Nederlandse

norm

NEN-EN 1988-2

Voedingsmiddelen - Bepaling van sulfiet -
Deel 2: Enzymatische methode

Foodstuffs - Determination of sulfite - Part 2: Enzymatic method

maart 1998
ICS 67.040

Vervangt NEN-EN 1988-2:1995 Ontw.

Als Nederlandse norm is aanvaard:
- EN 1988-2:1998

Normcommissie 370 275 "Voedingsmiddelenanalyse - Horizontale methoden"

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Nederlands Voorwoord

Voor de in deze norm vermelde normatieve verwijzingen bestaan in Nederland de volgende equivalenten:

Vermelde norm	Nederlandse norm	Titel
EN ISO 3696	NEN-EN-ISO 3696	Water voor analystische laboratoriumdoeleinden - Eisen en beproevingsmethoden (ISO 3696:1987)

EUROPEAN STANDARD
NORME EUROPÉENNE
EUROPÄISCHE NORM

EN 1988-2

February 1998

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Descriptors: food products, chemical analysis, determination of content, sulphites, enzymatic methods

English version

Foodstuffs - Determination of sulfite - Part 2: Enzymatic method

Produits alimentaires - Dosage des sulfites - Partie 2: Méthode enzymatique

Lebensmittel - Bestimmung von Sulfit - Teil 2: Enzymatisches Verfahren

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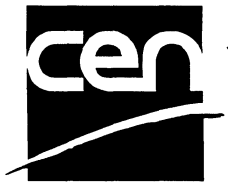

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Ref. No. EN 1988-2:1998 E

Contents

Foreword

This European Standard has been prepared by Technical Committee CEN/TC 275 "Food analysis - Horizontal methods", the secretariat of which is held by DIN.

This European Standard shall be given the status of a national standard, either by publication of an identical text or by endorsement, at the latest by August 1998, and conflicting national standards shall be withdrawn at the latest by August 1998.

According to the CEN/CENELEC Internal Regulations, the national standards organizations of the following countries are bound to implement this European Standard: Austria, Belgium, Czech Republic, Denmark, Finland, France, Germany, Greece, Iceland, Ireland, Italy, Luxembourg, Netherlands, Norway, Portugal, Spain, Sweden, Switzerland and the United Kingdom.

This European Standard "Foodstuffs - Determination of sulfite", consists of the following parts:

Part 1: Optimized Monier-Williams method

Part 2: Enzymatic method

Introduction

Sulfite can be used as a preservative in foodstuffs. In order to minimize possible negative health effects, many countries have regulated the use of sulfite in foods. This has resulted in the development of several methods of analysis to detect the presence and quantity of sulfite in a great variety of foods.

1 Scope

This European Standard specifies an enzymatic method for the determination of the sulfite content, expressed as sulfur dioxide, in foodstuffs. Other sulfur-containing substances such as sulfate, sulfide or thiosulfate do not interfere with the determination. Carbonyl-sulfite complexes react as free sulfites. Isothiocyanates occuring in, e.g. mustard interfere with the determination. The method is not applicable to cabbages, dried garlic, dried onions, ginger, leeks and soy protein[1]). It has been shown that the analysis of isolated soy protein leads to false positive results.

Specific products, for which European Standards for the determination of the sulfites exist, are excluded from the scope of this horizontal European Standard.

2 Normative references

This European Standard incorporates by dated or undated reference, provisions from other publications. These normative references are cited at the appropriate places in the text and the publications are listed hereafter. For dated references, subsequent amendments to or revisions of any of these publications apply to this European Standard only when incorporated in it by amendment or revision. For undated references the latest edition of the publication referred to applies.

EN ISO 3696 Water for analytical laboratory use - Specification and test methods (ISO 3696:1987)

3 Principle

Oxidation of sulfite to sulfate in the presence of sulfite oxidase with the liberation of hydrogen peroxide at the same time.

$$SO_3^{2-} + O_2 + H_2O \xrightarrow{\text{sulfite oxidase}} SO_4^{2-} + H_2O_2$$

Reduction of hydrogen peroxide and conversion of NADH to NAD^+ in the presence of NADH peroxidase.

$$H_2O_2 + NADH + H^+ \xrightarrow{\text{NADH peroxidase}} 2\,H_2O + NAD^+$$

Conversion of NADH to NAD^+ is determined spectrometrically and is proportional to the concentration of sulfite, see [1] to [6] in annex A.

4 Reagents

During the analysis, unless otherwise stated, use only reagents of recognized analytical grade and only water of at least grade 3 as defined in EN ISO 3696.

4.1 Ammonium sulfate

4.2 Ethylenediamine - N,N,N',N' - tetraacetic acid (EDTA)

4.3 Sodium hydrogen carbonate

4.4 Sodium sulfite

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[1]) It has been shown that the analysis of isolated soy protein leads to false positive results in the range of 20 mg/kg to 30 mg/kg expressed as sulfur dioxide. Therefore, when analysing foodstuffs containing isolated soy proteins a proportional enhancement of the result may be obtained and is taken into account.

4.5 Ammonium sulfate solution, substance concentration $c[(NH_4)_2SO_4]$ = 2 mol/l

4.6 Sodium hydroxide solution, c(NaOH) = 0,1 mol/l

4.7 Sodium hydroxide solution, c(NaOH) = 2 mol/l

4.8 Triethanolamine buffer solution [2], $c(C_6H_{15}NO_3)$ = 0,6 mol/l, pH 8,0

Dissolve 5,57 g of triethanolamine hydrochloride in 40 ml of water in a beaker. Adjust to pH 8,0 with the sodium hydroxide solution (4.6). Transfer the solution to a 50 ml volumetric flask and dilute to the mark with water and mix. The buffer is stable for 1 year at +4 °C.

4.9 NADH solution [2] (Reduced nicotinamide-adenine dinucleotide) c(NADH) = $7 \cdot 10^{-3}$ mol/l

Dissolve 25 mg of ß-nicotinamide-adenine dinucleotide disodium salt (ß-NADH-Na_2) and 50 mg of sodium hydrogen carbonate (4.3) in 5,0 ml of water and mix. The solution is stable for at least 4 weeks at +4 °C.

4.10 NADH peroxidase suspension [2] (EC 1.11.1.1) (see [7] of annex A)

Make a suspension of 10 enzyme units/ml (U/ml) [3] in the ammonium sulfate solution (4.5), pH approximately 7. The suspension is stable for 1 year at +4 °C.

4.11 Sulfite oxidase suspension [2] (EC 1.8.3.1) (see [7] of annex A)

Prepare a suspension of 2,5 enzyme units/ml in ammonium sulfate solution (4.5), pH approximately 7. The suspension is stable for 1 year at +4 °C.

4.12 Reference solution

Weigh 0,6 g of sodium sulfite (4.4) (equivalent to about 300 mg of sulfur dioxide), to the nearest 0,1 mg, and 37 mg of EDTA (4.2) and dissolve in water. Transfer the solution quantitatively to a 1 000 ml-volumetric flask, dilute to the mark with water and mix. Take 100 µl of this solution as reference sample and analyse the sulfite content within 30 min. The coefficient of variation for the reference values shall not exceed 0,06.

4.13 Polyvinylpyrrolidone cross linked (Polyvinylpolypyrrolidone)

4.14 Ascorbate oxidase, e.g. as ascorbate oxidase spatula, of defined activity.

4.15 Bentonite

5 Apparatus

Usual laboratory apparatus and in particular the following:

5.1 Water bath, capable of being controlled at 60 °C ± 2 °C

5.2 Homogenizer

5.3 Graduated micropipettes 10 µl, 20 µl, 50 µl and 100 µl. If mechanical pipettes with disposable ends/capillaries are used, it is of the utmost importance that they are calibrated.

5.4 pH-meter

5.5 Spectrometer suitable for measurements at a wavelength of 340 nm

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[2] These reagents are included in commercially available test kits. If these test kits are used, the manufacturers' instructions should be followed.

[3] This unit (often called the International unit or Standard unit) is defined as the amount of enzyme which will catalyse the transformation of 1 µmol substrate per minute under standard conditions.

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