

Methods of Chemical Analysis of Green Tea

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Methods of chemical analysis of tea have been studied since many years ago, and published in the Bulletin (in Japanese) of Tea Experiment Station of Japan in 1925. The AOAC also standardized analytical methods for tea.

Chemical analysis requires accuracy. In addition, simple and efficient procedures with minimum personal errors are desirable. From this point of view, various modifications and improvements of the methods have been made, and the methods were re-examined to know whether they are suitable or not as standard methods for analysis of green tea.

In this paper, methods for general analysis are described, not including methods which require particular instruments.

Extraction apparatus

Extraction apparatus shown in Fig. 1 was originally devised for the analysis of caffeine¹⁾, but it is presently used for tannin, sugar, pectin, etc. Sample (1 g) of pulverized tea is placed in the extraction tube, inside the adapter. Organic solvent contained in the flask, i.e. 100 ml-volumetric flask, is heated to carry out continuous extraction. In case of hot water extraction, sample is placed in the flask, not using the extraction tube. The author used to use a support which accommodates eight extraction apparatus at a same time. Heaters, water baths, and condensers for recovering solvent are equipped to the support. It is 25 × 30 × 40 cm of size, compact and convenient to use.

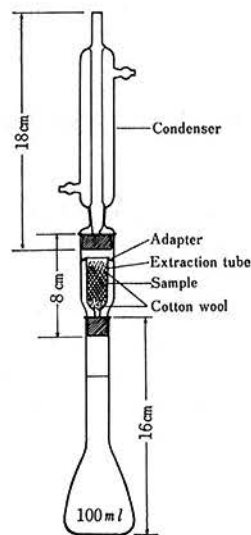


Fig. 1. Extraction apparatus

Accuracy and personal errors

The same samples were distributed to a number of research scientists working in different stations and they carried out chemical analysis on various items repeatedly for 10 times. Statistical analysis of these results is shown in Table 1¹⁾⁻⁵⁾. For caffeine, only data obtained by one person are given. For tannin determination, the Löwenthal method has been widely used. However the result showed large personal errors with this method, so that the colorimetric method using ferrous tartrate was adopted as an official procedure. Analytical methods which were examined by these cross-check tests were published officially, but those not yet examined are described as tentative methods in this paper.

Table 1. Errors in the analytical methods of green tea

| Item | Number of analyst | Average content (dry wt. %) | Error of the method (C. V. %) | Error with a single determination by an analyst (C. V. %) |
|------------------|-------------------|-----------------------------|-------------------------------|---|
| Moisture | 13 | 5.344 | 1.24 | 4.84 |
| Nitrogen | 12 | 5.576 | 0.65 | 2.02 |
| Tannin | | | | |
| Löwenthal | 13 | 9.257 | 3.83 | 15.95 |
| Ferrous tartrate | 9 | 10.457 | 1.52 | 5.42 |
| Water extract | 11 | 39.57 | 1.63 | 3.17 |
| Caffeine | | 2.46 | 0.54 | |

C. V.: Coefficient of variance

Official analytical methods⁶⁾

1) Preparation of samples

Pulverize sample by a mill, screen it through 0.5–1 mm sieve, and mix thoroughly.

2) Moisture (water content)

Take 2 g of sample into a weighing bottle of known weight, and dry in an oven at 98–100°C until reaching a constant weight. A decrease of weight represents the quantity of water.

3) Total nitrogen (Kjeldahl method)

Place 0.08–0.1 g of sample into a 100 ml digestion flask. After adding 1.5 g of catalyst ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}:\text{K}_2\text{SO}_4=1:9$) and 3 ml of conc. H_2SO_4 , heat the flask. Continue the heating for 1.5–2 hr after the content of the flask become transparent blue-green color. After adding 30% NaOH to the digested solution to make it basic, submit to steam distillation for 10 minutes. NH_3 released is absorbed by 10 ml of N/25 H_2SO_4 solution. Residual acid is titrated by N/25 NaOH. Indicator is a mixture of equal volume of 0.2% alcoholic methyl red and 0.1% alcoholic methylene blue.

4) Caffeine

Take 0.5 g of sample into a small dish, add 5 ml of 5% Na_2CO_3 to make it wet, and allow to stand for 1 hr. Then submit the sample to the extraction apparatus. Place 20 ml

of chloroform into the flask and heat it so that chloroform drops at a rate of 2 drops/sec for 2–3 hr. Evaporate the chloroform in the flask by using a water bath, add 40 ml of hot water and boil it to remove chloroform completely. Add water up to 3/4 volume of the flask, and add 2.5 ml of 20% CuSO_4 solution and 2.5 ml of 1N NaOH solution. Fill up to 100 ml with water. Filter the solution through dry filter paper, and take 25 ml of the filtrate into a separatory funnel. Shake 2 times with 30 ml and 20 ml of chloroform. After collecting the chloroform into a Kjeldahl flask, evaporate the chloroform at temperature lower than 90°C of a water bath. Add 0.1 g of catalyst and 3 ml of conc. H_2SO_4 , and determine nitrogen by the Kjeldahl method. Caffeine is calculated as $\text{N} \times 3.464$.

5) Water extract

Place 2 g sample in a 500 ml Erlenmeyer flask, add 200 ml of boiled water, and reflux gently for 2 hr. After cooling, fill up to 500 ml and filter through dry filter paper (Toyo-Roshi No. 2). Take 50 ml of the filtrate into a weighing bottle of known weight, and evaporate to dryness on a steam bath. Dry at 98–100°C to a constant weight.

6) Tannin

The method described below is not applicable to tannin of black tea.

Prepare ethyl gallate solutions at five different concentrations ranging 5–25 mg/100 ml at 5 mg intervals. Take 5 ml of each of these

solutions into 25 ml volumetric flasks, and add 5 ml of ferrous tartrate solution (100 mg of $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ and 500 mg of Rochelle salt dissolved in 100 ml of water). Fill up to 25 ml by adding Sørensen's M/15 phosphate buffer of pH 7.5. Use water in place of ethyl gallate to prepare a blank solution. Measure 540 nm absorbance against the blank to prepare a calibration curve. Absorbance of 1 mg of ethyl gallate corresponds to that of 1.5 mg of tea tannin (catechins).

Take 0.5 g of sample into the flask of extraction apparatus (Fig. 1), add 50–60 ml of hot water, and heat for 30 min at above 80°C in a water bath. After cooling, dilute with water to 100 ml, and filter through dry filter paper. Discard the initial filtrate of about 20 ml, and take 5 ml from the subsequent aliquot of the filtrate for measurement. In the same manner as with ethyl gallate solution, determine the absorbance at 540 nm. Calculate the quantity of tannin as 1.5 times of the value determined by the calibration curve.

Tentatively decided analytical methods

1) Pectin⁷⁾

Place 0.5–1 g sample into the extraction apparatus shown in Fig. 1, and extract for 2 hr with 70% ethanol. Colorless powder thus obtained is dried at below 60°C overnight.

Transfer the powder into a beaker, add 60 ml of water, stir thoroughly, and allow to stand overnight at 20°C. On the next day, dilute it to 100 ml and filter it. The filtrate is used to determine water soluble pectin.

Add 300 ml of water to the residue, allow to stand for a while, and discard the supernatant. Add 10 ml of 4% sodium hexametaphosphate ($(\text{NaPO}_3)_6$) to fill up to 100 ml, and allow to stand for 2 hr at 20°C. The filtrate is used to determine phosphate-soluble pectin.

This residue is again washed by water as previously done, and transferred into a 100 ml Erlenmeyer flask. Add 2 ml of 1N HCl and

80 ml of water, and reflux for 1 hr in a boiling water bath. After cooling, fill up to 100 ml, and filter it. The filtrate is used to determine HCl-soluble pectin.

Three pectin extracts prepared as above were diluted to 2–4 times volume (10–50 $\mu\text{g}/\text{ml}$). Take 2 ml of the solution into a 25 ml stoppered test tube. As a blank, 2 ml of water is used in stead of the extract. Add 12 ml of conc. H_2SO_4 (high special grade) while chilling the test tube in ice-water.

Heat the test tube for 10 min in a boiling water bath, and cool. Add 0.05% alcoholic carbazole solution, mix, and allow to stand at room temperature for 1.5 hr for colour development.

Calibration curve is prepared by using 10–60 $\mu\text{g}/\text{ml}$ solution of D-galacturonic acid. Absorbance at 520 nm is measured and the quantity of pectin is expressed in terms of galacturonic acid by use of the calibration curve.

2) Ascorbic acid⁸⁾

Place 1 g of sample into a mortar together with sand, and grind while moistening with 2% metaphosphoric acid. Transfer the ground sample into a 100 ml volumetric flask by applying metaphosphoric acid solution and fill up to 100 ml. Add 1 spoonful of purified kaolin, and filter through dry filter paper.

Pipet 2 ml of the filtrate into each of two stoppered test tubes (2×20 cm), and add one drop of 0.2% solution of 2,6-dichlorophenol-indophenol. After pink color developed, add 2 ml of thiourea solution (2 g of thiourea dissolved in 100 ml of 8% metaphosphoric acid) and mix well. Add 1 ml of DNP reagent (2 g of 2,4-dinitrophenylhydrazin dissolved in 100 ml of 9N H_2SO_4 solution) to one test tube whereas another tube is used as blank without DNP reagent application.

Keeping in a water bath at 37°C for 6 hr, place the test tubes in ice-water and add 5 ml of 85% H_2SO_4 dropwise, while chilling. To the blank test tube, add 1 ml of DNP reagent. Allow to stand at room temperature for 30

minutes and measure the absorbance (E_1) at 540 nm against the blank.

Pipet 2 ml of the filtrate, mentioned above, into each of two test tubes, add 2 ml of thiourea solution, and to one test tube add 1 ml of DNP reagent, whereas no addition to another tube (blank). After heating as above, measure the absorbance at 540 nm (E_2).

The calibration curve is prepared as follows: Prepare 0.001, 0.002, and 0.003% solution of L-ascorbic acid in metaphosphoric acid, and pipet 2 ml of each into two test tubes. Add one drop of indophenol solution and then 2 ml of thiourea solution. To one test tube add DNP reagent, and measure absorbance at 540 nm, after the same procedure as above, and produce the calibration curve. Then,

$$\text{Ascorbic acid} = E_1 - E_2$$

$$\text{Dehydroascorbic acid} = E_2$$

3) Reducing sugar⁹⁾

Using the extraction apparatus shown in Fig. 1, extract the sample (0.5–1 g) with 80% ethanol for 3 hr. Evaporate ethanol from the extract on a water bath, add 2 ml of basic lead acetate solution (430 g of neutral lead acetate and 130 g of PbO in 1 l of water is boiled for 30 min, and after cooling the supernatant is diluted with water to give specific gravity of 1.25) to precipitate tannin, and dilute with water to 100 ml. Filter through dry filter paper and receive the filtrate in a beaker containing enough solid

sodium oxalate. Remove by filtering lead oxalate produced, and determine sugars by the Hanes method.

References

- 1) Torii, H. & Ōta I.: Studies on the caffeine determination. II. An improved semimicro method. *Study of Tea*, No. 7, 29 (1952).
- 2) Iwasa, K., Ōta, I. & Torii, H.: Improvement of official chemical analysis of tea. IV. Examination of moisture determination. *Study of Tea*, No. 40, 74 (1970).
- 3) Torii, H. & Ōta, I.: Improvement of official chemical analysis of tea. I. Examination of total nitrogen determination. *Study of Tea*, No. 31, 124 (1965).
- 4) Iwasa, K., Ōta, I. & Torii, H.: Improvement of official chemical analysis of tea. III. Examination of tannin determination. *Study of Tea* No. 40, 69 (1970).
- 5) Iwasa, K., Sakamoto, Y. & Torii, H.: Improvement of official chemical analysis of tea. II. Examination of water extract determination. *Study of Tea*, No. 33, 69 (1966).
- 6) Chemistry Laboratory: Official methods of analysis of tea. *Bull. Tea Res. Station*, No. 6, 167 (1970).
- 7) McComb, E. A. & McCreedy, R. M.: Colorimetric determination of pectic substances. *Anal. Chem.*, 24, 1630 (1952).
- 8) Tamura, S. & Suzuki, S.: The 2,4-dinitrophenylhydrazine method for the determination of L-ascorbic acid, dehydro-L-ascorbic acid and 2,3-diketo-L-gluconic acid in plant tissues and processed foods. *J. Agr. Soc. of Japan*, 29, 492 (1955) [In Japanese].
- 9) Iwasa, K., Nakajima, S. & Nakagawa M.: Comparison of the clarifying reagents for the quantitative determination of sugars in tea. *Study of Tea*, No. 44, 44 (1972).