

BUREAU OF INDIAN STANDARDS

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भारतीय मानक मसौदा
खाद्यानों की विश्लेषण पद्धति
भाग 5 यूरिक एसिड का निर्धारण
[आई एस (4333 भाग 5) का पहला पुनरीक्षण]

Draft Indian Standard
Methods of Analysis for Foodgrains
Part 5 Determination of Uric Acid
[First revision of IS 4333 (Part 5)]

ICS 67.060

Foodgrains, Allied products, and other
agricultural Produce Sectional Committee, FAD 16

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FOREWORD

(Formal clause would be added later)

Owing to the large production of foodgrains and inter-state transactions in the country as well as development of infrastructure for handling foodgrains, the assessment of their quality assumes great significance. For proper assessment, it is necessary that only uniform methods of test are adopted and only such terms are used in test reports which have been defined properly. This standard, based on national and international practices was formulated accordingly, to ensure the adoption of uniform terminology and methods of test for foodgrains throughout the country. The standard has been published in five parts covering methods for tests for different physico-chemical characteristics of foodgrains.

This standard (Part 5) covers test method for determination of uric acid content in foodgrains. The other parts published in this series are:

Part 1 Refractions

Part 2 Determination of moisture content

Part 3 Determination of bulk density called mass per hectolitre (Routine Method)

Part 4 Determination of the mass of 1000 grains

Various methods are employed for assessing insect damage in foodgrains. The methods consist of utilizing a number of physical or chemical indices for estimating the degree of insect infestation. Since uric acid is the main constituent of the insect excreta, estimation of this characteristic is most often taken as an index of the degree of insect infestation.

This standard was first published in 1970. This revision (*first*) has been undertaken to include the spectrophometric method for analyzing uric acid in foodgrains and their products. The standard has also been brought out in the latest style and format of Indian Standards and references to Indian Standards, wherever applicable, have been updated.

In reporting the result of a test or analysis made in accordance with this standard, if the final value, observed or calculated, is to be rounded off, it shall be done in accordance with IS 2 : 2022 'Rules for rounding off numerical values (second revision)'.

1 SCOPE

This standard prescribes the method for the determination of uric acid in cereals and cereal products.

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 is encouraged to investigate the possibility of applying the most recent editions of the standards indicated below:

<i>IS</i>	<i>TITLE</i>
IS 14818 : 2017/ ISO 24333 : 2009	Cereal and cereal products – Sampling (<i>first revision</i>)

3 METHODS

3.0 Two methods namely, photometric method (**3.1**) and spectrophotometric method (**3.2**) may be used for determination of uric acid in cereal and cereal products. In case of dispute, the spectrophotometric method shall be used as the referee method.

3.1 Photoelectric Method

3.1.1 Principle of Method

Proteins of a known weight of the sample are precipitated by sodium tungstate and sulphuric acid. An aliquot part of the protein-free filtrate is treated with uric acid reagent and sodium cyanide under standard conditions and the blue colour contained is compared colorimetrically against a similarly treated standard.

3.1.2 Apparatus

- a) *Photo-electric colorimeter*
- b) *Volumetric flask – 50 ml capacity*
- c) *Burette*
- d) *Nessler's tubes*

3.1.3 Reagents

- a) *Sodium tungstate solution – 10 percent (w/v)*
- b) *Standard sulphuric acid solution – 0.667 N*

- c) *Benedict's uric acid reagent* – Prepared by first dissolving 100 g of pure sodium tungstate in 600 ml of water. Then add 5 g of arsenic acid (As_2O_5) followed by 25 ml of 85 percent phosphoric acid and 20 ml of concentrated hydrochloric acid. Boil the mixture for 20 minutes, cool and make volume up to 1 litre.
- d) *Sodium cyanide solution* – 5 percent (w/v) solution containing 2 ml of ammonia per litre. This solution requires to be prepared anew after about 6 weeks.
- e) *Standard uric acid solution (Benedict's)*
 - i) *Stock solution* – Prepared by dissolving 9 g of disodium hydrogen phosphate and 1g of sodium di-hydrogen phosphate in about 200 to 300 ml of hot water. If the solution is not clear, filter and make up the volume to 500 ml with hot water. Weigh 200 mg of pure uric acid in one-litre volumetric flask and add a few millilitres of water to suspend the uric acid. Now add the solution made earlier and shake till the uric acid dissolves completely. Cool and add 1.4 ml of glacial acetic acid, dilute to mark and mix. To prevent bacterial or mould growth add 5 ml of chloroform. Five millilitres of this stock solution contains 1 mg of uric acid.
 - ii) *Working standard solution* – Prepared by diluting 50 ml of stock solution [see 3.1.3 (e) (i)] containing 10 mg of uric acid with 400 ml of distilled water in a 500-ml volumetric flask. Add 25 ml of dilute hydrochloric acid (1 volume of concentrated hydrochloric acid and 9 volumes of water). Make the solution up to the mark and mix. This working standard solution should be prepared from stock solution [see 3.1.3 (e) (i)] which is more than 10 days old.

3.1.4 Procedure

3.1.4.1 Weigh 50 g of the sample and pulverize it finely. Take from 4 to 20 g of the powder, expected to contain about 1 to 5 mg of uric acid and suspend in 200 ml of water. Allow the mixture to stand for two hours and then mix in Waring Blender for 10 minutes and centrifuge at about 2 000 rev/min for 10 minutes. To 100 ml of the clear centrifugate add 10 ml of sodium tungstate solution and mix. Then add 10 ml of standard sulphuric acid solution to precipitate the proteins present in the extract. Mix and allow it to stand for five minutes and filter. Take an aliquot of the filtrate (containing between 0.15 and 0.3 mg of uric acid for every 10 ml of the filtrate) in the 50-ml volumetric flask and add 5 ml of sodium cyanide solution followed by 1 ml of Benedict's uric acid reagent. Mix by gentle shaking and make up to the mark with distilled water.

3.1.4.2 Take 10 ml of standard uric acid solution [see 3.1.3 (e)] containing 0.2 mg of uric acid in a 50-ml flask and add 5 ml of sodium cyanide solution and 1 ml of Benedict's uric acid reagent. Dilute to the mark after 5 minutes and determine the intensity of colour either in a photoelectric colorimeter using a 520 nm filter or by visual comparison in Nessler's tubes.

3.1.4.3 In case the determination is carried out by visual comparison, it may be necessary to have a number of standards containing varying proportions of uric acid for matching with the colour developed in the sample under test.

3.1.4.4 A parallel test using the same quantity of uninfested grains, as the sample under test should be run as the 'control'.

3.2 METHOD 2 Spectrophotometric Method

3.2.1 Principle of Method

Samples are treated with hydrochloric acid, incubated, and neutralized with sodium hydroxide. Uric acid is extracted with sodium acetate and is determined with glutathione solution as the color reagent. Uric acid is destroyed with uricase, and the spectrophotometric analysis is repeated for a blank measurement.

3.2.2 Apparatus

- a) *UV-Visible spectrophotometer*;
- b) *Centrifuge* – Desk centrifuge with multiple head to hold 15 ml polyethylene test tube; and
- c) *Incubator or water bath* – Maintaining temperature of $37\text{ }^{\circ}\text{C} \pm 1\text{ }^{\circ}\text{C}$.

3.2.3 Reagents

- a) *Uric acid standard solution*, 100 $\mu\text{g/ml}$ – Dissolve 100 mg uric acid in 1 litre 5% CH_3COONa solution. (If necessary, warm in water bath at 60°C - $70\text{ }^{\circ}\text{C}$). Filter and store in brown bottle; discard after 1 week.
- b) *Sodium borate buffer*, 1.01 M, pH 9.2 – Dissolve 3.8 g sodium borate in distilled water and dilute to 1 litre.
- c) *Sodium acetate solution*, 5% – Dissolve 100 g anhydrous sodium acetate in distilled water and dilute to 2 litre. Adjust pH to 8.8-9.2 with acetic acid and/or sodium hydroxide.
- d) *Glutathione solution*, 10 mg/ml in distilled water
- e) *Uricase solution* – Prepare suspension of 10 mg dried uricase in 50 ml 0.01 M Sodium borate buffer. Use within 1 hour. (Clean all glassware that comes in contact with uricase enzyme with chromic acid solution; adsorbed uricase on glass surface produces low results).

3.2.4 Preliminary tests

3.2.4.1 *Test for purity of reagents* – Dilute 5.0 ml uric acid standard solution to 25 ml with 5% CH_3COONa solution. Place 5 ml in each of three test tubes. To one tube add 5 ml sodium borate buffer, invert several times, and measure *A* (absorbance) at 292 nm. *A* should be 0.72, which corresponds to 0.072 unit/ μg uric acid/ml final solution.

3.2.4.2 *Test for efficiency of urease solution* – Label remaining two tubes in **3.2.4.1** as No. 1 and No. 2, and label a third test tube No.3. Add 5 ml uricase solution to tube No. 1 and No.3. Close mouth of tube No. 1 with piece of cellophane sheet under thumb and invert. Stopper all three tubes with clean rubber stoppers and incubate for 2 h at $37\text{ }^{\circ}\text{C}$. After incubation, mix

contents of tubes No. 2 and 3 by repeatedly pouring (6 times) from one tube to other, and immediately (within 60 s) read A of combined solution at 292 nm, using solution in tube No. 1 as blank. A should be 0.648 for 90% of theoretical efficiency of uricase. If efficiency is less than 90%, incubate for 4 h. If increased incubation does not increase efficiency to 90%, discard uricase.

3.2.5 Preparation of Standard Curve

Pipette 1.0 ml, 2.5 ml, 5.0 ml, 10.0 ml and 15.0 ml uric acid standard solution into separate beakers (corresponds to 0.0 μg , 1.0 μg , 2.0 μg , 4.0 μg and 6.0 μg uric acid/ml in final solution, respectively), and perform all steps as in **3.2.6**.

3.2.6 Procedure

3.2.6.1 Add 25 ml of 1M HCl and 5 ml glutathione solution to 4 g flour in 250 ml beaker. Mix well with glass rod and let it stand overnight (approximately, 16 hours).

3.2.6.2 Add 25 ml of 1M NaOH with stirring and adjust $p\text{H}$ to 9.0 – 9.3 with 1M NaOH or 1M HCl. Transfer to 100 ml glass stoppered graduate cylinder, carefully scraping all material sticking to sides of beaker with glass rod. Rinse beaker with 6 small portions of 5% CH_3COONa . Shake gently by inverting graduate several times every 10 min for 1 h. (Vigorous shaking tends to produce turbid solution). Transfer aliquot to 15 ml polyethylene test tube and centrifuge 30 min at 3000 rpm.

3.2.6.3 Decant supernatant into small Erlenmeyer flask, mix well, and pipet 4 ml sodium borate buffer and mix by rotating between palms of hands. (Mix solution with sodium borate buffer within 15 min to avoid turbid solution). Label third tube as No. 3. Add 5 ml uricase solution to tubes No. 1 and No. 3. Mix content of tube No. 1 as in **3.2.4.2**. Stopper all 3 tubes with rubber stoppers and incubate for 2 h at 37 °C. Combine solutions in tubes No. 2 and No. 3, as in **3.2.4.2**, and read A immediately (within 60 s) at 292 nm against solution No. 1 (blank). [If flour extract appears very turbid after centrifuging, dilute centrifuged extract with sodium borate buffer in the ratio of 1:4, and pipet 5.0 ml into each of two test tubes, No. 1 and No. 2. Add 5 ml uricase to each test tube (No. 1 and No. 3) and proceed with determination as above].

3.2.7 Calculation

$$U = A \times D$$

Where,

U = Amount of uric acid in test sample

A = Reading A , amount of uric acid obtained from standard curve

D = Dilution factor

3.2.7 Precautions

3.2.7.1 Use glutathione solution within 30 minutes.

3.2.7.2 Do not use commercial uric acid standard solutions; as they may contain uricase inhibitors.

3.2.7.3 Test standard uric acid solution daily.