R&D REPORT NO. 14

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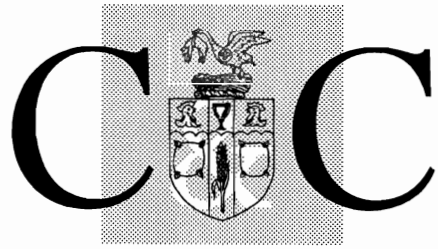

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Development of a Simple Screening Test to Detect and Determine the Microbiological Quality of Irradiated Foods

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SUMMARY

The direct epifluorescent filter technique/aerobic plate count (DEFT/APC) method is a recognised technique for the screening of irradiated foods. When the APC of an irradiated sample is compared with the DEFT count on the same sample, the APC is found to be considerably lower than that obtained by the DEFT, thus indicating that the sample could have been irradiated.

Since the development of the DEFT/APC screening method, the technique has been tested with a limited range of food products. Previous work has indicated that the storage of irradiated foods can, in certain circumstances, allow microorganisms to grow, and thus compromise the ability of the DEFT/APC method to discriminate between irradiated and unirradiated samples. In some cases the method has been shown to give high DEFT count and low APC with food samples that have not been irradiated. Potentially, foods which have undergone a food processing treatment could give a high DEFT count compared to an APC and be erroneously identified as having been irradiated.

The work reported here is aimed at analysing a range of irradiated samples (meat, poultry, fish, seafood, herbs and spices), stored under different conditions, to evaluate the applicability of the screening method for use with such products. The effects of other food processes on the DEFT/APC results were also investigated.

The initial part of the project was aimed at finding a suitable sample pre-treatment to give the best results in terms of ease of sample filtration through a DEFT membrane, clarity of the slide preparation when viewed microscopically and accuracy of the count when directly compared to the APC of the same sample. A universal pre-treatment incorporating a combination of a filter stomacher bag, Triton X 100 and trypsin was selected for all meat, poultry, fish and seafood. For herbs and spices pre-filtration using Whatman No. 4 filter paper and a 10μm filter was found to give the best results.

The second part of the work involved irradiation and storage of samples. Meat, poultry, fish and seafood were irradiated at three dose levels (0.5, 5 and 10 kGy) and tested using the DEFT/APC method during chilled and frozen storage. Samples irradiated at 0.5 kGy did not show a large reduction in APC. Foods irradiated with such doses, therefore, could not be identified by the DEFT/APC method. For foods irradiated at 5 kGy, the detection method would appear to be applicable for use with samples stored chilled for up to ten days (minced beef and beef), six days (chicken), and one day (cod and prawns). Foods stored frozen for eight weeks or more after irradiation at 5kGy were all detected in the study and so could be used with the DEFT/APC screening method. Chilled and frozen

stored meat, poultry, fish and seafood samples irradiated at 10 kGy could be readily identified by the method up to the end of storage of 15 days (chilled) and eight weeks (frozen) with the exception of chilled prawns which were only identifiable for up to six days of storage.

A total of twelve herbs and spices were irradiated at three dose levels (0.5, 5 and 10 kGy) and tested using the DEFT/APC method. In general, herb and spice samples irradiated at 5 kGy and 10 kGy were able to be detected using the screening method. In five sample types either the APC was not sufficiently reduced or the DEFT/APC log unit differentials fluctuated due to sample variation.

The final part of the work involved the effect of other food processes on the DEFT/APC method. Meat, poultry, fish and seafood were treated within the 200-300 MPa range for pasteurisation treatment for meats. The results for high pressure were more variable between samples than those seen for irradiation. Results showed that minced beef treated with high pressure could be misidentified as having been irradiated when using the DEFT/APC method. Much more work is required using the screening method with high pressure before any conclusions can be made.

For herbs and spices, the process applied to untreated samples was heat. Heat treatments of herbs and spices have previously been reported to reduce the viable count of the samples significantly and hence give similar DEFT counts and APC's to irradiated samples. This study showed that a dry heat treatment at 80°C for up to 60 min did not give a substantial decontamination of the samples, with one exception. With parsley, DEFT/APC results were similar to those obtained with irradiation.

The DEFT/APC screening method is a cost effective, easy to use technique for the detection of irradiated foods. From the data reported the method would be applicable for use with stored irradiated meat, poultry, fish and seafood, particularly during frozen storage, and ambient stored herbs and spices. The applicability of the method, however, has limitations with different food types, particularly during storage. Therefore, the method should be used with those food types used in this study to identify potentially irradiated foods. Other food processes can give rise to results that mimic those from irradiation on some occasions. It is therefore recommended that the technique is used as a rapid, easy to use, economical screening method, and that foods identified as having been potentially irradiated are confirmed using another method.

CONTENTS

INTRODUCTION

Food irradiation is recognised as having the potential to ensure the hygienic quality of food and to reduce post harvest food losses, thus aiding expansion of trade of certain food items. The process could give rise to considerable public health benefits. The majority of microorganisms and parasites are inactivated at the irradiation doses permitted for foods. As well as prolonging product life by inactivation of spoilage bacteria, microbial and parasitic pathogens could also be eliminated. The diseases caused by such agents were the most frequent cause of death (35%) worldwide in 1990; the majority of these occurred in developing countries (Loaharamu and Murrell, 1994). Irradiation would undoubtedly improve the safety of food and have significant health related economic benefits for the whole society. In the USA, the FDA has approved irradiation of poultry to kill *Salmonella*, and in France, Camembert cheese is treated with irradiation to ensure that it is free from pathogens (Scoular, 1994).

The process of irradiation treatment involves exposure of foods to ionising radiation. This results in the production of free radicals through the breakdown of water, and the subsequent reaction with the DNA of living insects and microorganisms ultimately causes their death. Ionising radiation can also cause direct damage to DNA by causing strand breaks.

Four sources of radiation have the potential to produce the predictable, precise amount of ionising radiation required for the treatment of food, namely cobalt-60, caesium-137, and electron beam and X-ray generators (WHO, 1994). Two sources are used in commercial irradiation plants. Gamma irradiation plants usually use cobalt-60 as the radioactive source. Caesium-137 is not available in sufficiently large quantities to play a role in commercial food irradiation. A major characteristic of gamma radiation is its high penetration which facilitates its use in treatment of bulk items such as chickens and drums of food. The second main source of ionising radiation is high energy electrons generated by electron beam machines. Such machines have the advantages that they can be switched off when not in use, leaving no residual radiation hazard. A major limitation of electron beam radiation for food use is the limited penetration depth of up to 8cm. A third source, the conversion of electrons to X-rays, is not yet commercially viable but would combine the advantages of high penetrating power with the switching on/off capability (Kilcast, 1990).

The applied radiation dose is measured in kilograys (kGy) and the maximum dose recommended by FAO/WHO for food irradiation is 10 kGy (Codex Alimentarius Commission, 1992). Dose levels are dependent not only on the food itself but also on a

balance between the desired effect and the amount of radiation that the product can tolerate without suffering unwanted changes. Irradiation has many potential applications. At very low doses (< 1 kGy) sprouting in products such as potatoes and onions is inhibited and insect manifestation, in for example grains and citrus fruit, is prevented (Kilcast, 1990). The use of irradiation in either case can eliminate the need for chemical treatments, many of which are now suspected of carrying toxicological hazards. Low irradiation doses can also delay ripening of certain fruits which is of particular importance in maintaining high quality in imported tropical fruits. At slightly higher doses (1-3 kGy) the numbers of microorganisms present in foods can be reduced. Reduction in the normal spoilage microflora can extend the shelf life of foods such as soft fruits, meat and fish. Food poisoning organisms such as *Salmonella* are slightly more resistant but a reduction in counts of practical value can be achieved within this dose range. At higher doses (3-10 kGy) pathogens frequently found in refrigerated products such as prawns and chickens can be destroyed and dried ingredients such as spices, natural gums and animal and vegetable proteins can be sterilized. There are essentially three dose levels of irradiation:

1. Radappertization

This is an application to foods of a dose of ionizing radiation sufficient to decrease the number and/or activity of microorganisms to such an extent that very few, if any, are viable. Doses used are typically greater than 10 kGy and the resulting foods can be stored at room temperature (Anon, 1983).

2. Radicidation

This is an application to foods of a dose of ionizing radiation sufficient to decrease the number of viable specific non-spore-forming pathogenic bacteria. Doses are usually 2-8 kGy, but are lower (0.1-1 kGy) for parasites. The products are usually kept under refrigeration.

3. Radurization

This is an application to foods of a dose usually sufficient to enhance its keeping quality by causing a substantial decrease in the number of viable, specific spoilage organisms. Processing takes place at dose levels generally in the range of 0.4-10 kGy, and the processed foods usually must be stored under refrigeration as with radicidized food.

Although the microbiological safety of irradiated food has been conclusively demonstrated, the process still remains an emotive subject. Concerns that standards of food hygiene and manufacturing practises would be allowed to slip and that food quality could be compromised are amongst the many fears that consumers hold (Scoular, 1994).

Despite some problems with its acceptance by the public, by 1994 food irradiation had been approved by 37 countries for a variety of foods. Of these countries, 25 were using it to treat a variety of food/food ingredients for commercial purposes, particularly spices and seasonings where the technology is used as a replacement for the banned fumigant, ethylene oxide (Loaharamu, 1994). In view of the restricted use of fumigants, the demand for safe, nutritious and convenient food and the increase in consumption of imported exotic food from developing countries the trend towards the practical application of irradiation is increasing and practices will have to be strictly controlled. UK regulations require that all irradiated foods or food ingredients are explicitly labelled. To ensure that this can be enforced, foods treated with ionizing radiation will need to be identifiable.

Detection Methods

To date there is no single recommended method available for the detection of post-irradiated foods. Over the last decade, numerous methods based on DNA changes, and on physical, chemical and microbiological methods have been proposed. Although the proposed methodologies for the detection of irradiated foods are numerous, the majority of the techniques have limitations. The problem of specificity is common, e.g. similar results to those obtained from irradiated foods can be seen from foods which have undergone an alternative treatment such as heat processing, fumigation or freeze-thawing.

DNA detection methods are principally based on the fact that the discernable changes, through base damage and strand breaks in the DNA, occur after irradiation. As most foods contain DNA, such techniques would have a wide applicability and a number of methods have been developed to detect these changes in irradiated DNA. The more intensive areas of research have concentrated on methods including enzyme linked immunosorbant assays to detect markers such as thymidine glycol and dihydrothymidine, although the formation of the latter has been found to be reduced if the food is irradiated in the presence of oxygen (Delincée, 1992). Another drawback of dihydrothymidine is that it is formed on exposure of the DNA to UV light. Microgel electrophoresis of DNA fragments formed during irradiation is seen as a promising method for detection due to its simplicity and speed. Mistreatment of frozen samples, however, can lead to DNA fragmentation and so results may be similar to those expected

from foods that have been irradiated. A more recent electrophoretic method, pulsed field gel electrophoresis, is now being studied for its applicability for detecting foods that have been irradiated. Gene probes for detection of specific nucleic acids are also being researched.

Physical detection methods including thermoluminescence and electron spin resonance spectroscopy have been used successfully in a range of food products, particularly shellfish. Photostimulated luminescence is now commercially available for the detection of irradiated herbs and spices, with a wider food application possible in the future (Sanderson, 1991).

Chemical approaches for irradiated food detection include methods based on the volatile compounds formed from irradiated lipids, namely long chain hydrocarbons and 2-alkylcyclobutanones. These methods offer the most promise but, as yet, they have been mainly used for the identification of irradiated meat, particularly chicken (McMurray *et al*, 1994). Another chemical method which has been the subject of a number of studies is that based on the formation of σ-tyrosine. The amounts of this compound in foods, however, has been shown to vary considerably in unirradiated samples and so is not thought to be specific enough to differentiate between irradiated and unirradiated samples (Stevenson, 1992).

In the field of microbiology, two methods have been widely researched, the limulus amoebocyte lysate (LAL) test and the direct epifluorescent filter technique (DEFT). The LAL test is based on the ability of the contents of cytoplasmic granules from a horseshoe crab amoebocyte to react specifically with lipopolysaccaride (LPS) present in the cell wall of all Gram negative bacteria (GNB) (Scotter *et al*, 1992). The LAL test detects all LPS whether derived from viable or non-viable GNB and, when compared to a viable Gram negative plate count, foods which have undergone a process, such as heat treatment or irradiation, can be identified. When a high LAL titre is obtained in the absence of significant numbers of viable GNB, this indicates the presence of a large population of dead bacteria and may be indicative of processing or treatment of the food before testing. The LAL test has been used with poultry, herbs and spices and seafood to test its applicability with a range of irradiated foodstuffs.

The Direct Epifluorescent Filter Technique (DEFT)

The Deft is a method developed originally for the rapid enumeration of microorganisms in raw milk samples (Pettipher *et al*, 1983). the method is based on the pre-treatment of a milk sample in the presence of a proteolytic enzyme and surfactant at 50°c, followed by a

membrane filtration that captures the microorganisms. The pre-treatment is designed to lyse somatic cells and solubilize fat that would otherwise block the filter membrane. after filtration the membrane is stained with the fluorescent nucleic acid binding dye, acridine orange, then rinsed and mounted on a microscope slide.

The filter membrane is viewed with an epifluorescent microscope which illuminates the membrane with ultra violet light, causing any bound dye to emit visible light that can be viewed through the microscope. The dye binds to nucleic acids (DNA and RNA) within microbial cells; thus any organisms on the membrane can be easily visualised and counted. The complete pre-treatment and counting can take as little as 30 minutes.

The types of food with which DEFT can be used has increased since the early work on raw milk. Reports now cover the use of the method with frozen meat and vegetables, alcoholic beverages, confectionery and dried foods as well as in hygiene testing.

Betts *et al* (1988) proposed that the DEFT could be used for the detection of irradiated foods by the direct comparison of a DEFT count with a conventional aerobic plate count (APC). The APC is a microbiological method of estimating the total number of aerobic mesophilic organisms present in a food sample. It is done by homogenising a sample, diluting and then dispersing in a solid nutrient. The agar plates are then incubated to allow the individual organisms dispersed through the medium to grow into colonies that can be visually counted.

When the APC of an irradiated sample is compared with the DEFT count on the same sample, the APC is found to be considerably lower than that obtained by DEFT, indicating that the sample could have been irradiated. The difference in the counts is due to microorganisms being inactivated by the irradiation process; these cells are not detectable by the APC method. The dead cells can, however, be seen microscopically and are counted. The DEFT count of a sample is the same before and after irradiation. Use of the DEFT is seen to have the added advantage of allowing the determination of the viable microbial population in the sample before irradiation; thus an indication of the microbiological food quality can be obtained.

APPLICATION OF THE DEFT/APC METHOD

Betts *et al* (1988) proved that the DEFT/APC method was a qualitative method for the detection of a number of irradiated foods. The results of DEFT counts were found to correlate well with APC from unirradiated samples of bacterial cultures and raw foods (meats and milk). The DEFT counts of the samples carried out before and after

irradiation showed little difference. The APC of the irradiated samples, however, exhibited a substantial reduction when compared with the APC of unirradiated controls and the DEFT counts of irradiated samples. Since these trials, the technique has been used successfully with a range of irradiated foods.

It has been generally accepted that for detection of raw food, irradiated with a dose of between 5-10 kGy, the difference between the DEFT count and the APC count should be in excess of three log units (Wirtanen and Sjöberg, 1992). A recent report by VTT Biotechnology and Foods Research (Anon, 1994), however, has suggested that this should be reduced to two log units. With herbs and spices, however, a difference of 3.5 log units has been quoted to indicate irradiation has taken place (Boisen, 1992, Wirtanen *et al*, 1993). This is due to the fact that the correlation between DEFT count and APC in non-irradiated spices is not in such close agreement as that observed with raw foods (Sjöberg *et al*, 1990). The DEFT count from unirradiated spices can be seen to be significantly higher than those obtained using the APC technique. It is possible that certain procedures in the manufacture of spices (e.g. drying) may lead to a reduction in the viable count of the finished product (Pettipher, 1983). It is also possible that the APC technique used is not able to support germination and growth of all the microorganisms present in the sample. Another theory is that anti-bacterial agents, released by the grinding of certain spices, may reduce the viable count (Antai, 1988).

Much of the work evaluating the DEFT/APC technique has concentrated on the detection of irradiated herbs and spices. The method has now been proposed as a screening method for irradiated herbs and spices as part of routine microbiological quality control systems (Wirtanen *et al*, 1993).

The development of the DEFT/APC method for herbs and spices has involved investigation into the sample pre-treatment procedures to enable efficient filtration through the polycarbonate DEFT membrane and to remove excessive debris. The exact nature of the pre-treatment is dependent on the type of food and should aim to have little or no effect on the microbiological count of the sample. For herbs and spices, pre-filtration using Whatman No. 4 filter paper has proved to be superior to other pre-treatments such as use of a glass microfibre filter and centrifugation (Manninen and Sjöberg, 1991). For certain spices, however, pre-filtration has been shown to present difficulties due to blockage of the filter paper (Liberti and Aureli, 1992). In particular, Manninen and Sjöberg (1991) found marjoram powder to be too fine to filter, and filtration of paprika powder, cut basil and cut majoram were found to be slower than other samples tested by Boisen (1992). Where slow pre-filtration was observed in these herbs and spices, the subsequent DEFT staining and rinsing filtration procedures were

also found to be slow. In such samples, more fluorescing non-microbial particles were seen in the microscope field of view than for other samples. Debris can make the slide preparation difficult to interpret as fluorescing particles can either mask the cells or can make it hard to distinguish between debris and microorganisms. This can make the counts inaccurate, particularly when an automatic DEFT image analyzer is used for enumeration.

The applicability of the DEFT/APC method has been evaluated on a range of other food products including deep frozen irradiated mechanically deboned poultry, meat, liquid egg and parsley stored at -18°C for up to 12 months (Copin *et al*, 1993). The results showed that the method was able to detect the irradiated samples during the whole storage period with deep frozen foods. With chilled foods, however, storage of chicken for nine days after irradiation reduced the ability of the DEFT/APC method to detect the irradiated samples. With such chilled foods, detection of irradiated samples was only achieved immediately after treatment (Copin and Bourgeois, 1992). The reason for these problems was due to microbial growth occurring in the irradiated samples during chilled storage causing an increase in the APC. This significantly reduced the difference between the APC and the DEFT counts in all irradiated samples and appeared to cast doubt on the use of the DEFT/APC method with such samples. The applicability of the technique using frozen prepared shrimps was also shown to be limited where low irradiation doses and extended storage times were used (Wirtanen and Sjöberg, 1992).

Another limitation of the DEFT/APC method is that it has been shown to be affected by other food processes. The method cannot be used to differentiate between heat treated, fumigated and irradiated herbs and spices, although fumigated samples can be identified by chemical analysis. Potentially all foods which have undergone a food treatment could give an erroneously high DEFT count compared to an APC. Conclusive evidence of irradiation therefore relies on the knowledge that the sample has not been previously treated.

The aim of this evaluation is to extend the work previously undertaken to cover a range of food types (red meat, poultry, seafood and herbs and spices) stored under various conditions with a view to evaluating their suitability for use with the screening method. The areas highlighted from previous research include the optimization of sample pre-treatment prior to the DEFT to obtain a good slide preparation, and sample storage after irradiation. As previously discussed, microbial growth with time has been shown to compromise the ability of the DEFT/APC to discriminate between irradiated and unirradiated samples. Further work is also needed on other irradiated samples stored under different conditions (chilled, frozen and ambient storage) to evaluate the

applicability of the screening method for use with such products. Finally the effects of other food processes on the DEFT/APC counts must be understood to determine the specificity of the technique.

MATERIALS AND METHODS

Food Samples

The food samples used in this study are shown in Table 1.

Meat, poultry, fish and seafood samples were purchased from retail stores on the day before irradiation. Samples (20g) were weighed into sterile filter stomacher bags (Seward Medical), sealed and stored at 4°C before transportation to the irradiation plant.

Untreated herb and spice samples were supplied by a manufacturer and stored at room temperature. Samples (5g) were weighed into sterile stomacher bags, sealed and stored at room temperature before transportation to the irradiation plant.

Samples, including unirradiated controls, were transported to the irradiation plant and back to the laboratory at chilled temperatures in an insulated box.

Irradiation

Samples were irradiated at Isotron (Elgin Industrial Estate, Swindon) using gamma radiation from a cobalt-60 source. The samples were irradiated at doses of 0.5, 5 and 10 kGy, and the doses were monitored with dosimeters. The colour changes of the dosimeters caused by the irraditation were measured spectrophotometrically and the minimum and maximum doses achieved were calculated (Table 2).

Storage and Sampling of Irradiated Samples

a) Frozen Storage

Irradiated meat, poultry, fish and seafood samples were frozen and stored at -20°C for up to 8 weeks. Two samples of each food type and dose level were removed after 0, 1, 2, 3, 4 and 8 weeks and allowed to thaw overnight at 2-4°C before testing.

b) Chilled Storage

Irradiated meat, poultry, fish and seafood samples were stored between 2-4°C for up to 15 days. Two samples of each food type and dose level were removed after 0, 1, 3, 6, 10 and 15 days and tested immediately.

c) Storage at Room Temperature

Irradiated herb and spice samples were stored at ambient temperature (18-23°C) for up to 8 weeks. Two samples of each herb and spice at each dose level were tested after 0, 1, 2, 3, 4 and 8 weeks.

Direct Epifluorescent Filter Technique (DEFT)

Pre-treatment of Samples

a) Meat and Fish Samples

Samples (20g) in the filter stomacher bags were macerated in a stomacher for 1 min in Maximum Recovery Diluent (180ml) (MRD, Oxoid). A sample of filtrate (10ml) was removed and filtered through a 10μm pore size polypropylene membrane (Gelman Sciences) in a 25mm Swinnex filter holder (Gelman Sciences). An aliquot (1ml) of the second filtrate was added to (0.25ml) DEFT Trypsin (Difco) and 1ml of filter sterilised 0.5% (v/v) Triton X 100 (BDH). The solution was mixed by vortexing for 30 sec before incubating in a water bath at 50°C for 20 min.

b) Herb and Spice Samples

Herb and spice samples (5g) were shaken in 45ml of MRD for 10 sec and filtered through a Whatman No. 4 filter paper.

Preparation of the DEFT Apparatus

Towers and membrane supports were washed in Lypsol solution (LIP Equipment Ltd.) and rinsed in tap water. The DEFT apparatus was assembled and connected to a vacuum pump.

Towers were cleaned and pre-washed by rinsing three times with filter sterilised 1% Triton X 100 (10ml) at 80°C and three times with filter sterilised deionised water (10ml) at 80°C, before mounting the DEFT membrane filters (0.6μm pore size polycarbonate membrane filters, Difco). For herb and spice samples a 10μm pore size polypropylene membrane was mounted directly on top of the DEFT membrane to effect a pre-filtration.

Preparation of DEFT Slides

a) Meat, Poultry, Fish and Seafood Samples

Immediately after incubation at 50°C (see Pre-treatment of Samples), samples were filtered through DEFT membrane filters. The remaining sample was rinsed out of the tube with 0.1% (v/v) Triton X 100 (5ml) at 50°C, mixed by vortexing, then filtered through the same DEFT membrane.

b) Herb and Spice Samples

Pre-filtered samples (1ml), or appropriate dilutions, were filtered simultaneously through 10μm pore size polypropylene membranes and 0.6μm pore size polycarbonate membranes, the former being mounted on top of the latter to effect a prefiltration. The top (10μm) membrane was removed before staining the lower 0.6μm membrane.

Staining Procedures

The membranes were stained with 2ml acridine orange solution (Difco) for up to 2 min depending on the sample type. Herbs and spices were stained for 30 s except for basil samples which were stained for only 15 s to prevent undesirable staining of sample debris. Meat, poultry, fish and seafood samples were stained for 2 min. After staining, filters were rinsed immediately with 2ml DEFT buffer pH 3.0 (Difco) and with 2ml isopropanol (Rathburn). The alcohol rinsing was carried out rapidly to prevent decolorisation of the bacteria on the membrane. Membranes were dried in air before mounting onto slides. A drop of immersion oil was placed on a slide and the membrane was mounted on top of the oil. Another drop of immersion oil was placed on the membrane and a coverslip was placed on top and pressed down firmly to remove any air trapped between the membrane and the coverslip. Slides were stored in the dark until ready for examination.

Counting of DEFT Slides

Slides were examined under a x 100 oil immersion objective on a fluorescence microscope.

Fluorescing (orange) cells were counted in 20 randomly chosen fields. If the number of units per field was greater than 100 then further dilutions were made from the macerated sample and an appropriate dilution was pre-treated and another slide prepared.

Slides were counted using an automated counter (Bio-Foss Automated Microbiology

System, Foss Electric, York, UK) which was adjusted manually to count only fluorescing cells. The cfu/ml of sample was calculated automatically by the apparatus and results converted to cfu/g of food sample. The results from duplicate samples were converted to average cfu/g of sample.

Aerobic Plate Counts

a) Meat, Poultry, Fish and Seafood Samples

After filtration through 10μm pore-size polypropylene membranes, filtrates from the meat, poultry, fish and seafood samples were serially diluted in MRD. Aliquots (1ml) of appropriate dilutions were plated in duplicate in Plate Count Agar (PCA, Oxoid) using a pour plate technique. Plates were incubated at 30°C for 72 h.

Duplicate plates with between 30 and 300 colonies were counted and the cfu/g of each sample was calculated. Results from duplicate samples were converted to average cfu/g of sample.

b) Herb and Spice Samples

After filtration through Whatman No. 4 filter papers, filtrates from herb and spice samples were serially diluted in MRD and appropriate dilutions plated onto PCA. Plates were incubated and counted as described for meat and fish samples.

Heat Treatment of Herbs and Spices

Untreated herbs and spices, stored at room temperature for approximately 4 months, were used in this part of the study. Samples (5g) were weighed into sterile stomacher bags and sealed. Two samples of each herb and spice were heated in an oven at 80°C for 15 min and 80°C for 60 min while two samples remained untreated. Samples were analysed immediately after heating. Samples were pre-treated and aerobic plate counts and DEFT slides prepared and counted as previously described.

High Pressure Treatment of Meat, Poultry, Fish and Seafood Samples

Samples of minced beef, beef, chicken, cod and prawns were purchased from retail stores on the day of treatment. Samples (20g) were weighed into sterile stomacher bags and vacuum sealed. Two samples of each food type were processed in an indirect hydraulic compression system (National Forge) at 200 and 300 MPa for 5 min and two samples

remained unprocessed. The samples were analysed immediately after processing.

After high pressure treatment, samples were transferred to sterile filter stomacher bags and MRD (180ml) was added to each (10^{-1} dilution). Pre-treatment of the samples, preparation of DEFT slides and aerobic plate counts were carried out as previously described.

TABLE 1
FOOD SAMPLES

Meat, Poultry, Fish and Seafood	Herbs and Spices
Minced beef Beef (steak) Chicken (breast) Cod (fillets) Prawns	Basil (leaf) Black pepper (ground) Black peppercorns (whole) Chilli powder Garam masala (ground) Garlic powder Marjoram (leaf) Onion powder Oregano (leaf) Paprika (ground) Parsley (leaf) Thyme (leaf)

TABLE 2
LEVELS OF RADIATION APPLIED TO SAMPLES OF MEAT, POULTRY, FISH, SEAFOOD, HERBS AND SPICES

Sample type	Target dose kGy	Actual dose kGy	
		Minimum	Maximum
Minced beef Beef	0.5	0.79	0.92
Chicken Cod	5	4.82	5.96
Prawns	10	10.2	13.9
Garam masala Chilli powder	0.5	0.59	0.72
Black pepper (ground) Basil	5	4.82	5.16
Thyme Onion powder	10	10.1	10.2
Garlic powder Oregano	0.5	0.83	0.85
Black peppercorns (whole) Paprika	5	5.27	5.50
Marjoram Parsley	10	10.0	10.1

RESULTS AND DISCUSSION

Pre-treatment of Meat, Poultry, Fish and Seafood

The initial work with meat, poultry, fish and seafood was aimed at finding an optimum pre-treatment for the samples prior to filtration using the DEFT.

Different combinations of pre-treatments were tested using samples of meat, poultry, fish and seafood. A universal method for these samples was selected that was designed to lyse somatic cells and solubilise fat that would otherwise block the DEFT membrane filter.

A combination of three pre-treatments (filter stomacher bag, pre-filtration (10μm filter) and a Trypsin/Triton X-100 (0.5%) treatment) gave the best results in terms of ease of sample filtration through a DEFT membrane, clarity of the slide preparation when viewed microscopically and accuracy of the count when directly compared to the APC of the same sample (this is fully detailed in the Materials and Methods section). With the prawn samples, some debris was evident in the slide preparations; however, these did not appear to mask any cells, or to fluoresce to an extent that made it difficult to distinguish between the background and microbial cells.

The value of using a combination of pre-treatments to improve the filtration of food samples through DEFT membranes has previously been shown (Pettipher, 1983).

The pre-treatment method selected was used for all meat, poultry, fish and seafood samples tested, thus standardising the pre-treatment used with the DEFT.

Irradiation of Meat, Poultry, Fish and Seafood

It has been indicated that in order to determine if a sample has been irradiated the difference between the DEFT count and APC would be in excess of 2 log units (Copin and Bourgeois, 1992). A paper published recently by VTT Biotechnology and Food Research reporting work on irradiated frozen poultry has verified that a difference of at least 2.0 log units can be considered as the limit value to indicate potential irradiation treatment of such food samples (Anon, 1994).

Work described in this report has shown that foods irradiated with 5 kGy and 10 kGy generally had in excess of 2.0 log units difference between the DEFT count and APC immediately after treatment (day 0). When foods were irradiated with a 0.5 kGy, a 2.0 log unit difference was only seen in samples containing microorganisms that were

apparently susceptible to radiation: beef, chicken and cod. In prawn and minced beef samples, however, the low irradiation dose had little effect on the viable count of the samples and thus the difference between the DEFT count and APC was less than 2.0 log units. Where a value of 2.0 log units difference between DEFT count and APC is used to indicate irradiated foods the results for samples irradiated at 0.5 kGy would be inconclusive. It is unlikely, however, that doses as low as 0.5 kGy would have any practical use in terms of microbial decontamination in meats, poultry, fish and seafood and so the DEFT/APC method would not be used to identify foods treated with 0.5 kGy.

Storage of Irradiated Meat, Poultry, Fish and Seafood

The investigation into storage of irradiated foods involved irradiation of all foods at three dose levels: 0.5 kGy, 5 kGy and 10 kGy. The types and duration of storage were chosen to reflect the usual storage practices used for the food types. Meat, poultry, fish and seafood were stored chilled (2-4°C) and tested after storage for 0, 1, 3, 6, 10 and 15 days. The foods were also stored frozen (-20°C) and tested during two months of storage (0, 1, 2, 3, 4 and 8 weeks).

The results for meat, poultry, fish and seafood showed similar trends between the samples for the three irradiation dose levels and for the two storage conditions used. The effects of irradiation at 0.5 kGy, 5 kGy, and 10 kGy, followed by subsequent storage, on the DEFT count and APC of minced beef are described:

The DEFT count and APC obtained from minced beef before irradiation correlated well at levels of 8.38 and 8.29 log count/g respectively. When minced beef was irradiated with a dose of 0.5 kGy the APC count was reduced by 1.49 log units to 6.80 log count/g. The DEFT count, however, remained over 8 log count/g to give a difference between the DEFT count and APC of 1.21 log count/g (Fig. 1a). During chilled storage (4°C) the viable count remained at this level over a period of six days. After this time the viable count increased such that at day ten, the APC was as high as that found in the sample before irradiation and at day fifteen the APC had surpassed it.

The DEFT count of the minced beef samples irradiated at 0.5 kGy remained relatively static at 8 log counts/g for up to ten days of chilled storage. This is due to the fact that the DEFT counts both live and dead cells and so is not affected by the irradiation process. After day ten however, microbial growth during chilled storage gave a count higher than that in the initial unirradiated sample. The DEFT count increased to a level corresponding to the high APC obtained from the same sample and at the final day of storage, the DEFT count was 9.24 log count/g and the APC was 9.61 log count/g.

When irradiated minced beef (0.5 kGy) was stored under frozen conditions (-20°C) (Fig. 2a) the APC of the sample did not increase; the viable counts remained at the same level during the eight weeks of storage with the exception of some minor variation due to the uneven distribution of microflora in different portions of the test sample. The DEFT count remained at approximately 8 log count/g with no increase during the testing period. The difference between the DEFT count and APC ranged from 0.05 - 1.21 log units throughout the eight weeks of storage.

The practicality of using the DEFT/APC method with minced beef irradiated at 0.5 kGy can be determined from the data. At such low doses regardless of storage conditions, a log difference of two was not seen in the minced beef samples. The DEFT/APC could not be used to confidently identify minced beef irradiated at 0.5 kGy.

At a higher dose of 5 kGy the viable count was reduced to a greater extent than with the 0.5 kGy dose (Fig. 3). Immediately after irradiation (day 0) the APC was reduced by 4.13 log units to 4.16 log count/g. The DEFT count, however, was not reduced and the difference between the DEFT count and APC increased to 3.86 log units compared to 1.21 log units at 0.5 kGy. During chilled storage, the DEFT count remained at the same level. The APC, however, further decreased over the next day to 3.31 log count/g, increasing the difference between the DEFT count and APC to 4.78 log units (Fig. 1b). The further decline in the viable count could have been due to the death of radiation stressed or injured cells during initial chill storage. Following the initial drop in the APC after chilling, the remaining viable cells appeared to recover and grow, until at day ten the difference between the DEFT count and APC was 3.27 log units and at day fifteen this had been further reduced to a 1.36 log units.

The results of storing minced beef samples previously irradiated at 5 kGy, under frozen conditions are shown in Fig. 2b. The viable cell count of 4.16 log count/g obtained after irradiation further declined very gradually over the eight weeks of frozen storage presumably due to death of cells that had been sublethally damaged by irradiation. Minor fluctuations in the APC results during the storage period were attributed to a variation in microbial load in the different samples tested. The DEFT count, however, remained relatively static at over 8 log count/g. At week eight, the difference between the DEFT count and APC had increased to 5.40 log units.

The DEFT/APC method could thus be used to screen minced beef irradiated at 5 kGy and stored chilled for up to ten days or stored frozen for at least eight weeks. Experimental data on frozen storage covered an eight week period. It is not, however, believed that the

microbial levels in the frozen storage would vary over a longer storage time and thus the DEFT/APC method has utility in samples stored frozen for longer periods.

A 10 kGy dose gave a difference between the DEFT count and APC in excess of four log units (Fig. 3). The APC of the sample was reduced from 8.29 log count/g in the unirradiated sample to 3.62 log count/g. The treatment did not affect the DEFT count. Subsequent testing of the samples stored chilled, showed that no significant microbial growth occurred during storage (Fig. 1c). There was in fact a slight decline in the viable count over the fifteen day sampling period and the APC was reduced to 2.83 log count/g at the fifteenth day of storage. The higher irradiation dose of 10 kGy appeared to have injured or stressed the remaining viable cells to an extent that after chilling they could not recover and multiply. The DEFT counts of the samples remained at the same level throughout storage, the difference between the DEFT count and APC increasing to 5.44 log units at the end of the storage period.

Frozen storage of the minced beef seemed to impose an additional stress on the cells over that observed with chilling (Fig. 2c). Further cell death was evident up to two weeks of storage and the difference between the two counts increased to 5.63 log units. Subsequently the APC stabilised and a log difference in excess of five log units was maintained between the DEFT count and APC. The DEFT count remained at the same level throughout chilled and frozen storage.

Samples irradiated at 10 kGy had in excess of a 2.0 log difference between the DEFT count and APC throughout both the chilled and frozen storage period. The data showed that the DEFT/APC screening test for the detection of irradiated minced beef is applicable to samples receiving a dose of 10 kGy or higher when stored under refrigerated or frozen conditions.

Similar results were obtained for beef, chicken, cod and prawns. For foods irradiated with 5 kGy, the DEFT/APC screening method would appear to be applicable for use with samples stored chilled for up to ten days (beef), six days (chicken) and one day (cod and prawns). Foods stored frozen for eight weeks or more after irradiation with a 5 kGy dose were all identified in the study and so could be used with the screening method. Chilled and frozen stored beef, chicken, cod and prawn samples irradiated with 10 kGy could be readily identified up to the end of 15 days chilled storage and eight weeks frozen storage with the exception of chilled prawns in which a 2.0 log unit difference between the DEFT count and APC was maintained up the sixth day of storage.

The effects of irradiation at 0.5, 5 and 10 kGy followed by chilled storage at 4°C on the DEFT count and APC of minced beef

Figure 1a.

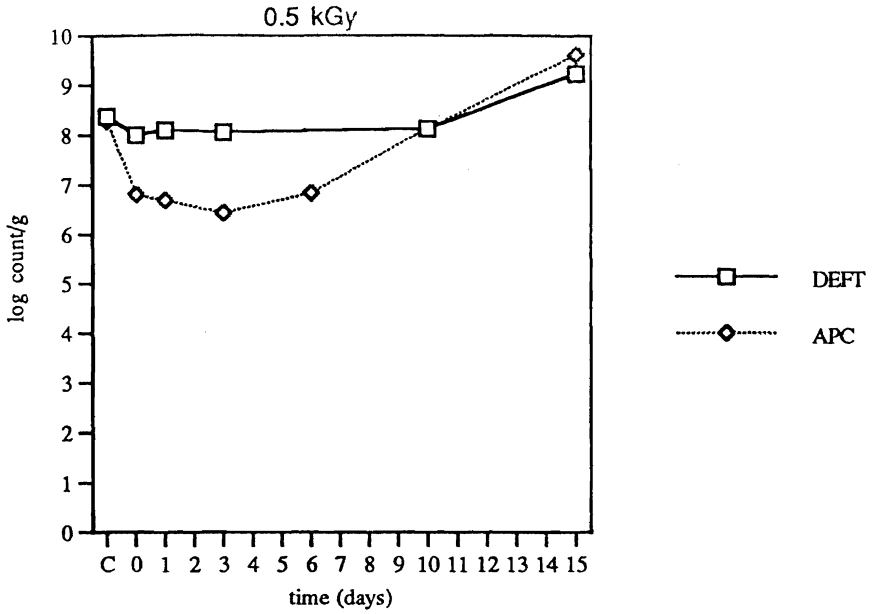


Figure 1b.

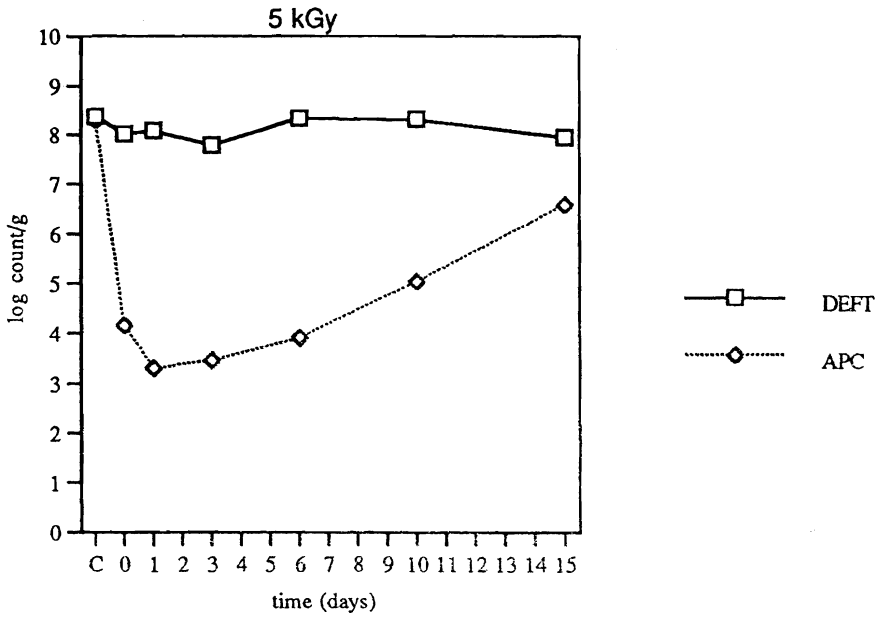
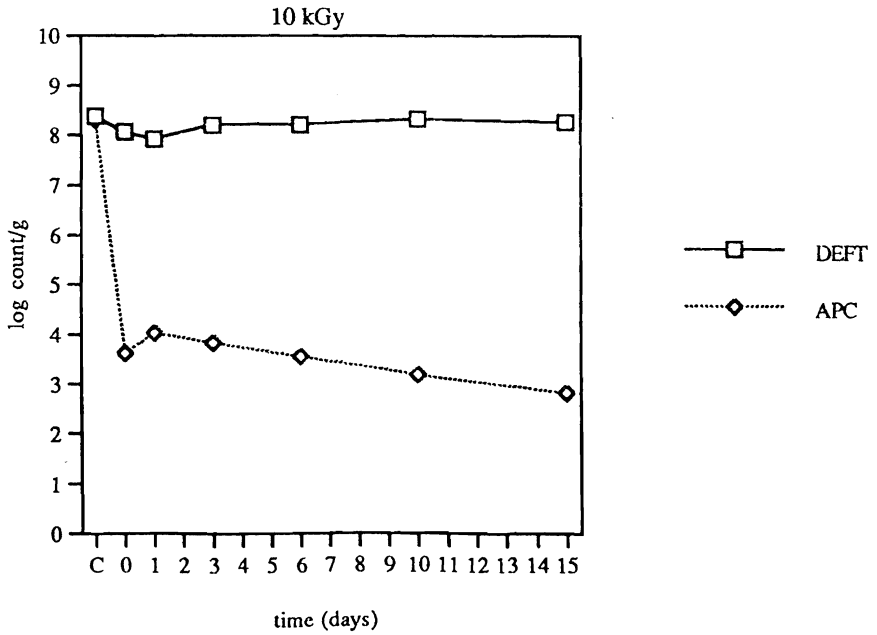


Figure 1c.



The effects of irradiation at 0.5, 5 and 10 kGy followed by frozen storage at -20°C on the DEFT count and APC of minced beef

Figure 2a.

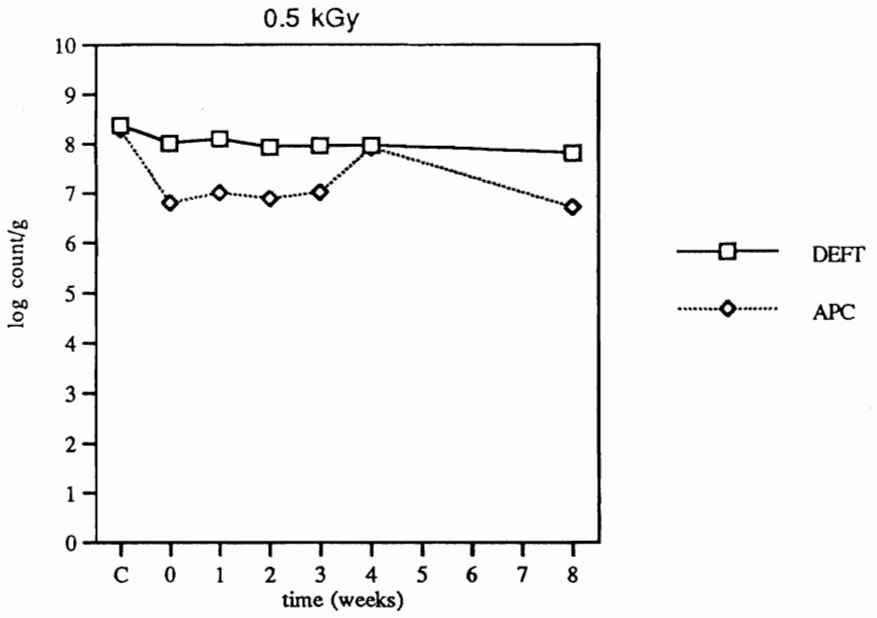


Figure 2b.

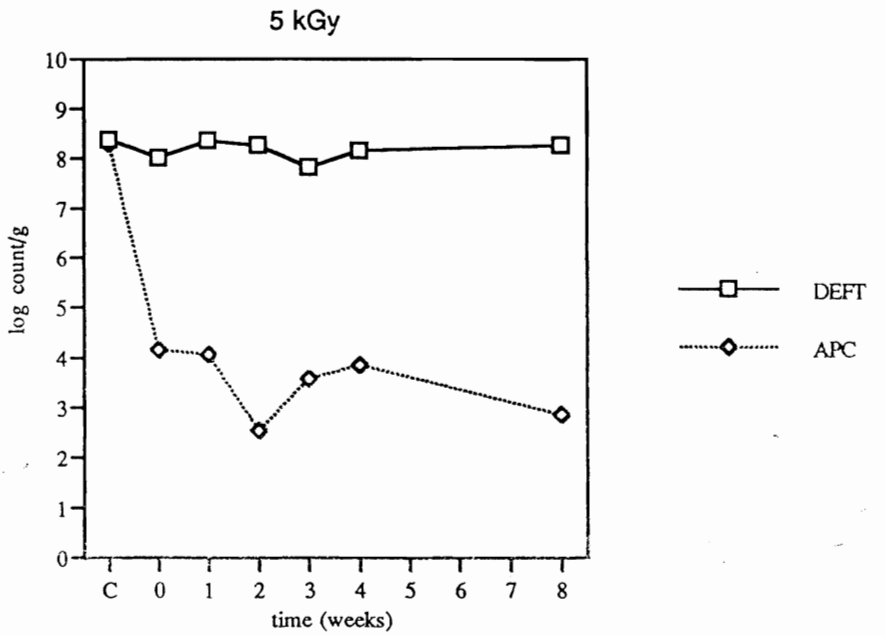
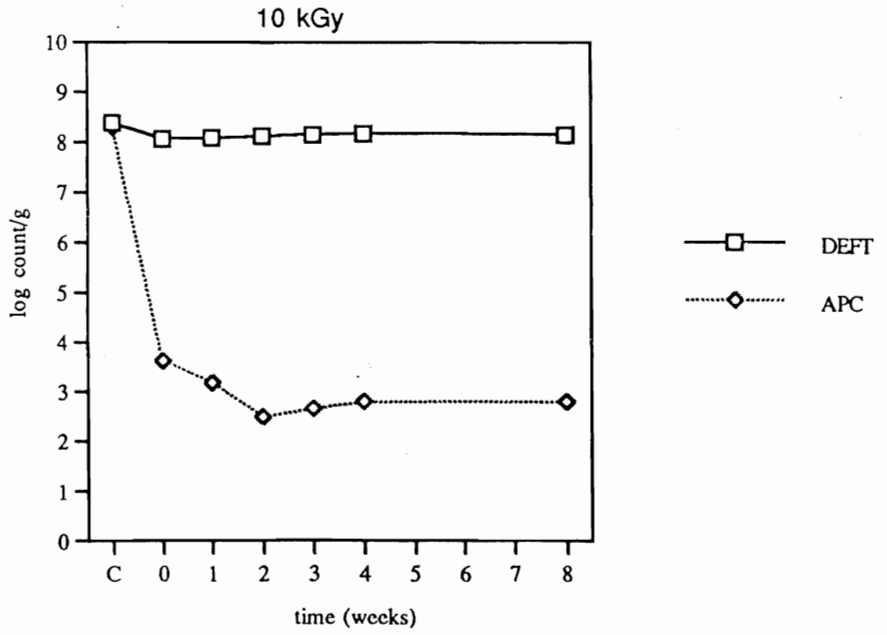
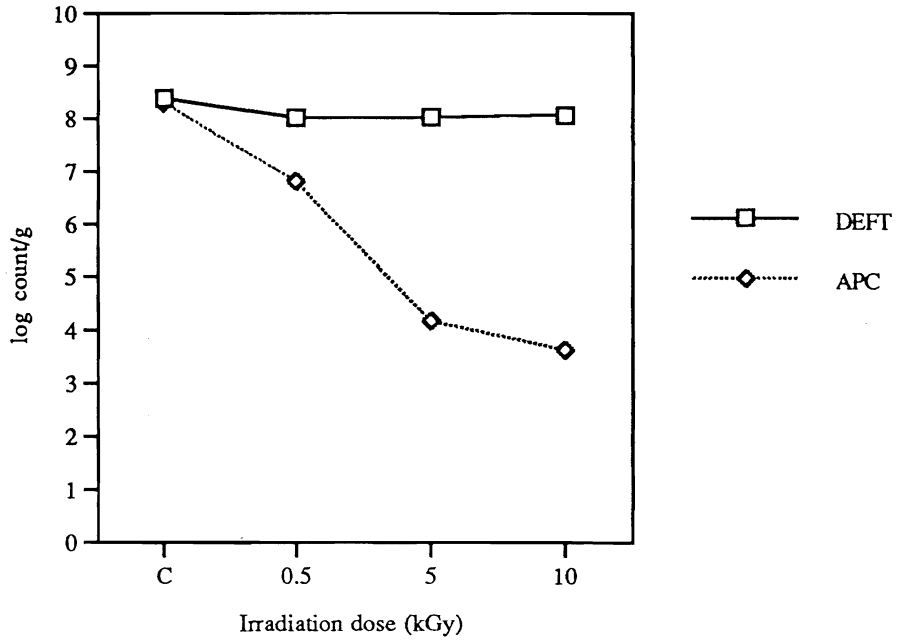


Figure 2c.



The effects of irradiation doses of 0.5, 5 and 10 kGy on the DEFT count and APC of minced beef

Figure 3.



Pre-treatment of Herbs and Spices

The preliminary work on herbs and spices was designed to evaluate and develop an optimum pre-treatment for use with samples before filtration through the DEFT membrane. As mentioned previously, the pre-treatment must allow a good correlation between the DEFT count and the APC of a sample prior to irradiation, and give a clear slide preparation with the minimum amount of debris.

Results obtained from combinations of pre-treatments tested indicated that a filtration of the sample through a Whatman No. 4 filter and passage of the filtrate through a 10μm filter mounted immediately on top of the DEFT membrane appeared to give the best correlation of the DEFT count with the APC and the clearest clear slide preparations.

The DEFT pre-treatment of herbs and spices as noted above was effective, except in the case of some leaf herbs (basil, marjoram, oregano) where pre-filtration was very slow. The slow filtration was also noted in subsequent DEFT filtration and in rinsing and staining procedures. As a result the microscopic examination of membrane preparations from such leaf herbs was difficult to interpret due to stained debris masking the microbial cells. With basil samples, the reduction in contact time of acridine orange with the filter helped to reduce the fluorescing background debris. This facilitated the enumeration of microbial cells.

Paprika also caused problems when tested with the DEFT. The paprika powder was very fine and difficult to pre-filter prior to preparation of the DEFT slide. When viewed, the slide contained fluorescing debris that were of similar size to microbial cells. Discrimination between debris and cells could not be achieved using the DEFT image analyser and so the counts had to be carried out manually, making it a time consuming process.

Generally the DEFT count and APC before herb and spice samples were irradiated did not appear to give such a good correlation as that seen with the meat, poultry, fish and seafood. Variations between the counts ranged from 0.49 to 2.30 in herbs and 0.80 to 2.62 in spice samples.

Herbs and spices in which the DEFT count was substantially higher than the APC were parsley, marjoram, garam masala and garlic powder. Similar discrepancies between the DEFT count and APC have been previously reported in herbs and spices by Sjöberg *et al.* (1990) and Boisen (1992).

Such differences in count are due to either the DEFT enumeration of cells inactivated in the drying process, or by undercounting in the APC due to the antimicrobial nature of some herbs and spices inhibiting cell growth and thus colony formation.

Irradiation of Herbs and Spices

Wirtanen *et al.* (1993) noted that herbs and spices irradiated with doses between 5 kGy and 10 kGy generally had a difference in count between DEFT and APC in excess of 3.5 log units. This difference is much greater than those seen in unirradiated samples, and thus the 3.5 log unit value can be used as a marker for irradiated samples.

In this study, samples irradiated at 0.5 kGy did not have a difference between DEFT counts and APC's of more than 3.5 log units and thus the method could not be used to identify such samples. Irradiation of herbs and spices at this dose level, however, appears to have no microbiological advantage in terms of a reduction in the viable number of microorganisms and it is thus unlikely that these type of samples would be treated with such low doses.

Storage of Irradiated Herbs and Spices

The results for the twelve herb and spice samples showed similar trends within the three irradiation dose levels (0.5 kGy, 5 kGy, and 10 kGy) stored for up to two months at ambient temperature (18-23°C). The results for irradiated black peppercorns (whole) are described.

Counts from unirradiated black peppercorns showed a 2.62 log unit discrepancy between the DEFT count and the APC. The DEFT count was 6.22 log count/g whilst the APC was lower at 3.60 log count/g.

Irradiation of the peppercorns at 0.5 kGy did not have a large effect on the APC of the sample, the count being reduced to 3.48 log count/g (Fig. 5). The DEFT count decreased slightly to 5.89 log count/g to give an initial difference in count of 2.41 log count/g. During storage, the DEFT count and APC remained relatively static. The difference between the counts varied from 2.41 to 3.14 log count/g due to sample to sample variation (Fig. 4a).

Irradiation at 5 kGy reduced the viable count from 3.60 log count/g in the unirradiated sample to 1.18 log count/g. The DEFT count remained over 6 log count/g, to give an overall difference between counts of 4.85 log units. This difference was maintained over the eight week storage period (Fig. 4b).

Irradiation of the samples at 10 kGy reduced the viable count to 1.00 log count/g whilst the DEFT count was 6.19 log count/g. The difference between the counts was thus 5.19 log count/g (Fig. 5). This difference of over five log units between the DEFT counts and APC was maintained throughout storage (Fig. 4c).

Irradiation of samples at 0.5 kGy did not give a 3.5 log difference between the APC and the DEFT count. Black peppercorns irradiated at 5 kGy and 10 kGy were decontaminated sufficiently to give over a four log unit difference between the DEFT and APC. Thus the DEFT/APC screening method could be used with confidence to identify irradiated black peppercorn samples throughout the eight week storage period.

Similar results were obtained for the remaining eleven herbs and spices tested. Irradiation at 0.5 kGy did not give a 3.5 log difference between the DEFT and APC with any of the herb and spice samples, with the exception of parsley, and so could not be used with the DEFT/APC method. Parsley had in excess of a 3.5 log difference after irradiation and storage of up to two weeks. This was due, in part, to a large difference between the DEFT count and APC before the irradiation.

At 5 kGy, the majority of herbs and spices were sufficiently decontaminated to bring about a 3.5 log unit difference between the DEFT count and APC. The exceptions were thyme, basil, chilli, and garlic powder. With basil, the differences between the DEFT count and APC during storage never achieved 3.5 log units. The 5 kGy dose was not sufficient to cause a substantial reduction in the viable count. With chilli and thyme, a log difference in excess of 3.5 log units was achieved after irradiation, but subsequently some log unit differences fell below this level during storage. With garlic powder, despite the initial poor correlation between the DEFT count and APC prior to irradiation, a log difference of 3.5 units did not occur after irradiation. During storage, some points exceeded the 3.5 log unit criteria for irradiated herb and spice detection. The fluctuations in the log differences appeared to mainly due to variations in the APC because of the inhibitory nature of the garlic powder. It is therefore possible that garlic powder irradiated with 5 kGy could be detected using the DEFT/APC method during storage.

All of the herbs and spices irradiated with a 10 kGy dose were able to be detected using the DEFT/APC method with one exception. On one sampling day with oregano, the difference between the two counting techniques fell below 3.5 log units. The screening method, therefore, would be suitable for herbs and spices irradiated at 10 kGy.

The effects of irradiation at 0.5, 5 and 10 kGy followed by ambient storage on the DEFT count and APC of black peppercorns (whole)

Figure 4a.

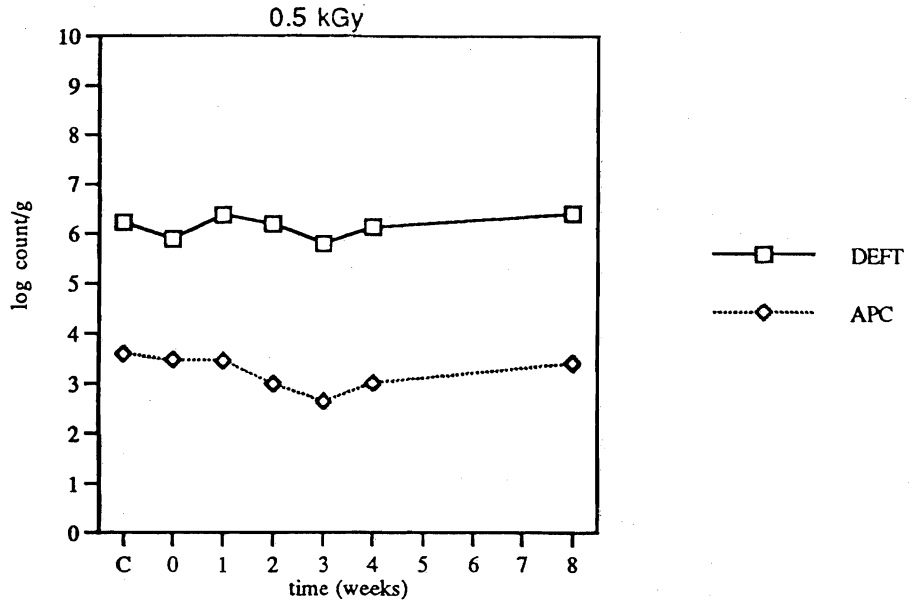


Figure 4b.

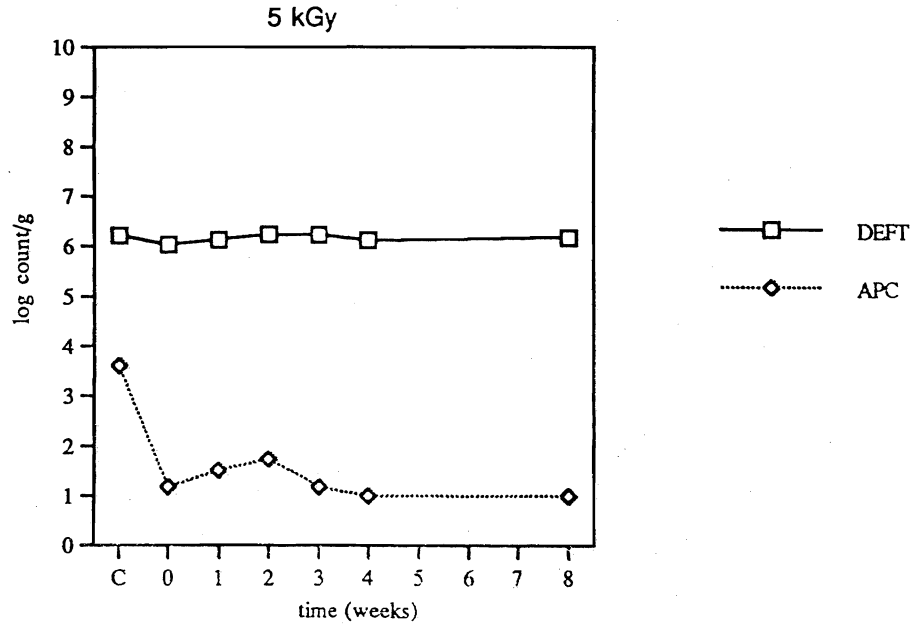
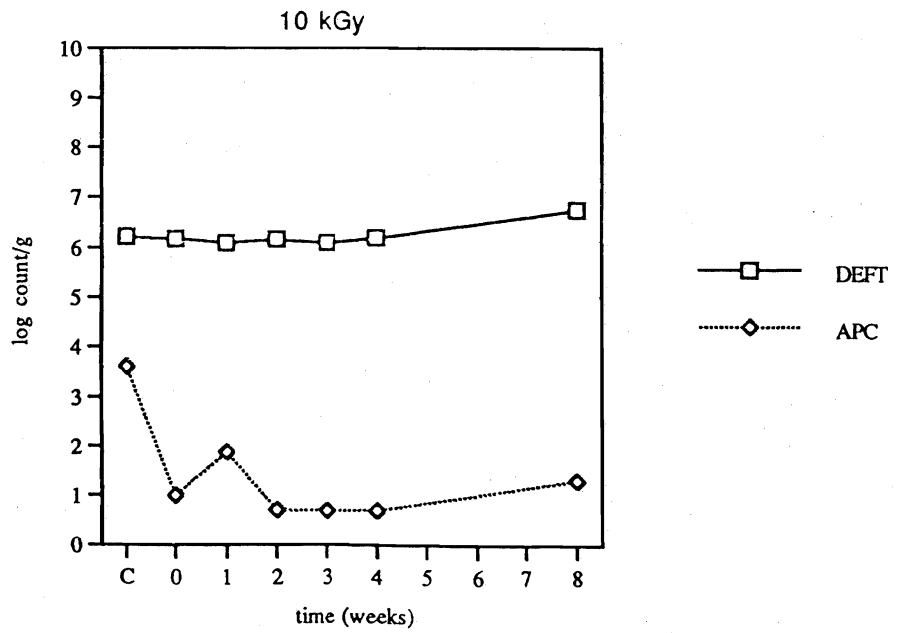
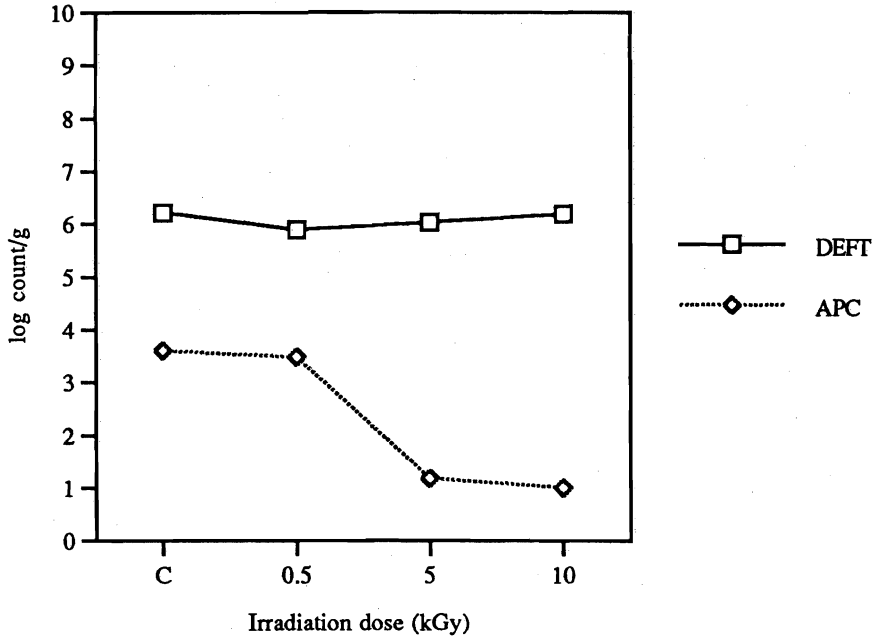


Figure 4c.



The effect of irradiation doses of 0.5, 5 and 10 kGy on the DEFT count and APC of black peppercorns (whole)

Figure 5.



Effects of Other Processes on DEFT/APC Count

High Pressure Treated Meat, Poultry, Fish and Seafood

Various foods were treated with high pressures in order to determine the effect of this procedure on the DEFT/APC differential count. If a high DEFT count, low APC were noted then it would be possible to mis-identify such a sample as having been irradiated.

High pressure is recognised as being useful for the purposes of processing and preservation of foods. The advantages of high pressure are that foods retain good organoleptic properties and nutrients, and the process can be considered to be cost effective and clean in comparison with the use of heat (Hayashi, 1992).

For more than a century it has been recognised that bacteria generally become inactive when they are placed under high pressure. During the past six years, research work has been concentrated on the use of high pressure as a food processing aid.

The resistance of microorganisms to high pressure is very variable. Gram positive bacteria are more resistant than Gram negative bacteria. Vegetative bacterial cells in the growth phase, together with yeasts and moulds, are perhaps the most sensitive microbial structures. Bacterial spores and viruses appear to have a high resistance to pressure (Cheftel, 1992).

There has been a limited amount of research on the effect of high pressure on the ultrastructure and metabolism of particular bacteria and little is known concerning the exact mechanisms of bacterial destruction. Various morphological changes have been observed such as compression of gas vacuoles, cell lengthening, separation of the cell membrane from the cell wall, contraction of the cell membrane, modification of the nucleus and of intracellular organelles and the release of intracellular material into the extracellular spaces.

In experiments reported here the meat, poultry, fish and seafood samples were treated with pressures of between 200-300 MPa for 5 min. This is within the 200-400 MPa range of pasteurisation treatments recommended for meats (Richard Earnshaw, Personal Communication).

The results for high pressure on the DEFT count and APC were more variable between samples then seen for irradiation. The counts obtained for minced beef and chicken, treated with 200 MPa and 300 MPa, are shown in Figures 6 and 7.

The effect of high pressure treatments of 200 and 300 MPa for 5 min on the DEFT count and APC of minced beef and chicken

Figure 6.

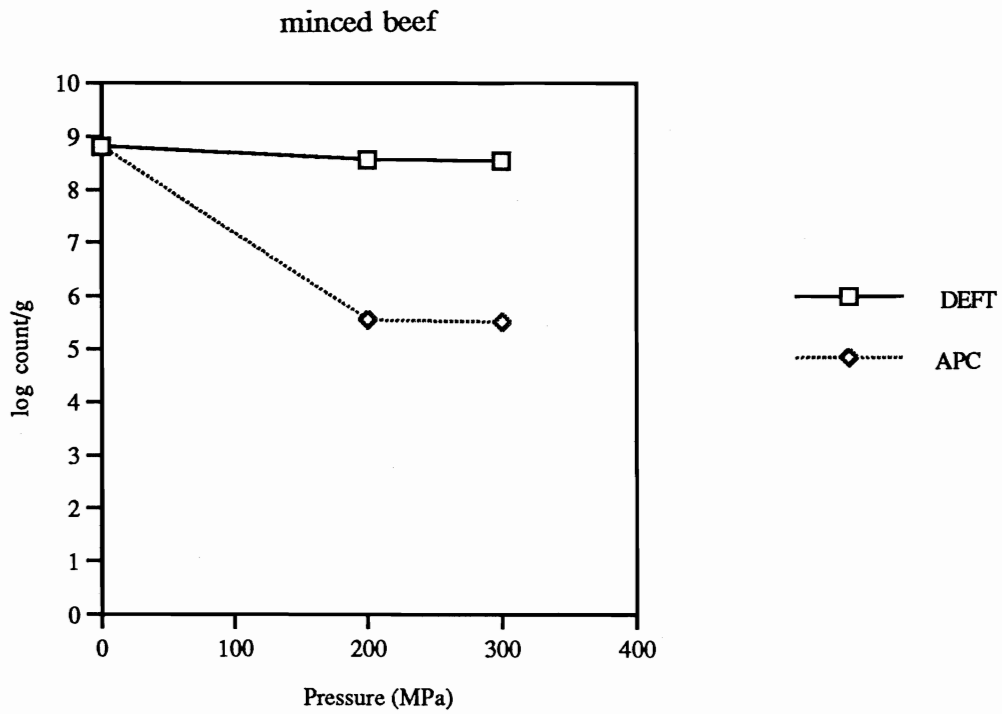
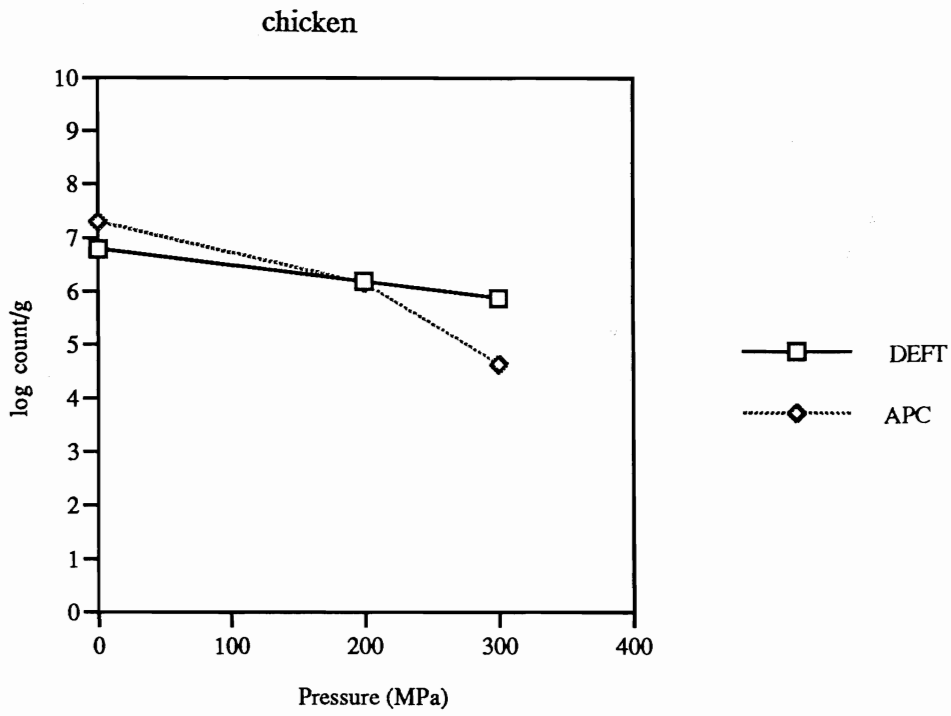


Figure 7.



In minced beef before treatment, the DEFT count and the APC showed an exact correlation of 8.81 log count/g. When subjected to pressure of 200 MPa the APC of the samples was reduced by 3.26 log units from 8.81 log count/g to 5.55 log count/g (Fig. 6). The corresponding DEFT count, however, only decreased slightly to 8.56 log count/g to give a difference between the counts of 2.99 log units.

The counts were not further reduced by an increased pressure treatment of 300 MPa. In this case the DEFT count was 8.54 log count/g and the APC was 5.51 log count/g. Further work using increasing pressures would be required to verify the kinetics of the pressure treatment on the APC of the sample.

After high pressure treatment the count from the DEFT showed a very slight decrease. It was noted that there was a higher number of green, unstained cells in the pressure treated samples when compared to the control samples. This was not seen in irradiated samples. It is possible that the high pressure treatment caused a change in the cell surface properties of the microbial cells that ultimately had an effect on the staining procedure. The acridine orange may have not been taken up by some of the cells, presumably due to loss of their structural intergrity.

At a pressure of 200 MPa and above, the difference between the DEFT count and the APC was between 2.99 - 3.01 log units. This log difference fits in with the criteria for the detection of irradiated foods using the DEFT/APC screening method, in that the difference was in excess of 2 log units. It would therefore be possible for high pressure treated minced beef to be identified as having potentially been irradiated when screened with the DEFT/APC method.

In chicken, the DEFT count and APC from untreated samples were 6.79 and 7.30 log count/g respectively. In chicken treated with a pressure of 200 MPa, the APC of the sample was reduced by 1.15 log units from 7.30 log count/g to 6.15 log count/g (Fig. 7). The DEFT count obtained after 200 MPa was reduced by 0.61 log count/g to 6.18 log count/g. The difference between the DEFT count and APC was 0.03 log units.

A greater reduction in viable count was noted when the pressure was increased to 300 MPa. The APC was reduced by a further 1.52 log units to 4.63 log count/g. The DEFT count at 300 MPa also showed a further decline and the count was reduced to 5.88 cell count/g. The difference between the DEFT count and APC was increased to 1.25 log units.

At high pressure treatments of up to 300 MPa, the maximum log difference between the DEFT count and APC was 1.25 log units due to decrease in both the DEFT counts and

APC after treatment. The results would not be confused with a sample having being irradiated.

The results of high pressure treatment on foods was much more variable than the effects of irradiation. Factors that need to be taken into consideration include the food structure, the protein content and type, ionic strength of the surrounding medium, water activity, temperature, compressibility of the food and local associated adiobatic heating. All of these factors can influence the effect of the pressure treatment on the food.

It is apparent that high pressure can also affect the staining properties of the remaining viable cells when analysed using the DEFT. After treatment in some food samples, the DEFT count decreased due to a reduction in the number of cells fluorescing after staining. This occurred in all the samples tested with high pressure, but the degree of the DEFT count reduction before and after treatment was dependent on the food type and pressure treatment.

The fact that the DEFT count was affected by the treatment added a further variable to experiments with high pressure treated food. From the data it can be seen that such a treatment has the capacity to cause a change in the DEFT count and APC, and can ultimately give results similar to those expected from irradiated foods.

Further work would be required to elucidate and verify the potential effects of high pressure treatment on foods.

Heat Treated Herbs and Spices

Fumigation as a method of decontamination for herbs and spices has been banned in many countries and heat treatment is now the most common method used for sterilisation of these samples.

Herbs and spices were initially treated at 80°C (dry heat) for 15 minutes to mimic a dry heat decontamination method. From the results it was apparent that the heat treatment had little effect on the viable count of the samples. With the exception of parsley the APC of all samples was reduced by less than 1 log unit compared to untreated samples.

In parsley, the APC was reduced by 1.68 log units by the heat treatment. The correlation between the DEFT count and APC of untreated parsley was shown to be poor and a 2.61 log unit difference between the DEFT count and APC occurred. Due to this difference in untreated samples, and the reduction in viable count caused by the heat treatment, the

difference between the DEFT/APC analysis was 4.30 log units. Parsley treated with dry heat at 80°C for 15 min, therefore, gave results similar to those expected from a sample irradiated at 5 kGy or more.

Due to the low decontamination levels achieved by the heat treatment, the difference between the DEFT count and APC remained less than 3.5 log units for all other samples tested. The results from the majority of the herbs and spices treated at 80°C for 15 min would not therefore give results similar to samples that had been irradiated at 5 kGy or higher.

Due to the limited effect of the heat treatment on the APC, the treatment time of herbs and spices at 80°C was extended to 60 min. As with the previous treatment, however, the viable count was not greatly affected by the heat process. The increased treatment time did not appear to be more effective at decontamination of the herbs and spices than the previous treatment.

The viable count in parsley appeared to be the most susceptible to the higher heat treatment, the APC being reduced by 1.99 log units. The 80°C heat treatment for 60 min, thus, had a slightly greater effect on parsley than that for 15 min which reduced the APC by 0.75 log units. Taking into consideration the 3.51 log unit difference between the DEFT count and APC of the untreated control, the difference between the counts after heat treatment was 5.45 log units. The results were therefore similar to those that could be expected from an irradiated sample.

In many cases the heat processes used here did not greatly decontaminate the herb and spice samples tested. Commercial processes for herb and spice decontamination will be more effective than laboratory methods; e.g. the Prima Pura method uses superheated steam for the sterilisation of products (Reimerink and den Uijl, 1992). Other methods are being developed such as use of a vacuum treatment which can be applied before steam treatment to maximise the penetration of steam (Grufstedt, 1990). The results from the current study, together with reports from other workers (Manninen and Sjöberg, 1991), indicate that the heat treatment of herbs and spices gives results that could mimic irradiation treatment, and it is thus necessary to confirm that a sample has been irradiated if a high DEFT count, low APC differential is obtained. The DEFT/APC procedure does however still provide an effective screening test for rapidly assessing whether samples could have been irradiated, as long as samples found positive are 'confirmed' using another test.

CONCLUSIONS

The DEFT/APC method is able to show the microbiological quality of the product at the time of analysis (APC count), and in addition it also provides information about the microbiological history of the product (DEFT count). Conclusive evidence of irradiation, however, relies on the knowledge that the sample had not undergone any prior process or treatment.

From the results it is apparent that the DEFT/APC screening method for the detection of irradiated foods is a qualitative rather than quantitative method. The dose of irradiation cannot be determined using the technique. The results of log count/g obtained immediately after irradiation at different dose levels indicate that the extent of cell death is not proportional to the dose given.

In cases where the DEFT/APC method has been used to indicate suspected food irradiation this should be confirmed by a second detection method such as thermoluminescence.

The DEFT/APC screening method is a cost effective, easy to use technique for the screening of a range of irradiated foods. It should be recognised, however, that the applicability of the method has limitations with different food types, particularly during storage as discussed. The DEFT/APC method has the potential to be used with stored irradiated meat, poultry, fish and seafood, particularly during frozen storage, and with ambient stored herbs and spices.

Herbs and spices have already been demonstrated to have been reliably detected using the DEFT/APC method in a recent BCR trial (Wirtanen *et al*, 1993). An interlaboratory trial using irradiated, stored meat, poultry, fish and seafood is required to demonstrate the reliability and accuracy of the screening method in realistic laboratory situations.

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