

Foodstuffs — Determination of vitamin B6 by microbiological assay

ICS 07.100.30

National foreword

This British Standard is the UK implementation of EN 14166:2009. It supersedes DD ENV 14166:2001 which is withdrawn.

The UK participation in its preparation was entrusted to Technical Committee AW/-/3, Food analysis - Horizontal methods.

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

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**Foodstuffs - Determination of vitamin B6 by microbiological
assay**

Produits alimentaires - Détermination de la vitamine B6 par
essai microbiologique

Lebensmittel - Mikrobiologische Bestimmung von Vitamin
B6

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Foreword

This document (EN 14166:2009) 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of an identical text or by endorsement, at the latest by November 2009, and conflicting national standards shall be withdrawn at the latest by November 2009.

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1 Scope

This European Standard 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.

This method has been validated in an inter-laboratory test on fortified and non-fortified samples such as wholemeal flour, milk powder, mixed vegetables and pigs liver at levels from 0,5 mg/100 g to 1,9 mg/100 g. For further information on the validation data, see Annex B.

2 Normative references

The following referenced documents are indispensable for the application of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

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 vitamin 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 a pyridoxine hydrochloride standard, 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 according to 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.

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