[CONTRIBUTION FROM THE PHYTOCHEMICAL LABORATORY, BUREAU OF CHEMISTRY, U. S. DEPARTMENT OF AGRICULTURE.]

AN IMPROVED METHOD FOR THE QUANTITATIVE DETERMINATION OF CAFFEINE IN VEGETABLE MATERIAL.

BY FREDERICK B. POWER AND VICTOR K. CHESNUT.

Received June 2, 1919.

The methods that have heretofore been employed for the quantitative determination of caffeine in vegetable products, especially coffee and tea, are exceedingly numerous and varied in character.[1] Several of the older methods have previously been shown to give inaccurate results, which may be attributed to a number of causes. Among these there may be mentioned, in the first instance, the incomplete extraction of the caffeine from the crude material or its partial decomposition by the use of lime in the process of extraction, while a loss of caffeine may also be incurred by the evaporation of large amounts of aqueous extract and the employment of such agents for its purification as lead acetate or animal charcoal. The methods which depend upon the use of an aliquot portion of a chloroform extract of the crude material would also appear to be subject to considerable error through a loss of solvent by volatilization in the operations of filtering and weighing or measuring the liquid, with a consequent alteration of the calculated relations between the latter and the amount of material which it is supposed to represent.

As an investigation undertaken by the authors, which is described in a separate communication,[2] required the determination of caffeine in various kinds of vegetable material, the methods which have heretofore been suggested were subjected to a critical examination. Some of the methods were thus found to be inherently inaccurate, while others are more or less complicated in their manipulative details. It was therefore sought to establish a method which would be both simple and accurate, and which also could be employed, without any considerable modification, for determining the presence or amount of caffeine in material of different physical character.

Although the method to be described embodies some of the features of other methods, the principles and details of procedure, and consequently the results obtained, are so different from most of them as to render it practically new. Nevertheless, we have purposely designated it as an improved method, rather than as a new one. The only recorded method with which it may to some extent be compared is that ascribed to Paul and

[1] Compare Beilstein's *Handbuch d. org. Chem.*, III Auflage, Bd. II, p. 957. Also Ergänzungsband, III, p. 704. Czapek, *Biochemie der Pflanzen*, 1905, Bd. II, p. 244. An especially complete and critical review of methods of assay, in chronological order, is given by Lendrich and Nottbohm, *Z. Nahr. Genussm.*, 17, 241–651 (1909). See also Fendler and Stüber, *Ibid.*, 28, 9–20 (1914).

[2] THIS JOURNAL, 41, 1307 (1919).

Cownley for determining caffeine in tea, which, as given in Allen's *Commercial Organic Analysis*,[1] is essentially as follows:

"Five grams of finely powdered tea are well mixed in a mortar with 2 grams of ignited magnesia,[2] the mixture thoroughly moistened with hot water, again triturated, and then dried at 100°. It is next extracted with boiling alcohol, and the resultant liquid evaporated nearly to dryness. The residue is boiled with 50 cc. of water, and treated with a few drops of dil. sulfuric acid. When cold, the liquid is filtered and repeatedly shaken with chloroform until exhausted. The united chloroform solution is then agitated with a very dilute solution of sodium hydroxide, which removes a little coloring matter, so that on subsequently distilling off the chloroform in a weighed flask the caffeine is obtained perfectly pure and colorless, or at most with a faint green tinge."

A trial of the above-described method for the assay of Maté or Paraguay tea did not give very satisfactory results.

A consideration of the entire subject rendered it evident that the first object to be attained was the complete removal of the caffeine from the crude material, and for this purpose the direct extraction of the latter with hot alcohol appeared to be the best mode of procedure. The elimination from the extract of such plant constituents as are only soluble in water would thus primarily be effected, and whatever may be the form in which the caffeine is contained in the vegetable material, it was ascertained that it could be completely removed by alcohol. It has been assumed by some investigators that in certain drugs the caffeine is partly in the free state and partly in combination, either with tannic acid or, in the case of coffee, as caffeine-potassium chlorogenate, to which the formula $C_{32}H_{36}O_{19}K_2.(C_8H_{10}N_4O_2)_2 + 2H_2O$ has been ascribed.[3] The existence of the latter compound does not appear as yet to have been completely established,[4] and the recorded analyses are not in very close agreement with the accepted formula. The use of lime, magnesia, ammonia, or other alkali in the various methods of assay is evidently with the primary object of liberating the caffeine from its combinations in the drug, but apart from this purpose a weak alkali, such as magnesia, may also serve for removing the tannin or other acidic substances from solution, as in the method of procedure adopted by us. The use of lime is considered objectionable on account of the observation that, under certain conditions, it may cause some

[1] 4th edition, Vol. 6, p. 611.

[2] In the original paper by Paul and Cownley, *Pharm. J.*, [3] **18**, 417 (1887), to which reference is made in the above-mentioned work by Allen, the authors direct for 5 g. of tea the use of 1 g. of "hydrate of lime," and no mention is there made of magnesia.

[3] Gorter, *Ann.*, **358**, 327–348 and **359**, 217–244 (1908).

[4] Compare Lendrich and Nottbohm, *Loc. cit.*, p. 264.

decomposition of the caffeine with the formation of ammonia and methylamine, but it has been shown that this is not the case with magnesia.[1]

Method of Assay.—After a large number of experiments with different kinds of material in order to overcome the various minor difficulties that were experienced, and also to insure the accuracy of the results, the following general method of procedure was finally adopted:

Ten g. of the finely ground material, previously moistened with a little alcohol, is extracted for about 8 hours in a Soxhlet apparatus with hot alcohol. The alcoholic extract is then added to a suspension of 10 g. of heavy magnesium oxide in 100 cc. of water, contained in a porcelain dish, the flask being rinsed with a little hot water, and this liquid added to the mixture. The mixture is allowed to evaporate slowly on a steam-bath or water-bath, with frequent stirring, until all the alcohol is removed and a nearly dry, powdery mass is obtained. This is mixed with sufficient hot water to enable it to be brought on a filter, which preferably should be smooth, and, after thoroughly cleaning the dish by means of a glass rod, to which a piece of rubber tubing is attached, the contents of the filter are washed with successive portions of hot water until about 250 cc. of filtrate is obtained. To the filtrate, contained in a flask of one-liter capacity, is added 10 cc. of a 10% solution of sulfuric acid, which causes the liquid to become much lighter in color, and with some kinds of material, such as *Ilex* leaves, a considerable precipitate is produced. In some cases, as with tea and guarana, it was found necessary to use 20 cc. of the above-mentioned acid in order to prevent the formation of an emulsion on subsequently extracting with chloroform. After the addition of the acid, a small funnel is placed in the neck of the flask, and the liquid, which is at first gently heated until any frothing ceases, is kept in a state of active ebullition for half an hour. This treatment is for the purpose of hydrolyzing any saponin that may be present. After being allowed to cool, the liquid is passed through a double moistened filter into a separatory funnel, the flask and filter being washed with small portions of about 0.5% sulfuric acid. The clear acid filtrate is then shaken with 6 successive portions of chloroform of 25 cc. each, which usually separates sharply and quickly, but, if not, can be made to do so by gently rotating the separatory funnel, or, if necessary, by the use of somewhat larger portions of chloroform. The united chloroform extracts are brought into another dry separatory funnel and shaken with 5 cc. of a 1% solution of potassium hydroxide, which serves to remove coloring matter. After complete subsidence of the chloroform solution it is passed through a small, dry filter into an Erlenmeyer flask, the alkaline liquid remaining in the separatory funnel being subsequently washed with two successive portions of chloroform of 10 cc.

[1] Tassilly, *Bull. soc. chim.*, [3] 17, 596 (1897). Compare also Maly and Andreasch, *Monatsh.*, 4, 369–386 (1883).

each. These washings of the alkali are passed through the previously mentioned filter, and, after washing the latter with a little chloroform, they are added to the first chloroform solution. The chloroform is finally removed by distillation from a water-bath, the residual caffeine brought by means of a little chloroform into a tared beaker, and, after the solvent has been allowed to evaporate spontaneously, the caffeine is dried for half an hour in a water-oven and weighed. On heating for another half hour there is usually a further slight diminution of weight, and this second weighing may be considered to represent the correct amount of caffeine, which, when multiplied by ten, denotes the percentage. As so obtained the caffeine is nearly colorless, and possesses a quite satisfactory degree of purity.

Control Experiments.—In order to ascertain that no caffeine was lost in any of the described operations a number of control experiments were made.

I.—Fifty g. of finely ground coffee was thoroughly extracted in a Soxhlet apparatus with warm alçohol, after which the material was dried on a steam-bath, moistened with 25 cc. of water, and allowed to stand for an hour. It was then extracted for 6 hours in a Soxhlet apparatus with warm chloroform, and the chloroform extract further treated according to the method of Lendrich and Nottbohm,[1] but no trace of caffeine was obtained. It was thus evident that the caffeine had previously been completely removed by extraction with alcohol.

To 50 g. of maté, which in two assays by the above-described method had been found to yield 1.33 and 1.34%, respectively, of caffeine, was added an alcoholic solution of 0.5 g. of anhydrous caffeine, and the whole completely extracted with hot alcohol in a Soxhlet apparatus. This extract was added to a suspension of 25 g. of heavy magnesium oxide in 500 cc. of water, as in the case of all assays when 50 g. of material was used. After evaporating the mixture to dryness, the residue was treated with water, brought on a filter, and washed repeatedly with hot water until about one liter of filtrate was obtained. A portion of the aqueous filtered liquid, representing 10 g. of the original material, was heated with dil. sulfuric acid, again filtered, and extracted with chloroform, when 0.2353 g. of caffeine was obtained. By deducting the amount of added caffeine, corresponding to 0.1 g., the yield of the latter from the maté was 0.1353 g. or 1.35%. This result afforded a further indication that the caffeine originally contained in the drug had been completely removed, and that no loss had occurred in the process of assay.

II.—As the second step in the process consisted in evaporating the alcoholic extract of the drug with a suspension of magnesia, it was deemed important to obtain the assurance that there was no loss of caffeine by

[1] *Loc. cit.*

adsorption or otherwise during this treatment. It may be considered, however, that in most cases the magnesia would be to a large extent withdrawn from action on the caffeine through its combination with the tannin and other acidic substances of the drug.

To 5 g. of heavy magnesium oxide, mixed with 100 cc. of water, was added 0.2084 g. of caffeine, the mixture evaporated to dryness, and subsequently treated as in the described method of assay. The amount of pure caffeine recovered by extraction with chloroform was 0.2045 g. In another experiment 0.2118 g. of caffeine was added to a suspension of 10 g. of magnesia in 100 cc. of water, and the mixture treated as before. The amount of caffeine recovered was 0.2117 g. It will thus be seen that under the prescribed conditions the use of magnesia involves no loss of caffeine.

It was observed in the course of the investigation that somewhat lower yields of caffeine were obtained by the use of light magnesia than with the heavy form, which is evidently due to the more gelatinous character of the mixture formed with the light magnesia, and the greater difficulty of removing the caffeine completely from it by washing. It was also found that the heavy magnesium oxide is of variable character, and some specimens designated as "chemically pure" contained considerable quantities of sodium carbonate. In one sample the amount of sodium carbonate, calculated as anhydrous salt, was 13.9%, and much sulfate and chloride was also present. Other samples contained 7.0 and 8.2%, respectively, of the alkali carbonate.

In order to ascertain whether sodium carbonate in the proportion contained in the most strongly alkaline sample of magnesia caused any decomposition of the caffeine in the process of assay, the following experiment was conducted: Such an amount of anhydrous sodium carbonate as would be contained in 10 g. of the magnesia (1.39 g.) was dissolved in 100 cc. of water, and 0.2003 g. of caffeine added. The solution was then evaporated to dryness, the residue dissolved in water, and the filtered liquid, amounting to 200 cc., extracted with 6 successive portions of chloroform of 25 cc. each. The amount of caffeine recovered, dried at 100°, was 0.1894 g. Although the loss in this instance was not very considerable, it is nevertheless deemed desirable that for the determination of caffeine by the proposed method the magnesium oxide should contain no appreciable amount of sodium carbonate, and it is also particularly important that the heavy form of magnesia be employed.

III.—As one of the essential operations in the proposed method of assay consists in the addition of dil. sulfuric acid to the aqueous liquid obtained by extracting the dry magnesia mixture with water, and maintaining the acid liquid in active ebullition for half an hour, it was considered important to ascertain that no loss of caffeine was thus incurred.

To a solution of 0.2136 g. of caffeine in 200 cc. of water 20 cc. of 10%

sulfuric acid were added, and the liquid kept at the boiling temperature for half an hour. It was then allowed to cool and extracted with 6 successive portions of chloroform of 25 cc. each. The amount of caffeine obtained was 0.2119 g. It was thus evident that no loss of caffeine had been caused by this treatment.

IV.—An experiment was finally conducted for the purpose of determining that no loss of caffeine resulted by shaking its chloroform solution with a dil. alkali.

0.2000 g. of anhydrous caffeine was dissolved in chloroform, and the solution shaken with 5 cc. of a 1% solution of potassium hydroxide. After separating the chloroform the alkaline liquid was washed with two small successive portions of chloroform, the united chloroform solutions passed through a small, dry filter, the solvent removed, and the residual caffeine dried at 100°. The amount of caffeine recovered was 0.1982 g., which indicates that in the process of assay there is no loss of caffeine by the use of alkali for its purification.

The degree of purity of the caffeine obtained by the proposed method of assay was ascertained by nitrogen determinations, using the Kjeldahl method. As a control experiment a specimen of commercial caffeine, designated as U. S. P., was examined.

Subs., 0.1500 g. (anhydrous) gave ammonia equivalent to 30.8 cc. of 0.1 *N* HCl. Found: 0.0431 N or 0.1493 g. caffeine. Purity 99.5%.

The general applicability of the proposed method for the quantitative determination of caffeine has been ascertained by the examination of a number of vegetable products which differed greatly in their physical characters and composition. Although it is not desirable as a rule to employ more than 10 g. of material for the purpose of assay, in some instances 50 g. or even larger amounts were used in order to confirm the accuracy of the results. With such material as the so-called "caffeine-free coffee," which usually contains a very small proportion of caffeine, it is important that at least 20 g. should be used, and 50 g. would be preferable. The value of the method is believed to have been shown by the close concordance of the results obtained in several assays, and even with the larger amounts of material the figures are in remarkably close agreement.

The material examined in the course of this investigation, and the observations pertaining thereto, may be described as follows:

1. *Green Tea.*—A sample of fair quality yielded in two 10 g. assays 1.98 and 2.01%, respectively, of caffeine.

2. *Black Tea* (Oolong).—A sample of ordinary quality yielded in two 10 g. assays 2.43 and 2.44%, respectively, of caffeine. The caffeine obtained from tea was quite white, and evidently of a high degree of purity.

3. *Maté or Paraguay Tea.*—(From species of *Ilex.*) Two 10 g. assays yielded, respectively, 1.44 and 1.46%, and in a 50 g. assay 1.38% was obtained.

4. *Coffee, Roasted Java.*—The yield in a 10 g. assay was 1.22%, and in two 50 g. assays 1.24 and 1.25%.

5. *Coffee, Roasted Rio.*—A 10 g. assay yielded 1.12% and a 50 g. assay 1.07%. From 200 g. of the same coffee 1.10% was obtained.

6. *Coffee, Roasted Santos.*—A 20 g. assay yielded 0.96%.

7. *Coffee, Natural Santos.*—A sample of this coffee was available which consisted of a mixture of (*a*) green and (*b*) dark colored beans. As the latter are rejected in the process of sorting, it was deemed of interest to ascertain the difference in caffeine content of the two kinds of beans.

(*a*) *Green Beans.*—A 20 g. assay yielded 0.97% of perfectly white caffeine.

(*b*) *Dark Colored Beans.*—A 20 g. assay yielded 0.71% of nearly white caffeine.

Although the dark colored beans contain an appreciably smaller proportion of caffeine than the selected ones, they would still appear to possess considerable value for the production of this substance.

8. "*Caffeine-free Coffee.*"—A sample of this preparation, in a 20 g. assay, yielded 0.08% of caffeine, somewhat dark in color.

Through the courtesy of Dr. Walter H. Evans, Chief of Insular Experiment Stations, U. S. Department of Agriculture, and Mr. T. B. McClelland, horticulturist of the Porto Rico Experiment Station at Mayaguez, we have been able to obtain a quantity of coffee leaves and the pulp of the coffee berry. Mr. McClelland has kindly communicated the following information concerning them:

"As the coffee plants are not given much pruning, the amount of leaves as a waste product would be negligible if large quantities were desired. A small amount of leaves could be obtained from November to January, as such pruning as is done is given immediately after the picking of the crop."

"Coffee pulp, immediately after breaking, represents approximately $^1/_3$ of the total weight of the coffee berries as picked. About 5 pounds of coffee berries are required to furnish one pound of market coffee. As the Porto Rican crop varies roughly from thirty million to fifty million pounds, the amount of fresh pulp would run from fifty to eighty million pounds. The water content of this would be very high, and at present it is used only for manuring purposes. It would be available from October to February."

9. (*a*) *Coffee Leaves.*—Several determinations of the amount of caffeine in air-dried leaves gave the following results: Two 10 g. assays yielded, respectively, 0.85 and 0.86%, a 50 g. assay gave 0.85%, and from 100 g. of the leaves 0.82% was obtained. The stems of the plant, separated from the leaves, were likewise examined, but in a 50 g. assay yielded only 0.087%. On extracting a larger portion (about 2 kg.) of the stems with hot alcohol a considerable quantity of a handsome crystalline substance was obtained. This substance, after repeated crystallization from hot alcohol, separated in snow-white, felted needles, much resembling caffeine in appearance, but it gave no reaction for the latter compound by the extremely delicate murexide test, and differed otherwise in its properties. Its more complete examination will be undertaken for the purpose of determining its composition.

9. (*b*) *Coffee Pulp.*—This material, when thoroughly dried, yielded in a 10 g. assay 0.88% and in a 50 g. assay 0.90% of perfectly white caffeine. A preliminary

examination of the other constituents of coffee pulp has shown that they possess chemical interest, and it is intended to pursue their investigation.

It may incidentally be noted that in the *National Standard Dispensatory*, third edition, 1916, p. 356, the statement occurs in connection with coffee substitutes that "the pulp of the berry (*sarcocarp*) is practically devoid of caffeine." This is not in accordance with our observations, inasmuch as we have found the pulp to contain such an amount of caffeine as to render its utilization for the production of this substance worthy of consideration, especially in view of the enormous amounts of material available.

10. *Guarana.*—In order to obtain as much evidence as possible concerning the general applicability of the proposed method of assay, it was deemed desirable to determine the amount of caffeine in guarana, and for this purpose 10 g. of material was employed. As previously noted, it was found to be important, as in the case of tea, to heat the aqueous filtrate from the magnesia mixture with 20 cc. of 10% sulfuric acid, in order to effect the hydrolysis of the saponin substances before extracting with chloroform. In the latter operation on account of the high percentage of caffeine, 10 portions of chloroform of 25 cc. each were employed. The yield of perfectly white caffeine was 4.20%.

For the purpose of comparison a determination of caffeine in the same material was made by the U. S. Pharmacopoeia method of assay, when 4.27% of caffeine was obtained. The results by the two methods are therefore seen to be in very close agreement, but the newly proposed method is believed to possess some advantages over that officially adopted. One of these advantages consists in avoiding the use of a definite volume of a filtered chloroform solution, which is assumed to represent a certain proportion of the drug, since an appreciable error may be caused through volatilization of the solvent. There is, furthermore, a more economical use of chloroform, for by the proposed method this solvent is recovered, whereas the Pharmacopoeia directs it to be evaporated, and it is consequently lost.

The results obtained by the examination of the above-described material are summarized in the following table, in which are recorded the yields of caffeine by direct gravimetric determinations and also by the determination of nitrogen in the products.

The leaves of several plants have at various times and in different countries been used as a substitute for tea. An opportunity was afforded us of examining some of these tea substitutes in order to ascertain whether they contain caffeine.

Ehretia macrophylla, Wall. (Fam. *Borraginaceae*).—A small quantity of the leafy twigs of this tropical plant was available through the kindness of Mr. Geo. F. Mitchell, Supervising Tea Examiner of the Treasury Department, who had received it from Adn. Hernandez, Director of Agriculture in the Bureau of Agriculture of the Philippines. This plant is known in the Philippines as "Forest Tea," or by the vernacular name of

Chaa-bundoc. It is stated by Mr. Mitchell that the beverage prepared from it, while not objectionable, has no pronounced or characteristic flavor that would commend it to Americans. No trace of caffeine could be detected in it, and neither the leaves nor the stems of the plant responded to the general tests for an alkaloid.

	Percentage yield of caffeine, gravimetric.		Percentage yield of caffeine, calculated from nitrogen.	
Name of material.	10 g. assay.	50 g. assay.	10 g. assay.	50 g. assay.
1. Green Tea	1.98; 2.01	.....	1.95 ..	.....
2. Black Tea (Oolong)	2.43; 2.44	.....	.. 2.39	.....
3. Maté (Paraguay Tea)	1.44; 1.46	1.38	1.33; 1.35	1.32
4. Coffee, Roasted Java	1.22	1.24; 1.25	1.15	1.16; 1.18
5. Coffee, Roasted Rio	1.12	1.07	1.05	1.00
6. Coffee, Roasted Santos	0.96	..	0.93	..
7. Coffee, Natural Santos:				
(*a*) Green beans	0.97	..	0.94	..
(*b*) Dark colored beans	0.71	..	0.69	..
8. "Caffeine-free Coffee"	0.08	..	0.06	..
9. Coffee Leaves and Pulp:				
(*a*) Leaves	0.85	0.85	0.77	0.77
(*b*) Pulp	0.88	0.90	0.80	0.85
10. Guarana	4.20	..	..	..

NOTE.—A 200 g. portion of Rio Coffee yielded 1.10% of caffeine and a 100 g. portion of coffee leaves yielded 0.82%.

Ceanothus Americanus, Linné (Fam. *Rhamnaceae*).—The leaves of this plant, commonly known as "New Jersey Tea," are said to have been used as a substitute for tea during the American Revolution. A small quantity of the leaves was collected by Dr. R. M. Harper on October 1 in woods near Washington. No caffeine could be found in the leaves, which, however, gave decided general reactions for the presence of an alkaloid.

Psychotria undata, Jacq. (Fam. *Rubiaceae*).—Through the courtesy of Mr. Charles T. Simpson, of Little River, Fla., a small quantity of the leaves and fruit of this plant was collected for us. Inasmuch as it belongs to the same botanical family as that of the coffee plant, and is locally known as "wild coffee," it seemed desirable to consider the possible presence of caffeine, but none of this substance could be found in either the leaves or the fruit.

WASHINGTON, D. C.