Test method for Edible-birdnest (EBN) - Determination for nitrite (NO$_2^-$) and nitrate (NO$_3^-$) contents - Ion Chromatography

ICS: 67.050

Descriptors: edible-birdnest, test method, nitrite, nitrate, ion chromatography

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DEPARTMENT OF STANDARDS MALAYSIA
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Committee representation

The Industry Standards Committee on Agricultural (ISC A) under whose authority this Malaysian Standard was developed, comprises representatives from the following organisations:

Department of Agriculture Malaysia
Department of Fisheries Malaysia
Department of Standards Malaysia
Department of Veterinary Services
Farmers' Organisation Authority
Federal Agricultural Marketing Authority
Federation of Livestock Farmers’ Associations of Malaysia
Forest Research Institute Malaysia
Malaysia Fruit Exporters Association
Malaysian Agricultural Research and Development Institute
Malaysian Association of Standards Users
Malaysian Palm Oil Association
Malaysian Rubber Board
Ministry of Agriculture and Agro-Based Industry Malaysia
Ministry of Plantation Industries and Commodities
National Seed Association Malaysia
SIRIM Berhad (Secretariat)
Universiti Putra Malaysia

The Working Group on Edible-birdnest (EBN) test method which developed this Malaysian Standard consists of representatives from the following organisations:

Department of Chemistry
Department of Veterinary Services
Department of Veterinary Services (Southern Regional Veterinary Laboratory)
Department of Veterinary Services (Eastern Regional Veterinary Laboratory)
Ministry of Health Malaysia
Permulab Sdn Bhd
SIRIM Berhad (Industrial Biotechnology Research Centre)
SIRIM Berhad (Secretariat)
Sunshine Region Sdn Bhd
Universiti Putra Malaysia (Faculty of Veterinary Medicine)
Foreword

This Malaysian Standard was developed by the Working Group on Edible-birdnest (EBN) test method under the authority of the Industry Standards Committee on Agriculture.

Compliance with a Malaysian Standard does not of itself confer immunity from legal obligations.
Test method for Edible-birdnest (EBN) - Determination for nitrite (NO$_2^-$) and nitrate (NO$_3^-$) contents - Ion Chromatography

1 Scope

This Malaysian Standard describes the test method for nitrite (NO$_2^-$) and nitrate (NO$_3^-$) in Edible-birdnest (EBN). The test shall be based on the use of ion chromatographic method.

2 Normative references

The following normative references are indispensable for the application of this standard. For dated references, only the edition cited applies. For undated references, the latest editions of the normative references (including any amendments) apply.

MS 954: Part 14, Methods of test for meat and meat products, Part 14 : Determination of nitrite content

MS 954: Part 15, Methods of test for meat and meat products, Part 14 : Determination of nitrate content

MS ISO/IEC 17025:2005, General requirements for the competence of testing and calibration laboratories

GB 5009.33-2010, Determination of Nitrite and Nitrate in Foods

3 Terms and definitions

3.1 Raw-Clean EBN

EBN that has undergone cleaning process may include but not limiting to sorting, drying, soaking, picking of feathers and impurities, moulding, drying, grading and packing.

3.2 Raw-Unclean EBN

EBN harvested from caves and ranches which may include but not limiting to sorting, drying, grading, trimming, weighing and packing but, without any cleaning process. There are still visible feathers and impurities.

3.3 Nitrite

Nitrite is an anion with the chemical formula NO$_2^-$.

3.4 Nitrate

Nitrate is an anion with the chemical formula NO$_3^-$.
3.5 **Ion chromatography**

A process that allows the separation of ions and polar molecules based on their affinity to the ion exchanger.

3.6 **Eluent**

Mobile phase used to extract solute or ion bound to a matrix as shown in the figure below:

![Diagram of ion chromatography](image)

3.7 **Reagents**

A reagent is a substance or compound that is added to a system in order to bring about a chemical reaction, or added to see if a reaction occurs.

3.8 **Ultra-pure water (UPW)**

Water, where, organic and inorganic compounds, dissolved and particulate matter, volatile and non-volatile, reactive and inert, hydrophilic and hydrophobic, and dissolved gases have been removed. Its conductivity of 18.2 MΩ.cm is maintained.

4 **Principle**

An EBN aqueous extract is subjected to ion exchange chromatography. The ion exchange column is eluated using potassium hydroxide. The eluate is measured using conductivity detector and quantified.

5 **Reagents**

Use only reagents of recognised analytical grade, unless otherwise stated.

5.1 **Ultra-pure water (UPW)**

5.2 **Eluent generator for Potassium hydroxide (KOH).**

5.3 **Nitrite ion (NO₂⁻) stock solution (1000 mg/l, aqueous solution).**

5.4 **Nitrate ion (NO₃⁻) stock solution (1000 mg/l, aqueous solution).**
5.5  **Mixed Standard Solution, Nitrite and Nitrate.**

5.5.1  Pipette 10 ml of nitrite ion (NO$_2^-$) stock solution and 10 ml nitrate ion (NO$_3^-$) stock solution into 100 ml volumetric flask, adjust to the mark with water.

5.5.2  Mix well
1 ml of this mixed solution contains 100 µg of nitrite ion and 100 µg of nitrate ion.

5.6  **Working Mixed Standard Solution**

Prepare a working mixed standard solution between 0.1 mg/l to 10 mg/l.

6  **Apparatus**

6.1  **Ion Chromatography System Dionex** inclusive of a conductivity detector, suppressor or equivalent (refer to manufacturer procedure).

6.1.1  **Automated Sampler.**

6.1.2  **Anion exchange column (Analytical Column: AS19 or equivalent)** (4 x 250mm, 5 µm particle diameter)

6.1.3  **Guard Column: AG 19 or equivalent** (4 x 50 mm, 5 µm particle diameter) - a column used before the separator column to protect it from contaminants, such as particulate matter or irreversibly attained materials.

6.1.4  **Software (Chromleon or equivalent)** for data analysis

6.2  **Dry mixer**

6.3  **Analytical balance**: readability 0.1 mg and 1 mg.

6.4  **Centrifuge**: 10000 rpm with 5 ml or 10 mL centrifuge tubes.

6.5  **Membrane filter**: 0.45 µm

6.6  **Syringe**: 1 ml and 5 ml

6.7  **Polypropylene plastic ware**

Should be soaked in distilled water and dried.

7  **Test specimen**

**Preparation of EBN sample**

7.1.  **Pre-treatment**

The EBN samples shall be a representative from a single batch. The samples must be treated as received. There are 2 techniques of sample preparation available as below:

7.1.1  **Sample preparation technique 1**
7.1.1.1 Weigh 0.25 to 2.5 g into centrifuge tube and add 10 ml to 100 ml UPW.
7.1.1.2 Vortex the sample solution vigorously to ensure complete mixing of the samples.
7.1.1.3 Heat the sample solution at 70 °C to 90 °C in a waterbath shaker for 15 minutes.
7.1.1.4 Centrifuge the solution from 5 000 rpm to 10 000 rpm for 10 minutes at room temperature.
7.1.1.5 Filter the supernatant with membrane filter (0.45 micron).
7.1.1.6 The sample ready for analysis (minimum 5 ml per injection).
7.1.1.7 The sample should be analysed within a day.

8  Determination

8.1   Chromatographic conditions for reference

8.1.1 Chromatographic column

High capacity anionic exchange column compatible to gradient elution capable of separating anions (NO₃⁻, NO₂⁻).

8.1.2 Elution solution

8.1.2.1 KOH * solution with its concentration of 6 mmol/l-70mmol/l, elution gradient is 6 mmol/l for 30 minutes, 70 mmol/1 for 5 minutes and 6 mmol/1 for 5 minutes. Flow rate is 1.0 ml/min.

Note: *the solution depends on the column capacity and type.

8.1.2.2 Sample volume: 20 µl to 25 µl (enabled to be modified according to the content of ion to be measured).

8.1.3 Determine and calibrate a standard curve

8.1.3.1 Inject 20 µl to 25 µl of the prepared working standard solution.

8.1.3.2 Inject 20 µl to 25 µl of blank sample (ultrapure water).

8.1.3.3 Plot and verify the linearity of the standard curve (R²=0.99 to 1.0).

NOTE: Calibration of the equipment based on MS ISO/IEC 17025:2005

8.2   Quality control procedure

8.2.1. Prepare blank sample or known samples in triplicate.

8.2.2 Prepare spike sample by adding known concentration of standard in triplicate.
8.3 Preparation of test sample

8.3.1 Inject 20 µl to 25 µl of blank sample (ultrapure water).

8.3.2 Inject 20 µl to 25 µl of test sample.

9 Calculation for nitrite and nitrate amount

The amount of nitrite ($NO_2^-$) and nitrate ($NO_3^-$) in samples are calculated using the formula below:

$$A = \frac{(c - c_0) \times V \times F \times 1000}{w \times 1000}$$

where

$A$ the amount of nitrite or nitrate in the sample, mg/kg;
$c$ the amount of nitrite or nitrate in the samples obtained from the curve calibrated, mg/l;
$c_0$ the amount of nitrite or nitrate in blank solution, mg/l;
$V$ the volume of sample solution, ml;
$F$ dilution factor of sample solution;
$w$ weight of sample, g.

NOTE. The amount of nitrite ($NO_2^-$) in the sample, expressed as sodium nitrite is calculated as a multiply with 1.5.

The amount of nitrate ($NO_3^-$) in the sample, expressed as sodium nitrate is calculated as $A$ multiply with 1.37.

10 Test report

10.1 Report the test result to the nearest milligram per kilogram.

10.2 The test report shall also contain the following information:

10.2.1 reference to this national standard;

10.2.2 all details required for the complete identification of the sample;

10.2.3 any departure from the specified procedure or any circumstances which affected the results.
Annex A
(informative)

The diagram in determination of nitrite content

1. Spin the sample, then filter, pipette, and take 15 ml (V1).
2. Pipette 40 ml (V3) of the solution, then filtrate.
3. Add water and reagents, then incubate, etc.
4. Add reagents for color formation.

For Public Comment
Annex B
(informative)

The diagram in determination of nitrate content

Reduction and determination of nitrate

For Public Comment
Acknowledgements

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