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IS 15344 (2003): Green Tea [FAD 6: Stimulant Foods]



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“Knowledge is such a treasure which cannot be stolen”

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भारतीय मानक
हरी चाय — विशिष्टि

Indian Standard
GREEN TEA — SPECIFICATION

ICS 67.140.10

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BUREAU OF INDIAN STANDARDS
MANAK BHAVAN, 9 BAHADUR SHAH ZAFAR MARG
NEW DELHI 110002

AMENDMENT NO. 1 JUNE 2011
TO
IS 15344 : 2003 GREEN TEA — SPECIFICATION

[Page 2, clause 7.1.1(c)] — Substitute 'Net quantity' for 'Net mass'.

[Page 2, clause 7.1.2(c)] — Substitute 'Net quantity' for 'Net mass'.

[Page 2, clause 7.1.2(e)] — Substitute 'Tare quantity' for 'Tare mass'.

(FAD 6)

Reprography Unit, BIS, New Delhi, India

FOREWORD

This Indian Standard was adopted by the Bureau of Indian Standards, after the draft finalized by the Stimulant Foods Sectional Committee had been approved by the Food and Agriculture Division Council.

Tea is a popular beverage consumed all over the world. It is an important commodity in the international trade and India is one of the major tea producing and exporting countries in the world.

Earlier both Black Tea and Green Tea were covered in IS 3633 : 1972. On revision of IS 3633 technical committee felt the need to bring out a separate standard for green tea, in line with the practice of ISO, which is in the process of developing a separate standard for Green Tea.

The objects of this standard are to specify the plant source from which Green Tea is to be manufactured and to set requirements for certain chemical characteristics which, if met, are an indication that the Green Tea has been subjected to recognized good production practice. To save time and expenses, the quality of Green Tea is also assessed by tea taster, who from his experience can assess whether a given Green Tea would comply with the chemical requirements or not.

In the preparation of this standard, due consideration has been, given to the *Prevention of Food Adulteration Act*, 1954 and the Rules framed thereunder and the *Standards of Weights and Measures (Packaged Commodities) Rules*, 1977. The standard is, however, subject to the restrictions imposed under these, wherever applicable.

For the purpose of deciding whether a particular requirement of this standard is complied with, the final value, observed or calculated, expressing the result of a test or analysis, shall be rounded off in accordance with IS 2 : 1960 'Rules for rounding off numerical values (*revised*)'. The number of significant places retained in the rounded off value should be the same as that of the specified value in this standard.

Indian Standard

GREEN TEA — SPECIFICATION

1 SCOPE

This standard prescribes the requirements, methods of test and sampling for Green Tea.

2 REFERENCES

The following standards contain provisions, which through reference in this text, constitute provision of this standard. At the time of publication, the editions indicated were valid. All standards are subject to revision and parties to agreements based on this standard are encouraged to investigate the possibility of applying the most recent editions of the standards indicated below:

<i>IS No.</i>	<i>Title</i>
1070 : 1992	Reagent grade water (<i>third revision</i>)
2491 : 1998	Food hygiene — General principles — Code of practice (<i>second revision</i>)
3611 : 1975	Method of sampling for tea (<i>first revision</i>)
3633 : 2003	Black Tea (<i>second revision</i>)
10226 (Part 1) : 1982	Method for determination of crude fibre content: Part 1 General method
11123 : 1984	Method for determination of copper by atomic absorption spectrophotometry
11773 : 1986	Method for determination of ethion residues in food commodities, soil and water
12074 : 1987	Method for determination of lead by atomic absorption spectrophotometry
13852 : 1994	Tea — Preparation of ground sample of known dry matter content
13853 : 1994	Tea — Determination of loss in mass at 103°C
13854 : 1994	Tea — Determination of total ash
13855 : 1993	Tea — Determination of water-soluble ash and water-insoluble ash
13856 : 1993	Tea — Determination of alkalinity of water-soluble ash
13857 : 1993	Tea — Determination of acid-insoluble ash
13859 : 1993	Instant tea in solid form — Determination of moisture content (loss in mass at 103°C)
13862 : 1998	Tea — Determination of water extract (<i>first revision</i>)
14629 : 1999	Method for determination of dicofol residues in agricultural and food commodities

<i>IS No.</i>	<i>Title</i>
14437 : 1997	Method for determination of quinalphos residues in agricultural and food commodities

3 TERMINOLOGY

Green Tea means the tea derived solely and exclusively and produced by acceptable processes, notably enzyme inactivation, rolling or comminution and drying, from the leaves, buds and tender stems of varieties of the species *Camellia sinensis* (Linnaeus) O. Kuntze, known to be suitable for making Green Tea for consumption as a beverage.

4 REQUIREMENTS

4.1 Description

The Green Tea shall produce a liquor of characteristic flavour, colour and taste. The Green Tea shall have no taint and shall be free from extraneous matter, added colours and non-permitted flavours. Green Tea shall be evaluated in accordance with the procedure prescribed in Annex A of IS 3633.

4.2 Iron filings in the product shall not exceed the limit of 250 mg/kg, and their size shall not be greater than 2.0 mm when tested by the method given in Annex C of IS 3633.

NOTE — No limit is specified for the moisture content of the Green Tea. If desired, the actual loss in mass at $103 \pm 2^\circ\text{C}$ of the sample as received may be determined and the result recorded in the test report. In such cases, the determination shall be carried out by the method described in IS 13853.

4.3 Hygiene Requirements

Green Tea shall be manufactured and packed under hygienic conditions as per IS 2491.

4.4 Chemical Requirements

4.4.1 Green Tea shall also comply with the requirements specified in Tables 1, 2 and 3 in which all the requirements are expressed on the basis of the material oven-dried at $103 \pm 2^\circ\text{C}$ by the method described in IS 13852.

5 ADDITIONAL REQUIREMENT FOR ECO-MARK

The additional requirement for ECO-Mark are same as given in Annex B of IS 3633.

6 PACKING

Green Tea shall be packed in closed, clean and dry

containers made of material, which does not affect the Green Tea, or in accordance with the customary trade practices so as to allow the Green Tea to retain its freshness.

Table 1 Requirements for Green Tea
(Clause 4.4.1)

Sl No.	Characteristic	Requirement	Method of Test, Ref to	
			IS No.	Annex of this standard
(1)	(2)	(3)	(4)	(4)
i)	Water extract, percent by mass, <i>Min</i>	32.0	13862	—
ii)	Total ash, percent by mass,	4.0-8.0	13854	—
iii)	Water-soluble ash, of total ash, percent by mass, <i>Min</i>	45	13855	—
iv)	Alkalinity of water-soluble ash (as K ₂ O), percent by mass	1.0-2.2	13856	—
v)	Acid-insoluble ash, percent by mass, <i>Max</i>	1.0	13857	—
vi)	Crude fibre, percent by mass, <i>Max</i>	16.5	10226 (Part 1)	—
vii)	Total catechins, percent by mass, <i>Min</i>	9.0	—	Annex A

**Table 2 Additional Requirements —
Metallic Contaminants**
(Clause 4.4.1)

Sl No.	Characteristic	Requirement	Method of Test, Ref to
(1)	(2)	(3)	(4)
i)	Lead, mg/kg, <i>Max</i>	10.0	IS 12074
ii)	Copper, mg/kg, <i>Max</i>	150.0	IS 11123

**Table 3 Additional Requirements —
Pesticide Residues**
(Clause 4.4.1)

Sl No.	Characteristic	Requirement	Method of Test, Ref to
(1)	(2)	(3)	(4)
i)	Dicofol, mg/kg, <i>Max</i>	5.0	IS 14629
ii)	Ethion, mg/kg, <i>Max</i>	5.0	IS 11773
iii)	Quinolphos, mg/kg, <i>Max</i>	0.01	IS 14437

7 MARKING

7.1 The information given in 7.1.1 or 7.1.2 shall be clearly and indelibly marked on the package.

7.1.1 Retail Package

- Name and address of the manufacturer;

- Name of the product;
- Net mass of contents;
- Month and year of manufacture;
- Batch, code number or any other identification No.;
- The words 'Best before.....' (Month and year to be indicated);
- Tea board registration number, if flavour is added;
- Declaration, if flavours and flavouring substance added; and
- Any other requirements given under the *Prevention of Food Adulteration Rules*, or the *Standards of Weights and Measures (Packaged Commodities) Rules*, 1977.

7.1.2 Wholesale Package

- Name and address of the manufacturer;
- Name of the product;
- Net mass of contents;
- Gross mass of contents;
- Tare mass of contents;
- Month and year of manufacture;
- Batch, code number or any other identification No.;
- The words 'Best before' (Month and year to be indicated);
- Tea board registration number, if flavour is added;
- Declaration, if flavours and flavouring substance added; and
- Any other requirements given under the *Prevention of Food Adulteration Rules*, or the *Standards of Weights and Measures (Packaged Commodities) Rules*, 1977.

7.2 BIS Certification Marking

The product may also be marked with the Standard Mark.

7.2.1 The use of the Standard Mark is governed by the provisions of *Bureau of Indian Standards Act*, 1986 and the Rules and Regulations made thereunder. The details of conditions under which the licence for the use of the Standard Mark may be granted to manufacturers or producers may be obtained from the Bureau of Indian Standards.

8 SAMPLING

The ground sample of the material shall be prepared in accordance with the procedure outlined in IS 13852 before undertaking the analysis for various chemical characteristics. The method of drawing representative samples of the material and the criteria for conformity shall be as prescribed in IS 3611.

ANNEX A

[Table 1 Item (vii)]

DETERMINATION OF CATECHINS IN GREEN TEA — METHOD USING HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY**A-1 PRINCIPLE**

The total catechin content is extracted from a test portion of finely ground tea with 70 percent methanol at 70°C, and instant tea is dissolved in hot water with 10 percent (v/v) acetonitrile added to stabilize the extract. The resulting extracts are then assayed for individual intact catechins by HPLC on a phenyl bonded column using a linear acetonitrile gradient elution and detection by UV absorbance at a wavelength of 278 nm.

A-2 REAGENTS

A-2.1 Water — in accordance with IS 1070.

A-2.2 Acetonitrile — HPLC Grade.

A-2.3 Methanol

A-2.4 Glacial Acetic Acid — HPLC Grade.

A-2.5 EDTA (Ethylenediaminetetraacetic Acid Disodium Salt, Dihydrate)

A-2.6 L-ascorbic Acid — Free acid.

A-2.7 Methanol/Water Extraction Mixture, 70 percent v/v Methanol — Add 700 ml of the methanol to a 1 litre mark volumetric flask. Dilute to the mark with water and mix.

A-2.8 HPLC Mobile Phase — Caution wear gloves, eye protection and dispense reagents in a fume cupboard.

A-2.8.1 Mobile Phase A — Add 180 ml of acetonitrile and 40 ml acetic acid to a 2 litre mark volumetric flask. Dilute to the mark with water, mix, and filter through a filter of 0.45 µm pore size.

A-2.8.2 Mobile Phase B — Add 800 ml acetonitrile to a 1 litre mark volumetric flask. Dilute to the mark with water, mix and filter through a filter of 0.45 µm pore size.

A-2.9 Stabilizing Solution

Weigh, to the nearest 0.01 g, 0.25 g of EDTA and 0.25g of ascorbic acid into a 1 litre mark volumetric flask and dissolve in approximately 500 ml water. Add 100 ml acetonitrile dilute to the mark with water and mix. Prepare fresh stabilizing solution on the day of use.

A-2.10 Stock Standard Solutions

A-2.10.1 General — When standards are available in

sufficient quantities (that is > 20 mg) an analytical balance capable of weighing to at least 0.1 mg is required, whereas for limited quantities (that is < 20 mg) an analytical balance capable of weighing to 0.01 mg is required.

Weighed amounts of standards are transferred to separate one-mark volumetric flasks and dissolved in stabilizing solution, gently warming, if required (maximum 40°C). The cool solution is diluted to the mark with stabilizing solution. Stock standard solutions are prepared in accordance with A-2.10.2 to A-2.10.8.

A-2.10.2 Gallic Acid Stock Standard Solution — corresponding to 2.00 mg/ml.

A-2.10.3 Caffeine Stock Standard Solution — corresponding to 2.00 mg/ml.

A-2.10.4 (+)–Catechin, + C, Stock Standard Solution — corresponding to 1.00 mg/ml.

A-2.10.5 (–)–Epicatechin, EC, Stock Standard Solution — corresponding to 1.00 mg/ml.

A-2.10.6 (–)–Epigallocatechin, EGC, Stock Standard Solution — corresponding to 2.00 mg/ml.

A-2.10.7 (–)–Epigallocatechingallate, EGCG, Stock Standard Solution — corresponding to 2.00 mg/ml.

A-2.10.8 (–)–Epicatechingallate, ECG, Stock Standard Solution — corresponding to 2.00 mg/ml.

A-2.11 Dilute Gallic Acid Standard Solution — corresponding to 200 µg/ml. Using a pipette transfer 10 ml of the gallic acid stock standard solution to a 100 ml one-mark volumetric flask. Dilute to the mark with stabilizing solution and mix.

A-2.12 Mixed Working Standard Solutions

A-2.12.1 Prepare three mixed working standard solutions, with concentrations selected to cover the range of compositions typically found in tea.

A-2.12.2 Following Table 4, carefully pipette the given aliquots of dilute gallic acid standard solution and stock standard solutions into three separate 20 ml one-mark volumetric flasks, dilute to volume with stabilizing solution and mix. These mixed working standard solutions correspond to the nominal concentrations shown in Table 5. Use the actual standard weights taken

Table 4 Composition of Mixed Working Standard Solutions Standard 1 to Standard 3
(Clause A-2.12.2)

SI No.	Component	Solution	Aliquot, ml		
			Standard 1	Standard 2	Standard 3
(1)	(2)	(3)	(4)	(5)	(6)
i)	Gallic acid	200 µg/ml dilute stock standard solution	0.5	1.0	2.5
ii)	Caffeine	2.00 mg/ml stock standard solution	0.5	1.0	1.5
iii)	+C	1.00 mg/ml stock standard solution	1.0	2.0	3.0
iv)	EC	1.00 mg/ml stock standard solution	1.0	2.0	3.0
v)	EGC	2.00 mg/ml stock standard solution	1.0	2.0	3.0
vi)	EGCG	2.00 mg/ml stock standard solution	1.0	2.0	4.0
vii)	ECG	2.00 mg/ml stock standard solution	0.5	1.0	2.0

to obtain the actual concentrations at each standard level.

Table 5 Nominal Concentrations in Mixed Working Standard Solutions Standard 1 to Standard 3
(Clause A-2.12.2)

SI No.	Component	Nominal Concentration		
		Standard 1	Standard 2	Standard 3
(1)	(2)	(3)	(4)	(5)
i)	Gallic acid	5	10	25
ii)	Caffeine	50	100	150
iii)	+C	50	100	150
iv)	EC	50	100	150
v)	EGC	100	200	300
vi)	EGCG	100	200	400
vii)	ECG	50	100	200

A-2.12.3 Pipette 1.0 ml aliquots of each mixed standard solution into labelled small amber glass vials, gently flush with nitrogen prior to sealing and store frozen at -20°C .

NOTES

1 Mixed working standard solutions are stable for at least 2 months when stored frozen at -20°C .

2 Only thaw sufficient mixed working standard solution vials for each chromatographic run. Discard any remaining solution, do not re-freeze.

A-3 APPARATUS

A-3.1 Analytical Balance — capable of weighing to an accuracy of ± 0.0001 g.

A-3.2 Water Bath — regulated to a temperature of $70 \pm 1^{\circ}\text{C}$.

A-3.3 Dispenser — set at 5 ml for methanol/water extraction mixture (see A-2.7).

A-3.4 Centrifuge — capable of 3 500 rev/min.

A-3.5 Vortex Mixer — for efficient mixing during extraction.

A-3.6 Extraction Tubes — glass, 10 ml capacity, stoppered and able to withstand centrifugation.

A-3.7 Graduated Tubes — glass, 10 ml capacity with 0.1 ml graduations.

A-3.8 Automatic Pipettes — to cover the volume range for standard and sample extract dilutions.

A-3.9 Filters — membrane filter units of $0.45 \mu\text{m}$ pore size.

A-3.10 High-Performance Liquid Chromatograph — equipped to perform binary gradient elution, with a thermostatically controlled column compartment and an ultraviolet detector, allowing measurements to be made at a wavelength of 278 nm.

A-3.11 Data Collection/Integration System

A-3.12 Chromatographic Column for HPLC

NOTES

1 Phenyl bonded phases give additional selectivity over reversed phase packings, and result in improved resolution of the catechins.

2 In this standard the chromatographic conditions and composition of the mobile phase specified are suitable for a Phenomenex Luna $5 \mu\text{m}$ Phenyl-Hexyl column of dimensions $250 \text{ mm} \times 4.6 \text{ mm}$ fitted with a Phenomenex Security Guard $4 \text{ mm} \times 3.0 \text{ mm}$ Phenyl-Hexyl cartridge. If other types of column are used, an alternative mobile phase and alternative chromatographic conditions may be necessary.

A-4 SAMPLING

It is important that the laboratory receives a sample which is truly representative and has not been damaged or changed during transport or storage.

A-5 PREPARATION OF TEST SAMPLES

Grind the sample of Green leaf tea or Black leaf tea in accordance with IS 13852. Store all samples in well sealed containers, protected from light, and cool.

NOTE — Grinding of instant tea is only required for samples with a coarse granular structure.

A-6 PROCEDURE

A-6.1 Determination of Dry Matter Content

Calculate the dry matter content from the moisture content (loss in mass at 103°C) determined on a portion of the test sample in accordance with:

- a) IS 13852 for Green or Black Tea; and
- b) IS 13859 for instant tea.

A-6.2 Test Portion

A-6.2.1 Instant Tea

Weigh, to the nearest 0.000 1 g, 0.5 g of the test sample (see A-5) into a 50 ml one-mark volumetric flask.

A-6.2.2 Green and Black Tea

Weigh, to the nearest 0.000 1 g, 0.2 g of the test sample (see A-5) into an extraction tube.

A-6.3 Extraction

A-6.3.1 Instant Tea

A-6.3.1.1 Add, to the instant tea in the flask from 6.2.1, approximately 25 ml hot water (maximum temperature of 60°C), mix to dissolve the sample and allow to cool to room temperature.

A-6.3.1.2 Add, 5 ml acetonitrile, dilute to the mark with water and mix.

A-6.3.2 Green and Black Tea

A-6.3.2.1 Place the methanol/water extraction mixture contained in the dispenser into the waterbath set at 70°C, and allow 30 min for the extraction mixture to reach temperature.

A-6.3.2.2 Place the extraction tube containing the tea sample from A-6.2.2 into the water bath set at 70°C. Add 5 ml hot methanol/water extraction mixture from the dispenser, stopper the tube and carefully mix on the vortex mixer.

NOTE — It is important to mix samples thoroughly to ensure complete extraction.

A-6.3.2.3 Continue heating the extraction tube in the water bath for 10 min, mixing on the vortex mixer at 5 min and 10 min.

A-6.3.2.4 Remove the extraction tube from the water bath, and allow to cool to room temperature. Remove stopper and place in the centrifuge at 3 500 rev/min for 10 min.

A-6.3.2.5 Carefully decant the supernatant into a graduated tube.

A-6.3.2.6 Repeat extraction steps A-6.3.2.2 to A-6.3.2.5. Combine extracts, make up to 10 ml with cold methanol/water extraction mixture and mix contents.

NOTE — The extract from A-6.3.2.6 is stable for at least 24 h if stored at 4°C. Allow extract to reach room temperature before proceeding with the assay. Resuspension of the small amount of fine particulate material settled during storage is not necessary.

A-6.4 Dilution

Using a pipette, transfer 1.0 ml of the sample extract (see A-6.3.1.2 for instant tea and A-6.3.2.6 for leaf tea) into a graduated tube and dilute to 5 ml with stabilizing solution. Mix solution then filter through 0.45 µm filter.

A-6.5 Determination

A-6.5.1 Adjustment of the Apparatus

Set up the chromatograph in accordance with the manufacturer's instructions and adjust it as follows:

- a) Flow rate of the mobile phase: 1.0 ml/min
- b) Binary gradient conditions: 100 percent mobile phase A for 10 min, then over 15 min a linear gradient to 68 percent mobile phase A, 32 percent mobile phase B and hold at this composition for 10 min. Then reset to 100 percent mobile phase A and allow to equilibrate for 10 min before next injection.
- c) Temperature of the column: 35 ± 0.5°C.

NOTES

1 Column temperature control is recommended (chromatography column oven or recirculating water jacket) if major drifts in retention times are to be avoided. UV detector setting: wavelength 278 nm.

2 Ensure that the detector sensitivity range selected is able to keep all peaks from the highest mixed working standard (Standard 3) within the scale of the data collection system used.

A-6.5.2 HPLC Analysis

A-6.5.2.1 Once the flow rate of the mobile phase and temperature are stable, condition the column with a blank gradient run. Then inject onto the column 10 µl of each of the mixed working standard solutions Standard 1, Standard 2 and Standard 3 followed by an equal volume of the diluted test solution. Repeat injection of the mixed working standard solutions at regular intervals (typically after six test solutions). Collect and record the data for the peaks of all standards and test samples.

A-6.5.2.2 After each day's use and prior to storage, wash the column with approximately 50 percent acetonitrile, replacing the column sealing plugs after disconnection.

A-7 CALCULATION

A-7.1 Identify and measure the peak areas or heights (area is preferable) for all standards and test samples. Construct linear calibration graphs for all components in the standards of concentration (µg/ml) against peak areas or heights and obtain the individual standard

response factors (RF) automatically using a data collection/integration system or manually from a selected point on the calibration graph.

$$RF = \frac{c_{std}}{A_{std} \text{ or } h_{std}}$$

where

RF = standard response factor;

c_{std} = concentration of the standard ($\mu\text{g/ml}$);

A_{std} = peak area of the standard; and

h_{std} = peak height of the standard.

A-7.2 Calculate response factors for all the individual components, that is gallic acid, caffeine and the individual catechins EGC, +C, EC, EGCG and ECG. Calibration information obtained from a data collection/integration system will include an intercept value when the calibration is not forced through the origin and this should be included in the calculation.

A-7.3 The concentration of the individual components expressed as a percentage by mass on a sample as received basis is given by the formula:

$$\text{Percent individual component (m/m) (as received basis)} = (A_{\text{samp}} \text{ or } h_{\text{samp}}) RF \times \frac{Vd}{10\,000 m}$$

where

A_{samp} = peak area for the test sample;

h_{samp} = peak height for the test sample;

RF = response factor for the individual component;

V = sample extraction volume (50 for instant tea or 10 for leaf tea);

d = dilution factor (*see A-6.4*), typically 5; and

m = mass, in g, of the test sample.

Percent total catechins (m/m) (as received basis) = (percent EGG) + (percent +G) + (percent EG) + (percent EGGG) + (percent EGG)

$$\begin{array}{l} \text{Percent total catechins} \\ \text{(m/m) (dry matter basis)} \end{array} = \frac{\text{Percent total catechins (m/m)} \\ \text{(as received basis)} \times 100}{w}$$

where

w = dry matter content of the test sample, determined in accordance with A-6.1.

Bureau of Indian Standards

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Review of Indian Standards

Amendments are issued to standards as the need arises on the basis of comments. Standards are also reviewed periodically; a standard along with amendments is reaffirmed when such review indicates that no changes are needed; if the review indicates that changes are needed, it is taken up for revision. Users of Indian Standards should ascertain that they are in possession of the latest amendments or edition by referring to the latest issue of 'BIS Catalogue' and 'Standards: Monthly Additions'.

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Amendments Issued Since Publication

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