SLOVENSKI STANDARD
SIST EN 1785:1998

01-november-1998

Živila - Detekcija obsevane hrane, ki vsebuje maščobe - Plinsko kromatografska/masno spektrometrijska analiza 2-alkilciklobutanonov

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

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

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ICS:

67.050	Splošne preskusne in analizne metode za živilske proizvode	General methods of tests and analysis for food products

SIST EN 1785:1998 **en**

2003-01.Slovenski inštitut za standardizacijo. Razmnoževanje celote ali delov tega standarda ni dovoljeno.

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EUROPEAN STANDARD

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Descriptors: foodstuffs, irradiated foodstuffs, ionizing radiation, food analysis, detection of irradiation treatment, fat containing foodstuffs, gas chromatography, mass spectrometry

English version

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

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

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

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CEN

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

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Foreword

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

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

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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 June 1997, and conflicting national standards shall be withdrawn at the latest by June 1997.

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

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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 tests on raw chicken, pork, and liquid whole egg, [4] to [6].

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.

EN ISO 3696 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], [7] to [11]. 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.

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The 2-alkylcyclobutanones may alternatively be detected using liquid chromatography (LC)-GC-MS coupling [12].

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4 Reagents

4.1 General

During analysis use only reagents of recognized analytical grade, the purity of which has to be tested regularly by analysis of reagent blank samples. Use only water in accordance with at least grade 3 of EN ISO 3696.

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.

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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]) The National Standardization Organizations inform on 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.

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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 absorbent 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.

4.8 Nitrogen for concentrating solutions

4.9 Helium as carrier gas

5 Apparatus

Usual laboratory apparatus and, in particular, the following:

5.1 Electric blender

5.2 Soxhlet apparatus, with suitable flask of 250 ml and extractor of 100 ml.

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

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

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

5.6 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.

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

5.8 Rotary evaporator

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5.9 Apparatus for concentration of solutions under nitrogen.

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5.10 Gas chromatograph (GC) glass vials

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

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

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 homogenize in an electric blender (5.1). 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 (5.3), mix and plug with cotton wool (5.4). Extra sodium sulfate may be used if necessary. 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 250 ml flask (5.2) 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 (5.5) 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 solvent from the flask to a 100 ml-stoppered 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.

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

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7.3 Determination of lipid content

Dry duplicate flasks for at least 4 h or overnight at 100 °C. Cool and weigh. Pipette an aliquot of lipid extract into each flask, rotary evaporate 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 into a glass vial, the weight of which has been determined. Evaporate the solvent under a stream of nitrogen. Reweigh. Repeat the process until the weight is constant. Calculate the volume of extract required to provide approximately 200 mg of lipid. Record the exact weight of lipid applied to the column.

7.4 Florisil® column chromatography

Prepare a Florisil® column (20 cm to 21 cm) using a chromatographic tube (5.6), 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 which provides approximately 200 mg of lipid and concentrate if necessary. The final volume should not exceed 5 ml.

Apply the lipid extract, rinse the flask with approximately 5 ml of n-hexane and apply to the column. 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 the separating funnel (5.7) on top of the column, elute at 2 ml/min to 5 ml/min and collect the eluent in a suitable 250 ml flask.

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 the 1 % diethyl ether fraction at 40 °C, using minimum reduced pressure, 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).

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7.5 Separation and detection

Separate the 2-alkylcyclobutanones using a suitable capillary column (5.12) and identify using a mass spectrometer (5.11) operating in the selected ion monitoring mode for ions of 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.5 show typical chromatograms for irradiated chicken, pork and egg.

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 200 µl and 100 µl of 10 µg/ml 2-dodecylcyclobutanone and 2-tetradecylcyclobutanone in n-hexane or isooctane (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 both ions monitored 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 cyclobutanones.

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8.2 Calculation of the content of 2-alkylcyclobutanones

Measure a number of standard solutions (e. g. 3) containing 2-dodecyl- and 2-tetradecylcyclobutanone (see 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:

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

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:

$$\rho_{cy/s} = \frac{A_{cy/s}}{A_{is/s} \times F_{av} \times 5} \quad \dots (2)$$

where:

$A_{cy/s}$ is the peak area of ion m/z 98 corresponding to 2-alkylcyclobutanone in the sample;
$A_{is/s}$ is the peak area of ion m/z 98 corresponding to the internal standard in the sample;
F_{av} is the average of all ratios F as calculated using equation 1.

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Correction for lipid by equation 3:

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$$w_{cy} = \frac{\rho_{cy/s}}{m_O} \times 1000 \quad \dots (3)$$

where:

$\rho_{cy/s}$ is the mass concentration of 2-alkylcyclobutanone in the sample in micrograms per 200 microlitres, as calculated using equation 2;
m_O is the weight of the lipid taken for the Florisil® column, in milligrams;
w_{cy} is the mass fraction of the corresponding 2-alkylcyclobutanone, in micrograms per gram lipid.

8.3 Identification of irradiated samples

Samples are considered to be irradiated when:

a) at least one 2-alkylcyclobutanone has been positively identified (see 8.1) and

b) the estimated concentration (see 8.2) exceeds the concentration equivalent to a signal to noise ratio of 3 to 1 in the least sensitive ion.

9 Limitations

Detection of irradiated raw chicken has been validated for doses of approximately 0,5 kGy and above. The detection of irradiated liquid whole egg and raw pork has been validated for doses of approximately 1 kGy and above. Validation at these doses covers the majority of commercial applications. Detection limits and stability of the 2-alkylcyclobutanones are not significantly influenced by heating or storage [9], [10].

Usually, the DCB:TCB ratio reflects the palmitic acid:stearic acid ratio. This was not found for irradiated raw pork [3], [6].

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While in principle a range of fat extraction procedures may be acceptable, solvent extraction procedures using diethyl ether or pentane/2-propanol have been found to give unsatisfactory results and therefore should not be used.

Since the internal standard is added after the Florisil® column chromatography step the quantitative data may contain an unknown error unless a correction is made for the percentage recovery.

10 Validation

The method was tested in an interlaboratory test carried out by the Community Bureau of Reference (BCR) [5]. Five laboratories quantified 2-dodecylcyclobutanone in 15 coded samples of chicken which were either not irradiated or irradiated with doses of approximately 0,5 kGy, 3,0 kGy or 5,0 kGy, one and six months after irradiation (see table 1).

Table 1: Interlaboratory data

Time after irradiation	No of samples	No of false negatives[1])	No of false positives[2])
1 month 6 months	74 60[3])	0 2	0 0

[1]) The false negatives were associated with samples given the 0,5 kGy dose. False negatives are irradiated samples identified as unirradiated.
[2]) False positives are unirradiated samples identified as irradiated.
[3]) One laboratory did not provide results after the six months period.

The method was also tested in an interlaboratory trial carried out by the Food and Agricultural Organization of the United Nations (FAO) and the International Atomic Energy Agency (IAEA) [6]. Eleven laboratories used 2-dodecylcyclobutanone and 2-tetradecylcyclobutanone to detect 9 coded samples of chicken, and liquid whole egg while 8 laboratories analysed pork. The samples were either unirradiated or given doses of 1,0 kGy or 3,0 kGy (see table 2).

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Table 2: Interlaboratory data

Sample	No of samples	No of false negatives	No of false positives
Chicken Liquid whole egg Pork	99 99 72	1 [1]) 0 0	0 0 0

[1]) Radiation-induced hydrocarbons were also not detectable in this sample so it was concluded that it had been miscoded.

11 Test report

The test report shall include at least the following:

a information necessary for identification of the sample;
b a reference to this European Standard or to the method used;
c the results;
d date of sampling and sampling procedure (if known);
e date of receipt;
f date of test;
g any particular points observed in the course of the test;
h any operations not specified in the method or regarded as optional which might have affected the results.