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# SECTION 2. 2. IDENTIFICATION

## CONTROL OF IRRADIATED FOOD: RECENT DEVELOPMENTS IN ANALYTICAL DETECTION METHODS.

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### ABSTRACT

An overview of recent international efforts, i.e. programmes of "ADMIT" (FAO/IAEA) and of BCR (EC) towards the development of analytical detection methods for radiation processed foods will be given. Some larger collaborative studies have already taken place, e.g. ESR of bones from chicken, pork, beef, frog legs and fish, thermoluminescence of insoluble minerals isolated from herbs and spices, GC analysis of long-chain hydrocarbons derived from the lipid fraction of chicken and other meats, and the microbiological APC/DEFT procedure for spices. These methods could soon be implemented in international standard protocols.

### KEYWORDS

Food irradiation; irradiation identification; electron spin resonance; free radicals; thermoluminescence; radiolytic products; microbiological test

### INTRODUCTION

At the 7. IMRP, 1989 at Noordwijkerhout (Netherlands) a session dealing with identification of irradiated food was introduced. Progress in commercialisation of food irradiation and increase in international trade of food products seemed to create the need for methods by which the radiation processing of a variety of foods could be recognized (Swallow, 1990). A number of analytical detection methods showing considerable promise were already presented (Bögl, 1990). At that time an international conference on "The Acceptance, Control of, and Trade in Irradiated Food" has just taken place in Geneva (December 1988), and delegates from about 60 countries recommended that "governments should encourage research into methods of detection of irradiated foods so that administrative control of irradiated food once it leaves the facility can be supplemented by an additional means of enforcement, thus facilitating international trade and reinforcing consumer confidence in the overall control system" (Anon., 1989).

This advice of the Geneva conference has been followed up by several international efforts. On a European Community (EC) level, the Community Bureau of Reference, has started a collaborative project to establish analytical methods to assess whether food has been treated by irradiation or not. The implementation of the European Single Market on Jan. 1, 1993 provides a further impetus to harmonize legislation governing the production and trade of irradiated food. The availability of common detection methods will contribute considerably to this purpose. Experts from the EC Member States, who were convened in three meetings organized in Brussels (2 June and 3-4 October 1989) and in Cadarache (13-15 February 1990), proposed to carry out intercomparisons on the use of ESR and thermoluminescence and to set up a concerted research action covering 4 areas: DNA based methods, microbiological and biological methods, physical methods and chemical methods during the years 1991-92 (Raffi and Belliardo 1991). On a worldwide level, the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture has initiated a co-ordinated research programme on the analytical detection methods for irradiation treatment of food (ADMIT), the first meeting of the about 20 participating laboratories being held in Warsaw/Jachranka (25-29 June 1990). This meeting recommended the establishment of several research groups devoted to ESR, luminescence, other physical methods, chemical methods, respectively. It was agreed at the Meeting that promising methods of analysis could eventually be subjected to collaborative testing procedures by International Organisations, such as IUPAC, ISO, AOAC, and the Codex Alimentarius Commission (Anon., 1990).

In addition to this international efforts, several countries e.g. France, United Kingdom, Germany, established national research programmes, in which a limited number of laboratories from other countries can also participate. Our Federal Research Centre for Nutrition in Karlsruhe is participating actively in both international and national programmes.

## NEED FOR ANALYTICAL METHODS TO IDENTIFY IRRADIATED FOODS

Although in principle, the administrative control of facilities licensed for food irradiation, and compulsory labelling of treated foods as proposed by Codex Alimentarius (Anon., 1984), supported by an international inventory of facilities (Loaharanu 1992), should provide a reliable control of irradiated food, it seems desirable to have an additional means of detection of the radiation treatment by analysing the food itself. Food irradiation herewith takes up a particular position in processing of foodstuffs. Other kinds of food like "organic vegetables" or "kosher food" are only inspected at the producing facility and certified by labelling. Here the consumer rely on the labelling, although he is as little able to observe whether the "organic" food has been produced according to the specified guidelines as he is to recognize whether the food has been exposed to a radiation treatment. However, due to the enormous amount of knowledge gathered by the in-depth investigations of the radiation processing of food during the last four decades, it now seems possible to identify directly in the food itself whether the food has been irradiated or not. This ability clearly demonstrates, how thoroughly the process of food irradiation has been researched. The request for detection methods for irradiated food, once judged by some as a disadvantage for the application of the process, now turns up as an advantage offering the consumers the confidence that inspection authorities are able to properly control the correct application of the radiation process. Particularly for imported foods or foods sold at the retail level, direct identification offers advantages. Non-labelled irradiated foods have already appeared on the market, and these batches can now be identified and enforced to correct labelling or be rejected. Although properly irradiated foods are safe and wholesome, the consumer should be able to make his own free choice between irradiated and non-irradiated food. It should also be mentioned, that although food irradiation is approved in about 40 countries, in which more than 50 different kinds of food, ranging from spices to grains to fruits and vegetables to meat and sea-food are exposed to radiation processing, there are still countries in which food irradiation for what-so-ever reasons is prohibited. The latter countries, e.g. Germany, want to control the ban of all irradiated foods. Other countries just may wish to control specific foods for their being irradiated: The main reasons for the development of analytical detection methods are summarized in Table 1.

Table 1: Need for analytical methods to identify iradiated foods

| | |
|---|---|
| - | to facilitate international trade with food |
| - | to check the compliance with existing regulations, e.g. enforcement of labelling, control of prohibition |
| - | to enhance consumer confidence in the correct application of the radiation process and its proper control by the inspection authorities |
| - | to protect the consumers' freedom of choice between irradiated or unirradiated food products |

However, it should still be emphasized that proper inspection of irradiation facilities is unavoidable to ensure that good irradiation practices are been followed.

## CRITERIA FOR ANALYTICAL DETECTION METHODS

Experiments in the past have shown that it is not a simple venture to develop analytical detection methods. About 10 years ago for most foodstuffs no practical method was available. The status was such that many laboratories had recorded some tiny changes in irradiated samples directly compared with control samples, but it should be kept in mind that this criterion alone is not sufficient for a detection method. In practice, an identical but unirradiated control sample will not be available. The response to be measured must therefore be large enough or specific to irradiation. Only in recent years, practical methods have emerged which show high potential for identification of foods which have been radiation processed. Some general criteria for an identification method are shown in Table 2.

Table 2: Some requirements for an identification procedure of irradiated food

| | | |
|---|---|---|
| ♦ | Discrimination | radiation-induced response should be distinct and separable |
| ♦ | Specificity | comparable response not induced by other processing, different breeds or varieties, different growth or storage conditions |
| ♦ | Robustness | insensitive or predictable response for:<br>- variation of radiation parameters (dose-rate, temperature, gaseous environment etc.)<br>- presence of other food components<br>- further processing |
| ♦ | Reliability | reproducible, accurate, validated |
| ♦ | Stability | throughout storage life |
| ♦ | Confidence | no falsification possible |
| ♦ | Practicability | rapid, simple, low cost, no complicated instruments, small sample size, applicable to a wide range of foods |
| ♦ | Dose-dependence | estimation of the absorbed dose |
| ♦ | Proof in court | |

In practice, however, all these requirements are difficult to achieve. Up to now, no universal detection method for all kinds of food has been found. Consequently, as many types of tests as possible should be developed and if necessary combined together to form test procedures with a higher probability of identification.

It should be stressed that at present the main aim of the detection methods is the qualitative question: irradiated ? Yes or No. The quantitative estimation of the applied radiation dose seems only of secondary importance, although of course in the radiation plant correct dosimetry is of utmost importance to ensure the proper treatment of the food. For most foods irradiation is a self-limiting process, higher doses leading to unacceptable sensory properties whereas a certain minimum dose is necessary to achieve the desired effect (e.g. in the case of chicken Salmonella destruction). Therefore, the applied dose will only vary between narrow limits and needs no additional estimation. A further dose limitation is caused by economic restraints, since higher doses are simply more costly. Dose estimation in the food itself will mostly be hindered by the unknown previous history of the food. The exact treating radiation parameters and duration of storage of the sample will in practice not be known. The qualitative estimation in thus of primary importance, and if the sample is judged to be irradiated, it may be traced back to the radiation facility and the dose controlled by its documentary records.

Analytical detection methods should be rigorously standardized and validated by collaborative studies. It would be of advantage to produce a handbook of internationally acknowledged analytical methods for the detection of irradiated foods.

## STATE-OF-THE-ART OF DETECTION METHODS

Numerous analytical methods for the identification of radiation processed foods have been investigated over the years, and a review of the current literature up to 1990 has appeared as an IAEA technical document within the framework of the ADMIT programme (Delincée 1991). Recent reports include the proceedings of the BCR meeting in Cadarache 13-15 Feb.1990 (Raffi and Belliardo 1991), the British Symposium in Belfast 10-11 April 1990 (Johnston and Stevenson 1990), and the ADMIT meeting at Warsaw/Jachranka 25-29 June 1990 (Anon. 1990). In addition several reviews have appeared (Hasselmann and Marchioni 1991, Meier 1991, Schreiber et al. 1992, Delincée 1992). Both BCR and ADMIT had very recent meetings from which detailed information will soon be available, i.e. the BCR meeting in Ancona 24-26 Sept. 1991 (Leonardi et al. 1992) and the ADMIT meeting in Budapest 15-19 June 1992 (Anon. 1992). In this paper only a very short overview of the various methods will be presented with emphasis on the international inter-laboratory studies. According to the detected changes in the irradiated food, the following tables are divided into physical, chemical or biological methods.

Table 3. Physical methods for detection of irradiated foods and their proximity to usability.

| | Status | Remarks |
|---|---|---|
| Changes in physical properties | | |
| electrical impedance | B | For potatoes |
| viscosity of suspensions | B | For pepper |
| thermal analysis, differential scanning calorimetry (DSC) | A | |
| near infra-red spectroscopy (NIR) | A | |
| nuclear magnetic resonance (NMR) | ? | |
| Formation of free radicals | | |
| electron spin resonance (ESR) | E | For meat and poultry bones |
| | D | For fish bones, nutshells and dehydrated fruits |
| | C | For fresh fruits (seeds, stones or stalks), shellfish and some spices |
| | B | For dehydrated mushrooms, egg shells, snails |
| luminescence | | |
| chemiluminescence | D | For some spices, herbs or dehydrated vegetables |
| thermoluminescence | E | For spices and herbs measuring minerals |
| | D | For fruits and vegetables measuring minerals |
| | B | For shellfish |
| photo-stimulated luminescence | A | |

Status

| | |
|---|---|
| ? | insufficient information |
| A | concept promising |
| B | encouraging experiments in one or several laboratories, needing further evaluation in more laboratories |
| C | ready for international testing |
| D | collaborative study already carried out |
| E | ready for implementation in routine use |

Inter-laboratory comparisons under the auspices of international organisations employing ESR have been carried out by BCR (bone samples from chicken, beef, trout bones, sardine scales, dried papaya and grapes, shells from pistachio nuts) a large trial in which 22 European laboratories participated, and by ADMIT (3 trials with bone samples from chicken, pork and frog legs), with 11 participants.

ESR collaborative studies have also been carried out at a national level in e.g. UK and Germany. The conclusion of these trials is that ESR for analysis of meat bones is ready for implementation in a recommended standard protocol. Some further research is required on fish bones, and this pertains also to shells from molluscs and crustaceae, to seed and stones from fresh fruits, to dehydrated fruits and vegetables, to nut shells and to some spices.

A preliminary trial with thermoluminescence (TL) of spices measuring concomitant silicate minerals has been organized by BCR, 10 laboratories participitating. In addition, a large collaborative study is being carried out in Germany, also including fresh fruits and vegetables. The results indicate that isolation of adhering minerals from the food products and measuring their TL response, eventually combined with a re-irradiation step to normalise the TL signals, greatly improves identification. At least for herbs and spices is the TL analysis ready for international implementation.

Table 4. Chemical methods for detection of irradiated foods

| | Status | Remarks |
|---|---|---|
| Changes in chemical compounds | | |
| proteins | | |
| o-tyrosine | B | For meat, shrimps |
| crosslinks | ? | |
| lipids | | |
| long chain hydrocarbons | E | For meat and poultry |
| | B | For fish, shrimps, egg products, spices |
| long chain aldehydes | B | |
| 2-alkylcyclobutanones | D | For poultry meat |
| | C | For egg products |
| immunoassay of cyclobutanones | ? | very promising concept |
| evolution of carbon monoxide | B | For meat |
| lipid hydroperoxides | B | For meat, egg products |
| cholesterol oxidation products | A | |
| carbohydrates | | |
| optical isomers | ? | |
| nucleic acids | | |
| base damage, immunoassay | ? | Development of antibodies towards dihydrothymidine |
| strand breaks | | |
| alkaline filtration | A | Interference with storage |
| agarose electrophoresis of mt DNA | B | |
| pulsed field gel electrophoresis | B | |
| microgel electrophoresis | B | Small preliminary trial on frozen chicken successful. |
| other food constituents | | |
| evolution of $H_2$ | A | |
| optical isomers | B | For liquor |

Status legends as in Table 3.

Larger international collaborative tests have so far only been carried out with the GC/MS measurements of lipid-derived hydrocarbons and alkylcyclobutanones in irradiated meat (chicken) in the framework of BCR with 12 participants. Although no 100 % correct classification using hydrocarbon analysis was obtained in the first stage of the trial, results were very promising. Using 2- dodecylcyclobutanone analysis all samples were correctly identified. Promising results were also obtained in a German intercomparison (17 participants) using hydrocarbon analysis of chicken, pork and beef. The hydrocarbon method may thus be ready for international implementation, and protocols should be submitted to the corresponding organisations. As soon as experience in more laboratories is gained with the handling of the cyclobutanones, also this method seems ready for routine use.

Promising resullts were also achieved in a BCR small preliminary blind trial employing micro gel electrophoresis with single cells to detect changes in DNA. This is a rapid and simple test, but more research is needed about the effects of storage of the food and further processing.

Table 5. Biological methods for detection of irradiated foods

| | Status | Remarks |
|---|---|---|
| Biological changes | | |
| histological/morphological characteristics | | |
| inhibition of cell division | A | For bulbs and tubers |
| lack of wound periderm formation | B | For potatoes |
| inhibition of seed germination (shooting, rooting) | B | For citrus fruits, cereals |
| chromosomal aberations | A | |
| electron microscopy | A | For fruits, shrimps |
| changes in substrate suspectibility to bacterial spoilage | B | For fish and meat |
| changes in microflora | | |
| radioresistance | A | For meat |
| direct epifluorescent filter technique (DEFT) | D | For spices |
| | C | For meat |
| endotoxin (Limulus Amoebocyte Lysate test) | B | For meat |
| changes in insects | A | |

Status legends as in Table 3

A BCR collaborative study with the direct epifluorescent filter technique (DEFT) combined with an aerobic plate count (APC), by which the number of non-viable micro-organisms inactivated by radiation (or other treatments) can be identified, showed good results with irradiated spices ( 8 European laboratories). It should be kept in mind, however, that the APC/DEFT technique is not specific for irradiation, thus have to be supplemented by other test procedures. On the other hand, the DEFT technique gives information of the hygienic quality of the food prior to treatment, and therefore about the use of GMP and good irradiation practices.

## CONCLUSION

Reliable identification of a number of irradiated foods now seems feasible. The international co-operation in this field has led to rapid progress, and it now appears that it will be possible to identify the radiation treatment of those foods which are already, or will shortly be, processed in commercial amounts. To achieve international adoption of identification methods, collaborative studies should be pursued, and procedures harmonised by international organisations.

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