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of an identical text or by endorsement, at the latest by February 2004, and conflicting national standards shall be withdrawn at the latest by February 2004.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.

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 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.

3 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, raw pork, salmon and Camembert 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 in these products are not significantly influenced by heating or storage [11], [12], [20].

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

Results of an interlaboratory trial [8] with mangoes and papayas, using their seeds as the source of lipid, were not considered satisfactory and hence validation is not extended to these products. In the case of papayas, considerable difficulty was encountered by most participating laboratories in the detection of irradiated samples. This may be indicative of a lack of stability of the 2-alkycyclobutanones in the seeds of this product as indicated by previous studies [15], [20]. For mangoes, a small number of false positives were reported [8]. However, these were attributed to analytical difficulties encountered by two participating laboratories as 2-alkylcyclobutanones have never been detected in unirradiated samples of this product [15], [20].

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.

4 Validation

The method was tested in an interlaboratory trial carried out by the Community Bureau of Reference (BCR) [5], [7]. 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[a)]	No of false positives[b)]
1 month	74	0	0
6 months	60[c)]	2	0

a) The false negatives were associated with samples given the 0,5 kGy dose. False negatives are irradiated samples identified as unirradiated.

b) False positives are unirradiated samples identified as irradiated.

c) 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], [7]. 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).

Table 2 — Interlaboratory data

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

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

The method was further validated by means of an interlaboratory trial carried out by the Ministry of Agriculture, Fisheries and Food (MAFF) in the UK [8]. Seven laboratories used 2-dodecylcyclobutanone and 2-tetradecylcyclobutanone to detect 9 coded samples each of salmon and Camembert. The samples were either unirradiated or given doses of 1,0 or 3,0 kGy (see table 3).

Table 3 - Interlaboratory data

Sample	No of samples	No of false negatives [a)]	No of false positives [b)]
Camembert	63	1	0
Salmon	63	1	0

[a)] The false negatives are associated with samples given the 1,0 kGy dose. False negatives are irradiated samples identified as unirradiated.

[b)] False positives are unirradiated samples identified as irradiated.

Bibliography

[1] Stevenson, M.H.: Progress in the identification of irradiated food. In: Trends in Food Science and Technology, 1992, **3,** 257-262.

[2] Crone, A.V.J., Hand, M.V., Hamilton, J.T.G., Sharma, N.D., Boyd, D.R. and Stevenson, M.H.: Synthesis, characterisation and use of 2-tetradecylcyclobutanone together with other cyclobutanones as markers for irradiated liquid whole egg. In: J. Sci. Food Agric., 1993, **62**, 361-367.

[3] Stevenson, M.H.: Detection of irradiated food. In: Food Technol., 1994, 141-144.

[4] Meier, W. and Stevenson, M.H.: Determination of volatiles and o-tyrosine in irradiated chicken. Results of an intercomparison study. Edited by: Leonardi, M., Raffi, J.J., and Belliardo, J.J., In: Recent Advances on the Detection of Irradiated Food. BCR Information, 1993, Luxembourg: Commission of the European Communities, 1993, 211-218, (Report EUR/14315/en).

[5] Stevenson, M.H., Meier, W., and Kilpatrick, D.J.: A European Collaborative Blind Trial Using Volatile Hydrocarbons and 2-Dodecylcyclobutanone to Detect Irradiated Chicken Meat. BCR Information, 1994, Luxembourg: Commission of the European Communities, 1994, (Report EUR/15969/en).

[6] Stevenson, M.H., Kilpatrick, D.J. and McMurray, C.H.: A European Collaborative Blind Trial Using 2-Dodecylcyclobutanone and 2-Tetradecylcyclobutanone to Detect Irradiated Chicken, Pork and Liquid Whole Egg, FAO/IAEA Report, Vienna, Austria, 1994.

[7] Stevenson, M.H.: Validation of the cyclobutanone protocol for detection of irradiated lipid containing foods by interlaboratory trials. In: Detection Methods for Irradiated Foods - Current Status. Edited by C.H. McMurray, E.M. Stewart, R. Gray, and J. Pearce. Royal Society of Chemistry, Cambridge, UK, 1996, 269-284.

[8] Stewart, E.M.: An international collaborative blind trial using 2-dodecylcyclobutanone and 2-tetradecylcyclobutanone to detect irradiated Camembert cheese, salmon, mango and papaya. Report to the Ministry of Agriculture, Fisheries and Food (MAFF), London, UK, 2001.

[9] Stevenson, M.H., Crone, A.V.J. and Hamilton, J.T.G.: Irradiation detection. In: Nature, 1990, **344**, 202-203.

[10] Boyd, D.R., Crone, A.V.J., Hamilton, J.T.G., Hand, M.V.,Stevenson, M.H. and Stevenson, P.J.: Synthesis, characterisation and possible use of 2-dodecylcyclobutanone as a marker for irradiated chicken. In: J. Agric. Food Chem., 1991, **39**, 789-792.

[11] Crone, A.V.J., Hamilton, J.T.G. and Stevenson, M.H.: Effect of storage and cooking on the dose response of 2-dodecylcyclobutanone, a potential marker for irradiated chicken. In: J. Sci. Food Agric., 1992, **58**, 249-252.

[12] Crone, A.V.J., Hamilton, J.T.G. and Stevenson, M.H.: The detection of 2-dodecylcyclobutanone in radiation-sterilised chicken stored for several years. In: Int. J. Food Sci. Technol., 1992, **27**, 691-696.

[13] Raffi, J., Delincée, H., Marchioni, E., Hasselmann, C., Sjöberg, A.-M., Leonardi, M., Kent, N., Bögl, K.W., Schreiber, G.A., Stevenson, M.H., Meier, W.: Concerted Action of the Community Bureau of Reference on Methods of Identification on Irradiated Foods. BCR-Information, 1994, Luxembourg: Commission of the European Communities, 1994, (Report EUR/15261/en).

[14] Meier, W., Artho, A., Nägeli, P.: Detection of irradiation of fat containing foods by on-line LC-GC-MS of alkylcyclobutanones. In: Mitt. Geb. Lebensm. Hyg., 1996, **87**, 118-122.

[15] Stewart, E.M., Moore, S., McRoberts, W.C., Graham, W.D., and Hamilton, J.T.G.: 2-Alkylcyclobutanones as markers for exotic fruits. In: Food Sci. Technol. Today, 1998, **12**, 103-106.

[16] Ndiaye, B., Jamet, G., Miesch, M., Hasselmann, C., and Marchioni, E.: 2-Alkylcyclobutanones as markers for irradiated foodstuffs. II. The CEN (European Committee for Standardization) method: field of application and limit of utilization. In: Rad. Phys. Chem., 1999, **55**, 437-445.

[17] Ndiaye, B., Horvatovich, P., Miesch, M., Hasselmann, C., and Marchioni, E.: 2-Alkylcyclobutanones as markers for irradiated foodstuffs.III. Improvement of the field of application on the EN 1785 method by using silver ion chromatography. In: J. Chromatogr. A, 1999, **858**, 109-115.

[18] Horvatovich, P., Miesch, M., Hasselmann, C., and Marchioni, E.: Supercritical fluid extraction of hydrocarbons and 2-alkylcyclobutanones for the detection of irradiated foodstuffs. In: J. Chromatogr. A, 2000, **897**, 259-268.

[19] Lee, H-J., Byun, M-W., and Kim, K-S.: Detection of radiation-induced hydrocarbons and 2-alkylcyclobutanones in irradiated perilla seeds. In: J. Food Protect., 2000, **63**, 1563-1569.

[20] Stewart, E.M., Moore, S., Graham, W.D., McRoberts, W.C., and Hamilton, J.T.G.: 2-Alkylcyclobutanones as markers for the detection of irradiated mango, papaya, Camembert cheese and salmon meat. In: J. Sci. Food Agric., 2000, **80**, 121-130.

[21] Stewart, E.M., McRoberts, W.C., Hamilton, J.T.G. and Graham, W.D.: Isolation of lipid and 2-alkylcyclobutanones from irradiated food by supercritical fluid extraction. J AOAC Int., 2001, **84(3)**, 976-986.