and 138, respectively.

The emulsions were kept in 9-cm. Petri dishes during storage at constant temperature and during reflectance measurements. During this storage the emulsions were exposed to atmospheres saturated with water vapor so that evaporative losses were minimized.

During the time the emulsions were developing brown color, reflectance measurements were made with a filter tristimulus colorimeter, the Colormaster Differential Colorimeter (Manufacturers Engineering and Equipment Corporation, Hatboro, Pa.). Petri dishes were placed over a reflectance port so that the instrument viewed the emulsion surface lying against the bottom of the Petri dish. Black shielding served to obviate spurious reflectance and stray light effects. Standardized white tiles were used as a reference standard throughout all determinations. The possibility of serious interference with measurement of the reflectance of an emulsion because of reflection by the Petri dish itself was ruled out by measurements made before the emulsion was added to the dishes.

The reflectance readings for the samples of emulsions were obtained successively through each of the three filters; these readings have a simple relationship to C.I.E. tristimulus values. From these readings the total color difference, $\triangle E$, was calculated. The magnitude of $\triangle E$ values is expressed in National Bureau of Standards units; the latter is a measure of distance within uniform color space. In all our experiments $\triangle E$ represents the deviation of the color of an emulsion from its initial color.

For studies of active carbonyl-amine condensation reaction during the initial oxidation of the menhaden oil-water emulsion, the reaction system consisted of 1 ml. of menhaden oil and 3 ml. of 0.02 M phenylacetic acid or phenylalanine, which was rapidly shaken in a Warburg vessel in a 50°C.-bath. Unreacted phenylacetic acid and phenylalanine were extracted with water and determined by their spectral absorbance in the 260 mμ region.

Results and Discussion

Preliminary experiments involved visual comparison of colors, using a set of reference standards of Maerz and Paul (13). Although the use of the Colormaster permits much more objective study, certain effects were sufficiently pronounced in these preliminary investigations to justify their presentation here.

The formation of brown color in emulsions of menhaden and herring oils with aqueous dispersions of egg albumin, lactalbumin, and blood fibrin was studied at pH values covering the range 4 to 10.

That the formation of brown color was most rapid in menhaden emulsions is consistent with the greater unsaturation of this oil and shows the importance of unsaturated lipide oxidation.

The egg albumin emulsions were most stable and formed brown color fastest; blood fibrin yielded the most unstable emulsions and formed brown color the slowest; and the behavior of the lactalbumin emulsions was intermediate in both respects. In all cases the rate of formation of brown color was a function of increase in pH. These observations would be consistent with both of the postulated reaction mechanisms of oxypolymerization of lipide, especially if it involved base-catalyzed carbonyl condensations, and of carbonyl-amine browning.

Effect of Temperature Upon the Rate of Formation of Brown Color. Over the temperature interval of 20–55°C. emulsions of menhaden oil displayed a progressive increase of browning rate with rising temperature. Reflectance measurements performed on these emulsions yielded $\triangle E$ values that were plotted

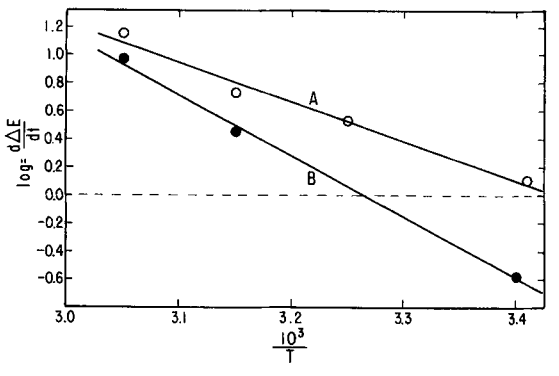

FIG. 1. The effect of temperature upon the rate of formation of brown color: A. sodium alginate and menhaden oil; B. egg albumin and menhaden oil.

TABLE I
Apparent Activation Energies

System	Activation energy	Method	Reference
	K cal./g. mole		
Active carbonyl-amine browning			
Casein-glucose	29.0	Free amino N	12
Bovine serum albumin-glucose	30.3	Color	15
Unsaturated lipide oxidation			
Alkali linoleate-linoleic acid	15.2	O_2 Uptake	12
Ethyl linoleate	17.2	O_2 Uptake	1
Methyl linoleate	16.2	O_2 Uptake	17
Emulsions, this investigation			
Egg albumin-menhaden oil	19.7	Color	....
Sodium alginate-menhaden oil	12.8	Color	....

versus time to give curves whose initial slopes are represented by the points appearing in Figure 1. Activation energies corresponding to these Arrhenius plots are given in Table I together with some values taken from the literature for comparison.

In the emulsion system consisting of sodium alginate and menhaden oil the only known mechanism for the formation of brown color is the oxypolymerization of the menhaden oil. The alginate was chosen as an inert polymer which gave an emulsion of physical properties similar to those prepared with proteins like egg albumin. The activation energy of 12.8 kg. cal./mole for this oxypolymerization of menhaden oil is lower than the available literature values for unsaturated lipide autoxidations.

In the emulsion system consisting of aqueous egg albumin and menhaden oil either of the two well-known mechanisms, oxypolymerization of the lipide or active carbonyl-amine browning, may lead to the formation of brown color. If oxypolymerization of the lipide is the dominant mechanism, then an activation energy similar to those for unsaturated lipide oxidation and the oxypolymerization of menhaden oil-alginate emulsions would be expected. If active carbonyl-amine browning were the dominant mechanism, then the characteristically high activation energy of about 30 kg. cal./mole would be expected. The experimental value of 19.7 kg. cal./mole indicates that active carbonyl-amine browning is not the dominant mechanism. This activation energy is of magnitude similar to those for unsaturated lipide oxidation and the oxypolymerization of menhaden oil-alginate emulsions and therefore cannot be cited as evidence against these mechanisms.

Effect of Emulsion Composition, Catalysts, and Inhibitors Upon the Rate of Formation of Brown Color. Introduction of various reagents into the menhaden oil emulsions results in measurably different initial colors. In order to compensate for these differences use is made of the deviation ratio

$$R = \triangle E / \triangle E_{max}$$

where $\triangle E_{max}$ represents the distance, in Bureau of Standards units, between the original emulsion color and pure black. The extreme values obtained for $\triangle E_{max}$ were 76.3 (cupric ion) and 85.4 (butylated hydroxy toluene).

Control emulsions did not behave identically on account of the influence of a number of variables, *e.g.*, time elapsed between color measurements and changes that were made in emulsion composition. Thus, in order to obtain a basis for comparison, all kinetic data have been plotted in terms of the deviation ratio difference $R-R_c$, where R is the ratio for a specified emulsion and R_c the ratio for its control. By arbitrarily equating the behavior of all control emulsions, as has been done in Figure 2, the effects of various treatments can be compared.

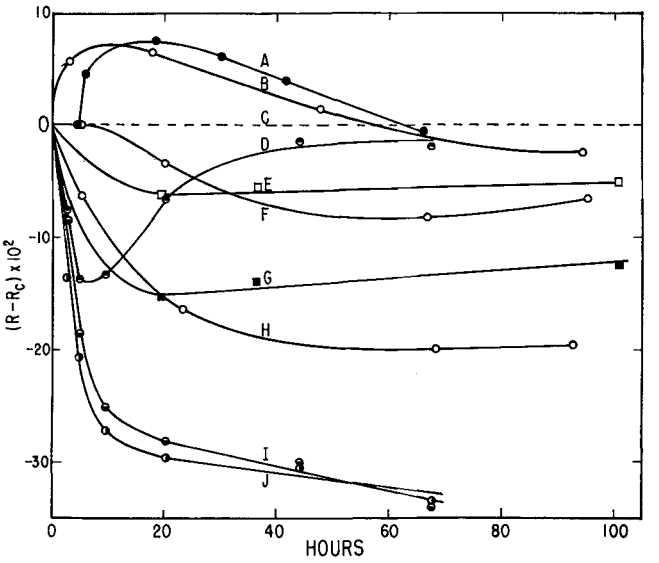

FIG. 2. The effect of emulsion composition, catalysts, and inhibitors upon the rate of formation of brown color: A. acetylated egg albumin; B. sodium bisulfite (13 mg.); C. control, menhaden oil-egg albumin emulsions in air; D. nordihydroguaiaretic acid (50 mg.); E. hemoglobin (1 mg.); F. cupric chloride (13 mg.); G. hemoglobin (5 mg.); H. oxygen; I. santoquin (1,2-dihydro-2,2,4-trimethyl-6-ethoxyquinoline (50 mg.); and J. butylated hydroxytoluene (50 mg.).

If the initial rates of formation of brown color are considered, it is observed that two gross effects are produced by altering the composition of the emulsions. If acetylated egg albumin is used to replace the unacetylated material or if sulfite ion is added to an otherwise unaltered emulsion, the browning rate is increased with respect to the controls. Upon addition of either of two classes of substances, *viz.*, unsaturated lipide antioxidant or unsaturated lipide pro-oxidant, the rate of formation of brown color is diminished with respect to that of the control emulsions.

It is of interest to note that the decrease of the rate under pro-oxidative conditions received additional confirmation from the observation that the top surfaces of the emulsions were less colored than the bottom surfaces whenever oxidation was promoted as in the use of hemoglobin cupric ion, or pure oxygen atmospheres.

To reconcile all of these observations it appears most reasonable to postulate the following systems of reactions, which to some extent compete with each other for reactants or reaction intermediates: a) relatively rapid, unsaturated lipide oxidation, b) comparatively slow, unsaturated lipide oxypolymerization, c) active carbonyl-amine browning.

Conditions augmenting the oxidation of an emulsion at a given temperature favor reaction a), which leads to a more lightly colored emulsion. The rate of lipide oxidation was increased by the replacement of air by pure oxygen and by the use of cupric ion and hemoglobin as catalysts.

The inhibition of active carbonyl-amine browning was achieved by acetylation of the egg albumin to make amino groups unavailable for browning or

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 quantitate 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

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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":