SLOVENSKI STANDARD
SIST EN 1785:2003

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Nadomešča:
SIST EN 1785:1998

Živila - Pepoznavanje obsevane hrane, ki vsebuje maščobo - Plinska kromatografska/masno spektrometrijska analiza 2-alkilciklobutanona

Foodstuffs - Detection of irradiated food containing fat - Gas chromatographic/mass spectrometric analysis of 2-alkylcyclobutanones

Lebensmittel - Nachweis von bestrahlten fetthaltigen Lebensmitteln - Gaschromatographisch / massenspektrometrische Untersuchung auf 2-Alkylcyclobutanone

Produits alimentaires - Détection d'aliments ionisés contenant des lipides - Analyse par chromatographie en phase gazeuse / Spectrométrie de masse des 2-alkylcyclobutanones

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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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This European Standard was approved by CEN on 20 June 2003.

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COMITÉ EUROPÉEN DE NORMALISATION
EUROPÄISCHES KOMITEE FÜR NORMUNG

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Contents

Foreword

This document (EN 1785:2003) 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of an identical text or by endorsement, at the latest by February 2004, and conflicting national standards shall be withdrawn at the latest by February 2004.

This document supersedes EN 1785:1996.

This European Standard was elaborated on the basis of a protocol during a concerted action of the European Commission (DG XII C.5). Experts and laboratories from E.U. and EFTA countries, contributed jointly to the development of this protocol.

The predecessor of the present standard (EN 1785:1996) has been elaborated following a mandate of the European Commission.

Annex A is informative.

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, Hungary, Iceland, Ireland, Italy, Luxembourg, Malta, Netherlands, Norway, Portugal, Slovakia, Spain, Sweden, Switzerland and the United Kingdom.

1 Scope

This European Standard specifies a method for the identification of irradiation treatment of food containing fat. It is based on the mass spectrometric (MS) detection of radiation-induced 2-alkylcyclobutanones after gas chromatographic (GC) separation [1] to [3].

The method has been successfully tested in interlaboratory trials on raw chicken, pork, liquid whole egg, salmon and Camembert [4] to [8].

Other studies demonstrate that the method is applicable to a wide range of foodstuffs [9] to [21].

2 Normative references

This European Standard incorporates by dated or undated references, 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 (including amendments).

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

3 Principle

During irradiation, the acyl-oxygen bond in triglycerides is cleaved and this reaction results in the formation of 2-alkylcyclobutanones containing the same number of carbon atoms as the parent fatty acid and the alkyl group is located in ring position 2. Thus, if the fatty acid composition is known, the 2-alkylcyclobutanones formed can be predicted.

The 2-alkylcyclobutanones which were analysed in interlaboratory studies were 2-dodecylcyclobutanone (DCB) and 2-tetradecylcyclobutanone (TCB) which are formed from palmitic and stearic acid, respectively, during irradiation. To date, there is no evidence that the 2-alkylcyclobutanones can be detected in unirradiated foods [4],

[9] to [21]. The 2-alkylcyclobutanones are extracted using n-hexane or n-pentane along with the fat. The extract is then fractionated using adsorption chromatography prior to separation using gas chromatography and detection with a mass spectrometer. Other 2-alkycyclobutanones, e.g. 2 (tetradec-5'-enyl) cyclobutanone derived from oleic acid, have also beenidentified in irradiated foodstuffs [8], [15] to [20].

NOTE As an alternative procedure for extraction and purification of the 2-alkylcyclobutanones, supercritical fluid extraction (SFE), has been successfully employed [18], [21]. Argentation chromatography has been used effectively for the detection of foods irradiated at very low doses or containing irradiated ingredients [17]. Liquid chromatography (LC)-GC-MS coupling has been used successfully as an alternative procedure for purification and detection [14]. It should, however, be noted that these alternative procedures have not been validated by interlaboratory trials.

4 Reagents

4.1 General

All reagents and materials used shall be of recognized analytical grade the purity of which has to be tested regularly by the analysis of blank samples. Water shall be of at least grade 3 according to EN ISO 3696:1995.

4.2 n-Hexane [1)]

4.3 **Sodium sulfate,** anhydrous

4.4 Diethyl ether

4.5 Stock standard solutions

n-hexane or isooctane may be used to prepare solutions of 2-cyclohexylcyclohexanone (5 µg/ml), and 2-dodecylcyclobutanone[2)] and 2-tetradecylcyclobutanone[2)] (100 µg/ml). Store at –20 °C.

4.6 Working standard solutions

n-hexane or isooctane may be used to prepare solutions of 2-cyclohexylcyclohexanone (0,5 µg/ml) (internal standard), 2-dodecylcyclobutanone and 2-tetradecylcyclobutanone (10 µg/ml). Store at –20 °C.

4.7 Florisil®[3)], 150 µm to 250 µm (60 mesh to 100 mesh), pesticide residue analysis grade.

Before use, activate the adsorbent by heating at 550 °C for at least 5 h or overnight. Cool in a desiccator. Keep well sealed after cooling.

Prepare deactivated Florisil® by adding 20 parts of water to 100 parts of adsorbent (m/m). Approximately 30 g of activated Florisil® is required to prepare sufficient deactivated adsorbent for each column. Ensure that the deactivated Florisil® contains no lumps and that the powder flows freely. Leave to equilibrate overnight. Use within one week.

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1) n-Hexane was the solvent used to validate the method. However, it is also possible to use n-pentane on health grounds provided it can be shown to lead to the same result.

2) Contact the National Standardization Organizations for the availability of reference standards

3) Florisil® is an example for a suitable product available commercially. This information is given for the convenience of users of this standard and does not constitute an endorsement by CEN of this product.

4.8 Nitrogen, for concentrating solutions

4.9 Helium, as carrier gas

5 Apparatus

5.1 General

Usual laboratory apparatus and, in particular, the following:

5.2 Electric blender

5.3 Soxhlet apparatus, with suitable flask of e.g. 250 ml and extractor of e.g. 100 ml.

5.4 Cellulose extraction thimbles, e. g. of length 80 mm to 100 mm, with an internal diameter of 30 mm. Extraction with n-hexane prior to use may be necessary.

5.5 Cotton wool, non-absorbent, washed in n-hexane prior to use.

5.6 Electric heating mantle or **water-bath**

5.7 Chromatographic tube, made of glass, having a length of 300 mm and with an internal diameter of 20 mm, fitted with a frit, a polytetrafluoroethylene (PTFE) stopcock and a ground glass joint at the top.

5.8 Separating funnel, or **dropping funnel**, e. g. of 250 ml, with a ground glass joint.

5.9 Rotary evaporator, with evaporation flask and a water bath capable of being controlled at 45 °C (low vacuum, approximately 25 kPa).

5.10 Apparatus for concentration of solutions under nitrogen.

5.11 Gas chromatograph (GC) glass vials

5.12 Gas chromatograph (GC) linked to a **mass spectrometer (MS).**

5.13 Capillary column, with suitable performance characteristics, see Annex A.

5.14 Laboratory oven, capable of being maintained at 100° C

6 Sampling technique

When taking samples, give preference to those parts of the food which have a high fat content (e. g. chicken skin). Keep the sample in a sealable glass vessel or in a fat-free metal foil.

7 Procedure

7.1 Sample preparation

Coarsely chop the samples of food and then, with the exception of Camembert, homogenize in an electric blender (5.2). Camembert should be cut into small cubes prior to extraction. For liquid whole egg, ensure that the sample is thoroughly mixed prior to sampling.

7.2 Fat extraction

Weigh 20 g of anhydrous sodium sulfate (4.3) and 20 g of well mixed homogenized sample into an extraction thimble (5.4), mix and plug with cotton wool (5.5). Extra sodium sulfate may be used if necessary. For foodstuffs having a low fat content, it may be necessary to increase the size of the test sample and quantity of anhydrous sodium sulfate accordingly. It is recommended that liquid egg is dried at 100 °C for 12 h prior to extraction. A thin film of egg partially dried (2 h at 100 °C) has given comparable results. Alternative drying procedures, e. g. freeze-drying, may be used provided recovery of 2-alkylcyclobutanones is checked (see 7.6).

Pour 100 ml of n-hexane (4.2) into a suitable flask (5.3) and place extractor on top. Place extraction thimble in the extractor and add 40 ml of n-hexane. Place the flask on the heating mantle (5.6) and condenser on top of the extractor. Reflux and extract gently for 6 h. The solvent should siphon over four times in approximately 1 h. Remove the flask from the heat and dispose of the thimble and the n-hexane in the extractor. Transfer the lipid extract from the flask to a 100 ml-stoppered glass measuring cylinder and adjust the volume to 100 ml with more solvent. Add 5 g to 10 g of anhydrous sodium sulfate, stopper, mix and leave overnight.

Alternative fat extraction procedures may be used if they can be shown to lead to the same results.

7.3 Preparation of the lipid extract

7.3.1 Determination of lipid content – method I

Dry duplicate flasks for at least 4 h or overnight at 100 °C. Cool and weigh. Pipette an aliquot of lipid extract (7.2) into each flask and rotary evaporate (5.9) to dryness. Dry for at least 4 h or overnight at 100 °C and reweigh. Alternatively, to provide a more rapid measurement of lipid content, pipette an aliquot of lipid extract (7.2) into a pre-weighed glass vial. Evaporate the solvent under a stream of nitrogen (5.10) to constant weight. Calculate the volume of extract required to provide approximately 200 mg of lipid.

7.3.2 Determination of lipid concent – method II

Alternatively to 7.3.1, concentrate the whole lipid extract (7.2) to a few millilitres (2 ml to 3 ml) using a rotary evaporator (5.9). Transfer the concentrated lipid extract to a small (e. g. 10 ml) sealable pre-weighed glass vial. Dry the sample under a stream of nitrogen (5.10) to constant weight.

7.4 Florisil® column chromatography

Prepare a Florisil® column (20 cm to 21 cm) using a chromatographic tube (5.7), deactivated Florisil® (4.7) and n-hexane (4.2). Allow the n-hexane level to drop to just above the top of the Florisil®.

Take a volume of the extract (7.3.1) which provides approximately 200 mg of lipid and concentrate if necessary. The final volume should not exceed 5 ml. Alternatively, dissolve 200 mg of the lipid obtained in 7.3.2 in not more than 5 ml of n-hexane.

Apply the lipid extract quantitatively to the column (record exact weight of lipid applied) and allow the n-hexane level to drop to just above the top of the Florisil® and add 5 ml to 10 ml of n-hexane. Place the remaining n-hexane (150 ml in total) in a separating funnel (5.8) on top of the column, elute at 2 ml/min to 5 ml/min and collect the eluent in a suitable flask e. g. 250 ml.

When the funnel is empty (take care that the column does not run dry), change the collection flasks and elute with 150 ml of 1 % diethyl ether (4.4) in n-hexane. Rotary evaporate (5.9) the 1 % diethyl ether fraction at 40 °C, using minimum vacuum, to 5 ml to 10 ml and transfer to a test tube. Concentrate to dryness under a stream of nitrogen at 40 °C ensuring that the sample is not left under nitrogen flow once it is dry. Resuspend in 200 µl of a solution of 2-cyclohexylcyclohexanone (4.6) and transfer to a glass vial (5.11).

7.5 Separation and detection

Separate the 2-alkylcyclobutanones using a suitable capillary column (5.13) and using a mass spectrometer (5.12) operating in the selected ion monitoring mode, measure ion currents at mass/charge (m/z) 98 and m/z 112. In annex A, an example of the GC-MS conditions used with an Ultra I column is given. Figures A.1 and A.2 show the typical electron impact mass spectra of 2-dodecylcyclobutanone and 2-tetradecylcyclobutanone. Figures A.3 to A.7 show typical chromatograms for irradiated chicken, pork, liquid whole egg, salmon and Camembert, respectively.

7.6 Internal quality control

Test the system using an unirradiated control sample of the same type as the unknown sample and a duplicate control spiked with 2-dodecylcyclobutanone and 2-tetradecylcyclobutanone at concentrations expected in the test samples. For example, products validated by interlaboratory trials [5] to [8] were spiked with 200 µl and 100 µl of 10 µg/ml 2-dodecylcyclobutanone and 2-tetradecylcyclobutanone (4.6), respectively. Spiking should be done immediately after sample preparation (7.1) and prior to further treatment. Treat these samples in the same way as the unknown samples. Calculate the percentage recovery using the spiked sample.

Check the linear range regularly using standard solutions of suitable concentrations (e. g. 0,25 µg/ml up to 2 µg/ml of 2-dodecylcyclobutanone or 2-tetradecylcyclobutanone) with 0,5 µg/ml 2-cyclohexylcyclohexanone.

8 Evaluation

8.1 Identification of 2-alkylcyclobutanones

2-Dodecylcyclobutanone produces peaks of ions m/z 98 and m/z 112 in a ratio approximately 4,0 - 4,5 to 1 while for 2-tetradecylcyclobutanone the corresponding ratio is approximately 3,8 - 4,2 to 1. The ratios in samples should reflect those found in standards analysed at the same time. Both ions m/z 98 and m/z 112 have to be present and be in the correct ratio to give a positive identification. The signal to noise ratio of each of these ions should be greater than 3:1 and the relative ion intensities should be within ± 20 % of those obtained from injection of a standard of similar concentration run on the same day. In the case of positive results, scan, e. g. between ions m/z 95 and m/z 115, to confirm that ions m/z 98 and m/z 112 are the major ions present at the retention times of the standard 2-alkylcyclobutanones.

8.2 Calculation of the content of 2-alkylcyclobutanones

Measure a number of standard solutions (e. g. 3) containing 2-dodecylcyclobutanone and 2-tetradecylcyclo-butanone (7.6) either side of the samples.

Calculate the relative response F of each 2-alkylcyclobutanone in relation to the internal standard (4.6) by equation (1):

$$F = \frac{A_{cy}}{A_{is} \times \rho_{cy}} \qquad (1)$$

where:

A_{cy} is the peak area of ion m/z 98 of 2-alkylcyclobutanone;

A_{is} is the peak area of ion m/z 98 of the internal standard (see 7.6);

ρ_{cy} is the mass concentration of 2-alkylcyclobutanone, in micrograms per millilitre.

Average all the responses F to get F_{av} for each of the 2-alkylcyclobutanones.

Calculate the mass concentration, $\rho_{cy/s}$ in micrograms per 200 microlitres of both 2-alkylcyclobutanones, respectively, by equation (2):