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A study of the Kjeldahl method and a modification for the determination of nitrogen in alcoholic distillates.

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A STUDY OF THE KJELDAHL METHOD AND A MODIFICATION FOR THE DETERMINATION OF NITROGEN IN ALCOHOLIC DISTILLATES

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INTRODUCTION AND HISTORY

Introduction and History

For a long while after its introduction the Kjeldahl method for the determination of nitrogen was almost exclusively used in clinical analysis on such samples as blood, urine, various body fluids, etc. More recently, however, it has found its way into industry, particularly in agricultural research, and it is becoming one of the routine procedures in the industrial laboratory. It was used in agricultural chemistry to determine the nitrogen, mainly protein in nature, in cereal grains and today modifications of the Kjeldahl method have been standardized and placed among the official agricultural tests by the United States Department of Agriculture. Coleman, Fellows, and Dixon (1)* of this department, made a study of various modifications of the Kjeldahl method to determine the uniformity of results obtained from each by various workers. They obtained a uniform grain sample and sent it to forty-five different laboratories, made up of the following four types: (a) research or experimental, (b) mill, (c) elevator and (d) commercial. The size of the samples, after being ground, varied at the different laboratories from six to sixty grams. The quantity of acid used in the digestion mixture varied from fifteen to thirty cubic centimeters. The catalytic agents were numerous, e.g., sodium sulfate, potassium sulfate, metallic mercury, yellow oxide

*These numbers, throughout the thesis, correspond to the references on pages 37 to 39.

of mercury, mercuric sulfate, metallic copper, copper sulfate, etc., each of the laboratories using various amounts of the catalytic agent. Both gas and electricity were used as heat source and digestion time varied from twenty-five to one hundred eighty minutes. Three receiving acids were used: sulfuric, hydrochloric and boric acids. The strength of the two former acids varied and the usual 4% boric acid was used. The authors found that many methods of analysis were used but the chief ones were: (a) Kjeldahl or some modification, (b) Gunning, (c) and a method devised by the Kansas City Referee Board. The majority of the workers used the Gunning method in that it is more economical than the others. From the results of the study, it was found that the maximum difference with any one sample was 1.22% and the minimum difference was 0.58%.

The diverse methods which have evolved from the original Kjeldahl procedure are many and wide in scope. The foregoing study shows this to some extent, but it also shows that even with all manner of working conditions and manipulations, the results obtained are quite concordant. The method of Kjeldahl was made public information for the first time in a lecture delivered to the Chemical Society of Copenhagen on March 7, 1883 (2). Despite the fact that the Kjeldahl method is usually classified as the first of its kind, there were others which preceded the Kjeldahl by a number of years. These methods I would like to review at this point.

In 1841, a method discussed and described by Oesper (3), was suggested by Will-Varrentrop but it has now practically passed into disuse. The method is as follows: The sample together with soda lime is placed in a tube drawn out to a point at one end, the other end being joined to a receiver charged with acid. The contents of the tube are carefully heated, the nitrogen is evolved as ammonia (or related bases) and after the reaction is over, the tip of the tube is broken off and the residual gases are drawn through the acid by aspiration. This method of Will-Varrentrop falls into the class of procedures known as dry methods. Originally the procedure was completed by determining the ammonia produced as chloroplatinate or platinum, but with the development of volumetric analysis it was found more convenient to absorb the ammonia in standard acid. Many criticisms and modifications of the Will-Varrentrop method were offered but few were of any value. The method, however, was an important step in the development of organic chemistry, even though it was abandoned when a simpler, quicker, more convenient and less expensive method was devised. It served its purpose well and gave good results when skillfully handled.

The Dumas procedure, discussed by Oesper (3), devised in 1831, is doubtless the most reliable and accurate of all present day procedures. It requires, however, careful handling and the analyst must be aware of the errors attendant in gasometric nitrogen determination. Even though the Dumas method preceded that of Will-Varrentrop by ten years, the latter was more generally used because of the uncertainty of

performance of the former.

A few problems that had to be solved before the Dumas method could be successfully employed were the discovery of:

(a) tractable sources of carbon dioxide,

(b) method of completely removing the air from the combustion tube,

(c) sure means of deoxidizing nitrogen oxides,

(d) impeccable reducing agents for the copper spiral, since hydrogen was found to be absorbed and later released,

(e) simple yet trustworthy azotometers. (3)

All these things have not yet been perfected but the Dumas method yields excellent results.

No matter what improvements and modifications are offered the Dumas and Will-Varrentrop methods remain expensive, tedious and difficult, unsuited to large scale routine demands. Samples, to be best analyzed, must be finely divided or they cannot be properly mixed, as is necessary in dry methods with soda lime, copper oxide, etc., and analysis of liquids, also, presents difficulties. Workers searched constantly to find wet methods to substitute for dry ones. If wet methods were devised, it would obviate the constant supervision, necessary in heating dry samples. Most of the methods suggested were so limited in scope that for general use they were valueless. The Will-Varrentrop method was satisfactory only with compounds which were direct derivatives of ammonia,

but it did point to the right direction since mere oxidation of samples gave indifferent success. It was concluded that complete ammonification can be brought about only after destroying the organic matter. Alkali permanganate gives incomplete decomposition but, nevertheless, this formed the basis of Wanklyn's method (1877) of determining the protein content of vegetable material. In 1878 Grete (4) used boiling sulfuric acid in the analysis of agricultural samples and stated that the nitrogen was in the form of ammonium sulfate. In 1883 Dreyfuss used concentrated sulfuric acid in the initial treatment of wool, horn, leather, and fertilizers to put these resistant substances in a form which the soda lime could attack. Then the Will-Varrentrop procedure was used.

Kjeldahl knew that the simple apparatus and easy technique of the Will-Varrentrop method were suited to his needs (he was doing research on grains and required a method for nitrogen determination) so he set about to modify the procedure. He first used dilute sulfuric acid and an excess of permanganate; then the ammonia was liberated by alkali and distilled out. There was much fluctuation in his results due to incomplete conversion of the nitrogenous bodies by dilute acid. After much effort and laborious research, he found that better results could be obtained on his materials by boiling with concentrated sulfuric acid until the solution cleared and the nitrogen existed as ammonium sulfate or in such form that oxidation

by the powdered permanganate completed the ammonification. The original Kjeldahl procedure required the following (4):

.2 to .7 gram sample

10 cubic centimeters of concentrated sulfuric plus fuming sulfuric acid or phosphorus anhydride

powdered potassium permanganate (Czeczetka in 1886 and Malfatti in 1903 used permanganate in solution since it produced, when powdered, such a violent reaction.)

titration by iodometric method

After the method was ready for inspection, it was tested, primarily in Germany, and the verdict was favorable.

Thereafter, the Kjeldahl procedure gained widespread notice and was modified in some of the following ways: (3)

(a) the use of mercuric and cupric compounds, etc., as accelerators in the digestion. In 1885 the use of accelerators was first suggested by Wilfarth (4).

(b) the inclusion of potassium sulfate in the digestion mixtures by Gunning in 1889. The potassium sulfate elevates temperature and increases reaction speed.

(c) in 1884, multiple digestion and distillation equipment were designed by Heffter, Hollrung, and Morgan.

(d) Various still heads or splash bulbs, among the first was that constructed by Reitmair and Stutzer in 1885. (Kjeldahl, himself, suggested a method for clearing the ammonia vapors of sodium hydroxide.)

(e) Modification of the method to apply to compounds requiring treatment before destructive action of the acid is effected.

Although Kjeldahl realized that various compounds such as nitrates, alkaloids, nitro bodies, etc., would not

respond to his method, he found that some of the non-amide nitrogen is converted into ammonia by sulfuric acid. For example, a mixture of potassium nitrate and sugar gave a sixty to eighty percent yield of ammonia (3). On the basis of this fact, Asboth (4) in 1886 found that by adding cane sugar to some of these resistant compounds fairly good results were obtained. He also added benzoic acid and was able to analyze nitrates. Jodlbauer in the same year substituted phenol, which is more easily nitrated, for the benzoic acid and the method was greatly improved and the manipulation, more successful. On pages 19 and 20 are the tables (4) which give the main modifications emerging from the original Kjeldahl method. There are two forms of procedure:

(a) oxidation

(b) reduction (for compounds such as nitrates, nitrites, nitro, etc.)

Methods of reduction are used when the compounds are attacked with difficulty, e. g. oxygenated compounds. In 1894 Deniges wrote that the oxidizing agents gave less favorable results than the reducing agents. Villiers and Moreau-Talon, in 1918, believed that the violent oxidation destroyed some of the ammonia formed and they stated that the use of oxidants must be limited to the end of the oxidation. In an article, Metrezat states that the oxidants have practically fallen into disuse since 1903.

In 1908, the Association of Official Agricultural

Chemists (5) adopted officially, a modification of the Gunning method for the determination of nitrogen which prescribed the use of .1 to .3 grams of crystalline copper sulfate in addition to potassium sulfate. (In place of potassium sulfate, Latshaw (6) proposed the use of sodium sulfate on the grounds that it is less expensive. In 1918, Dowell and Friedeman (7) wrote that either anhydrous or hydrated sodium sulfate can be used, since the time of clearing is not appreciably affected by water of crystallization.) The agricultural method was not generally used, however.

Later another modification known as the Kjeldahl-Gunning-Arnold method appeared. This method differs from the Gunning method, only in the use of metallic mercury instead of copper sulfate. Trescott (8) made a comparison of the Gunning and Kjeldahl-Gunning-Arnold methods and concluded that the latter gives more concordant and reliable estimations than do official Gunning or Kjeldahl methods. He, also, found that with the Kjeldahl-Gunning-Arnold method, the digestion time was decreased by half. Jensen (5) substantiates the results of Trescott but prefers the Gunning method because of advantages in manipulation. Pickel (9), also, established the fact that considerably less time was required in digestion with the Kjeldahl-Gunning-Arnold procedure. He declared that thirty minutes of digestion yielded excellent results.

Blumenthal and Plaisance (10), however, maintained that thirty minutes were not sufficient to fix all the nitrogen. They ascertained that after longer digestion a gain of 3% in protein was apparent.

Gerritz and St. John (11) made a study of various rapid methods of digestion since the Kjeldahl-Gunning-Arnold method, even though it gives good results, requires a long period of digestion, especially if adequate heat is not provided. Some of the following methods were cited by these authors: Their own method was to change potassium phosphate or equivalent reagents in part for sodium sulfate. Mears and Hussey and Bimmerman and Frank used perchloric acid to accelerate Kjeldahl digestion. Kleeman and Huess found the period of digestion shortened when 30% hydrogen peroxide was added. Sborowsky and Sborowsky and Richards, independently, reported more rapid digestion by substitution of mercurous iodide for mercuric oxide. Hassig, however, critized the use of the iodide because it sublimes on the neck of the flask. Parri found the use of vanadium pentoxide and cupric oxide, together, produced a better digestion accelerator than either of the two when used alone. Lepper used copper sulfate, potassium sulfate, and sulfuric acid with shortened digestion time.

Much discussion has arisen in the past few years on the subject of catalysis by selenium and some of its compounds. Lauro (12) proposed the use of selenium in the

Kjeldahl reaction. He obtained digestion of one gram cereal samples in twelve to fifty-five minutes. The subject engaged the attention of cereal chemists and a number of papers have appeared supporting his contention. Sandstedt (13) and Rich (14) reported rapid digestion with the aid of selenium, catalyzing the reaction with mercury and selenium together and using electric heat. Messman (15) obtained digestion in twenty minutes. Osborn and Krasnitz (16) preferred the use of selenium coupled with mercury. Snider and Coleman show that, though lower temperatures prevail in the digest containing selenium, the "critical point" occurs sooner, accompanied by a rise in temperature. They maintain that mercury and selenium combined are unsatisfactory as catalysts; mossy zinc in combination with selenium causes frothing and may give rise to noxious fumes (selenium selenide) creating a danger to health.

Recently, more critical papers have appeared, chief among which is one by Davis and Wise (17) who state that indications favor a lower result with its use and that the use of selenium combined with mercury should be discouraged. Nevertheless, they admit, it may prove satisfactory in the hands of some. Snider and Coleman (18) found that selenium as a catalyst gave low results and although the solution cleared rapidly it did not necessarily indicate completed digestion. However, Wieninger (19) obviates the use of mercuric sulfate in conjunction with selenium without any loss of time by employing a mixture of anhydrous

sodium sulfate, anhydrous copper sulfate, and metallic selenium. His work was done on barley, malt, wort, and beer. The fact is that all evidence seems to point to the mercury-selenium combination as inexpedient but that copper and selenium form a desirable catalyst. Schwoegler, Babler, and Hurd (20) inferred, that since the latter was so satisfactory, a single compound instead of two substances would simplify the procedure. They suggested copper selenite dihydrate ($CuSeO_3 \cdot 2H_2O$) which is easily prepared, does not deteriorate or alter upon standing and is soluble in sulfuric acid. I used this catalyst in my research plus potassium persulfate; the latter I discarded later. For my purpose the copper selenite has proven very successful.

Davisson and Parsons (21), in an article, describe the Gunning-Jodlbauer, the Forster, and the Ulsch modifications of the Kjeldahl method. The Gunning-Jodlbauer modification entails the addition of salicylic acid to the sulfuric acid plus zinc dust and potassium sulfate to convert nitrates to ammonia. The original Kjeldahl method failed in this respect. Forster used sodium thiosulfate as a reducing agent in the above when complete recovery of nitric nitrogen was not effected. The Ulsch modification consists of the reduction of nitric nitrogen by hydrogen, evolved from the action of sulfuric acid and reduced iron. Winston (21) used the Gunning-Jodlbauer modification and found more consistant results were ob-

tained when the mixture of acids and sample was allowed to stand two hours previous to adding the zinc dust. The mixture was then digested for two hours and the digestion completed after adding potassium sulfate. Sherman (21) tested Winston's modification and concluded that it gave better results than either of the other procedures. Duggar and Davis (21) employed Forster's modification of determining total nitrogen in studies of nitrogen fixation. They found it necessary to use three grams of sodium thiosulfate and to allow from ten to fifteen minutes in order to obtain complete recovery of the nitrogen.

As I have said, numerous attempts have been made to shorten the Kjeldahl method because it involves such a great expenditure of time. In 1885, Wilfarth, mentioned herein before, initiated the application of catalysts to the method by using copper oxide and mercuric oxide. The next important modification, suggested by Gunning (1889), was the addition of potassium or sodium sulfate and later, in 1919, the introduction of sulfuric and phosphoric acids and copper sulfate with the addition of ten percent ferric chloride solution to hasten digestion by Folin and Wright (22). In 1935, Lundin, Ellburg, and Riehm (23) published a method utilizing the acid mixture of Folin and Wright, i. e., three parts of sulfuric and two parts of phosphoric acids for decomposition of resistant substances in thirty

minutes or less. Copper, mercuric sulfate, and hydrogen peroxide were added to facilitate complete destruction of the organic matter. Still more recently the combination of acid mixtures and potassium persulfate technique in 1926 by Van Slyke (24), as well as the foregoing methods mentioned, have contributed materially to shortening the length of time necessary for digestion. After experimenting with the persulfate, suggested by Van Slyke, San Yin Wong (25) recommended this material, if used with special precautions, especially in the direct nesslerization method (to be discussed later). In the first place, upon adding the persulfate late in the digestion when no moisture is present, it is decomposed. On the other hand, when added very early in the digestion, it is no better than the plain sulfate recommended by Gunning.

When one compares the original Kjeldahl procedure with present day schemes of analysis one finds the latter vary only in such details as the use of the same flask for distillation and digestion, a safer method for adding the alkali in liberating the ammonia, the use of paraffin and zinc to prevent foaming, the use of varied accelerators with the omission of the permanganate and last the use of back titration of the standard acids with alkali in place of the iodometric titration of Kjeldahl. In 1914, Winkler simplified the method by using boric acid to absorb the am-

monia distilled over. Some debate has arisen as to whether the method using boric acid compares favorably with the standard acid-base method or not. H. D. Spears (26) favors the former method and states that the results obtained from the use of boric acid are comparable to the ordinary procedure using sulfuric acid, with the added advantages that the boric acid may be measured only approximately and only one standard solution is needed, i. e., the sulfuric acid for titrating. Any number of workers have found the results obtained from the utilization of boric acid solution very gratifying. Spears suggests the use of bromophenol as the indicator in titration. A wide range of indicators have been used in the Kjeldahl titration. The use of several blue and greenish blue dyes in conjunction with methyl red or sodium alizarin sulfonate by Johnson and Green (27), effects an increase in the sensitivity with which the endpoint can be determined. Mestrezat (4) recommended the use of sodium alizarin sulfonate as early as 1918. Taylor (28) favors titrating two hundredths normal hydrochloric acid and sodium hydroxide with Tashirio's indicator instead of methyl red alone, because the endpoint of the former indicator is sharper. This indicator is made up of methylene blue and methyl red in such proportions that the endpoint is a lavender or pink color with acid, changing to a green or yellowish green on the addition of the alkaline solution.

The last group of modifications that I shall discuss are not so closely related to the original Kjeldahl procedure; but, nevertheless, they were made possible by and are derivatives of the latter. They are the colorimetric methods which vary from the Kjeldahl in that the nitrogen is determined in the final analysis by color comparison rather than titration. In the last decade or two, colorimeters have come into general use and their value perceived. The same can be said of the spectrophotometer. Folin and Denis (29) began investigation of a colorimetric method for the determination of nitrogen. Their digestion mixture consisted of sulfuric acid, phosphoric acid and copper sulfate. The Duboscq colorimeter, Nessler's reagent and standard ammonium sulfate were the remaining materials. In 1912, Folin and Farmer introduced a colorimetric method for the determination of total nitrogen in urine. Gulick (30) discusses their method as follows: The sample is digested with acids and the ammonia is freed by aeration. The most troublesome part of this procedure is the quantitative aspiration of the ammonia out of the original mixture into a new solution. Folin regretted that he had to employ this operation but did not find it possible to avoid the use of it. When the ammonia was nesslerized in the oxidation mixture, the colored solution was very apt to be turbid and unfit for colorimetric determination. However, Gulick (30) improved upon this method by avoiding the necessity of as-

piring the products of oxidation, thus, abbreviating the method considerably. The method used is as follows: The sample is oxidized with acid and potassium sulfate, then diluted, nesslerized and the amount of nitrogen determined colorimetrically. Bock and Benedict (31) stated that there are many sources of error in the Folin-Farmer method. The method has been shown by experimentation as usually agreeing with the Kjeldahl method within two or three percent. The authors say that the Folin-Farmer method is not to be regarded as equivalent to the ordinary Kjeldahl procedure in accuracy or reliability. Harding and Warneford (32) comment, also, on the errors in the Folin-Farmer method and the amount of divergence in the results.

The destruction of the carbonaceous matter is much the same in the Folin-Farmer method as in modified Kjeldahl methods. After the ammonia has been aspirated over into a little acid, it is estimated colorimetrically. Nessler's solution, which is the chief reagent used in the estimation of nitrogen by colorimetry, is made up as follows: (33)

(a) 300 cubic centimeters double iodide (75 grams potassium iodide in 50 cubic centimeters of water plus 100 grams of mercuric iodide)

(b) 200 cubic centimeters of 10% sodium hydroxide

(c) 500 cubic centimeters of water

In this reaction, a strongly colored reddish or orange brown solution is produced by the action of ammonia upon

mercuric potassium iodide in alkaline solution. The color produced is compared with a standard solution of ammonium sulfate which has been nesslerized. Modifications of Nessler's solution have been offered, among which are those of Winkler (34) and Folin-Wu (22).

It seems that the gravest difficulty in the determination of nitrogen colorimetrically, is the production in some cases of a turbid solution which makes color comparison impossible. Hubbard (35) used Rochelle salt to prevent precipitation of the Nessler reagent. Oftentimes the solution contains particles of silica which separate from the glassware during digestion when phosphoric acid is used. Koch conquered this difficulty by using hydrogen peroxide and Wong did likewise, using potassium persulfate (36). Both of these men employed sulfuric acid alone and avoided the danger of attack on the glassware. Looney and Folin (36) found that gum ghatti, reported by Folin in 1929, could be used as a satisfactory protective agent in the direct nesslerization of ammoniacal solutions. Daly (36) advises the use of sodium citrate and gum ghatti to prevent precipitation of the color reagent when solutions, turbid from tungstic acid, are nesslerized. Doneen (37) used gun ghatti, as a protective colloid, in the analysis of wheat for nitrogen estimation. In 1928, Chiles (38), also, cites a protective colloid prepared from gum arabic. The limit of accuracy for colorimetric determination of nitrogen, accord-

ing to Chiles, appears to be the accuracy of the colorimetric readings. Since the colorimetric method is subject to such personal errors, Allen and Davisson (34) prefer the ordinary titrimetric method. This, I believe, is quite true since, whenever comparison of one substance with another to establish the amount of an unknown quantity is involved, it must, of necessity, be more subjective than other analytical forms of procedure and the results, in turn, will vary with the subjective factor. Bock and Benedict (31) do not consider the colorimetric method on a par with the standard Kjeldahl procedure in accuracy and reliability.

From the foregoing discussion, one becomes aware of the important role played by the Kjeldahl method in analytical chemistry. Myriads of techniques for nitrogen determination have arisen from this source. I have chosen some of the modifications mentioned and applied them to the work which is described in the ensuing pages.

	H_2SO_4	Fuming H_2SO_4	P_2O_5	K_2SO_4	Cata-lysts	Oxida-tion Oxido-Cata-lysts	Reduc-tion Reducto-Cata-lysis	Nature of the Adju-vants
Brunnenmann and Seyfert (1320)	+	+	+					Destruction by Acid alone
Gunning (1889)	+			+				Elevation of temperature
Kjeldahl(1883)	+	+	(+)			$KMnO_4$		Oxydants
Kreusler (1884)	+		+⅕			$KMnO_4$		"
Wilfarth (1885)	+		+⅓			Diverse Oxides, HgO,CuO.		"
Dafert (1887)	+		+			$KMnO_4$		"
Martinotti(1889)	+		+			MgO $KMnO_4$		"
Kruger (1894)	+					$K_2Cr_2O_7$		"
Malfatti (1903)	+					Dissolved $KMnO_4$		"
Milbauer (1903)	+					Persul-fate		"
Gunning-Koefoed (1910)	+			+		CuO		"
Hoppe-Seyler (-)	+			+	$CuSO_4$			Catalysers
Sislig (1889)	+			+	$CuSO_4$			"
Koefoed (1910)	+				$CuSO_4$			"
Walter Jones (1910)	+			+	$CuSO_4$			"
Rona and Ottenburg (1910)	+				$PtCl_4$			"
Dakin and Dudley (1917)	+				Cu SO_4			"
Folin (1919)	+			+	$CuSO_4$			"
Arnold and Wedemeyer (1892)	+			+	$CuSO_4$	HgO		Oxido-Catalysts
Ulsch (1892)	+		+⅕		$PtCl_4$ $CuSO_4$	CuO $KMnO_4$		"
Kutscher and Stendel (1903)	+				$CuSO_4$	$KMnO_4$		"
Sorensen and Pedersen (1903)	+				$CuSO_4$	$KMnO_4$		"
Margoscher and Lang (1915)	+				Tung-sten	HgO		"
Kulisch (1886)	+	+	+⅕				Hg	Reducto-Catalysts
Arnold (1892)	+		+⅕			CuO	Hg	" "
Bottscher (1892)	+		+⅕				Hg	" "
Wedemeyer (1898)	+		+	+			Hg	" "
Argutinsky(1900)		+					Hg	" "
Berger Fengerling & Morgan	+		+				Hg	" "
Villiers & Moreantalon	+			+			Hg	" "

METHODS BY REDUCTION

A - Two Successive Operations

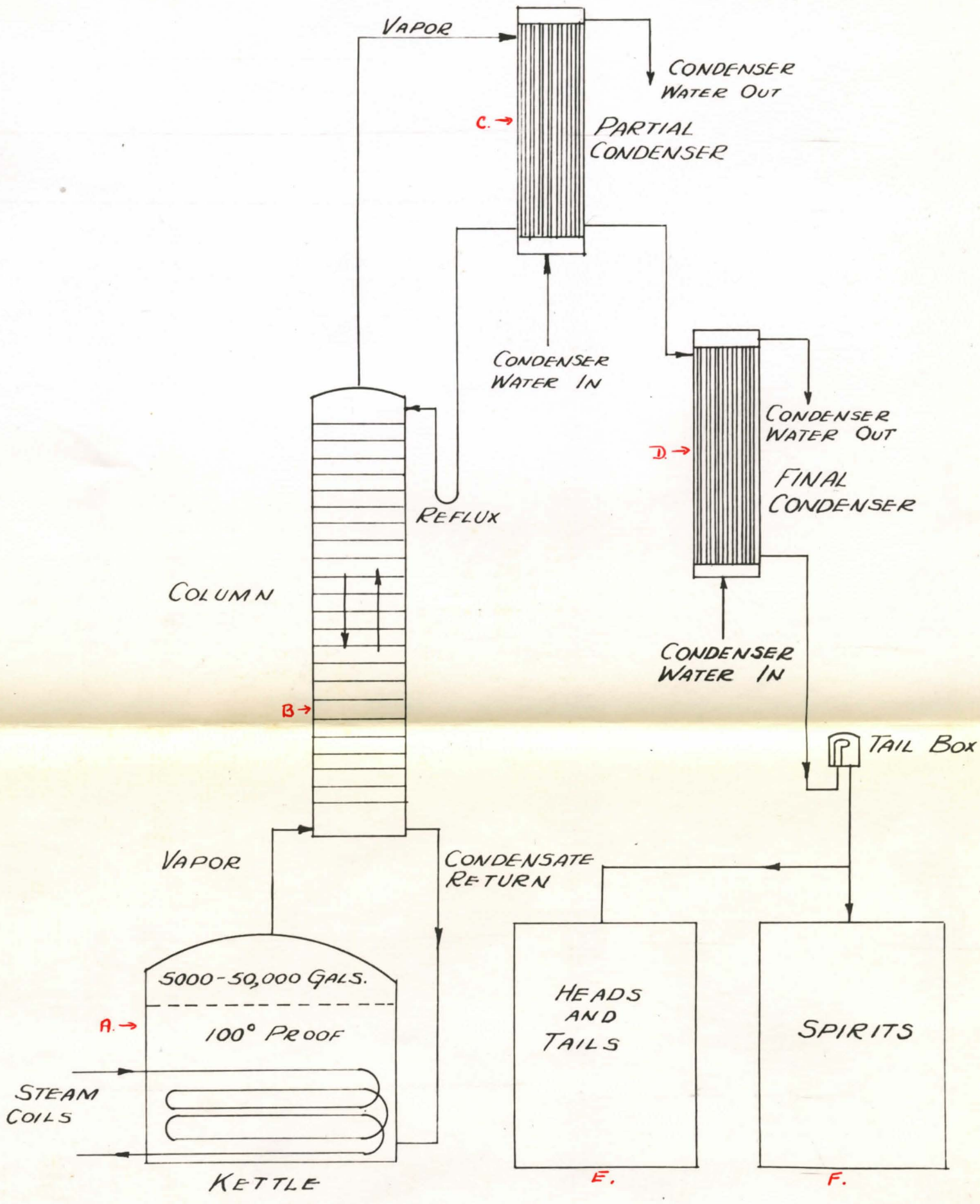
	1st Part		2nd Part
Jodlbauer (1886)	(H_2SO_4) (C_6H_5OH)	Zn	$PtCl_4$
Dafert (1887)	H_2SO_4	Zn	Hg
Jodlbauer-Martinatti (1889)	(H_2SO_4) (P_2O_5) (C_6H_5OH)	Sn	(H_2SO_4) $(K_2Cr_2O_7)$
Krieger (1894)	(H_2O) (HCl)	Sn	(H_2SO_4) $(K_2Cr_2O_7)$
Milbauer (1903)	(H_2O) (H_2SO_4)	Zn	Hg K Persulfate
Falmond & Prayer	(C_2H_5OH) (H Cl)	Zn	(H_2SO_4) $(Cu\ SO_4)$ (K_2SO_4)

B - A Single Operation

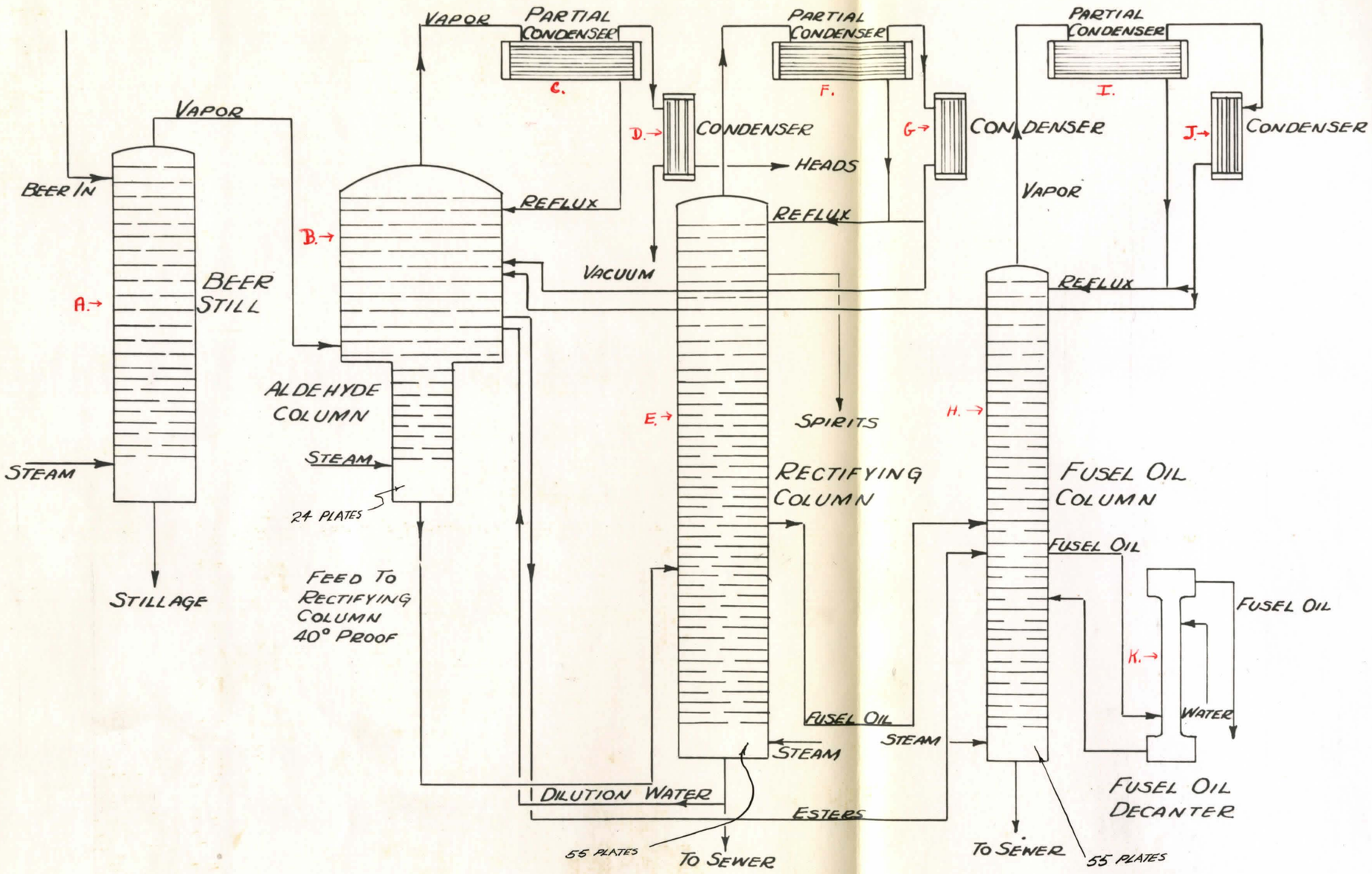
Asboth (1886)	H_2SO_4 C_6H_5OH		Cane Sugar
Foerster (1889)	H_2SO_4 C_6H_5OH	Sodium Hg	Hyposulfite
Foerster-Arnold & Wedemeyer (1892)	H_2SO_4 C_6H_5OH	Sodium $Cu\ SO_4$	Hyposulfite HgO
Arnold & Wedemeyer (1892)	C_6 1. Co_2H 2. OH		H_2SO_4 or $C_6H_5Co_2H$ with or without K_2SO_4
Deniges (1894)	H_2SO K Oxalate		

DISCUSSION OF THE MANUFACTURE OF VARIOUS SAMPLES

ATMOSPHERIC BATCH SPIRITS STILL



VACUUM CONTINUOUS SPIRITS STILL



Discussion of the Manufacture of Various Samples

Two main classes of samples were used in the research. On the one hand, those samples derived from vacuum* distillation and on the other hand, samples which were produced from a batch atmospheric still. The equipment required in the manufacture of the various samples handled is sketched on pages 21 and 22.

I will endeavor now to elucidate the prints by discussion. The batch atmospheric distillation equipment is pictured on page 21. The kettle A is heated by a system of steam coils and contains a mixture of 100 proof. From the top of the kettle A, which contains from 5,000 to 50,000 gallons of liquid, the vapor passes into column B; this part of the procedure is called rectification, i.e., increasing the proof of the alcohol. Column B is made up of a number of perforated copper plates (fifty to fifty-five). The three top plates have bell-shaped caps over the perforations. The alcoholic vapors pass into a partial condenser C, which is water cooled. The very low proof liquid runs back into kettle A. From partial condenser C, a portion of the alcohol (that of high proof) is stripped out and flows into condenser D. That part of the distillate which does not distill from partial condenser C into final condenser D, goes back into column B at the top. The distillate from final condenser D is collected into tanks E

* low temperature

and F; the low boilers are received in tank E as heads and the high boilers, as tails. The rest of the distillate is considered the spirits (approximately 192 proof) and these are collected in tank F. The heads contain the aldehydes and esters and the tails are mainly fusel oil.

The second type distillation is the vacuum* or continous still, pictured on page 22. The beer still A is composed of perforated copper plates, eighteen to twenty-five in number. The beer, which contains grain, water, alcohol (190 proof or higher), aldehydes, esters and fusel oil (consisting of the higher alcohols from iso-propyl through amyl), flows into the column near the top and steam is passed in at the bottom. As the steam rises and the beer forms layers on the perforated plates, the alcohol is stripped out and the vapor proceeds at 120 to 140 proof into aldehyde column B. The grain and some water from the beer come off at the bottom of column A as stillage. Fusel oil, the high boiling esters and 40 proof alcohol leave at the bottom of the aldehyde column and feed into the rectifying column E, which has between fifty and fifty-five copper plates. The aldehydes and esters form a layer at the top of the aldehyde column B and distill into partial condenser C and are either refluxed back into the aldehyde column or pass into final condenser D and are drawn off as heads. A vacuum is set up on condenser D. Steam is introduced into the rectifying column E at the bottom and the vapors are carried over into a partial condenser F; part

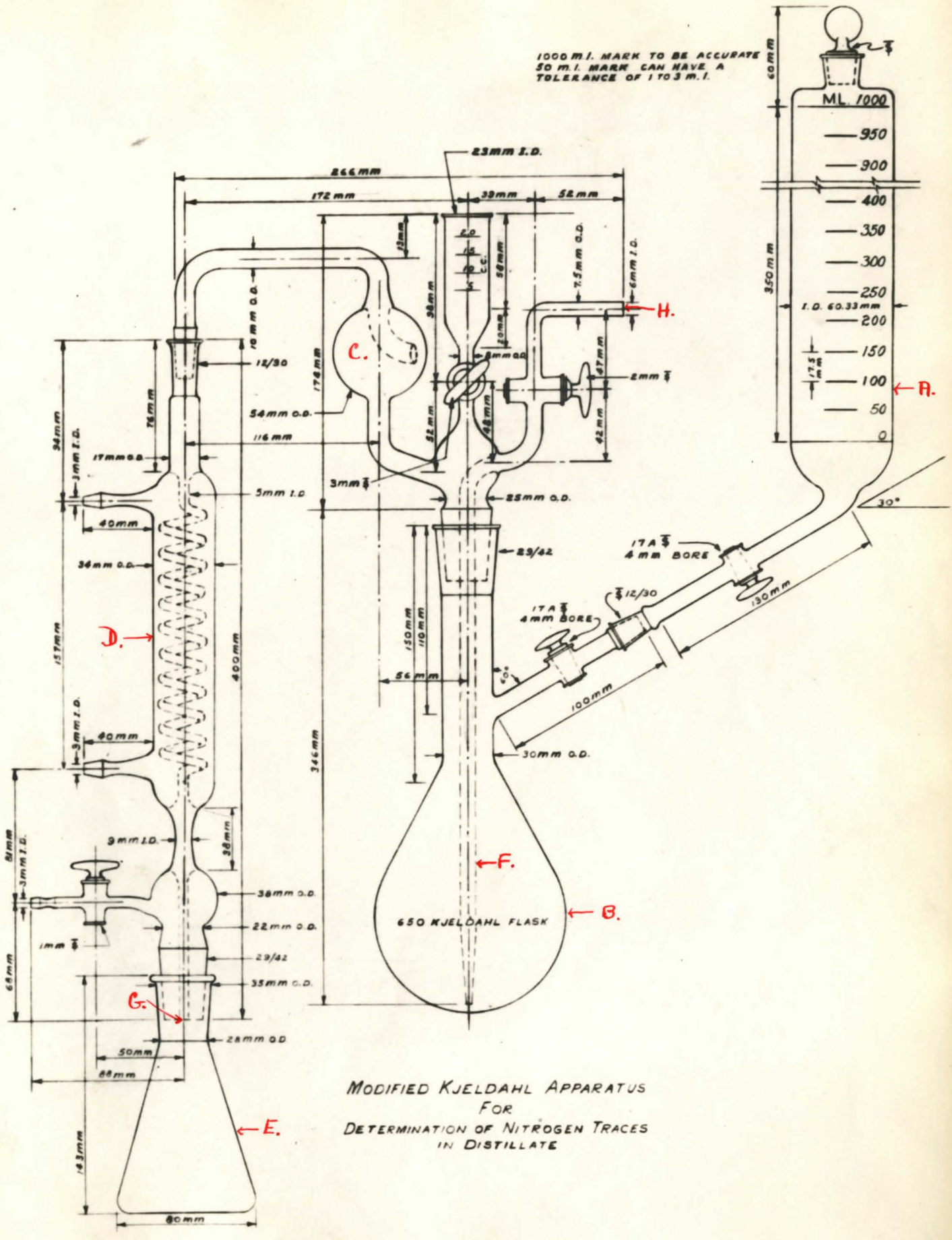
* low temperature distillation

of the distillate refluxes back into column E and the rest passes into a condenser G which returns the alcohol to the aldehyde column B. Spirits of 192 proof are finally drawn off from the rectifying column E and the fusel oil flows into tank H, called the fusel oil column. As with the vapors from the rectifying column E, the same refluxing occurs with those from the fusel oil column H. They pass into a partial condenser I; thence into final condenser J and on into the aldehyde column B again. The fusel oil is collected from the fusel oil column into the fusel oil decanter K; the residue from the fusel oil column H goes to the sewer.

The samples, labelled Gin A and Gin B in the table on page 35, are, respectively, vacuum* and atmospherically distilled.

* low temperature distillation

MODIFIED KJELDAHL APPARATUS USED IN THE NITROGEN DETERMINATION OF ALCOHOLIC DISTILLATES



MODIFIED KJELDAHL APPARATUS
FOR
DETERMINATION OF NITROGEN TRACES
IN DISTILLATE

Modified Kjeldahl Apparatus Used in the Nitrogen Determination of Alcoholic Distillates

The apparatus used in the determination of nitrogen in spirits and other alcoholic distillates is shown with the various parts fitted together and with the measurements on each part, on page 26. This apparatus grew out of a suggestion offered by Engineer Josef Stastny (39), Director of Research, Distilling Institute of Prague, in a personal communication with Dr. Paul Kolachov, Director of Research, of Joseph E. Seagram and Sons, Incorporated, of Louisville, Kentucky and modified by myself.

The apparatus does not vary greatly from the conventional Kjeldahl apparatus currently used. It consists of a modified Kjeldahl flask (labelled B on the print on page 26) equipped with a side arm and stopcock. To this side arm is connected, by means of a ground glass joint, a burette type cylinder, calibrated to a thousand cubic centimeters at fifty cubic centimeter intervals. The burette, labelled A, contains, like the flask, an arm furnished with a stopcock. This double stopcock allows easier manipulation and more adequate control when the sample addition is made. The opening at the top of the burette is fitted with a ground glass stopper, which is left in place during all the distillation to prevent undue evaporation and to protect the sample from dust particles, etc.

A splash bulb (labelled C) is inserted into the flask B with a ground glass joint, directly above which is a calibrated cylinder, used when small samples are involved. A tube (labelled F) forms a continuous part of the splash bulb apparatus and extends down to the bottom of the flask. When the alkali is added to the diluted acid digest, a funnel is joined to this tube at point H with a rubber connection. The tube of the funnel is bent at right angles, allowing the alkali to flow gently into the flask. Condenser D is connected with the bulb by a ground glass joint. The former is a coiled glass type of condenser, found very efficient since a more extensive cooling surface and a more uniform flow of distillate is provided than in a longer condenser with the straight glass tube. At point G on the condenser, in that part of the procedure when the ammonia is distilled over, a piece of glass tubing, which extends down into the boric acid in the receiving flask, is added by a rubber connection. The receiving flasks used were the regular Erlenmeyer flasks of 250 cubic centimeter capacity. In that part of the technique, however, where the original sample is reduced to a small volume, an Erlenmeyer flask (labelled E) with a ground glass joint is employed and fits on condenser D.

The flame of the bunsen burner was subject to such variation in heat intensity by air draughts that it was necessary to use a small metal chimney. The cone-shaped

chimney screwed on the cylinder of the burner and a steady heat was obtained, when the flame was so protected. The chimney could be raised or lowered to accommodate any size flame.

PROCEDURE AND TECHNIQUE FOR DETERMINING NITROGEN IN SPIRITS

Procedure and Technique for Determining Nitrogen in Spirits

A sample of material from 200 to 2000 cubic centimeters is used in the analysis; when working with spirits and gin, a liter sample was utilized but only 200 cubic centimeters samples of whiskey could be employed because the congenerics become too concentrated and form a solid mass making the digestion of the mixture highly difficult. The samples were obtained as uniformly as possible to insure close results.

One hundred cubic centimeters of the spirits and thirty cubic centimeters of concentrated sulfuric acid are measured by means of a graduated cylinder into the special Kjeldahl flask, marked B in the diagram. A few grains of alundum are added to prevent bumping and heavy frothing. Alundum, a kind of abrasive grain, was found to be much more efficient than glass beads and less likely to be attacked by the concentrated alkali added. Glass beads are, also, ineffective with substances containing large quantities of alcohol.

Of the mixture in the flask, approximately 50 cubic centimeters are distilled into the receiving flask E. Then another 100 cubic centimeter addition of sample is made by measuring with the calibrated apparatus A. When the liter of sample has been exhausted the flask is disconnected and some of the remaining water boiled off to restore the con-

centration of the sulfuric acid in order to insure subsequently complete digestion of the organic matter. To the remaining residue (about 35 to 40 cubic centimeters in volume), a small pinch of copper selenite is introduced for catalysis. The digestion requires between fifteen and forty-five minutes, depending upon the nature of the sample and the efficiency of the combustion equipment.

After the digestion is completed, as is evidenced by the clearing of the liquid and the incipient formation of a salt, the digested mixture is allowed to cool and then is diluted with about 200 cubic centimeters of nitrogen-free distilled water. The nitrogen-free water is prepared by introducing into distilled water, free from carbon dioxide, permutit, a Folin reagent obtained from the Sargent Company. The permutit removes the nitrogen from the water and after a day or two, settles to the bottom of the water bottle. The water flask must be shaken at intervals and the permutit allowed to settle again to insure that the water is always free from nitrogen. The permutit may be recovered, dried, and heated to expel the ammonia and reused.

After the digested mixture is diluted, the flask is replaced on the original distillation apparatus and a 50% solution of sodium hydroxide is added through a funnel, attached to tube F. The alkali forms a layer on the bottom. The flask is then gently agitated until the two layers are mixed and quickly attached to the apparatus, C, to prevent

loss of nitrogen, escaping as ammonia gas, when the sodium hydroxide neutralizes the acid producing heat and bubbling.

The ammonia is then distilled into 4% boric acid solution (10 to 15 cubic centimeters) until approximately half of the liquid of the flask has distilled over. The ammonia is then directly titrated with one hundredth normal sulfuric acid, using three drops each of tetrabromphenol blue and methyl red as a double indicator. The endpoint is determined as a blank, using the same amount of carbon dioxide and nitrogen-free water as distillate.

Calculation of Kjeldahl nitrogen:

The number of cubic centimeters of one hundredth normal sulfuric acid multiplied by .00014, equals the grams of Kjeldahl nitrogen per liter sample.

Principle of the Kjeldahl method:

The principle of this method is the conversion of the various nitrogenous bodies into ammonium sulfate by boiling with concentrated sulfuric acid, the subsequent decomposition of the ammonium sulfate by means of a fixed alkali, (sodium hydroxide) and the collection of the liberated ammonia in an acid (4% boric acid). Finally, the distillate is titrated with an acid of known strength, which the ammonia neutralizes.

DISCUSSION OF RESULTS AND CONCLUSIONS

Table of Results

Type of sample	Number of millegrams per liter at 100 proof		
Low Temperature Distilled A	I	II	Average
Sample I	.229	.229	.229
Sample II (Final Distillate)	.181	.181	.181
Sample III (Heads)	.084	.084	.084
High Temperature Distilled			
Sample I	.265	.265	.265
Sample II (Final Distillate)	.212	.212	.212
Sample III (Heads & Tails)	.965	.965	.965
Low Temperature Distilled B			
Sample I	.217	.217	.217
Sample II (Final Distillate)	.145	.145	.145
Sample III (Heads)	.077	.077	.077
Fresh Distillate of Whiskey (green whiskey)	.434	.434	.434
Blended Whiskey (37½%, 5 yrs old plus 62½% grain alcohol previously aged in reused cooperage)	4.521	4.521	4.521
Straight Whiskey (3 yrs old)	4.221	4.221	4.221
Gin A (Low Temperature Distillation)	.476	.476	.476
Gin B (High Temperature Distillation)	.672	.672	.672

Discussion and Conclusions

In adjusting the apparatus to meet the requirements for the determination of nitrogen in alcoholic distillates, the same sample was used throughout, namely, the sample of vacuum distilled spirits listed as vacuum*A in the table on page 33. Different types of flasks were considered and tried but the one shown in the print was found most effective in shape and size.

The sample after digestion was diluted to various amounts, and I found that if not sufficient water is added and if the mixture is not iced (merely as a precautionery measure), the loss of nitrogen through violent evolution of heat and bubbling when the acid and alkali were mixed was great. The distillation takes somewhat longer when a larger volume of water is added but the loss of nitrogen is negligible and it affords greater accuracy.

At the incipiency of the experimentation, the most pronounced difficulty encountered was the sucking back of the boric acid into the condenser in the very early stages of distillation due to the fact that the flame was at the time subject to air draughts and the level of the liquid in the receiving flask and the distilling flask were too nearly the same. When the chimney, described on page 28, was used to protect the flame and the glass tubing extending from the end of the condenser into the receiving li-

* low temperature distillation

quid was lengthened, the sucking back was eliminated.

After the impediments in manipulation and apparatus were overcome, repeated runs were performed on the vacuum* sample until the apparatus was adjusted and the checks were perfect and no flaws were apparent, either in technique or equipment. Other samples of alcoholic nature which were available were used. It was found that those samples which were distilled at low temperatures possessed a lower nitrogen content than the samples atmospherically distilled. Green whiskey contained slightly higher nitrogen content than spirits.

Blended whiskey was a trifle higher in nitrogen than straight whiskey. The difference in ageing time between the whiskies is two years and for this reason the blended whiskey will remain in contact, for a longer period of time, with the cooperage drawing from it various substances; in addition to the above fact, the grain alcohol, used in blended whiskey, is aged previously.

The manufacture of gin is the redistillation of spirits with aromatic ingredients (coriander seed, lemon peel, orange peel, etc.). Gin, lower than straight whiskey, but higher than spirits, gave a yield near that from green whiskey. Low temperature distillation appears to result in a smaller yield of nitrogen. The amount of nitrogen in all the samples tested was very small. At least three trials were made on each sample listed in the table of results. Blanks were run on all the materials used and the necessary corrections were made.

* low temperature distilled

SUMMARY

Summary

I. The Kjeldahl method is finding wide application today in both clinical and industrial laboratories. Although numerous modifications and methods have evolved, in principle the Kjeldahl method is almost intact.

II. When modified, the Kjeldahl method is applicable to alcoholic distillates containing only a very small amount of nitrogen in a large volume. Copper selenite was a satisfactory catalyst for digestion and the use of 4% boric acid as the receiving acid gave good results and eliminated the use of two standard solutions. A double indicator of tetrabromphenol blue and methyl red was successfully used.

III. For the major part, vacuum * distilled samples possessed lower nitrogen content than atmospherically distilled samples.

* low temperature

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