

Foodstuffs — Determination of vitamin B₆ by microbiological assay

ICS 07.100.30; 67.050

National foreword

This Draft for Development is the official English language version of ENV 14166:2001.

This publication is not to be regarded as a British Standard.

It is being issued in the Draft for Development series of publications and is of a provisional nature in order to assess the method. It should be applied on this provisional basis, so that information and experience of its practical application may be obtained.

Comments arising from the use of this Draft for Development are requested so that UK experience can be reported to the European organization responsible for its conversion into a European Standard. A review of this publication will be initiated 2 years after its publication by the European organization so that a decision can be taken on its status at the end of its three-year life. The commencement of the review period will be notified by an announcement in *Update Standards*.

According to the replies received by the end of the review period, the responsible BSI Committee will decide whether to support the conversion into a European Standard, to extend the life of the prestandard or to withdraw it. Comments should be sent in writing to the Secretary of BSI Technical Committee AW/-/3, Food analysis – Horizontal methods, at 389 Chiswick High Road, London W4 4AL, giving the document reference and clause number and proposing, where possible, an appropriate revision of the text.

A list of organizations represented on this committee can be obtained on request to its secretary.

Cross-references

The British Standards which implement international or European publications referred to in this document may be found in the BSI Standards Catalogue under the section entitled “International Standards Correspondence Index”, or by using the “Find” facility of the BSI Standards Electronic Catalogue.

This Draft for Development, having been prepared under the direction of the Consumer Products and Services Sector Policy and Strategy Committee, was published under the authority of the Standards Policy and Strategy Committee on 19 December 2001

Summary of pages

This document comprises a front cover, an inside front cover, the ENV title page, pages 2 to 13 and a back cover.

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English version

Foodstuffs - Determination of vitamin B6 by microbiological assay

Produits alimentaires - Détermination de la vitamine B6 par
essai microbiologique

Lebensmittel - Mikrobiologische Bestimmung von Vitamin
B6

This European Prestandard (ENV) was approved by CEN on 29 September 2001 as a prospective standard for provisional application.

The period of validity of this ENV is limited initially to three years. After two years the members of CEN will be requested to submit their comments, particularly on the question whether the ENV can be converted into a European Standard.

CEN members are required to announce the existence of this ENV in the same way as for an EN and to make the ENV available promptly at national level in an appropriate form. It is permissible to keep conflicting national standards in force (in parallel to the ENV) until the final decision about the possible conversion of the ENV into an EN is reached.

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EUROPEAN COMMITTEE FOR STANDARDIZATION
COMITÉ EUROPÉEN DE NORMALISATION
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Foreword

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

Annexes A and B are informative.

This standard includes a Bibliography.

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

1 Scope

This European Prestandard specifies a method for the determination of total vitamin B₆ in foodstuffs by microbiological assay (MBA). Vitamin B₆ is determined as the mass fraction of pyridoxine, pyridoxal and pyridoxamine, including their phosphorylated or glycosylated derivatives. It is usually expressed as milligram vitamin B₆ per 100 g of foodstuff. The method is applicable to samples that can be rendered homogeneous and do not contain high concentrations of antibiotics or other interfering substances.

2 Normative references

This European Prestandard 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 Prestandard only when incorporated in it by amendment or revision. For undated references the latest edition of the publication referred to applies (including amendments).

EN ISO 3696:1995, *Water for analytical laboratory use — Specification and test methods (ISO 3696:1987)*.

3 Principle

Pyridoxine, pyridoxal and/or pyridoxamine are extracted from foodstuffs by acid hydrolysis. The hydrolysis step liberates the B₆ vitamers from proteins and carbohydrates in the sample and hydrolyses the phosphates to the free vitamers. The total Vitamin B₆ content in the sample extract is then determined by comparing the growth response of the assay test organism against growth obtained from appropriate standards, see [1].

4 Reagents

4.1 General

During analysis, unless otherwise stated, use only reagents of recognised analytical grade and water of at least grade 1 as defined in EN ISO 3696:1995. The water used for reagent preparation shall be glass distilled. Once distilled, water shall be used within five days or discarded.

4.2 Chemicals and solutions

4.2.1 Sulfuric acid solution, substance concentration $c(\text{H}_2\text{SO}_4) = 0,22 \text{ mol/l}$

4.2.2 Sodium hydroxide solution, $c(\text{NaOH}) = 4 \text{ mol/l}$.

4.2.3 Wort agar, (Difco¹⁾ or suitable alternative)

Dissolve the agar in glass distilled water according to the manufacturer's instructions. Heat to boil. Dispense 5 ml aliquots into glass bottles, cap and autoclave at 121 °C for 15 min. Cool at an angle for slopes to form. Store in a refrigerator for up to three months.

4.2.4 Basal medium (Difco, Pyridoxine Y assay medium¹⁾ or suitable alternative)

The concentration of the assay medium should be chosen, depending upon the assay format used, to ensure that the manufacturer's recommended concentration is obtained in the final assay volume.

4.2.5 Liquid culture medium

Dilute basal medium (4.2.4) with an equal volume of water containing 2,0 ng/ml pyridoxine, pyridoxamine and pyridoxal. Add 10 ml portions to screw-topped tubes and autoclave at 121 °C for 5 min and cool rapidly. Store in refrigerator for up to one month.

4.2.6 Inoculum rinse

Dilute basal medium (4.2.4) with an equal volume of water. Add 10 ml portions to screw-topped tubes and autoclave at 121 °C for 5 min and cool rapidly. Store in refrigerator for up to one month.

4.2.7 Sodium chloride, mass fraction $w(\text{NaCl}) \geq 98,0 \%$ **4.2.8 Sterile saline solution**

Dissolve 0,9 g of sodium chloride (4.2.7) in 100 ml of glass distilled water. Autoclave at 121 °C for 15 min.

4.2.9 Hydrochloric acid, $c(\text{HCl}) = 0,1 \text{ mol/l}$ **4.2.10 Sodium hydroxide solution**, $c(\text{NaOH}) = 0,1 \text{ mol/l}$ **4.3 Test organism**, *Sacchromyces Uvarum ATCC 9080*. (Freeze-dried yeast)**4.3.1 Test organism maintenance (stock culture)**

The test organism is maintained by weekly transfers onto agar maintenance medium (4.2.3) using the following procedure:

Prepare 50 ml portions of Pyridoxine basal medium (4.2.4) and place in a 100 ml thick-walled glass bottle or suitable flask. Add 2 ml of Pyridoxine calibration solution 20 (4.8.1), cap and autoclave at 121 °C for 5 min. Cool as rapidly as possible in cold water to below 30 °C. Aseptically, add 1 ml of autoclaved medium to the freeze dried culture (4.3) and add 0,5 ml of the resultant suspension to the remaining medium using a sterile pipette. Incubate at 30 °C for 16 h. After incubation, the organism should show thick growth. Transfer the medium to suitable sterile, centrifuge tubes and centrifuge at 2000 *g* for 5 min. Discard the supernatant and wash the cell residue with two 50 ml portions of sterile saline solution (4.2.8), centrifuging between washes. Re-suspend the cells in 50 ml of sterile saline solution.

Using a sterile loop, transfer cells from this suspension onto three agar slopes (4.2.3) in a cross pattern and incubate for 16 h to 20 h at 30° C. After incubation, the cross should show visible growth and there should be no growth in the surrounding areas. Store the organism in a fridge. The organism should be transferred to fresh agar slopes on a weekly basis. It is essential to maintain aseptic conditions during preparation and transfer of solutions.

1) This information is given for the convenience of users of this Prestandard and does not constitute an endorsement of the product named. Equivalent products may be used if they can be shown to lead to the same results.