SLOVENSKI STANDARD
SIST EN 1988-2:1999

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Živila - Določevanje sulfita - 2. del: Encimska metoda

Foodstuffs - Determination of sulfite - Part 2: Enzymatic method

Lebensmittel - Bestimmung von Sulfit - Teil 2: Enzymatisches Verfahren

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

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67.050	Splošne preskusne in analizne metode za živilske proizvode	General methods of tests and analysis for food products

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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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EUROPEAN COMMITTEE FOR STANDARDIZATION
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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of an identical text or by endorsement, at the latest by August 1998, and conflicting national standards shall be withdrawn at the latest by August 1998.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.

5.6 Quartz cells with an optical path length of 1 cm. Disposable cells/cuvettes can also be used.

5.7 Centrifuge, suitable for centripetal acceleration of 2 000 *g* with blender cups or glass tubes of suitable capacity

6 Procedure

6.1 Preparation of the sample test solutions

6.1.1 General

Remove high concentrations of ascorbic acid of more than 100 mg/kg or 100 mg/l of sample (see 6.1.2.3).

If the concentration of sulfite in the sample test solution is higher than 0,3 g/l, dilute the sample test solution prior to the determination or take a smaller sample volume.

6.1.2 Liquid samples

6.1.2.1 White wine, brandy and beer

Analyse white wine and brandy directly. Beer should be filtered to remove carbon dioxide. It may be necessary to decolorize beer. For the decolorization, add not more than about 0,7 g of bentonite (4.15) to 10 ml of filtered beer in a 50 ml glass beaker, stir the mixture for 2 min using a magnetic stirrer and then filter the solution into another 50 ml glass beaker.

For the enzymatic determination (6.2) take 100 μl to 200 μl of wine or 500 μl of brandy or beer respectively.

6.1.2.2 Red wines

Adjust 25 ml of red wine to pH 7,5 to 8,0 with the sodium hydroxide solution (4.7) in a beaker. Transfer the solution into a 50 ml volumetric flask and dilute to the mark with water and mix. It is often necessary to decolorize red wine. This can be done as described in 6.1.2.3 for fruit juices.

6.1.2.3 Fruit juices

Centrifuge cloudy juices at approximately 2 000 *g*. Add 5 ml of juice into a beaker and adjust the pH to 5 to 6 with the sodium hydroxide solution (4.7). Remove asorbic acid by adding approximately 40 units of ascorbate oxidase (4.14) in solution to the juice and leave the sample for 10 min, or remove the ascorbic units by stirring for 3 min with an ascorbate oxidase spatula (4.14). Then adjust the pH to 7,5 to 8,0 with the sodium hydroxide solution (4.7). In the case of coloured juices, add approximately 0,25 g of polyvinylpolypyrrolidone (4.13) and stir the mixture for 1 min. Transfer the mixture to a 10 ml volumetric flask and dilute with water. Filter the solution and take 200 μl for enzymatic determination (6.2).

6.1.3 Solid foodstuffs

Homogenize the sample thoroughly and extract with water at 60 °C for 5 min. Shake occasionally. Cool the sample to ambient temperature before analysing. Vary the sample size depending on the amount of sulfite. In the case of e.g. potato flakes, take 5,0 g of homogenized material into a 50 ml volumetric flask. Add 40 ml of water. Close the flask and extract in a water bath (5.1) at 60 °C for 5 min. Shake occasionally. Cool the volumetric flask, either by letting it stand for at least 15 min, to ambient temperature, or in a water bath of 20 °C, and dilute to the mark with water (V_3 = 50 ml). If necessary centrifuge the solution.

The following sample quantities of some other foods are suggested:

Dried fruit	1,0 g of sample/50 ml of water
Jam	5,0 g of sample/50 ml of water
Spices	0,1 g of sample/50 ml of water
Dried potato products	2,0 g of sample/50 ml of water

Take 100 μl to 500 μl of these solutions for enzymatic determination (see 6.2).

6.2 Determination

Perform the determination according to table 1 at a temperature of 20 °C to 25 °C in a quartz cell (5.6) usually with a sample volume of 100 µl. If the sample volume is different from 100 µl, adjust the volume of added water so that the final volume of water and sample is 2,00 ml.

Table 1

Fluids pipetted into the cells	Sample cell	Blank cell
Triethanolamine buffer solution (4.8) NADH solution (4.9) NADH peroxidase suspension (4.10) Sample test solution Water	1,00 ml 0,10 ml 0,01 ml 0,10 ml 1,90 ml	1,00 ml 0,10 ml 0,01 ml - 2,00 ml
Mix gently. Measure the absorbance A_1 of the sample cell and of the blank cell after 5 min (without a cell in the reference path), then start the reaction by adding the following:		
Sulfite oxidase suspension (4.11)	0,05 ml	0,05 ml
Mix and read the absorbance A_2 after approximately 30 min. Take an additional reading after 5 min to check that no further change in the absorbance has taken place.		

If the reaction has not stopped, continue to read the absorbance at intervals of 2 min until the change in absorbance is constant. If the absorbance decreases constantly, extrapolate the absorbance back to the time of addition of the sulfite oxidase suspension to estimate the A_2 to be used.

7 Calculation

Calculate the absorbance of the sample test solutions from:

$$A_{sample} = A_{1sample} - A_{2sample} \quad (1)$$

$$A_{blank} = A_{1blank} - A_{2blank} \quad (2)$$

$$A = A_{sample} - A_{blank} \quad (3)$$

The absorbance value of the sample A_{sample} shall exceed 0,05, if not, use a larger sample test solution volume.

Calculate the mass concentration of (i.e. sulfur dioxide) sulfite, ρ, in grams per litre, or the mass fraction, w, in grams per kilogram, in the sample from equation (4) or (5):

$$\rho = \frac{V_1 \times M \times Ax \times F}{\varepsilon \times d \times V_2 \times 1000} \quad (4)$$

$$w = \frac{V_1 \times M \times A \times V_3}{\varepsilon \times d \times V_2 \times m \times 1000} \quad (5)$$

where:

V_1 is the final volume of the solutions in the cells, in millilitres (here: 3,16 ml);

V_2 is the sample volume taken for the enzymatic analysis, in millilitres, (here: 0,1 ml up to 0,5 ml);

V_3 is the total volume of sample test solution for solid samples, in millilitres (here: 50 ml);

M is the relative molecular mass of sulfur dioxide (64,1 g/mol);

d is the light path of the cell, in centimetres (here: 1 cm);

ε is the absorption coefficient of NADH at 340 nm (6,3 l · $mmol^{-1}$ · cm^{-1});

m is the sample mass, in grams of solid samples (6.1.3);

F is the dilution factor if the sample has been diluted during the sample preparation (see 6.1, 6.1.2.2 or 6.1.2.3)

8 Precision

8.1 General

Details of the interlaboratory test according to ISO 5725:1986 (see [8] of annex A) of the precision of the method are summarized in annex B. The values derived from the interlaboratory test may not be applicable to analyte concentration ranges and matrices other than given in annex B.

8.2 Repeatability

The absolute difference between two single test results found on identical test material by one operator using the same apparatus within the shortest feasible time interval will exceed the repeatability limit r in not more than 5 % of the cases.

The values are:

Wine	$\bar{x}$ = 75 mg/l	r = 8 mg/l
Dried apples	$\bar{x}$ = 800 mg/kg	r = 298 mg/kg
Dried apples	$\bar{x}$ = 960 mg/kg	r = 358 mg/kg
Lemon juice	$\bar{x}$ = 270 mg/l	r = 37 mg/l
Sultanas	$\bar{x}$ = 260 mg/kg	r = 45 mg/kg
Beer	$\bar{x}$ = 4,9 mg/l	r = 0,8 mg/l

8.3 Reproducibility

The absolute difference between two single test results on identical test material reported by two laboratories will exceed the reproducibility limit R in not more than 5 % of the cases.

The values are:

Wine	$\bar{x}$ = 75 mg/l	R = 16 mg/l
Dried apples	$\bar{x}$ = 800 mg/kg	R = 311 mg/kg
Dried apples	$\bar{x}$ = 960 mg/kg	R = 374 mg/kg
Lemon juice	$\bar{x}$ = 270 mg/l	R = 79 mg/l
Sultanas	$\bar{x}$ = 260 mg/kg	R = 129 mg/kg
Beer	$\bar{x}$ = 4,9 mg/l	R = 1,6 mg/l

9 Test report

The test report shall contain at least the following:

- all information necessary for the identification of the sample;
- a reference to this European Standard or to the method used;
- the results and the units in which the results have been expressed;
- date and type of sampling procedure (if known);
- date of receipt;
- date of test;
- any particular points observed in the course of the test;
- any operations not specified in the method or regarded as optional which might have affected the results.