British Standard

BRITISH STANDARD

BS EN 1784:2003

Foodstuffs — Detection of irradiated food containing fat — Gas chromatographic analysis of hydrocarbons

The European Standard EN 1784:2003 has the status of a British Standard

ICS 67.050

British Standards

National foreword

This British Standard is the official English language version of EN 1784:2003. It supersedes BS EN 1784:1997 which is withdrawn.

The UK participation in its preparation was entrusted to Technical Committee AW/-/3, Horizontal analysis, which has the responsibility to:

— aid enquirers to understand the text;

— present to the responsible international/European committee any enquiries on the interpretation, or proposals for change, and keep the UK interests informed;

— monitor related international and European developments and promulgate them in the UK.

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

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Foodstuffs - Detection of irradiated food containing fat - Gas chromatographic analysis of hydrocarbons

Produits alimentaires - Détection d'aliments ionisés contenant des lipides - Analyse par chromatographie en phase gazeuse des hydrocarbures

Lebensmittel - Nachweis von bestrahlten fetthaltigen Lebensmitteln - Gaschromatographische Untersuchung auf Kohlenwasserstoffe

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

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This European Standard exists 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 Management Centre has the same status as the official versions.

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Contents

Foreword

This document (EN 1784:2003) 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of an identical text or by endorsement, at the latest by February 2004, and conflicting national standards shall be withdrawn at the latest by February 2004.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 1784:1996.

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

The predecessor of the present standard (EN 1784:1996) has been elaborated following a mandate of the European Commission.

Annex A is normative. Annex B 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 which contains fat. It is based on the gas chromatographic (GC) detection of radiation-induced hydrocarbons (HC). The method has been successfully tested in interlaboratory trials on raw chicken, pork and beef [1] to [4] as well as on Camembert, avocado, papaya and mango [5], [6].

Other studies demonstrate that the method is applicable to a wide range of foodstuffs [7] to [28].

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 (including amendments).

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

3 Principle

During irradiation chemical bonds are broken in primary and secondary reactions. In the fatty acid moieties of triglycerides breaks occur mainly in the α and ß positions with respect to the carbonyl groups resulting in the respective C_{n-1}[1)] and the $C_{n-2:1}$[2)] HC. To predict these chief radiolytic products, the fatty acid composition of samples has to be known (see tables A.1 and A.2).

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1) C_{n-1}: HC which has one carbon atom less than the parent fatty acid.

For detection of HC the fat is isolated from the sample by melting it out or by solvent extraction. The HC fraction is obtained by adsorption chromatography prior to separation using gas chromatography and detection with a flame ionization detector (FID) or a mass spectrometer (MS).

NOTE As alternative procedures for extraction and/or purification of hydrocarbons, miniaturized solid phase extraction (SPE) [18], [22], supercritical fluid extraction (SFE) [27] and argentation chromatography [22] have been successfully employed. Argentation chromatography [22] has been used effectively for identification of radiation treatment of avocados with 0,025 kGy, and for processed samples containing low amounts of irradiated ingredients. The method was also beneficial in reducing matrix effects e. g. with irradiated paprika powder. Liquid chromatography (LC)-GC-MS coupling has been used successfully as an alternative procedure for purification and detection [8], [17], [20]. 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 Sodium sulfate, anhydrous, calcined at 650 °C.

4.3 Florisil®[3]), 150 µm to 250 µm (60 mesh to 100 mesh), deactivated by addition of water.

Heat at 550 °C for at least 5 h or overnight (5.10) and store it in a tightly stoppered container (5.22). If it is not used within the next 3 days, heat the Florisil® at 130 °C for at least 5 h (5.21) and allow to cool in a desiccator (5.11). Add 3 parts of water to 100 parts of the adsorbent (m/m) for deactivation. Shake this mixture for at least 20 min, and then store it in a stoppered container for at least 10 h to 12 h for equilibration. Use the deactivated adsorbent which is further stored in a stoppered container in the course of the next 3 days; after that reheat to 130 °C and follow the same procedure as described above.

2) $C_{n-2:1}$: HC which has two carbon atoms less than the parent fatty acid and an additional double bond in position 1.

3) Florisil® is an example of 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.

4.4 n-Pentane

4.5 n-Hexane [4]

4.6 2-Propanol

4.7 Isooctane

4.8 Nitrogen, for concentrating solutions.

4.9 Hydrogen, nitrogen or helium as carrier gas.

4.10 HC standard solution with concentrations of about 1 µg/ml to 4 µg/ml to be prepared by dissolving in n-pentane, n-hexane or isooctane:

1-dodecene (optional)	1-12:1
n-tridecane (optional)	13:0
1-tetradecene	1-14:1
n-pentadecane	15:0
n-hexadecane (optional)	16:0
1-hexadecene	1-16:1
1,7-hexadecadiene[5]	1,7-16:2
n-heptadecane	17:0
8-heptadecene[5]	8-17:1
n-octadecane (optional)	18:0
1-octadecene (optional)	1-18:1

and if available

1,7,10-hexadecatriene	1,7,10-16:3
6,9-heptadecadiene	6,9-17:2

4.11 n-Eicosane solution (internal standard) with a concentration of about 1 µg/ml to 4 µg/ml of solvent (n-pentane, n-hexane or isooctane).

5 Apparatus

5.1 General

Usual laboratory equipment and, in particular, the following:

5.2 Electric blender and **Homogenizer**.

5.3 Centrifuge with swing out rotor and suitable tubes, e.g. 100 ml, capable of producing a centrifugal force of at least 900 *g* at the outer end of the tubes.

5.4 Water bath, capable of being maintained at 50 °C ± 5 °C.

5.5 Soxhlet apparatus, with round bottomed flask of e.g. 250 ml and an extractor of e.g. 100 ml.

4) 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 results.

5) The National Standardization Organizations inform on the availability of 1,7-16:2 and 8-17:1

5.6 Extraction thimbles, e. g. of cellulose, solvent washed for decontamination, or of glass fibre, heated to 470°C over night for decontamination, 25 mm × 100 mm.

5.7 Reflux apparatus, e.g. 250 ml flask with condenser.

5.8 Stoppered graduated cylinders, e.g. of 100 ml capacity.

5.9 Sealable glass tubes, e.g. of 10 ml capacity.

5.10 Muffle furnace, capable of being maintained at 550°C and 650 °C.

5.11 Desiccator

5.12 Chromatographic tube, made of glass, having a length of 200 mm to 300 mm and an internal diameter of 20 mm, fitted with a frit, a polytetrafluoroethylene (PTFE) stopcock and a ground glass joint at the top.

5.13 Graduated dropping funnel, e.g. of 100 ml capacity with pressure compensation.

5.14 Pear-shaped flask, e.g. of 100 ml capacity.

5.15 Graduated conical-bottom test tubes, e.g. of 10 ml capacity.

5.16 Volumetric flask or GC flask, e.g. of 1 ml capacity.

5.17 Rotary evaporator, with evaporation flask and a water bath capable of being controlled at 45 °C.

5.18 Apparatus for concentration of solutions under nitrogen.

5.19 Gas chromatograph (GC) equipped with flame ionization detector (FID) or mass spectrometer (MS).

5.20 Capillary column, with suitable performance characteristics, see Annex B.

5.21 Laboratory oven, capable of being maintained at 100° C to 130° C

5.22 Stoppered container

5.23 Round bottom flasks, e.g. of 250 ml.

5.24 Filter paper, solvent washed for decontamination

6 Sampling technique

When taking samples, give preference to those parts which have a high fat content (e. g. chicken skin). Keep the sample in a sealable glass tube or in fat-free metal foil. Foils having a wax coating or packing materials made of polyethylene should not be used.

7 Procedure

7.1 Reagent blank

Prepare a reagent blank for every analysis series.

The impurities which are encountered are mainly saturated HC, which have been detected in particular in Florisil®, solvent, filter paper and extraction thimbles (for Soxhlet extraction). To remove impurities, wash filter paper and extraction thimbles of cellulose with solvent until no impurities can be detected. Solvent blank solutions should be concentrated before analysing them for contamination. Plastics materials should not be used for the analyses. Only thoroughly clean glassware should be used.

7.2 Fat extraction procedures

7.2.1 Extraction of fat from meat samples

7.2.1.1 General

Coarsely chop the sample (chicken meat, pork or beef) and homogenize it in a blender (5.2).

Any of the following fat extraction methods may be used since a particular method is not believed to affect classification.

7.2.1.2 Extraction by melting

This procedure is particulary suitable for samples having a high fat content (chicken, pork). The risks of contamination (7.1) are considered to be very low.

After homogenizing the sample (7.2.1.1), place a suitable amount (see 7.3) (up to 50 g, depending on the fat content) in glass centrifuge tubes (5.3) and heat it in the water bath (5.4) at 50 °C. The phase separation may be facilitated by adding a small amount (e. g. 2 ml to 5 ml) of water at this stage. Stir it occasionally with a glass rod, until the fat phase has completely visibly liquefied.

To separate the phases, centrifuge the heated homogenate for 10 min at 900 *g* (5.3), then remove the upper oil phase using a Pasteur pipette, taking care not to disturb the aqueous phase (otherwise, it will be necessary to centrifuge the sample again). If the amount of fat extracted is too low, loosen up the solid phase (meat) using a glass rod and repeat the heating and centrifugation as described above.

7.2.1.3 n-Pentane/2-propanol extraction

Homogenize equal parts of the chopped sample (7.2.1.1) (up to 100 g, depending on the fat content) and a n-pentane/2-propanol mixture (3 + 2 parts by volume) (4.4), (4.6) in a blender (5.2), transfer the homogenate to glass centrifuge tubes (5.3) and centrifuge for 10 min at 900 *g*. Combine the upper clear oily phases and, if necessary, extract the residues once more using one third of the amount of solvent previously taken.

To remove the solvent, concentrate the combined oily phases to a few millilitres in a vacuum rotary evaporator (5.17) at not more than 45 °C. Then add about 20 ml of n-pentane (4.4) and dry the extract over sodium sulfate (4.2) for at least 1 h with occasional shaking. Filter off the sodium sulfate and completely remove the solvent in a rotary evaporator at not more than 45 °C.

7.2.1.4 Extraction using a soxhlet apparatus

Weigh 10 g of sodium sulfate (4.2) into an extraction thimble (5.6). Mix about 20 g of a well mixed and homogenized sample (7.2.1.1) with a further 10 g of sodium sulfate and add to the thimble. For samples of high water content the amount of sodium sulfate should be increased to bind all the water. For foodstuffs having a low fat content, it may be necessary to increase size of the test sample and quantity of anhydrous sodium sulfate accordingly.

Pour 100 ml of solvent (n-hexane (4.5) or n-pentane (4.4)) into a flask (5.5) and a further 40 ml of solvent into the extractor (5.5). Reflux gently for 6 h. Remove from heat when the extractor is nearly filled with solvent. Discard the thimble and the solvent in the extractor. Transfer the lipid extract of the flask into a stoppered graduated cylinder (5.8) and dilute to a known volume with more solvent. Add approximately 5 g to 10 g of sodium sulfate (4.2), stopper the cylinder, mix gently and leave until sodium sulfate is sedimented.

7.2.1.5 Extraction with n-hexane under reflux

Mix 20 g of homogenized sample (7.2.1.1) with 20 g of sodium sulfate (4.2). For samples of high water content the amount of sodium sulfate should be increased to bind all the water. 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.

Transfer the mixture into a flask (5.7) and reflux with 100 ml of n-hexane (4.5) for 60 min. Add 5 g of sodium sulfate, mix gently and filter the solution after 15 min through decontaminated filter paper. Wash the flask and the sodium sulfate once with 25 ml of additional n-hexane. Combine the filtered solutions and remove n-hexane by rotary evaporation (5.17) to a volume of less than 100 ml. Transfer the solution to a stoppered graduated cylinder (5.8) and dilute to a known volume (e. g. 50 ml up to 100 ml) by adding n-hexane. Add approximately 5 g to 10 g of sodium sulfate, stopper the cylinder, mix gently and leave at room temperature overnight.

7.2.1.6 Further preparation (if using 7.2.1.4 or 7.2.1.5)

7.2.1.6.1 General

For the determination of the lipid content, use one of the following methods:

7.2.1.6.2 Determination of lipid content– method I

Dry duplicate flasks to a constant weight. Pipette a known volume of lipid extract (e. g. 5 ml) into each flask, rotary evaporate (5.17) to dryness. Dry for at least 4 h or overnight at 100 °C and reweigh. Calculate the volume of extract required to provide 1 g of lipid.

7.2.1.6.3 Determination of lipid content– method II

Pipette 1 ml of the lipid extract in a weighing boat after determination of weight. Evaporate the solvent by leaving it for some minutes under the fume cupboard. Dry the sample under a stream of nitrogen (5.18) to constant weight. Weigh the boat back and calculate the volume of extract required to provide 1 g of lipid.

7.2.1.6.4 Determination of lipid content – method III

Concentrate the whole lipid extract to 2 ml to 3 ml by rotary evaporation (5.17) (waterbath, 45 °C, low vacuum [approximately 25 kPa]). Transfer the concentrated lipid extract to a pre-weighed sealable glass tube (5.9). Dry the sample under a stream of nitrogen (5.18) to constant weight.

7.2.2 Extraction of fat from cheese and fruit samples

7.2.2.1 Homogenization

7.2.2.1.1 Camembert

Homogenize Camembert and weigh 60 g of the homogenate and 40 g of sodium sulfate (4.2) into a beaker. Mix well, add 100 ml of n-hexane (4.5) and blend for about 2 min.

7.2.2.1.2 Avocado

Homogenize the fruit pulp of an avocado and weigh 40 g of the homogenate and 60 g of sodium sulfate (4.2) into a beaker. Mix well, add 100 ml of n-hexane (4.5) and blend for about 2 min. In the case of unripe avocados, use the whole fruit pulp and increase the amount of sodium sulfate and n-hexane proportionally.

7.2.2.1.3 Papaya

Halve two papayas, take the seeds and remove the attached fruit pulp as thoroughly as possible. Homogenize all the seeds with sodium sulfate (4.2) (1:1, m/m). Blend the homogenate in a beaker with about 150 ml of n-hexane (4.5) for about 2 min.

7.2.2.1.4 Mango

Remove the fruit pulp of three mangoes, crack the kernels (e. g. along the length with a knife, if not possible with a hammer), take the seeds and remove the seedcase as thoroughly as possible. Homogenize all the seeds with sodium sulfate (4.2) (1:1, m/m). Blend the homogenate in a beaker with about 150 ml of n-hexane (4.5) for about 2 min.

7.2.2.2 Further preparation

Transfer the n-hexane/sample mixtures to centrifuge tubes (5.3). After centrifugation (5 min at 900 *g*) (5.3), pool the extracts by cautiously decanting into a round-bottom flask (5.23). In the case of papaya and mango, the residues may be re-extracted with half of the solvent volume to achieve higher fat yields. Concentrate the extracts to 2 ml to 3 ml by rotary evaporation (5.17) (waterbath, 45 °C, low vacuum [approx. 25 kPa]). Transfer the concentrated lipid extract to a preweighted sealable glass tube (5.9). Dry the sample fat under a stream of nitrogen (5.18) to constant weight.

7.3 Application of fat to the Florisil® column

7.3.1 Addition of internal standard

7.3.1.1 Using pure lipids

Dissolve 1 g of fat (7.2.1.2, 7.2.1.3, 7.2.1.6.4 or 7.2.2.2) in 1 ml of n-eicosane solution (4.11).

7.3.1.2 Using lipid extracts

After extraction of fat (7.2.1.4 or 7.2.1.5), mix 1 ml of n-eicosane solution (4.11) and the volume of fat extract required to provide 1 g of lipid (7.2.1.6). If the total volume is more than 5 ml, concentrate by rotary evaporation.

7.3.2 Florisil® column chromatography

Isolate the HC by Florisil® column chromatography using about 20 g of deactivated Florisil® (4.3) for each sample. Fill about 20 g of deactivated Florisil® into a chromatographic tube (5.12). n-Hexane (4.5) is recommended as eluent although n-pentane (4.4) may also be used.

NOTE In interlaboratory studies on cheese and fruit, n-hexane was mandatory [5], [6].

Apply the fat after the addition of the internal standard (7.3.1) quantitatively to the chromatographic tube (5.12) and elute the HC with 60 ml of the eluent at a flow rate of about 3 ml/min.

Concentrate the eluate to about 3 ml at 40 °C in a pear-shaped flask (5.14) on the rotary evaporator (5.17) at about 25 kPa for n-hexane or for n-pentane without applying vacuum. If considered necessary, add about 1 ml of isooctane to the eluate to prevent inadvertent evaporation to dryness. Transfer the concentrated eluate to a graduated test tube (5.15). Concentrate the solution to about 1 ml under a stream of nitrogen which then comprises the test solution. Transfer it to a volumetric flask (5.16).

7.4 Separation and detection

Separate the HC by GC (5.19) using a suitable capillary column (5.20). "Splitless" or "on column" injection is advisable. The HC can be detected by a FID or a MS (see figures B.5 to B.8). Where there is any ambiguity in the recognition of the radiation-induced HC pattern using FID, mass spectrometric detection is essential (see table A.3, figures B.1 to B.4 and if necessary [19] which provides additional spectra).

8 Evaluation

8.1 General

Identification of irradiated samples depends on the detection of the expected radiation induced C_{n-1} and $C_{n-2:1}$ HC (see tables A.1 and A.2). The relative proportions of the unsaturated HC usually reflect the proportion of the parent fatty acid of the total amount of triglycerides.

8.2 Calculation of the hydrocarbon content

Recovery experiments should be carried out with each set of analyses.

Calculate the mass fraction of each HC, w_{HC} in micrograms per gram of fat according to equation (1):

$$w_{HC} = \frac{A_{HC} \times w_{20:0}}{A_{20:0}} \times F_i \qquad (1)$$

where

A_{HC} is the peak area of the hydrocarbon in the sample;

$A_{20:0}$ is the peak area of the internal standard in the sample;

$w_{20:0}$ is the mass fraction of the internal standard in the sample in micrograms per grams of fat;

F_i is the response factor for each HC in relation to the internal standard (4.11).

8.3 Identification

Identify which of the expected C_{n-1} and $C_{n-2:1}$ HC are clearly detectable above blanks (typically, this corresponds to a signal to noise ratio of greater than 3 to 1.). On the basis of the amounts of the $C_{n-1}/C_{n-2:1}$ pair derived from the unsaturated main fatty acid, calculate the expected amounts of the C_{n-1} and $C_{n-2:1}$ HC derived from the other fatty acids listed in tables A.1 and A.2 for each particular food. Identify a sample as irradiated if all $C_{n-1}/C_{n-2:1}$ HC which should be above detection limit are clearly detected in the expected proportions.

Special case: The radiolytic yield of 1-tetradecene 1-14:1 in pork is unusually low [3].

9 Limitations

Saturated HC are frequently present both as contaminants and as naturally occuring compounds in food. Therefore, their presence in a sample is only used in combination with the presence of other expected unsaturated HC for the identification of an irradiated sample.

Detection of irradiated raw meat and Camembert has been validated for doses of about 0,5 kGy and above which covers the majority of commercial applications.

Detection of irradiated fresh avocado, papaya and mango has been validated for doses of approximately 0,3 kGy and above.

The concentration of HC derived from fatty acids which are of low concentration in the particular fat will be low and might be below the detection limit in the case of low radiation doses. Particularly in fruit the applied doses might be lower than the doses used in the interlaboratory trial (see Table 3 in clause 10).

The detection limits are not influenced by usual applied commercial storage time [3], [5].

10 Validation

The method has been tested in four interlaboratory trials.

In an interlaboratory trial carried out by the Community Bureau of Reference of the European Commission (BCR), 4 laboratories quantified HC in 15 chicken samples irradiated with about 5 kGy and in 3 unirradiated samples about 2 weeks and 6 to 8 weeks after irradiation, respectively. Radiation-induced HC were detected in all irradiated samples [1].

In a second interlaboratory trial carried out by BCR, 8 laboratories quantified HC in 15 coded samples of chicken meat which were either unirradiated or given doses of approximately 0,5 kGy, 3,0 kGy or 5,0 kGy, 1 and 6 months after irradiation, respectively [2] (table 1).

Table 1 — Interlaboratory data

Time after irradiation	No. of samples	No. of false negatives [a]	No. of false positives [b]
1 month	119	7	2
6 months	120	8	0

[a] The false negatives were all associated with samples given approximately 0,5 kGy. False negatives are irradiated samples identified as unirradiated.

[b] The false positives were due to misinterpretation of the data. False positives are unirradiated samples identified as irradiated.

In an interlaboratory trial carried out by the German Federal Health Office (Bundesgesundheitsamt, BGA), 17 laboratories identified coded chicken, pork and beef samples which were unirradiated or irradiated with 0,8 kGy, 2,8 kGy or 7 kGy (mean doses) three and six months after irradiation, respectively [3], [4] (table 2).

Table 2 — Interlaboratory data

Species	Time after irradiation	No. of samples	No. of false negatives [a]	No. of false positives[b]
Chicken	3 months	160	0	0
Chicken	6 months	126	1	0
Pork	3 months	153	1	3
Pork	6 months	140	1	4
Beef	3 months	149	2	2
Beef	6 months	136	1	0

[a] The false negatives were associated with samples given approximately 0,6 kGy to 0,8 kGy (except one sample which received approximately 2,8 kGy).

[b] The false positives were due to either contaminations, mix up of samples or misinterpretation of data.

In a second interlaboratory trial carried out by the BGA/BgVV (Bundesinstitut für gesundheitlichen Verbraucher-schutz und Veterinärmedizin, German Federal Institute for Consumer Protection and Veterinary Medicine), 22 laboratories identified coded Camembert samples which were either unirradiated or irradiated with a dose of approximately 0,5 kGy or 1 kGy and coded avocado, papaya and mango samples either unirradiated or irradiated with doses of approximately 0,3 kGy, 0,5 kGy or 1 kGy [5], [6] (table 3).

Table 3 — Interlaboratory data

Product	No. of samples	No. of false negative [a]	No. of false positives [b]
Camembert	126	1	0
Avocado	103	1	0
Papaya	104	0	0
Mango	98	5	1

[a] Out of seven false negatives, four came from one laboratory. The remaining three false negatives were reported from three different laboratories and were all associated with mango samples irradiated with approximately 0,3 kGy.

[b] The false positive was due to a mix up with a sample irradiated with about 1 kGy.

11 Test report

The test report shall contain at least the following data:

a) all information necessary for the 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) any particular points observed in the course of the test;

g) any operations not specified in the method or regarded as optional which might have affected the results.

Annex A
(normative)

Tables

Table A.1 — Main fatty acids in chicken, pork and beef examined in interlaboratory studies and their radiation-induced C_{n-1} and $C_{n-2:1}$ HC

Fatty acid		Approx. content (%) per total fat			Radiation-induced hydrocarbons	
		Chicken	Pork	Beef	C_{n-1}	$C_{n-2:1}$
Palmitic acid	(C 16:0)	21	25	23	15:0	1-14:1
Stearic acid	(C 18:0)	6	11	10	17:0	1-16:1
Oleic acid	(C 18:1)	32	35	43	8-17:1	1,7-16:2
Linoleic acid	(C 18:2)	25	10	2	6,9-17:2	1,7,10-16:3

Table A.2 — Main fatty acids in Camembert, avocado, papaya and mango examined in interlaboratory studies and their radiation-induced C_{n-1} and $C_{n-2:1}$ HC

Fatty acid		Approx. content (%) per total fat				Radiation-induced hydrocarbons	
		Camembert	Avocado	Papaya	Mango	C_{n-1}	$C_{n-2:1}$
Myristic acid	(C 14:0)	10 to 15	---	---	---	13:0	1-12:1
Palmitic acid	(C 16:0)	30 to 40	10 to 15	15 to 20	5 to 10	15:0	1-14:1
Stearic acid	(C 18:0)	10 to 15	---	2 to 6	30 to 45	17:0	1-16:1
Oleic acid	(C 18:1)	20 to 25	55 to 65	60 to 80	40 to 50	8-17:1	1,7-16:2
Linoleic acid	(C 18:2)	---	10 to 15	2 to 6	5 to 10	6,9-17:2	1,7,10-16:3

Table A.3 — Typical hydrocarbon masses encountered in mass spectrometric detection using electron impact ionization (EI)

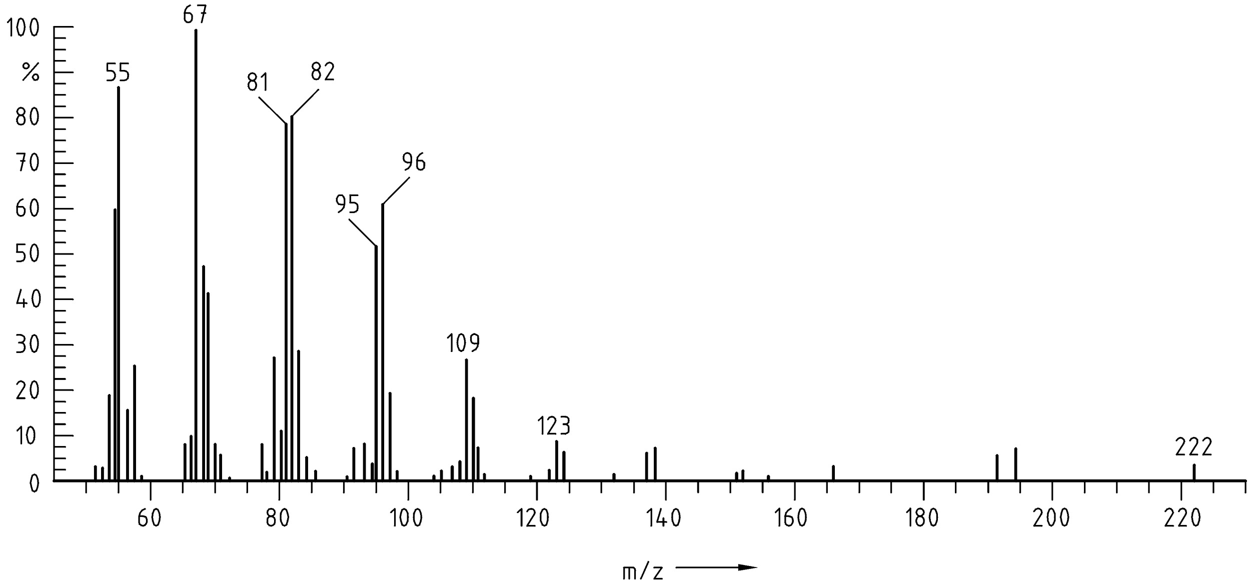
Hydrocarbon		Fragmentation products	M^+
Alkanes	13:0	57-71-85-99-113-127	184
	15:0		212
	17:0		240
	20:0		282
Alkenes	1-12:1	55-69-83-97-111-125	168
	1-14:1		196
	8-17:1		238
Alkadienes	1,7-16:2	67-55-81-82-95-96-109-110-123	222
	6,9-17:2		236
Alkatrienes	1,7,10-16:3	67-55-81-82-95-96-109-110-121	220

If the identification in EI mode is not sufficient, it is recommended to try the positive chemical ionisation (PCI) mode using the following conditions which result in the respective main fragments $M+H^+$ and $M-H^+$.

Gas: Methane, of purity 4.5
Ionization energy: 150 eV
Ion source temperature: 150 °C

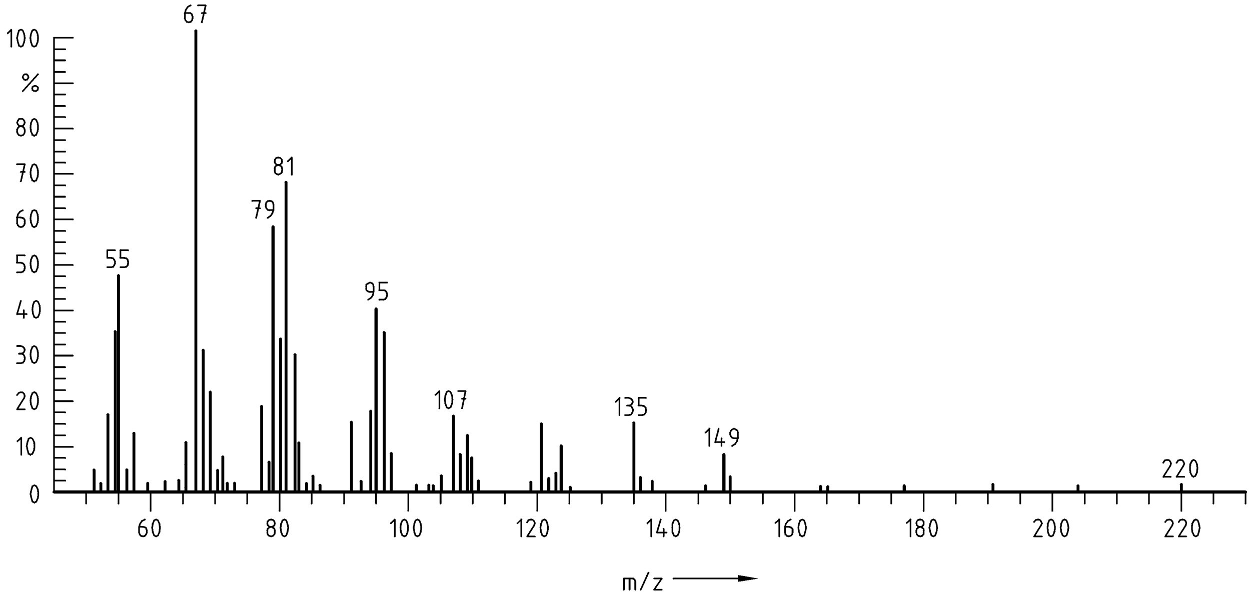
Annex B
(informative)

Figures



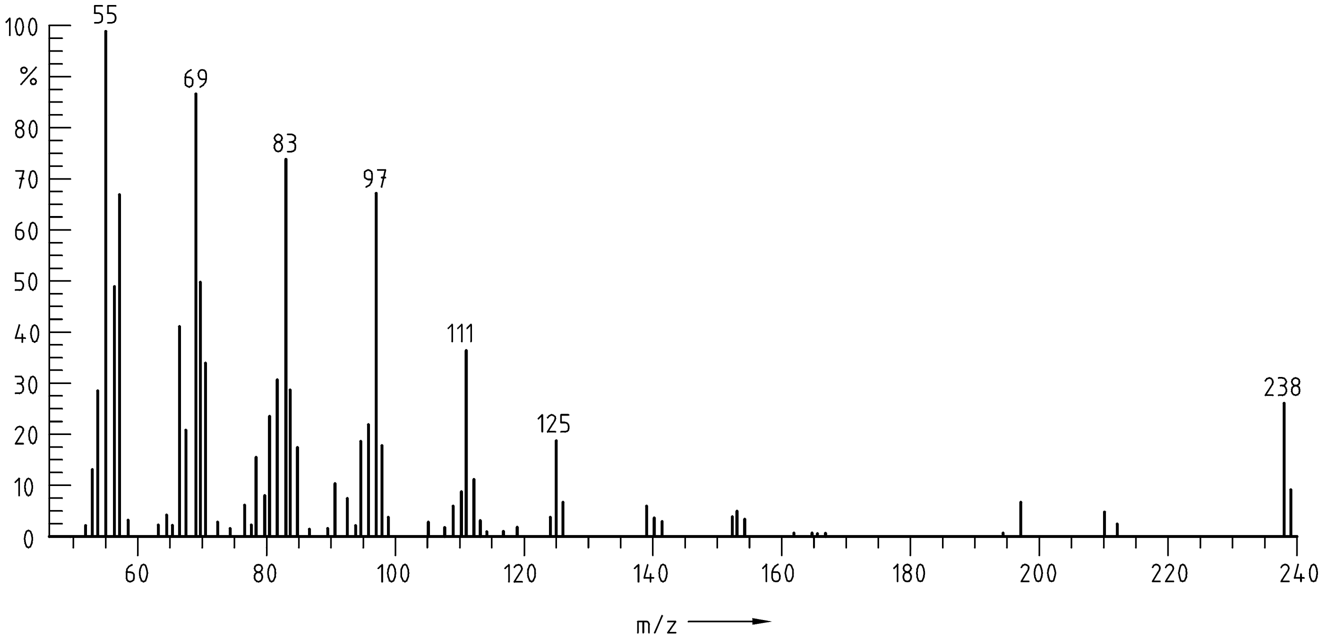
(m/z = mass/charge)

Figure B.1 — Typical mass spectrum of hydrocarbon 1,7-16:2



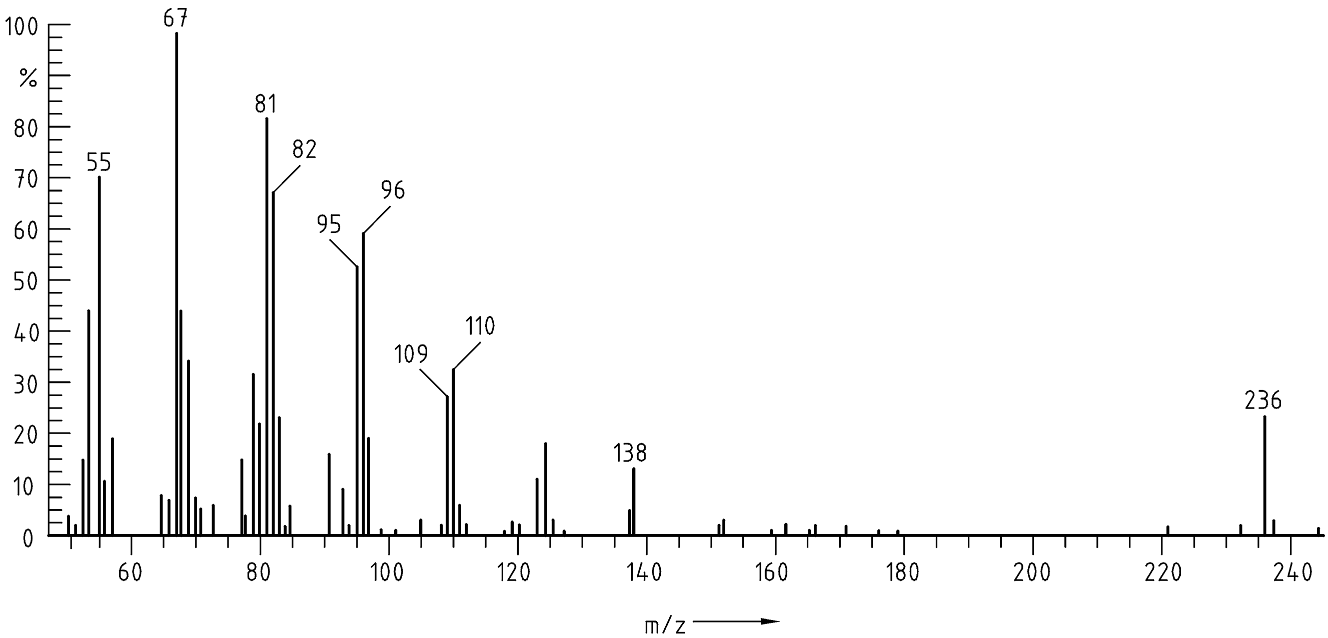
(m/z = mass/charge)

Figure B.2 — Typical mass spectrum of hydrocarbon 1,7,10-16:3



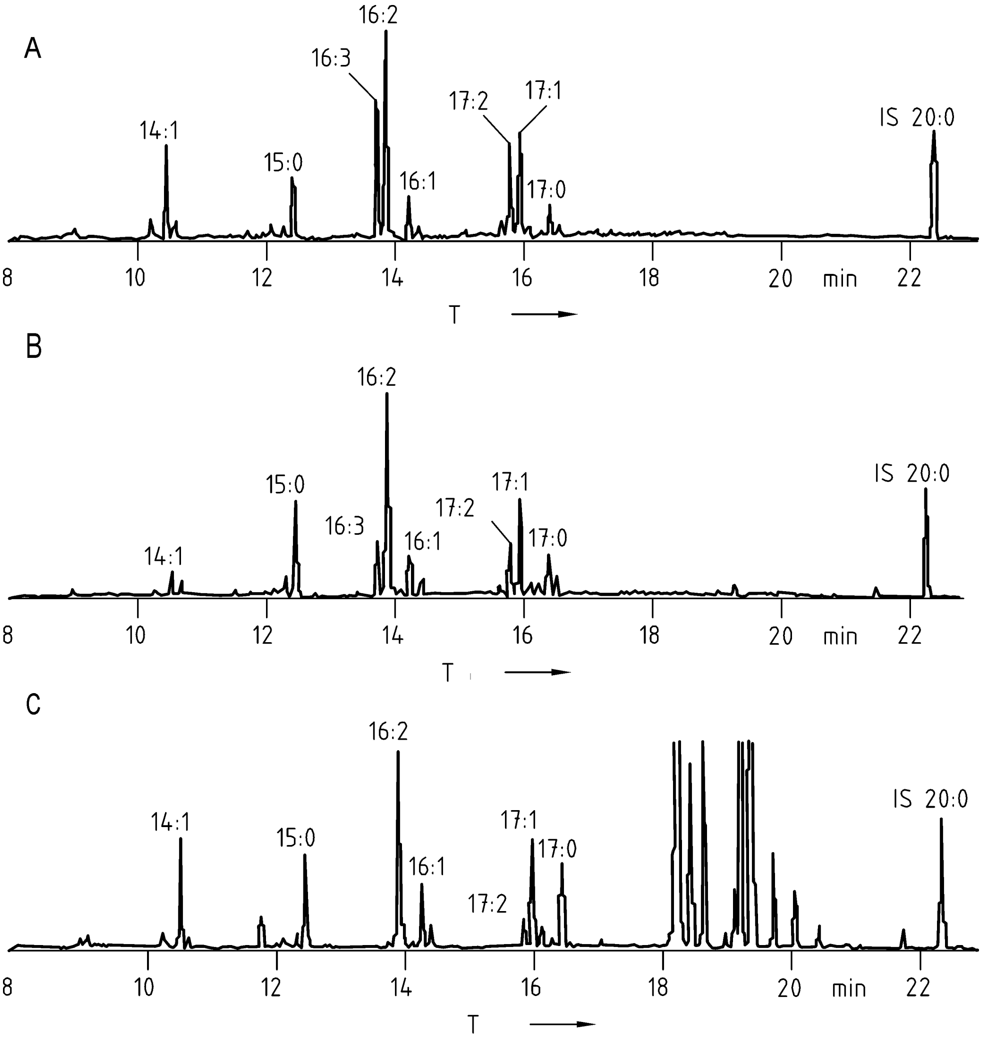
(m/z = mass/charge)

Figure B.3 — Typical mass spectrum of hydrocarbon 8-17:1



(m/z = mass/charge)

Figure B.4 — Typical mass spectrum of hydrocarbon 6,9-17:2



Key

A Chicken
B Pork
C Beef
T Time

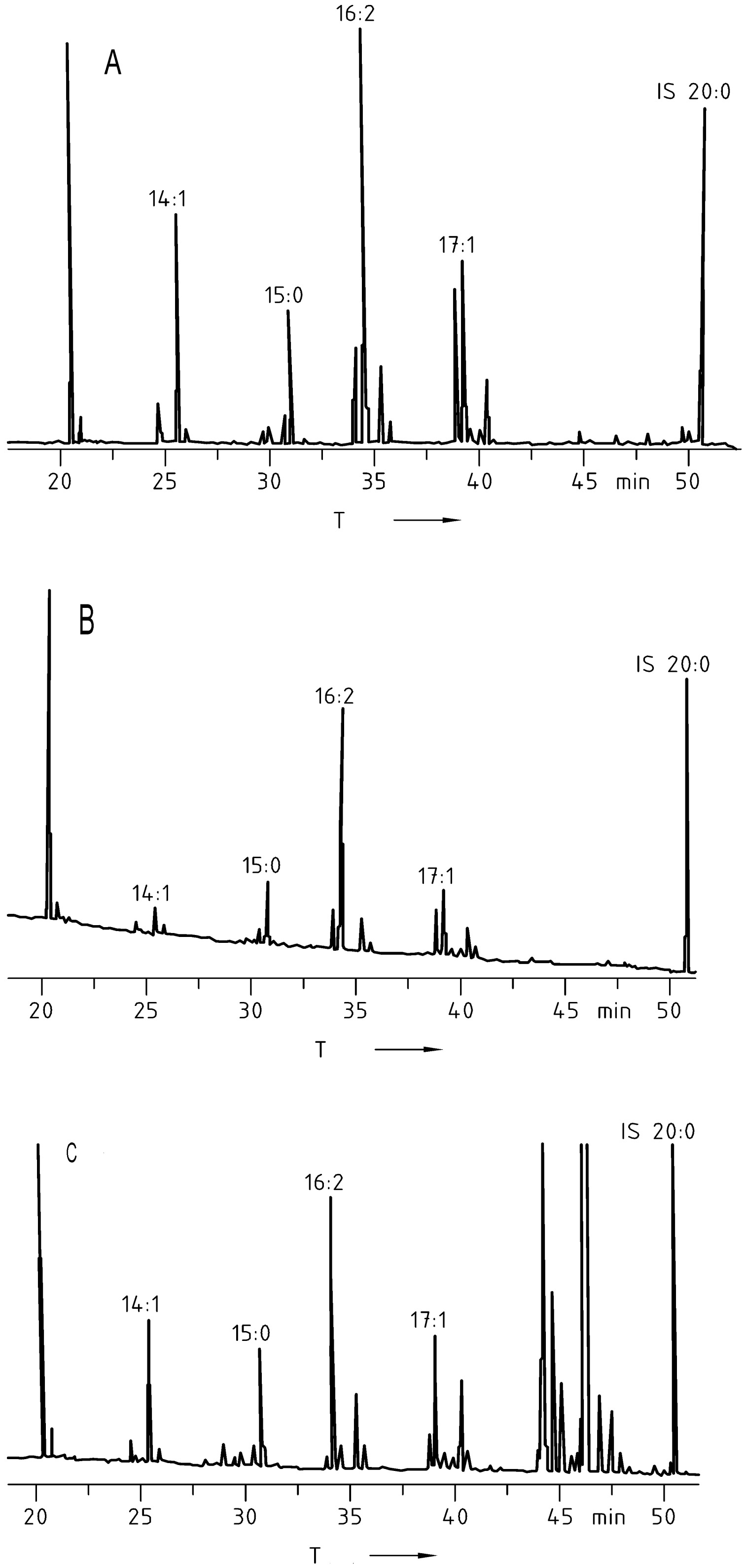
Figure B.5 — Typical gas chromatograms of hydrocarbons from meat samples irradiated with a dose of approximately 3 kGy detected with a mass spectrometer [14]

In the case of Figure B.5 the hydrocarbons were separated on a Hewlett-Packard® Ultra 2[6)] column, 12 m × 0,22 mm with a 0,33 µm stationary phase (5 % diphenyl, 95 % dimethyl polysiloxane), on a Hewlett-Packard® 5890 GC[6)] gas chromatograph directly linked to a Hewlett-Packard® 5970 B[6)] mass selective detector (MSD). Conditions were as follows:

Injector temperature:	200 °C;
Transfer line temperature:	270 °C;
Initial column oven temperature:	50 °C isotherm for 1 min,
First ramp:	10 °C/min to 130 °C and
Second ramp:	5 °C/min to 200 °C;
Injection volume:	1 µl;
Injection mode:	splitless;
Carrier gas:	helium;
MSD mode:	fullscan 50 atomic mass units (amu) to 300 amu

It should be noted that the elution order of hydrocarbons might be different on columns of other polarity.

6) Hewlett Packard® Ultra 2 column, 5890 GC gas chromatograph and 5970 B mass selective detector are examples of suitable products available commercially. This information is given for the convenience of users of this standard and does not constitute an endorsement by CEN of these products.



Key

A	Chicken
B	Pork
C	Beef
T	Time

Figure B.6 — Typical gas chromatograms of hydrocarbons of meat samples irradiated with a dose of approximately 6,8 kGy detected by a flame ionization detector[7)]

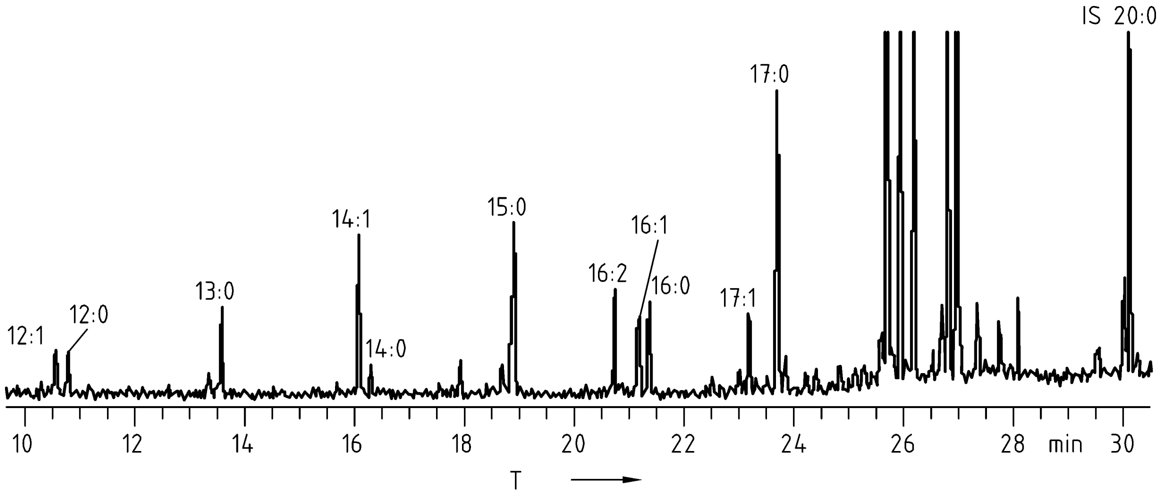
7) Kindly supplied by Dr. C Gemperle, Kantonales Laboratorium, Aargau, Switzerland

In the case of Figure B.6 the hydrocarbons were separated on a Hewlett-Packard® SE 54[6)] column, 25 m × 0,32 mm with a 0,25 µm stationary phase (5 % diphenyl, 95 % dimethyl polysiloxane), on a Carlo-Erba® 5300 gas chromatograph[6)] equipped with a flame ionization detector.

Conditions were as follows:

Injector temperature:	230 °C;
Initial column oven temperature:	40 °C for 2 min;
First ramp:	10 °C/min to 70 °C;
Second ramp:	2,5 °C/min to 170 °C;
Third ramp:	10 °C/min to 280 °C;
Final temperature:	is hold for 5 min;
Injection volume:	1 µl;
Injection mode:	splitless;
Carrier gas:	hydrogen;
Detector temperature:	280 °C.

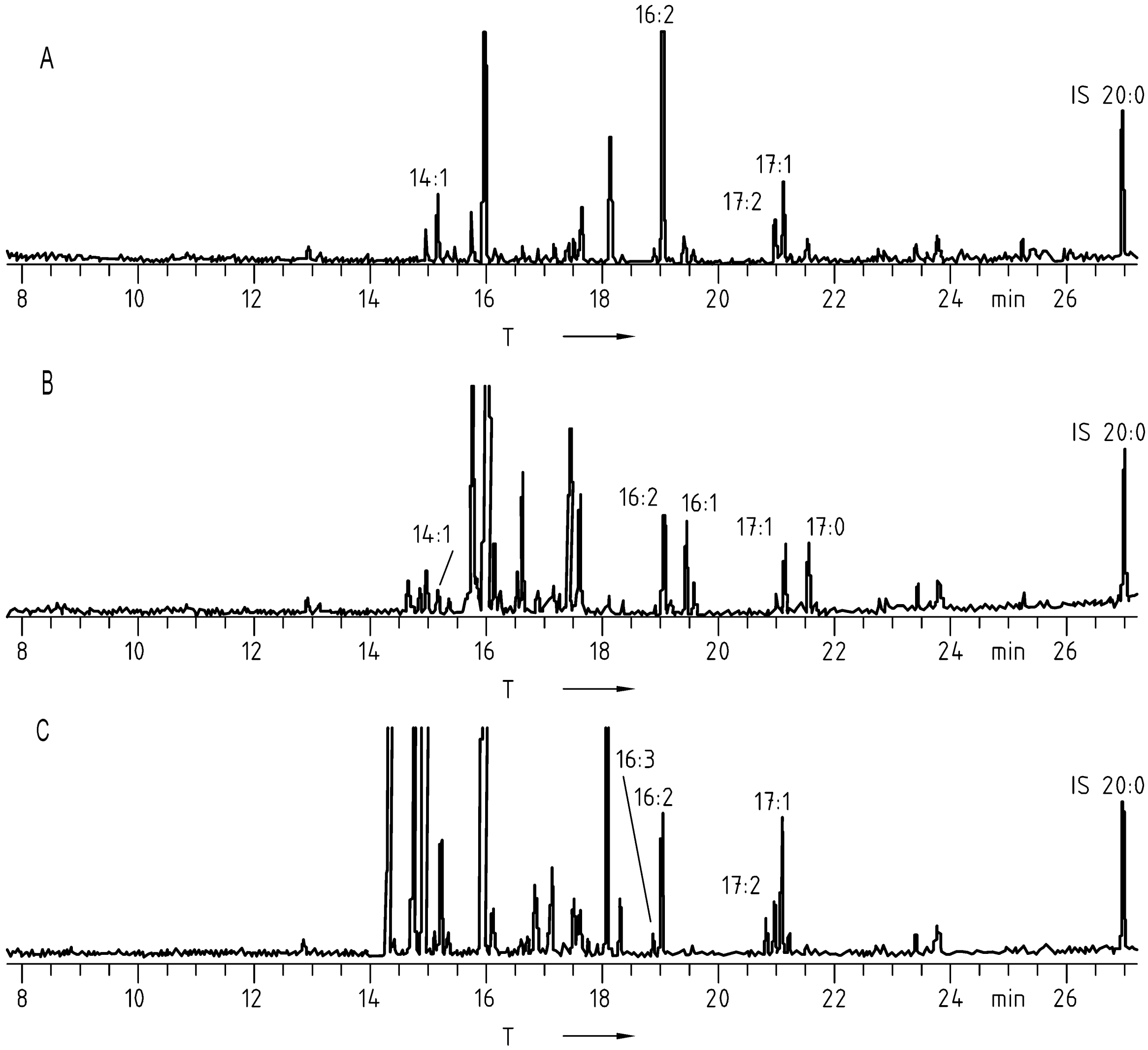
It should be noted that the elution order of hydrocarbons might be different on columns of other polarity.



Key

T Time

Figure B.7 — Typical gas chromatogram of hydrocarbons from a Camembert sample irradiated with a dose of approximately 1 kGy detected with a mass spectrometer [15]



Key

A	Papaya
B	Mango
C	Avocado
T	Time

Figure B.8 — Typical gas chromatograms of hydrocarbons from avocado, papaya and mango samples irradiated with a dose of approximately 1 kGy detected with a mass spectrometer [15]

In the case of Figures B.7 and B.8, the hydrocarbons were separated on a Hewlett-Packard® Ultra 2[6)] column, 25 m x 0,2 mm with a 0,33 µm stationary phase (5% diphenyl, 95 % dimethyl polysiloxane) on a Hewlett Packard 5890 GC[6)] gas chromatograph directly linked to a Hewlett Packard 5970 B[6)] mass selective detector (MSD). Conditions were as follows:

Injector temperature:	200 °C
Transfer line temperature:	270 °C
Initial column oven temperature:	55 °C for 2 min
First ramp:	12°C/min to 155 °C
Second ramp:	5 °C/min to 230 °C
Injection volume:	1 µl
Injection mode:	splitless
Carrier gas:	helium
MSD mode:	fullscan 50 amu to 300 amu

It should be noted that the elution order of hydrocarbons might be different on columns of other polarity.

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