

**Foodstuffs - Determination of vitamin E  
by high performance liquid  
chromatography - Measurement of  
alpha-, beta-, gamma- and delta-  
tocopherols**

Foodstuffs - Determination of vitamin E by high  
performance liquid chromatography - Measurement  
of alpha-, beta-, gamma- and delta-tocopherols

## EESTI STANDARDI EESSÕNA

## NATIONAL FOREWORD

<p>Käesolev Eesti standard EVS-EN 12822:2000 sisaldab Euroopa standardi EN 12822:2000 ingliskeelset teksti.</p> <p>Käesolev dokument on jõustatud 19.07.2000 ja selle kohta on avaldatud teade Eesti standardiorganisatsiooni ametlikus väljaandes.</p> <p>Standard on kättesaadav Eesti standardiorganisatsioonist.</p>	<p>This Estonian standard EVS-EN 12822:2000 consists of the English text of the European standard EN 12822:2000.</p> <p>This document is endorsed on 19.07.2000 with the notification being published in the official publication of the Estonian national standardisation organisation.</p> <p>The standard is available from Estonian standardisation organisation.</p>
--	---

<p><b>Käsitlusala:</b></p> <p>This draft European Standard specifies a method for the determination of Vitamin E in foodstuffs by high performance liquid chromatography (HPLC). The determination of Vitamin E content is carried out by measurement of alpha-tocopherol.</p>	<p><b>Scope:</b></p> <p>This draft European Standard specifies a method for the determination of Vitamin E in foodstuffs by high performance liquid chromatography (HPLC). The determination of Vitamin E content is carried out by measurement of alpha-tocopherol.</p>
--	--

ICS 67.040

Võtmesõnad:

ICS 67.040

English version

Foodstuffs – Determination of vitamin E by high  
performance liquid chromatography

Measurement of  $\alpha$ -,  $\beta$ -,  $\gamma$ -, and  $\delta$ -tocopherols

Produits alimentaires – Dosage de la  
vitamine E par chromatographie  
liquide haute performance – Dosage  
des  $\alpha$ -,  $\beta$ -,  $\gamma$ - et  $\delta$ -tocophérols

Lebensmittel – Bestimmung von  
Vitamin E mit Hochleistungs-Flüssig-  
chromatographie – Bestimmung von  
 $\alpha$ -,  $\beta$ -,  $\gamma$ - und  $\delta$ -Tocopherol

This European Standard was approved by CEN on 2000-01-02.

CEN members are bound to comply with the CEN/CENELEC Internal Regulations which stipulate the conditions for giving this European Standard the status of a national standard without any alteration.

Up-to-date lists and bibliographical references concerning such national standards may be obtained on application to the Central Secretariat or to any CEN member.

The European Standards exist in three official versions (English, French, German). A version in any other language made by translation under the responsibility of a CEN member into its own language and notified to the Central Secretariat has the same status as the official versions.

CEN members are the national standards bodies of Austria, Belgium, the Czech Republic, Denmark, Finland, France, Germany, Greece, Iceland, Ireland, Italy, Luxembourg, the Netherlands, Norway, Portugal, Spain, Sweden, Switzerland, and the United Kingdom.

**CEN**

European Committee for Standardization  
Comité Européen de Normalisation  
Europäisches Komitee für Normung

**Central Secretariat: rue de Stassart 36, B-1050 Brussels**

## Contents

	Page
Foreword.....	2
Introduction .....	2
1 Scope.....	3
2 Normative references .....	3
3 Principle.....	3
4 Reagents .....	3
5 Apparatus .....	5
6 Sampling.....	6
7 Procedure .....	7
8 Calculation.....	9
9 Precision .....	9
10 Test report .....	10
Annex A (informative) Examples of HPLC chromatogrammes .....	11
Annex B (informative) Precision data.....	12
Annex C (informative) Alternative HPLC-Systems .....	14
Bibliography .....	15

### 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 2000, and conflicting national standards shall be withdrawn at the latest by August 2000.

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 provide the base for the analytical methods. It is intended to serve as a frame in which the analyst can define his own analytical work in accordance to the standard procedure.

### Introduction

As this European Standard method deals with the measurement of the mass fraction of  $\alpha$ -,  $\beta$ -,  $\gamma$ - and  $\delta$ -tocopherol in foodstuffs, reference is made to the literature for the calculation and expression of the vitamin E content in terms of biological activities [1], [2], [3].

## 1 Scope

This European Standard specifies a method for the determination of Vitamin E in foodstuffs by high performance liquid chromatography (HPLC). The determination of Vitamin E content is carried out by measurement of  $\alpha$ -,  $\beta$ -,  $\gamma$ - and  $\delta$ -tocopherol.

The vitamin E activity can be calculated from the tocopherol content assuming appropriate factors as given in the introduction.

## 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 draft 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)

EN ISO 5555      Animal and vegetable fats and oils - Sampling (ISO 5555:1991)

## 3 Principle

Determination of  $\alpha$ -,  $\beta$ -,  $\gamma$ - and  $\delta$ -tocopherol in an appropriate sample solution by high performance liquid chromatographic (HPLC) separation and subsequent photometric (UV-range) or preferably fluorometric detection. In most cases a saponification of the test material followed by an appropriate extraction is necessary. Identification is carried out on the basis of the retention times, and quantitative determination by the external standard method using peak areas or peak heights. Internal standard methods can also be used if the corresponding recovery tests have proven the same behaviour of the internal standard during the analysis as the analyte itself [4] to [13].

## 4 Reagents

During the analysis, unless otherwise stated, use only reagents of recognized analytical grade and water of at least grade 1 according to EN ISO 3696.

### 4.1 Methanol

**4.2 Ethanol, abs.**, volume fraction  $\varphi(\text{C}_2\text{H}_5\text{OH}) = 100\%$

**4.3 Ethanol**,  $\varphi(\text{C}_2\text{H}_5\text{OH}) = 96\%$

**4.4 Sodium sulfate**, anhydrous

**4.5 KOH solutions for saponification**, in suitable concentrations, e.g. (KOH) = 50 g/100 ml or 60 g/100 ml, or alcoholic solutions, e.g. 28 g KOH in 100 ml of an ethanol/water mixture (9+1)(V+V).

**4.6 Antioxidants**, such as ascorbic acid (AA), sodium ascorbate, pyrogallol, sodium sulfide ( $\text{Na}_2\text{S}$ ), hydroquinone or butylated hydroxytoluene (BHT).

**4.7 Solvents and extraction solvents** such as diethyl ether (peroxide-free), dichloromethane, light petroleum (boiling range of 40 °C to 60 °C), *n*-hexane, ethylacetate or appropriate mixtures thereof.

**4.8 HPLC mobile phase:** Appropriate mixtures expressed as volume fractions of e.g. 3 % 1,4-dioxane or 0,5 % 2-propanol, 3 % *tert*-butyl methyl ether in *n*-hexane or *n*-heptane for normal phase chromatography (NP) or 1 % to 10 % water in methanol for reversed phase chromatography (RP).

For alternative HPLC-systems see Annex C.