THE STUDY AND DETERMINATION OF ACCURATE MICRO METHODS OF CAFFEINE ANALYSIS IN DECAFFEINATED COFFEE

THESIS FOR THE DEGREE OF M. S.

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THESIS

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By

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APPRECIATION

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INTRODUCTION

The analysis of caffeine in coffee, tea and cocoa has been accomplished by many varied methods. This has been due, partially at least, to the somewhat long and troublesome methods often employed and their comparative inaccuracies, resulting in the constant endeavor to perfect methods which are comparatively short and easy to run, and at the same time capable of giving accurate and consistent results.

In the analysis of decaffeinated coffee for caffeine by the Fendler and Stuber method, as well as by the Power and Chesnut method it is found that a 10 g. sample of Kaffee Hag or Sanka Coffee contains on the average from 1 to 3 mgs. of caffeine, determined either by the nitrogen content of the final residue of caffeine, or by sublimation of this residue. This small amount of caffeine present necessitates the employment of methods more accurate than is necessary in the case of the caffeine analysis of ordinary coffee.

Practically all of recognized methods in use up to the present time calculate the percentage of caffeine on the dried weight of the final, more or less crude caffeine residue which is left after evaporating off the solvent (usually chloroform) and drying in an oven at 100°C. for about 30 minutes. Most of the methods make it optional for the analyst to determine the purity of this residue by running a nitrogen determination on it and multiplying by the factor 3.464 for caffeine.

But this has not been made compulsory and the percentage of caffeine may be based upon the weight of the residue alone and be considered correct. The error involved here, due to the oily and waxy impurities present with the caffeine, is perhaps negligible in the case of caffeine analysis of ordinary coffee, and the residue will often run 95% pure caffeine. However in the case of decaffeinated coffee the residue of caffeine will usually run from 20 to 40% pure caffeine. Hence basing the final results in this case upon the weight of the residue would be entirely erroneous.

It is the object of the analytical and experimental date given in this thesis to thoroly prove the above statement. In addition to this, an attempt was made to accomplish the following:

1. To find possible losses of caffeine in the various steps of the analysis. And to make suitable modifications or additions in order to make a more adaptable micro method.

2. To further purify the final caffeine residue.

3. To accurately determine the percentage of caffeine in this residue.

It is needless to say that to fully cover these accomplishments in the time already spent on this work is an impossibility, and further investigations will be made on phases of this problem not touched upon in this thesis.

A great deal of the experimental work already covered has to do with the accurate determination of the percentage of caffeine in the caffeine residue, by two methods:(1) by a micro nitrogen determination, (2) by sublimation.

In view of the fact that the Fendler-Stuber and the Power-Chesnut methods are the only recognized methods in use today, all of the analytical and experimental results given in this work involve the use of these two methods, with modifications in some cases.

HISTORICAL

Caffeine was first isolated in the pure form in 1821; when it was obtained almost simultaneously by Runge, Pelleter and Caventon, and Robiquet. Oudry discovered a similar compound in tea in 1827 and called it theine, but which was later identified by Berzelious, together with the alkaloid of guarana, as being identical with caffeine. (Allen's Comm. Org. Analysis, 5th. edition, vol.7, p. 307).

The method of Paul and Cownley (Pharm.J.(iii),18, 417, 1887) for caffeine analysis was prominent among the earlier methods. It consisted in drying the moistened sample with ignited magnesia and extracting with alcohol. At about the same time E. D. Smith (Chem.Centr.1270-71,1887, from Zeit. Oster. Apoth.Ver. 41, 359) brought out a method for caffee consisting in boiling the sample in successive portions of water in the presence of magnesium oxide. Both methods separated and purified the caffeine by evaporating off the solvent, taking the residue up in hot water, filtering and shaking the aqueous filtrate with successive portions of chloroform. The solvent is evaporated off, the residue dried & weighed.

The determination of caffeine in coffee has held the attention of the Association of Official Agricultural Chemists since 1908. And the various methods have been very thoroly investigated by the Association. Prior to 1915 the Fuller method and the Gorter method were studied (J.Assoc. Official Agr. Chem. 1,No.2,206-8,1915-16) and also the Hilger & Fricke method (U.S.Bur.Chem.Bul.107,Rev.P.153) for the purpose of being adopted as provisional or tentative methods by the Ass'n. The Gorter method involves Soxhlet extraction with $CHCl_3$. Purification consists simply in taking up the residue, after evaporating off the $CHCl_3$, in hot water, filtering, and finally shaking out the aqueous solution with successive portions of $CHCl_3$, and evaporating off the solvent. It was found that the caffeine was in a very impure condition, and had to be further purified, or to base the results only upon the nitrogen determination. The Fuller method uses water extraction, slightly acidified. Separation and purification of the caffeine consists in precipitating the alkaloid as the periodide, and then decomposing the iodine, filtering and shaking out the filtrate with $CHCl_3$ as usual. The method of extraction and filtration is laborious and incomplete, which is shown by the consistently lower results obtained in comparison with the other methods.

In 1915 the Stahlschmidt method (J.A.O.A.C.,3,No.1, 21-24, 1917) was adopted as a provisional method after being given certain modifications. Extraction is accomplished by boiling the sample with water, and purification is made by the addition of dry basic lead acetate. Excess lead is removed in the filtrate by passing in H_2S and refiltering. The aqueous solution is shaken out with $CHCl_3$ as in the other methods. The inconvenience of using large volume of water is eliminated in the modifications by using aliquots. There is often a tendency for the liquids to emulsify slightly when shaking the final aqueous solution out with $CHCl_3$. The final

Caffeine residue is quite pure in comparison with Fuller method.

In 1917 and 1918 the Fendler - Stuber method (J.A.O.A.C.,5 No.2,267-73,1921) was critically studied in comparison with the Stahlschmidt method. This method involves extraction of the caffeine by shaking out with $CHCl_3$, the sample being first dampened with dilute ammonia. Purification of the dried residue consists in oxidation with 1% $KMnO_4$ at room temp. and removing the excess permanganate with 3% H_2O_2. After filtration, the aqueous solution is shaken out with $CHCl_3$, and the resulting dried residue of caffeine is of exceptional purity, in comparison with that obtained by some other methods. It was found that there is about a 1% loss of caffeine by oxidation by the $KMNO_4$ at room temperature. This can be partially eliminated by keeping the flask in an ice bath, but this lowers the purity of the final residue of caffeine. In 1919 this method was adopted tentatively by the Ass'n. because of its rapidity, ease of manipulation, and the production of an exceptionally pure caffeine residue.

Also, in 1919 the Power-Chesnut method (J.Amer.Chem. Soc.,14,1298,1919)and (J.A.O.A.C.5,No.2,267-73,1921) was thoroly studied by the Ass'n. of Official Agr. Chemists. Extraction is accomplished here by the Soxhlet extractor with alcohol. The extract is purified by evaporating to dryness in an aqueous suspension with magnesium oxide, pulverizing, making into a paste and washing thoroly with water on a filter. The filtrate is boiled with dilute H_2SO_4, cooled, filtered and shaken out with $CHCl_3$. Considerable coloring matter is removed by washing the combined extracts with 1% KOH. Because of its scientific accuracy, wide applicability and consistent results in close agreement with these obtained in the Fendler-Stuber method, the Power-Chesnut method was adopted as an official method by the A.O.A.C. in 1319.

Resulting from the thoro and critical study of the different methods of caffeine analysis for coffee, the Fendler-Stuber and the Power-Chesnut methods are the only ones which, have stood the test, and are recognized today by the Assoc. of Official Agr. Chemists.

HISTORICAL (cont'd)

1. PROPERTIES OF CAFFEINE.

In order to better study the various methods of analysis, and to suggest modifications for caffeine analysis of decaffeinated coffees, it will be well to give some of the properties of caffeine. (Allen's Commercial Organic Analysis, fifth edition, Vol.7.Veg. Alkaloids, P.308 to 315).

A. Physical Properties.

Caffeine is a solid at ordinary temperatures, and is a very stable compound, It forms long, white silky needles when crystalized from such solutions as water or chloroform. The crystals present a characteristic appearance when magnified 100 to 300 times.

The melting point of caffeine is 235 to 237 degrees Centigrade, after drying. (U.S. Pharmacopaeia).

It sublimes very slowly when heated over 100 degrees, and is gradual but continuous at 120°. Complete quantitative sublimation takes place at 178 to 180°. Caffeine can be repeatedly evaporated with water at 100 degrees in an open vessel without more than the very slightest loss of the alkaloid, and no loss with chloroform even after repeated evaporations.

Caffeine crystallizes from aqueous solutions with one molecule of water. When heated to 100 degrees C. it loses its water of crystallization, the loss amounting to not more than 8.5%, and usually not much over 7.00% with the average samples of commercial caffeine. The anhydrous crystals of caffeine are opaque and quite friable, and dissolve without turbidity in chloroform. Caffeine also becomes anhydrous by prolonged exposure over H_2SO_4 at ordinary temperatures. And under certain conditions anhydrous crystals are deposited from solutions of alcohol and ether.

The Solubility of caffeine in various solvents is given in the following table:

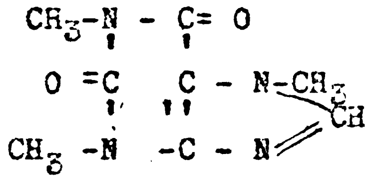
	Parts of solvent required for one of caffeine. (Anhydrous caffeine)		
Solvent	A. Commaille (Compt.rend.1875,81,817)		U.S.P.
	At 15 to 17 deg.C.	At B.pt.of solvent	At 25 Deg.C.
Water.	74.0	2.2	46.0
Rectified spirit	44.0		66.0
Abs. alcohol	165.0	32.0	
Commercial ether	526.0		
Pure anhydrous ether	2288.0	277.0	530.0
Chloroform	7.7	5.25	5.5
Carbon disulfide	1709.0	220.0	
Petroleum spirit	4000.0		
	(Gockel,Chem.Centr.. 1897,2,401)		
Benzene	109.8	18.9	100.0
Carbon tetrachloride	1123.0	142.4	
Acetone			50.0

	Gms. caffeine per 100 gms. of satd, soln. (Seidell, J.Amer. Chem.Sox. 1907,29,1091)	
	Temp. deg.C.	Solubility per 100 gms. satd. soln.
Water.	25.	2.14 gms
Ether	25.	0.27
Chloroform	25.	11.00
Acetone	30.	2.18
Benzene	30.	1.22
Benzaldehyde.	30.	11.62
Amyl acetate	30.	0.72
Analine	30.	22.89
Amyl Alcohol	25.	0.49
Acetic acid	21.5	2.44
Xylene.	32.5	1.11
Toluene	25.	0.57

The solubility of caffeine in water is increased by adding sodium benzoate, sodium bromide, sodium cinniminate, sodium silicate and antipyrine.

B. Chemical Properties.

Caffeine is a methylated Oxypurine compound, or 1, 3,7, tri-methyl Xanthine. It has the following structural formula:



It acts as a very feeble base, having a dissociation constant of 4×10^{-14}.

HCl has no action on caffeine at temperatures below 200 deg.C., but when it is heated with conc. HCl. at 250 deg. under pressure for 6 to 12 hours oxidation products are formed which include ammonia, methylamine, sarcosine, carbon dioxide and water, and sometimes formic acid.

Dilute alkali hydroxides when warmed with caffeine change the alkaloid to an acid. $C_8H_{12}O_3N_4$. but at first adding on H_2O. This is easily split up into CO_2 and the base caffeidine, $C_7H_{12}ON_4$. This is upon further heating decomposed to CO_2., HCOOH.,NH_3.,CH_3NH_2., and sarcosine (CH_2NH_2 COOH). These are practically the same end products as when decomposed with a conc. acid.

Caffeine is easily decomposed by heating with lime water, or heating with soda lime at 180 deg. (forming a large quantity of cyanide, thus distinguishing caffeine from piperine, morthine, quinine, and cinchonine). If ignited with soda lime ammonia is evolved, and cyanide is formed as only an intermediate product. However caffeine may be boiled with an aqueous suspension of MgO. or PbO. without the slightest loss

or decomposition. This fact is made use of in several methods of analysis as a means of purifying the alkaloid.

The well known murexide reaction, especially as a qualitative test for uric acid, gives very good results with caffeine or theobromine (3,7 dimethyl xanthene). The alkaloid may be oxidized several ways:

1. Evaporated to dryness with Br. water.
2. Evaporate to dryness with conc. HCl. and a crystal of $KClO_3$.
3. Evaporate to dryness with conc. HNO_3.

Upon adding a few drops of conc. NH_4OH to the yellow residue a bright reddish purple is produced. The addition of NaOH will cause immediate decoloration, while with uric acid the color turns blue. This test is a very delicate qualitative one.

All methylated xanthenes give the murexide reaction as well as uric acid. Tho the color produced is the same the reactions for caffeine and uric acid are slightly different, as is shown:

Caffeine--Oxidized to Amalic acid $(C_8H_2(CH_3)_4O_8N_4)$ --add ammonia, gives- Murexoin. $(NH_4C_8(CH_3)_4O_6N_5)$.

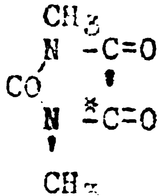
Uric acid-Oxidized to Alloxantin $(C_8H_6O_8N_4)$--- add ammonia, gives- Murexide. $(NH_4C_8H_4O_6N_5)$

Caffeine can be distinquished from theobromine according to F. P. Stoup (Ann.J.Pharm., 1919,91,598) by treating a sample on a tile with one part $K_2Cr_2O_7$ and twenty parts of conc. H_2SO_4. If it is caffeine the yellow color of the reagent is turned almost immediately to a bluish green. While if it is theobromine the color changes from yellow thru purplish green and olive green to the same bluish green of caffeine.

Potassium-iodo-bismuthate, if freshly prepared, gives an orange colored ppt. with caffeine and theobromine. Old reagents due to traces of hydriotic acid give different results (M.Malmy.,J.Pharm.Chim.,1921,23,630). This test also distinguishes between the two alkaloids. It is as follows: 0.05 gm. of the alkaloid is dissolved in 10 cc.of H_2O and 0.5 cc. of the fresh reagent and 5 drops of 10% iodine soln. co containing about 1% hydriotic acid are added. Caffeine gives a red ppt., while theobromine gives a brown ppt. which changes in about 30 min. to a chocolate brown.

A very delicate test for purines in general consists in boiling the alkaloid with conc. HNO_3 and $K_3Fe(CN)_6$. A ppt. of Prussian blue is formed upon dilution.

Cholestrophane or dimethylparabanic acid, is formed when caffeine is boiled with an excess of conc. HNO_3 or chromic acid mixture. It is a compound which crystallizes in pearly laminae, having a melting point of 145.5° and A B.pt. of 275 to 277°. This compound is very easily decomposed with alkalies into symmetrical dimethyl carbamide and oxalic acid. Hence on adding NH_4OH and $CaCl_2$ to its aqueous soln. and warming the liquid calcium oxalate is ppt.



Neutral iodized potassium iodide and Mayers soln. do not ppt. caffeine. This property distinguishes caffeine and theobromine from nearly all other alkaloids. However an acid soln. of iodine and Kl. ppts. caffeine quantitatively.

Phosphomolybdic acid produces a yellow ppt. with caffeine soluble in warm Na acetate soln., the liquid depositing free caffeine upon cooling.

Mercuric chloride ($HgCl_2$) gives a characteristic qualitative test with all methylated purines by forming large white rosettes of crystals in an aqueous soln. These crystals are $C_8H_{10}O_2N_4HgCl_2$. They are formed immediately in a saturated soln., and after a few days in a soln. containing one part of aklaloid to 4000 parts of water. This compound is soluble in about 260 parts of cold water, hence it is not adapted for the quantitative precipitation of caffeine. (R.H. Davies., Pharm.J., 1890,253, (LLL),21.).

Gallotannin precipitates moderately diluts solns. of caffeine. A variation in temperature, changes the solubility and the kind of precipitate formed.

Even tho caffeine is a feeble base and will form salts with acids they are very easily decomposed by water, alcohol and ether. This is due to the fact that salts of caffeine are hydrolytically dissociated upon dilution. Hence it is possible to completely remove the alkaloid from an acidified solution of water by shaking out several times with benzene or chloroform.

Caffeine hydrochloride crystallizes in colorless prismatic needles. The acid is lost at 75°C. Caffeine sulfate is deposited from a hot alcoholic solution in shining needles, and is not decomposed below 100°C. Caffeine nitrate is found as fine transparent crystals, becoming opaque in water and changing into pseudomorphs, or microscopic needles of the free alkaloid.

C. O. Johns has prepared isomers of caffeine and theobromine by alkylating with dimethylsulfate.

According to Gomberg (J.Amer.Chem.Soc., 347,1896,18)

caffeine gives rise to a number of perhalides which are noteworthy on account of their insolubility in water. This is accomplished by adding a solution of iodine and potassium iodide to an aqueous solution of the alkaloid acidified with HCl. A ppt. of $C_8H_{10}O_2N_4Hl.l_4$ is immediately formed; and this crystallizes into dark blue prisms when put into methyl alcohol.

Caffeine can be completely precipitated by silico tungstic acid in the presence of 5% HCl. (C.A.Vol.16,P2833, 1922. & A. Azadian, Bull.Soc.Chim.Belg.15, 31.1922). When dried at 30°C. the composition of the precipitate is $12\ Wo_3.SiO_2.2H_2O.3(C_8H_{10}N_4O_2)$ plus $6H_2O$. After igniting this there is left $12\ Wo_3.SiO_2$ which, multiplied by the factor 0.2646 gives the equivalent of caffeine. While this method might be used for the quantitative determination of caffeine when considerable of the alkaloid is present, it would not by practical or accurate when there is present only one or two milligrams of caffeine. Several alkaloids including caffeine can be titrated with the above compound, using malachite green as the indicator. Malachite green in HCl has a reddish or brownish orange color. The green color is at once restored by silico tungstic acid. As long as the alkaloid is in excess the solution remains yellow, but as soon as the reagent is in excess the green color appears. Volumetric silico tungstic acid is standardized against the pure caffeine to be titrated. (C.A.Vol.19.P. 792,1925. E.O.North, and G.D. Beal, J.Am.Pharm.Assoc.13,889, 98,1001-9,1924).

According to I.M. Kolthoff (Z.Inorg.Allgem.Chem. 112,196 to 208. 1920 and C.A.15, 1670,1921) alkaloids and their salts may be titrated with satisfactory accuracy by the

electrometric method in very dilute solutions. The alkaloids themselves are titrated with acid; the conductivity curve rises steadily from the commencement of the titration, and at the point where salt formation is complete the rate of increase of conductivity augments. The alkaloidal salts are titrated with alkali, and there is a sharp break at the neutral point. Titration of alkaloids or their salts with indicators generally give uncertain results because a solvent such as alcohol or chloroform has to be used. While for electrometric titrations no solvents need be used in the caseoof alkaloidal salts, and 55% alcohol can be successfully used for the free alkaloids. Caffeine and theobromine have a dissociation constant of approximate 1 $10^{-14}$and 1 10^{-10} respectively, and the latter can be exactly estimated in a mixture of the two alkaloids by dissolving in an alkali and titrating back with an acid. The break in the conductivity curve is quite distincty.

C. Physiological Properties.

While having no direct bearing upon this problem, it may be of general interest to give a few of the physiological properties of caffeine.

This alkaloid is odorless, but has a bitter taste. When given in excessive doses it possesses decidedly poisonous properties. When administered to frogs it produces tetanas and rigor of the voluntary muscles. One fourth of a gm. of caffeine killed a cat in 35 minutes in one trial. In all experiments it caused increased frequency of the heart's action and repeated emptying of the bladder and intestines. After death the alkaloid has been detected in the blood, bile and the urine. In man, caffeine causes increased heart action by stimulating the cardiac muscles, and excites the nervous system, and it has been stated to be an antidote for nicotine poisoning. The toxicity of caffeine may fluctuate between 0.10 to 0.30 gm. per kilo. wt. of animal. (Allen's Comm. Org. Analysis,5th edition, vol. 7,p.210).

A neutral compound of caffeine and aminoacidylphenetddine is stated to be suitable for sucutaneous injections. Stable solutions of caffeine are prepared by dissolving in sodium or ammonium benzoate, cinnimate, or salicylate, and are used for hypodermic injections.

The continued use of stimulating drinks containing caffeine results in the establishment of a tolerance for the alkaloid in the body. The absence of this tolerance is easily demonstrated by observing the effects of drinking tea or coffee after having abstained from such stimulants for a considerable length of time. However caffeine differs from most other alkaloids in that the same dose will continue to give the same amount of stimulation and effect upon the nervous system, after once becoming accustomed to it. This is called the "habit tolerance" of caffeine. While such alkaloids as morphine and cocaine are taken in increasingly larger doses to get the same amount of stimulation, with the inevitable fatal result.

Some authorities claim that the injection of caffeine results in the increased ability of the body to take care of foods containing a large percentage of protein. This perhaps is partially due to the diuretic effect of the alkaloid.

It is questionable whether caffeine is changed to uric acid to any extent in the body before being excreted. Most of it appears to be excreted in the urine as unchanged caffeine. (E.L.Wardell & V.C. Meyers.Proc.Soc.Expetl.Biol. Med. 23,828-30, 1926).

HISTORICAL (cont'd)

II. METHODS OF CAFFEINE ANALYSIS

The caffeine found in coffee and tea, being entirely soluble in hot water, alcohol and chloroform, has been extracted by these solvents in practically all of the earlier methods of caffeine analysis, as well as in the best methods in use at the present time. Ether and carbon tetrachloride has been used in a few of the less important methods.

The methods of purifying the extracted caffeine have been quite varied. Lime was used in a few of the earlier methods, but due to the fact that it rapidly decomposes caffeine when heated above 100° it was soon replaced by magnesium oxide, which has no destroying effect upon the alkaloid at the temperature of boiling water. Some of the simpler methods remove the fatty and oily impurities by simply adding hot water to the dried residue after evaporating the solvent off, filtering and reextracting the caffeine from the aqueous solution by shaking out with succesive portions of chloroform. Further purification is extremely necessary in the case of decaffeinated coffee. Basic lead acetate is employed successfully as a purifying substance. The excess lead is precipitated as PbS. by passing H_2S thru the filtrate. Aluminum acetate is used in one or two methods. The use of 1% $KMnO_4$ solution and precipitating the excess with 3% H_2O_2 is as popular way of purifying the caffeine residue as the use of MgO. if not more so, because it is a simpler and a quicker procedure of the two. However there is a slight loss of caffeine when $KMnO_4$ is used especially if the temperature is much over 20° C., or if the sample is let stand over 15-20 minutes.

Hydr lysis of some of the impurities, such as the saponins, by boiling the crude extracted caffeine in dilute acid is employed in combination with other steps in purification in a few of the methods.

In all of the recognized methods the separation of the caffeine from the aqueous solution, after purifying and filtering, is accomplished by shaking out with from three to six portions of chloroform, and then evaporating off the solvent on a steam bath.

The calculation of the final residue of caffeine in determining the percentage of caffeine is accomplished in one of five ways in the various methods used up to the present time:

1. Drying the residue at 100°C. and weighing as pure caffeine.
2. Determining nitrogen in the residue of caffeine.
3. Subliming the caffeine and weighing the sublined crystals.
4. Precipitation of caffeine as periodide, and titrating the excess iodine left.
5. Precipitation of caffeine with silicotungstic acid, igniting and weighing.

In studying the different methods of caffeine analysis it can be readily observed that there are four distinct steps in practically every method.

These are:

1. Extraction with a suitable solvent. (Usually wetting with water or ammonia if solvents other than water or alcohol are used).

2. Purification of the caffeine after removal of the solvent. (By absorption, oxidation, hydrolysis, precipitation, or selective solubility).
3. Separation of the caffeine from the aqueous solution, after purification & filtration, by shaking out with chloroform.
4. Determination of the purity of the caffeine residue.

These four steps will be used as a basis for classification of the different methods of caffeine analysis in coffee and tea. The majority of the methods are applicable to decaffeinated caffee, if greater care is observed in the analysis, especially in the fourth step. The majority of the methods will be described briefly, while the others will be merely classified with the accompanying references.

I. CAFFEINE EXTRACTED WITH HOT WATER.

A. Purification methods include the use of magnesium oxide.

1. <u>E. D. Smith</u>. Improved method of estimating caffeine in coffee. (J.Amer.Chem.Soc.54,583. & Chem. Centr. 1870-71,1887., from Zeit.oster.Apoth.Ver.41,353).

The sample is mixed with a small amount of MgO, boiled 5 min. filtered. This is repeated. Made up to 300 cc. by percolation. Combined filtrates are evaporated to 20 cc. Residue treated with alcohol. This is evaporated off & the residue dissolved in a small amt. of water. The aqueous soln. is shaken out with $CHCl_3$ in the usual manner.

2. <u>Camilla & Tertusi. Method</u>. (Camilla, S. & Tertusi, C. Research on the determination of basic xanthines and their derivatives. (C.A.6,3479,1912. Giorn. Farm.Chim., 61,385-91).

This is somewhat similar to the Bailey & Andrew method except that the sample is boiled in an acid soln. neutralized with MgO., conc. to 80 cc. acidified, extracted with CCl_4. Residue is put into suspension with water, filtered, treated again with MgO, evaporating to a powder & re-extracting with $CHCl_3$.

3. <u>Bailey and Andrew method</u> (R.E. Andrew & E.M.Bailey. Determination of caffeine in tea and coffee. C.A. 17,1068,1923. Conn.Agr.Extt.Sta.,Bull. 227,256-8 1921.Analyst 46,405).

(1) Grind sample to pass 1/25 in. sieve.

(2) To 5 gm. tea in a 500 cc. grad. flask add 10 g. heavy MgO & 200 cc. water.

(3) Boil gently for 2 hrs. using a small bore tube

(4) Cool, dilute to volume & filter thru a dry filter.

(5) To a 300 cc. aliquot in a 1 L. Erlenmeyer flask add 10 cc. of 10% H_2SO_4& evap. by gentle boiling to 100cc.

(6) Filter into a separatory funnel, washing the flask with small portions of 1% H_2SO_4.

(7) Shake out 6 times with $CHCl_3$ (25,20,15,10,10,10,cc. portions).

(8) Treat the combined exts. with 5 cc. of 1% KOH. Shake thoroly.

(9) Draw off the $CHCl_3$layer, and wash the KOH with 2, 10 cc portions of $CHCl_3$ and add to the main bulk of the extd.

(10) Distill off the $CHCl_3$to a small amt. & transfer to tared flask. Evap. to dryness, dry at 100° to constant wt. and weigh.

(11) Transfer residue to a digestion flask with small amt. of H_2SO_4& Deter. N. by Kjeldahl method. Calc. caffeine from N. by the factor 3.464.

4. Tassily. (Tassily E. Estimation of caffeine in caffee. Bull. Soc.Chim.(iii),17,761-768, & J.Amer.Chem.Soc., 76,134).

B. Purification of the extracted caffeine by oxidation with $KMnO_4$.

1. Savini. (Savini G. The analysis of coffee and coffee substitutes. An accurate method of determining caffeine. Ann. Chim. Applicata 13,217-20,1923. C.A.18,132,1924).

In this method the aqueous extract is made alkaline with ammonia and extracted with $CHCl_3$ in a Marino extractor. The solvent is evaporated off and the residue boiled with

sufficient 0.5% KMnO4 in 50 cc. of water to give a permanent violet color. It is reextracted with $ChCl_3$ in the Marino extractor. The solvent evaporated off, and the residue dried at 98° for 15 to 20 min. and weighed as pure caffeine.

C. Purification of the extracted caffeine by precipitation of impurities with basic lead acetate, or basic aluminum acetate.

1. Modified Stahlschmidt Method. (J.A.O.A.C. 3,No.1,22, 1917. & Allen's Comm. Organic Analysis,Vol.3,Pt.2,P.49).

(1) Grind sample to pass a 40 mesh sieve & weigh out 3.125 g.

(2) Add 225 cc. of water to the sample in a 500 cc. flask, attach a reflux condenser and boil for 3 hrs.

(3) Add 2g of dry basic lead acetate & boil 10 min. Cool to room temp.

(4) Transfer to a 250 cc. graduated flask. Make up to the mark, thoroly mix & filter thru a dry paper.

(5) Measure 200 cc. of the filtrate into a 250 cc. graduated flask & pass H_2S thru to remove the excess lead. Make up to the mark and filter.

(6) Measure out 200 cc. of the filtrate, representing 2 g. of the sample, into an evaporating dish & conc. on a steam bath to about 40 cc.

(7) Wash the conc. soln. with as little water as possible into a separatory funnel & shake out 4 times with $CHCl_3$ (25,20,15 & 10 cc portions). In the case of coffee the soln. is made slightly alkaline with NH_4OH before extraction with $CHCl_3$. Run the separate portions of $CHCl_3$ thru a 5 cm. filter paper into a small Erlenmeyer flask.

(8) Remove the chloroform, dry the residue to a constant wt. at 100°, and weigh.

(9) If the caffeine residue is not pure, determine N. by the Kjeldahl method & multiply by the factor 3.464 for caffeine.

2. <u>Hilger and Juckenack method</u>. (Hilger A. & Juckenack A. The estimation of caffeine in tea and coffee. J.Pharm. 1897 (vi),6 184 -188. Apoth.Zeit.1897,12,145 & 422).

(1) 20 g. of finely ground coffee or tea are digested for several hours with 900 cc. of water at room temp. & then boiled three hrs. for green coffee & 1½ hrs. for roasted coffee, replacing the water lost by evaporation.

(2) After cooling to 70° 7.5 to 8.g.of basic aluminum acetate in solution is run in, and then 1.9 g. of $NaHCO_3$ is gradually added while the mixture is well stirred.

(3) It is boiled for 5 min., cooled, water added to make the total wt. 1020 g. and filtered.

(4) 750 g. of the filtrate coresponding to 15 g. of the sample, to which 10 g. of dried & powdered aluminum hydroxide & a little filter paper have been added, are evaporated to dryness.

(5) The residue is extracted for 8 hrs. with CCl_4.

(6) The solvent is evaporated off & the residue dried, weighed & calculated as caffeine.

3. <u>Azadian</u>. (Bul.Soc.Chim.Belg.31,15, 1922 & Allen's Comm.Org.Analysis 5th. edition,vol.7,P.340).

(1) After extracting the sample with water, the soln. is cleared with basic lead acetate.

(2) The caffeine is precipitated in a 5% HCl soln. of

HCl with silicotungstic acid, and let stand several hrs. or over night.

(3) The liquid is filtered off and the ppt. is dried ignited and weighed. It has the composition of $12\ WO_3.SiO_2$., which multiplied by the factor 0.2646 gives the equivalent of ca caffeine.

D. No purification or separation of impurities from the caffeine other than shaking out the aqueous solution with $CHCl_3$, in an acid or alkaline soln.

1. Tatlock and Thomson. (Analyst,1910,35,105).

The aqueous filtrate is evaporated to a small vol. NaOH soln. added and then extracted with $CHCl_3$ etc. in the usual manner.

2. Guillot. Rapid estimation of caffeine in tea and coffee. (Cham.Centr.1.8 865,1898. & Apoth.Zeit.,8,122).J.Am. Chem.Soc.64,808).

This method simply consists in boiling the sample in successive portions of water, filtering, and shaking out with 3 portions of $CHCl_3$.

E. Methods involving the use of iodine & KI soln. precipitating the caffeine as a periodide.

1. Caffeine determined by titrating the excess iodine in an aliquot of the filtrate after precipitating the caffeine as the periodide.

Gomberg's Process. (Allen's Comm. Org. Analysis,5th edition,vil.7,p 233 & J.Amer.Chem.Soc.18,331,1896 & J.Amer.Chem.Soc.19,279,1897)

(1) 5 g. of tea are boiled for ½ hr. with 400 cc. of

water, and then digested for another ½ hr. with an excess of freshly ppt. ferric hydroxide.

(2) The liquid is cooled and made up to a suitable vol. An aliquot is taken and filtered, acidified with dil.HCl.

(3) The caffeine is ppt. by a known vol. of standardized iodine soln., & the ppt. allowed to settle after being made up to a convenient vol.

(4) An aliquot part of the clear supernatent liquid is drawn off, and the excess of iodine determined as usual. The amount of caffeine obtained from the value 1 part iodine = o.2834 caffeine, or 1cc. N/10 sodium thiosulfate = o.00485 caffeine. The most accurate results are obtained when the iodine soln. is used in large excess.

This method was devised for the estimation of caffeine in drugs. It depends upon the fact that when a soln. of caffeine containing HCl is treated with a soln. of iodine and potasium iodide the whole of the caffeine is ppt. as periodide $C_8H_{10}O_2N_4HI,I_4$.

2. Caffeine separated and purified by precipitating as the periodide and then decomposing the iodine, reextracting the caffeine, dried & weighed as in other methods.

H. C. Fuller. Fuller method for tea and coffee. (J. Assoc. Official Agr. Chem.1,No.2,203-8,1915-16).

(1) A 10 g. sample is put into an Erlenmeyer flask 100 cc. water & 10 cc. of 10% HCl, and heated under a reflux condenser for 2 hrs.

(2) It is cooled & the liquid decanted thru a filter. The solid material is treated with 3-50 cc. portions of hot water, washing thru the filter. And then the filter is washed

with 50cc. of boiling water.

(3) The entire filtrate is evaporated down to 150cc. It is transferred to a Squibb type separatory funnel, made alkaline with 5cc. of strong ammonia, and shaken out with 5-50cc. portions of $CHCl_3$. A second separation may have to be made if the liquids emulsify considerably. If there is still an emulsion remaining in the second separatory funnel a few cc's of C_2H_5OH is added and shaken. Then the separated $CHCl_3$ is added to the main portion of the solvent and shaken out the alcohol layer with a little $CHCl_3$.

(4) The $CHCl_3$ extract is evaporated off on a steam bath, the last portion being driven off by a current of air.

(5) To the dried residue is added 10cc. of 10% HCl & 50cc. of water and warmed until the caffeine is thoroly dissolved. It is cooled & 50cc. of iodine soln. added (10 g. iodine & 20 g. of KI in 100cc. of water). The flask is stoppered and let stand over night.

(6) The soln. is filtered, refiltering if necessary, and the flask and ppt. washed with iodine soln. but not necessarily removing all of the ppt. to the filter.

(7) The filter paper is put into the flask containing the rest of the ppt. & 6.5 g. of sodium acid sulphite, or sodium sulphite, 3cc. of 10% H_2SO_4 & 15cc. of water are added, and warmed until all of the iodine is decomposed. More of the salt is added if the amount is insufficient to decolorize.

(8) The soln. is filtered into a separatory funnel, an excess of strong NH_4OH is added & then shaken out with 5-15 cc. portions of $CHCl_3$. The combined extracts are washed with water, discarding the latter.

(9) The $CHCl_3$ is evaporated down to 10 to 15 cc. and dry animal charcoal is added and allowed to stand for 1 hour with occasional shaking.

(10) The soln. is filtered thru a small filter paper into a tared dish, washing the flask and filter 4 times with 5cc. portions of $CHCl_3$.

(11) The $CHCl_3$ is evaporated off, the residue dried in a desiccator & weighed.

II. CAFFEINE EXTRACTED WITH CHLOROFORM.

A. Purification methods include the use of magnesium oxide.

1. J.Katz (J.C.S.86,ii,301. & Arch.Pharm. 242,42048,1904).
Amount of caffeine in the coffee used as
a beverage.

(1) A 10 g. sample is wet with 10 cc of ammonia, and shaken mechanically with 200 cc. of $CHCl_3$ for ½ hour.

(2) After the solid has settled 150 cc. of the chloroform is filtered off thru a Sander's "cigarette filter". The $CHCl_3$ is now distilled off.

(3) The residue is mixed with 10 cc of o.5% HCl & a few cc's. of ether. About o.5 g. of paraffin are added, and the ether evaporated on a water bath.

(4) The residue is heated until the paraffin has melted thoroly, cooled and filtered thru a wet filter. The residue is warmed twice in succession with 10 cc. of 0.5% HCl and filtered.

(5) The united filtrates are extracted for 2 hrs. in a Katz percolator, with $CHCl_3$. The solvent is evaporated and the crude residue weighed.

(6) The crude caffeine is dissolves in 10 cc. of water (plus a few drops of ether) which are then boiled off) .7 the hot soln. is heated for 10 min. with a suspension of lead hydroxide in water (1 to 20) and mixed with about 0.2 g. of calcined magnesia, then filtered.

(7) The residue is washed with water, extracted with $CHCl_3$ in the percolator for 2 hrs.

(8). The $CHCl_3$ is evaporated off, the pure caffeine dried & weighed.

2. <u>C. Virchow</u> (The determination of caffeine in roasted coffee. Chem.Ztg.24,1037. & C.A.5,542,1911 & Chem.Zeit.34,1037-8,1910 & J.S.C.I.29,1224).

(1) 10 g. of finely ground coffee, 2.5 g. of MgO. & 10 g. of water are allowed to stand for 2 hours.

(2) This mixture is shaken with 3,100 cc. portions of chloroform, shaking each one about a minute.

(3) The extract is treated with 1 g. of paraffin & distilled. The last portion of the chloroform being removed with a gentle current of air.

(4) 25 cc. of hot water are added to the flask on the steam bath and occasionally shaken until the wax and fat is melted.

(5) The soln. is poured into a beaker, washing the flask out thoroly with hot water, and heated until the wax and fats have completely melted.

(6) The soln. is cooled and filtered, and the filtrate evaporated down to dryness. The residue is dried to constant wt. and weighed.

(7) To purify the crude caffeine, the residue is washed into a porcelain dish and partially evaporated on a hot water bath. Finely powdered MgO is added and the mixture evaporated to dryness.

(8) The residue is powdered as finely as possible and extracted with $CHCl_3$ in 3 portions, decanting thru a small filter into a weighed flask.

(9) The solvent & washings are distilled off, the residue dried and weighed.

3. G. Bonifazi. The determination of caffeine in decaffeinated coffee. (Mitt.Lebensm.Hyg. 17,307-15,1926 & Ann.Chim.anal.Chim. appl.9,208,1927).C.A.21,1156,1927).

(1) Add 5 cc. of 5% NH_4OH soln. to a 25 g. sample (finely ground) in a Soxhlet apparatus, and extract 3 to 4 hrs. with $CHCl_3$ or ether.

(2) Distill off the solvent and add 80 cc. of hot water to the residue. Boil for 5 minutes.

(3) Add 1 g. of MgO suspended in a little water and continue boiling for another 5 minutes.

(4) Filter thru a moistened filter and wash 4 to 5 times with 20-25 cc. of boiling water.

(5) Evaporate to dryness, & heat in an oven at 100° for 1 hour.

(6) Add 10 cc. of boiling water to the residue. If it is not entirely soluble filter, wash and again evaporate to dryness.

(7) Take up in about 50 cc. of water and add 5 cc. of 1% $KMnO_4$ soln. Stir for 15 minutes and then decolorize with 3% H_2O_2 containing 1% acetic acid.

(8) Filter, if necessary, evaporate to dryness and heat for an hour at 100° in an oven.

(9) Extract the residue with boiling $CHCl_3$ 4 or 5 times, rubbing the residue with a stirring rod, and filtering thru a small filter.

(10) Evaporate off most of the $CHCl_3$, and then sublime the caffeine according to the directions of Vautier.

(11) Weigh the sublimate after drying 1 hr. at 100°.

B. Purification methods include the use of potassium permanganate.

1. <u>Fendler and Stuber</u>. The determination of caffeine in coffee. (C.A.8,2533,1914 & J.A.O.A.C.4,5[illegible].1914. & Berlin Z.Nahr.Genussm.28,9-30,1914).

(1) After grinding the sample to pass a 1 mm. sieve (later modified to 0.5m) 10 g. are put into a glass stoppered bottle, and 10 cc. of 10% NH_4OH are added. Let stand a short time until the sample is thoroly wet.

(2) 200 g. of $CHCl_3$ are added and shaken vigorously for ½ hr. then chilled in an ice bath.

(3) The contents are filtered on a filter large enough to hold the entire contents of the flask, covering the flask with a watch glass.

(4) 150 g. of the filtrate or more are collected, having the funnel resting directly on the neck of the flask, and the latter surrounded with ice. Assoon as the $CHCl_3$ ceases to run from the funnel the flask is stoppered and weighed.

(5) The chloroform is distilled off on a steam bath, removing the last of the $CHCl_3$ with a current of air.

(6) The residue is digested with 50 cc. of water on the steam bath for 10 minutes and let cool.

(7) 20 cc. (for roasted) & 10 cc. (for green coffee) of 1% $KMnO_4$ are added and the mixture let stand for 15 minutes shaking occasionally.

(8) 2 cc. of 3% H_2O_2(cont'g.1 cc. of glacial acetic acid per 100 cc. free from acetanalid) are added, and then 1 cc. portion at a time until the red color of the $KMnO_4$ is destroyed.

It is then placed on the steam bath and 0.5 cc. portions of H_2O_2 added until the solution becomes no lighter in color.

(9) It is cooled, filtered thru a gooch crucible, washing with cold water.

(10) The filtrate is transferred to a separatory funnel and shaken out with six portions (of 25 cc.each) of $CHCl_3$.

(11) The combined washings are evaporated down to a small vol. and transferred to a weighed beaker, evaporated to dryness, and dried to constant wt. in an oven at 100° for about 20 minutes. The residue of caffeine is then weighed.

(12) To test the purity of the residue, the nitrogen determination is made by the Kjeldahl method and multiplying by the factor 3.464 for caffeine.

2. <u>G.Bonifazi.</u> This method is described under II A. having a double purification using both MgO & $KMnO_4$.

C. The final caffeine residue is sublimed. MgO and $KMnO_4$ not used.

1. <u>J.Burmann</u>. The determination of caffeine in teas and roasted coffees. (C.A.5,1949,1911. & Ann. Fals. 4,99-101).

(1) 5 g. of a finely ground sample is dried to a constant wt. at 100°.

(2) It is shaken 10 min. with 50 cc. of petroleum ether (b.p.60°) and decanted thru a filter paper. This is repeated using 25 cc. of ether. And then the whole sample is thrown on the paper and washed with 25 cc. of ether. Fatty matters may be determined by evaporating off the ether drying the residue and weighing.

(3) The residue on the filter paper is air dried and transferred to a 200 cc glass stoppered flask. 150 g. of $CHCl_3$ are added and then 5 g. of a 10% NH_4OH soln. and shaken for at least 30 minutes.

(4) The extract is filtered thru a wet paper, and the solvent distilled off on a steam bath. Residue is dried and weighed thus estimating the crude caffeine.

(5) The residue is dissolved in a little $CHCl_3$ and transferred to a small sublimation of the following description: Length 18 cm., dia. 18 mm. sealed at the bottom, drawn down to a dia. of 3 mm. 4 cm from each end.

(6) The $CHCl_3$ is evaporated off on a steam bath, dried at 100°.In the lower constriction an asbestos plug is placed.

(7) The tube is kept in a paraffin bath for at least 3 hours at 210° to 240°.

(8) The upper bulb is now filed off and the caffeine crystals washed out with $CHCl_3$ into a weighing bottle, evaporated, dried and weighed. A second sublimation will give absolutely pure caffeine. This method is applicable to all substances containing caffeine.

D. No purification methods used other than adding hot water, filtering and shaking the aqueous solution with chloroform.

1. <u>F. Adams.</u> The determination of caffeine in coffee.
(C.A.5,1470,1911. & Arch.Chem.Micros.2,213-15).

This is essentially a double extraction method using the Katz extractor and adding paraffin to the aqueous solution.

2. <u>A.C.Rottigger.</u> A micro method for the determination of
caffeine in coffee. (C.A.21,2747.1927. &
Z. Untersuch.Lebensm.53,146-51,1927).

3. C.Wolff. The estimation of caffeine in raw coffee. (J.C.S.90,ii,507. & Zeit.Offentl.Chem.12, 186-9,1906).

This method involves soxhlet with $CHCl_3$ instead of the usual shaking. And the nitrogen determination is simply made on the first residue left after evaporating the $CHCl_3$ off from the Soxhlet extraction.

4. E. Vautier. The determination of caffeine in coffee and coffee substitutes and in decaffeinated coffee. ([illegible],804. & C.A.12,1875, 1918. & Mitt. Lebensm.Hyg. 9,54-7,1918).

The author recommends nitrogen determination of the first Crude caffeine obtained, or further purification and then determining N, or by sublimation.

5. Gorter Method. (C.A.4,2817,1910. & A.G. Woodman,Proc. A.O.A.C.,1909; U.S.Dept. Agr. Bur. Chem.Bull.132,134-6,C.A.4,2479,1909).

(1) 11 g. of finely ground coffee is moistened with 6 cc. of water, and let stand ½ hour.

(2) It is then extracted for 3 hrs. in a Soxhlet extractor with $CHCl_3$.

(3) The solvent is evaporated off and the residue of fat & caffeine is treated with hot water.

(4) The solution is filtered thru a cotton plug, and washed with hot water.

(5) The filtrate and washings are made up to 55 cc. 50 cc. are taken and extracted with 4 portions of $CHCl_3$.

(6) The extract is evaporated down to dryness in a tared flask, the caffeine dried at 100° and weighed.

6. E. Leger. Modified process for the determination of caffeine and theobromine in coffee. (Pharm. Chim. 18,57-61,1903 & J.S.C.I.22,1154).

This method has essentially the same procedure as the Gorter method.

III. CAFFEINE EXTRACTED WITH ETHYL ALCOHOL

A. Purification methods include the use of magnesium oxide.

1. <u>Paul and Cownley</u>. (Pharm.J.(iii),18,417,1887. & Allen's Comm. Org. Anal. 5th edition. Vol.7,p 228.).

(1) 5 g. of a finely ground sample are mixed in a mortar with 2 g. of MgO., moistened with hot water again triturated and then dried at 100°.

(2) It is extracted with boiling alcohol and the resulting liquid evaporated nearly to dryness.

(3) The residue is boiled with 50 cc. of water and treated with a few drops of dilute H_2SO_4.

(4). When cold the soln. is shaken with $CHCl_3$ until exhausted.

(5) The combined extracts are washed with a little dil. soln. of NaOH which removes some of the coloring matters.

(6) The $CHCl_3$ is distilled off leaving the caffeine pure and colorless or at the most a slight green tinge.

2. <u>Power and Chesnut</u>. An improved method for the quantitative determination of caffeine in vegetable material. (F.B.Power and V.K.Chesnut.J. Amer.Chem.Soc.41,1298-1306,1919. & Official & Tentative Methods of Analysis.A.O.A.C.2nd. edition, p.234.& C.A.13,2564,1919).

(1). A 10 g. sample (ground to pass a 0.5 mm. sieve) is moistened with a little alcohol and is extracted for 8 hrs. in a Soxhlet extractor.

(2).The extract is added to 10 g. of heavy MgO in 100 cc. of water and evaporated with frequent stirring on a water bath to a dry powder.

(3). It is finely pulverized and made into a paste with hot water, then transferred with hot water to a smooth filter, cleaning the dish with a rubber tipped glass rod. It is filtered into a 1 L. flask and the filter is washed until there is 250 cc. of filtrate.

(4). 20 cc. of 10% H_2SO_4 is added and boiled gently for 30 minutes with a funnel in the neck of the flask.

(5). The soln. is cooled and filtered thru a moistened filter into a separatory funnel, washing the flask and filter with small portions of 0.5% H_2SO_4.

(6). The aqueous soln. is shaken out with 6,25 cc. portions of $CHCl_3$.

(7). The combined extract of $CHCl_3$ is shaken in a separatory funnel with 5 cc. of 1% KOH. The $CHCl_3$ is filtered into an Erlenmeyer flask; and the KOH soln. is washed with 2,10 cc. portions of $CHCl_3$, adding to the extract.

(8). The final $CHCl_3$ extract is distilled on the steam bath to a small vol., and then transferred with $CHCl_3$ to a small weighed beaker, carefully evaporated, dried for 30 min and weighed.

(9). To test the purity of the caffeine residue, the N. determination may be made, and multiply by the factor 3.464 for caffeine.

B. Purification methods include the use of potassium permanganate.

1. (Mrs.) S.Cobert. Determination of caffeine in tea and coffee.(C.A.21,363,1927. & Ann. Fals.19, 586-94,1926).

This method involves shaking the sample (previously wet with ammonia) with 4 portions of alcohol. It differs from the usual methods in that after each shaking with alcohol the mixture is cenrifuged. The dried residue after distilling off the solvent is treated with hot water, filtered, treated with 1% $KMnO_4$ etc. in the usual manner (Fendler and Stuber method).

IV. CAFFEINE EXTRACTED WITH CARBON TETRACHLORIDE.

A. Purification methods include the use of Magnesium oxide.

1. <u>I.F.Camilla and C.Pertuse.</u> The determination of basic xanthines in cocoa, tea, coffee and their derivatives. (C.A.6,3479, 1912. & Giorn.Farm.Chim. 61,337-44).

With this method the finely ground sample is extracted with CCl_4 in a Soxhlet extractor for several hrs. The solvent is then shaken with water acidulated with H_2SO_4. This removes the theobromine, leaving the caffeine in the CCl_4, which is evaporated off and the residue taken up in water. MgO is added and the mixture evaporated down to dryness, powdered and transferred to a flask under a reflux condenser and extracted 4 to 5 times with boiling $CHCl_3$. The solvent is distilled off, and the caffeine residue dried to constant weight and weighed.

B. Purification methods include the use of potassium permanganate.

1. <u>K.Lendrich and E.Nottbohm.</u> A method for determining caffeine in coffee (C.A.3, 1433,1909.& Staat.Hyg.Inst. Hamburg.Z.Nahr.-Genussm. 17,241,-65).

(1). A 20 g. sample of coffee (fineness of 1 mm.) is moistened with 10 cc. of water, and let stand with occasional stirring for ½ hour. After transferring to a paper capsule it is extracted for 3 hrs. with CCl_4.

(2). 1 g. of paraffin is added to the CCl_4 extract, the solvent distilled off and the residue extracted with boiling water (50,25,25,25 cc. portions).

(3). The combined aqueous extract is filtered thru a moistened filter, the latter washed, and the filtrate treated with 10 -20 cc. of 1% $KMnO_4$.

(4). After standing 15 min. the Mn. is ppt. as hydrated oxide by means of a little H_2O_2 (contg. 1% CH_3COOH). It is heated 15 minutes on a steam bath, filtered, washed, evaporated to dryness, and finally dried at 100°.

(5). The residue is extracted with warm $CHCl_3$ by decantation. The $CHCl_3$ is distilled off, the residue dried for 30 minutes at 100° and weighed.

V. CAFFEINE EXTRACTED WITH ETHER.

A. Impurities separated only by treating residue with boiling water and filtering.

1. <u>E. Vautier.</u> Determination of caffeine in coffee.
(Ann.Chim. Analyt.,23,207,-10,1918.,
& J.S.C.I.37,711).

(1). 5 g. of a finely ground sample is moistened with 5 cc. of ammonia in an extraction thimble and extracted for 4 hours in a Soxhlet apparatus with ether.

(2). The extract is evaporated, and the residue treated with boiling water. The soln. is filtered and the insoluble material washed with boiling water until 150 cc. have been collected.

(3). The filtrate is evaporated, and the residue dissolved in a small quantity of boiling water. The soln. is again filtered, the insoluble portion washed, and the filtrate evaporated to a small vol. as before.

(4). The residue thus obtained is heated and the sublimed caffeine collected on a watch glass and weighed.

EXPERIMENTAL

I. METHODS

The methods of caffeine analysis employed in this work are, as stated in the introduction, the official Power-Chesnut method and the tentative Fendler-Stuber method which are given in the Official and Tentative Methods of Analysis of the Assoc. of Official Agr. Chemists, second edition, p. 334-335.

The procedure for the Power-Chesnut method was followed exactly as given, and each step was performed very thoroly when analyzing decaffeinated coffee.

Procedure in the Fendler-Stuber method.

In the majority of the analyses made by the Fendler-Stuber method a few minor modifications were adopted, which were thought to add to the accuracy and convenience of the method. Because of the preference of this method over the Power-Chesnut method, for the caffeine analysis of decaffeinated coffee,(the reasons for which will be given later), it will be well to give a brief discussion and description of the various steps of the method..Steps No. 1,11,12 and 13 are also applicable to the Power-Chesnut method.

1. The sample is ground to pass a 0.5 mm. sieve. The necessity of grinding to this degree of fineness is shown in table No. 1. It is difficult to grind coffee to this degree of fineness in most mills because of its oily nature.

The Wiley mill will not do satisfactorily if it is desired to grind finer than the 1.0 mm. size. A pebble mill

works well if considerable time is available. A small Enterprise #24 coffee mill was used to do most of the grinding. And it was found that by setting the plates quite closely together the most of the sample is ground to an even greater degree of fineness than that which will just pass the 0.5 mm. sieve. Hence it was found unnecessary to run the samples thru a sieve after grinding.

2. A 10 g. sample is wet with 10 cc. of 10% NH_4OH. This is a large enough sample even for the analysis of decaffeinated coffee. A larger sample results in more inconvenience in handling with no greater accuracy in the results.

TABLE NO. I.

THE EFFECT OF FINENESS OF GRINDING UPON THE EFFICIENCY OF EXTRACTION OF CAFFEINE FROM DECAFFEINATED COFFEE.

(FENDLER - STUBER METHOD)

Sample.	% of caffeine by nitrogen determination.		
	1.0 mm. sieve.	0.5 mm. sieve.	
Kaffee Hag No. 1.	0.0122 %	0.0145 %	
" " " "	0.0121	0.0133	
" " " "	0.0106	0.0147	
" " " "	0.0100	0.0149	
" " " 8.	0.0173	0.0183	(Power-Chesnut method)
" " " 9.	0.0186	0.0161	" " "
Average-	0.0135 %	0.0154 %	

TABLE NO. 2.

COMPARATIVE RESULTS OBTAINED BY USING WATER INSTEAD OF 10% NH_4OH TO WET THE SAMPLE BEFORE EXTRACTING WITH CHLOROFORM

(Fendler-Stuber method).

Sample.	% caffeine by nitrogen determination.	
	Wet with water.	Wet with ammonia.
Kaffee Hag No. 1a	0.0147 %	0.0145 %
" " " "	0.0149	0.0153
" " " 1b.	0.0106	0.0122
" " " "	0.0100	0.0121
" " " 2.	0.0108	0.0091
" " " 4.	0.0257	0.0246
" " " 5.	0.0340	0.0330
" " " 6.	0.0219	0.0130
" " " 7.	0.0108	0.0111
" " " 8.	0.0162	0.0171
" " " 9.	0.0122	0.0130
" " " 10.	0.0127	0.0116
" " " 11.	0.0129	0.0133
" " " 12.	0.0214	0.0224
Sanka Coffee 1.	0.0224	0.0208
Average-	0.0167 %	0.0164 %

It is preferred to wet the sample with 10% NH_4OH instead of water, not because of obtaining higher or more accurate results as is shown in Table No. 2, but because of a greater convenience in filtering (the residue being in a more granular state), and the securing of a more colorless aqueous solution in the next step.

3. Approximately 150 g. of comm. redistilled $CHCl_3$ are added to the wet sample in a suitable glass stoppered bottle. As the entire $CHCl_3$ is used, it is unnecessary to weigh the solvent, and a mark can be made on the side of the bottle so that in later analyses the bottles can be filled very rapidly. A rubber band is placed around the bottle and over the glass stopper to hold it in place while shaking.

It was found that ordinary comm. $CHCl_3$ used possessed no caffeine destroying power as is shown on table No. 3. But in order to safeguard the possibility of securing $CHCl_3$ of varying composition all of this solvent used was redistilled in the presence of caffeine.

4. The bottle is shaken in a shaking machine for at least ½ hour (usually 1 hour). For absolutely thoro extraction of the caffeine, Soxhlet extraction with $CHCl_3$ is recommended. However this will result in a still more impure final caffeine residue, than when shaking is used.

5. By carefully holding the stopper partially in place the $CHCl_3$ extract is poured into a 4 in. funnel having a small cotton plug in the neck (instead of the usual 24 cm. filter paper). Thus the extract is filtered into a 200 cc. Florence flask without getting practically any of the residue into the funnel. The residue left in the bottle is shaken out with 3

TABLE NO.3

COMPARISON OF ORDINARY COMM. CHLOROFORM WITH ALCOHOL FREE, REDISTILLED IN THE PRESENCE OF CAFFEINE CHLOROFORM IN EFFICIENCY OF CAFFEINE EXTRACTION.

(Fendler-Stuber method)

	% of caffeine by nitrogen determination	
Sample.	Commercial $CHCl_3$	Redistilled $CHCl_3$
Kaffee Hag No. 1.	0/0121 %	0.0122 %
" " " 2.	0.0111	0.0091
" " " 4.	0.0239	0.0246
" " " 5.	0.0299	0.0330
" " " 6.	0.0212	0.0130
" " " 7.	0.0118	0.0111
Average-	0.0183 %	0.0182 %

TABLE NO. 4.

DIFFERENCE IN USING AN ALIQUOT OR THE ENTIRE AMOUNT (AND WASHING THE FIRST CHLOROFORM EXTRACT.

(Fendler-Stuber method)

	% of caffeine by nitrogen determination	
Sample	Aliquot.	Entire
Kaffee Hag No. 1a.	0.0145 %	0.0133 %
" " " "	0.0133	0.0145
" " " 1b.	0.0106	0.0115
" " " "	0.0100	0.0114
" " " 1c.	0.0122	0.0121
" " " 4.	0.0246	0.0251
" " " 5.	0.0330	0.0336
" " " 6.	0.0190	0.0212
" " " 7.	0.0111	0.0108
" " " 2f.	0.0091	0.0132
Average-	0.0158 %	0.0167 %

approx. 15 cc. portions of $CHCl_3$. The comparative results shown on table No. 4 prove that the same and just as accurate results are obtained when the entire extract is used, as when an aliquot is taken. This simplifies and slightly shortens the method by eliminating two weighings and the care in cooling the extract in ice to prevent evaporation of the solvent, and only requires the thoro washing of the residue. The use of a cotton plug instead of a filter paper reduces the possibility of a slight loss of caffeine by being retained by the filter.

6. The $CHCl_3$ is distilled off on a steam bath. The addition of a couple small beads to the $CHCl_3$ greatly facilitates uniform boiling and prevents bumping and subsequent loss of material. The last portion of $CHCl_3$ is driven off with a current of air.

7. 80 cc. of hot water are added, digested on the steam bath for 10 minutes with occasional shaking, and then cooled to as near 20° C. as possible.

8. 20 cc. of 1% $KMnO_4$ are added and let stand for 15 minutes. Then 3 cc. of 3% H_2O_2 (free of acetanilide, and containing 1 cc. of glacial acetic acid per 100 cc. of solution) are added and the flask shaken. 1 cc. portions of the H_2O_2 are added until the $KMnO_4$ has been all reduced and precipitated as MnO_2, and the entire disappearance of the red color to an almost colorless solution. The flask is now put on a steam bath, and after being thoroly heated o.5 cc. portions of the H_2O_2 solution are added until the liquid becomes no lighter in color. Digestion on the steam bath is continued for 15 minutes.

9. After the contents of the flask have cooled it is filtered thru a No. 50 moistened filter paper, into a 250 cc.

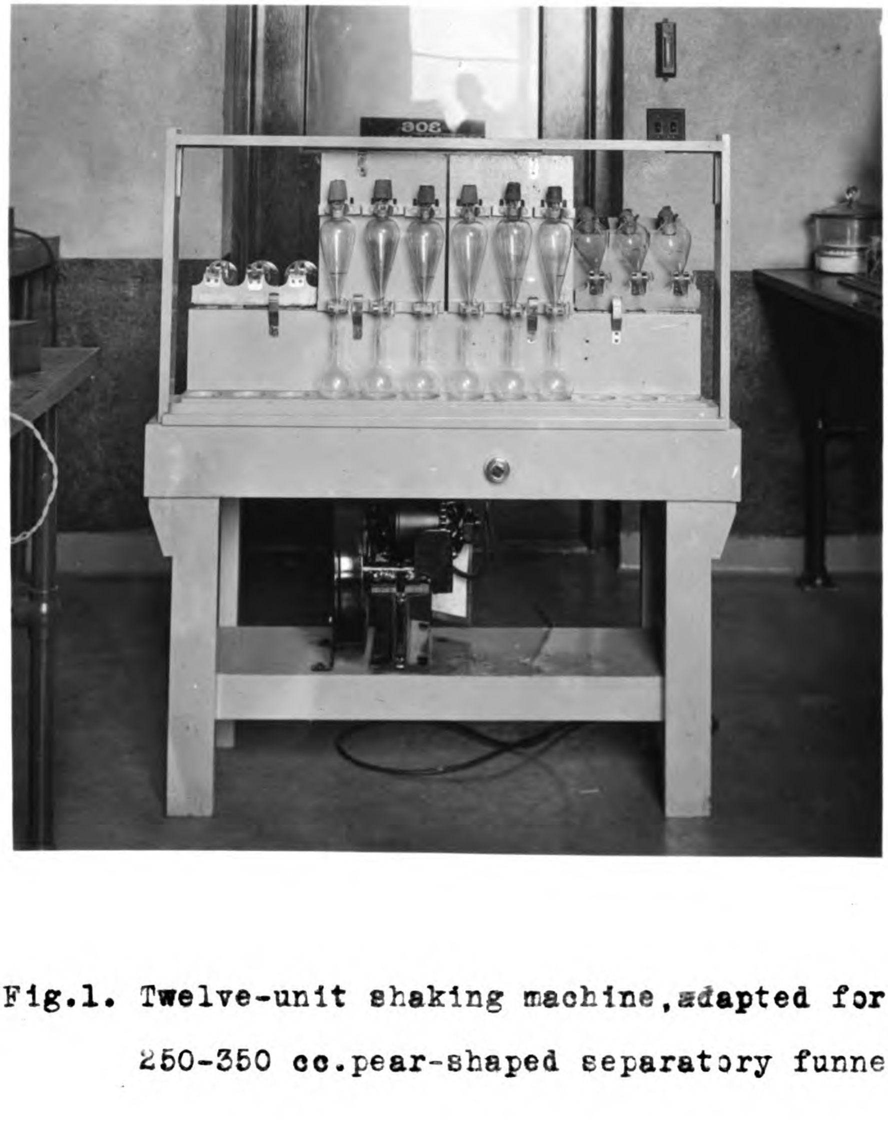


Fig.1. Twelve-unit shaking machine, adapted for 250-350 cc. pear-shaped separatory funnels.

pear shaped separatory funnel (into which has been added the first 25 cc. portion of $CHCl_3$ to shake out the aqueous solution after filtration is completed). The flask and filter are washed at least three times with small portions of cold distilled water.

10. The aqueous solution is shaken out with six 25 cc. portions of $CHCl_3$. For this purpose a 12 unit shaking machine is used, especially adapted for this work and which is shown in the accompanying illustration. Each portion is shaken for about two minutes. The portions of $CHCl_3$ are filtered thru small filters when running out of the separatory funnels as a safeguard to prevent any sediment and filmy material as well as water from getting into the $CHCl_3$ extract. This extract is run into the same 200 cc.flasks which contained the aqueous solution (cleaned and dried).

11. The $CHCl_3$ is again distilled off on the steam bath, and the last trace of the solvent is driven off with a gentle current of air.

12. In samples where the weight of the residue is desired for comparison, the residue is transferred into a small weighing bottle with several small portions of $CHCl_3$, the solvent is carefully evaporated, and the residue dried in an oven at 100° C. for about 30 minutes. It is then cooled in a dessicator and weighed. If the caffeine is to be sublimed, the residue is transferred directly into the lower half of the sublimation tube, dried and weighed (with a counterpoise).

13. The amount of caffeine in the final residue is determined in two ways in the analyses made thus far:

A. By the nitrogen determination.

(1). Using a micro Kjeldahl method, and apparatus.

(2). Using the ordinary macro Kjeldahl appartus, but with micro methods.

(3). The employment of direct nesslerization.

B. By subliming the caffeine.

(1) Weighing the sublimed crystals.

(2) Determining nitrogen on the sublimed caffeine.

The discussion of these two methods will now be taken up in detail.

A. Methods of determining nitrogen in the caffeine residue.

1. The nitrogen determination by a modification of Pregyls micro Kjeldahl method. (Pregyl, F. Quantitative Organic Microanalysis, 1924, translated from the German edition 2, p. 94-102).

This method is essentially a refinement of the official Kjeldahl-Cunning-Arnold procedure, using steam distillation in a special apparatus adapted for micro analysis only. However the method was modified and a special apparatus was made which resulted in greater simplicity and convenience as well as a wider adaptability for the analysis of the residue from decaffeinated coffee as well as from ordinary coffee than is possible with Parna's and Wagner's modification of Pregyl's apparatus. The procedure is as follows:

(a). 10 cc. of a digestion mixture (containing 2 cc's of conc. H_2SO_4, 1.0 g. of K_2SO_4 and 0.02 g. of $CuSO_4$ per 10 cc's.) is added to the 200 cc flask containing the caffeine residue after evaporating off the $CHCl_3$. It takes only a few minutes to evaporate the water, and such a mixture is very convenient to use, and insures the same mixture in every sample thus making possible more constant blank determinations. A low flame is used in the digestion, and run a few minutes after the mixture is entirely clear. It usually does not take over 15 minutes for complete digestion.

2. 30-50 cc. of freshly boiled distilled water are added to the digested mixture after it is cool. The flask is stoppered if it is not to be distilled immediately.

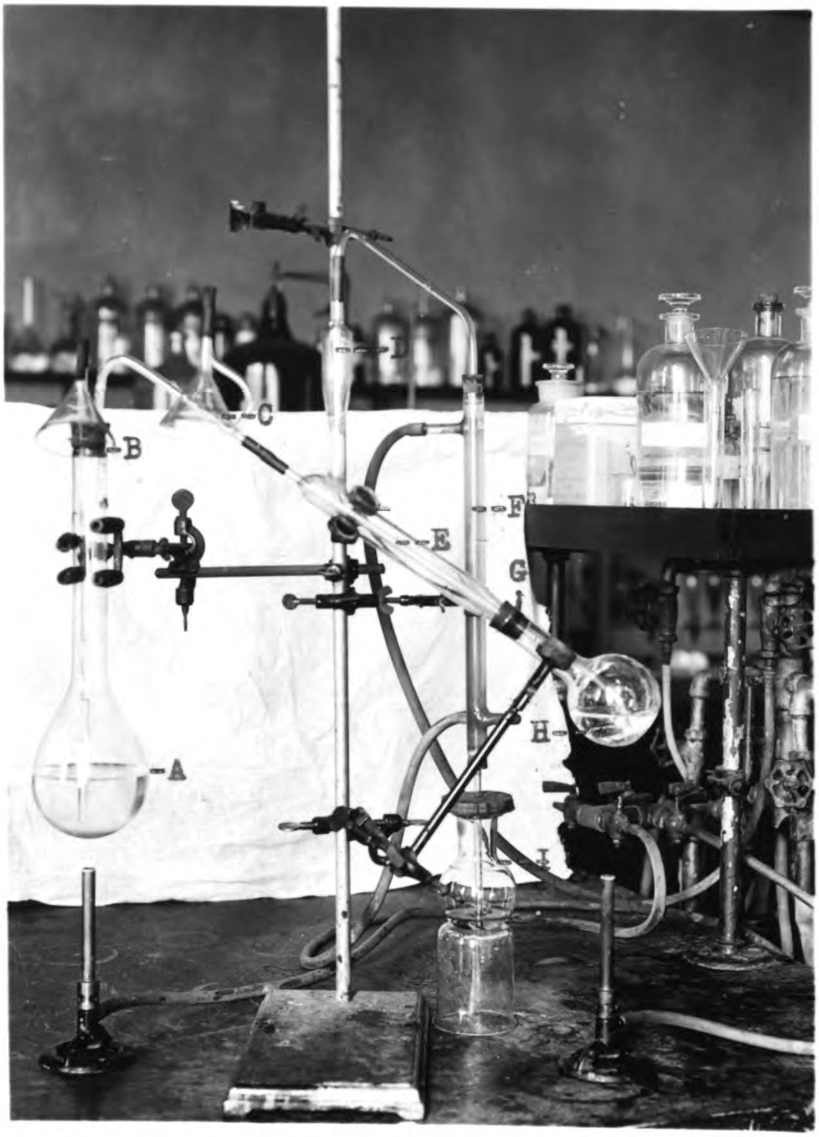


Fig.2. Micro-Kjeldahl distillation apparatus.

A-500 cc.Kjeldahl flask used as a steam generator.
B-Funnel thru which water is added.
C-Funnel thru which NaOH is added.
D-Safety trap made from a 25 by 200 mm. pyrex test tube.
E-made from a 25 by 200 mm. pyrex test tube.
F-Small condenser made of pyrex tubing.
G-Rubber stopper for joint.
H-200 cc. flask containing digested sample.
I-150 cc.receiving flask containing N/100 acid.

3. The flask is now placed into position on the steam distillation apparatus made especially for this work, the design of which can best be studied by observing the accompanying illustration. The main body of this apparatus is made from a 25 mm. by 200 mm. pyrex test tube, which is drawn out slightly on the open end so that a rubber stopper can be fitted and still be small enough to fit inside the neck of the 800 cc. flasks used for digestion. The bulb on the upper portion, which is connected with the upper portion of the condenser tubing is also made from the same size pyrex test tube. The condenser tubing, as well as the tubing running from the steam generator (560 cc. Kjeldahl flask) down into the bottom of the digestion flask is made of 6 mm. pyrex tubing. In fact, the entire apparatus is made of pyrex glass.

10 cc. of N/100 H_2SO_4 are measured from 10 cc. micro burettes into a 150 cc. wide mouth flask, 10 drops of methyl red indicator added, and placed under the receiving end of the small condenser. A large cork is put onto the tube of the condenser so that it just rests on the top of the receiving flask when in position.

4. Heat is applied to the steam generator, and then about 13 cc. of 42 % NaOH are added to the digested mixture thru the funnel connected with the tube about half way between the steam generator and where it enters the test tube.

5. Heat is applied to the steam generator and the digestion flask in such a manner that about 100 cc. of liquid distill over in 12 minutes, and yet the volume of liquid in the digestion flask remains practically the same.

The receiving flask is now lowered so that the end of the condenser is about 2 cm. above the liquid. The end of the condenser is washed down with a small stream of water. Distillation is continued at an increased rate for about 2 minutes. The funnel (thru which the alkali is added) is raised before the heat is removed, to prevent suction.

6. The contents of the receiving flask are titrated with N/100 NaOH from 10 cc. micro buretes. The end point is taken when the methyl red becomes a permanent canary yellow color.

Nearly every day, or every time a group of samples are run a blank determination is made by using the same digestion mixture, etc. as with samples and a small bit of filter paper to digest. This serves as a safe check on the apparatus and the standard solutions. The blanks usually run from 0.20 to 0.25 cc of N/100 H_2SO_4. But they will not vary more than 0.05 cc. for one particular time. The No. of cc's N/100 NaOH used subtracted from the 10 cc's of N/100 H_2SO_4 (minus the blank) gives the No. of cc's which neutralized the NH_3 driven over. And 100 cc. of N/100 H_2SO_4 equals 0.000485 gms. of caffeine. This multiplied by 10 (when the entire sample is used) gives the percentage of caffeine in the 10 gm. sample.

The N/100 H_2SO_4 and NaOH are standardized against N/100 HCl. which is made from constant boiling point HCl. They are checked against freshly prepared N/100 HCl from time to time to insure them being correct. This is very important when analyzing samples containing an average of less than one milligram of nitrogen.

2. Ordinary Macro Kjeldahl procedure.

In many laboratories a micro nitrogen determination apparatus similar to that just described will not be available. However quite accurate and reliable results can be obtained with the ordinary Macro Kjeldahl apparatus using 300 or 500 cc. Kjeldahl flasks.

TABLE NO.5.

COMPARATIVE ACCURACY OF THE ORDINARY MACRO KJELDAHL METHOD OF NITROGEN DETERMINATION IN SAMPLE CONTAINING ONE MILLIGRAM OR LESS OF NITROGEN.

(Using a digestion mixture containing 2 cc. of conc.H_2SO_4; 1 gm.K_2SO_4; and 0.02 gm. $CuSO_4$; and adding approx. 15 cc.conc. NaOH before distilling. 500 cc. Kjeldahl flasks are used).

---

1. Blank determinations showing the necessity of thoroly steaming out the outfit just before using. (cc. of N/100 H_2SO_4 used).

Not steamed at all.	Steamed 15 min. just before using.(used the day before)	Steamed 30 min. 3 hrs before using.	Steamed 1 hr. just before using.	After running outfit intermittently for a day.
2.70 cc.	0.77 cc.	1.23 cc.	0.55 cc.	0.43 cc.
1.71	0.50	0.63	0.59	0.50
0.73	0.45	0.88	0.86	0.48
2.98	0.50	1.21	0.74	0.58
5.00	0.37	0.75	0.84	0.51
1.09		0.66	0.62	0.46
1.95		0.55	0.68	0.35
0.60		0.71	0.76	
0.50			0.65	
Ave. 1.67 cc.	0.52 cc.	0.84 cc.	0.70 cc.	0.49 cc.

2. Caffeine in samples of Kaffee Hag and in pure caffeine determined by both the macro and the micro methods of nitrogen deter. and multiplying by the factor 3.464.

Sample.	By the macro-Kjeldahl method. Steamed thoroly.	Steamed 30 min. just before using.	Steamed 1 hr.3 hrs. before using.	By the micro-Kjeldahl method (Pregl's modification)	
2.02 mg.caff.	2.02 mg.	1.86 mg.	1.91 mg.	2.02 mg	Blank deter. on micro app.
" " "	2.05	1.87	2.09	2.03	
" " "	2.11	1/84	2.08	2.01	
" " "	1.99	2.01	1.99	2.06	
		1.82			0.23 cc
Ave.	2.04	1.88	2.02	2.03	0.16
					0.20
Kaffee Hag					0.19
No. 16	0.0126 %			16. 0.0147	0.21
17	0.0134			17 0.0148	0.19
18	0.0113			18 0.0135	
23	0.0273			23 0.0259	
23	0.0280				
24	0.0165			24 0.0153	
24	0.0148				
25	0.0136			25 0.0164	
25	0.0136				
Average -	0.0167 %		Average -	0.0166 %	

The comparative accuracy of the ordinary Kjeldahl procedure, when the same digestion mixture, N/100 standard solutions etc. are used as with the micro procedure, can be observed in the results obtained which are given in table No. 5.

It is of course taken for granted that a nitrogen deter. apparatus will be steamed a short time.

These results show the absolute necessity of thoroly steaming the apparatus for at least one hour, or until fairly constant black determinations are obtained which do not vary by more then 0.20 of a cc. of N/100 acid. And this must be done immediately before using. If these precautions are not followed the variation in blank determinations will oftimes be greater than the acid neutralized by the ammonia given off by the sample.

3. Direct Nesslerization.

Table No. 6 shows the results obtained by using direct nesslerization (Koch and McMeekin method. J.Am.Chem.Soc. 46,2066,1924), in the determination of nitrogen in the caffeine residues. While this method would no doubt be applicable as a rapid method for control work, for extreme accuracy and dependability it does not compare with the micro-Kjeldahl method.

TABLE NO. 6.

COMPARATIVE RESULTS OF NITROGEN DETERMINATIONS MADE BY DIRECT NESSLERIZATION AND BY MICRO KJELDAHL METHODS.

Sample.	Percentage of caffeine by direct nesslerization	Percentage of caffeine by micro-Kjeldahl.
Kaffee Hag No. 1	0.0175 %	0.0121 %
" " " 2	0.0096	0.0111
" " " 4.	0.0189	0.0251
" " " 5.	0.0247	0.0226
" " " 6.	0.0148	0.0212
" " " 7	0.0095	0.0111
Average-	0.0158 %	0.0173 %

Fig.3. Sublimation apparatus for the quantitative determination of caffeine.

A-Paraffin bath.
B-30 by 200 mm.test tube.
C-6 mm.pyrex tube with a 1 mm. opening in the end.
D-Upper half made from a 25 by 200 mm.pyrex test tube.
E-Tight fitting ground glass joint.
F-Lower half made from a 25 by 200 mm. pyrex test tube.

B. Sublimation of caffeine.

After making several types of tubes for the quantative siblimation of caffeine in the endeavor to get as near 100% recovery as possible and at the same time prevent the contamination of the sublimed crystals by impurities subliming along with them, the apparatus shown in the accompanying illustration was found to be the most satisfactory.

The sublimation tube is made from a 25 by 200 mm. pyrex test tube, which is drawn out slightly in the middle, cut in two. The broken end of the upper portion is enlarged and fitted over the constricted end of the lower portion. The glass joint thus made is ground in until a tight fit is assured. However it is made tapering enough so that the upper portion may be lifted off without the lower part coming with it. The outside diameter of the joint is not any larger than the rest of the tube, and is about 30 mm. in length. The lower tube is 100 mm. in length. The upper tube is also 100 mm. in length, but it also has a 6 by 80 mm. tube sealed onto the upper end after drawing the top of the test out quite sharply to a small diameter. The top of the small tube is heated until but a very small opening remains. This was found more satisfactory then putting a plug of cotton in the end. Both tubes are thoroly dried before being used.

The caffeine residue is washed into the lower tube with 3 to 4 about 3 cc. portions of $CHCl_3$. The solvent is carefully evaporated off on the steam bath.

When there is but 1 or 2 cc's of $CHCl_3$ left, the tube is rotated in an almost horizontal position so that the residue will be deposited in a very thin layer around the inside of the tube. This was found to somewhat hasten the complete sublimation of the alkaloid. The last traces of $CHCl_3$ are removed by a gentle current of air.

3. The two tubes are now put together and placed into a 30 by 200 mm. test tube. A rather loose fitting cork is put into the end of the outside tube, the small end of the upper sublimation tube extending about 20 mm. above the cork.

4. The assembled apparatus is placed into a paraffin bath so that the joint between the two tubes is at least 20 mm. below the surface of the paraffin. The bath is kept at a temperature of 180 to 190° C. for about 10 hours. This temperature was found to be the highest which could be safely maintained and prevent other materials from subliming with the caffeine to any extent.

Practically all references found regarding the sublimation of caffeine for quantitative analysis (J.Burmann. Ann. Fals., 4,99-101 & E. Phillippe, Mitt. Lebensm. Hyg.6, 177-91,228-47,1915. & J.Burmann,Bull. Soc. Chem.,7,233), specify a temperature of 230 to 240° C. It was found if the temp. was allowed to remain above 190° C. for any length of time the crystals of caffeine became contaminated with other materials which sublimed above this temp. And there appeared small droplets of brownish material just above the caffeine crystals. Even at a temp. of 185° some samples showed a discoloration of the crystals, but to a much less degree, and there was an entire absence of the droplets of brownish substance. The comparative difference in wt. of the sublimed caffeine at these different temperatures can be studied on table No. 7 . Tho it was not tried out, perhaps a still more pure product could be secured if the temperature was held at 175° for at least 20 hours. To prove that all of the caffeine had sublimed after the 10 hours at about 185° C., the caffeine was washed out of the upper portion of the apparatus, reassembled and the temperature maintained at 235 to 240° for about 2 hours. No crystals whatsoever were observed in the upper tube.

5. After sublimation is complete the upper tube is immediately removed, cooled in a dessicator and weighed with a counterpoise. The caffeine is thoroly washed out of the tube into the 200 cc. flask,if the nitrogen determination is to be made. The empty tube dried in an oven at 100° for 15 to 20 minutes, cooled and reweighed. In the majority of the samples the difference in wt. is a little over 1 mg. Hence the weighing must be done very accurately.

6. It is realized that gravimetric methods are as a rule hot as accurate for micro work as volumetric and colormetric methods. However much can be learned by the sublimation method, especially if a nitrogen determination is made on the sublimed caffeine, which gives results that are more nearly correct of any method or combination of methods yet tried out for the caffeine analysis of decaffeinated coffee. By running the nitrogen determination on the residue left in the lower tube after sublimation,the amount of nitrogen from non-caffeine substances can be estimated, and gives us the error involved when the results are based upon the nitrogen determination of the final caffeine residue.

7. In the endeavor to have the caffeine sublime in as pure state as possible, or in other words to have the weight

of the sublimed caffeine check with the nitrogen determination, magnesium oxide, sodium carbonate and quartz sand were each separately mixed with the residue in the subliming tube with the idea of holding the impurities from subliming with the caffeine. The results which appear on table No. 7 indicates that every one does more harm than good, as it prevents a small amount of the caffeine from subliming and does not increase the purity of the sublimed crystals. Na_2CO_3 offered the most possibility by slightly increasing the rate of sublimation at a minimum temp. with no appreciable loss of caffeine. However the final product was no better than when it was sublimed alone.

TABLE NO.7.

THE EFFECT OF ADDING SMALL AMOUNTS OF MAGNESIUM OXIDE, SODIUM CARBONATE OR IGNITED QUARTZ SAND TO THE CAFFEINE RESIDUE BEFORE SUBLIMING FOR THE PURPOSE OF PREVENTING IMPURITIES FROM SUBLIMING WITH THE CAFFEINE CRYSTALS

Sample.	Not sublimed. (by N.deter.)	Sublimed alone. By wt. of crystals.	Sublimed alone. By N.deter. of crystals.	Sublimed in the presence of 0.2 g. MgO. By wt. of crystals	Sublimed in the presence of 0.2 g. MgO. By N. deter.	Sublimed in the presence of 3.0 g.quartz sand. By wt.of crystals.	Sublimed in the presence of 3.0 g.quartz sand. By N. deter.
		(Temp. 230-240° C. for 6 hrs.)					
Kaffee Hag No.							
13.	0.0179%	0.0700%	0.0104%	0.0640%	0.0096%		
14.	0.0200	0.0540	0.0127	0.0110	0.0107		
15.	0.0145		0.0185	0.0190	0.0076		
16.	0.0167			0.0120	0.0050		
18.	0.0145	0.0340	0.0093				
Average	0.0167%	0.0526%	0.0115%	0.0265%	0.0081%		
Pure caffeine.	0.0020 g.	0.00210g.	0.00183 g.	0.00200g.	0.00188g.	0.00200g.	0.00175
"	"	0.00220	0.00192	0.00190	0.00160	0.00270	0.00157
"	0.0030			0.00310	0.00242	0.00340	0.00254
"	0.0010	0.00100	0.00105	0.00150	0.00075		

(Temp. 180-190° C. for 10 hrs.)

Sample.	Not sublimed. (by N.deter.)	Sublimed alone. By wt. of crystals.	Sublimed alone. By N.deter. of crystals.	Sublimed in the presence of 0.5 g. + 1.0 g.Na_2CO_3 By wt.	Sublimed in the presence of 0.5 g. + 1.0 g.Na_2CO_3 By N. deter.
Pure caffeine.	0.0010 g.	0.0012 g.	0.00093 g.		
"	"	0.0010	0.00100		
"	0.0020	0.0021	0.00194	0.0019 g.	0.00198 g.
"	"	0.0022	0.00196	0.0022	0.00187
"	"	0.0022	0.00194	0.0022	0.00212
"	"	0.0022	0.00200		
"	"	0.0022	0.00198		
SankaC.3,	0.0461	0.049 %	0.0403 %	0.045 %	0.0437 %
Kaffee Hag No.					
32.	0.0114 %	0.011 %	0.0063 %	0.021 %	0.0051 %
33	0.0143	0.014	0.0102	0.018	0.0087
34	0.0150	0.020	0.0099	0.015	0.0088
35	0.0123	0.012	0.0099	0.016	0.0095
36	0.0129	0.015	0.0082	0.007	0.0067
Average	0.0186 %	0.0185 %	0.0141 %	0.0203 %	0.0121 %

Precautions observed to secure the greatest accuracy;

1. All apparatus used is made of pyrex glass, and also all glass ware as far as possible.

2. All distilled water used is boiled about 15 minutes immediately before using, and it is tested often to insure its being neutral.

3. Comm. $CHCl_3$ is redistilled in the presence of a small amount of caffeine, to remove any possible caffeine destroying power it may have.

4. Heavy MgO,U.S.P. is used at all times in the Power-Chesnut method.

5. The H_2O_2 used in the Fendler-Stuber method is acetanilide free.

6. The samples are ground as finely as possible. At least 95% will go thru a 0.5 mm. sieve.

7. The samples are moistened thoroly before extraction in either method.

8. N/100 H_2SO_4 and NaOH are standardized against N/100 HCl, which is made from constant boiling point HCl. These solutions are checked very often against freshly prepared N/100 to insure them being correct. And also nitrogen determinations are made often on usually 2 mg. samples of caffeine of known purity as another check on the procedure.

9. 10 cc. micro burets are used for measuring out the acid and in titrating with the alkali.

10. Blank determinations are always made when a number of samples are run, and the distillation apparatus is always steamed at least 15 minutes before a sample is distilled.

EXPERIMENTAL

II. ANALYTICAL RESULTS

The tabulated data, followed in some cases by brief conclusions drawn from the results obtained, is given in the following order:

A. Table No. 8. *Percentage of caffeine in samples of Kaffee Hag*.

This includes the analysis of 35 samples of Kaffee Hag, as well as 5 samples of Sanka Coffee. It also give the average % of caffeine removed.

B. Table No. 9. *Determination of the percentage of caffeine removed in samples of Kaffee Hag. Analyzed by the Fendler-Stuber method*.

Analysis is made of 7 samples of coffee in 3 stages: The green and undecaffeinated coffee, roasted but not decaffeinated, and the roasted and decaffeinated coffee. Moisture determinations are made and the results are based upon the moisture free basis.

C. Table No. 10. *Comparative results of caffeine analysis of decaffeinated coffee by weight of the caffeine residue, and by nitrogen determination. Analyzed by the Fendler-Stuber and the Power-Chesnut methods*.

This table gives the analysis of 21 samples. And the average results obtained are very conclusive in showing the importance of the nitrogen determination.

D. Table No. 11. *Comparative results of caffeine analysis obtained by sublimation and by the nitrogen*

determination of the caffeine residue.

The results contained in this table and in table No. 13 are considered by the writer to be the most important of any given in this thesis. For while it was easy to show the necessity of the nitrogen determination, it was not so easy to show how much of the nitrogen thus determined came from caffeine. These results are very consistent and give the % of nitrogen which really comes from the caffeine alone, and not from the impurities.

E. Table No. 12. Distribution of nitrogen, calculated as percentage of Caffeine, in the sublimed crystals and in the remaining residue.

F. Table No. 13. Comparative results of the nitrogen determinations, calculated as percentage of caffeine, in the residue from the first $CHCl_3$ extract, and the residue from the final $CHCl_3$ extract with and without the oxidation step of adding 1 % $KMnO_4$ to the aqueous solution.

This table clearly shows the amount of nitrogenous substances other than caffeine which is extracted in the Fendler-Stuber method.

G. Table No. 14. Effect of further purification of the final caffeine residue by adding 20 cc. of water and 0.2 g. MgO, digesting a short time on a steam bath, filtering and shaking the aqueous solution with $CHCl_3$ (Fendler-Stuber method).

H. Table No. 15. Comparative results in adding 10 g. of MgO to the aqueous solution, digesting on the steam bath, filtering and cooling before oxidizing

with $KMnO_4$ (Fendler-Stuber method).

I. Table No. 16. Comparative results obtained in shaking out the final chloroform extract with 1 % KOH, as in the Power-Chesnut method. (F.& S.method)

Tables No. 14,15 and 16 give some interesting results, But they are of questionable value except perhaps the method employed in table No. 16.

J. Table No. 17. Loss of caffeine in the analysis, determined by adding 2 g. of pure caffeine to the residue left after the first extraction and repeating the analysis. (Both methods).

K. Table No. 18. Comparative results obtained when extracting by Soxhlet apparatus with alcohol in the Fendler-Stuber method, and by shaking with $CHCl_3$ in the Power-Chesnut method.

This is a very good table showing the comparison between the two methods, and especially the two methods of extraction.

L. Table No. 19. The comparative effectiveness of carbon tetrachloride as a solvent for caffeine extraction in Kaffee Hag (Fendler-Stuber method).

This also gives the ratio of distribution of caffeine in water and CCl_4.

M. Table No. 20. Comparative results obtained by using Soxhlet chloroform extraction instead of shaking, in the Fendler-Stuber method.

TABLE NO. 8.

PERCENTAGE OF CAFFEINE IN SAMPLES OF KAFFEE HAG.

(Average of the Fendler-Stuber and the Power-Chesnut methods, and based upon the nitrogen determination of the caffeine residue.)

Sample No.	Year 1929 Month Purchased.	Purchased at		% of caffeine
1.	January	Adams Grocery	E. Lansing, Mich.	0.0109 %
2	February	" "	" "	0.0105
4.	"	Kellogg Company	Battle Creek "	0.0245
5.	"	" "	" " "	0.0328
6.	"	" "	" " "	0.0198
7.	"	" "	" " "	0.0034
8.	April	Adams Grocery	E. Lansing, "	0.0175
9.	"	A. & P. "	" " "	0.0141
10.	"	" "	Detroit, "	0.0139
11.	"	" "	Clinton "	0.0147
12.	"	Arbaughs Dept. ST.	Lansing, "	0.0280
13.	May	Rite Way Store	Jackson "	0.0193
14.	"	A. & P. Grocery	E. Lansing, "	0.0194
15.	June	Delicatessen Grocery	Lansing, "	0.0145
16.	"	Goosens Groc.	" "	0.0147
17.	"	Kroger "	" "	0.0148
18.	"	C. Thomas Stores	E. Lansing "	0.0125
19.	"	E. R. Quigley Groc.	Clinton "	0.0143
20. (1927)	"	Kroger Store	Flwlerville, "	0.0948
21.	"	Rite Way System	Detroit, "	0.0135
22.	July	Warner Stores	Lansing, "	0.0103
23.	"	Fox Bros. Groc.	E. Lansing, "	0.0259
24.	"	Kroger Store	Charlotte, "	0.0155
25.	"	Rite Way System	Bellvue "	0.0170
26.	"	Aldrich Grocery	Tecumseh "	0.0161
27.	"	Kroger Store	Howell "	0.0117
28.	"	A. & P. Store	Brighton "	0.0110
29.	"	" "	Mason "	0.0149
30.	August	C. Thomas Stores	North Lansing "	0.0160
31.	"	F. A. Rouse Store	" " "	0.0150
32.	"	Bopp's Market	Lansing, "	0.0121
33.	"	Piggly Wiggly St.	" "	0.0143
34.	"	Economy Store	Garrett, Indiana	0.0164
35.	"	A. & P. Store	Angola "	0.0134
36.	"	American Stores	Coldwater, Mich.	0.0132
			Average-	0.0180 %

Percentage of caffeine removed, based upon the undecaffeinated coffee containing an average of 1.20% caffeine - 98.50 %
This is well above the company's guarantee of 97% removed.

Sanka Coffee

No. 1.	April, 1929	Hunter's Grocery	Lansing, Mich.	0.0230 %
2.	June "	Fox Bros. "	E. Lansing, Mich.	0.0393
3.	August "	" " "	" " "	0.0440
			Average-	0.0354 %

Percentage of caffeine removed, based upon the undecaffeinted coffee containing an average of 1.20% caffeine-97.05 %

TABLE NO. 9.

DETERMINATION OF THE PERCENTAGE OF CAFFEINE REMOVED IN SAMPLES OF KAFFEE HAG. ANALYZED BY THE FENDLER-STUBER METHOD.

Sample No.	% of Moisture	Wt. of moisture free sample.	% of caffeine based upon the 10 gm. wt.	% of caffeine based upon the moisture free wt.	% of caffeine removed. Based upon moisture free wts.	
Roasted & Decaffeinated					From green coffee	From roasted coffee.
61.	4.77	9.523 gm.	0.0122	0.0128	98.84	98.80
62.	4.59	9.541	0.0160	0.0167	98.27	98.50
63.	4.09	9.591	0.0209	0.0217	98.04	98.10
64.	4.22	9.578	0.0184	0.0192	98.00	98.31
65.	4.32	9.568	0.0124	0.0129	98.88	98.88
66.	4.20	9.580	0.0127	0.0132	98.83	98.84
1461	3.86	9.614	0.0148	0.0153	98.26	
Average-	4.29 %	9.570 %	0.0153 %	0.0160 %	98.46 %	98.57 %
Green & not Decaffeinated.						
61.	8.38	9.162	1.005	1.096		
62.	8.68	9.132	0.933	1.021		
63.	8.41	9.159	1.014	1.107		
64.	8.14	9.186	0.883	0.961		
65.	7.87	9.213	1.062	1.152		
66.	7.75	9.225	1.036	1.123		
1461.	8.63	9.137	0.803	0.878		
Average-	8.26 %	9.173 %	0.962 %	1.048 %		
Roasted & not Decaffeinated.						
61.	4.10	9.590	1.020	1.063		
62	3.74	3.626	1.066	1.110		
63	4.20	9.580	1.095	1.143		
64	4.07	9.593	1.091	1.137		
65	4.07	9.593	1.103	1.150		
66.	3.86	9.614	1.086	1.129		
Average-	4.00 %	9.599 %	1.077 %	1.122 %		
Green Decaffeinated						
1461.	7.09	9.291	0.0212	0.0228	97.41 %	

TABLE NO. 10

COMPARATIVE RESULTS OF CAFFEINE ANALYSIS OF DECAFFEINATED COFFEE BY WEIGHT OF THE CAFFEINE RESIDUE, AND BY NITROGEN DETERMINATION. ANALYZED BY THE FENDLER & STUBER AND THE POWER & CHESNUT METHODS.

Sample No.	Percentage of caffeine.			
	Fendler & Stuber method.		Power & Chesnut method.	
	By wt. of Residue.	By nitrogen determination.	By wt. of residue.	By nitrogen determination.
Kaffee Hag No.				
17.	0.034 %	0.0148 %	0.062 %	0.0143 %
18.	0.030	0.0125	0.058	0.0164
19.	0.062	0.0143	0.065	0.0204
21.	0.034	0.0135	0.059	0.0203
22.	0.024	0.0103	0.058	0.0176
23.	0.053	0.0259	0.075	0.0318
24.	0.064	0.0153	0.057	0.0157
25.	0.058	0.0164	0.060	0.0177
26.	0.048	0.0146	0.056	0.0176
27.	0.040	0.0106	0.055	0.0129
28.	0.040	0.0098	0.048	0.0123
29.	0.042	0.0129	0.054	0.0159
30.	0.046	0.0157	0.048	0.0163
31.	0.048	0.0141	0.045	0.0158
32.	0.058	0.0114	0.039	0.0128
33.	0.045	0.0143	0.048	0.0144
34.	0.054	0.0150	0.058	0.0179
35.	0.044	0.0123	0.059	0.0145
36.	0.040	0.0129	0.043	0.0136
Ave.	0.045 %	0.0141 %	0.0551 %	0.0167 %
Sanka Coffee No.				
2.	0.075	0.0424	0.060	0.0363
3.	0.084	0.0461	0.077	0.0420
Ave.	-0.0795 %	0.0442 %	0.0685%	0.0391 %
Total ave.	-0.0487 %	0.0170 %	0.0564 %	0.0189 %
Percentage of caffeine in the final residue.	34.9 %		33.5 %	

Conclusions- These results prove conclusively that to base the % of caffeine upon the wt. of the residue of caffeine would be entirely erroneous, and would give results about three times too high, no matter which method is used.

TABLE NO. 11.

COMPARATIVE RESULTS OF CAFFEINE ANALYSIS OBTAINED BY SUBLIMATION, AND THE NITROGEN DETERMINATION OF THE CAFFEINE RESIDUE.

Sample.	Percentage of caffeine.					
	Fendler-Stuber method.			Power - Chesnut method.		
	By sublimation.		By nitrogen deter.	By sublimation		By nitrogen deter.
	By weight.	By N.deter.		By weight.	By N. deter.	
Kaffee Hag No.						
17.	0.014	0.0102	0.0165	0.012	0.0100	0.0168
18.	0.012	0.0097	0.0127	0.010	0.0072	0.0120
19.	0.014	0.0133	0.0153	0.015	0.0097	0.0185
21.	0.012	0.0131	0.0157	0.022	0.0114	0.0209
22.	0.010	0.0080	0.0111	0.012	0.0063	0.0104
23.	0.030	0.0253	0.0273	0.024	0.0222	0.0270
30.	0.018	0.0113	0.0157	0.030	0.0096	0.0163
31.	0.007	0.0104	0.0141	0.015	0.0086	0.0158
32.	0.011	0.0063	0.0114	0.021	0.0052	0.0128
33.	0.014	0.0102	0.0143	0.015	0.0074	0.0144
34.	0.020	0.0121	0.0163	0.016	0.0125	0.0173
35.	0.012	0.0096	0.0128	0.020	0.0104	0.0145
36.	0.015	0.0093	0.0123	0.014	0.0095	0.0126
Sanka Coffee No.						
2.	0.034	0.0369	0.0424	0.037	0.0298	0.0363
3.	0.039	0.0403	0.0461	0.043	0.0310	0.0420
Average-	0.0174%	0.0150 %	0.0186 %	0.0208 %	0.0127%	0.0195%

Chase & Sanburn Coffee	By wt.	By sublimation. Wt. of crystals	By N.deter. of crystals	N.deter.of residue(calc'd as % caffeine).	Total N.deter. (calc'd as % caffeine)
	1.21 %	1.066 %	1.057 %	0.0704 %	1.1274 %

Sublimed

	By wt.		By N.Deter.	
0.0020 gms. Pure caffeine-	0.00210	gms	0.00134	gms
" " " "	0.0022	"	0.00136	"
" " " "	0.0022	"	0.00194	"
" " " "	0.0022	"	0.00200	"
" " " "	0.0023	"	0.00198	"
0.0010 " " "	0.0012	"	0.00099	"
" " " "	0.0010	"	0.00103	"

TABLE NO. 12

DISTRIBUTION OF NITROGEN, CALCULATED AS PERCENTAGE OF CAFFEINE, IN THE SUBLIMED CRYSTALS AND IN THE REMAINING RESIDUE.

Sample.	Percentage of caffeine.			
	Fendler - Stuber method.		Power - Chesnut method.	
	In the crystals	In the residue.	In the crystals.	In the residue
Kaffee Hag no.				
17	0.0102 %	0.0030 %	0.0100 %	0.0069 %
18.	0.0097	0.0030	0.0072	0.00485
19.	0.0133	0.0020	0.0097	0.0088
21.	0.0131	0.0026	0.0114	0.0095
22.	0.0080	0.0031	0.0063	0.0044
23.	0.0253	0.0020	0.0222	0.00485
24.	0.0113	0.0044	0.0096	0.0067
31.	0.0104	0.0037	0.0086	0.0072
32.	0.0063	0.0051	0.0052	0.0076
33.	0.0102	0.0041	0.0074	0.0070
34.	0.0121	0.0042	0.0125	0.0054
35.	0.0096	0.0022	0.0104	0.0041
36.	0.0093	0.0033	0.0095	0.0041
Sanka Coffee No.				
2.	0.0369	0.0055	0.0298	0.0065
3.	0.0403	0.0058	0.0310	0.0110
Average-	0.0150 %	0.0036 %	0.0127 %	0.0066 %
Average total % of caffeine-	0.0186 %		0.0133 %	
% of non caffeine nitrogenous material in the unsublimed caffeine residue-		19.3 %		34.2 %
% of nitrogen from caffeine only -	80.7 %		65.8 %	

Conclusions from tables 11 & 12. -

In the Fendler-Stuber method the % caffeine by wt. of the sublimed crystals is about 0.0011% less than the % Caffeine by nitrogen determination of the residue before sublimation. Whereas in the Power-Chesnut method it is 0.0017% more. It was found to be a little more difficult to obtain the pure colorless sublimed crystals from the residue obtained by the Power-Chesnut method than it was by the Fendler- Stuber method.

It is a striking fact that the % by wt. of the sublimed caffeine corresponds very closely with the nitrogen determination of the unsublimed residue. There are consistently lower results obtained when the results are based upon the nitrogen determination of the sublimed caffeine than when they are based upon the wt. of the sublimed caffeine. This is also true when pure caffeine is sublimed. There is a greater possibility for error in weighing then in running the nitrogen determination.

It will also be observed that while the Power-Chesnut method usually gives higher results than the Fendler-Stuber method when they are based upon the nitrogen determination of the caffeine residue, it gives lower results when based upon the nitrogen determination of the sublimed caffeine (Average 0.0021% less). And the nitrogen calculated as % of caffeine left in the residue after sublimation is 0.003 % higher in the Power-Chesnut method than in the Fendler-Stuber method.

Therefore these figures indicate that the Power-Chesnut method extracts caffeine slightly less efficiently than does the Fendler-Stuber method, but extracts more non caffeine nitrogenous materials, making it more difficult to get the pure caffeine crystals by sublimation.

The biggest fact that these tables bring out is that 19.3% of the nitrogen determined and calculated as caffeine in the final caffeine residue in the Fendler-Stuber method, and 34.2% in the Power-Chesnut method, is not from caffeine at all, but from other nitrogenous materials.

Of course, the question may be asked if it is not possible for a small amount of caffeine to be held by the residue even after subliming a long time. This is possible, but not very probable for the following reasons:

1. Later tables show that non caffeine mitrogenous materials are extracted in the first $CHCl_3$ extraction, but are more or less eliminated in the various steps of purification. And it is reasonable to conclude that the impurities that escaped the purification process would also contain nitrogen.

2. The sublimation of pure caffeine results in nearly a 100 % recovery, and at a much shorter period of time than the samples are run.

3. Continued heating at a much higher temperature (235-245°C.) does not cause even a trace of caffeine to sublime.

TABLE NO. 13.

COMPARATIVE RESULTS OF THE NITROGEN DETERMINATIONS, CALCULATED AS PERCENTAGE OF CAFFEINE, IN THE RESIDUE FROM THE FIRST $CHCl_3$ EXTRACT AND THE RESIDUE FROM THE FINAL $CHCl_3$ EXTRACT, WITH AND WITHOUT THE OXIDATION STEP OF ADDING 1 % $KMnO_4$ TO THE AQUEOUS SOLUTION. (Fendler-Stuber method).

Sample.	Percentage of caffeine by nitrogen determination.		
	First residue.	Final residue not oxidized.	Final residue oxidized.
Kaffee Hag			
No. 8.	0.0859 %	0.0431 %	0.0163 %
9.	0.0807	0.0405	0.0121
10.	0.0734	0.0330	0.0125
11.	0.0804	0.0238	0.0129
Sanka Coffee			
No. 1.	0.0769	0.0403	0.0225
Postum Cereal			
No. 1.	0.0672	0.0276	0.0059
Average-	0.0774 %	0.0346 %	0.0137 %

Conclusions-

Over half of the non caffeine nitrogenous materials are removed by simply putting the residue in solution with water, filtering and shaking the aqueous solution with 6 portions of $CHCl_3$. But about five sixth's of it is removed if the complete Fendler-Stuber procedure is followed.

Therefore it is reasonable to conclude that the impurities in the final residue of caffeine, which is only about 34% pure caffeine, also contains non caffeine nitrogenous substances.

TABLE NO. 14.

EFFECT OF FURTHER PURIFICATION OF THE FINAL CAFFEINE RESIDUE BY ADDING 20cc. OF WATER AND 0.2 Gm. of MGO, DIGESTING A SHORT TIME ON THE STEAM BATH, FILTERING AND SHAKING THE AQUEOUS SOLUTION WITH $CHCl_3$. (Fendler-Stuber method).

Sample.	Percentage of caffeine by nitrogen determination.		
	ORDINARY F. & S. METHOD.	F.& S. METHOD,RESIDUE PURIFIED (Twice)	
Kaffee Hag No. 2		By wt. of residue.	By nitrogen deter.
Kaffee Hag No. 2.	0.0039 %	0.0280 %	0.0079 %
" " " 8.	0.0162	0.036	0.0160
" " " 9.	0.0122	0.066	0.0100
" " " 10.	0.0127	0.022	0.0033
" " " 11.	0.0123	0.030	0.0103
" " " 12.	0.0214	0.030	0.0145
Sanka Coffee" 1.	0.0224	0.024	0.0158
Postum Cereal 1.	0.0057	0.016	0.0035
Average.	0.0141	0.031	0.0085

CONCLUSIONS-

The consistently lower results obtained by purifying the caffeine residue the second time with magnesium oxide may be due to two reasons.

1. The possible slight caffeine destroying power of the MgO.

2. The removal of non caffeine nitrogenous substances.

Other ext. indicate that the caffeine destroying power of MgO is negligible. Also, the supposedly caffeine content of Postum Cereal, which has no caffeine in it whatsoever, shows

about one half the amount of nitrogen as it does by the ordinary Fendler and Stuber method.

The final caffeine residue appears to be nearly pure, as observed in the bottom of the flask, showing the characteristic white needle shaped crystals with practically no fatty or waxy material present.

While this extra step would probably not be practical for commercial analysis, it shows that some such method even better than this perhaps, should be employed if we are to estimate all nitrogen as coming from caffeine in the final results. It is probable that from 10 to 40 % of the nitrogen determined when the ordinary methods are used is from non-caffeine nitrogenous substances.

TABLE NO. 15.

COMPARATIVE RESULTS IN ADDING 10 G. MGO TO THE AQUEOUS SOLUTION, DIGESTING ON THE STEAM BATH, FILTERING AND COOLING BEFORE OXIDIZING WITH POTASSIUM PERMANGANATE.

Sample.	Percentage of caffeine. Ordinary F.and S. procedure. By wt. of residue	Ordinary F.and S. procedure. By nitrogen deter.	F. and S. Method with MgO. By wt. of residue.	F. and S. Method with MgO. By nitrogen deter
Kaffee Hag No. 16.		0.0147 %	0.0190 %	0.0213 %
17.	0.034	0.0148	0.0100	0.0106
18.	0.030	0.0125	0.0160	0.0097
19.	0.062	0.0143	0.0200	0.0127
20.(1926)	0.236	0.0948	0.0860	0.0925
21.	0.034	0.0135	0.0100	0.0120
22.	0.0130	0.0103	0.0170	0.0065
23.	0.053	0.0259	0.0420	0.0219
24.	0.064	0.0153	0.027	0.0115
25.	0.058	0.0164	0.022	0.0141
Average-	0.065 %	0.0235 %	0.027 %	0.0213 %
Pure caffeine	0.00131 g.		0.00121 g.	0.00133 g.
"	"			0.00162

TABLE NO. 16.

COMPARATIVE RESULTS OBTAINED IN SHAKING OUT THE FINAL CHLOROFORM EXTRACT WITH 1 % KOH, AS IN THE POWER-CHESNUT METHOD.

Sample.	Percentage of caffeine. Ordinary F. & S.method. By wt. of residue.	Ordinary F. & S.method. By nitrogen deter.	F. & S. method plus above step. By wt. of residue.	F. & S. method plus above step. By nitrogen deter.
- 19.	0.062 %	0.0143 %	0.031 %	0.0153 %
21.	0.034	0.0135	0.028	0.0157
22.	0.024	0.0103	0.024	0.0080
23.	0.053	0.0259	0.037	0.0253
24.	0.064	0.0153	0.034	0.0143
25.	0.058	0.0164	0.047	0.0153
26.	0.048	0.0146	0.026	0.0142
Average-	0.049 %	0.0157 %	0.034 %	0.0154 %

Conclusions from tables 15 and 16.-

The digestion of the aqueous solution with MgO and filtering before proceeding with the usual step of oxidation with $KMnO_4$ greatly increase the purity of the final caffeine residue. In some cases the crystals of caffeine in the bottom of the flask had nearly the same appearance as pure caffeine. However some samples will have over twice the % of caffeine by wt. as by nitrogen determination. Consequently the wt. of the final residue could not be relied upon for calculating the % of caffeine. There is also a slight loss of the alkaloid by being absorbed by the MgO.

Washing the final $CHCl_3$ extract with 5 cc. of KOH, as in the Power-Chesnut method, improves the purity of the caffeine to quite an extent, but not as much as does the use of MgO. It has the advantage of not having any destroying power on the caffeine. The use of this extra step on samples that are to be sublimed considerably improves the purity of the sublimed crystals. The result in table 16 show a decrease of 0.015 % caffeine by wt. by the use of KOH, whereas table 15 shows a decrease of 0.028 % caffeine by the use of MgO.

TABLE NO. 17.

LOSS OF CAFFEINE IN THE ANALYSIS, DETERMINED BY ADDING 2 Mg. OF PURE CAFFEINE TO THE RESIDUE LEFT AFTER THE FIRST EXTRACTION AND REPEATING THE ANALYSIS.

	Gms of caffeine by the nitrogen determination.	
Sample.	Power-Chesnut method.	Fendler-Stuber method.
		(First $CHCl_3$ filtered thru cotton plug.)
0.00200 g.	0.00160 g.	0.00202
"	0.00139	0.00187
"	0.00120	0.00192
Average-	0.00140 g.	0.00193 g.
		(First $CHCl_3$ extract filtered thru a 24 cm. filter paper).
0.00200 g.	($CHCl_3$ evaporated off without shaking with Kaffee Hag residue).	0.00181 g. (New filter paper) 0.00194 g. (Old filter paper).
Percentage loss of caffeine-	30.0 %	3.5 %

Conclusions-

While it would not be justifiable perhaps to draw conclusions obtained in the above table, the results shown in some of the other tables involving the use of MgO strongly indicates that the MgO has a slight retentive or destroying power on the caffeine. And there is no doubt that lower results would be obtained, in nearly every trial, with the Power-Chesnut method than with the Fendler-Stuber method.

The explanation might be made that perhaps the reason the Fendler-Stuber method gave such a high recovery of caffeine was because caffeine had not been completely extracted from the Kaffee Hag. This is not true as the last two samples were

not added to the Kaffee Hag residue at all, and they gave nearly as high results as the others. It is also interesting to observe that the highest recovery was obtained when the $CHCl_3$ extract was filtered thru a filter that had previously filtered a $CHCl_3$ extracts and had been thoroly washed.

TABLE NO. 18.

COMPARATIVE RESULTS OBTAINED BY EXTRACTING WITH SOXHLET APPARATUS WITH ALCOHOL IN THE FENDLER-STUBER METHOD, AND BY SHAKING WITH CHLOROFORM IN THE POWER-CHESNUT METHOD.

Sample.	Percentage of caffeine by nitrogen determination.			
	Ordinary F.& S.method	F.& S.method with alcohol extraction.	Ordinary P.& C.method.	P.&.C. method with $CHCl_3$ (shaking) extraction.
Kaffee Hag #1.				0.0114 %
No. 1.	0.0122 %	0.0160 %	0.0091 %	0.0109 %
1.	0.0121	0.0103	0.0091	0.0116
2.	0.0111	0.0107	0.0067	0.0099
4.	0.0245	0.0391	0.0246	0.0225
5.	0.0326	0.0319	0.0330	0.0300
6.	0.0207	0.0155	0.0190	0.0177
7.	0.0112	0.0157	0.0076	0.0101
7.	0.0111	0.0181		
8.	0.0162	0.0294		
9.	0.0122	0.0221		
10.	0.0127	0.0236		
11.	0.0129	0.0335		
12.	0.0214	0.0301		
Sanka Coffee No. 1	0.0224	0.0273		
(Sample 1 to 7) Average	0.0178 %	0.0199		
Total Average	0.0166 %	0.0231 %	0.0156 %	0.0155 %

Conclusions-

These results point toward the same conclusions as are reached in some of the other tables, namely: The Power-Chesnut method gives the same and uniformly lower results no matter which method of extraction is employed. The reason for this can well be explained by the slight caffeine retentive power of the MgO used for purification. Also, this method with alcohol extraction removes more non caffeine nitrogenous materials than does the shaking method with $CHCl_3$. Thus the Fendler-Stuber method gave 0.0065 % higher results when alcohol Soxhlet extrac-

TABLE NO. 19.

THE COMPARATIVE EFFECTIVENESS OF CARBON TETRACHLORIDE AS A SOLVENT FOR CAFFEINE EXTRACTION IN KAFFEE HAG. (FENDLER & STUBER METHOD).

Sample.	Percentage of caffeine by nitrogen determination.			
	Ordinary F.& S. method (using 200 gm. $CHCl_3$ wet with cc. 10% NH_4OH shaking 1 hour).	F.& S. method, (using 200 gm. CC14. wet with 5 cc. alcohol shaking 1 hour)	Soxhlet extraction with CC14. for 10 hours Sample wet with 10 cc water.	% caffeine by wt.of residue
Kaffee Hag No. 13.	0.0179 %	0.0065 %		
" " " 14.	0.0200	0.0079		
" " " 15.	0.01348	0.0056		
" " " 16.	0.0147	0.0068	0.0109%	0.022%
" " " 17.	0.0148	0.0063	0.0103	0.033
" " " 18.	0.0125	0.0084	0.0101	0.032
" " " 19.	0.0143		0.0116	0.025
" " " 20.	0.0948		0.0754	0.084
" " " 21.	0.0135		0.0087	0.024
Average of first six samples	0.0155 %			
Average of last 6 samples	0.0274 %	0.0069 %	0.0211%	0.0366%

Conclusions-

The extraction of caffeine by CC14. by the shaking method is very incomplete. And the final residue of caffeine is only about one half caffeine by its appearance. Hence, this modification can be regarded as unsatisfactory.

We find much different results when Soxhlet extraction with CC14. is used. And while the appearance of the caffeine crystals shows the presence of some oily material it is less than when the ordinary F. & S. method is used.

This is also proven by comparing the last two columns which shows that the % caffeine by wt. is about double that determined by nitrogen. Whereas, it is from three to four times as

great in the ordinary Fendler & Stuber methodl

As undoubtedly, the foreign material present in the final residue of caffeine contains nitrogen, the elimination of the most of this material will give us more correct results. The agreement of the above figures with other methods of purifying this residue will help greatly in the solution of this problem, as well as show that the caffeine is not destroyed in the modifications or additional steps used.

The ratio of distribution of caffeine in solution in equal volumes of water and carbon tetrachloride after being shaken thoroly was found to be: 3.95 parts to 1.00 part in water and CCl_4. respect. and: 1.00 part to 21.00 parts in water and $CHCl_3$ respect.

TABLE NO. 20.

COMPARATIVE RESULTS OBTAINED BY USING THE SOXHLET CHLOROFORM EXTRACTION INSTEAD OF SHAKING, IN THE FENDLER AND STUBER METHOD

Samples.	Percentage of caffeine by nitrogen determination.	
	Shaking with $CHCl_3$	Soxhlet $CHCl_3$ extraction.
Kaffee Hag No. 8.	0.0162 %	0.0234 %
" " " 9.	0.0122	0.0231
" " " 10.	0.0127	0.0263
" " " 11.	0.0129	0.0335
" " " 12.	0.0214	0.0301
Senka Coffee. 1.	0.0224	0.0273
Average.	0.0163	0.0278

Conclusions-

The consistently higher results obtained by the Soxhlet extraction does not necessarily prove that the shaking out method is inadequate in extracting all of the caffeine. But because of the increased amount of material carried thru in the final residue when the Soxhlet extraction was used, and also its darker color, it would seem more logical to conclude that the Soxhlet extraction method removed much non caffeine nitrogenous substances than does the shaking out method. This may be also true in the Power-Chesnut method where alcohol Soxhlet extraction is used.

SUMMARY

I. METHODS

A. The Fendler-Stuber method is preferred to the Power-Chesnut method for the caffeine analysis of decaffeinated coffee for the following reasons:

1. It gives fully as accurate and consistent results.
2. This method is slightly more efficient in the extraction of caffeine.
3. It extracts slightly less non caffeine nitrogenous substances, and less is carried thru into the final caffeine residue.
4. The method is much shorter, more convenient and results in less possibility for error, especially with minute amounts of caffeine present.
5. The necessary apparatus may be found in every laboratory.
6. The method of purification employed removes a smaller percentage of caffeine when analyzing decaffeinated coffee.
7. The sublimed caffeine crystals are more nearly pure caffeine, as determined by the nitrogen content and their appearance.

B. A micro Kjeldahl procedure, similar to Pregyl's method using steam distillation, is one of the most accurate methods of determining nitrogen in the caffeine residue obtained from decaffeinated coffee.

Macro Kjeldahl apparatus gives fairly reliable results if micro methods are employed.

C. If the caffeine residue from decaffeinated coffee is sublimed at a temperature not exceeding 185° C. for at least 10 hours, results can be obtained which agree very closely with the nitrogen determination of the caffeine residue. The nitrogen determinations of the sublimed caffeine gives the most accurate and true results of any procedure yet tried.

Sublimation proves that the final caffeine residue contains non caffeine nitrogenous materials.

SUMMARY

II. ANALYTICAL RESULTS (Averages)

A. 85 samples of Kaffee Hag analyzed gave an average of 98.5% caffeine removed, based upon the undecaffeinated coffee containing 1.2% caffeine.

B. Kaffee Hag was 98.46% and 98.57% caffeine removed when based upon the green and roasted coffee respectively from which it was taken, in 7 samples.

C. 84.9% of the final residue consists of caffeine (By N.deter.) by the Fendler-Stuber method, and 83.5% by the Power-Chesnut method.

D. % of caffeine by weight of sublimed caffeine and by nitrogen determination was practically the same no matter which method was used. But % of caffeine by nitrogen determination of sublimed caffeine was less in the Power-Chesnut method than in the Fendler-Stuber method.

E. 80.7% of the nitrogen determined in the caffeine residue comes from the caffeine only, when run by the Fendler-Stuber method, and 65.8% when run by the Power-Chesnut method.

F. There was found to be over 5 times as much nitrogen in the residue from the first $CHCl_3$ extract as there was in the final caffeine residue when purified and oxidized, and over twice as much when not oxidized as when it is oxidized. Hence there must be a small amount of non caffeine in nitrogenous material in the final residue.

G. The use of MgO to further purify the final caffeine residue does more harm than good, in retaining a small amount of caffeine.

H. The use of MgO in purification before oxidation with $KMnO_4$ considerably increases the purity of the caffeine, but it is not consistent enough so that the % of caffeine can be based upon the weight of the residue.

I. Washing the final $CHCl_3$ extract with 1% KOH lowered the impurities about 50. % and materially increases the purity of sublimed caffeine.

J. There was a 30.% loss of caffeine (2 mg. sample) by the Power-Chesnut method, and only a 3.5% loss with the Fendler-Stuber method. This supports other results pointing toward the slight caffeine retaining power of MgO especially noticeable with minute quantitaties of caffeine.

K. Alcohol extraction with the Soxhlet apparatus does remove more non caffeine nitrogen substances than does shaking with $CHCl_3$. The final results are somewhat evened up in the two methods (when based upon the nitrogen determination) by the slight caffeine retaining power of the MgO.

L. Extraction of caffeine with CCl_4 was very incomplete by the shaking method, but it was practically complete when the Soxhlet extractor was used, and resulted in a more pure caffeine residue than when the regular Fendler-Stuber procedure was used.

M. Soxhlet extraction with $CHCl_3$ resulted in much higher results by the nitrogen determination than by the shaking method. This is undoubtedly due to the extraction of considerably more impurities which contained nitrogen, as the caffeine residue was in a very impure condition.

RECOMMENDATIONS

It is recommended that the procedure of caffeine analysis for coffee by the official Power-Chesnut and the tentative Fendler-Stuber methods, as given in the Official and Tentative Methods of Analysis of the Assoc. of Official Agr. Chemists, second edition, 1924, be given the following modifications applying specifically to the caffeine analysis of decaffeinated coffee:

1. The weight of the final residue of caffeine shall be entirely disregarded in calculating the percentage of caffeine.
2. The percentage of caffeine shall be based upon the accurate nitrogen determination of this caffeine residue.
3. Micro Kjeldahl methods shall be used for the nitrogen determination. The use of ordinary Kjeldahl apparatus shall be permissible, providing at least N/70 standard solutions, and an indicator as sensitive as methyl red are used, and also making it necessary that blank determinations be made just before samples are run that do not vary more than 0.30 cc. of N/100 acid used.
4. The use of sublimation procedure shall be recognized if it can be proven to be complete; and shall be especially recommended if combined with the nitrogen determination of the sublimed caffeine.

Finally, the choice of an accurate micro method of caffeine analysis for decaffeinated coffee is the Fendler-Stuber method with the suggested modifications, employing micro procedure in all of the steps, and basing the percentage of caffeine upon the micro nitrogen determination of the sublimed caffeine.