INTERNATIONAL STANDARD

ISO 11702

Second edition
2016-07-15

Animal and vegetable fats and oils — Enzymatic determination of total sterols content

Corps gras d'origines animale et végétale — Détermination enzymatique de la teneur en stérols totaux

Reference number
ISO 11702:2016(E)

© ISO 2016



COPYRIGHT PROTECTED DOCUMENT

© ISO 2016, Published in Switzerland

All rights reserved. Unless otherwise specified, no part of this publication may be reproduced or utilized otherwise in any form or by any means, electronic or mechanical, including photocopying, or posting on the internet or an intranet, without prior written permission. Permission can be requested from either ISO at the address below or ISO's member body in the country of the requester.

ISO copyright office
Ch. de Blandonnet 8 • CP 401
CH-1214 Vernier, Geneva, Switzerland
Tel. +41 22 749 01 11
Fax +41 22 749 09 47
copyright@iso.org
www.iso.org

Contents

Page

Foreword ... **iv**
1 Scope ... **1**
2 Normative references ... **1**
3 Terms and definitions ... **1**
4 Principle ... **1**
5 Reagents ... **2**
6 Apparatus ... **2**
7 Sampling ... **3**
8 Preparation of the test sample ... **3**
9 Procedure ... **3**
10 Result of the determination ... **4**
11 Precision of the method ... **5**
11.1 Interlaboratory test ... 5
11.2 Repeatability limit ... 5
11.3 Reproducibility limit ... 5
12 Test report ... **6**
Annex A (informative) **Results of an interlaboratory test** ... **7**
Bibliography ... **8**

© ISO 2016 – All rights reserved

Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see www.iso.org/patents).

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

For an explanation on the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT) see the following URL: www.iso.org/iso/foreword.html.

The committee responsible for this document is ISO/TC 34, *Food products*, Subcommittee SC 11, *Animal and vegetable fats and oils*.

This second edition cancels and replaces the first edition (ISO 11702:2009), of which it constitutes a minor revision. The scope has been revised to state that the document is not applicable to milk and milk fat products.

© ISO 2016 – All rights reserved

Animal and vegetable fats and oils — Enzymatic determination of total sterols content

1 Scope

This International Standard specifies a method for the quantitative determination of the total sterols content by means of an enzymatic staining test. The method is applicable to free and esterified sterols in animal and vegetable fats and oils, fatty foods and related products. The determination is applicable to sample quantities of 1 g to 2 g of fat.

The method is not applicable to dark coloured fats and oils. The enzyme is not specific for cholesterol, but also oxidizes other 3-hydroxysterols. The method has not been tested for products fortified with sterols at higher levels.

Milk and milk products (or fat coming from milk and milk products) are excluded from the scope of this International Standard.

NOTE The method is technically equivalent to IUPAC method 2.404[8] and DGF standard method F-III 2 (91).[7]

2 Normative references

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

ISO 661, *Animal and vegetable fats and oils — Preparation of test sample*

ISO 3696, *Water for analytical laboratory use — Specification and test methods*

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

3.1
total sterols content
w_{sterols}
mass fraction of sterols determined by the method specified in this International Standard

Note 1 to entry: For vegetable fats and oils, the sterols content is expressed as β-sitosterol; for animal fats, as cholesterol.

Note 2 to entry: The total sterols content is expressed in milligrams per 100 g of fat.

4 Principle

The test sample is saponified and the sterols in the unsaponifiable matter are determined enzymatically. Cholesterol is oxidized by cholesterol oxidase to cholestenone. The equimolar amount of hydrogen peroxide produced in the process oxidizes in the presence of catalase methanol to formaldehyde. In the presence of ammonium ions, with acetylacetone, it forms a yellow lutidine dye (3,5-diacetyl-1,4-dihydrolutidine). The latter is determined spectrophotometrically in the visible range at 405 nm. The concentration of the dye is equivalent to the amount of sterols.

NOTE Cholesterol oxidase oxidizes cholesterol and other sterols having a hydroxy group in the 3β-position. Therefore, phytosterols like stigmasterol and sitosterol are also determined.

© ISO 2016 – All rights reserved