Thus K_w for 0.5 N — NaCl solution is about twice that for pure water,[20] which means that the OH^- ion concentration is about 1.5 times as great. But since the H^+ ion concentration is also 1.5 times as great, the pH is actually lower in the salt solution, even though it is still "neutral."

It is obvious how difficult the interpretation of "pH" becomes at temperatures above room temperature, or in solutions containing salts. For this reason, it is far simpler and much more easily comprehensible for practical purposes, to express the alkalinity of a soap solution in terms of the concentration of NaOH which at the same temperature will produce the same e.m.f. reading with the hydrogen electrode. The standard used is then available to all and is understood by all, and if such a standard were in universal use, the literature would not be cluttered up with so-called "pH" readings, the validity and meaning of which will never be known by any but the author.

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THE PIGMENTS OF RYE GERM OIL[*]

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IT IS the purpose of this communication to present the results of preliminary work, based upon the experience of others, on attempts at the development of a procedure for the determination of the carotenoid or polyene pigments of rye germ oil, one which, it is hoped, may find ready adaption to the examination of the germs of other members of the Gramineae as well as to fatty oils in general.

Investigations in this field divide themselves roughly into two classes. One collectively determines all the pigments under one general head as "gasoline color value" or at best utilizes but one of the several components as a measure of the pigments present. The other, an extremely exhaustive one, is time-consuming and, therefore, hardly applicable in the average laboratory. The first group has been developed by Schertz,[1, 2] Bailey and associates,[3-5] Guilbert,[6] and Miller.[7-9] Their methods, however, are restricted by several factors which make them inapplicable for the objective in question. As already indicated, often only the total pigment concentration is determined, or at most but two components thereof, such as xanthophyll and *beta*-carotene. Furthermore, since these methods were primarily developed for the examination of pigmented material of low fat content, we have found them to be unsatisfactory when applied to the problem in hand. The second group includes the contributions of Willstätter and Escher,[10] Palmer,[11, 12] and Kuhn and others,[13, 14] investigators to whom collectively belongs the credit for the greater portion of the information now available on the properties, structure, and methods of isolation of the polyene pigments. Since these methods require large amounts of raw materials, with the consequent handling of large volumes of solutions in laboratory glassware of unusual sizes, they hardly lend themselves to analytical procedures although they do have the advantage of successful application to the qualitative determination of all of the known carotenoids.

The polyene pigments, as the name suggests, owe their coloration to an extended system of conjugated double bonds. Just as is the case with many such conjugated systems, these pigments are very susceptible to the action of chemical reagents. Their susceptibility to oxidation makes necessary the elimination of atmospheric oxygen in all quantitative procedures, proscribes the use of any solvent, such as diethyl ether, which has not been freed of peroxides immediately before use, and demands that all analytical procedures require as little time as possible. The polyene pigments readily add other "double bond" reagents and are particularly susceptible to acidic compounds. Thus, if the chromatographic adsorption method of Tswett[15] is employed, it is best to avoid the use of acid adsorbants. Heat and light are quite effective in isomerizing and decomposing the carotenoid pigments, especially in the presence of air and moisture. Therefore, temperatures higher than 45° C. are rarely used when handling these compounds. The polyenes are soluble in the organic solvents; hence, for effecting solution diethyl ether, petroleum ether, chloroform, and carbon disulfide have been most frequently used. Solutions of the pigments in these solvents, with the exception of diethyl ether, are generally conceded to be stable[1, 2] when stored under refrigeration away from light in an inert atmosphere. Carbon disulfide is one of the most efficient of the solvents for the pure pigments, and it was this fact, used in conjunction with the knowledge that carbon disulfide yields an oil high in color value and with a relatively large amount of unsaponifiable matter,[16] which led to the adaption of Miller's method of isolation as a basis

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for the preliminary investigations.

Both Gill[17] and Miller[9] have successfully applied carbon disulfide to the extraction of the aqueous solution of the soap resulting from the saponification of the fatty material. However, in the present case it was found that carbon disulfide and the alkaline soap solution emulsified with extreme ease, and the resulting emulsions were recalcitrant to such methods of breaking as freezing, centrifuging, and moderate heating. The emulsions were successfully, but laboriously, broken by treatment with a warm, saturated solution of sodium chloride. The salting-out effect of the sodium chloride on the soaps rendered this method undesirable, and for these reasons the use of carbon disulfide was abandoned.

Zechmeister[18] and Boekenoogen[19] have adsorbed the pigments directly from the unsaponified oil by the use of aluminum oxide. In their experiments the oil was run through a Tswett column either in the pure state or diluted with petroleum ether, as the individual case required. Nevertheless, this simple and direct case did not work successfully in the case of rye germ oil even though the test portions were successively run through as many as five freshly prepared columns.

A qualitative examination of the action of the common solvents indicated that petroleum ether was the most suitable solvent for the extraction of the saponified fats. In order to avoid the formation of emulsions, the soaps were first dried in an inert atmosphere of nitrogen under reduced pressure (this procedure is time-consuming and is best applied in those cases where there is a large amount of soap to be extracted) and the coloring matter of the dried soap extracted with petroleum ether. Here it was found that the potassium soaps were preferable to the sodium derivatives since the latter became viscous when treated with the solvent, thus making efficient extraction impossible.

After separation from the soaps, the carotenoids were partitioned between the petroleum ether and methyl alcohol (89 per cent) by repeated extraction with the latter, the xanthophylls accompanying the methyl alcohol, the carotenes remaining in the petroleum ether. For purposes of identification the latter are designated as solution I, the former solution II. At this point it was deemed advisable to remove the sterols which remained in the petroleum ether solution with the carotenes since Zechmeister[18] has shown that the sterols interfere with the smooth adsorption of the pigments in the Tswett[15] method of analysis, which was to be the next step in the procedure. The sterols were removed from the solution by crystallization and filtration at a temperature of —50° C. Attempts at removing the sterols both at this point and in the case of the original unsaponified oil by the use of alcoholic digitonin solution did not prove to be a satisfactory alternative for the crystallization procedure. The elimination of the sterols is unnecessary when relatively small samples are being handled, but is advisable when large amounts of raw material (such as 500 gs.) are employed, as is often the case with the lightly colored oils.

The next step in the procedure was the application of Tswett's[15] chromatographic adsorption analysis for the separation of the isometric carotenes. In a certain sense, Tswett's method of isolation is incompatible with the attainment of quantitative results, for it is well known that complete recovery is never attained by this method. Yet in certain cases this procedure offers the only means of separation of the interfering impurities from the main constituents.

The solution of the carotenes was concentrated to exactly 100 cc. and divided into two portions of 25 and 75 cc. each. The smaller aliquot after further concentration was used to determine the number and kind of isomers present by adsorption on a column of magnesium or aluminum oxide. If the color, number and position of the rings indicated but one or two carotenes, uncontaminated by oxygenated derivatives, the larger aliquot was used directly for the spectrophotometric determination of the constituents. If the qualitative test, however, showed the presence of considerable amounts of impurities, recourse was had to the use of the Tswett[15] column for purification of the solution.

In the case at hand, the preliminary assay indicated the presence of *alpha* and *beta*-carotenes with possible traces of the *gamma* isomer. The latter was present in such small amounts that its existence was unconfirmable by other means, and its presence or absence must be determined by the use of a larger sample.

Solution II, containing the oxygenated carotene derivatives or xanthophylls in hydro-methyl alcohol, was evaporated under reduced pressure in an atmosphere of nitrogen, and the pigments taken up in petroleum ether and brought to a volume of 100 cc. Here again aliquots were taken and a qualitative test run on one portion to determine the number of constituents by the Tswett method. The presence of two pigments, xanthophyll (lutein) and zeaxanthine, in relatively high concentrations were indicated, while a third was present in trace amounts only. Its nature was not conclusively established. Both magnesium oxide[20] and powdered sugar[21] were successfully applied to this separation. The former gave very firm adsorption and sharply defined bands but tended towards slow filtration; in this respect the powdered sugar was far superior.

All of the Tswett columns employed in this work were prepared by the suspension method of Winterstein and Stein[21] as this method appeared consistently to give more uniform results than the dry packing methods previously employed by other investigators.

The quantitative determination of the more important pigments of the rye germ is being continued at the present time by the use of the spectrophotometer (Bausch and Lomb, Littrow type). This method has been chosen in preference to the more rapid colorimetric procedure because it yields more accurate data.

In conclusion, the method briefly outlined above has been used for the isolation and characterization of the carotenoid pigments of rye germ oil. Two carotenes, the *alpha* and *beta* isomers, accompanied perhaps by the *gamma* form, have been demonstrated to be present. Two xanthophylls, lutein and zeaxanthine, were found to be present, and the existence of a third unidentified pigment of this class has been tentatively suggested.

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1937 Report A. O. C. S. Committee On Soap Wrapper Paper

A BALLOT was sent to members of this committee late in August to ascertain their opinion concerning methods of testing or evaluating soap wrappers, on which this committee has done collaborative work in previous years.

Replies were received from eight of the twelve members of the committee.

Summary of Replies to Ballot:

1. Do the collaborative results to date justify, in your opinion, the recommendation by this committee of any tentative method for evaluating soap wrap paper?

Yes—4; No—3; Not voting—1

2. Are you in favor of doing more collaborative work before submitting any tentative methods to the American Oil Chemists' Society?

Yes—3; No—4; Not Voting—1

3. Which method or methods do you favor to be used in evaluating soap wrap papers?

(a) Alkali spot test only	0
(b) Extraction test only	1
(c) Soap contact test only	2
(a+b) Spot & extraction tests	0
(a+c) Spot & soap contact tests	1
(b+c) Extraction & soap contact tests	3
(a+b+c) Spot & extraction & contact tests	1

4. Additional comments and recommendations:

W. H. Burkhart:

"Although much work remains to be carried out on this problem the results obtained in my opinion justify the issuance of the methods tested, as tentative standards. Such publication should promote wider discussion and experimentation."

T. Linsey Crossley:

"After the members of a committee have done work on a committee project, they tend to a sort of short-sightedness to flaws in their processes. Hence having laid out a method and put it through its paces, it seems advisable to turn it loose among the members at large, and the best way of doing that is to propose it as a tentative standard, subject to revision after a year or so."

"My personal feeling is that the spot test should not be recognized."

"With reference to the notes made by two members of the committee as to difficulty in yellow and red elements in the extraction method, I did a lot of work trying to make a scheme that would match each case, but found that it would require a separate combination of tints for every type of paper and sometimes for two lots of the same type. Hence I came to the conclusion that it was not practical and further that intensity was far more readily noted than exact tint match. In the case of the Nesslerizing of water there is often this tint difficulty, but only in the cases where the figure is very low, and a similar thing happens with very small amounts of chlorides and silver chromates."

Frank Libby:

"There is a very good article relating to this subject under the title of 'The Discoloration of Soap-Wrap Paper' by W. R. Keating. This was published Sept. 2, 1937, in Volume CV No. 10, of the Paper Trade Journal. The paper was originally presented at the February meeting of T. A. P. P. I. in New York."

A. S. Richardson:

"I think that A.O.C.S should hesitate to give formal approval to performance tests as distinguished from methods of analysis and of determining physical and chemical constants. The word 'evaluating' as used above compels me to vote "no" on question (1) and refrain from voting on question (2). I do not mean to question the value of the work of our committee, results of which can be published in Oil & Soap to be used at discretion of the reader. I prefer 'extraction' test (b), which I think that we can sponsor properly as a chemical test without calling it a method of evaluation."

H. L. Roschen:

"All three methods appear to rate the several papers in much the same order, the soap contact method giving best results. I am almost inclined to vote for submission of the contact tests as tentative methods; however, I object to the method of grading the results as 'slight,' 'marked,' etc. If we submit a method permitting such designations, it is apt to give rise to difference of opinion on the part of buyer and seller due solely to differences in idea of what may constitute 'slight' or 'marked.' So before we submit methods, I should like to see more coöperative work. Perhaps some standard colors or stains should be introduced, or perhaps the contact methods could be combined with the spot test method, reporting the color produced in the contact test as equivalent to the color of N/10, N/2, N, 2N alkali on the same paper.

"Since it is a quantitative method, I should like to see the extraction test adopted. However, I feel that the agreement between collaborators on the same sample has not been sufficiently good and that the method which we used might be improved. I believe it would be well to work on the T.A.P.P.I. method, using more dilute color solutions so that greater spread in numerical values will be obtained, and also introducing a cold filtration of the extract. This latter step would eliminate difficulty with suspended matter from the paper, and might obviate the difficulties which some members of the Committee have had with milky or cloudy solutions. I am assuming that the cloudiness and milkiness is due to wax on paper K-30 W-35 and that this could be removed by cold filtration of the extract."

M. L. Sheely:

"We agree with your comments cocerning the extraction method. We believe that further collaborative work is necessary on this method before it should be adopted even tentatively."

Conclusions

The votes indicate a small majority in favor of recommending tentative methods for testing soap wrap paper.

Analysis of the replies to question No. 3 of the ballot as to which