BS 770 : Part 9 : 1985
ISO 4099-1984
UDC 637.3.07 : [546.173 + 546.175]

British Standard Methods for

Chemical analysis of cheese

Part 9. Determination of nitrate and nitrite contents. Cadmium reduction and photometry method

Méthodes d'analyse chimique du fromage
Partie 9. Détermination des teneurs en nitrates et en nitrites.
Méthode par réduction au cadmium et photométrie

Verfahren für die chemische Analyse von Käse
Teil 9. Bestimmung des Nitrat- und Nitritgehalts.
Reduktion von Kadmium und photometrisches Verfahren

NOTE. This Part should be read in conjunction with Part 1 'General introduction', published separately.

National foreword

This revision of this Part of BS 770, which has been prepared under the direction of the Dairying Standards Committee, is identical with ISO 4099-1984 'Cheese — Determination of nitrate and nitrite contents — Method by cadmium reduction and photometry', prepared by ISO/TC 34, Agricultural food products, of the International Organization for Standardization (ISO). It supersedes BS 770 : Part 9 : 1980, which is withdrawn.

Terminology and conventions. The text of the international standard has been approved as suitable for publication as a British Standard without deviation. Some terminology and certain conventions are not identical with those used in British Standards; attention is drawn especially to the following.

The comma has been used as a decimal marker. In British Standards it is current practice to use a full point on the baseline as the decimal marker.

Where the words 'International Standard' appear, referring to this standard, they should be read as 'British Standard'.

The spelling 'sulfur' is used throughout for that element and its derivatives. In British Standards it is current practice to use the spelling 'sulphur'.

Cross-reference. The Technical Committee has reviewed the provisions of ISO 707-1984, to which reference is made in the text, and has decided that they are acceptable for use in conjunction with this standard. A related standard is BS 809 'Methods for sampling milk and milk products', which is in course of revision.

Additional information. With reference to **8.4**, for some hard cheeses, it is often advantageous to grate the cheese, rather than grinding, prior to mixing (kneading).

NOTE. *Typographical error.* In **5.1**, the chemical formula for cadmium sulfate octahydrate should read '$3CdSO_4 \cdot 8H_2O$'.

Compliance with a British Standard does not of itself confer immunity from legal obligations.

British Standards Institution

1 Scope and field of application

This International Standard specifies a method by cadmium reduction and photometry for the determination of the nitrate and nitrite contents of cheese.

The method is suitable for hard, semi-hard and soft cheeses of various ages and for processed cheese.

The detection limits of the method are 5 mg of nitrate per kilogram and 0,5 mg of nitrite per kilogram.

2 Reference

ISO 707, *Milk and milk products — Methods of sampling.*

3 Definition

nitrate and nitrite contents of cheese : The contents of substances determined by the procedure specified in this International Standard and expressed respectively as milligrams of nitrate ion (NO_3^-) and of nitrite ion (NO_2^-) per kilogram.

4 Principle

Extraction of the cheese with warm water, precipitation of the fat and proteins, and filtration.

Reduction of the nitrate in a portion of the filtrate to nitrite, by means of copperized cadmium.

Development of a red colour, in portions of both unreduced filtrate and of the reduced solution, by addition of sulfanilamide and *N*-1-naphthyl-ethylenediamine dihydrochloride, and spectrometric measurement at a wavelength of 538 nm.

Calculation of the nitrite content of the sample and of the total nitrite content after reduction of nitrate, by comparing the measured absorbances with those of a series of standard sodium nitrite solutions; calculation of the nitrate content from the difference between these two contents.

5 Reagents

All reagents shall be of recognized analytical grade. The water used shall be distilled or deionized water, free from nitrate and nitrite.

NOTE — In order to avoid possible inclusion of small gas bubbles in the copperized cadmium column (6.10), the distilled or deionized water used for the preparation of the column (8.1), for checking the reducing capacity of the column (8.2), and for regeneration of the column (8.3) should preferably be freshly boiled and afterwards cooled to room temperature.

5.1 Cadmium, granules, diameter 0,3 to 0,8 mm.

If cadmium granules are not available commercially, they may be prepared as follows.

Place a suitable number of zinc rods in a beaker and cover with a 40 g/l solution of cadmium sulfate octahydrate ($CdSO_4 \cdot 8H_2O$). From time to time, scrape the cadmium sponge from the rods over a period of 24 h. Remove the zinc rods and decant the liquid until only sufficient remains to cover the cadmium. Wash the sponge two or three times with water. Transfer the cadmium to a laboratory blender together with 400 ml of 0,1 mol/l hydrochloric acid, and blend for a few seconds to obtain granules of the required size. Return the contents of the blender to the beaker and leave to stand for several hours, occasionally stirring to remove bubbles. Decant most of the liquid and immediately copperize as described in 8.1.1 to 8.1.5.

5.2 Copper(II) sulfate, solution.

Dissolve 20 g of copper(II) sulfate pentahydrate ($CuSO_4 \cdot 5H_2O$) in water and dilute to 1 000 ml.

5.3 Buffer solution, pH 9,6 to 9,7.

Dilute 50 ml of concentrated hydrochloric acid [ϱ_{20} 1,19 g/ml; about 38 % (m/m) hydrogen chloride solution] with 600 ml of water. After mixing, add 135 ml of ammonium hydroxide [ϱ_{20} 0,91 g/ml; about 25 % (m/m) ammonia solution]. Dilute to 1 000 ml with water and mix.

NOTE — If ammonium hydroxide of this concentration is not available, an equivalent amount of a more concentrated solution may be used, for example 100 ml of 35 % (*m/m*) solution (ϱ_{20} 0,88 g/ml).

Adjust the pH to 9,6 to 9,7 if necessary.

5.4 Hydrochloric acid, about 2 mol/l.

Dilute 160 ml of concentrated hydrochloric acid (ϱ_{20} 1,19 g/ml) to 1 000 ml with water.

5.5 Hydrochloric acid, about 0,1 mol/l.

Dilute 50 ml of the hydrochloric acid (5.4) to 1 000 ml with water.

5.6 Solutions for precipitation of proteins and fat.

5.6.1 Zinc sulfate, solution.

Dissolve 53,5 g of zinc sulfate heptahydrate ($ZnSO_4 \cdot 7H_2O$) in water and dilute to 100 ml.

5.6.2 Potassium hexacyanoferrate(II), solution.

Dissolve 17,2 g of potassium hexacyanoferrate(II) trihydrate [$K_4Fe(CN)_6 \cdot 3H_2O$] in water and dilute to 100 ml.

5.7 EDTA, solution.

Dissolve 33,5 g of disodium ethylenediaminetetraacetate dihydrate ($Na_2C_{10}H_{14}N_2O_8 \cdot 2H_2O$) in water and dilute to 1 000 ml.

5.8 Solutions for colour development.

5.8.1 Solution I.

Dissolve, by heating on a water-bath, 0,5 g of sulfanilamide ($NH_2C_6H_4SO_2NH_2$) in a mixture of 75 ml of water and 5 ml of concentrated hydrochloric acid (ϱ_{20} 1,19 g/ml). Cool to room temperature and dilute to 100 ml with water. Filter if necessary.

5.8.2 Solution II.

Dilute 450 ml of concentrated hydrochloric acid (ϱ_{20} 1,19 g/ml) to 1 000 ml with water.

5.8.3 Solution III.

Dissolve 0,1 g of *N*-1-naphthyl-ethylenediamine dihydrochloride ($C_{10}H_7NHCH_2CH_2NH_2 \cdot 2HCl$) in water. Dilute to 100 ml with water. Filter if necessary.

The solution may be stored for up to 1 week in a well-stoppered brown bottle in a refrigerator.

5.9 Sodium nitrite, standard solution corresponding to 0,001 g of nitrite per litre.

On the day of use, dissolve in water 0,150 g of sodium nitrite ($NaNO_2$), dried to constant mass at 110 to 120 °C, dilute to 1 000 ml with water in a one-mark volumetric flask and mix.

Dilute 10 ml of this solution with 20 ml of the buffer solution (5.3) and dilute further to 1 000 ml with water in a one-mark volumetric flask. Mix.

1 ml of this standard solution contains 1,00 µg of NO_2^-.

5.10 Potassium nitrate, standard solution corresponding to 0,004 5 g of nitrate per litre.

Dissolve in water 1,468 g of potassium nitrate (KNO_3), dried to constant mass at 110 to 120 °C, dilute to 1 000 ml with water in a one-mark volumetric flask and mix.

On the day of use, dilute 5 ml of this solution with 20 ml of the buffer solution (5.3) and dilute further to 1 000 ml with water in a one-mark volumetric flask. Mix.

1 ml of this standard solution contains 4,50 µg of NO_3^-.

6 Apparatus

All glassware shall be thoroughly cleaned and rinsed with distilled water to ensure that it is free from nitrate and nitrite.

Usual laboratory apparatus, and in particular

6.1 Analytical balance.

6.2 Appropriate grinding device.

6.3 Suitable laboratory mixer/homogenizer, with glass containers of capacity 250 or 400 ml.

6.4 Conical flasks, of capacity 250 ml.

6.5 Volumetric flasks, of capacities 100; 500 and 1 000 ml, complying with the requirements of ISO 1042, class B.

6.6 Pipettes, to deliver 2; 4; 5; 6; 8; 10; 12; 20 and 25 ml, complying with the requirements of ISO 648, class A, or ISO 835/1.

NOTE — Where appropriate, burettes may be used instead of pipettes.

6.7 Graduated cylinders, of capacities 5; 10; 25; 100; 250; 500 and 1 000 ml.

6.8 Glass funnels, of diameter about 7 cm, with short stem.

6.9 Filter paper, medium grade, of diameter about 15 cm, free from nitrate and nitrite.

6.10 Reduction column (for example as shown in the figure).

6.11 Spectrometer, suitable for measuring absorbance at a wavelength of 538 nm, with cells of optical path length 1 to 2 cm.