THE PREPARATION OF FATTY ACIDS Susceptible to Oxidation and a Study of the Variables Influencing Iodine Number Determinations.

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By

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A portion of this thesis was presented by Dr. W. F. Geddes at the fifteenth annual convention of the Canadian Chemical Association, June, 1932. The paper was prepared in collaboration with Mr. D. S. Binnington.

In the course of an investigation (unpublished) into the chemical and physico-chemical changes induced in wheat germ by ageing, it was found necessary to determine with considerable precision the iodine numbers of the fatty acids obtained from the extracted lipoid material. Samples of wheat germ were aged at various moisture levels by means of a slow current of air; samples were removed at intervals, the fat extracted with ether and iodine numbers determined. Contrary to expectations the iodine numbers of the extracted fats were found to increase with time of ageing, this increase being most marked in the case of the sample aged at a high moisture level. The only possible explanation of this apparent anomaly would be an increase in the fatty acid content of the fat due to hydrolytic splitting of the glycerides. This was verified experimentally by the increase of the acid value of the extracted fats. The fatty acids thus liberated would tend to mask any concurrent oxidation by increasing the iodine number, and it was therefore deemed nessessary to prepare and study the insoluble fatty acids rather than the mixture of acids and glycerides constituting the extracted oil. In this connection it may be pointed out that van Loon (18) has shown the iodine numbers of the fatty acids to be more characteristic than that of the parent oil.

In the early stages of this investigation it became evident that the changes were of a small order of magnitude and that the conventional methods of preparation and iodine

number determination were not sufficiently precise for the purpose.

PREPARATION OF FATTY ACIDS.

The essential steps in the preparation of insoluble fatty acids are; saponification with alcoholic alkali; removal of alcohol by distillation; dilution of resultant soap with water; decomposition of soap with mineral acid in the presence of ether; washing of ethereal solution of fatty acids with water until free from mineral acid; removal of water and drying of solution; filtration and removal of solvent. Customarily only the removal of the solvent is carried out in an inert atmosphere, the possibilities of oxidation in the earlier stages of the preparation being neglected.

Preliminary experiments made evident the fact that some oxidation was taking place, and an apparatus was therefore designed and constructed whereby the entire series of operations could be carried out in an atmosphere of inert gas.

APPARATUS.

The complete set-up of the apparatus as originally devised is illustrated in Fig. 1 and is herein described with the mode of operation. This apparatus was used for one portion of the investigation under discussion, but it was found necessary at a later stage to construct one of larger capacity, incorporat-

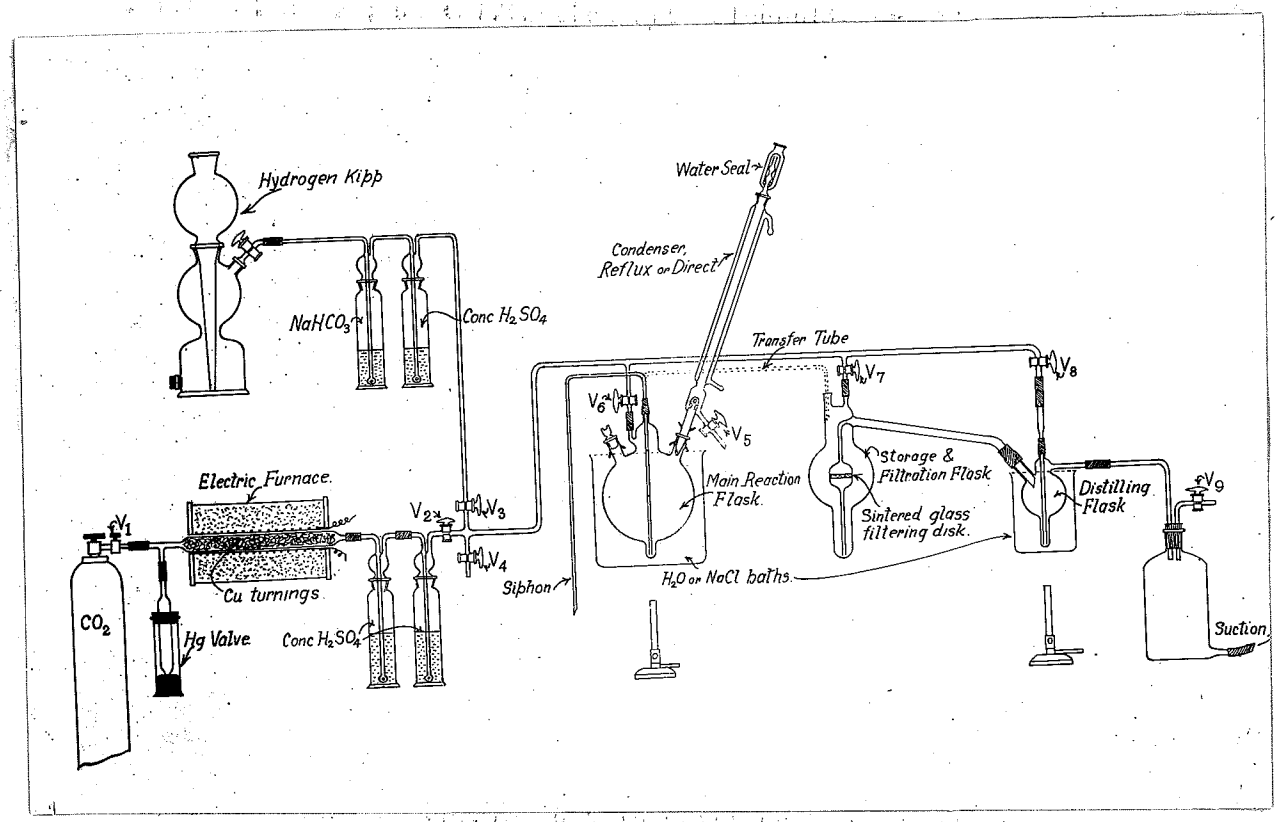


Fig. 1. — Diagram of apparatus for preparation of fatty acids in an inert atmosphere.

ing certain modifications making it capable of more general application. This modified apparatus will be described during the discussion of the investigation necessitating its construction.

As illustrated the set-up consists of purifying and drying trains for the hydrogen and carbon dioxide used, a main reaction flask, an intermediate storage and filtration vessel, and a special distilling flask for removal of the solvent, the entire apparatus being so constructed that it may be filled with hydrogen or carbon dioxide at will.

Hydrogen is prepared in a Kipp generator from zinc and hydrochloric acid, washed with saturated sodium bicarbonate solution, and dried by bubbling through concentrated sulphuric acid. Carbon dioxide under sufficient pressure is most conveniently obtained from a cylinder of the gas. As an analysis showed the presence of 0.05% oxygen, the gas is passed over heated copper turnings and afterwards dried by concentrated sulphuric acid. A precision needle valve V1 is employed for control of the gas flow, supplemented by a mercury blow-off valve for pressure regulation. Both gas trains are connected to a common manifold through stopcocks V2 and V3. Stopcock V4 may be used as a vent to provide additional pressure regulation within the system.

The main reaction flask is constructed from a 500 cc. round bottom Pyrex flask. Two extra side necks are provided, one

fitted with a ground stopper and the other with interchangeable ground stopper and reflux condenser. This condenser is of the convertible West type and may be used for either reflux or direct distillation by manipulation of stopcock V5. In the second and larger apparatus constructed stopcock V5 is replaced by a mercury valve. (Fig. 2.) The condenser is also provided with a liquid seal at the top allowing for the escape of gases while preventing the entry of air. The original neck of the reaction flask is drawn down to provide a sleeve fit for the interchangeable small bore siphon and transfer tubes employed, and is also provided with an inlet tube for inert gas. A narrow elongated cuplike depression at the bottom of the flask permits a sharp separation between ethereal and aqueous layers.

The intermediate storage and filtration vessel is constructed from a 200 cc. Pyrex flask with a large tubular elongation at the bottom, the neck being altered to provide inlets for inert gas from the manifold and introduction of solution from the main reaction flask. A sintered Pyrex (4) immersion filter is fitted internally, making connection through an external rubber sleeve with the distillation flask.

The distillation flask is designed so that small amounts of fatty acids may be recovered from large volumes of solvent and conveniently handled. It consists of a 100 cc. Pyrex flask with a long narrow tubulation at the base, a side

arm establishing connection with the filtration flask through a rubber sleeve, and a side arm for suction. The original neck of the flask is constricted to provide a sleeve fit for a narrow tube introducing inert gas to the bottom of the flask.

The three flasks are connected to a common manifold through stopcocks V6, V7 and V8. Transfer of solutions between the flasks is effected by gas pressure.

OPERATION OF APPARATUS.

A steady stream of hydrogen is allowed to flow through the main reaction flask during the introduction of the sample to be treated which may be either the oil itself or its ethereal solution as obtained by extraction. In the latter case the bulk of the ether is removed by distillation through stopcock V5, heat being applied by means of a water bath, hydrogen passing through the flask in the meanwhile. The siphon tube is, of course, closed during these operations. Alcoholic alkali is now introduced without interrupting the flow of gas, stopcock V5 closed and heat applied by means of a brine bath until the saponification process is complete, the condenser functioning as a reflux. Stopcock V5 is now opened, excess alcohol removed and the heating bath withdrawn. The contents of the flask are diluted with water, the condenser is removed and replaced by the stopper and the rate of hydrogen flow increased. Approximately 250 ccs. of ether are introduced and the soap is decomposed with an excess of dilute sulphuric acid. The reaction flask is

thoroughly shaken to ensure complete decomposition of the soap and to effect the transfer of the fatty acids to the ether. Any troublesome emulsions may usually be broken by running a few drops of concentrated acid down the sides of the flask or adding more ether in the same manner.

The hydrogen flow is now replaced by one of carbon dioxide, stopcock V3 being closed to prevent backing up in the hydrogen train. The aqueous layer is siphoned off as soon as clear, the siphon being started by gas pressure and controlled by rubber tubing and a pinchcock. The ethereal solution of fatty acids is now washed with successive portions of distilled water until the washings are free from sulphates, the last washing being drained off completely. Anhydrous sodium sulphate, wetted with ether is introduced and the flask thoroughly shaken, stoppered and allowed to stand until dehydration is complete.

Stopcock V6 is now closed, a gentle suction applied to the distillation flask and a rapid stream of carbon dioxide passed through the remainder of the apparatus via stopcocks V7 and V8. When the air in the system has been thoroughly swept out the suction is shut off, stopcock V8 closed, the stopper removed from the auxiliary neck of the filtration flask and a slow current of gas from stopcock V7 is allowed to escape through this opening. The ethereal solution is forced by gas pressure into the filtration flask by means of the transfer tube as indicated in Fig. 1, the transfer tube is removed, and the opening stoppered.

The salt bath under the distillation flask is now brought to a temperature of about 50°C. and a light suction applied by contral of stopcock V9. The pressure of gas in the filtration flask is adjusted so that the ethereal solution is forced through the sintered glass filtering disk and over into the distilling flask. The momentary pressure due to the volatilization of the ether temporarily forces the column of liquid back and through careful regulation of stopcock V7 this action may be caused to proceed automatically until all the ether has been evaporated and the fatty acids concentrated in the distilling flask.

Stopcock V7 is now closed and the temperature of the brine bath raised to within a few degrees of its boiling point- having first taken the precaution of using a film of paraffin on the surface of the bath to prevent water vapor interfering with later manipulations. A slow stream of carbon dioxide is bubbled through the fatty acids from stopcock V8 to remove the traces of solvent and water. Suction is stopped and the bath allowed to cool to about 50°C. maintaining the steady current of gas. The acids must not be allowed to cool too far lest crystallization occur. Stopcock V8 is closed, a gas inlet tube withdrawn and a pipette with a long capillary tip inserted in its place. A quantity of the fatty acids is drawn into the pipette which is then removed and the acids transferred to weighed bulbs which are immediately sealed in a fine oxygen-gas flame. The bulbs for this purpose are <u>freshly</u> blown on

thin-walled tubing 1 to 1.5 mm. in diameter and about 50 mm. long, the diameter of the bulbs being between 6 and 9 mm. These bulbs are heated in a temperature of 100°C. and are cooled and kept in a dessicator until immediately before required for use. The bulbs are immediately reweighed after sealing and stored in stoppered test-tubes in a cool dark place until required for the iodine number determination. Experience has shown that these samples may be stored for a period of at least several days without appreciable change and in all probability for longer, particularly in the case of samples of comparatively low iodine value. However, it is of course preferable to determine the constants on the fresh samples.

EXPERIMENTAL RESULTS.

The reliability of the above apparatus and procedure was determined by a number of test runs employing maize, linseed, perilla and extracted wheat germ oils. Excellent checks were secured between duplicate iodine number determinations. The results were then compared with those on fatty acids prepared from the same oils by the various conventional methods. The results are given in Table 1.

TABLE NO. 1.

THE IODINE VALUES OF FATTY ACIDS
PREPARED WITH VARIOUS PRECAUTIONS.

Oil	No Precautions	Dried in Current of CO_2	Dried in Vacuum Oven	Modified Method
Maize	122.4	122.5	122.9	127.4
	121.7	122.8	123.5	127.3
Perilla	181.8	183.8	183.9	185.0
	180.5	183.9	184.1	184.8
Linseed	181.9	184.2	187.1	189.3
	182.3	185.0	186.9	189.6

It should be noted that the iodine numbers of the maize, linseed and perilla oils given in this table are of interest for purposes of comparison only as the samples of oils from which the fatty acids were obtained were several years old and had undoubtedly undergone some oxidation.

It is obvious from Table 1 that, while fair checks are obtained in duplicate determinations by the other methods the values obtained fell considerably below those of the improved method, indicating that oxidation is taking place at some stage during the preparation of the fatty acids.

It will be noted that the values given include the unsaponifiable matter. As this is a small and constant factor, it was not deemed necessary to remove it for the purpose of the investigation. Such removal, if desired, could be readily accomplished by extraction of the soap solution with petroleum ether in the main reaction flask, removing the successive extracts by raising the siphon.

It was thought that a possible source of error not controllable within the apparatus might reside in the likelihood of oxidation of the fat during the extraction. A search of the literature revealed no information on this point and experiments were therefore conducted to determine definitely whether or not oxidation took place during prolonged extraction.

Diatomaceous earth was saturated with linseed oil of exceptionally high fatty acid content, distributed amongst four extraction thimbles and extracted for forty-eight hours with equal quantities of ether in an improved form of Soxhelt extraction apparatus (2). Two of the extraction flasks were provided with sealed-in gas inlet tubes through which a slow stream of dry carbon dioxide was passed during the entire time of extraction. At the

expiration of the forty-eight hours the ether was removed from all the flasks by a current of carbon dioxide. Samples of the oil were immediately sealed in bulbs and their iodine numbers determined. The results obtained and given below in Table No. 2 show conclusively that no change takes place during the ether extraction of fats, the ether vapor itself presumably displacing the air sufficiently well to prevent contact of the extracted fat with the oxygen of the atmosphere.

TABLE NO.2.

Data On Oxidation of Oil During Ether Extraction.

Extracted without special precautions.....	181.9 182.1
Extracted in a current of carbon dioxide.....	181.9 182.0

With particular reference to the wheat germ oil under consideration, the results of the investigation are given in Table 4, p.14. A few words of explanation may be necessary. The germ samples were stored in an electric refrigerator at about 7° to 10°C, immediately following the conclusion of the original research conducted by Winkler (19). It was assumed that any changes taking place would be retarded by the low temperature and would therefore not be sufficiently marked to invalidate the results of any further investigations on the same samples. A comparison of some of the analytical data obtained by Winkler at the conclusion of the accelerated ageing period and those obtained two months later would

serve to indicate that any further changes taking place are likely to be only those arising from slow ageing, and therefore of the same order but of diminished magnitude.

TABLE NO. 3.

ANALYTICAL DATA ON WHEAT GERM.

	Moisture		I.No. of Fat		Lipoid Phosphorus	
	E.S.	C.W.W.	E.S.	C.A.W.	E.S.	C.A. W.
Low	7.11%	7.0%	124.9	125.8	0.046%	0.048%
Original	9.45	----	123.3	123.0	0.002	0.014
High	12.81	13.0	130.7	129.4	0.068	0.104

The percentages of lipoid phosphorus have all dropped and, as it might be expected from Winkler's report, most rapidly in the high moisture level germ and least in the low moisture level. The behaviour of the ether extracted fats during the storage period is also in complete confirmation of the hypothesis first proposed to account for their apparently anomalous iodine numbers. The iodine numbers of the fat extracted from the low moisture level germ has dropped during storage. This would be apparently due to the fact that, the rate of hydrolysis being so slow, the concurrent oxidation of the glycerides and fatty acids has sufficed to produce a net lowering in iodine number. In the other two samples the rate of hydrolysis was sufficiently rapid to overbalance the effect of concurrent oxidation, producing a net increase in iodine number.

TABLE NO. 4.

ANALYTICAL RESULTS ON WHEAT GERM OIL

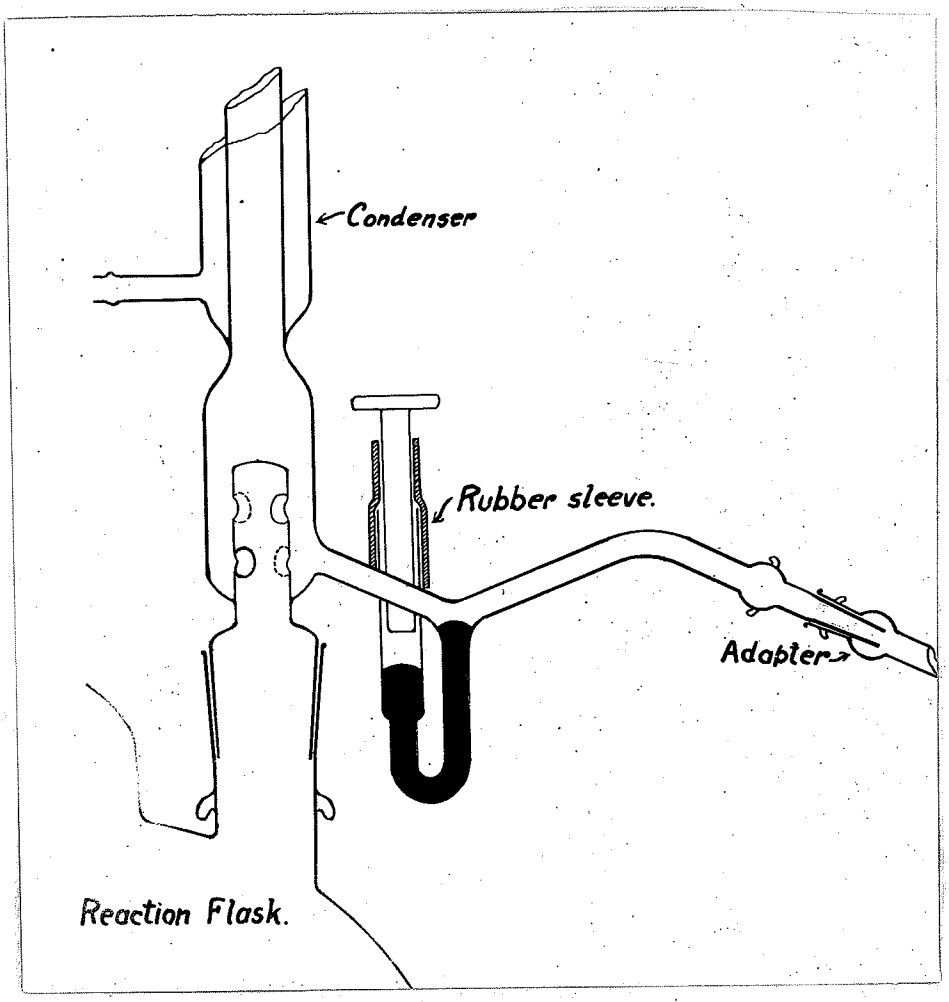
	Moisture	I.No. of Fat	I. No. of Fatty Acids	% Acid (as oleic)
Low	7.11%	124.0	129.0	13.71%
Original	9.45%	123.3	130.2	11.52%
High	12.81%	130.7	126.25	54.01%

The results obtained from the insoluble mixed fatty acids derived from the glycerides plus those present in the free condition are also in entire accord and may be explained on the basis of this hypothesis. This assumes that considerable hydrolysis of the glycerides has taken place resulting in a progressive increase of fatty acid content, this effect being expected to be most pronounced in the high moisture level where conditions would favor hydrolysis. The fatty acids obtained from the glycerides would indicate the actual extent of oxidation of the fats, and would therefore be expected to exhibit a decrease in iodine number on ageing, the decrease being most pronounced under conditions favorable to oxidation, i. e., high moisture content. An examination of the data given in Table 4 will indicate that these expectations are entirely fulfilled.

FURTHER APPLICATIONS OF THE APPARATUS.

As previously mentioned under the description of the apparatus, a second larger and somewhat modified form has been constructed which proves to be more adaptable. The capacity of this is approximately four times that of the original, the main reaction flask having an actual capacity of approximately two litres and a working capacity of about 1200 ccs. The West condenser is provided with a mercury cut-off valve, replacing stopcock V5, as illustrated in Fig. 2. The two gas trains, one for hydrogen and one for carbon dioxide, have been replaced by a nitrogen cylinder, equipped with a needle valve and followed by a purifying train. This train is

FIG. 2.



MERCURY VALVE and ADAPTER.
(modifications of apparatus illustrated in Fig. 1.)

identical with that provided for the carbon dioxide, the gas being first passed over heated copper turnings and then bubbled through concentrated sulphuric acid. The use of a single inert gas simplifies the procedure and reduces the complexity of the set-up. Stopcock V4 has been replaced with a three-way stopcock vent. Other minor changes have been made in various constructional features of the apparatus but these have no real bearing upon the operation of the apparatus.

In order to show that the results obtainable through the use of the original apparatus with the two gas trains are strictly comparable with those obtainable from the modified apparatus employing nitrogen as the inert gas, the iodine numbers of the fatty acids of linseed oil prepared by the two methods are compared.

TABLE NO. 5.

COMPARISON OF FATTY ACIDS PREPARED IN ORIGINAL AND IN MODIFIED APPARATUS.

Apparatus	Gas Trains	I. No. of Fatty Acids
Original apparatus	CO_2 & H_2	189.3 189.6
Modified apparatus	N_2	189.5

The use of the mercury valve (Fig. 2) permits the distillation of glacial acetic acid, this acid having been found to have no action even at its boiling point upon mercury. This is

particularly useful in the preparation of 100% acetic acid from commercial 99.5% glacial acetic acid as required for Kaufmann's thiocyanogen number determination (12). The commercial product is first refluxed on a sand bath with chromic acid or potassium permanganate until all the reducing agents and empyreumatic impurities in general have been oxidized. The acid is then distilled into a receiving flask through an adapter ground to fit the end of the mercury valve. This distillate is transferred back to the reaction flask, refluxed over phosphorus pentoxide, using a liquid paraffin bath, and finally distilled as before into a receiving vessel, the fraction boiling between 118° and 119°C. being collected. Since the acetic acid is very hygroscopic it is advisable to have the receiving flask connected to the outer atmosphere only through a drying tube filled with anhydrous barium perchlorate (Desicchlora). By far the largest portion of the glacial acetic acid prepared in this manner is found to have a boiling point of 118.0°C. (uncorrected) and a melting point of 16.6°C. - - - - corresponding to an acid containing less than 0.02% water.

The further adaptability of the apparatus may be illustrated in its use in the preparation of linolenic acid from the mixed fatty acids of linseed oil. The mixed fatty acids are conveniently prepared from a fresh sample of raw linseed oil according to the method given under 'Operation of Apparatus' p.6, but instead of evaporating off the ether in the last stage the acids are left in solution. The method of bromination devised by Bailey and Baldseifen (1) may be employed. The ethereal

solution of the fatty acids is brominated at about -17°C. using a solution of bromine in glacial acetic acid (1:5). After standing overnight in the refrigerator, the brominated solution is centrifuged several times, being washed well each time with cold ether. The insoluble linolenic hexabromide so prepared is then recrystallized twice from benzene and then dried in a vacuum dessicator. The melting point of the recrystallized hexabromide so prepared was found to be 183.0°C. The melting point is perfectly sharp. Comparing this with values found in the literature:

TABLE NO. 6.

MELTING POINT OF LINOLENIC HEXABROMIDE AS FOUND BY VARIOUS INVESTIGATORS.

Coffey (5)	185°C.
Erdmann & Bedford)7)	179°
Kimura (13)	183°
Lewkowitsch (14)	183°
Smith & West (17)	179.5-180°
Found by author	183.0°

The hexabromide is reduced, according to the method of Rollett (15) using zinc dust and a methyl alcoholic solution of hydrochloric acid. Zinc dust is added in about 67% calculated excess to a methyl alcoholic solution of the hexabromide in the main reaction vessel. The hydrochloric-alcohol solution (1:1) is

added from a dropping funnel fitted through a tinfoil wrapped cork, into one neck of the flask, while a rapid stream of nitrogen is passing over the surface of the liquid. With the condenser functioning as a reflux, the flask is warmed gently to increase the rate of reduction and allowed to stand until the evolution of hydrogen practically ceases. The major portion of the alcohol is distilled off and the solution is cooled to about 40°C. A large quantity of peroxide-free ether is added. (The peroxides are apparently reduced during the dehydration of ether with metallic sodium.) The ethereal solution is washed thoroughly and repeatedly with freshly boiled and cooled water until there is no silver nitrate test for chlorides. The solution is dehydrated over anhydrous sodium sulphate that has been wetted with ether before being introduced into the flask, allowing the solution to stand overnight. It is then transferred to the filtration flask, filtered, and the ether distilled off in the distillation flask, nitrogen being bubbled very slowly to remove the last traces of solvents. The acid is then removed by suction into a pipette-ampoule that has been heated to 100°C. and cooled over in an atmospher of nitrogen. One end of the ampoule is immediately sealed with a hand blow-torch, the other end being most conveniently closed with sealing-wax, care being taken to leave a minimum of space above the acid. Samples required for iodine number determinations are removed in a blanketing atmosphere of nitrogen as illustrated in Fig. 3, the nitrogen being passed in through the $\frac{1}{4}$" side tube.

The ethereal solution of the acid is water-white at first but by the time the filtration stage in the process is reached

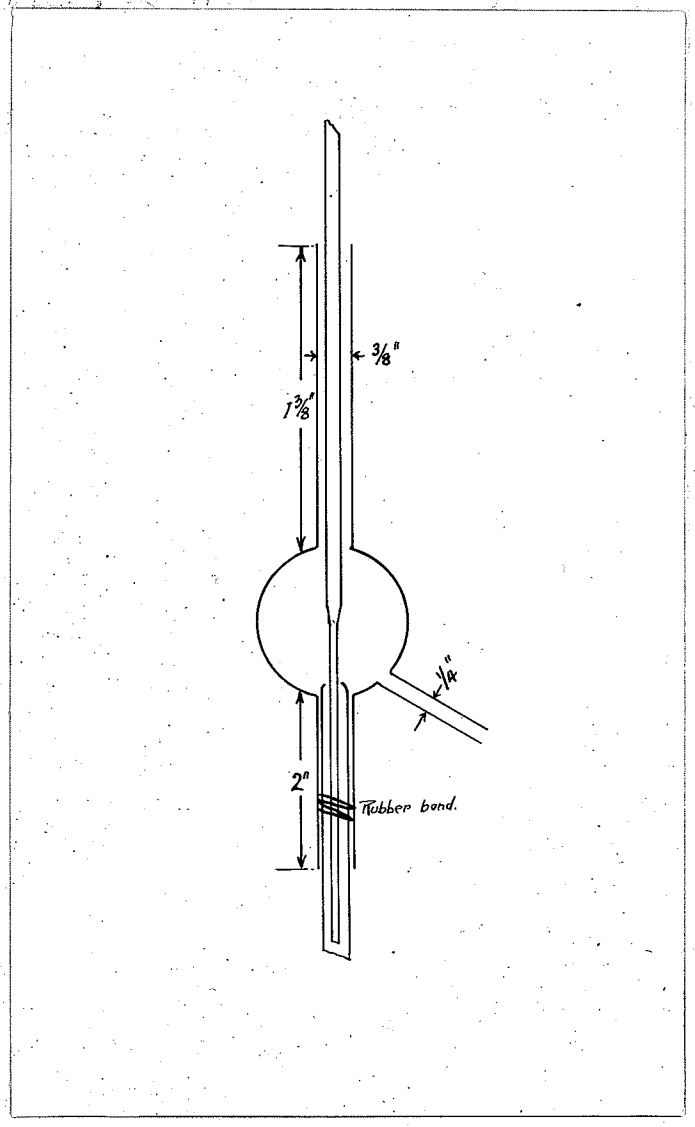


FIG. 111. Device for removal of samples of linolenic acid in an inert atmosphere.

it has become a faint yellow. When the free acid is drawn into the ampoule it is distinctly yellow. The appearance of the yellow color would seem to indicate auto-oxidation and, in the opinion of Erdmann (6), possibly polymerization. There is always the possibility of oxygen absorbed on the glass causing slow oxidation though all precautions are taken.

The iodine number of a freshly prepared sample has been found to be 269.1. A very rapid change in iodine number is evident even when the linolenic acid is sealed under nitrogen in the ampoule and stored in the dark. The yellow coloration increases markedly in the first twelve hours after preparation and the iodine number drops about six units. After this the color density does not appear to change appreciably, although the iodine number drops steadily but more slowly.

	Iodine Number.
Fresh sample.....	269.1
12 hrs.....	264.0
38 hrs.....	258.5
61 hrs.....	252.3

These values compare very favourably with those obtained by other workers (Table 3).

The density of a fresh sample of the acid determined at 20.0°C., using a Sprengel pyknometer, was found to be 0.9081. This value may be compared with those found by other workers (Table 7). It would appear that the sample of linolenic acid obtained by Erdmann (6) from what he terms Zn Linolenate is the purest so far prepared, despite the criticism of Coffey (5).

TABLE NO. 7.

COMPARISON OF PROPERTIES OF LINOLENIC ACID AS DETERMINED BY VARIOUS INVESTIGATORS.

Investigator	Iodine No.	Density
Theoretical	273.7	
Hazura (9)	241.8	------
Hehner & Mitchell (10)	245.	0.9228
Erdmann (6)	269-278	0.9046
Rollett (15)	---	0.9141*
Present investigation	269.1	0.9081

*This value for the density of linolenic acid is quoted in the International Critical Tables, Vol. 1.

TECHNIQUE OF THE IODINE NUMBER DETERMINATION.

During the course of this investigation it was found possible to introduce several refinements into the determination of iodine numbers by the Wijs method. The chief difficulty in this determination lies in that the technique is essentially more or less empirical in nature. All improvements must lie in the line of obtaining more reproducible results than have been obtained hitherto.

Iodine numbers in the literature are frequently expressed to the second decimal, that is, with an assumed accuracy of 0.05%. Schmidt-Nielsen and Owe (16), in a comparative investigation on the iodine numbers of fats, state that the experimental error in their tests did not exceed 0.05%. A simple calculation will demonstrate the absurdity of such a claim. An error of 0.2 mg. in weighing a 200 mg. sample would amount to 0.1 units in the case of an oil having an iodine number of 100.0. The experimental error in titration may be easily three or four times as great as this. The practice of weighing a heavy iodine flask directly on the balance would increase the error due to a lowering of the sensitivity of the balance. It is rather poor chemical practice to weigh samples of one or two hundred milligrams in a tare of eighty thousand milligrams. This error is minimized by the use of small sealed bulbs as previously described, which are broken under the solvent in the iodine number flask, thus obviating possible oxidation during weighing. The weight of these bulbs is of the same order of magnitude as the sample itself.

Another source of error not generally recognized is the effect of the low viscosity of Wijs solution when used in an ordinary pipette. Experience has shown that the delivery time of thepipette must be considerably increased in order to obtain reproducible results. Marked improvement can be effected by the use of a Bureau of Standards pipette delivering its contents in from 30 to 40 seconds, but still greater accuracy may be attained by a drainage time in excess of 60 seconds. The variation of Wijs solution delivery (in terms of N/10 sodium thiosulphate solution) has been determined for such a pipette and is less than 0.05 ccs. The extent of variation in delivery for a Bureau of Standards pipette is of a similar order and for an ordinary laboratory pipette in excess of 0.12 ccs. Calculated for a sample weight of 200 mgs. and an iodine value of 100.0 the possible error using even the improved slow delivery pipette may be as high as 0.6 units, if we allow the maximum deviation to both the blank determination and the actual run.

Wijs solution cannot be pipetted in the ordinary manner without excessive salivation, resulting in dilution of successive portions. Furthermore, as the pipette empties, moist air is drawn in and due to the extreme hygroscopicity of glacial acetic acid the moisture is rapidly absorbed by the thin film of Wijs solution left on the walls of the pipette. This results in further dilution of the solution. An examination of the literature has revealed a considerable number of automatic and semi-automatic pipettes specially designed for the accurate delivery of Wijs solution. With-

out exception these devices incorporate a stopcock through which the solution must flow. The satisfactory lubrication of a stopcock under such conditions is a practical impossibility.

In order to obviate the above difficulties the apparatus illustrated in Fig. 4 was constructed. The measuring pipette and its attached drying tube, containing anhydrous calcium chloride are rigidly attached to a stand, thereby eliminating temperature differences due to handling. To prevent checking of air into the pipette during adjustment the tip is made with a long parallel bore being tapered on the outside only and the extremity bevelled by grinding.

The Wijs solution is stored in an amber glass two liter bottle. Transfer of the solution to the pipette is accomplished as illustrated by means of a cup fitting snugly onto a stopper on the discharge tube of the pipette; this cup is attached to a narrow tube reaching almost to the bottom of the stock bottle and capable of vertical movement through a sliding sleeve. In operation the cup is brought directly under the pipette, raised, fitted tightly to the stopper and Wijs solution forced up by means of air pressure. The compressed air is conveniently furnished by a rubber bulb and is dried by passing over anhydrous barium perchlorate (Desiccchlora) in a drying tube. When the pipette is filled, the stopcock is closed and the cup lowered. Adjustment to the calibration mark is then effected by means of the stopcock. In order to facilitate

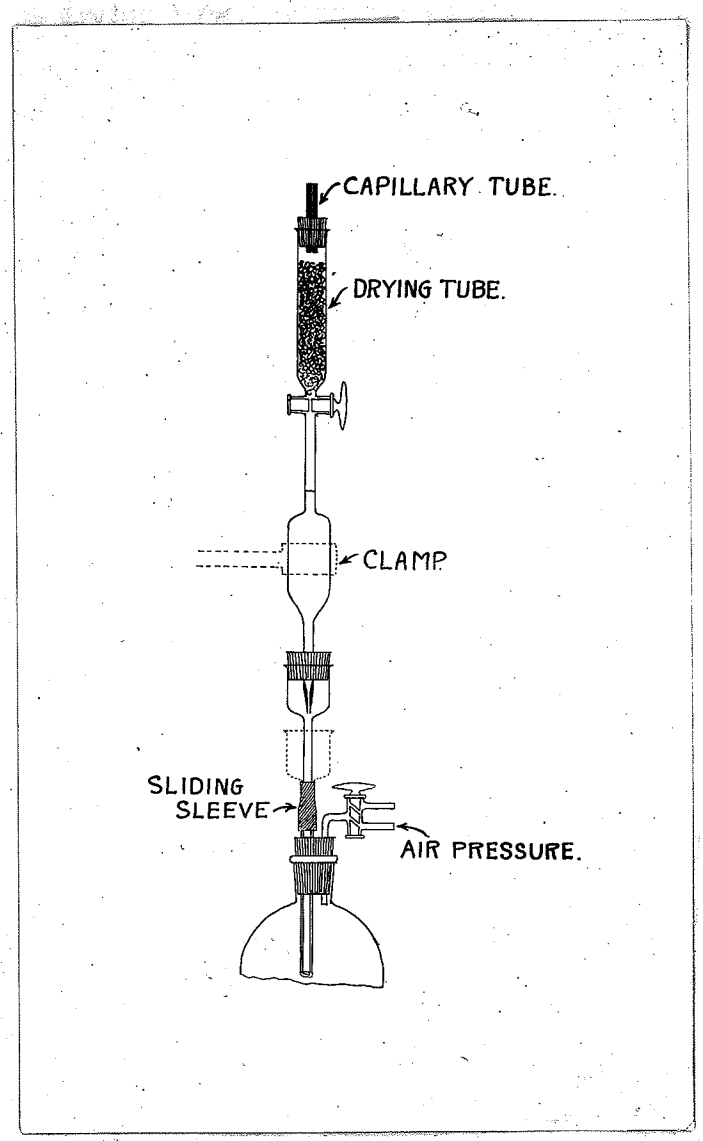


FIG. 4. Pipette for Wijs solution.

this adjustment the stopcock has a 'v' groove filed on one side of one end of the bore, and a fine bore capillary tube fixed at the end of the drying tube. Transfer of the Wijs solution to the iodine number flask is then effected by swinging the stock bottle to one side, swinging the flask into place under the pipette and delivering by opening the stopcock. Suitable rotating supports are arranged for both the stock bottle and the iodine number flasks. In addition to increasing the accuracy of the determination, this apparatus is more convenient and rapid in use than many apparently simpler methods.

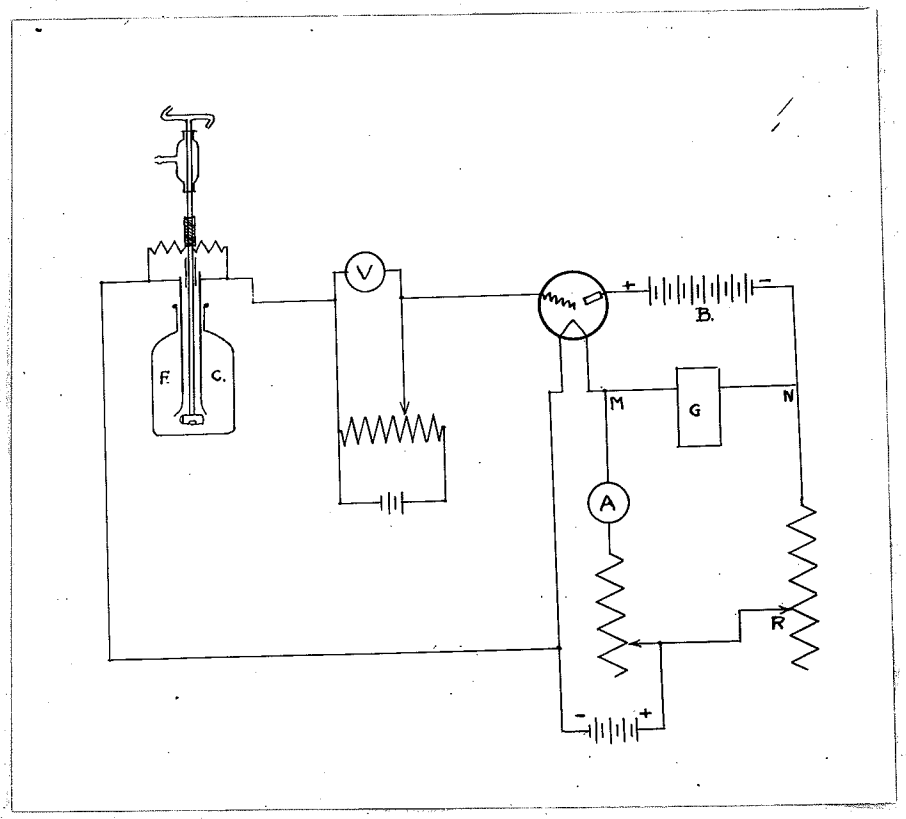
ELECTROMETRIC DETERMINATION OF IODINE NUMBERS.

An attempt was made to adapt electrometric titration methods to iodine number determination, but as a result of the experience so gained it is not believed that there is any material gain in accuracy. While electrometric methods may be employed to increase the accuracy of the titration through the more facile use of dilute solutions, in this case the additional accuracy so gained is offset by the fact that 'back-titration' is required if a convenient set-up is to be used. That is, a measured excess of thiosulphate solution is added to the iodine flask and the extent of the excess determined by 'back-titration' with iodine solution. However, this method of 'back-titration' was found useful in the investigation on the effect of temperature on iodine value.

The set-up used is a modification of that described by

Foulk and Bawden (8) and was devised by Kassner, Hunze and Chatfield (11). The apparatus is unique in its application of the vacuum tube to provide both amplification and a very precise and convenient indication of the end-point. A diagram of the "hook-up" is given in Fig. 5. Shunted electrodes are used, permitting only a very low potential difference between the electrodes this potential difference remaining constant up to the equivalence point. To attain this end, so low a potential (10 to 15 millivolts) is used between the electrodes that the back electromotive force of polarization balances it and no current flows. Under these conditions this electrometric titration apparatus may be employed in all cases where a sharp transition from the polarization of at least one electrode to the complete depolarization of both coincides with the end of the reaction. The grid current of R.C.A. 201A vacuum tube is employed in polarizing the bimetallic platinum electrodes. Part of the grid current, which is necessarily very small, is shunted around the electrodes. When the electrodes have been placed in the solution to be titrated and the resistance R adjusted so that the galvanometer reads zero, the points M and N are at the same potential. During the course of a titration the currents in the various circuits will remain unchanged as long as the potential difference between F and C remains constant. At the equivalence point there is a sharp change in the potential difference between F and C due to the depolarization of the cathode by the first trace of excess iodine. This produces a corresponding change in the positive potential impressed on the grid, causing a

FIG. 5.



KEY: B...225 volt storage battery
G...Galvanometer
A...Milliameter
V...Voltmeter
R...Resistance Box
F & C..Platinum electrodes sealed in glass.

ELECTROMETRIC TITRATION APPARATUS.

relatively large change in the filament plate current, which unbalances the system. M and N are no longer at the same potential and the galvanometer deflects. In actual practice we find a gradual deflection first to one side of the scale and then at the equivalence point, a sudden deflection to the other side.

This method is remarkable in that the different variables may be varied over a wide range without vitiating the results. It was found most convenient to use a shunt of about 35,000 ohms between the electrodes. In a personal communication the authors advised that instead of using a single rheostat of 5000 ohms, it was found more convenient to use three variable resistances in series - one of 5000 ohms, one of 250 ohms, and another of about 50 ohms. However, in this work a large dial resistance-box in series with a 50 ohm rheostat was employed.

Vigorous and very efficient stirring is particularly essential in this titration since there are present two immiscible solvents of quite different densities. An air-driven stirrer of the type devised by the Eastman Kodak Laboratories has been adopted; in order to minimize vibration and to prevent the exhaust air from blowing into the reaction flask the shaft is made in two sections joined by a rubber coupling with the lower portion of the shaft sleeved through a glass tube. The arrangement of the electrodes in relation to the stirrer is indicated in Fig. 5. Since it is impossible to fit the electrode and stirring unit into the conventional iodine flask, 250 cc. wide-mouth reagent bottles with

steppers reground with fine emery were employed. These were found to be quite satisfactory when the usual precaution was taken of running KI solution around the rim.

A comparison of the results obtained on a series of blanks of Wijs solution would show no appreciable difference between the results obtainable by the ordinary methods of titration and the electrometric:

	Electrometric	Starch Indicator
Solutions used	N/20 Thiosulphate N/20 iodine soln.	N/10 thiosulphate
	0.5993 gms. iodine	0.5994 gms. iodine
	0.5996	0.5997
	0.5997	0.5999
	0.5998	0.5999
Ave.	0.5996 gms. iodine	Ave0.5997 gms. iodine

We also note here in confirmation of other workers that the electrometric end-point is very slightly in advance of the starch end-point. The approach of the end-point in the electrometric method is indicated by the transitory straw-yellow color of the solution.

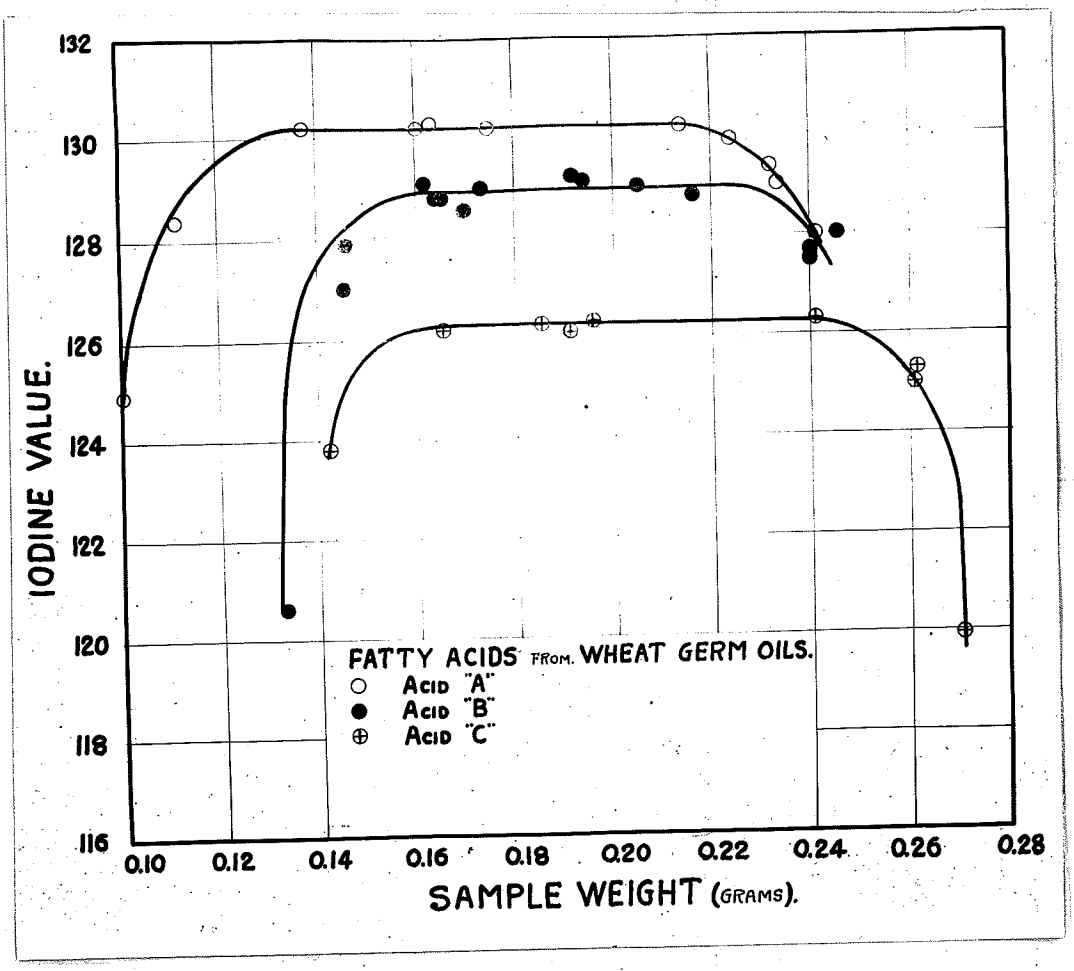
In conclusion then, we find that even with back-titration the electrometric method would seem to be fully as accurate as the conventional due to the use of the more dilute solutions. Doubtlessly if the rest of the technique could be more highly refined it might be advisable for very accurate work to use N/50 solutions, in which event this method of titration would be very useful. Another possible use that has not been investigated is in the micro-deter-

mination of iodine values. It does not appear likely, however, that an electrometric method will ever find general application in this particular determination since the apparatus is necessarily elaborate, and the other experimental errors so great in comparison. This method will be referred to again.

EFFECT OF SAMPLE WEIGHT.

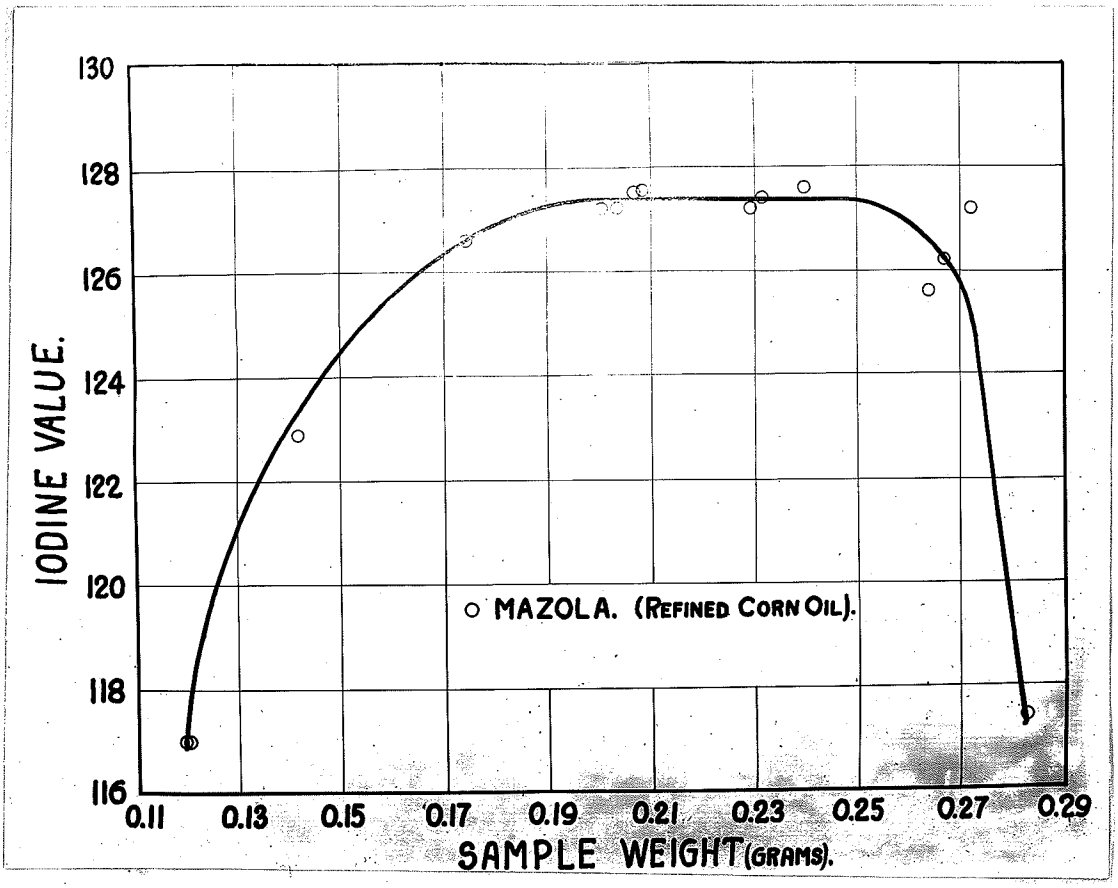
The necessity of varying sample weight in accordance with the iodine number of an oil is an established procedure. A set range of sample weight is usually specified, being for drying oils 0.1 to 0.2 grams, for semi-drying oils about 0.25 grams and for fats about 0.5 grams. In a private communication H. N. Brocklesby (3) drew attention to the marked variation in iodine number of highly unsaturated oils with change in sample weight. This variation is much greater than appears to be generally appreciated and promised to become a factor of considerable importance in this work, due to the fact that the sample weight could not be conveniently controlled within narrow limits. The method of plotting iodine numbers against sample weight was therefore tried. Extremely interesting results were obtained as illustrated in Figs. 6, 7, 8 and 9.

FIG. 6.



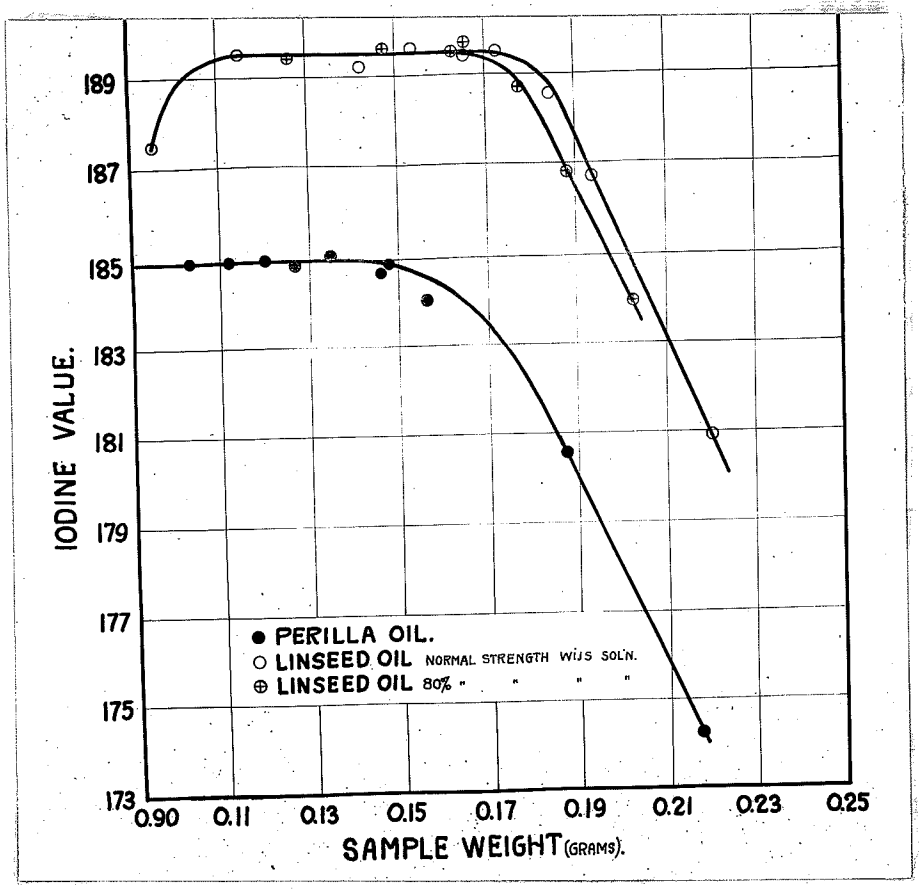
RELATION BETWEEN IODINE NUMBER AND SAMPLE WEIGHT.

FIG. 7.



RELATION BETWEEN IODINE NUMBER AND SAMPLE WEIGHT.

FIG. 8.



RELATION BETWEEN IODINE NUMBER AND SAMPLE WEIGHT.

From these graphs it is evident that there is a narrow range of sample weight in which the iodine number is constant and below or above this range varies abruptly. This range varies not only for different oils, but also for the same oil in different stages of oxidation and thus the usual method of assigning definite sample weights to broad groups of fats and oils cannot give reliable or consistent results. It would appear that the only method of securing accurate values for the iodine number of a given oil or fat is to make a series of determinations using varying sample weights, plotting the results as indicated and selecting the true values from the flat portion of the curve. Averaging the results of a number of determinations, particularly if the sample weights vary widely, is bound to give an erroneous figure, as it is inevitable that the lower values included will be out of the true range.

It will be noted that the flat portion of the curve exhibits a decided shift to the left with increasing degree of unsaturation. This is understandable since the greater the degree of unsaturation of the sample, the less will be required in order that the same excess of Wijs solution be present. The opinion is offered that a quantitative study of this phenomenon would be of interest since it is believed that it bears some interpretable relationship to the relative amounts of the different unsaturated acids present and also to their degree of oxidation.

STUDY OF TEMPERATURE EFFECT ON ACTION OF WIJS SOLUTION.

In a private communication H. N. Brocklesby (3) outlined a method for determining iodine numbers in which the reaction is allowed to proceed at a temperature considerably below normal room temperature. Since a search of the literature has furnished no evidence of any previous work on this, it has been thought advisable to find the effect of temperature on the action of Wijs solution.

A constant-temperature bath, capable of holding within $\pm 0.2^{\circ}$C., was employed for temperatures of 30°C. and 50°C. Since the heating bulbs of the bath emit a considerable quantity of light, it was thought necessary to provide for the absorption of the actinic rays which are known to effect the action of Wijs reagent. Benzopurpurin was found to suit the requirements since the solution in water exhibits high absorption in the blue and green portions of the spectrum and also because it forms a lake that has an additional screening effect. The bath was kept well covered to prevent the entry of external light. A bath of small ice chips at their melting point, i.e., 0°C., was employed to provide a low constant temperature without the use of a thermostatic regulator. This bath was well lagged and the ice supply replenished as occasion demanded.

Reagent bottles (250 ccs. capacity) were used rather than the conventional iodine number flasks since it was desired to make a simultaneous test of the electrometric titration method (q.v.), and also because they are more easily kept immersed. The bottles

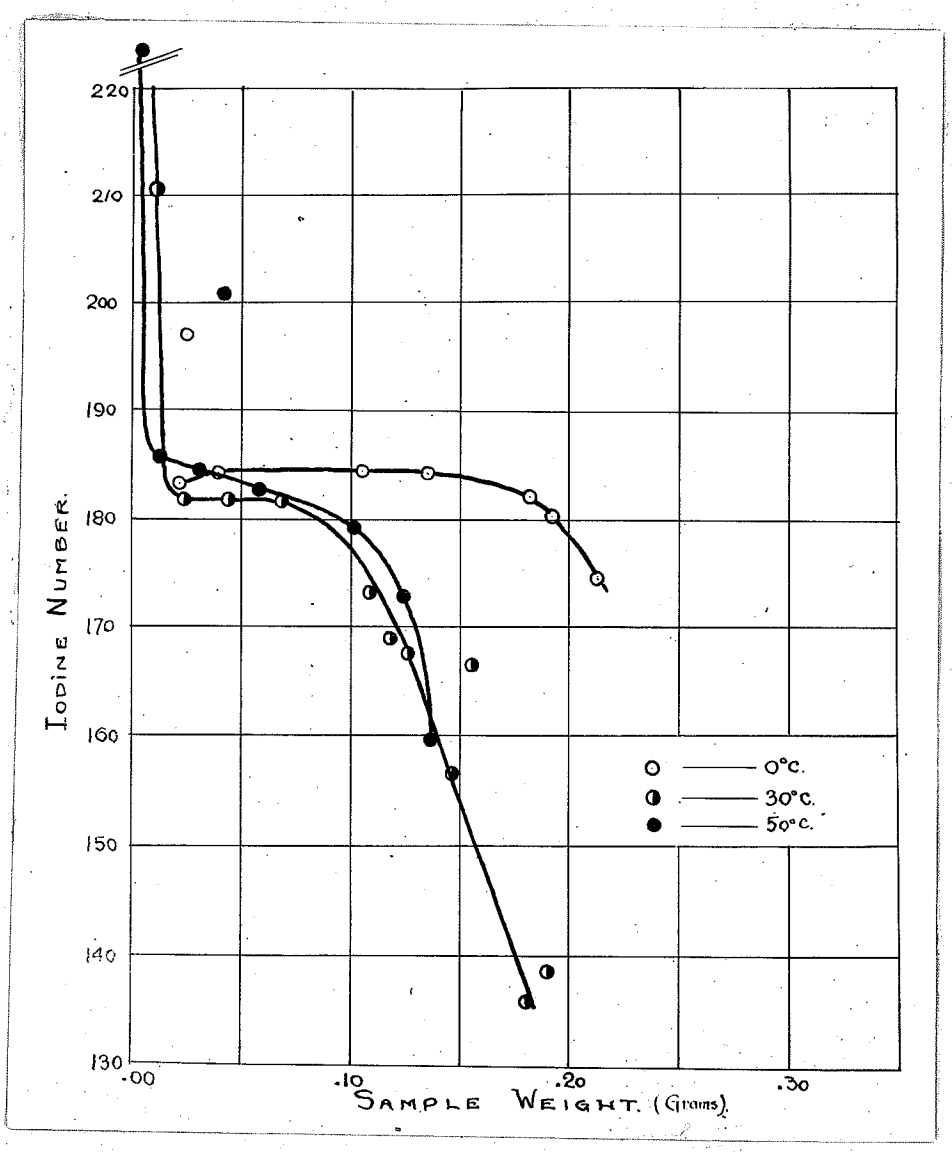
were supported in the bath on flat trays and kept in place by means of rings of perforated metal.

Since as little time lag as possible was desired the carbon tetrachloride was pipetted into the bottles about 15 minutes in advance and brought to the required temperature before the oil or the Wijs solution was added. It was considered neither advisable nor necessary to bring the Wijs solution to the temperature of the reaction. In any event the specific heat of Wijs solution is quite low - approximately 0.50 - so that the temperature lag would not be very marked.

At the conclusion of the thirty minute reaction period allowed the bottles were removed from the bath, the required amount of potassium iodide solution added and this immediately followed by 100 ccs. of N/20 sodium thiosulphate solution from a Bureau of Standards pipette. This precaution was particularly necessary in working at a temperature of 50°C. in order to prevent the loss of iodine as vapor. It is not necessary to follow this with any additional water. The excess of thiosulphate was then titrated back with N/20 iodine solution, using the electrometric method previously described. In this work the electrometric method yielded excellent results and was well worth the slight additional time required for the adjustments.

The results of a series of determinations using linseed oil are best considered from an examination of the isothermals obtained on plotting sample weight against iodine number. The

Fig. 2.



EFFECT OF TEMPERATURE ON RELATION BETWEEN IODINE NUMBER & SAMPLE WEIGHT.

reaction at 0°C. would appear to consist of addition alone, no matter what the excess may be. In fact, as was also found in Figs. 6, 7 and 8, too great an excess seems to inhibit even addition. Working at 30°C. there is a definite change in the direction of the curve indicating a region of sample weight in which addition is marked, and this is followed abruptly by substitution reactions on the addition of a larger excess of reagent. At 50°C. the range for addition is very limited and indefinite, grading into simultaneous addition and substitution.

While a difference in temperature causes only a slight difference in the final result, it has a marked effect on the excess of Wijs solution required. From the standpoint that a wider range of sample weight is desirable, working at low temperatures is therefore to be preferred.

CONCLUSIONS.

1. An apparatus has been constructed and a technique devised for the preparation of fatty acids susceptible to oxidation, the entire series of operations being performed in an inert atmosphere.

2. Linolenic acid of high purity has been prepared through the use of this apparatus.

3. A study has been made of the technique of iodine number determination and improvements have been suggested. In this connection a new pipette for the uniform delivery of Wijs solution has been constructed.

4. Electrometric titration methods have been applied to iodine number determinations.

5. The effect of sample weight on iodine number has been studied and found to be of more importance than generally considered. A graphical method of interpreting iodine numbers has been adopted.

6. The effect of temperature on the relationship between iodine number and sample weight has been studied. It has been found that the lower the temperature at which the reaction proceeds the greater the latitude in sample weight allowable. At low temperatures substitution of halogen would appear to be inhibited and, though only in the presence of a large excess of halogenizing agent, addition as well.

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