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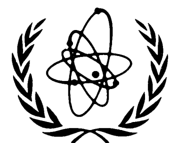
Radiation processing for safe, shelf-stable and ready-to-eat food

Proceedings of a final Research Co-ordination Meeting held in Montreal, Canada, 10–14 july 2000



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FOREWORD

The increasingly busy lifestyles of populations in many countries have driven the demand for safe, convenient and ready-to-eat food. Traditional food processes such as drying, canning or refrigeration offer a partial solution to this demand as the sensory quality of such food may be significantly affected or the products may be contaminated by pathogenic bacteria during preparation. For developing countries, safe shelf-stable food without the need for refrigeration would offer advantages. In addition, the increasing number of immuno-compromised populations in many countries requires a new approach to food safety to meet their needs.

Irradiation offers a potential to enhance microbiological safety and quality of food through shelf-life extension. The benefits of irradiation as a sanitary treatment of many types of food are well known, some of which are applied commercially in several countries. Little data were available, however, on the effect of irradiation on minimally processed food and composite food including prepared meals. A Co-ordinated Research Project (CRP) on the Development of Safe, Shelf-Stable and Ready-to-Eat Food through Radiation Processing therefore was implemented by the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture in 1996 to evaluate the role of irradiation for such food. The results were encouraging as irradiation offers promise as a sanitary treatment to ensure microbiological safety and shelf-life extension of several types of food products including pre-cut vegetables and some *sous-vide* meals, chilled ready-prepared meals, chilled ready-to-eat meat products, food for immuno-compromised patients/populations, sterile meals, ready-to-eat-food of intermediate moisture content.

This publication presents the research results reported at the final Research Co-ordiantion meeting on this CRP held in Saint Hyacinthe, Quebec, Canada, 10–14 July 2000.

E. Stewart of the Department of Food Science, the Queen’s University of Belfast, Northern Ireland assisted in finalizing this manuscript for publication.The IAEA officer responsible for it was P. Loaharanu of the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture.

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SUMMARY

1. INTRODUCTION

The widespread and increasing incidence in recent years of food-borne illness caused by pathogenic bacteria and parasites and the consequent social and economic impact on the human population have brought food safety to the forefront of public health concerns. Irradiation is widely recognised as an effective control measure for inactivating pathogenic bacteria and parasites from solid food, especially those which are eaten raw or minimally processed in the same manner which thermal pasteurisation has done successfully for liquid food, e.g. milk, fruit juices, etc. Small scale commercial application of irradiation to ensure hygienic quality of food of animal origin has been carried out in several countries including Belgium, France, China, Indonesia, the Netherlands, Thailand and the United States of America (USA) in the past two decades. Following the final approval of the US Food and Drug Administration (FDA) in 1997 and US Department of Agriculture (USDA)/Food Safety Inspection Service (FSIS) on the quality control program of irradiation of red meat in late 1999, large scale commercial application of irradiation of meat (mainly ground beef) commenced in the USA in mid–2000. Some 200 metric tonnes of ground beef per week have been irradiated by two industrial scale electron beam machines in the state of Iowa and marketed widely in some 250 supermarkets in several States of the USA in 2000. There appeared to be no consumer acceptance problem of any kind and irradiated meat was selling well. The market for irradiated beef has expanded significantly in 2001. As of June 2001, some 2000 supermarkets in 22 States in the USA are carrying irradiated ground beef on a routine basis.

Global production of irradiated food, while still small in quantities, is increasing steadily with some 250,000 metric tonnes being irradiated in 1999. The production of irradiated spices and dried vegetable seasonings to ensure their hygienic quality has increased significantly from 10,000 tonnes in 1990 to approximately 80,000 tonnes in 1999.

Irradiation is also increasingly recognised as an effective phytosanitary treatment especially against tephretid fruit flies, which can infest fresh fruits and vegetables from tropical and sub-tropical countries. Small scale commercial application of irradiation to control fruit flies in fruits from Hawaii has been successfully carried out in the US mainland since 1995. The USDA’s Animal and Plant Health Inspection Service (APHIS) published a Proposed Rule on Irradiation Phytosanitary Treatment of Imported Fruits and Vegetables in May 2000 which, when finalised, will open the USA market to irradiated commodities from other countries. A number of countries in Asia and the Pacific, especially ASEAN, have adopted a harmonised protocol on irradiation as a phytosanitary treatment for fruit fly control. In addition, the increasing pressure on global phasing out of methyl bromide, the most widely used fumigant for controlling insects in fresh and dried food, under the Montreal Protocol will have a positive impact on the application of irradiation as a phytosanitary treatment of many fresh and dried food commodities.

Data to demonstrate the effectiveness of irradiation as a sanitary and phytosanitary treatment for food is widely available on many individual food products, e.g. fruits, vegetables, grain, seafood, meat, potatoes, onions, spices, etc. Few data are available on the effect of irradiation on a more composite foods, minimally processed food and ready-prepared meals, especially those to be marketed at ambient temperature. Preliminary studies on irradiation of some of these types of food products conducted under the scope of the Co-ordinated Research Programme (CRP) on Combination Processes for Food Irradiation, which was finalised in 1995, showed that irradiation could offer potential benefits.

Therefore, a CRP on Development of Safe, Shelf-Stable and Ready-to-Eat Food through High-Dose Radiation Processing was initiated by the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, Vienna, in 1996. Special emphasis of the CRP was originally given to development of shelf-stable sterile food through high-dose irradiation (above 10 kGy) which could be kept without refrigeration for many months. However, it was soon realised that not many countries have the facilities and expertise to conduct experiments using high-dose irradiation of food.

Also, several types of composite food and ready-prepared meals, which require refrigeration for storage and distribution, could benefit from irradiation to ensure microbiological safety and shelf-life extension. The scope of the CRP was, therefore, slightly modified to “Development of Safe, Shelf-Stable and Ready-to-Eat Food through Radiation Processing”.

There are a number of factors which drive the demand for ready-to-eat composite food and ready-prepared meals with microbiological safety and enhanced shelf-life, both under refrigeration and at ambient temperature. In western countries and many developing countries, the busy lifestyle and changing social trends have resulted in less time for food preparation. There is an increasing production and marketing of convenience food throughout the world, mainly through freezing and frozen storage. There are technological and economic benefits for these products to be marketed either at chilled or ambient temperature. Irradiation and proper packaging could fulfil this requirement.

2. OBJECTIVES OF THE CRP

The CRP had the following specific objectives:

(1) Evaluate the use of irradiation to produce a variety of sterile, shelf-stable foods, either as individual food components or in combination as composite meals;

(2) Evaluate the acceptability of irradiated shelf-stable foods for use by specific target populations, e.g. hospital patients, immunocompromised (high-risk) individuals, pilgrims, certain sport/recreation activities, etc.;

(3) Evaluate the role of irradiation in combination with other processes to produce non-sterile shelf-stable foods (semi-dried meat, seafood, fruits, vegetables);

(4) Evaluate the role of irradiation on the microbiological safety and shelf-life extension of ready-prepared meals and composite foods, stored either at ambient or chilled conditions;

(5) Develop predictive microbiological models incorporating the effect of irradiation, and to encourage inclusion of such models into national and other databases; and

(6) Evaluate the effect of irradiation on various packaging materials required for shelf-stable, composite and ready-to-eat foods.

It was also agreed that good manufacturing practices (GMPs) which include Good Irradiation Practices (GIPs) and HACCP principles should be applied in any process employing irradiation to produce foods for consumption by the general public. Under GIP the following conditions for irradiation are required:

(1) Properly established dosimetry system;

(2) Temperature control before, during and after irradiation; and

(3) Appropriate packaging for specific products, e.g. fresh produce require gas penetrable packaging while sterile shelf-stable foods require impermeable packaging.

Applicable international standards documents, e.g. ASTM, Codex and ICGFI guidelines and appropriate national regulations should be consulted when establishing Standard Operating Procedures (SOPs).

3. ACHIEVEMENTS OF THE CRP

The achievements of work carried out under the scope of this CRP in the past 5 years may be summarised as follows:

3.1. Potential for food irradiation to improve the microbiological safety and quality of chilled convenience foods

3.1.1. Background

The demand for, and use of chilled convenience foods, by consumers is growing steadily. There is an increasing demand for foods which are fresh-like, or at least less drastically processed, even 'chef-like', which are either ready-to-eat or simply need to be reheated prior to consumption. In the United Kingdom (UK), e.g. the market for chilled ready meals that are cooked, stored chilled (0–3°C) and reheated has shown substantial growth, largely as a result of the consumer perception that chilled foods are closer to fresh than frozen foods. They are also perceived to be more nutritious. The reason for this growing market is due to a number of reasons. Firstly, the faster pace of life, which now exists, has resulted in less time being available to prepare foods. Thus, the availability of foods which require minimal processing in the home is necessary in order to save time. Secondly, in developed countries, in particular, greater affluence means that consumers are willing to pay more for convenience. The catering industry (hospitals, institutions, work canteens, etc.) is also interested in prepared, but minimally processed, non-frozen meals and meal components. Added advantages of such foods include less need for conventional kitchen space, equipment and personnel in food service operations and good palatability of the processed product. To satisfy these requirements the food industry has developed such groups of foods as minimally processed prepared vegetables, and chilled rather than frozen foods. In addition, a further extension of refrigerated shelf-life for prepared vegetables, chilled ready-prepared meals and ready-to-eat products is desirable for marketing and widespread distribution of products.

With regard to cook-chill ready meals, the main problems and limitations associated with cook-chill ready meals include a relatively short shelf-life (maximum of 5 days at 0–3°C, including the days of production and consumption), concerns about microbiological safety, a reduced sensory quality and a decreased nutritive value.

Regarding minimally processed prepared vegetables, chilling, often in combination with modified atmosphere packaging (MAP), may extend the shelf-life of peeled/pre-cooked produce. However, this technology can, potentially, involve a microbiological hazard due to the growth of pyschrotrophic, pathogenic bacteria frequently associated with fresh produce or as a result of contamination before packaging. These bacteria are not significantly inhibited by refrigeration and are able to multiply in the minimally processed, extended shelf-life products, especially due to lack of competition from spoilage microflora. Similar microbiological hazards may occur in chilled prepared meals due to possible contamination before packaging. In the complete meals a cross-contamination between components, e.g. contamination of meat from the vegetables, may produce a better growth environment for certain bacteria which otherwise will remain in a non-active stage. MAP may result in an extension of refrigerated shelf-life, but in itself MAP does not significantly reduce the numbers of psychroptrophic pathogens, and in some cases may even facilitate their growth.

In all cases, there is always a risk of temperature abuse during distribution, retailing or with the end user.

3.1.2. Objectives

To evaluate the role of irradiation to improve microbiological safety and extend shelf-life while maintaining nutritional quality of minimally processed fresh products or prepared meals and composite foods stored under chill conditions.

3.1.3. Rationale for using irradiation

The use of ionizing radiation at relatively low-doses may play a role in minimising the risks outlined previously. The technology could provide an additional benefit by giving an increased shelf-life of certain products without compromising their quality and safety. These benefits are offered by the process because:

- irradiation can be used to improve the microbiological quality without significantly affecting the physical state of the products;
- irradiation treatment of packaged foods avoids the risk of recontamination;
- most of the psychrotrophic microorganisms of importance to the microbiological safety or keeping quality of chilled foods are relatively sensitive to irradiation; and
- the incidence and contamination levels of pathogenic bacteria are usually relatively low.

Because of the benefits of the process, irradiation can potentially facilitate the availability of food for immunocompromised hospital patients and populations, which is safe to eat, and of acceptable nutritional and organoleptic quality. As food irradiation is a ‘cold process’ the treated food is close to the natural state both in appearance and taste unlike other processes involving heat treatment which may lead to unacceptable changes in the food. The use of food irradiation can give the consumer a greater choice as to the range of foods available on the market and may, e.g. ensure that seasonal vegetables are available all year round. It can also be used to reduce food spoilage and wastage and, therefore, is of economic benefit to food producers, retailers and consumers alike. Reduction in food wastage is of major benefit to developing countries where a significant proportion of food is destroyed annually due to spoilage.

It is, however, important that irradiation be used as an additional preservation hurdle rather than as a necessity due to lack of GMP. It would also be desirable to have predictive models for inactivation of pathogenic bacteria in food by irradiation and other inhibiting factors to facilitate the work on microbiological safety of chilled convenience foods.

3.1.4. Achievements to date

3.1.4.1. Fresh produce such as pre-packed, prepared vegetables and some sous-vide meals

Various aerobically packaged pre-cut vegetables, heavily inoculated with *Listeria monocytogenes*, were irradiated with 1 kGy of gamma rays without changes in the sensory quality of the product. This low-dose was able to improve the microbiological safety and stability of the refrigerated products stored at various temperatures. The irradiation treatment diminished remarkably the population of *L. monocytogenes*. The growth temperature requirement of its surviving cells also increased. Losses of the vitamin C content as a direct effect of low-dose irradiation proved to be not higher than those which occurred in the non-irradiated samples during their shelf-life. The studies underlined the importance of good temperature control in the chill chain. Package conditions of minimally processed, low-dose irradiated chilled vegetables must be aerobic enough to prevent growth of non-proteolytic *Clostridium botulinum*.

Experimental batches of prepared meals such as chopped beef in tomato sauce, chopped beef in paprika gravy, and smoked-cured pork in stewed beans sauce were inoculated with spores of psychrotrophic *Bacillus* spores, more heat- and radiation-resistant than spores of non-proteolytic *C. botulinum*. After vacuum packaging, the meals were treated with combinations of pasteurising heat treatments and gamma irradiation in the dose range of 2.5 to 5 kGy. Prior to and after treatments, and periodically during storage at 10°C, total aerobic and total anaerobic viable cell counts, and selectively, the viable cell counts of *Bacillus cereus* and sulphite-reducing clostridia were determined.

The effects of the treatment order as well as addition of nisin to enhance the preservative efficiency of the physical treatments were also studied. Sensory testing with uninoculated samples proved that the combination-preserved meals were of acceptable quality at the beginning of their storage. The microbiological investigations showed that the sous-vide cooking, in combination with medium-dose gamma irradiation and/or nisin addition, may increase considerably the microbiological safety and keeping quality of the meals studied.

3.1.4.2. Chilled ready-prepared meals

For the purposes of this research ready meals were obtained from a catering production unit which produces 45,000 chilled meals per week for hospitals, day care centres, residential homes for the elderly, etc. Meals consisting of roast pork, gravy, mixed vegetables (green beans and garden peas) and boiled potatoes were obtained and either irradiated (1, 2 or 3 kGy) or left unirradiated. The meals were stored at either 3°C or 10°C and sampled at intervals for up to 15 days for microbiological quality. The meals were either analysed without further heating or were reheated. A dose of 2 kGy was found to be sufficient to extend the microbiological shelf-life of the meals by at least 7 days. Storage temperature significantly affected the growth of surviving microorganisms and it was shown that numbers increased rapidly at 10°C compared to 3°C. It was, therefore, concluded that the irradiated meals need to be stored under good refrigeration (0–3°C) in order to achieve the maximum benefit from the irradiation treatment. Vitamin analysis was also carried out on the above meals. The pork was analysed for thiamine (vitamin B_1) while the mixed vegetables and potatoes were analysed for ascorbic acid (vitamin C). Irradiation significantly affected the vitamin content of the meal components but was found to be no more detrimental than cooking or storage both of which resulted in a diminution in concentration. It was also worthy of note that the overall vitamin content of the meals investigated was low prior to irradiation and some consideration should, therefore, be given to vitamin supplementation.

The effect of ionizing radiation on the nutritional and microbiological quality of ready prepared pureed meal components was also studied. Such meals are used for individuals with digestion problems. Pureed chicken and gravy, gardens peas and potatoes were treated in a similar fashion to the meals described previously and analysed for total viable counts (TVCs), thiamine (chicken and gravy) and ascorbic acid (peas and potatoes). Results from TVCs showed that irradiation at a dose of 2 or 3 kGy followed by storage at 3°C was most appropriate for achieving shelf-life extension. In the case of the pureed chicken and gravy, irradiation dose significantly affected thiamine content while storage and cooking had no significant effect. The difference in concentration of thiamine for 2 kGy samples compared to non-irradiated controls was approximately 13%. Irradiation, cooking and storage all resulted in a diminution in ascorbic acid content of the pureed peas and potatoes. It should, however, be noted that these pureed meals were of poorer nutritional quality prior to irradiation than the meals previously tested.

In order to determine if irradiation treatment of chilled ready meals could be detected, samples of pureed chicken and gravy given 1, 3 or 5 kGy or left unirradiated were analysed for 2-alklycyclobutanones (European Standard EN1785) while the packaging was analysed for irradiation treatment by ESR spectroscopy (European Standard EN1788). The cyclobutanones were readily detected in irradiated chicken and gravy and were absent in unirradiated samples. The characteristic ESR signal for cellulose was detected in the cardboard packaging of the irradiated meals being absent in unirradiated samples. It was, therefore, concluded that these validated detection methods can be readily used for the identification of irradiated chilled ready-meals.

3.1.4.3. Chilled ready-to-eat meat products

Smoked sausage

In Thailand, smoked sausages were packed in normal packaging with low density polyethylene (LDPE) pouches for control samples, vacuum packaging in nylon polyethylene ionomer resin (NPI)

plastic pouches and MAP in laminated films of polyvinylidene chloride-coated nylon and polyethylene (PVNP). All samples for vacuum packaging were vacuum-sealed by a LAPACK 450 while those for MAP were flushed with 20% CO_2 and 80% N_2. Sausage samples were treated with doses of 0, 2 or 4 kGy, stored in chilled room (5 ± 2°C) and then taken every week for quality evaluation.

Results showed that by using irradiation combined with packaging, the shelf-life of smoked sausages was extended from six weeks to more than two months with the minimum dose of 2 kGy. By using MAP, bacterial growth was hindered and shelf-life was extended to a period of time similar to vacuum packaging although it was one week longer. The initial bacterial count was reduced by 28.5% and 68.9% for the application of 2 kGy in vacuum packaging and MAP samples, respectively. A decrease of 71.1% in bacterial density was found in vacuum-packaged sample with a dose of 4 kGy dose. The moisture content of products was in the range of 58.4–63.3%. For drip loss, between vacuum and MAP, smoked sausages packed in vacuum resulted in a higher loss than MAP but those in normal packaging with LDPE gave the greatest loss of 2.9%. Smoked sausages exhibited promising values for the colour attributes although sausages packaged in LDPE pouches, both in vacuum and modified atmosphere, had lower redness values when compared with the other treatments. Packaging in vacuum produced a more stable colour quality than MAP while the application of irradiation at 2 and 4 kGy created some fluctuations in the intensity of the different colour attributes. For texture, vacuum-packed sausages treated with 2 kGy exhibited higher hardness values than those treated with 4 kGy dose and all unirradiated samples.

Sliced ham

A study was also undertaken on the shelf-life extension of sliced ham by using a combination of irradiation with normal packaging material (LDPE). Sliced ham (100 g) was packed in flexible pouches of NPI at a vacuum of 90 psi. Samples were irradiated at doses of 0, 2, 4 or 6 kGy and then stored in a chill room (2 ± 2°C) prior to quality determination.

The results showed that the samples with normal packaging and without irradiation had a bacterial count of 1.8×10^4 CFU/g at the beginning of storage and increased to 4.6×10^6 CFU/g on week 4 and were spoiled by week 7. Irradiation was very effective in the reduction of microorganisms. The significant decrease was shown especially at the 6 kGy dose.

3.1.4.4. Food for immunocompromised patients/populations

A number of meals that could benefit from irradiation treatment were identified among diets recommended by nutritionists in Argentina, for different categories of immunocompromised patients. The irradiation treatment employed for this purpose should not necessarily render sterile products, but result in low-microbe, pathogen free meals.

Vegetable salad

Fresh raw meals of high nutritional value, considering their vitamin and mineral content, like vegetable and fruits salads, are generally not included in the diet of immunocompromised patients because microbial contamination is greater than the limits that can be tolerated. These meals can also offer the patient a variation from routine intake consisting of cooked and even overcooked, colourless foodstuffs, prepared according to instructions to avoid microbial risks. Ionizing radiation could be a useful tool for attaining microbial decontamination without altering the fresh condition of such products.

Carrot and tomato salads, packed in polypropylene trays wrapped with PVC (polyvinylchloride) film, the method usually employed by vegetables processors to extend shelf-life, were irradiated with minimum doses of 2 and 4 kGy. Storage conditions were 2 ± 1°C and 70 ± 5% relative humidity (RH). In the control salad samples, total bacterial counts (TBCs) were about 10^6 CFU/g, which was well above the limits permitted in a “clean diet”. Mould and yeast counts were about10^3 CFU/g. Coliforms

were also present. In 2 kGy samples, this bioburden was adequately reduced by about 3 log cycles, and coliforms could not be detected. No significant sensory differences were observed between control and irradiated salad samples. Scores were always above the acceptability threshold for a one week period, which would be adequate for kitchen hospital provision.

Fruit salad in gelatine

Apples, pears, bananas, cut into pieces, soaked in fresh orange juice and afterwards covered with gelatine, were packed into polypropylene trays with lids, irradiated with a minimum dose of 2 or 4 kGy, and stored at 2 ± 1°C and 70 ± 5% RH.

In control samples, the procedures followed during preparation (low acidity along with the temperature of the liquid gelatine being 50°C) most likely led to the low microbial load observed with TBCs and moulds and yeasts being 10^2 CFU/g. Total and faecal coliforms were absent. However, the yeast and mould bioburden could be of concern in relation to diarrhoea production. Considering this, irradiation at a dose of 2 kGy could be useful, as it reduced mould and yeast counts to less than 50 CFU/g. This is of importance mainly if the product could be stored for periods longer than a week, in supermarkets for instance.

Every fruit salad was of acceptable sensory quality following one week of storage. The only significant differences were in colour as irradiation destroyed to some extent the artificial red and yellow pigments in the gelatine, which also influenced the aspect at the 4 kGy dose but not at 2 kGy.

Vanilla ice-cream

Ice cream was included in the list of foods that could benefit from irradiation treatment not only for its nutritional value but also to serve a psychological purpose, as a 'tit-bit', to raise spirits and enhance appetite. This is especially important when immunocompromised patients are children or the elderly.

Vanilla ice cream packed in polystyrene foam boxes was treated at minimum irradiation doses of 3, 6 and 9 kGy, and stored at –20 ± 3°C.

The ice cream control samples fulfilled the microbiological requirements of the national legislation (Argentine Alimentary Code) regarding TBCs being 10^5 CFU/g for milk ice creams. Coliforms were, however, present in the samples. The 3 kGy dose was sufficient to attain an acceptable microbiological condition, reducing TBCs by 3 log cycles and destroying coliforms to undetectable levels. Using a dose of 6 kGy, counts were close to those of sterility. This condition was maintained for at least a 2 month storage period.

Regarding sensory evaluation, significantly lower values were only found in colour, flavour and general acceptability for 9 kGy samples, which was found to be an evident over-dose. Lower dose samples were acceptable throughout storage. A greasy, waxy after-taste in the 6 and 9 kGy samples was occasionally noticed. Therefore, chemical fat analysis was carried out. No significant differences could be found between control and irradiated ice cream fat samples using acid and peroxide values, thin layer chromatography or ultraviolet spectra absorption patterns. Absorbance in the visible region showed carotenoid destruction to some extent, in proportion to the applied dose, which was also noticed by the sensory panel.

"Cannelloni" (Fresh stuffed pasta)

This product consisted of kneaded wheat flour pasta rolled over a cylindrical filling made of casein, egg, salt and nutmeg. Samples were packaged into polypropylene boxes with a lid, suitable for microwave cooking. Fresh tomatoes were liquidized in a domestic blender and used as a sauce covering the whole of the pasta mass, to avoid superficial desiccation upon microwave cooking. Samples were irradiated at minimum doses of 2 or 4 kGy and afterwards stored at 4 ± 2°C.

Although there are no national microbiological standards available for food destined for immunosuppressed patients, counts observed in control and even in 2 kGy samples were considered excessive to fit a "clean diet": TBCs were from 10^6 to 10^8 CFU/g and mould and yeast counts 10^2 to 10^3 CFU/g with total and faecal coliforms also being present. On the contrary, counts corresponding to the 4 kGy dose were found to be acceptable, with a 4 log cycle reduction in the TBC, moulds and yeasts, and coliforms being absent.

After microwave cooking, no survivors were detected in control samples. However, the irradiation treatment justifies considering that temperature can vary during the cooking process. So, it appears advisable to offer a meal microbiologically safe already in the raw state, so as to avoid the risk of under-cooking.

Although no significant differences in sensory quality were observed between the control and irradiated fresh stuffed pasta, the latter were generally more preferred in flavour and general acceptability.

3.1.4.5. Production and application of edible films and coating in relation to irradiation preservation of convenience foods

The combined effect of gamma irradiation and antimicrobial coating on the shelf-life of pre-cooked shrimp (*Penaeus spp.*) and oven-ready all dressed pizza was investigated. Antimicrobial coatings were obtained by incorporating various concentrations of thyme oil and trans-cinnamaldehyde in protein solutions prepared from soy and whey protein isolates. Coated shrimps and pizza were stored at 4°C under aerobic conditions and evaluated periodically for total aerobic plate counts (APCs) and *Pseudomonas putida*. Sensory evaluation for appearance, odour and taste was also performed.

Pre-cooked shrimp

Results for pre-cooked shrimp showed a significant ($p \leq 0.05$) synergistic effect of gamma irradiation and coating in reducing the APCs and *P. putida* with at least 12 days for shrimps and over 18 days for pizza extension of shelf-life. The irradiation process resulted in a significant ($p \leq 0.05$) increase of lag periods before initiation of bacterial growth. For both uncoated and coated samples no viable colony forming unit was detected during the first 7 days of storage. Furthermore, at all the sampling days, total APCs in irradiated samples were significantly ($p \leq 0.05$) lower than corresponding unirradiated samples. Similarly, growth of *P. putida* increased significantly to reach maximum values of 10.8 to 12.2 CFU/g after 21 days of storage. Data indicated that without irradiation, the limit of acceptability was reached after 9 days for uncoated and samples coated with the base solution, and 14 days for those coated with a protein solution containing a mixture of thyme oil and trans-cinnamaldehyde (0.9 and 1.8%, respectively). With irradiation, the limits of acceptability ranged from 14 to more than 21 days, corresponding to a shelf-life extension of 5 to more than 12 days.

Pizza

Gamma irradiation of pizzas alone produced a 2 to 3.5 log unit reduction of APCs depending on the dose. Furthermore, growth rates during storage were significantly reduced ($p < 0.05$). Shelf-life periods obtained were 3 days for non-irradiated samples compared to 12 and 14 days for samples irradiated at 1 and 2 kGy, respectively. Combining irradiation with antimicrobial coating resulted in a synergistic inhibitory effect. Indeed, the shelf-life periods were extended to 21 days for coated samples irradiated at 1 kGy and more than 21 days for those irradiated at 2 kGy.

In conclusion, a significant synergistic effect of gamma irradiation and antimicrobial coating in reducing the growth of bacteria in peeled shrimp and refrigerated pizzas was observed. Shelf-life was extended by 5 days for shrimps and by 10–16 days for pizza with gamma irradiation. Gamma irradiation in combination with a protein-based coating extended the shelf-life by 11 days for shrimps and by over 18 days for pizza. However, concentrations used in the coating solutions were found to

affect odour and taste. Further work is necessary to optimise the hurdle antimicrobial effect provided by active compounds present in essential oils.

3.1.4.6. Development of predictive models for inactivation of pathogenic bacteria in food by irradiation and other inhibiting factors

Several interactive predictive models for the effects of irradiation on food-borne pathogens and normal flora in different food products stored at refrigeration and ambient temperature (from −76°C to +20°C) were developed, according to the following:

- *Salmonella typhimurium* ATCC 14028 on sterile, mechanically deboned chicken meat;
- *S. typhimurium* ATCC 14028 on mechanically deboned chicken meat or drumsticks;
- Indigenous microflora on chicken drumsticks;
- *Escherichia coli* O157:H7 on ground beef;
- *B. cereus* ATCC 33018 on ground beef;
- *L. monocytogenes* on beef; and
- *Staphylococcus aureus* on ground beef.

3.1.5. Recommendations

As the products described previously are not shelf-stable, proper refrigeration is essential to maintain the safety and quality. Research programmes should include shelf-life evaluation at both trial and abuse temperatures (the worst case scenario) to ensure that the process does not introduce an additional risk to microbiological safety. It is important to note that low-dose irradiation does not eliminate spores of *C. botulinum* or other pathogenic spore formers.

There are databases, available in several countries, on predictive modelling of growth, survival and inactivation of microorganisms as a function of various treatments and environmental stress factors. It would be worthwhile to use these existing models and those that have been newly developed to assist in the design of treatments for food irradiation applications in context with both shelf-stable and chilled convenience foods.

During production of ready prepared meals, an integrated approach is needed (multi-disciplinary work to include nutritional and sensory quality) along with consideration of the whole food chain.

Technological feasibility may depend on the sensitivity/suitability of various vegetable items used for the irradiation treatment even at the lower dose ranges. A literature survey is needed to identify potentially suitable products and to highlight products which still need to be investigated.

Nutritional quality, e.g. vitamins and pro-vitamins, of the products during post-processing storage needs to be monitored.

Dairy and other protein edible films can be used for shelf-life extension and nutrient stability of chilled convenience foods. It is possible to incorporate active compounds into the edible films for controlled release during storage.

(7) Future work needs to be carried out on the various chilled ready prepared and ready-to-eat meat products. This can be summarised as follows:

From a microbiology point of view, performance criteria need to be agreed on and the equivalency of various treatments need to be investigated in order to be able to develop process criteria for new combination treatment of foods, e.g. irradiation and sous-vide treated foods.

Further work is necessary to establish the effect of irradiation, storage and reheating on the microbiological, nutritional and sensory quality of a greater variety chilled ready meals.

Regarding food for immunocompromised patients, the scope of this work needs to be widened to look at a greater range of food products as well as investigating the use of irradiation in combination with other processes, e.g. coating with edible films. There is need to inform medical professionals dealing with such patients of the benefit of irradiation.

Further studies on edible films should involve the evaluation of the effectiveness of using combination treatments (active and bioactive compounds, edible cross-linked coating, acidification, reduction of water activity, etc.) with irradiation to extend the shelf-life and assure the innocuity of fresh food (meat, fish, shrimp).

3.2. Development of shelf-stable foods

3.2.1. Background

The demand for safe, convenient and shelf-stable foods is increasing world-wide due to rapid urbanisation and change in socio-economic status. Commercially available high moisture products are currently preserved by conventional methods such as canning or freezing. Drying is also used as a preservation technique, but has detrimental effects on several quality parameters.

Canning can result in diminished nutritional and sensory quality of foods. Freezing maintains sensory and nutritional quality, but production and storage of frozen foods has high-energy demands. In addition, frozen foods are not necessarily safe microbiologically. For developing countries, improved food security (the sufficient availability of affordable nutritious food at all times) requires shelf-stable food without the need for the cold chain. Development of safe, shelf-stable foods at ambient temperature is necessary to reduce the dependency on refrigeration facilities during storage, distribution and marketing of perishable food products, which are relatively very costly for developing countries. There is a need for the development of alternative technologies to overcome some problems of heat sterilisation or freezing and to provide shelf-stability at a lower energy demand than required by frozen products. A non-thermal technology that is able to effectively inactivate bacterial spores is of particular importance because of better end product quality.

3.2.2. Objectives

To investigate how ionizing radiation could satisfy the above requirements, eventually in combination with other appropriate treatments or hurdles to develop safe, shelf-stable foods.

3.2.3. Rationale for using irradiation

Important characteristics of food irradiation which makes it a valuable tool in this context are, for example:

Nutritional as well as sensory quality is generally less severely affected by irradiation than by heat treatment.

Ionizing irradiation can inactivate even heat-resistant bacterial spores.

Radiation processing requires less energy (therefore, it is more energy economical) than freezing and cold storage.

There are already established irradiation processes developed in countries such as the USA and South Africa for high-dose application, but these still need further optimisation in terms of quality and an increased product assortment.

The population of immunocompromised patients is increasing world-wide and they have an increased health-risk when exposed even to normal microbial loads in food products. Products sterilised using ionizing radiation can provide a variety of foods for such individuals.

Irradiation is an excellent agent for use in combination with other technologies providing efficient hurdles for cost-effective preservation in order to produce shelf-stable foods.

Irradiation has the potential either alone or in combination with other technologies, to produce a variety of shelf-stable products with improved microbiological safety without changing the sensory properties of the food.

Shelf-stable intermediate moisture foods may still be contaminated with food-borne pathogens; irradiation can improve the microbiological safety of these foods by inactivation of the pathogens.

Irradiation treatment may offer opportunities to extend the range of shelf-stable convenience foods which could not be prepared otherwise (e.g. if the food is sensitive to heating).

3.2.4. Achievements to date

3.2.4.1. Shelf-stable sterile products

Vacuum packed (in laminated pouches) fish-based and chicken-based ethnic dishes called pepes and other chicken products such as semur, opor and curry have been successfully developed in Indonesia as sterile shelf-stable foods using sterilisation treatments at cryogenic temperatures with a minimum dose of 45 kGy. Such products could be stored for at least 18 months without refrigeration and without significant changes in their quality.

A number of safe shelf-stable sterile meat products, the sensory quality of which is acceptable, have been developed in India. These are chicken chilli, kofta, loaf, salami and sausages, mutton chilli, mutton kebab, and pork products such as pork salami, hamburgers, luncheon meat and bacon. In order to achieve commercial sterilisation, the process involved steaming the products to inactivate autolytic enzymes, vacuum packaging to reduce lipid oxidation and irradiation at a minimum dose of 45 kGy at a temperature of –50°C.

Vacuum packaged shelf-stable high moisture meat and poultry dishes have been developed in South Africa using a minimum dose of 45 kGy and irradiating at temperatures between –40°C and –20°C. This dose was determined using *C. sporogenes* inoculated pack studies. The maintenance of the product temperature during irradiation between –40°C and –20°C can be achieved using dry ice and a polystyrene separator between the ice and the product in regular tote boxes. These systems are facility specific. It was also shown that other factors related to the quality of the raw material, such as the selection of the breed of cattle in the case of irradiated roast beef products, have a significant influence on the end product quality in terms of tenderness, flavour and other sensory properties. The tenderness and juiciness of roast beef slices sterilised at 45 kGy (in vacuum laminated pouches at –40°C to –20°C) can be optimised by the pre-treatment of the meat with polyphosphates and sodium chloride injection. Roast beef slices with a shelf-life of more than 24 months at ambient temperature of 24°C (in Pretoria, South Africa) were successfully produced.

3.2.4.2. Shelf-stable non-sterile products

Application of gamma irradiation (at a minimum dose of 10 kGy) in combination with reduced water activity (a_w of 0.86) and vacuum packaging resulted in shelf-stable and microbiologically safe intermediate moisture meat (buffalo, chicken and lamb) cubes. Ready-to-use shelf-stable mutton and chicken sheek kababs, mutton and chicken chilli were developed by reducing the a_w (≤ 0.85) either by grilling, or by hot air drying, vacuum packaging and irradiation. Microbiological analysis revealed a dose-dependent reduction in TVCs and in potentially pathogenic *Staphylococcus spp*. upon irradiation treatment (2.5, 5 and 10 kGy). The products subjected to irradiation at 10 kGy showed absence of viable microorganisms and also had a high sensory acceptability up to 9 months at ambient temperature.

3.2.5. Recommendations

(1) Further work is required with regard to challenge studies with pathogens in order to confirm the safety of irradiated intermediate moisture foods.

(2) A database for the inactivation of specific pathogens by combination treatments has to be developed for shelf-stable foods which can allow predictive modelling and diminished demands for challenge testing.

(3) Shelf-stable irradiated foods with a high lipid content tend to become rancid after the application of sterilising doses. Therefore, such foods must be optimised regarding the formulation (for instance, the use of appropriate antioxidants) and the selection of proper packaging materials.

(4) Products requiring texturising or thickening ingredients, such as starch, were found to be less stable after irradiation in terms of sensory acceptability and texture properties. Therefore, to increase the variety of shelf-stable irradiated products, more information on the modelling and assessment of combination treatments is necessary, especially in order to introduce cereal-based (containing starch) and vegetarian-style shelf-stable foods. Vegetable and fruit-based shelf-stable foods or foods containing a mixture thereof (e.g. sauces with meat, fish or chicken) could also be produced using these combination treatments.

(5) Certain unique products (e.g. semi-dried meats, baked products and pizzas) that cannot be heat treated to achieve sterility will have to be made shelf-stable by combination treatments such as water activity reduction, vacuum packaging and irradiation. The process for achieving the stability of such products needs to be evaluated.

3.3. Improving the shelf-life and microbiological quality of ready-to-eat food of intermediate moisture content stored at ambient temperature

3.3.1. Background

Different types of ready-to-eat food including semi-dried meat, semi-dried shrimp, semi-dried sausages, semi-dried vegetables and bean curd, are popular products in many countries. These products are prepared according to traditional recipes and processes and often include high amounts of chemical preservatives. They are normally produced in rural areas by cottage industry and marketed at ambient temperature. Also, the products are normally contaminated by spoilage microflora and possibly pathogenic bacteria. These products, although in high demand by the local population, are not standardised in terms of processing. Therefore, they are either too dry or too salty to ensure the keeping quality of the products or require additional hurdle/treatment to ensure microbiological safety. There is also a need to improve the palatability of these products through less drying and less preservatives while maintaining their safety. Such improvements may be achieved by treating the products with irradiation.

3.3.2. Objectives

To investigate the role of irradiation to ensure microbiological safety and shelf-life extension of semi-dried, ready-to-eat traditional food products marketed at ambient temperature.

3.3.3. Rationale for using irradiation

No other technology (heat, chemical preservatives, refrigeration) has been found to offer a solution at a reasonable cost to improve the palatability, microbiological safety and shelf-life of these traditional products which are marketed at ambient temperature.

Ionizing radiation in combination with other technologies can be used to develop processes that will result in increased product shelf-life if the synergistic effect of the combined technologies on the destruction of microorganisms is optimised without significant detrimental effects on product quality.

3.3.4. Achievements to date

3.3.4.1. Semi-dried pork

Irradiation of cooked, ready-to-eat semi-dried pork, with an approximate moisture content of 18% with chemical preservative and packaged under vacuum, with a dose up to 6 kGy resulted in no microbial activity even at the end of the experimental period of 8 weeks. The moisture content of the product was in the range of 15.4–18.3%. In this experimental work, an increase in rancidity was observed, but sensory analysis did not find any significant difference. There was some change at the end of 8 months of storage, mainly due to the rancidity of the product.

Experiments on semi-dried pork with irradiated spice and no chemical preservatives, but with increased moisture content up to 25%, were also conducted in an attempt to improve palatability. The moisture content of finished products was varied to three levels of 18, 22 and 25%. Spice used as the main ingredient was irradiated at 4 kGy before addition to products. All samples were packed in PVNP pouches under modified atmospheric conditions with 20% CO_2 and 80% N_2. Semi-dried pork samples made with irradiated and non-irradiated spice were irradiated at 6 kGy and stored at ambient temperature.

The experiment showed that irradiation treatment had an effect on the total bacterial count. Considering the effect of irradiating spice on semi-dried pork products, spice treated with irradiation had a lower microbial load and thus the shelf-life of products could be extended. However, the effect of spice irradiation was not significant, when irradiation was applied afterwards to semi-dried pork products. From these results, the application of irradiation (6 kGy) reduced the bacterial population significantly, hence, prolonging the shelf-life of treated semi-dried pork to 5 months or more.

3.3.4.2. Semi-dried shrimp

Semi-dried shrimp (*Penaeus* spp.) was prepared for better palatability with higher moisture content than commercial products by reducing drying time. The final moisture content of semi-dried shrimp was around 36% compared to 20–25% of dried shrimp sold in markets. The increase of moisture content would cause semi-dried shrimp to be more susceptible to spoilage. Water activity (a_w) of the products were 0.67–0.7. Samples were irradiated at 0, 2 and 4 kGy and kept at ambient condition for quality analysis.

During storage for 7 weeks, total plate and mould counts of the dried shrimp of not more than 5 and 2.3 $\log_{10}$ CFU/g were considered as the shelf-life index. For fungal count, control samples were spoiled following approximately 3 weeks of storage while the shelf-life of 2 and 4 kGy irradiated samples was 5 weeks and more than 7 weeks, respectively. Consequently, panelists rejected the control product after 10 days of storage, while the 2 and 4 kGy irradiated samples were acceptable following 5 and 7 weeks of storage. The irradiated samples showed higher rancidity than non-irradiated samples. Hardness of semi-dried shrimp treated with or without irradiation increased during storage. The redness of semi-dried shrimp during storage decreased due to oxidation of astaxanthin being less in irradiated samples.

3.3.4.3. Ready-to-eat foods of reduced water activity

Two studies have been conducted to determine the irradiation dose required to inactivate all spoilage microorganisms and non-sporing and sporing pathogenic microorganisms in ready-to-eat foods (meats, fish, bean curd, preserved radish, date and pickle in cooked, vacuum packaged). The minimum irradiation dose required to inactivate spoilage and pathogenic microorganisms depended on the variety of food and a_w. The results of the earlier studies showed that an average dose of 2.5 kGy could

inactivate all spoilage microorganisms in ready-to-eat foods in which a_w ranged from 0.87 to 0.90. Most of the food samples treated with 2.5, 5, 10 and 20 kGy could be stored at ambient temperature for 2 years. The study carried out later indicated that the minimum dose required to inactivate all spoilage microorganisms was 2.5 kGy for cooked, ready-to-eat fish, 10 kGy for pork and bean curd and 15 kGy for pickle. The a_w ranged from 0.85–0.89 for fish, 0.91–0.90 for pickle, 0.92–0.93 for bean-curd, and 0.93–0.94 for pork.

When 10^{2-4} CFU/g of *Salmonella paratyphi B* and *Shigella shigae* were inoculated in ready-to-eat food samples in vacuum packaging, the minimum irradiation dose required to inactivate S. shigae was 2.5 kGy. A dose of 5 kGy could eliminate *S. paratyphi B* in ready-to-eat food products. When food samples were inoculated with 4×10^2 cells of S. aureus and 4×10^3 cells of B. cereus, the minimum irradiation dose required to inactivate *S. aureus* in pork and fish was 2 kGy. A dose of 2.5 kGy could eliminate this pathogen in bean curd. A dose of 5 kGy was the minimum required to inactivate *B. cereus* in pork and pickle and 7.5 kGy was sufficient to kill the pathogen in fish and bean curd. Due to the fact that the inoculum of *Salmonella enteritidis* in ready-to-eat food samples was too low, the experiment on this pathogen should be repeated.

3.3.4.4. Smoked sausage – "Chorizo"

"Chorizo" is a Portuguese raw smoked sausage made exclusively from pork, lean meat and fat stored and marketed at ambient temperature. It is an intermediate moisture shelf-stable product and when traditionally processed contains a high load of a mixed microbial population, including some food-borne pathogenic bacteria.

The application of irradiation up to 10 kGy improved the hygienic quality of the "chorizo", with a reduction of 4 logs in total aerobic microflora. An absorbed dose of 4 kGy was enough to achieve a 3 log reduction of the faecal streptococci. An 8 kGy dose was not sufficient to destroy all the spores of sulphite-reducing clostridia when present. The D_{10} of faecal streptococci was 1.25 kGy. It appears that irradiation offers promise and a method to ensure hygienic quality of this ready-to-eat product to be marketed at ambient temperature.

3.3.4.5. Ready-to-eat smoked and cured fish

Irradiation, with a dose up to 11 kGy, of different types of smoked and cured fish from Ghana having different moisture contents, water activities, salt and fat contents and stored at ambient temperature, showed that a dose of 2 kGy resulted in a doubling of the shelf-life of lightly-smoked sardine. Irradiation of such smoked products with dose up to 7 kGy resulted in no microbial growth in a package stored for up to 12 weeks at ambient temperature. Different types of microorganisms that are responsible for spoilage of the product were identified. Doses of 7−11 kGy were found to control microbial activity in some smoked products during the 12 weeks storage period. However, some of these irradiated products had an unacceptable colour, flavour and texture. Irradiation of ready-to-eat marinated fish (a_w 0.91–0.94) with a dose of 10 kGy extended shelf-life from 3 to 7 days.

3.3.5. Recommendations

3.3.5.1. Product criteria

To define the performance criteria of intermediate moisture content products such as a_w, microbial index of spoilage.

3.3.5.2. Pre-treatment process

To investigate the effect of pre-treatment processes for a_w reduction such as percentage salt, sugar, vinegar or heat treatment process (smoking and drying) with the irradiation process on the quality and shelf-life of products.

3.3.5.3. Packaging material

To find a suitable packaging and make recommendations on the type of package required for preventing recontamination.

3.3.5.4. Safety criteria

To develop the safety criteria (such as HACCP) for the ready-to-eat products through irradiation processing and marketing at ambient conditions (including raw material quality, pre-treatment process, packaging type and storage conditions).

3.4. Packaging requirements

3.4.1. Background

Shelf-stable composite and convenience foods must have packaging specially designed to maintain the sanitary benefits obtained by irradiation without compromising the safety and sensory quality of food.

Irradiation presents also some potential for the development of new film formulations from biopolymers which could be used to increase the shelf-life of convenience foods.

3.4.2. Objectives

To assess the effects of irradiation on various packaging materials required for shelf-stable composite and convenience foods.

To develop new packaging systems based on biopolymers.

3.4.3. Rationale for the studies

Few packaging materials have obtained approval to be used for pre-packaging of irradiated foods. Most of them were approved in the 1960s and are typically single layer films. Nowadays, new packaging materials are designed to meet more demanding performance from the packaging; to ensure specific gas permeability for MAP and vacuum application, to absorb juice from meat products via adsorbing pads, to obtain improved barrier properties, etc. If some special pouches are already available for irradiation purposes in South Africa and in the USA, data are missing to assess the performance and safety of other modern packaging materials, particularly trays and lids for irradiation treatment.

New coatings and packaging systems from irradiated biopolymers should be studied in order to estimate their controlled release kinetics into the food and the way they can provide an additional protection against bacteria.

3.4.4. Achievements to date

3.4.4.1. Evaluation of packaging performance and acceptability

Synthetic polymer based films

Several packaging systems were studied:

HDPE tray + Nylon 6 lid
Nylon/PE/AI/PET/PE/LLDPE pouch (the one used by the US Army)
CPET
APET

PET Sealer® (lid)
PET Mylar OL® (lid)

Parameters evaluated were:

Global migration
Specific migration (water, ethanol, acetic acid, CH_2Cl_2)
Identification of migrants and volatile compounds by GC/MS, HPLC, LC/MS, DH/GC/MS
Volatile weight loss
Stability (DSC)
Structure (FT-IR, NMR)
Permeability
Degradation of antioxidants used in polyhydrocarbons
Impact on sensory quality (water for HDPE and CPET trays)
Methodology development
Microscopy (APET and CPET)
Chemical pathway leading to the production of 1,3-di-t-butyl benzene in HDPE
Chemical pathway leading to the production of acetaldehyde and 2-methyl-1,3-dioxolane

Some results and comments:

- For absorbed doses of 1 to 100 kGy, irradiation treatment induced the production of several degradation compounds from all the packaging materials monitored in the study (including from the pouch). The level and the variety was particularly large for HDPE antioxidants (Irgafos 168, Irganox 1076, Irganox 1010, Weston XP-1532).
- Different kinetics and mechanisms of gamma degradation of the antioxidant were found in solid plastic and in solution.
- The presence of an antioxidant in the solvent for extraction methods was necessary in order to stabilise sensitive extractables for their identification and quantification of compounds present in irradiated plastic packaging.
- There was evidence that the formation of higher molecular weight compounds and intra-molecular rearrangements could be involved in the production of 1,3–DTBB (specific to irradiation) and t-butanol from irradiated Irgafos 168 in PE.
- PET is not inert and the effect of irradiation on it depends on formulation and morphology.
- Sealing PET products are not pure and they are more affected by irradiation than PET itself.
- Acetaldehyde levels are highly significantly affected by irradiation dose and environmental conditions. 40 ppm was obtained from irradiation of CPET at 50 kGy, an amount high enough to provoke severe sensory problems.
- Effect of irradiation on water affects sensory perception at a dose of 10 kGy.
- Descriptive sensory profiles of packaging materials depend on the chemical structure of the packaging.
- For an absorbed dose of 10 kGy, HDPE generates highly significant hydrocarbon off-tastes and odours and CPET generates significant “heated plastic” off-tastes and odours.
- Doses of 5–100 kGy cause significant chain scissions in HDPE, not in PET.
- The destruction of antioxidant in HDPE following irradiation treatment contributes to decreased material resistance to heat, oxidation and radiation.
- For polymers containing antioxidants, the level and nature of migrants will depend on the dose required to destroy the secondary antioxidant.

Biopolymer based films

Several formulations of films and coatings from dairy proteins (caseinate and whey) alone or in combination with soya proteins were developed using gamma irradiation as a cross-linking process at doses ranging from 8 to 128 kGy. This investigation has clearly demonstrated the usefulness of gamma irradiation for making free-sterilised edible films. Moreover, gamma irradiation combined with thermal treatment improved significantly the puncture strength for all types of films. Best results

were obtained for caseinate and soya-whey protein films increasing the puncture strength by 35 and 20%, respectively. Electron microscopy showed that the mechanical characteristics of cross-linked films are closely related to their microstructures. Irradiated formulations also showed significant improvements of the barrier properties, namely water vapour permeability. Size-exclusion chromatography performed on the cross-linked proteins showed that gamma irradiation has a synergistic effect with heating to increase the molecular weight of calcium caseinate, whey and soya proteins. For cross-linked proteins, the molecular weight distribution was 2×10^6 kD. Structural analysis of biofilms was also investigated. No major alteration of the structural conformation of the proteins were observed by FTIR for biofilms obtained after heat treatment, while gamma irradiation induced some modifications in the protein structure. Cross-linking by gamma irradiation seems to modify to a certain extent the conformation of proteins that will adopt structures more ordered and more stable, as suggested by X ray diffraction analysis. A loss of protein-water interaction was observed for cross-linked protein by isothermal calorimetry. Biodegradability evaluation on cross-linked films showed that the net bacterial degradation was 86 and 3%, respectively, for films irradiated at minimum doses of 4 and 64 kGy, confirming that cross-links produced by gamma irradiation slowed the biodegradation of the material.

3.4.4.2. Quality assurance guidelines and protocols

Standard guidelines and protocols are increasingly necessary for QA of irradiated foods and their packaging, in view of the matureness of this R&D fields, the rapid increase of industrial implementation of food irradiation processing, and the concurrent liability issues.

A standard protocol has been created, for the process of Selection of Packaging Polymers for foods to be irradiated and full details are given in the final paper of this publication.

3.4.5. Recommendations

3.4.5.1. For packaging materials to be used for pre-packaged irradiated foods

To determine the effect of the following parameters on irradiated packaging materials; % freezing and cooling temperatures (–40 and 0°C),

% modification of inside atmosphere during long term storage,

% ageing and modification of volatile profiles with time.

Toxicological and sensory issues regarding 1,3-DTBB should be considered in food packaging containing Irgafos 168 and similar antioxidants.

High-dose food irradiation applications will require tight quality control of packaging materials in order to ensure product safety and acceptability.

To include in legislation about packaging not only the follow up of the components of packaging formulation but also the assessment of degradation compounds from packaging materials.

To use leading brands of plastic materials in order to assure highest quality and long-term availability.

To use quality assurance guidelines specifically developed for the use of packaging systems.

To develop the list of quality criteria required for food packaging.
To harmonise packaging design in R&D and eventually in industry, preferably, for doses above 5 kGy.

3.4.5.2. For the development of new films and coatings using irradiation technology

To evaluate the diffusion rate of active compounds from the cross-linked biopolymer films to the foods.

To study the potential of cross-linked biopolymers for active food-components (flavours, antimicrobial, antioxidants, etc.).

To extend the study to other biopolymers (soya proteins, polysaccharides, etc.) to evaluate their potential for specific applications in terms of permeability, resistance, elasticity, etc.

Development of cross-linked biopolymers for coating and film application and evaluation of the potential of immobilisation of bioactive compounds (antimicrobial, antioxidants, etc.) in cross-linked biopolymers. Stabilisation of the activity of the immobilised bioactive compounds using cross-linked biofilms and application during irradiation of food.

Evaluation of the potentiality to develop cross-linked biopolymer films using various materials for specific applications (permeabilty, mechanical resistance, physico-chemical properties, etc.).

3.4.5.3. For protocol

To encourage the development of an international protocol for packaging to be used for pre-packaged irradiated foods.

4. CONCLUSIONS

This CRP has addressed all specific objectives with the following encouraging results:

(1) High-dose irradiation in combination with other preservation processes can produce a number of high-quality shelf-stable ready-to-eat meals that can be marketed at ambient temperature; some of such shelf-stable meals were evaluated to meet the requirements of immunocompromised patients.

(2) Irradiation in combination with other preservation processes offers promise to develop high-quality shelf-stable intermediate moisture products which could be kept at ambient condition for many months.

(3) Irradiation can ensure microbiological safety and shelf-life extension of chilled prepared meals and a number of refrigerated, ready-to-eat food products.

(4) Predictive models for irradiation inactivation of various pathogens in food have been developed.

(5) A number of packaging materials required for various shelf-stable food, irradiated with doses up to 100 kGy were evaluated.

This CRP has demonstrated the effectiveness of irradiation as a method to ensure microbiological safety and shelf-life extension of a number of ready-to-eat food products, to be stored either at ambient temperature or under refrigeration. It should broaden the scope of food irradiation beyond what is currently practised which consists mainly of treatment of individual food items. The outcome of this CRP should be of interest to the food industry which needs an effective method to ensure microbiological safety and enhanced shelf-life of ready-to-eat food products. It should be noted that the US National Food Processor Association (NFPA) submitted a petition to the FDA in August 1999 to allow irradiation to be used for these purposes.

5. RECOMMENDATIONS

The CRP participants recognised that most of data to demonstrate the effectiveness of irradiation as a food processing method are on individual food items. Little data are available on the effectiveness of irradiation on prepared meals apart from preliminary work conducted in South Africa.

There is increasing demand for ready-prepared meals in advanced countries as well as in many developing countries because of the changing lifestyles and restricted time available for food preparation. A number of prepared meals are marketed in advanced countries in the frozen state, which are expensive and not highly palatable to many consumers. Prepared meals in developing countries are marketed fresh, often at ambient temperature, which results in limited shelf-life and involve risks from contamination of pathogenic microorganisms. Irradiation could render such prepared meals in advanced countries to be marketed under refrigeration instead of frozen, with a sufficient shelf-life and microbiological safety. Prepared meals in developing countries could be irradiated for shelf-life extension as well as ensuring microbiological safety during marketing, either under chilled condition or at ambient temperature.

The participants agreed that a logical follow up of the current CRP is to evaluate the effectiveness of irradiation to ensure microbiological safety and shelf-life extension of prepared meals to be marketed either under refrigeration or at ambient temperature. Data of such studies are urgently needed in view of the increasing demand for such products in many countries.

DEVELOPMENT OF PREDICTIVE MODELS FOR THE EFFECTS OF GAMMA RADIATION, IRRADIATION TEMPERATURE, pH, AND MODIFIED ATMOSPHERE PACKAGING ON *Bacillus cereus*, *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonella typhimurium* and *Staphylococcus aureus*

D.W. THAYER
Food Safety Research Unit, US Department of Agriculture,
Agricultural Research Service, North Atlantic Area,
Eastern Regional Research Center,
Wyndmoor, Pennsylvania, United States of America

Abstract

Predictive models incorporating the effects of temperature on the inactivation by gamma irradiation of the food-borne pathogens *Bacillus cereus, Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonella* spp., and *Staphylococcus aureus* were developed and converted to forms suitable for incorporation into the ARS Pathogen Modelling Program. Because industrial radiation processors might use dry ice with frozen samples and up to ambient temperature, a range of –76 to +20ºC was included in the study. In each case the survival of the pathogen was greater when the product was frozen at the time of irradiation. *L. monocytogenes* cells surviving 2 kGy irradiation could multiply on cooked but not on raw turkey meat stored at 7º C for 21 days.

1. INTRODUCTION

Three objectives were established for research by the Agricultural Research Service as a contribution to the IAEA Co-ordinated Research Programme on Development of Safe, Shelf-Stable and Ready-to-Eat Foods through Radiation Processing. The first objective was to convert previously developed equations for the inactivation of *Bacillus cereus, Escherichia coli* O157:H7, *Listeria monocytogenes, Salmonella typhimurium,* and *Staphylococcus aureus* by irradiation and the effects of processing temperature on their inactivation into user friendly, interrogative forms that can be provided as a compiled program in the USDA ARS Pathogen Modelling Program. New research was initiated expanding this data base to include the use of several isolates of *E. coli* O157:H7 and *S. aureus* and to allow the prediction of D-values, rather than just survival, over a wide range of temperatures from the deep frozen –76ºC to +20ºC. The second objective was to develop predictive models for the effects of gamma irradiation on the survival and/or growth of *L. monocytogenes* during refrigerated storage on either raw or cooked turkey. The third objective was to initiate studies of the effects of pH on the survival of *E. coli* O157:H7 and *Salmonella* in processed meats. With the exception of the third, these objectives have been accomplished.

2. INTEGRATION OF EXISTING PREDICTIVE MODELS INTO THE ARS PATHOGEN MODELLING PROGRAM

Six interactive predictive models for the effects of ionizing radiation on food-borne pathogens and normal flora on ground poultry meat or ground beef were incorporated into the USDA ARS Pathogen Modelling Program. This program may be downloaded from the web page for the Eastern Regional Research Centre at http://www.arserrc.gov. Models based on our published studies of the inactivation of *S. typhimurium* ATCC 14028 and normal flora on ground chicken or drumsticks and of *E. coli* O157:H7 on ground beef by gamma irradiation were modified to provide 95% confidence limits and converted into interactive forms for use in the Pathogen Modelling Program.

2.1. *S. typhimurium* ATCC 14028 on sterile, mechanically-deboned chicken meat

The effects of ionizing radiation dose, temperature, and atmosphere on the survival of *S. typhimurium* ATCC 14028 in sterile, mechanically-deboned chicken meat were described in the

first interactive models on irradiation in the ARS Pathogen Modelling Program [1]. The responses to irradiation doses from 0 to 3.6 kGy, at temperatures from –20ºC to +20ºC, and when irradiated either in the presence of air or when vacuum packaged were determined. A central composite design was used to obtain the data for the basic model. Samples were irradiated for these studies using a temperature controlled, self-contained, ^{137}Cs, gamma irradiation source with an activity of approximately 135,000 Ci and a dose rate of 0.12 kGy/min. The original model was challenged for accuracy with several additional studies and the new data used to improve it. As expected, gamma irradiation was more lethal at higher temperatures and in the presence of air. The model allows prediction of the log inactivation with considerable accuracy over the experimental range in the study. The following response-surface equations were developed for the response of S. *typhimurium* ATCC 14028 to gamma irradiation on sterile, mechanically-deboned chicken meat [1]:

(1) Air atmosphere:

S. typhimurium Log Survivors (cfu/g) = –0.166 – 0.010 × temperature – 2.552 × kGy – 0.010 × temperature × kGy + 0.002 × $(\text{temperature})^2$ + 0.068 × kGy^2 $R^2 = 0.931$

(2) Vacuum packaging

S. typhimurium Log Survivors (cfu/g) = –0.170 + 0.006 × temperature – 2.013 × kGy + 0.002 × $(\text{temperature})^2$ + 0.068 × kGy^2 $R^2 = 0.921$

Let: temperature = degrees Celsius (–20ºC to +20ºC), dose = 0 to 3.60 kGy

These models were converted into an interactive format with the 95% confidence limits of the prediction incorporated into the modelling program. Model (2) predicts that the number of viable *Salmonella* in vacuum-packed, contaminated, mechanically-deboned chicken would decrease by 4.29 logs if it were to be irradiated to 3.0 kGy at -20ºC or by 6.38 logs at +20ºC.

2.2. *S. typhimurium*^{r} ATCC 14028 on mechanically-deboned chicken meat or drumsticks

The second, third, and fourth models added to the ARS Pathogen Modelling Program described the results of studies of the inactivation of a streptomycin-resistant mutant of *S. typhimurium* ATCC 14028 on non-sterile, mechanically-deboned chicken meat or chicken drumsticks by gamma irradiation [2]. These studies tested the validity of the first model when applied to poultry products with a typical normal flora as well as *S. typhimurium*. The variables were temperature, atmosphere, and irradiation dose. A streptomycin-resistant strain was isolated from the original culture and designated as *S. typhimurium*^{r}. Its use allowed the isolation of surviving cells without using inhibitory culture media. Preliminary studies confirmed that this isolate of the original strain had the same radiation D-value. Models were developed describing the inactivation of the pathogen on either the mechanically-deboned meat or the drumsticks. Results were expressed as colony forming units (CFU) per gram or as CFU per square cm of the surface of the drumsticks. In either case the new predictive models were remarkably similar to those developed with sterile chicken.

2.2.1. S. typhimurium^r *ATCC 14028 on mechanically-deboned chicken meat*

Under these conditions, the results obtained with air or vacuum atmospheres were not significantly different and the following single, combined response-surface equation for the inactivation of *S. typhimurium*^{r} on non-sterile, mechanically-deboned chicken meat was developed:

(3) *S. typhimurium*^{r} Log Survivors (CFU/g) = –0.0943 – 0.0 129 × temperature – 1.8849 × kGy – 0.0182 × temperature × kGy + 0.0008 × (temperature)^2 – 0.0647 × kGy^2 R^2 = 0.947

Let: temperature = degrees Celsius (–20ºC to +20ºC), dose = 0 to 3.60 kGy

2.2.2. S. typhimurium^r ATCC 14028 on chicken drumsticks

The following response-surface equations were developed from the radiation survival data for the streptomycin-resistant isolate of *S. typhimurium*^{r} and the indigenous microflora on chicken drumsticks:

(4) *S. typhimurium*^{r} Log Survivors (CFU/cm^2) = 0.0475 – 0.0133 × temperature – 2.1139 × kGy – 0.0209 × temperature × kGy + 0.0005 × temperature^2 + 0.1466 × kGy^2 R^2 = 0.838

Let: temperature = degrees Celsius (–20ºC to +20ºC), dose = 0 to 3.60 kGy

Equation (3) predicts that an irradiation dose of 3.0 kGy would inactivate 3.47 logs CFU/g of *S. typhimuriums*^{r} and 6.17 logs CFU/g at –20ºC and +20ºC, respectively. These results are very similar to those predicted by equation (1). Equation (4) predicts that a dose of 3.0 kGy would inactivate 3.35 logs CFU/cm^2 of *S. typhimuriums*^{r} or 6.39 logs CFU/cm^2 at –20ºC or +20ºC, respectively. Considering that the total numbers of colony forming units were very different in the mechanically-deboned chicken and on the chicken legs, the results of the studies are remarkably similar.

2.3. Indigenous microflora on chicken drumsticks

The following response-surface equations were developed from the radiation survival data for the indigenous microflora on chicken drumsticks:

(5) Indigenous Microflora Log Survivors (CFU/cm^2) = 0.6852 – 0.0178 × temperature – 1.8167 × kGy – 0.0056 × temperature^2 + 0.3025 × kGy^2 R^2 = 0.838

Let: temperature = degrees Celsius (–20ºC to +20ºC), dose = 0 to 3.60 kGy

2.4. *E. coli* O157:H7 on ground beef

The fifth model that was incorporated into the USDA ARS Pathogen Modelling Program describes the response of *E. coli* O157:H7 on ground beef to gamma irradiation over a temperature range from –20ºC to +20ºC [3]. The following isolates were used to generate the data: ATCC 43895 and ATCC 25922. Each isolate was grown separately and pooled to provide an inoculum for the meat. The following response-surface equation was developed from the studies:

(6) *E. coli* O157:H7 Log Survivors (CFU/g) = 0.6410 – 1.2888 × kGy + 0.0015 × temperature – 0.0696 × kGy × temperature – 1.3010 × kGy^2 + 0.0020 × temperature^2 R^2 = 0.931

Let: temperature = degrees Celsius (–20ºC to +20ºC), dose = 0 to 2.00 kGy

This equation predicts that a dose of 1.5 kGy administered at –20ºC or +20ºC would inactivate 2.64 or 6.76 logs CFU/g, respectively.

2.5. *B. cereus* ATCC 33018 on ground beef

Sterile ground beef was inoculated with 2.32×10^8 CFU/g of enterotoxic *B. cereus* ATCC 33018 and its response determined when irradiated over a temperature range of -20 to +20°C and gamma-radiation doses from 0 to 3.0 kGy [4]. A modified, central-composite, response-surface design was used with two replicate vacuum-packaged 5.0 ± 0.05 g samples for each of the following combinations of irradiation temperature and dose: –20ºC, 0, 1.5, 3.0 kGy; –10ºC, 0, 0.75, 2.25 kGy; 0ºC, 0, 3.0 kGy; +10ºC, 0, 0.75, 2.25 kGy; +20ºC, 0, 1.5, 3.0 kGy. Five replicate samples treated at 0ºC with 1.5 kGy were analysed.

The equation developed from the analysis of variance predicting the response of *B. cereus* ATCC 33018 is as follows:

(7) *B. cereus* Log Survivors (CFU/g) = $-0.128 - 1.366 \times \text{kGy} + 0.008 \times \text{temperature} - 0.015 \times \text{kGy} \times \text{temperature} - 0.117 \times \text{kGy}^2 + 0.001 \times \text{temperature}^2$

This equation predicts that *B. cereus* vegetative cells on mechanically-deboned chicken irradiated to an absorbed dose of 3.0 kGy at –20, 0 or +20ºC would decrease in number by 4.20, 5.27, or 5.72 logs, respectively. The equation will be modified for incorporation into the ARS Pathogen Modelling Program.

2.6. *L. monocytogenes* on beef

Ground Longissimus dorsi beef muscle was inoculated with a mixture of isolates of *L. monocytogenes* ATCC 15313, 43256, 49594, and 7644. Inoculated meat samples received irradiation doses of 0 to 3.0 kGy in increments of 0.60 kGy at 5, 0, –5, –10, –15, and –20°C. All samples for each replicate study were inoculated from the same inoculum, and the study was repeated twice [5]. The survival of *L. monocytogenes* on beef when irradiated at a given temperature can be predicted from the following equation:

(8) *L. monocytogenes* Log Survivors (CFU/g) = $-0.1001 - 0.0650 \times \text{temperature} - 1.922 \times \text{kGy} - 0.0638 \times \text{temperature} \times \text{kGy} - 0.0036 \times \text{temperature}^2$ $R^2 = 0.947$

The data from this study also allowed the derivation of the D-values for the inactivation of *L. monocytogenes* at each temperature. The D-value increased from 0.445 kGy at +5ºC to 1.208 kGy at –20ºC. The D-values were found to form a linear regression described by Log D-value = $-3.539 + 917.3 \times (T^{-1})$ where the D-value is expressed as kGy and T is the absolute temperature. These equations will be modified for incorporation into the ARS Pathogen Modelling Program.

3. DEVELOPMENT OF NEW PREDICTIVE MODELS FOR GAMMA-RADIATION INACTIVATION OF *E. coli* O157:H7 AND *Staphylococcus aureus* ON GROUND BEEF FROM –76 TO +20ºC

3.1. *E. coli* O157:H7

Processing temperature is known to affect the survival of bacteria during irradiation processing. Because different methods of cooling may be used during irradiation processing ranging from the use of dry ice, to water ice there may be significant differences in the actual temperature of the product during processing. The following study was conducted to allow these effects to be

predicted. A mixture of equal amounts of *E. coli* O157:H7: ATCC 35150, 43889, 43894, 93-437, and ENT C9490 was used to inoculate radiation-sterilised ground beef containing 12.3% fat, 0.89% ash, and 19.7% protein. Samples were gamma irradiated at +20, +12, 0, –4, –12, –20, –30, and –76ºC (68 to –104.8ºF) to 0, 0.4, 0.8, 1.2, 1.6, 2.0 and 2.4 kGy. Two independent replications of the study were completed. Radiation D-values were calculated from the results and a response-surface equation was developed from the data, which predicts the inactivation of the pathogen at a given dose within the temperature range of the study. The D-values were 0.248, 0.290, 0.388, 0.370, 0.360, 0.606, 0.979, 1.07, 1.11 log CFU/g at +20, +12, +4, 0, –4, –12, –20, –30, and –76ºC, respectively.

3.2. *S. aureus*

The following study was conducted to determine the effect of irradiation temperature on the inactivation of *S. aureus*. A mixture of equal amounts of *S. aureus:* ATCC 25923, 13565, 14458, 27154, and B124 was used to inoculate radiation-sterilised ground beef. Samples were gamma irradiated at +20, +12, 0, –4, –12, –20, –30, and –76°C (68 to –104.8°F) to 0, 0.4, 0.8, 1.2, 1.6, 2.0, and 2.4 kGy. Two independent replications of the study were completed. Radiation D-values were calculated from the results, and a response-surface equation was developed from the data to predict the inactivation of the pathogen by a given dose with the temperature range of the study. The D-values were 0.416, 0.426, 0.506, 0.380, 0.476, 0.736, 0.875, 0.869, 0.851, and 0.822 log CFU/g at +20, +12, +4, 0, –4, –12, –20, –30, –40 and –76°C, respectively.

4. SURVIVAL AND MULTIPLICATION OF *L. MONOCYTOGENES* ON RAW AND COOKED TURKEY

The radiation-resistance and ability of *L. monocytogenes* ATCC 7644, 15313, 43256, and 49594 to multiply on irradiated air-packed, refrigerated raw or cooked turkey breast meat was investigated [6]. D-values were not significantly different on raw versus cooked ground turkey. During 21 days of storage at a modest abuse temperature of 7ºC, *L. monocytogenes,* inoculated to a level of ca. 3.6×10^3 CFU/g and then irradiated to a dose of 2 kGy, was unable to multiply on raw meat but did do so on cooked meat. Apparently the cooked meat provided critical nutrients for recovery of irradiated *L. monocytogenes.*

At the time that this paper is in preparation, additional work is required with the help of a statistician and a computer programmer to allow the integration of the new radiation-inactivation models into the USDA ARS Pathogen Modelling Program. The new models will emphasise the prediction of D-values for the pathogens rather than the logarithm of the number of pathogens that will be inactivated.

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STUDIES ON IRRADIATION OF PRE-PACKAGED PREPARED VEGETABLES AND IMPROVEMENT OF MICROBIOLOGICAL SAFETY OF SOME *SOUS-VIDE* MEALS BY GAMMA RADIATION*

J. FARKAS**, K. POLYÁK-FEHÉR**, C. MOHÁCSI-FARKAS***, L. MÉSZÁROS**, E. ANDRÁSSY**, T. SÁRAY**

** Department of Refrigeration and Livestock Products' Technology
*** Department of Microbiology and Biotechnology

Szent István University, Budapest, Hungary

Abstract

In the first part of the work, various aerobically packaged pre-cut vegetables, heavily inoculated with *Listeria monocytogenes* were gamma-irradiated with 1 kGy thereby not changing the sensory quality of the produce. This low-dose irradiation was able to improve the microbiological safety and stability of the refrigerated products stored at various temperatures. The radiation treatment significantly diminished the population of *L. monocytogenes* and increased the growth temperature requirement of its surviving cells. Losses of vitamin C as a direct effect of low-dose irradiation proved to be no higher than those which occurred in the non-irradiated samples during their shelf-life. The studies underlined the importance of good temperature control in the chill chain. Package conditions of minimally processed, low-dose irradiated chilled vegetables must be aerobic enough to prevent growth of non-proteolytic *Clostridium botulinum.* In the second part of the work, experimental batches of prepared meals such as chopped beef in tomato sauce, chopped beef in paprika gravy, and smoked-cured pork in stewed beans sauce were inoculated with spores of psychrotrophic *Bacillus cereus,* more heat- and radiation-resistant than spores of non-proteolytic *C. botulinum.* After vacuum packaging, the meals were treated with combinations of pasteurising heat treatments and gamma irradiation in the dose range of 2.5 to 5 kGy. Prior to and after treatments, and periodically during storage at 10°C, total aerobic and total anaerobic viable cell counts, and, selectively, the viable cell counts of *B. cereus* and sulphite-reducing clostridia were determined. The effects of the treatment order as well as addition of nisin to enhance the preservative efficiency of the physical treatments were also studied. Sensory testing with uninoculated samples proved that the combination-preserved meals were of acceptable quality at the beginning of their storage. The microbiological investigations showed that the quality friendly *sous-vide* cooking in combination with medium-dose gamma irradiation and/or nisin addition may increase considerably the microbiological safety and keeping quality of the meals studied.

1. EFFECTS OF LOW-DOSE GAMMA RADIATION ON SHELF-LIFE AND MICROBIOLOGICAL SAFETY OF PRE-CUT PACKAGED VEGETABLES

In the first part of this research work [1], pre-cut bell pepper, carrot cubes, shredded cabbage, and sliced radish were heavily inoculated with *Listeria monocytogenes*, then packaged aerobically in low-density polyethylene (LDPE) pouches. Half of the experimental batches were kept as non-irradiated controls and the other half irradiated with 1 kGy using gamma rays, a dose that did not change the sensory properties of the pre-cut vegetables. The effect of irradiation on the viable cell counts of the test organism and growth of its surviving cells were followed as compared to the untreated samples at three different storage temperatures (between 1 and 16°C), together with the fate of the naturally contaminating bacterial flora (aerobic plate count, presumptive lactic acid bacteria and *Enterobacteriaceae*). Microbiological changes were compared with keeping quality based on appearance of the vegetables. With the exception of carrots, *L. monocytogenes* grew readily in the non-irradiated produce. However, the irradiation treatment reduced the numbers of *Listeria* by several log-cycles, and the surviving *Listeria* population was shown to be more susceptible to low temperature storage than the non-irradiated population. Irradiation drastically reduced the viable load of spoilage bacteria, thereby, improving the microbiological shelf-life and extending the sensory keeping quality.

* Work performed within the Framework of the FAO/IAEA Co-ordinated Research Programme on the Development of Safe, Shelf-Stable and Ready-to-Eat Food through Radiation Processing.

In the irradiated samples the latter was not limited by bacterial growth but by desiccation and discoloration or other physiological deterioration of the vegetable tissues. Considering the extent of the decontamination which could be achieved, Good Manufacturing Practice (GMP) coupled with a 1 kGy irradiation dose could potentially eliminate non-spore-forming psychrotrophic pathogenic bacteria from the products investigated without diminishing sensory quality and without significant loss of β-carotene in carrot. The vitamin C losses of the other three vegetables studied were no higher than those that occurred during the shelf-life of the non-irradiated samples. Microbiological data demonstrated the importance of proper temperature control. Furthermore, package conditions of minimally processed, low-dose irradiated vegetables must be sufficiently aerobic to prevent growth of psychrotrophic spore formers (e.g. non-proteolytic *Clostridium botulinum*) the spores of which are not destroyed by low-dose irradiation. Detailed results and discussions of this work have been published elsewhere (See Refs. [2-5]), thus, are not repeated in this paper.

2. DEVELOPING SOME *SOUS-VIDE* MEALS BY COMBINATION OF IRRADIATION TREATMENT AND/OR NISIN ADDITION

2.1. Background and scope of the project

There is an increasing consumer trend for fresh-like, or at least less severely processed, still convenient, or ready-to-eat foods with reduced levels of chemical preservatives. The catering industry is also strongly interested in such 'minimally processed', non-frozen meals and meal components. In response to these demands, minimal processing of food is gaining importance and it implies lighter preservation methods than the conventional canning, freezing or dehydration technologies. Minimally processed foods have an enhanced but limited shelf-life and mainly rely on refrigeration as key preservation measure. The consumer perceives them to be of better quality as they are chilled rather than canned, dried or frozen [6]. In the second part of this research investigations were devoted to studies on the improvement of the microbiological safety and stability of some vacuum-packaged then mildly cooked/pasteurised refrigerated (so called *sous-vide*) meals treated with gamma irradiation and/or addition of the bacteriocin, nisin.

Advantages of *sous-vide* foods include convenience, less need for conventional kitchen space, equipment and personnel in a food service operation and the good palatability of the processed product [7]. They are packaged (raw or partially cooked) in heat stable, air impermeable bags under vacuum, the bags are sealed, and the product is cooked (pasteurised), then cooled and stored under refrigeration [8]. *Sous-vide* processing uses lower temperature heating (within the range of 65–95°C) to maximise the nutritional and organoleptic qualities which would be adversely affected by heating at a higher temperature and in the presence of atmospheric oxygen.

The European Chilled Food Federation concluded that *sous-vide* products should obtain heat treatments according to their intended shelf-life and these form two categories regarding safety assurance [9]:

– Mildly heated products and otherwise unpreserved, treated by a minimum heat process of 70°C for 2 min (or equivalent). In this case it is expected that the heat treatment inactivates vegetative pathogens (e.g. six decimal reduction of *L. monocytogenes* is achieved) [10]. Bacterial spores do, however, persist. Therefore, this type of process is acceptable only if the temperatures of storage are below 3°C and storage times are short (maximum 5 days) in order to avoid growth of the psychrotrophic strains of *C. botulinum.* (The much more heat-resistant mesophilic, proteolytic strains do not grow below 10°C).

– Products intended to have longer shelf-life at refrigerated storage and otherwise unpreserved must be heat processed by a temperature-time combination of 90°C for 10 min (or equivalent lethality) which will achieve a six decimal reduction of psychrotrophic (non-proteolytic) *C. botulinum* spores. However, more resistant spores, e.g. mesophilic (proteolytic) clostridia

and pathogenic strains of *Bacillus cereus*, a facultatively anaerobic spore-former, may persist. If heat treatments milder than the above are given, evidence must be provided that other preservative factors are present and operating (including reduced pH, and/or reduced water activity (a_w), confident control of temperature below 3°C, etc.). It is assumed that the growth of psychrotrophic strains of *C. botulinum* will be prevented in a chilled food even if it is packed in a reduced oxygen atmosphere, if the a_w is adjusted to below 0.97 or, the pH is reduced to below pH 5.0 [11].

A combination of factors ('hurdles') inhibiting or inactivating microorganisms may require less of each hurdle to control growth than would be expected from each individual factor. Combined effects of pH, salt concentrations, heat treatment and subsequent storage temperature on growth from spores of non-proteolytic *C. botulinum* have been comprehensively studied (see e.g. Refs [12,13]). Experience shows, however, that compositional variations may exist and the required low temperatures cannot always be guaranteed throughout distribution, retail and during transport and storage by the consumer [14]. It is clear from the above considerations that a quality-friendly, non-thermal microbicide treatment such as ionizing radiation might enhance the microbiological safety of the aforementioned cook-chill foods.

Unlike conventional cook-chill meals which have been investigated by several laboratories (see e.g. Refs [15-22]), little information has been reported yet on irradiated *sous-vide* products. The combined effect of irradiation and *sous-vide* cooking of chicken breast meat was investigated with respect to the survival and growth of *L. monocytogenes*, product shelf-life, thiamine content and sensory quality by Shamsuzzaman et al. [23]. Chicken breast meat was inoculated with *L. monocytogenes*, vacuum packed, irradiated using electron-beam up to a dose of 2.9 kGy and cooked to an internal temperature of 65.6°C. This *sous-vide* cooking had very little lethal effect on *L. monocytogenes* (only 0.35 log-unit of CFU reduction). However, after combining irradiation with *sous-vide* cooking, a reduction of more than 5.5 log-units of CFU was achieved and the pathogen remained undetectable in the product during an 8 week storage period at 2°C studied. The combination-preserved product had a shelf-life of at least 8 weeks at this storage temperature. The electron-beam treatment had little effect on odour and flavour, and the thiamine content was reduced by approximately 5%. In a second experiment [24], samples of chicken breast meat inoculated with *L. monocytogenes* (to approximately 6 logs CFU/g) were cooked to 71.1°C and stored at 8°C. The *sous-vide* cooking alone reduced *Listeria* counts in the inoculated samples only by 1.5 log cycles, the survivors multiplied quickly during storage and the non-irradiated *sous-vide* cooked samples spoiled within 2 weeks. The combined treatment (3.1 kGy irradiation and *sous-vide* cooking) reduced the *Listeria* count to an undetectable level (< 0.18 $\log_{10}$ CFU/g) without adversely affecting sensory quality. Reduction of the total viable counts (TVCs) by the combined treatment prevented microbial spoilage for at least 8 weeks. The reduction in thiamine content caused by the combined treatment varied from 23 to 46 %.

As far as is known, the effect of irradiation in combination with *sous-vide* processing on the survival and growth of bacterial spores has not been addressed prior to the present work. Therefore, the objective of these studies was to investigate the possibility of improving the microbiological safety of some *sous-vide* meals by gamma irradiation using spores of a psychrotrophic strain of *B. cereus* as the inoculum. Although *B. cereus* is much less harmful than *C. botulinum, B. cereus* spores are more heat- and radiation-resistant than those of non-protelolytic *C. botulinum* [11,25–29] and may be present more frequently than the latter [30,31]. Cooked meats have been implicated in outbreaks of food poisoning caused by *B. cereus* [32]. Studies by Grant and Patterson [33] on a meal (comprising of roast beef and gravy, roast and mashed potato, and cauliflower in a white sauce) have shown that *B. cereus* formed a significant proportion of the microflora before and after chill storage. Van Netten et al. [34] provided evidence for the occurrence of psychrotrophic toxigenic strains of *B. cereus* in cook-chill meals, and a review by Schofield [35] lists this organism as an emerging pathogen of concern in chilled foods. Information on the influence of factors as pH, a_w, etc. on the growth of *B. cereus* is rather incomplete in the scientific literature.

2.2. Materials and methods

2.2.1. Test organism

The test organism used for these investigations was *B. cereus* strain F46.26.90 obtained from the Agrotechnological Research Institute, ATO-DLO, Wageningen, The Netherlands. From a spore inoculum heat shocked at 60°C for 30 min, this strain showed 2 log cycles increase of its viable cell count in Brain Heart Infusion Broth (BHI) in 3 days at 5–7°C. At 10°C of incubation, 5 log-cycles growth was noted already after one week.

2.2.2. Spore suspension preparation

A spore suspension of *B. cereus* was prepared on nutrient agar plates fortified with minerals according to the composition of FNA medium as described by Johnson et al. [36]. FNA plates were surface inoculated with an overnight culture of *B. cereus* grown in Trypticase Soy Broth (TSB, Oxoid CM 129) at 30°C under shaking. The inoculated FNA plates were first incubated at 30°C for 3 days, then the plates were held at 5°C for 24 h and the extent of sporulation was checked by phase contrast microscopy.

Spores developed on the FNA plates were suspended in cold sterile distilled water by scraping the surface with a bent glass rod. To purify spores from vegetative debris, the suspension was treated with membrane sterilised lysozyme (concentration of Lysozyme C: 2100 U/mL) for 24 h at 5°C. Following the lysozyme treatment, the suspension was centrifuged five times for 20 min at 5°C in a JA-10 rotor of a Beckman J2-21 refrigerated centrifuge at 8000 rpm. Between each centrifugation the supernatant fluid was discarded and pellets were re-suspended in cold sterile distilled water. The final stock suspension containing 98.8% refractive spores was stored at a concentration of ca. 10^9 spores/mL in sterile distilled water in a refrigerator.

2.2.3. Estimation of heat- and radiation-resistance of B. cereus spores

The refrigerated stock spore suspension was centrifuged as above and the pellet was re-suspended in Sörensen's 1/15 M phosphate buffer of pH 7.0, adjusting the spore concentration to ca. 10^9/mL. This buffered spore suspension was heat activated at 60°C for 30 min and 3 mL aliquots were distributed into thin-walled glass vials. These sets were then heat treated in a NESLAB ultra-thermostat-controlled water bath, pre-warmed to 85, 90, 95, and 100°C, respectively. The survivors' viable counts were estimated by sampling at intervals and following a quick cooling of the samples. Calculation of the heating time started when the temperature of the samples reached the above values (the temperature of samples in the 100°C water bath reached 99.7°C).

Radiation treatment of the buffered spore suspension was performed in a self-shielded ^{60}Co gamma irradiator (RH-gamma-30) of the Central Food Research Institute, Budapest, at room temperature and at a dose rate of 4.6 kGy/h. The survival curve was estimated from irradiation doses of 0, 1.5, 3, 4.5, 6 and 7.5 kGy. The survival levels were determined by plate counting on BHI agar (Merck 1.10493, containing 1.5% agar) after 6 days of incubation at 30°C. The diluent for the serial dilutions contained 0.1% peptone and 0.9% NaCl in distilled water.

2.2.4. Preparation of meals for sous-vide and combined treatments

The ready-to-eat meals:

chopped beef with tomato sauce,
chopped beef with paprika gravy, and
smoked-cured pork with boiled bean sauce

were seasoned and pre-cooked according to their respective Hungarian kitchen formula.

2.2.5. Inoculated pack studies

Portions of the prepared meals mentioned above were vacuum packed using a MULTIVAC apparatus in WIPAK 'Multibarrier 4' foil pouches of 100 μm foil thickness and an oxygen-transmittance rate of 5 mL/24h (at 23°C, and 50% relative humidity (RH)). Before packaging, the sauce/gravy parts of the meal were inoculated with appropriate dilutions of heat-activated *B. cereus* spores and mixed with the meat-components in ca. 1:1 ratio (total average weight of 80–100 g per pouch). Uninoculated controls served for sensory testing and comparative analyses.

Experimental batches of the vacuum-packed meals were irradiated and/or heat treated. The irradiation doses and severity of cooking were applied according to the experimental design of the individual trials.

For *sous-vide* cooking, the vacuum-packed samples were immersed in a controlled temperature water-bath and the heat penetration followed by thermocouples with a recording instrument automatically calculating the heat treatment equivalent value for a selected reference temperature. After heat treatment, the pouches were cooled in cold water.

The experimental batches were transported to and from the irradiation source and the laboratory under refrigeration conditions at 7°C and stored overnight at this temperature between the heat and irradiation treatments.

The experimental batches for storage studies were kept for several weeks at 10°C, a typical abusive temperature, quite common in retail and household refrigerators. Microbiological parameters as well as the pH (by an electrical pH meter) and a_w (by a NOVASINA instrument as well as by the crystal liquefaction method) were estimated at the beginning of and at intervals throughout storage. For each investigation, duplicate samples per treatment were taken. Sensory evaluation and thiamine content were evaluated at the beginning of storage.

2.2.6. Microbiological testing

Meat and sauce/gravy components were homogenised for 1 min in a Stomacher homogeniser with sufficient liquid. Appropriate decimal dilutions were plated to estimate the:

– aerobic total viable cell counts (TGYE agar, Oxoid CM 325) at 30°C for 5 days;
– anaerobic total viable cell counts (TGYE agar, in anaerobic jar Oxoid H P11 system) at 30°C for 5 days;
– counts of sulphite-reducing clostridia (in DCA agar, Merck 1.10259, containing ferric-ammonium-citrate and sodium sulphite, in anaerobic jar) at 30°C for 5 days; and
– *B. cereus* counts on selective agar (Mossel's medium, Merck 1.05 267, with egg yolk and selective Polymyxin-B supplement) at 30°C for 48 h.

2.2.7. Sensory testing

Non-inoculated samples were used for sensory testing one day after the combination treatment by taste panels of 10-11 judges rating samples according to a 9 point hedonic scale for colour, smell, taste and texture of samples warmed up to the ready-for-consumption state. Hedonic scores were transformed to rank-sums and evaluated statistically by Kramer's method.

2.2.8. Estimation of concentrations of thiobarbituric acid reactive substances (TBARS)

As indices of lipid oxidation, the relative concentrations of thiobarbituric acid reactive substances (TBARS) were estimated from the meat component of the *sous-vide* products by the filtration and spectrophotometric method of Newburg and Concon [37]. The relative concentrations of TBARS were expressed as absorbance values/g sample at 532 nm wavelengths.

2.2.9. Estimation of thiamine content

The thiamine (vitamin B_1) content from non-inoculated untreated and combination-treated meals was determined in the third experimental series using smoked-cured pork in boiled beans sauce directly after the preservation treatment. The thiamine concentration was measured both according to György and Peason [38] by a microbiological method using *Lactobacillus fermenti* (ATCC 9338) as the test organism, and by a HPLC method according to Barna and Dworschák [39].

2.2.10. Application of nisin

In experiments where nisin was applied as additive, a Nisaplin preparate (containing 10^6 IU nisin/g) of the Applin and Barrett Ltd, UK was used. The stock suspension of the additive was prepared with 0.02 N HCl.

2.3. Results and discussion

2.3.1. Heat- and radiation-resistance of spores of the test organism

The heat-resistance curves were practically of the first order exponential (i.e. semi-logarithmically linear) in the heating periods investigated (at least in the first 4 log cycles of cell count reduction) providing the following D-values:

- $D_{85°C}$ = 38.6 min
- $D_{90°C}$ = 10.4 min
- $D_{95°C}$ = 2.5 min
- $D_{99.7°C}$ = 1.0 min.

From the log D *versus* temperature function, a *z*-value of 9.3°C was estimated by linear regression.

Johnson et al. [36], who investigated the heat-resistance of eight *B. cereus* strains, including atoxigenic as well as those associated with diarrheal and emetic food-borne illnesses, reported the following ranges of spore D-values: 32 to 106 min at 85°C, 6 to 50 min at 90°C, and 1.2 to 20 min at 95°C when heated in 25 mM sodium phosphate buffer, pH 7.0. The *z*-values for the eight strains ranged from 6.8 to 13.9°C. Apparently, various strains of *B. cereus* show a broad spread of heat-resistance. Findings from the current work are close to heat-resistance data reported recently in relation to two strains of psychrotrophic *B. cereus* heated in Tryptone Soya Broth [40].

The radiation survival curve of the spores of the test organism had a shoulder with an L value of ca. 1.5 kGy, and a $D_{exp.}$ value of ca. 2.0 kGy. This radiation-resistance is about the same as the literature data [26,29].

2.3.2. Sensory quality and microbiological safety and stability of combination-treated chopped beef in tomato sauce

For this product, two series of preservation experiments were performed. In the first series, a heat treatment of 32 min equivalent at 90°C was applied in combination with 4 kGy gamma irradiation whereas in the second series heat treatment of only 80°C for 11 min equivalent was combined with a 4 kGy dose. The tomato sauce component of this meal had an initial pH of 4.3, but during the preservation treatment and subsequent storage, the pH values of the beef and sauce gradually approached each other, and after one week of storage no significant differences were found (pH of meat-component = 5.4–5.6; pH of sauce-component = 5.4–5.5). During further storage, the overall pH values were always between 5.4 and 5.5. The a_w of the preserved meal was between 0.973 and 0.980 values at 20°C.

TABLE I. RESULTS OF SENSORY TESTING OF COMBINATION-PRESERVED *SOUS-VIDE* CHOPPED BEEF IN TOMATO SAUCE (heat treatment equivalent: 32 min at 90°C; irradiation dose: 4 kGy)

Samples	**Colour**		**Odour**		**Taste**		**Texture**	
	av. sc.	**rank s.**	**av. sc.**	**rank s.**	**av. sc.**	**rank s.**	**av. sc.**	**rank s.**
Prior combin. Treatment	8.22	11.5	7.78	15.0	5.89	23.5	6.33	22.5
KHS	6.67	25.0	6.22	19.5	6.78	16.5	8.0	15.0
KSH	7.56	17.5	6.22	19.5	7.44	14.0	7.89	16.5

where: av. sc. = average scores
rank s. = rank sums
KHS = uninoculated *sous-vide* meal first heated then irradiated
KSH = uninoculated *sous-vide* meal first irradiated then heated
Rank sums statistically not significantly different from each other
at 95% significance level: 13–23; at 99% significance level: 12–24.

The results of sensory testing performed on the second day after the combination treatments are given in Table I.

It can be seen from these data that the product was organoleptically acceptable after both orders of combination treatment. The statistical evaluation of rank sums showed that, as compared to the untreated meal, the colour scores diminished significantly during combination treatments but less so in samples where irradiation preceded the *sous-vide* cooking. On the other hand, the taste panellist gave, on average, higher taste-scores to the combination preserved meals than those that had not received the combination treatment.

The results of the microbiological tests are summarised in Table II.

One can conclude from Table II that when uninoculated, this ‘severely’ treated *sous-vide* meal, of relatively low pH and low a_w, had little surviving bacterial flora after the combination treatment. These flora were unable to grow during the 28 day storage period at 10°C and it even appeared that the counts were declining. If present, sulphite-reducing clostridia were under the detection limit throughout the storage.

In this meal inoculated to ca. 2.10^6 *B. cereus* spores/g, the irradiation treatment with 4 kGy caused an approximate 0.8 log cycle reduction in the *B. cereus* count whereas the heat treatment resulted in a 5 log cycle destruction of *B. cereus*. That is, the heat-resistance of the spores of the test organism was greater and the radiation-resistance was lower in this food product than in the phosphate buffer. Under the storage temperature, pH and water activity levels of this meal, the surviving *B. cereus* spores apparently did not grow out to vegetative cells during storage in the combination treated samples.

Regarding the effect of the order of treatments, even the irradiation dose of the above little lethality was able to sensitise the irradiated spores against the subsequent heat treatment resulting in 90% more reduction of the *B. cereus* count than the opposite order of treatment.

In the second experimental series, the *sous-vide* heat treatment was reduced to 10.8 min equivalent at 80°C. In this case, the pH of the combination-preserved product had a somewhat lower pH than in the first experimental series and the pH equilibrium during the *sous-vide* cooking resulted in a pH of 4.9–5.1, both in the meat-and sauce components. The a_w of the preserved meal was between 0.980 and 0.985 values at 20°C.

Unlike in the previous experimental series, the sensory testing showed that the combination treatments tended to reduce somewhat the taste scores only (Table III).

Table IV shows that the radiation treatment resulted in only a 0.5 log unit decrease, whilst the mild *sous-vide* cooking caused even less reduction in the viable count of the *B. cereus* spores. In spite of these small effects, a slight heat sensitisation could be noted in those combination-preserved samples which had been '*sous-vide*' cooked subsequent to irradiation as compared to the opposite order of treatments. Due to the low pH, the survivors were unable to grow during the 2 month storage at the 10°C. In general, the 'chopped beef in tomato sauce' type *sous-vide* meal, when so heat-treated as described above, seemed to be microbiologically safe and stabile for at least 4 weeks of refrigerated storage even without irradiation.

TABLE II. RESULTS OF THE MICROBIOLOGICAL ANALYSES OF *SOUS-VIDE* CHOPPED BEEF IN TOMATO SAUCE SAMPLES (heat treatment equivalent: 32 min at 90°C; irradiation dose: 4 kGy)

Samples	Storage time (days) at 10°C	Log CFU/g values			
		Aerobic total count	Anaerobic total count	Sulphite-red. clostridia	*B. cereus*
Tomato sauce	0	1.84	0.78	<0.48	–
KSH	0	0.48	<0.48	<0.48	–
KHS	0	0.48	<0.48	<0.48	–
B	0	6.30	6.32	<0.48	6.48
BS	0	5.71	5.78	<0.48	5.64
BH	0	1.43	1.52	<0.48	1.48
BSH	0	0.48	<0.48	<0.48	0.48
BHS	0	1.92	1.18	<0.48	1.48
BSH	7	1.08	0.48	<0.48	0.78
BHS	7	1.68	0.95	<0.48	1.68
BSH	14	<0.48	<0.48	<0.48	<1.48
BHS	14	1.43	0.48	<0.48	1.43
BSH	21	0.48	0.48	<0.48	<1.48
BHS	21	0.48	0.48	<0.48	0.48
BSH	28	<0.48	<0.48	<0.48	<1.48
BHS	28	1.48	0.78	<0.48	1.48
KSH	28	<0.48	<0.48	<0.48	–
KHS	28	<0.48	<0.48	<0.48	–

where: KSH = uninoculated *sous-vide* meal first irradiated then heated
KHS = uninoculated *sous-vide* meal first heated then irradiated
B = inoculated meal before combination treatment
BS = inoculated and irradiated meal before *sous-vide* cooking
BH = inoculated and cooked meal before irradiation
BSH = inoculated *sous-vide* meal first irradiated then heated
BHS = inoculated *sous-vide* meal first heated then irradiated.

2.3.3. Relative concentrations of TBARS in the meat component of the combination-preserved sous-vide beef in tomato sauce

These measurements were performed in the second experimental series of sous-vide beef in tomato juice meals. The TBARS absorbance values were estimated from triplicate samples at the beginning of and after 6 weeks of storage. They are summarised in Table V.

TABLE III. RESULTS OF SENSORY TESTING OF COMBINATION-PRESERVED *SOUS-VIDE* CHOPPED BEEF IN TOMATO SAUCE (heat treatment equivalent: 10.8 min at 80°C; irradiation dose: 4 kGy)

Samples	Colour		Odour		Taste		Texture	
	av. sc.	rank s.	av. sc.	rank s.	av. sc.	rank s.	av. sc.	rank s.
Untreated meal	7.6	18.5	7.5	17.5	7.2	15.5	7.6	18.5
KHS	7.5	18.5	7.1	22.5	6.4	22.5	7.5	20.0
KSH	7.3	23.0	7.2	20.0	6.5	22.0	7.3	21.5

Legends as in Table II
Rank sums statistically not significantly different from each other at 95% significance level: 15–25; at 91% significance level: 13–27.

TABLE IV. RESULTS OF MICROBIOLOGICAL ANALYSES OF *SOUS-VIDE* CHOPPED BEEF IN TOMATO SAUCES (heat treatment equivalent:10.8 min at 80°C; irradiation dose: 4 kGy)

Samples	Storage time (days) at 10°C	Log CFU/g values			
		Aerobic total count	Anaerobic total count	Sulphite-red. clostridia	*B. cereus*
Uninoculated prior combin. tr.	0	1.65	1.65	<0.48	-
KSH	0	<0.48	<0.48	<0.48	-
KHS	0	<0.48	<0.48	<0.48	-
B	0	6.32	6.30	<0.48	6.34
BS	0	5.84	5.77	<0.48	5.84
BH	0	5.95	6.04	<0.48	6.15
BSH	0	5.46	5.48	<0.48	5.58
BHS	0	5.60	5.59	<0.48	5.71
BSH	7	5.53	5.62	<0.48	5.41
BHS	7	5.52	5.48	<0.48	5.48
BSH	14	5.32	5.30	<0.48	5.34
BHS	14	5.53	5.57	<0.48	5.58
BSH	21	5.46	5.46	<0.48	5.20
BHS	21	5.71	5.60	<0.48	5.70
BSH	56	5.26	5.38	<0.48	5.30
BHS	56	5.23	5.38	<0.48	5.32

Legends: as in Table II.

where: KSH = uninoculated meal first irradiated (4 kGy) then heated (90°C, 32 min equivalent)
KHS = uninoculated meal first heated (90°C, 32 min equivalent) then irradiated (4 kGy).

TABLE V. RELATIVE TBARS CONCENTRATIONS AT THE BEGINNING AND AFTER 6 WEEKS OF STORAGE AT 10°C IN THE MEAT PART OF COMBINATION-PRESERVED *SOUS-VIDE* BEEF IN TOMATO SAUCE

Experimental batches	Relative TBARS concentrations (A_{532} nm/g)			
	1 day		42 days	
	Mean value	Standard deviation	Mean value	Standard deviation
KSH	0.228	0.05	0.177	0.04
KHS	0.138	0.004	0.101	0.05

It can be seen from Table V that the TBARS relative concentrations were statistically significantly higher in those samples that were first irradiated then cooked than in the case of the opposite sequence of treatments. No further increase, but some decrease, of the TBARS concentrations was observed in the stored samples.

2.3.4. Microbiological safety and stability of combination-preserved sous-vide chopped beef in paprika gravy

To gather experience with combination treatments of a 'more risky' product, one experiment was performed with *sous-vide* meal samples composed of chopped beef and 'paprika gravy', which was less acidic (pH = 5.9) and had a somewhat higher a_w (> 0.98) than those of the tomato sauce discussed previously. In this experiment a *sous-vide* cooking at 85°C for 10 min was combined with an irradiation dose of 2.5 kGy (either preceding or following the *sous-vide* cooking), and/or application of nisin at 10 µg/g concentration in the gravy. The inoculum level of *B. cereus* was approximately log cycles lower than the experiments described previously. The results of microbiological analysis are given in Table VI. This table shows that the 2.5 kGy dose resulted in only little destruction of *B. cereus*. At this, practically sub-lethal, dose level one could not expect a synergistic effect of the pre-irradiation combination with the heat treatment. Probably due to an overload of the cooking bath during this combination treatment, some of the BSH samples showed even erratically higher survival values in this experimental batch than the values in the BHS samples, which showed the additive effect expected from this type of combination treatment. According to the results of testing for the stored samples, the 2.5 kGy dose was not enough to increase the lethality of the moderate level of *sous-vide* cooking.

The addition of nisin proved to be very effective at the applied concentration, enhancing considerably the lethality of cooking, with the result that no survivors were detected during at least 4–5 weeks of storage at the abusive 10°C temperature.

2.3.5. Sensory quality and microbiological safety and stability of combination-preserved smoked-cured pork with boiled beans sauce

For this product four series of preservation experiments were performed. In the first experimental series, an average 29.5 min equivalent heat treatment was applied at 90°C either prior to or after an irradiation dose of 4 kGy. The pH values of the combination preserved samples were 6.14 for the boiled beans sauce and 6.32 for the meat component, directly after treatments, i.e. they were in close agreement to each other. In all samples during the 42 day storage period at 10°C, the pH was within the range of 6.12 and 6.22 in the product homogenised for the pH measurement. The a_w of both components of the combination preserved meal was practically the same value (a_w = 0.962 at 23°C). The results of sensory testing are given in Table VII. The table shows that the colour and taste scores of the combination-preserved samples were significantly lower than those of untreated meals. However, from the remarks written by the panellists one could note that the combination treated samples were distinguished from the untreated meal not because of an irradiation off-flavour, but because the bean sauce cooking extracted a more salty taste from the smoked-cured pork during the *sous-vide* cooking.

TABLE VI. MICROBIOLOGICAL EFFECTS OF GAMMA IRRADIATION AND/OR NISIN ON *SOUS-VIDE* CHOPPED BEEF IN PAPRIKA GRAVY (heat treatment equivalent: 10 min at 85°C; irradiation dose: 2.5 kGy; nisin in the gravy: 240 IU/g)

Samples	Storage time (days) at 10°C	Log CFU/g values			
		Aerobic total count	Anaerobic total count	Sulphite-red. clostridia	*B. cereus*
Uninoculated prior combin. tr.	0	1.97	1.78	<0.48	–
B	0	4.56(3.34*)	4.48(3.26*)	<0.48	4.62
BS	0	4.34	4.34	<0.48	4.34
BH	0	2.28	2.11	<0.48	2.38
BHN	0	<0.48	<0.48	<0.48	<1.48
BSH	0	3.08	3.11	<0.48	3.34
BHS	0	2.00	1.97	<0.48	2.16
BSHN	0	<0.48	<0.48	<0.48	<1.48
BHSN	0	<0.48	<0.48	<0.48	<1.48
BH	14	4.73	4.70	<0.48	4.89
BHN	14	<0.48	<0.48	<0.48	<0.48
BSH	14	4.65	4.69	<0.48	4.73
BHS	14	5.46	5.43	<0.48	5.53
BH	21	5.59	5.56	<0.48	5.65
BHN	21	<0.48	<0.48	<0.48	<1.48
BSH	21	5.56	5.46	<0.48	5.59
BHS	21	5.67	5.63	<0.48	5.75
BSHN	21	<0.48	<0.48	<0.48	<1.48
BHSN	21	<0.48	<0.48	<0.48	<1.48
BH	28	5.36	5.28	<0.48	5.43
BHN	28	<0.48	<0.48	<0.48	<1.48
BSH	28	5.60	5.49	<0.48	5.69
BHS	28	5.30	5.28	<0.48	5.38
BSHN	35	<0.48	<0.48	<0.48	<1.48
BHSN	35	<0.48	<0.48	<0.48	<1.48

where: *Spore count (surviving a heat treatment for 10 min at 80°C
BHN = inoculated *sous-vide* meal containing nisin
BSHN = inoculated *sous-vide* meal first irradiated then heated, containing nisin
BHSN = inoculated *sous-vide* meal first heated then irradiated, containing nisin
Other legends as in Table II

TABLE VII. RESULTS OF SENSORY TESTING OF COMBINATION-PRESERVED *SOUS-VIDE* SMOKED-CURED-PORK IN BOILED BEANS SAUCE (heat treatment equivalent: 29.5 min at 90°C; irradiation dose: 4 kGy)

Samples	Colour		Odour		Taste		Texture	
	av. sc.	rank s.	av. sc.	rank s.	av. sc.	rank s.	av. sc.	rank s.
Untreated meal	8.0	12.5	7.3	15.5	6.7	12.0	7.8	17.0
KHS	6.3	23.5	6.0	22.5	5.5	21.5	7.2	21.5
KSH	6.3	24.0	6.1	22.0	4.7	26.5	7.0	21.5

Legends as in Table III
Rank sums statistically not significantly different from each other
at 95% significance level: 15–25; at 99% significance level: 13–27.

TABLE VIII. RESULTS OF THE MICROBIOLOGICAL ANALYSES OF *SOUS-VIDE* SMOKED-CURED PORK IN BOILED BEANS SAUCE (heat treatment equivalent: 29.5 min at 90°C; irradiation dose: 4 kGy).

Samples	Storage time (days) at 10°C	Log CFU/g values			
		Aerobic total count	Anaerobic total count	Sulphite-red. clostridia	*B. cereus*
Uninoculated					
Untreated meal	0	5.51	5.36	<0.48	–
KSH	0	0.48	<0.48	<0.48	–
KHS	0	0.95	0.48	<0.48	–
B	0	6.93 (4.69*)	6.46	<0.48	6.93 (4.69*)
BS	0	4.99	4.86	<0.48	5.18
BH	0	1.48	1.32	<0.48	1.48
BSH	0	0.48	<0.48	<0.48	<1.48
BHS	0	1.73	1.32	<0.48	<1.48
BSH	7	<0.48	<0.48	<0.48	<1.48
BHS	7	0.78	<0.48	<0.48	<1.48
BSH	14	<0.48	<0.48	<0.48	<1.48
BHS	14	0.95	0.48	<0.48	<1.48
BSH	28	<0.48	<0.48	<0.48	<1.48
BHS	28	0.95	<0.48	<0.48	<1.48
BSH	42	<1.48	<0.48	<0.48	<0.48
BHS	42	<1.48	<0.48	<0.48	<0.48

*Spore count (surviving a heat treatment for 10 min at 80°C
Legends as in Table IV.

The results of the microbiological analyses are given in Table VIII. It shows that the bacterial flora of the untreated meal diminished as an effect of the combination treatments. Here again the 'first irradiation then heat treatment' sequence seemed to be more efficient than the opposite order of treatment. The irradiation treatment alone reduced the total counts by 2 log cycles, whereas the *sous-vide* cooking alone by approximately 5.5 log cycles. However, Table VIII shows that the (*B. cereus)* spore count was more than 2 log units lower than its TVC count. Therefore, one has to assume that more than 99% of the spores of the inoculum germinated in this low-acid product before the irradiation or heat treatment. In this product, the 4 kGy dose as a single treatment was ineffective in diminishing further the number of still dormant spores, whereas, the heat treatment reduced their number by ca. 3 log units. These observations showed that this product was quite radiation-protective for the spores as compared to the phosphate buffer but the heat-resistance was not influenced. In spite of this increased radiation tolerance, even this sub-lethal irradiation dose seemed to result in a 'heat sensitisation by irradiation effect' of more than 90% additional reduction of survivors in the case of the first irradiation then heat treatment combination. The survivors were unable to grow but declined somewhat during the 42 day storage at 10°C. Neither were any sulphite-reducing clostridia found over their detection limit in this product.

In the second experimental series with this product, a mild heat treatment of 14.5 min equivalent at 80°C was applied alone or in combination with a 4 kGy irradiation dose. The equilibrium pH of the combination-preserved product was 6.09 for the boiled beans sauce and 6.14 for the meat component. The a_w of both components was 0.988 at 21°C. The sensory testing (Table IX) once again showed some colour changes as compared to the untreated meal, but, due to the less severe *sous-vide* cooking, the taste scores were higher for the combination treated samples (less salty taste) than in the

previous experimental series. Thus, the sensory quality of all samples was found to be acceptable on the day following the combination preservation.

The microbiological analyses (Table X) showed that in this case the microbial count of the untreated meal was very high, but almost all of the bacterial flora was composed of heat sensitive cells (perhaps lactic acid bacteria from the smoked-cured meat component). In this case the majority (almost 99%) of the spore inoculum germinated again prior to the combination treatment. Both the 4 kGy dose and the mild *sous-vide* cooking were sub-lethal for the remaining dormant spores. In spite of this, the combination of the two sub-lethal treatment resulted already in some reduction of the count of the spores (0.3 log-unit in the first heated than irradiated, and 0.7 log unit decrease in the first irradiated than heated samples). The latter synergistic effect remained noticeable during storage when, due to the relatively high a_w, the permissive pH level and the abusive storage temperature of 10°C, the surviving fraction of *B. cereus* could grow to ca. 1000-fold level during one week, reaching a viable cell count of the order of magnitude of 10^7/g, still not producing any obvious spoilage symptoms in this meal.

TABLE IX. RESULTS OF SENSORY TESTING OF COMBINATION-PRESERVED SMOKED-CURED PORK IN BOILED BEANS SAUCE (heat treatment equivalent: 14.5 min at 80°C; irradiation dose: 4 kGy)

Samples	Colour		Odour		Taste		Texture	
	av. sc.	rank s.	av. sc.	rank s.	av. sc.	rank s.	av. sc.	rank s.
Untreated meal	8.09	13	7.55	18	7.55	16	7.18	20
KHS	6.00	27	7.18	21	6.82	22.5	7.00	22
KSH	6.18	26	6.18	27	5.91	27.5	6.55	24

Legends as in Table III
Rank sums statistically not significantly different from each other
at 95% significance level: 16–28; at 99% significance level: 15–29.

It was clear from these results that, for demonstrating more effectively the efficiency of a combination of gamma irradiation with *sous-vide* cooking, a more severe treatment should be applied than in the second experimental series. Therefore, in the third series of studies, a dose of 5 kGy instead of 4 kGy, and a *sous-vide* cooking of 11.4 min equivalent at 85°C (instead of 80°C) were applied. According to the literature [29], approximately 99% of the spores of non-proteolytic *C. botulinum* would be inactivated by such a heat treatment.

The pH values of the combination-treated meals were 6.15 for the boiled beans sauce, and 6.04 for the meat component, while the a_w was equilibrated to an a_w equal to 0.983 in both components when measured at 21°C.

Sensory testing (Table XI) showed that both the combination treated products were acceptable, although a colour change was noted as compared to the untreated meal, and the *sous-vide* cooking changed the taste by causing a loss of the ‘smoky’ taint and increasing the salty taste in the product.

TABLE X. RESULTS OF MICROBIOLOGICAL ANALYSES OF *SOUS-VIDE* SMOKED-CURED PORK IN BOILED BEANS SAUCE (heat treatment equivalent: 14.5 min at 80°C; irradiation dose: 4 kGy)

Samples	Storage time (days) at 10°C	Log CFU/g values			
		Aerobic total count	Anaerobic total count	Sulphite-red. clostridia	*B. cereus*
Uninoculated					
untreated meal	0	8.41	8.51	<0.48	–
KSH	0	0.95	0.78	<0.48	–
KHS	0	0.95	<0.48	<0.48	–
B	0	8.40 (4.32*)	8.41	<0.48	6.15 (4.32*)
BS	0	4.85	4.74	<0.48	4.70
BH	0	4.56	4.53	<0.48	4.30
BSH	0	3.65	3.54	<0.48	3.63
BHS	0	4.11	4.18	<0.48	4.00
BSH	7	5.49	5.48	<0.48	5.52
BHS	7	6.86	6.89	<0.48	6.82
BSH	14	6.74	6.65	<0.48	6.80
BHS	14	6.96	6.91	<0.48	7.00
BSH	21	6.93	6.84	<0.48	7.04
BHS	21	6.97	6.98	<0.48	7.23
BSH	28	6.34	6.34	<0.48	6.46
BHS	28	6.48	6.46	<0.48	6.58
KSH	28	0.78	0.95	<0.48	–
KHS	28	0.78	<0.48	<0.48	–

*Spore counts (surviving a heat treatment for 10 min at 80°C)
Legends as in Table IV.

TABLE XI. RESULTS OF SENSORY TESTING OF COMBINED *SOUS-VIDE* SMOKED-CURED PORK IN BOILED BEANS SAUCE (heat treatment: 11.4 min equivalent at 85°C; irradiation dose: 5 kGy)

Samples	Colour		Odour		Taste		Texture	
	av. sc.	rank s.	av. sc.	rank s.	av. sc.	rank s.	av. sc.	rank s.
Untreated meal	8.5	11.5	7.4	16.5	6.9	20.0	7.8	17.5
KHS	7.1	23.0	6.3	22.5	6.1	20.0	7.3	22.5
KSH	6.8	25.5	6.5	21.0	6.2	20.0	7.5	20.0

Rank sums statistically not significantly different from each other at 95% significance level: 15–25; at 99% significance level: 13–27.

TABLE XII. RESULTS OF MICROBIOLOGICAL ANALYSES OF *SOUS-VIDE* SMOKED-CURED PORK IN BOILED BEANS SAUCE (heat treatment equivalent: 11.4 min at 85°C; irradiation dose: 5 kGy)

Samples	Storage time (days) at 10°C	Log CFU/g values			
		Aerobic total count	Anaerobic total count	Sulphite-red. clostridia	*B. cereus*
Uninoculated					
untreated meal	0	8.53	8.36	<0.48	–
KH	0	2.40	1.18	<0.48	–
KSH	0	1.08	<0.48	<0.48	–
KHS	0	1.26	<0.48	<0.48	–
B	0	8.32 (3.48)*	8.38 (2.08*)	<0.48	4.00 (2.32)*
BS	0	2.48	2.38	<0.48	1.78
BH	0	2.23	1.71	<0.48	2.23
BSH	0	1.18	0.78	<0.48	<1.48
BHS	0	1.82	1.65	<0.48	<1.48
BH	7	6.89	6.80	<0.48	6.65
BSH	7	0.78	0.78	<0.48	<1.48
BHS	7	3.15	3.15	<0.48	3.15
BH	14	7.36	7.36	<0.48	7.30
BSH	14	0.78	<0.48	<0.48	<1.48
BHS	14	5.46	5.34	<0.48	5.41
BH	21	7.15	7.20	<0.48	7.40
BHS	21	6.45	6.51	<0.48	6.59
BH	28	6.91	6.86	<0.48	6.91
BSH	28	<0.48	<0.48	<0.48	<1.48
BHS	28	7.34	7.36	<0.48	7.30
BSH	56	1.26	0.78	<0.48	<0.48

*Spore counts (surviving a heat treatment for 10 min at 80°C)
Other legends as in Table IV.

The microbiological results are summarised in Table XII. It can be seen that from the initial level of 4 log units of *B. cereus* spores/g, 2.32 log units/g remained in the dormant (heat-resistant) state in the meal until the combination preservation. The irradiation treatment alone resulted in only a 0.5 log unit decrease of the remaining *B. cereus* spores while the heat treatment alone resulted in only a 0.1 log-unit (i.e. non-significant) decrease. At the same time, the *B. cereus* counts in the combination treated meals for both sequences of treatments were lower than its actual detection limit of 1.48 log units. Subsequently, however, both the *B. cereus* and the total bacterial counts showed completely different patterns during storage, depending on the order of the treatments. Although less quickly than in the 'cooked-only' samples, the total bacterial counts and the *B. cereus* counts of the 'first heated then irradiated' samples were increasing steadily during storage. On the other hand, no bacterial growth was observed during storage for 56 days in the 'first irradiated then heated' samples. The surviving *B. cereus* level was less than the 0.48 log unit/g (3 cells/g) even at the end of the 8 week storage period. After 4 weeks of storage both the only cooked and the 'first cooked then irradiated' samples had pH values of 5.97–6.00, while pH values of 6.09–6.16 were measured in the microbiologically stabile 'first irradiated than cooked' meals.

The fourth experimental series aimed to investigate how the application of a low concentration of nisin (80 IU nisin/g in the sauce component) could enhance the microbiological efficiency of a *sous-vide* cooking (at 90°C for 10 min), considered to be a heat treatment satisfactory for 6D-inactivation of non-proteolytic *C. botulinum*, if elimination of the more heat-resistant psychrophilic *B. cereus* spores would be also required. The effect of the same nisin addition on the efficiency of a '*sous-vide* cooking plus irradiation' type of combination treatment (using a dose of 5 kGy) was also investigated. The pH of the *sous-vide* meal was 6.0 whereas a_w was equal to 0.976 in this experimental series.

TABLE XIII. MICROBIOLOGICAL EFFECTS OF GAMMA IRRADIATION AND/OR NISIN APPLICATION ON *SOUS-VIDE* SMOKED-CURED PORK IN BOILED BEANS SAUCE (heat treatment equivalent: 10 min at 90°C; irradiation dose: 5 kGy; nisin in the sauce: 80 IU/g)

Samples	Storage time (days) at 10°C	Log CFU/g values			
		Aerobic total count	Anaerobic total count	Sulphite-red. clostridia	*B. cereus*
Uninoculated prior comb. tr.	0	7.80	7.74	1.38	–
B	0	7.89 (6.15*)	7.82	1.56	7.20 (6.36*)
BH	0	4.15	4.49	<0.48	4.40
BHN	0	3.36	2.58	<0.48	2.68
BS	0	6.04	5.98	<0.48	6.15
BHS	0	3.08	2.93	<0.48	3.18
BHSN	0	2.85	2.28	<0.48	2.56
BH	7	7.00	7.00	<0.48	7.08
BHN	7	3.76	3.54	<0.48	3.64
BHS	7	1.86 (*B.c.* 1.38)	1.26	<0.48	<1.48
BHSN	7	1.32 (*B.c.* 0.48)	0.48	<0.48	<1.48
BH	14	6.65	6.73	<0.48	6.71
BHN	14	5.51	5.56	<0.48	5.67
BHS	14	1.94 (*B.c.* 1.71)	1.08	<0.48	<1.48
BHSN	14	<0.48	<0.48	<0.48	<0.48
BH	21	6.81	6.76	<0.48	6.86
BHN	21	4.95	4.93	<0.48	4.86
BH	28	6.41	6.40	<0.48	6.38
BHN	28	3.79	3.73	<0.48	3.81
BHS	28	2.08 (*B.c.* 1.98)	1.91	<0.48	<1.48
BHSN	28	1.26 (*B.c.* 0.48)	<0.48	<0.48	<1.48
BHS	42	2.18 (*B.c.* 1.97)	1.73	<0.48	2.68
BHSN	42	0.95 (*B.c.* 0.48)	0.78	<0.48	<1.48

*Spore count (surviving a heat treatment for 10 min at 80°C).
Legends: as in Table VI.

The results of the microbiological analyses are given in Table XIII. It can be seen that the irradiation treatment alone caused only approximately one log reduction of the viable count of *B. cereus* in the product while the *sous-vide* cooking resulted in about 3 log-units inactivation. The presence of nisin in the bean sauce enhanced the proportion of *B. cereus* cells inactivated by the *sous-vide* cooking or the combination of the above physical treatments. Comparing the fate of the survivors during storage of the nisin-containing *sous-vide* meal with that of those in the combination-treated

meals showed that in the non-irradiated *sous-vide* cooked meal a slow re-growth of the survivors occurred in spite of the nisin additive. The survival level stagnated more or less in the irradiated *sous-vide* meal, whereas it declined so much during storage of the nisin containing combination-preserved samples that the product was microbiologically stabile and free from detectable *B. cereus* under the testing conditions even after 42 days storage at the abusive 10°C temperature.

2.3.6. Relative concentrations of TBARS in the meat part of the combination-preserved sous-vide smoked-cured pork in boiled beans sauce

These measurements were performed on the combination preserved samples from the second experimental series. The results (Table XIV) showed that no significant differences between the untreated and the combination-preserved meals at the beginning of storage, and during the 4 weeks of storage at 10°C no increase, but rather a decrease of the relative concentrations of TBARS values might occur.

TABLE XIV. RELATIVE TBARS CONCENTRATIONS AT THE BEGINNING AND AFTER 4 WEEKS OF STORAGE AT 10°C IN THE MEAT PART OF COMBINATION-PRESERVED *SOUS-VIDE* SMOKED-CURED PORK IN BOILED BEANS SAUCE

Experimental batches	Relative TBARS concentrations (A_{532} nm values)			
	1 day		28 days	
	Mean value	Standard deviation	Mean value	Standard deviation
Untreated meal	0.324	0.09	-	-
KSH	0.296	0.12	0.251	0.07
KHS	0.340	0.12	0.107	0.10

where:
KSH = uninoculated meal first irradiated (4 kGy) then heated (80°C, 14.5 min equivalent)
KHS = uninoculated meal first heated (80°C, 14.5 min equivalent) then irradiated (4 kGy).

TABLE XV. THE THIAMINE CONTENT OF SAMPLES OF *SOUS-VIDE* SMOKED-CURED PORK IN BOILED BEANS AS AFFECTED BY THE COMBINATION PRESERVATION

Samples	Thiamine content (μg/100g)			
	Microbiological estimation			
	Mean value	Standard deviation	Replicate number	HPLC measurement
Untreated meal	327	28.8	6	314
5 kGy + 85°C, 11.4 min	184	15.6	6	201
85°C, 11.4 min + 5 kGy	192	14.8	7	208

2.3.7. Effect of the combination preservation on the thiamine content of the sou-vide meal of smoked-cured pork in boiled beans sauce

Thiamine is the most radiation-sensitive of the water-soluble vitamins [41]. The thiamine content of the samples of *sous-vide* meals 'smoked-cured pork in boiled beans sauce' preserved by combination of 5 kGy irradiation and cooking at 85°C, 11.4 min equivalent are shown in Table XV in comparison with the untreated meals. The concentrations were estimated directly after the combination treatments.

The results revealed that, on average, an approximate 40% loss of thiamine occurred as an effect of combination treatments, and the residual levels of the vitamin were not significantly different between the two sequences of the individual treatments. The magnitude of the thiamine losses are

similar to what one could expect from those reported in the literature for other types of food (irradiated-fried and fried-irradiated bacon [42], irradiated-cooked cod [43], irradiated-cooked legumes [44]. Thayer et al. [42], however, noted greater losses of thiamine when irradiation preceded frying than when bacon was first fried and then irradiated.

3. CONCLUSIONS

It is remarkable that most of the meals prepared during the course of this experimental work did not contain sulphite-reducing clostridia as 'natural' contaminants above their detection limit (ca. 3 CFU/g), and if present at all, they remained under this level during all storage experiments.

From the experimental results gathered during these investigations, it can be concluded that medium-dose ionising irradiation of prepared meals prior to their *sous-vide* cooking may increase considerably their microbiological safety as tested using the psychrotrophic *B. cereus* and, if required, it can increase the keeping quality. It has to be noted, however, that in the case of thiamine, the most radiation-sensitive of the water-soluble vitamins, a substantial reduction in concentration may occur.

Each of the experiments undertaken showed that some heat-sensitisation of the bacterial spores also occurs in *sous-vide* meals by the preliminary irradiation treatment. This has been amply demonstrated already in the past *in vitro* with pure cultures. However, due to the complex nature of the bacteriological effect of the combination treatment, compositional differences, and diversity of stress factors occurring during processing and storage of the meals, the optimal combination treatment should be established on an 'item by item' basis.

Because the considerably large 'shoulder' part in the irradiation survival curve of *B. cereus* spores, low-dose irradiation does not seem to be effective enough for low acid *sous-vide* food products containing radio-protective organic components. All samples of the *sous-vide* meals were, however, of acceptable sensory quality even after the medium doses of 4 or 5 kGy were applied. Limited analytical tests did not reveal lipid oxidation of practical importance, probably due to the vacuum packaging and the 'antioxidative' effect of the vegetable garnishes. If the threshold doses for 'irradiation off-flavour are lower than these, and/or lower doses are necessary in order to avoid eventual thiamine losses, the microbiological efficiency of the treatments against spore formers could be increased by small concentrations of nisin, a sporostatic bacteriocin, thereby, reducing the dose requirement of the combination preservation.

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EFFECT OF GAMMA IRRADIATION ON THE SHELF-LIFE AND NUTRITIONAL QUALITY OF READY MADE MEALS

M.F. PATTERSON, E.M. STEWART

Food Science Division, Department of Agriculture and Rural Development &
Department of Food Science,
The Queen's University of Belfast, United Kingdom

Abstract

The effect of ionizing radiation on the microbiological and nutritional quality of chilled ready meals was assessed. The ready meals used for the purposes of this experimental work are normally produced for consumption in a number of hospitals in the Belfast area or for use as 'meals-on-wheels' for elderly people. The meals analysed consisted of roast pork and gravy, boiled potatoes and mixed vegetables or pureed portions of chicken and gravy, boiled potatoes and garden peas. Following arrival at the laboratory, the meals were either left unirradiated or irradiated with doses ranging from 1 to 3 kGy after which they were stored for up to 14 days at 3°C or 10°C being sampled at suitable intervals. Total viable counts (TVCs) were performed on the samples along with analyses for thiamine (vitamin B1) and ascorbic acid (vitamin C). The usefulness of the 2-alkylcyclobutanone and electron spin resonance (ESR) spectroscopy methods for the detection of irradiation treatment of the pureed meals was also assessed. Results showed that an irradiation dose of 2 kGy can be used to extend the shelf-life of ready meals for up to 14 days and that the irradiated meals must be stored under good refrigeration conditions (<3°C) in order to obtain maximum benefit from the irradiation treatment. In terms of nutritional quality, it was found that losses of thiamine and ascorbic acid due to irradiation treatment were comparable to losses induced by cooking. Storage over the 14 day period at 3°C was found to reduce the thiamine and ascorbic acid content of the roast pork and boiled potatoes, respectively, in the whole meals. The thiamine content of the pureed chicken and gravy was not affected by storage. The overall thiamine and ascorbic acid content of the whole and pureed meals, including the non-irradiated controls, was found to be low especially for the vegetables. The 2-alkylcyclobutanone method was successfully used to detect irradiation treatment in the pureed chicken and gravy with both 2-dodecylcyclobutanone (2-DCB) and 2-tetradecylcyclobutanone (2-TCB) being detected over the dose range of 1 to 3 kGy. Neither compound was detected in the non-irradiated control samples. Both 2-DCB and 2-TCB were found to increase linearly in concentration with increasing irradiation dose and there was no significant diminution in concentration when samples were stored for 14 days at 3°C. ESR spectroscopy was successfully used to detect irradiation treatment via the cardboard packaging of the pureed meals. ESR spectra typical for irradiated samples containing cellulose were derived from the packaging of samples irradiated over a dose range of 1 to 5 kGy and were absent in non-irradiated samples. The intensity of the ESR signals increased with increasing irradiation dose.

1. INTRODUCTION

The market for 'cook-chill' ready meals has expanded significantly in recent years. This specific category of food has been defined as a catering system based on the full cooking of food followed by fast chilling and storage in controlled temperature conditions (0–3°C) and subsequent thorough re-heating before consumption [1]. Such meals cover a wide range of commodities including meat, poultry, fish, vegetables, pasta and desserts [2] and are used at home by consumers and by the catering industry for use, for example, as hospital meals or meals-on-wheels. These products have a relatively short shelf-life with a recommended maximum shelf-life of 5 days at 0–3°C including the day of cooking. In addition, there are other concerns regarding microbiological quality [3–5], reduced sensory quality [6] and decreased nutritive value [7]. It has been suggested that low-dose irradiation could be used to extend the shelf-life of these products while at the same time reducing the risk of

food poisoning [8]. However, it should always be stressed that, as is the case for any application of irradiation, the technology should be used as an additional preservation hurdle rather than as a necessity due to lack of Good Manufacturing Practice (GMP).

Promising results into the use of the technology for the treatment of chilled ready meals have been reported by McAteer et al. [9] and Patterson et al. [10] who investigated the effects of gamma irradiation and chilled storage on the microbiological and sensory quality of non-irradiated and irradiated chilled ready meals. These meals, consisting of roast beef and gravy, cauliflower and white sauce together with roast and mashed potatoes, were assessed at intervals during 15 days storage at 2–3°C. The non-irradiated controls usually had unacceptable counts after 7 days of storage. In general, irradiation to 2 and 3 kGy initially reduced the total viable counts of the components to below the detection limit (100 cells/g) and counts did not increase significantly during storage. Sensory results, however, were less promising. Sensory profiling techniques, using a trained panel of assessors, showed significant differences between the non-irradiated and irradiated meal components during storage. The changes were most apparent in the cauliflower and potato components and most often occurred in the colour, appearance and textural attributes tested. For example, there was a fawn/brown discoloration and more mushy texture associated with the irradiated cauliflower, while irradiated mashed potato became more sticky and greyer in colour. These changes were undesirable and would limit the acceptance of the irradiated meals. Further work was carried out to select more appropriate meal components that would not be significantly affected by irradiation. The producer of the roast beef dinner also included carrot and broccoli in some of the other meal formulations. The effect of irradiation (2 kGy) on the sensory quality of these ready meal vegetables was investigated during chill storage. In general, there was little difference between the non-irradiated controls and the irradiated samples. Some of the flavour attributes, especially of carrots, were affected by irradiation treatment. However, these effects were variable and definitive effects of irradiation or storage were not apparent. It was concluded that carrot and broccoli would be suitable vegetables for inclusion in an irradiated meal. Kilcast [11] reported that the production of off-flavours was a limiting factor in the acceptability of a range of hot and cold irradiated (1–4 kGy) ready meals. Nevertheless, it was suggested that these changes could be kept to a minimum when items of strong flavour were included in the meals.

Stevenson et al. [12] reported on a consumer trial carried out to assess the acceptability of a chilled irradiated (2 kGy) and non-irradiated ready meal consisting of roast beef and gravy, Yorkshire pudding, carrot, broccoli and roast potato, 4 days after treatment. This work was carried out to establish if sensory changes could be detected by a large untrained taste panel. One hundred and seven consumers participated in this trial and found the irradiated meal moderately to very acceptable and not significantly different to the irradiated meal. The beef and gravy component of the meal was most liked by consumers and it was found that appearance and aroma were likely to be more important than flavour or texture in the overall assessment of the meals. It therefore appeared that untrained consumers may not be able to detect differences noted by trained taste panellists. Overall, it was concluded that irradiation (2 kGy) of the roast beef meal can give improved microbiological safety and extend shelf-life without significantly affecting sensory quality.

The nutritional quality of ready meals is becoming increasingly relevant as these products are becoming more important in the diet. Bognar [7] carried out a detailed study of the nutritive content of a variety of commercially prepared chilled, frozen, sterilised and fresh ready meals packaged into multi-portion trays. It was found that on the day after preparation, there were no significant differences in the protein, fat, carbohydrate, thiamine and riboflavin content of the meals. Any fluctuations that occurred were due largely to differences in the raw materials rather than being a result of the production and preservation method used. The major limiting nutritive factor of ready meals was reported to be their ascorbic acid content, with 30–90% lower levels occurring in chilled and frozen meals (especially vegetable portions) compared to freshly prepared meals. The losses could be minimised by rapid chilling from 80°C to 15°C within 2 h and from 15°C down to 2°C within 20 h. During storage at 2°C the ascorbic acid content of the chilled meals decreased by 4–16% per day, the thiamine content by 0.3–4.4% per day and the riboflavin content by 0.6–2.5% per day. The thiamine and riboflavin content remained practically unchanged during re-heating but the ascorbic acid in

chilled meals was reduced by 23–49%. It has also been well documented that a number of vitamins are sensitive to ionizing irradiation [13–17].

On the basis of the promising results obtained by McAteer et al. [9], Patterson et al. [10] and Stevenson et al. [12] it was decided to investigate the effect of irradiation on other types of ready meals. The work reported in this paper was carried out in collaboration with a local catering production unit which produces 45,000 chilled meals per week for local hospitals, day centres, residential homes in the Belfast area. In addition to microbiological studies, it was decided to include studies on the effect of irradiation and chilled storage on vitamin content of the meals as well as investigating suitable methods for detection of irradiation treatment.

2. MATERIALS AND METHODS

2.1. Effect of gamma irradiation and chilled storage on the microbiological quality and vitamin content of chilled ready meals consisting of roast pork and gravy and mixed vegetables

2.1.1. Experimental design

On three separate occasions, a batch of chilled ready meals, consisting of roast pork and gravy and mixed vegetables, were obtained from a local catering manufacturer. The components of the meal had been cooked and cooled separately prior to portioning into heat-resistant cardboard trays that were then sealed with a polyester film. The meals were obtained from the processor within 6 h of portioning and irradiated on the same day. Irradiation was carried out using a ^{60}Co source (Gammabeam 650, Nordion International Inc., Canada) at a dose rate of 0.942 kGy/h and at a temperature of 2–3°C. The meals were irradiated to 1, 2 or 3 kGy or left unirradiated to serve as controls. Gammachrome YR dosimeters (AEA Technology, Harwell, UK) were used to confirm the dose received by the meals. The change in absorbance at 530 nm was measured spectrophotometrically and the corresponding doses obtained from a calibration graph by the National Physical Laboratory (NPL), Teddington, UK [9]. Following irradiation treatment the meals were analysed for microbiological and nutritional quality as described in the following sections.

2.1.2. Microbiological quality

On each of three occasions, eight meals were irradiated to 1, 2 or 3 kGy or left unirradiated as controls (two at each treatment). They were then stored at either 3 or 10°C and sampled at intervals for up to 15 days (Figs 1 and 2). The meals were either analysed without further heating or were reheated according to the manufacturer's instructions (160°C for 25 min) before analysis.

The total viable counts (TVCs) (aerobic and anaerobic) at both 25°C and 37°C were obtained as described previously [9].

2.1.3. Vitamin content

On each three occasions, three batches of eight meals were selected for vitamin analysis. Within each batch, two meals were irradiated with a dose of 1, 2 or 3 kGy while two were left unirradiated to serve as controls. One batch of meals was analysed immediately after irradiation while the other two batches were stored for 7 or 14 days at 3 ± 1°C. One meal at each dose level was analysed in the fresh state while the other meal was reheated in a convection oven at 160°C for 25 min and allowed to cool at ambient temperature prior to analysis. Vitamin C analysis was carried out on both the potato and mixed vegetable components of each meal while the pork was analysed for thiamine content.

2.1.3.1. Vitamin C (ascorbic acid) determination

Sub-samples of potatoes and mixed vegetables weighing 45–50 g were taken for analysis. Green beans and peas were analysed for the meals received from the processor on the first occasion while peas only were used on the second and third occasions due to the absence of green beans.

Each sample was analysed for vitamin C content as described by Graham and co-workers [18,19]. Total ascorbic acid (TAA), ascorbic acid (AA) and dehydroascorbic acid (DHAA) concentrations were determined in duplicate for each sample using ion-exclusion high performance liquid chromatography (HPLC).

2.1.3.2. Vitamin B1 (thiamine) determination

The thiamine content of the pork component of each meal was carried out using a modification of the HPLC method of Finglas and Faulks [20] as outlined by Graham et al. [21]. Each sample was analysed in duplicate.

2.2. Effect of gamma irradiation and chilled storage on the microbiological quality and vitamin content of chilled pureed chicken and gravy, garden peas and boiled potatoes

2.2.1. Experimental design

This work was carried out on meals consisting of pureed chicken and gravy, garden peas and potatoes. The pureed meal components were packaged individually in laminated cardboard trays and sealed by a clear film.

The samples were given irradiation doses of 1, 2 or 3 kGy or left unirradiated to serve as controls and analysed following storage for 1, 7 or 14 days at 4°C. The meals were analysed in the chilled state and after re-heating according to the manufacturer's instructions as before. The pureed chicken and gravy was analysed for thiamine while the pureed peas and potatoes were analysed for ascorbic acid. Analysis for vitamins was carried out as described in previous sections.

TVCs (aerobic and anaerobic) at both 25°C and 37°C were obtained as described previously [9].

2.2.2. Detection of irradiation treatment using standardised methods

Pureed chicken and gravy meals in their final packaging, as described in Section 2.2.1., were given irradiation doses of 1, 3 or 5 kGy, or left unirradiated to serve as controls and stored for 1, 7 or 14 days storage at 4°C. Following storage the food samples were analysed for 2-alkylcyclobutanones according to European Standard EN1785 [22–24]. Samples were analysed for 2-dodecylcyclobutanone (2-DCB) and 2-tetradecylcyclobutanone (2-TCB).

The packaging of the pureed meals was analysed by electron spin resonance (ESR) spectroscopy according to European Standard EN1787 [25,26]. Since the packaging is produced from cardboard, which contains cellulose, it was envisaged that irradiation treatment should be easily detected by the presence of the characteristic ESR signal from cellulose. Three samples were analysed from each pureed meal package.

3. RESULTS AND DISCUSSION

3.1. Effect of gamma irradiation and chilled storage on the microbiological quality and vitamin content of chilled ready meals consisting of roast pork and gravy and mixed vegetables

3.1.1. Microbiological quality

There was considerable variation between batches in the initial microbiological quality of the meal. This may have been due to variation in processing time between the individual meal components being cooked before they were portioned. Overall, there was no significant difference (p>0.5) in the TVCs obtained either aerobically or anaerobically or at 25°C and 37°C. Therefore, the results for each sampling day were combined together. However, irradiation dose and storage temperature did have a significant effect on numbers during storage as can be seen from Figs 1 and 2.

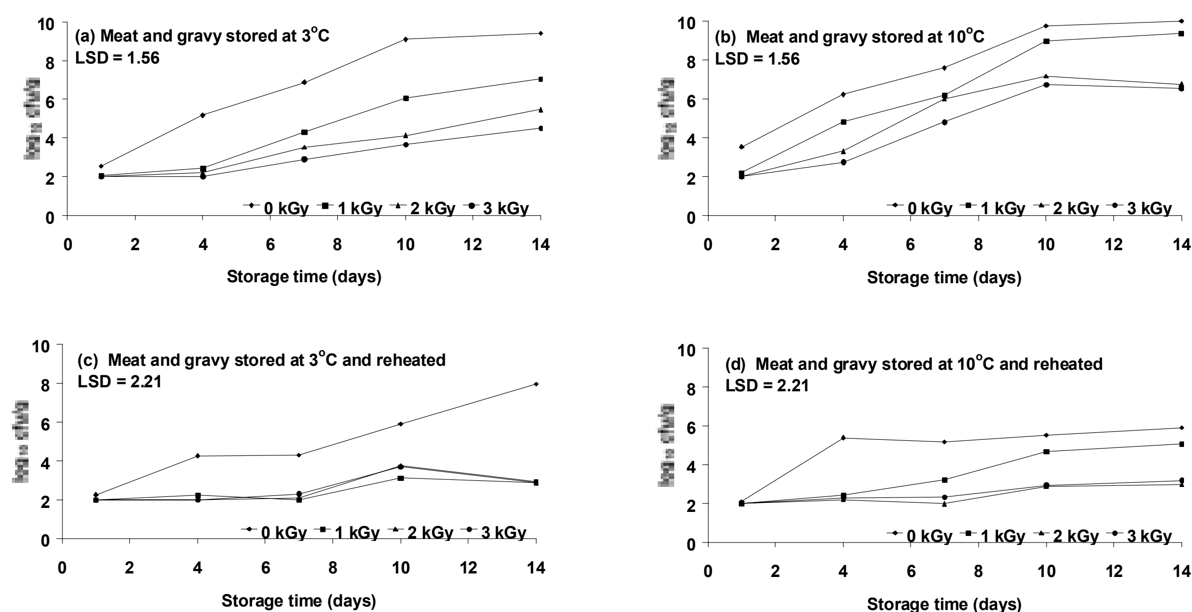

FIG. 1. Effect of irradiation dose and storage on the microbiological quality of the meat and gravy components in a chilled ready meal. LSD = Least Significant Difference (5% level).

Irradiation did significantly reduce microbial numbers (p<0.001) in the meat and gravy and vegetable components of the meals compared to the non-irradiated controls. Overall, there was no significant difference in results from meals treated with 2 and 3 kGy, although both were better than those given 1 kGy in controlling microbial numbers. Therefore, it was concluded 2 kGy should be used in future studies. Previous work has shown that this treatment will also be sufficient to kill vegetative pathogens and so the microbiological safety of the meals will also be improved [27].

Storage temperature had a significant effect on the growth of surviving microorganisms during storage (p<0.001). Numbers increased more rapidly at 10°C compared to 3°C, irrespective of irradiation treatment. The microbial numbers in the controls reached unacceptable levels by 6 and 4 days at 3°C and 10°C, respectively. Samples irradiated at 2 or 3 kGy and stored at 3°C remained acceptable, in terms of microbial numbers, throughout the 14 days of storage. However, irradiated samples stored at 10°C had unacceptably high microbial numbers by day 6. It was concluded that many of the beneficial effects of irradiation can be lost if the storage temperature is too high.

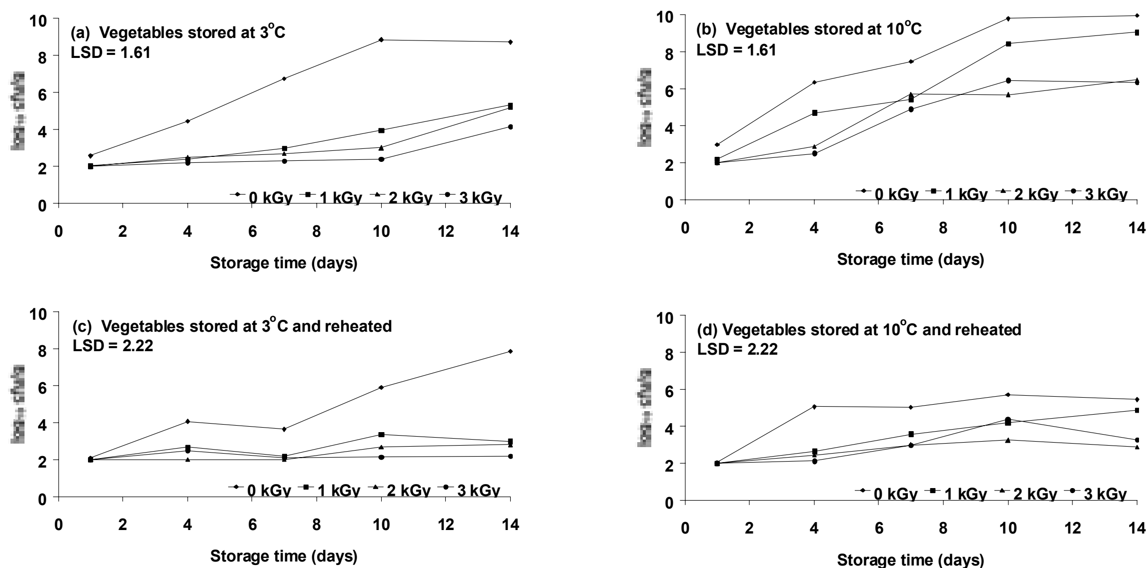

FIG. 2. Effect of irradiation dose and storage on the microbiological quality of the vegetable components in a chilled ready meal. LSD = Least Significant Difference (5% level).

As expected, cooking reduced microbial numbers. The effect was most marked in the 3°C irradiated samples and the differences in these samples compared to non-irradiated controls became more pronounced as storage time increased.

3.1.2. Vitamin content

3.1.2.1. Thiamine

Irradiation dose had a very highly significant ($p<0.001$) effect on the concentration of thiamine in the pork of the ready made meals (Table I). A 13% decrease in thiamine concentration was observed when the non-irradiated meals were given a dose of 1 kGy. A further reduction of 11% occurred when the meals received a dose of 2 kGy with an additional diminution of 10% being observed when a 3 kGy dose was used. Storage also had a very highly significant ($p<0.001$) effect on the thiamine content of the pork. It was noted that the greatest decrease occurred over the first 7 days of storage at 3°C when there was a 20% reduction in thiamine concentration. Between day 7 and 14 of storage there was only a further 6% diminution in thiamine levels. As expected, cooking significantly ($p<0.01$) affected the thiamine content of the pork with a 14% decrease in concentration being observed when the meals were reheated as per manufacturers instructions at 160°C for 25 min. It can be concluded from these results that storage has the greatest effect on the retention of thiamine in pork with the greatest losses being observed over the expected shelf-life of the ready made meal. Cooking was found to be as detrimental to thiamine concentration as irradiation which is in agreement with the findings of work published previously in this area [21,28,29]. Despite the fact that there are small reductions in the thiamine content of meats following irradiation, it should be noted that thiamine is even more sensitive to heat than to irradiation [30]. In addition, it should be borne in mind that the meals tested in the present work had been cooked prior to irradiation and reheating; therefore, the double heat treatment as such will have a significantly greater effect on thiamine concentration than treatment with ionising radiation.

3.1.2.2. Vitamin C

It was found that irradiation dose did not have a significant effect on the TAA content of the potatoes in the ready made meals (Table II). However, dose had a highly significant ($p<0.01$) effect on the concentration of AA and a very highly significant effect ($p<0.001$) on the DHAA content. Overall,

a 28% decrease in TAA was observed between non-irradiated samples and those that had received a dose of 1 kGy. There was a 51% difference in the TAA content observed between non-irradiated potatoes and those given 2 kGy, with a similar diminution of 54% being found for the 3 kGy dose. With regard to AA content, this decreased by 66% upon irradiation with a dose of 1 kGy. However, when potatoes were treated with doses of 2 and 3 kGy, the reduction in AA concentration was 91% and 94%, respectively.

TABLE I. EFFECT OF IRRADIATION DOSE, STORAGE AND REHEATING ON THE THIAMINE CONTENT (µg/100 g FRESH WEIGHT) OF PORK IN A READY MADE MEAL

Irradiation dose (kGy)	Storage time (days)	Uncooked (Not reheated)	Cooked (Reheated)
0	0	428.4	345.6
	7	459.0	299.1
	14	323.6	248.1
1	0	415.1	349.0
	7	262.7	255.1
	14	314.0	228.7
2	0	333.0	303.4
	7	254.7	237.1
	14	214.4	241.0
3	0	291.5	289.1
	7	214.4	224.8
	14	226.9	226.3

	SEM/Sig.
Irradiation dose (D)	14.6/***
Uncooked/Cooked (C)	12.6/**
Storage time (S)	10.3/***
D × C	20.6/NS
D × S	25.2/NS
C × S	17.8/NS
D × C × S	35.7/NS

where:

SEM = standard error of the mean; Sig. = statistical significance;
NS = no statistical significance; ** = p<0.01; *** = p<0.001

As expected, the DHAA concentration of the potatoes increased when irradiated. Potatoes treated with 1 kGy contained 54% more DHAA than their non-irradiated counterparts with similar increases of 49% and 51% being observed for the 2 and 3 kGy samples, respectively.

Storage significantly affected ($p<0.001$) the TAA, AA and DHAA content of the potatoes as can be seen from Table II. As was observed for the thiamine content of the roast pork, the greatest diminution in vitamin concentration occurred during the initial 7 days of storage. Thereafter, a more gradual reduction was observed. TAA decreased by 83% over the first week, AA by 95% while DHAA was reduced by 70%.

TABLE II. EFFECT OF IRRADIATION DOSE, STORAGE AND COOKING ON THE VITAMIN C CONTENT (mg/100 g FRESH WEIGHT) OF POTATOES IN A READY MADE MEAL

Irradiation dose (kGy)	Storage time (days)	Uncooked (Not reheated)			Cooked (Reheated)		
		TAA	AA	DHAA	TAA	AA	DHAA
0	0	8.64	7.39	1.25	2.99	2.97	0.03
	7	1.74	0.63	1.11	0.19	0.11	0.09
	14	0.38	nd	0.38	nd	nd	nd
1	0	6.78	2.53	4.25	1.53	1.27	0.26
	7	1.43	nd	1.43	nd	nd	nd
	14	0.28	nd	0.28	nd	nd	nd
2	0	5.10	0.85	4.25	0.34	0.18	0.16
	7	1.04	nd	1.04	nd	nd	nd
	14	0.30	nd	0.30	nd	nd	nd
3	0	5.17	0.49	4.68	0.22	0.12	0.10
	7	0.77	nd	0.77	nd	nd	nd
	14	0.32	nd	0.32	nd	nd	nd

TAA

	SEM/Sig
Irradiation dose (D)	0.43/NS
Uncooked/Cooked (C)	0.30/***
Storage time (S)	0.37/***
D × C	0.61/NS
D × S	0.74/NS
C × S	0.53/***
D × C × S	1.05/NS

DHAA

	SEM/Sig
Irradiation dose (D)	0.25/***
Uncooked/Cooked (C)	0.18/**
Storage time (S)	0.22/***
D × C	0.36/NS
D × S	0.44/NS
C × S	0.31/NS
D × C × S	0.62/NS

AA

	SEM/Sig
Irradiation dose (D)	0.49/**
Uncooked/Cooked (C)	0.34/**
Storage time (S)	0.34/***
D × C	0.69/NS
D × S	0.69/NS
C × S	0.49/NS
D × C × S	0.97/NS

where:

SEM = standard error of the mean;
Sig. = statistical significance; nd = not detected; NS = no statistical significance;
** = p<0.01;*** = p<0.001

Reheating the meals had a highly detrimental effect on the levels of vitamin C in the potatoes (Table II). There was a resultant 83% decrease in TAA concentration, a 61% reduction in the content of AA present and a 97% diminution in DHAA.

The results observed for the vitamin C content of the potatoes were not unexpected taking into account the results of previous work by Graham and Stevenson [18]. These workers observed that irradiation, storage and cooking all significantly affected TAA, AA and DHAA concentrations in potatoes. When reporting vitamin C levels it is important to take into account the fact that ionising radiation can cause a partial conversion of AA to DHAA. This indeed was the case in this experimental work where the DHAA content increased upon irradiation while the concentration of AA exhibited a simultaneous decrease. Since both these compounds have vitamin C activity in the body, it is important that both are measured otherwise any reported losses may be exaggerated [18,31].

Cooking the meals proved to be more harmful to the vitamin C content of the potatoes than irradiation as has been reported in earlier findings of Graham and Stevenson [18]. The latter work showed that cooking did not decrease the vitamin C content of irradiated potatoes to any greater extent than that of their non-irradiated counterparts. As for the pork, the potatoes tested in this experimental work were cooked prior to being incorporated into the meals. Therefore, vitamin C content would have been reduced prior to being irradiated with a further decrease resulting from re-heating. Thus, the effects of irradiation would be minimal compared to the effect of cooking.

Storage was also highly detrimental to the vitamin C content of the potatoes over the expected shelf-life of the meals (Table II). Moreover, a combination of cooking and storage significantly affected ($p<001$) the TAA content of the potatoes.

The concentration of vitamin C in the mixed vegetables component of the meals, i.e. the garden peas and runner beans, was very low even before the samples were irradiated, reheated or stored, being present at a level of approximately 2.95 mg/100 g. A combination of irradiation, re-heating and storage virtually eliminated the vitamin C content of these vegetables. For example, reheating decreased the TAA content by 95%, the AA concentration by 85% and DHAA was reduced to a trace level. However, it is highly likely that the treatment of the vegetables prior to their incorporation into the ready meals had reduced the vitamin C content to such low levels that any further treatment resulted in substantial reductions.

3.2. Effect of gamma irradiation and chilled storage on the microbiological quality and vitamin content of chilled pureed chicken and gravy, boiled potatoes and garden peas

3.2.1. Microbiological quality

Irradiation reduced the total bacterial load of the pureed meals. As for the irradiated meals referred to in Section 3.1, irradiation doses of 2 or 3 kGy were found to be better for controlling microbial numbers than 1 kGy as can be seen in Fig. 3. No significant reduction in the bacterial count was found one day post-irradiation. However, following 7 and 14 days storage at 4°C the irradiated pureed meals had significantly lower bacterial counts than their non-irradiated counterparts.

3.2.2. Vitamin content

Results (Table III) showed that irradiation had a significant effect ($p<0.001$) on the thiamine content of the chicken and gravy. A 13% and 20% decrease in thiamine concentration was observed between irradiated samples given 2 and 3 kGy, respectively, and the non-irradiated controls while a dose of 1 kGy resulted in a diminution of only 2%.

Storage and cooking did not appear to have a significant effect on the thiamine content of the pureed chicken and gravy. With regard to the pureed peas and potatoes (results not presented), the

vitamin C content of these was extremely poor. Vitamin C was present in only one of the three batches of pureed potatoes obtained for analysis and once stored at 4°C for 7 or 14 days this vitamin could not be quantified. Similarly, only one batch of the pureed peas contained measurable amounts of vitamin C which decreased upon irradiation, storage and re-heating. It therefore appears that these pureed meals require supplementation with vitamin C in order to improve their nutritional value.

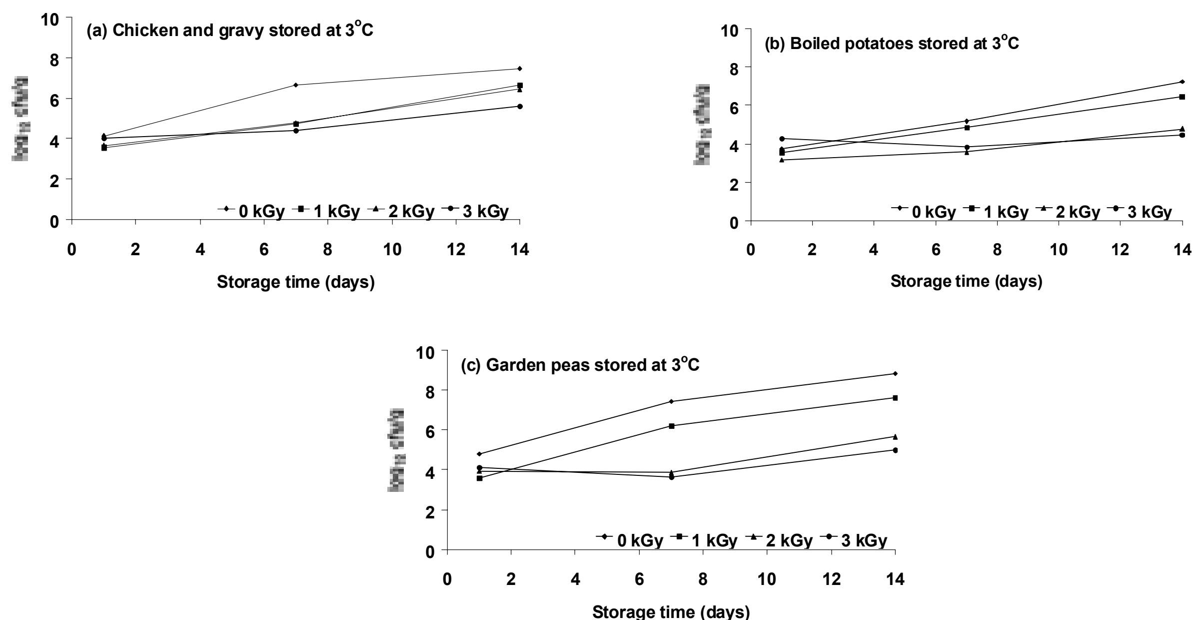

FIG. 3. Effect of irradiation dose and storage on the microbiological quality of pureed chicken and gravy, boiled potatoes and garden peas.

3.2.3. Detection of irradiation treatment

Both 2-DCB and 2-TCB were readily detected in the irradiated pureed chicken and gravy and were not detected in the non-irradiated samples (Fig. 4). Irradiation dose had a very highly significant effect ($p<0.001$) on the concentrations of both compounds which were observed to increase linearly with increasing dose (Table IV). These irradiation markers were readily detectable over the 14 day storage period at 3°C. It was somewhat surprising to observe that both 2-DCB and 2-TCB increased in concentration with storage, although this was most likely due to sample variation. It can, therefore, be concluded that the 2-alkylcyclobutanone methodology can be successfully used for the identification of such irradiated products. ESR spectroscopy (EN1787) was successfully employed to determine whether or not the laminated cardboard packaging of the pureed meals could be used as an indicator of irradiation treatment. ESR spectra derived from samples of packaging from irradiated meals were found to show the characteristic cellulose signal (Fig. 5a) which was absent from the non-irradiated samples (Fig. 5b). The intensity of the radiation-induced signal increased with increasing irradiation dose over the range 1 to 5 kGy (Fig. 6).

ESR examination of samples of packaging has the advantage of being relatively quick and simple, but it should be borne in mind that the presence of the characteristic signal due to irradiation treatment only indicates that the packaging has been treated. It is not evidence that the contents have been irradiated, but it would appear to suggest that the contents at least merit further investigation [10].

TABLE III. EFFECT OF IRRADIATION DOSE, STORAGE AND COOKING ON THE THIAMINE CONTENT (mg/100 g FRESH WEIGHT) OF PUREED CHICKEN AND GRAVY

Irradiation dose(kGy)	Storage time (days)	Cooking treatment	
		Uncooked (Not reheated)	Cooked (Reheated)
0	0	3.898	3.713
	7	3.727	4.052
	14	3.651	3.918
1	0	3.651	3.738
	7	3.858	3.984
	14	3.713	3.780
2	0	3.394	3.301
	7	3.624	3.000
	14	3.410	3.282
3	0	3.343	2.968
	7	3.319	2.806
	14	2.915	3.169

	SEM/Sig.
Irradiation dose (D)	0.067/***
Uncooked/Cooked (C)	0.095/NS
Storage time (S)	0.082/NS
D × C	0.135/NS
C × S	0.117/NS
D × S	0.165/NS
D × C × S	0.233/NS

where:

SEM = standard error of the mean; Sig. = statistical significance;
NS = no statistical significance; *** = p<0.001

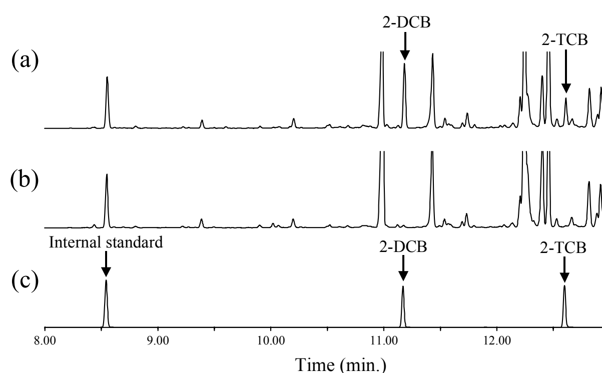

FIG. 4. Selected ion monitoring (SIM) of the sum of ions m/z 98 and m/z 112 from pureed chicken and gravy irradiated at 2 kGy (a), and left non-irradiated (b) and 2-dodecylcyclobutanone (2-DCB) and 2-tetradecylcyclobutanone (2-TCB) standards (c).

TABLE IV. EFFECT OF IRRADIATION DOSE AND STORAGE AT REFRIGERATION TEMPERATURE ON THE 2-DODECYLCYCLOBUTANONE (2-DCB) AND 2-TETRADECYLCYCLOBUTANONE (2-TCB) CONTENT (µg/g LIPID) OF PUREED CHICKEN AND GRAVY

Irradiation dose (kGy)	Storage time (days)					
	2-DCB			2 TCB		
	1	7	14	1	7	14
0	nd	nd	nd	nd	nd	nd
1	0.17	0.20	0.20	0.06	0.05	0.06
3	0.41	0.54	0.50	0.16	0.17	0.18
5	0.76	0.82	0.82	0.32	0.26	0.31

	2-DCB	2-TCB
	SEM/Sig.	SEM/Sig.
Irradiation dose (D)	0.014/***	0.005/***
Storage time (S)	0.014/**	0.005/*
D × S	0.025/NS	0.009/NS

where:

SEM = standard error of the mean; Sig. = statistical significance; nd = not detected; NS = no statistical significance; * = p<0.05; ** = p<0.01; *** = p<0.001

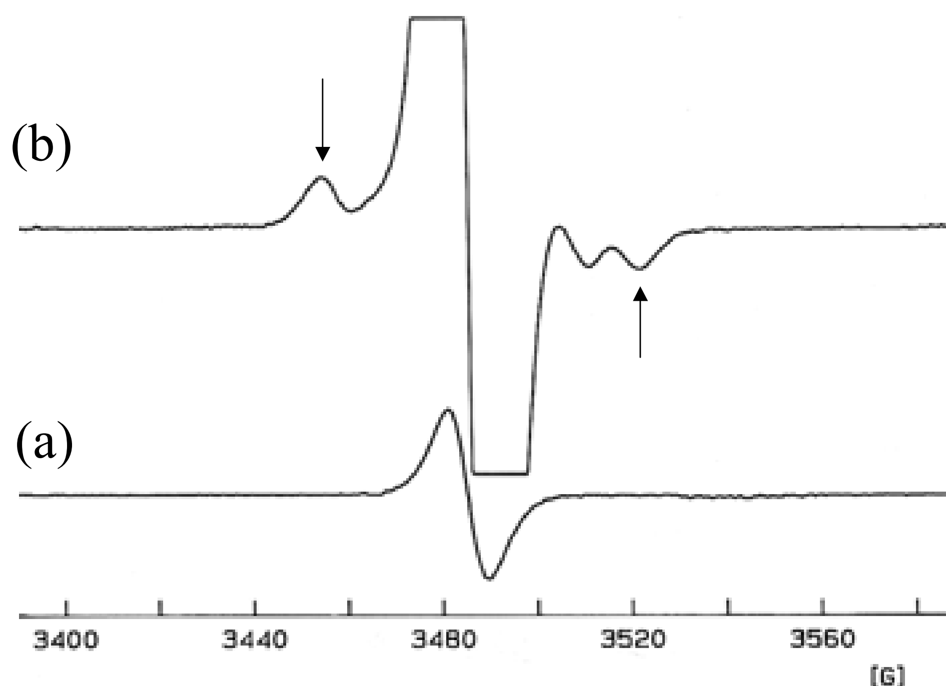

FIG. 5. ESR spectra derived from non-irradiated (a) and irradiated at 5 kGy (b) packaging from ready made meals.

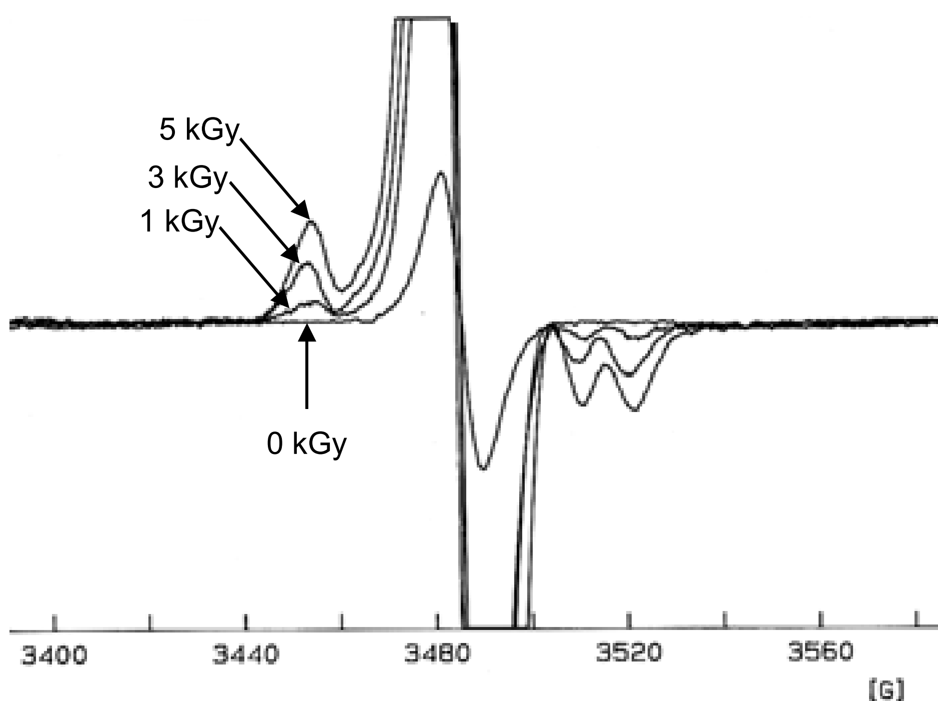

FIG. 6. ESR spectra derived from ready made meal packaging irradiated at different doses.

4. CONCLUSIONS

From the results of this research it can be concluded that an irradiation dose of 2 kGy can be used to extend the shelf-life of refrigerated ready meals up to 14 days. However, it is important that the meals are stored under good refrigeration conditions (<3°C) in order to obtain maximum benefit from the irradiation treatment. Vitamin losses due to treatment with ionizing irradiation are comparable to losses induced by cooking and storage. Overall, it was found that the vitamin C and thiamine content of both the whole meals (roast pork, boiled potatoes, mixed vegetables) and pureed meals (chicken and gravy, boiled potatoes, garden peas), including the non-irradiated controls, was low. As these meals are prepared mainly for consumption by the elderly and infirm, the manufacturers may wish to consider vitamin supplementation of the meals in order to improve their nutritional value. If ready made meals are to be irradiated, they must be labelled accordingly under EU regulations [32]. Thus, it is important that reliable methods are available to determine the irradiation status of such products. From the results obtained in the present experimental work, it can be concluded that the 2-alkylcyclobutanone (EN1785) and ESR spectroscopy (EN1787) standard methods can be used to detect irradiation treatment.

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FEASIBILITY OF OBTAINING SAFE, SHELF-STABLE, NUTRITIVE AND MORE VARIED WHOLE RATIONS FOR IMMUNOSUPPRESSED PATIENTS BY GAMMA IRRADIATION

P. NARVAIZ*, P. GIMÉNEZ**, C. HORAK*, M. ADEIL PIETRANERA*,E. KAIRIYAMA*, D. GRONOSTAJSKI**, A.M. RIBETTO***

* Technological and Agricultural Applications, National Atomic Energy Commission,
Ezeiza Atomic Centre,
Pcia. de Buenos Aires, Argentina

** Nutrition Service, Piñero Hospital, Buenos Aires, Argentina

*** CITECA (Meat Research and Industrial Technology Center),
INTI (National Institute of Industrial Technology),
Pcia. de Buenos Aires, Argentina

Abstract

An immunosuppressed patient is a person whose biological defences are below what are considered to be “normal limits” due to illness, a biological condition, or situations generating risks. As examples of this, several patients categories can be stated: stressed after complex surgery; suffering from Acquired Immunodeficiency Syndrome (AIDS) or tuberculosis; oncological; transplants; malnourishment. These can enhance the probability of acquiring microbial food borne diseases, caused by pathogens such as *Listeria, Salmonella* and *E. coli*. Traditionally, immunosuppressed patients in hospitals were isolated from the environment and had their food intake sterilised by a variety of treatments, including irradiation. Literature on foods sterilised by ionising irradiation indicates that it is a good method for treating meats but is not so promising for fruits, vegetables and starches, mainly due to losses in sensory quality. Taking this into account, and the fact that medical criteria at present has moved from using “sterile foods” to “clean diets”, in this experimental work ionizing radiation was used at sub-sterilising doses as a means of enhancing the variety of available meals for vulnerable patients, allowing some products normally considered as “high risk” to be available for consumption. Nutritionists working at the Nutrition Service in a local hospital in Buenos Aires, Argentina, elaborated suitable diets for patients immunocompromised to different degrees, and advised on the most interesting meal types to be studied. Tomato and carrot salad, fruit salad in gelatine, ice cream, fresh stuffed pasta in tomato sauce, and burgers, were the “ready-to-eat” foodstuffs selected for investigation. Irradiation was carried out at a semi-industrial ^{60}Co irradiation facility using the following doses: 2 and 4 kGy for salads and pasta; 3, 6 and 9 kGy for ice cream; 45 kGy for burgers. Microbiological and sensory analyses were carried out on control and irradiated samples stored for up to 7 days for salads and pasta, 45 days for ice cream, and up to 2 and 7 days for burgers. Chemical tests were also performed on ice cream and burgers. Samples were stored at 2 ± 1°C and 70 ± 5% RH, with the exception of ice cream which was stored at –20 ± 3°C while burgers were kept at room temperature. Results showed that the purpose of “cleaning” the meal without impairing quality was fulfilled using the following doses: 2 kGy for salads; 3 kGy for ice cream; 4 kGy for fresh stuffed pasta; 45 kGy for burgers. A seminar on food irradiation and results of this work directed to nutritionists and advanced students of that career was held, along with tasting of ice creams, fruit in gelatine, and burgers. The irradiated products were found to be generally acceptable and the technology promising.

1. INTRODUCTION

An immunosuppressed patient is a person whose biological defences are below those which are considered as “normal limits” due to illness, a biological condition, or situations generating risks. Several categories can be stated as examples of this:

- stressed patients, after complex surgery;
- patients suffering from Acquired Immunodeficiency Syndrome (AIDS);
- oncological patients;
- transplant patients;
- tuberculosis patients;
- malnourished patients; and
- patients under treatment related to pathologies compromising their nutrition such as osthomies, anorexia, long-treatment digestive tract pathologies.

Such patients may suffer from aplastic anaemia, granulocytopenia, secondary effects after the application of chemotherapy or radiotherapy as oncological treatments, or intentional and temporary drug immunosuppresssion to reduce the risk of transplant rejection. The intensive treatments following a bone-marrow transplant can usually damage the oral and gastrointestinal mucosa, causing nausea, vomiting and diarrhoea. Many times these patients are also malnourished. These conditions can enhance the probability of acquiring microbial food-borne diseases, caused by pathogens such as *Listeria*, *Salmonella*, *E. coli*.

Traditionally, immunosuppressed patients in hospitals were maintained under "reverse barrier methods" meaning that they were isolated from the environment by a plastic tent or laminar flow room. Often, their intestinal tract was sterilised through oral antibiotic therapy to prevent infections from common gut microorganisms and their food intake was also sterilised by different treatments [1,2].

Methods usually employed to sterilise the food included autoclaving and irradiation. Most canned foods were also considered acceptable. Other possible methods, such as steam sterilisation, encapsulation and extrusion, were also used but these can only be applied to a limited range of foodstuffs. Sterilising methods employing heat, although microbiologically safe, can lead to changes in taste and texture and to some nutritional losses. Irradiation has less detrimental effects while at the same time offering similar microbiological security [3]. In addition, meals may be irradiated in their final packaging and then reheated by microwave cooking at the bedside. At sterilising irradiation doses, nutritional losses may be such that it is necessary to supplement the diet with vitamins, as for any other food sterilisation method. This, however, can be minimised by selecting appropriate irradiation and packaging conditions.

At present medical opinion differs on the "reverse barrier" approach, due to the cost and specialised requirements, uncertainties about the clinical benefits, and the psychological convenience of treating these patients in a more normal way. Thus, the tendency nowadays seems to be moving from "sterile diets" to "low microbe diets" (or "clean diets"). Low microbe diets have been defined by some authors as containing <500 *Bacillus* colony-forming units (CFU) per gram of food, or <1000 CFU/g of coagulase-negative staphylococci or *Streptococcus viridans* and <10,000 CFU/mL *Bacillus* spp., diphteroids or *Micrococcus* spp. [1].

It is a common belief that fresh food, when adequately cooked, is microbiologically safe. However, the risk of causing crossed-contamination by bringing contaminated foodstuffs into the kitchen exists and has been evidenced fairly frequently. Ionizing radiation at sub-sterilising doses (radicidation) could be of great benefit for the production of safe meals destined for immunosuppressed people, either in hospitals or at home. This method could widen the variety of available foods for such vulnerable patients, allowing some products normally considered as "high risk" to be used, e.g. spices and herbs, poultry and salads.

A literature search was carried out to determine international experience on the subject of feeding radiation sterilised diets to immunosuppressed patients and, in fact, very few references were found. However, some countries have applied the irradiation technology for this purpose:

(1) United Kingdom (UK), since the early 1960's at Hammersmith Hospital, London. Irradiation was carried out at the UK Atomic Energy Authority's ^{60}Co irradiator at Wantage, Oxfordshire. From 1970 to 1988 a service was offered by Irradiated Products Limited (now Isotron) firstly at Wantage and then at Swindon. Some irradiated spices and tea were destined for patients at the Charring Cross Children's Hospital, London, until its closure in 1993. In 1995 the only reported place in the UK in which this process takes place was Scotland, where a number of hospitals were using irradiation to provide clean diets.

(2) United States of America (USA). At the Fred Hutchinson Cancer Research Centre in Seattle, a broad group of foods was irradiated for bone marrow transplanted patients between 1974 and 1988. More recently, it was reported that irradiated food was served in a Florida hospital to immunosuppressed patients [2].

(3) The International Consultative Group on Food Irradiation (ICGFI) reported that irradiated food for hospital patients is exempt from regulatory control in Finland and The Netherlands, although there is no evidence of such use in either country [2]. Indeed, allowances for irradiated food include "sterile meals" in Croatia, Finland, Korea, the UK and The Netherlands, as well as "sterile meats" in South Africa [4].

2. MATERIALS AND METHODS5

2.1. Definition of whole food rations suitable to be afforded to immunosuppressed patients, according to their particular illness requirements

Three levels of dietotherapic attention were defined:

(1) *Hospitalised patient, with serious health problems*: Very restricted feeding, requiring permanent adequacies. Diet progression must be very slow, as well as food and special preparations intake.

(2) *Patient under recovery, in an intermediate evolution state*: With food intake of higher calorific value than the former, but still taking extreme care on its acceptance so as not to cause improvement regression.

(3) *Patient allowed to leave hospitalisation, although sometimes this permission is transitory*: The diet is more complete and free, resembling that of normal feeding, but still respecting its acceptance and adequacy to his/her state.

In general, the appearance of a more or less serious impairment to eat and absorb food is usual, due to the bad condition of the oral and gastrointestinal mucosa. These patients suffer from nausea, diarrhoea and vomiting. Meals should be degluted more mild or cold than hot.

Coloured foodstuffs are preferred, because they are more cheerful, and also, patients miss this kind of nourishment as meals are generally served to them cooked and even overcooked so as to minimise the risk of microbial transmission. Excessive sugar and fat is not well tolerated while frozen foods are generally not appreciated by nutritionists, as they can be of lower nutritional value compared with fresh food.

It is also important to respect cultural feeding habits. Immunosuppresed patients often view their condition as a distinct partition from the healthy population, in every sense. Therefore, it would be advisable to offer them the same meals they formerly ate, when feasible.

Accordingly, four diets were elaborated taking into consideration the different patient categories described above. The following diet is shown as an example (Table I) to represent those kinds of foodstuffs which could benefit from treatment with irradiation, and are likely to be offered to patients described under (2) or (3). Diets for patients referred to under (1) are mainly based on pharmaceutical foods ("nutrotherapics") the microbiological quality of which is safe.

DIET DISTRIBUTION (Example):

- ✓ **Breakfast**
  Tea or mate ("Paraguay tea") or malt coffee infusions, with skim milk and sugar; water biscuits with skim soft cheese and marmalade.
- ✓ **Mid-morning**
  A fruit cooked with sugar.
- ✓ **Lunch**
  Vegetables "A" soup; chicken, boiled or oven-cooked; vegetables B and C, boiled; milk dessert (rice boiled in sweet milk); white bread.

- ✓ **Mid-afternoon**
  Infusion with milk, like at breakfast; bread and butter.
- ✓ **Supper**
  Semolina soup; hake fillet with vegetables B and C pureed; fruit salad; water biscuits.

Drinks: water, light tea or herbal infusions, stewed fruit juices, vegetable broth.

Condiments: salt, marjoram, laurel, nutmeg, romero, thyme, parsley, celery, lemon, vanilla extract, cinnamon, apple vinegar.

TABLE I. DIET TYPE I-IMMUNOSUPPRESSED PATIENT UNDER REST

Foods employed per day	Amount (g)	Foods employed per day	Amount (g)
Milk (skim)	200	Vegetables (type C)[d]	150
Cheese (whole)[a]	10	Fruits[e]	300
Cheese (skim)	30	Cereals	30
Eggs	25	Bread (white)	80
Meat (beef)	70	Water biscuits	40
Poultry	50	Jam	30
Fish	30	Sugar	40
Vegetables (type A)[b]	200	Oil	30
Vegetables (type B)[c]	200	Butter	10

Sex: Masculine; Age: 30 years; Weight: 60 kg; Height: 1.75 m
Diet characteristics: Soft consistency and flavour
Total calorific value: 1900 kcal
Carbohydrates: 256 g; Proteins: 70.4 g; Fats: 60.2 g

[a]Semi-hard paste cheeses, not very salty or spicy, e.g. Fontina or Pategrás
[b]Eggplant, asparagus, spinach, leek, squash, tomato
[c]Artichoke, onion, parsley, red beet, carrot
[d]Potato, sweet potato, mandioca
[e]Peach, pear, apple, banana. When possible, according to the patient adequacy, fruits should be offered ripe, raw and peeled, or cooked, with or without skin.

2.2. Choose, among those rations defined above, those to which combination treatments including irradiation could theoretically be successfully applied

Literature [3,5-9] indicates the beneficial effects of using radappertization for meats but not for fruits, vegetables and starches, mainly due to losses in sensory quality. Taking this into account, and the fact that the medical criteria at present has moved from using “sterile foods” to “clean diets”, the following foods were proposed to commence the experimental work with:

(1) Vegetable salads: Carrot and tomato were considered as the most usual in the Argentine habits, disregarding lettuce and onion, not recommended by nutritionists due to the intestinal problems they can cause. Vegetables should be peeled.
(2) Fruit salads: Due to the winter season and consumption habits, apples, pears and bananas were chosen.
(3) Ice creams.
(4) Fresh stuffed pasta.
(5) Burgers (beef and chicken).

2.2.1. Vegetable salad

Irradiation was carried out at the ^{60}Co facility of the Ezeiza Atomic Centre, with an activity of 380,000 Ci at a dose rate was 0.10 kGy/min. Silver dichromate dosimetry was employed [10].

Eight kilograms of both carrots and tomatoes ("Tommy" long-life variety) were purchased at a local green grocers. Carrots were hand-peeled, washed, drained and hand-grated. Tomatoes were washed, drained, peeled, the seeds extracted, and the flesh cut with a knife into small pieces. This was performed at the Food Irradiation Section laboratory of the Ezeiza Atomic Center, without taking special precautions to avoid contamination, considering that one of the aims of the work was to observe the effect of gamma irradiation on the microbial flora.

The salad was portioned and packed into 80 polypropylene disposable dishes, each containing approximately 100 g, which were then wrapped with PVC (polyvinylchloride) film, which allows selective gas permeation (this is the method usually employed by vegetables processors to extend shelf-life). The samples were divided into three batches, keeping one as a control (0 kGy) while the others were irradiated with 2 and 4 kGy. Samples were stored at 2 ± 1°C at a relative humidity (RH) of 70 ± 5%.

In a second experiment, samples prepared as described above were sprayed with a 2% calcium chloride solution in water before being packed. The dose received by these samples was 2 kGy.

2.2.2. Fruit salad in gelatine

Six kilograms of apples ("Granny Smith" variety), 6 kg of pears ("Packam" variety), 4 kg of bananas ("Bonita" variety, from Ecuador), and 6 kg of oranges were purchased at a local green grocers. The fruits were washed, drained, hand-peeled, and hand-cut into small pieces, with the exception of oranges which were only used for juice extraction. Eighty disposable polypropylene dishes with lids were half-filled with the fruit mixture so that the remaining capacity could be filled with gelatine. The fruits were soaked in orange juice which was afterwards filtered and incorporated into a water dilution as a cold component to produce the jelly frame. The gelatine used in this experiment was a food-grade, commercial brand which was peach flavoured and coloured. It was added to fruit salad at a temperature of about 50°C. Irradiation and storage conditions were as described for the vegetable salad.

In a second experiment, one batch of fruit was soaked in fresh orange juice containing 2% calcium chloride while another batch was soaked in orange juice without additives. Once the fruits were drained, they were placed into polypropylene disposable trays. Then gelatine (strawberry flavoured) was prepared, using hot water and the remaining orange juice to build the jelly frame. Colourings were incorporated into the gelatine (mixed with the orange juice). These were "Punzo 4-R Red" (CI 16255) and "Allura AC Red" (CI 16035) in a 100 mg/L concentration. Other samples without any addition were kept as controls to evaluate colour improvement. Half of the fruit salads were irradiated with 2 kGy while the others were left as non-irradiated controls.

In a third experiment, in which fruit salad was destined for sensory evaluation at the Nutrition School, samples were prepared as in the second experiment, but apple was the only fruit served due to the season (late Spring) which resulted in lack of availability. The irradiation dose used was 1.5 kGy and "Allura AC Red" was incorporated at a concentration of 200 mg/L.

2.2.3. Ice creams

A local commercial brand of ice cream was selected for purchase due to its 30 year tradition of manufacture employing mainly natural components and few additives, which seemed important from a nutritional point of view. In a first experiment, "vanilla" flavour was chosen as it was

supposedly composed of milk, milk cream, and egg, all of which ingredients are of high nutritional value. Such a foodstuff, which is consumed raw, is generally not included in the diet of an immunosuppressed patient due to the usually high bioburden and possible pathogenic microorganisms supported by the rich ingredients.

Ice creams were included in the list of foods which could benefit from irradiation treatment not only for nourishment purposes but also to serve a psychological purpose, as a "titbit", to raise the patient's spirits and enhance appetite. This is especially important when the immunosuppressed patients are children or the elderly.

Vanilla ice cream (20 kg) was purchased packed in 20 polystyrene trays each containing 1 kg. The ice cream had been stored at –30°C overnight in order to permit a 30 km car transport to the laboratory without melting, which was carefully avoided during further manipulations. Commercially, this product is maintained at a storage temperature ranging between –12°C to –15°C while it is served to the consumer. On arriving at the laboratory, the ice cream was placed in a freezer at –20 ± 3°C.

A 12 kg sample was portioned into individual disposable polystyrene foam trays with lids. Each cup contained 100 g of sample so that there was no space between the ice cream and the lid. These samples were destined for microbiological and sensory analyses. The remaining 8 kg of ice cream were used for chemical tests. All the samples were divided into four batches. Each batch was placed into a polystyrene box (50 × 30 × 25 cm) with a lid, which contained 5 kg bricks of dry ice homogeneously distributed on the bottom and walls. One box was reserved as a non-irradiated control and kept out of the freezer while the rest were being irradiated. This was done so as to avoid differences in temperature exposures between control and irradiated samples. The remaining boxes were gamma-irradiated at doses of 3, 6 and 9 kGy using a dose rate of 0.125 kGy/min. The bulk density was 0.17 kg/dm^3. Dosimetry measurements were carried out by means of silver dichromate for the three applied doses with potassium nitrate also being employed for the 9 kGy dose [11].

In a second experiment, the following flavoured ice creams were portioned into individual disposable polystyrene foam cups with lids: (1) chocolate, (2) raspberry, (3) white chocolate, (4) peach, (5) milk jam ("dulce de leche") with banana, and (6) milk cream with whole cherries. Ice creams (2) and (4) are water-based and the remainder were cream-based. Half of the samples were irradiated with 4 kGy and the rest were left as controls.

2.2.4. "Cannelloni" (fresh stuffed pasta)

This product was purchased at a local factory with a well-known long tradition in the market regarding their home-like, natural cooking. The cannelloni consisted of kneaded wheat flour pasta (resembling that of "spaghetti") rolled over a cylindrical filling made of ricotta cheese, egg, salt and nutmeg. The rolled pasta cover could also have been a pancake but it was not used since frying is included in its preparation, which is not advisable for immunosuppressed patients. This filling was chosen due to the high nutritional value of caseinates (in the cheese) as well as its good digestibility, and the light but pleasant flavour of nutmeg.

Fifty cannelloni, each weighing 100 g, were purchased and each cut in half. Each half was placed into a disposable polypropylene box (8 × 10 × 4 cm) with a lid, suitable for microwave cooking. Fresh tomatoes ("Perita" variety) (15 kg) were hand-peeled, de-seeded, liquified in a domestic blender, and used as sauce, covering the whole of the pasta mass, to avoid superficial desiccation on microwave cooking. The 100 pasta samples, maintained in a refrigeration chamber (4 ± 2°C) throughout the storage time, were divided in three batches, placed into three large polystyrene boxes (50 × 30 × 25 cm) with lids, one of which was kept as a control and the others irradiated at 2 and 4 kGy. Silver dichromate dosimetry was employed. Eighteen samples were destined for microbiological determinations, and the rest, for sensory testing. Several trials were carried out to determine the optimum cooking conditions in a BGH Quick Chef microwave oven. Point 6 heating

("Oven Cooking") was chosen, with times ranging from 2 to 10 min. A cooking time of 10 min was found to be sufficient to cook the pasta to the desired extent.

In a second experiment, two different gums were added to the sauce as stabilisers or thickeners in a 0.1% proportion (some samples with no gum addition were kept as controls). These were (1) "THIXOGUM S IRX 55405" (Colloïdes Naturels International brand, French origin), which combines the emulsifying power of acacia gum and the stabilising power of xanthan gum, and (2) "FRUTAGEL 175 GG FARVAS" (Argentine origin), another type of xanthan gum industrially used for this purpose. Sauces were poured into disposable polypropylene boxes with lids which were suitable for microwave cooking. Half of samples were irradiated with 4 kGy and the others were left as controls.

2.2.5. Burgers

2.2.5.1. Product preparation

For beef burgers, bovine hind quarter premium muscle was used while for chicken burgers, breast meat without skin, cartilage, bones or any visible fat was employed. The meat was minced, and 0.75% ClNa, 0.5% TPP (Sodium Tripolyphosphate), and 0.2% dehydrated "Provenzal" (garlic and parsley) were added. After mixing for 10–15 min with a domestic food processor, 150 burgers of each meat type, each weighing approximately 100 g, were moulded.

2.2.5.2. Enzyme inactivation

Enzyme inactivation was achieved by pre-cooking the burgers in an electric oven to an internal temperature of 70 to 75°C for 15 min.

2.2.5.3. Vacuum packaging

The burgers were packed in CRYOVAC® EOD 909A (PET/Aluminium/ Polyethylene) pouches. The vacuum packaging machine used for this purpose was a VAC-STAR®, MINIVAC model, containing a high-performance vacuum pump (BUSCH, 99.95%). The temperature during the whole process was maintained below 10°C. After 24 h at 3–5°C, the entire batch of vacuum packed pouches was checked for the adherence of the pouch to the burger. In the case of leaks being detected, the pouch was re-opened and the product re-packed.

2.2.5.4. Freezing

The burgers were frozen within 48 h of being vacuum packed. Samples were placed at –80°C for 30 min and then at –20°C until irradiated.

2.2.5.5. Irradiation in the frozen state (45 kGy) and storage

The pouches were placed in polystyrene boxes and covered with dry ice. At the end of the irradiation process, temperature was measured with a maximum-minimum thermometer placed inside the box. The dosimeters used were NO_3K (inside the box), and $Cr_2O_7K_2$ on the outside for measuring absorbed dose halfway through the irradiation treatment. The samples were stored at ambient temperature.

2.3. Microbiological analysis

Total mesophilic aerobic bacteria count, mould and yeast count, total and faecal coliforms, or *E. coli* type I, *Salmonella* spp., and *Staphylococcus* spp. counts were performed using standard procedures [12,13]. *Staphylococcus aureus* identification was performed on Vogel-Johnson agar, followed by Gram coloration.

The content of five trays of each sample at each irradiation treatment was used for analysis at each storage period.

For burgers only, 40 pouches from each of two batches, that is the beef and chicken treated at 45 kGy were microbiologically analysed at CITECA (Meat Research and Industrial Technology Center), INTI (National Institute of Industrial Technology). Procedures consisted of storing pouches in culture stoves at 35°C for 15 days, after which they were examined to detect vacuum losses. Sampling consisted of taking five pouches from each batch and analysing for:

(1) aerobic mesophiles and thermophiles, on dextrose-bromocresol purple broth for 5 days.
(2) anaerobic mesophiles and thermophiles, on liver broth for 5 days.

2.4. Sensory analysis

A panel of 20 persons belonging to the Industrial Radiation Applications Section was formed, the collaboration of whom is gratefully acknowledged. The taste panel evaluated aroma, aspect, colour, flavour, and general acceptability on a 7 point attribute scale (increasing from 1 to 7 in the particular attribute intensity or quality, with 4 being the acceptability threshold). For, burgers, tenderness and juiciness were also evaluated.

The burgers were heated for 15 min each side inside the pouch with its corners cut, in an electric oven previously heated to 85°C. This manner of cooking was considered to be the best way of assuring microbial sterility for intake by immunosuppressed patients. The sensory evaluation experiment was carried out according to references [14-16]. Results were evaluated by analysis of variance, Dunnett test, where p=95% confidence [14]. Sensory evaluation was performed at the Nutrition School of the Buenos Aires National University along with a seminar on food irradiation and on the experimental work. Forty-one nutritionists and advanced nutrition students evaluated beef and chicken burgers, apple pieces in gelatine, and vanilla, peach, and milk jam ice creams. It was estimated that about 30% of the audience left early and so thus did not participate in the sensory evaluation.

2.5. Chemical analysis

2.5.1. Vanilla ice cream

Fat was extracted according to a standard procedure [17], which were also applied to milk cream, margarine and egg yolk in order to verify the manufacturer's statements about the natural composition of this ice cream.

The following determinations were performed:

(1) Peroxide value, according to techniques described in Ref. [18].
(2) Acid value [17].

(3) Thin layer chromatography [19], using Silica Gel G as adsorbent; an equal volume mixture of petroleum ether (30–60°C) and diethyl ether as sample dissolution solvent; petroleum ether (60–80°C) – diethyl ether – acetic acid, in the proportions: 90–10–1 in the first case; 40–60–1 in the second case, as developing solvents, in a saturated chamber. Detection was carried out by exposing plates to iodine vapours. Sample volume in each run: 10 mL. Running time: 30 min. Running length: 12–14 cm.
(4) Absorption in the visible and ultraviolet regions, using a Beckman spectrophotometer. These regions were swept in search of absorption maxima. Sample concentration: stock solution: 1 g/100 mL of an equal volume mixture of petroleum ether (30–60°C) and diethyl ether. For measurements in the visible region, a 1/5 dilution was made while in the ultraviolet region a 1/100 dilution was used.

2.5.2. Burgers

2.5.2.1. Vitamin B1 (thiamine)

Five pouches of each batch of burgers: (1) 0 kGy, beef; (2) 45 kGy, beef; (3) 0 kGy, chicken; (4) 45 kGy, chicken, were taken. Analysis was carried out at a private laboratory ("Food Control", Buenos Aires). Contents from each batch were mixed, so that the resulting sample was about 500 g. This was extracted with 0.1 N HCl, autoclaved and derivatives were detected by fluorescence using a Shimadzu Liquid Chromatographer, Pumps: LC-10ATVP (X2), Detector: RF-10Axl. Chromatographic conditions: Column: Hamilton PRP-1/250 mm × 4.1 mm × 10 µm; Mobile phase: methanol: phosphate buffer (10 mM, pH=7) (45:55); Flow: 1 mL/min; Loop: 20 µL; Detection: Exc. 350 nm/Em. 435 nm; Temperature: 40°C.

3. RESULTS

3.1. Dosimetry

Dosimetry results are presented in Table II.

TABLE II. DOSIMETRIC RESULTS

Minimum irradiation dose required	Minimum irradiation dose received	Maximum irradiation dose received	D_{max}/D_{min}
	Salads		
2	2.02	2.67	1.32
4	4.51	5.36	1.19
	Ice Cream		
3	3.14	3.52	1.13
6	5.98	7.10	1.19
9	9.12	11.84	1.30
	"Cannelloni" (fresh stuffed pasta)		
2	2.16	2.44	1.13
4	4.02	4.82	1.20
	Beef burgers		
45	47	54	1.15
	Chicken burgers		
45	50	68	1.4

3.2. Microbiology

Results are summarised in Tables III to VI. In control salad samples, total aerobic mesophiles, moulds and yeasts were well above the limits permitted in a "clean diet". Coliforms were also present. This bioburden was adequately reduced by a 2 kGy irradiation dose (Table III). The 4 kGy dose was used so as to verify sensory quality stability of the product when part of the produce being treated (usually considered not exceeding 5%) is exposed to the maximum dose delivered during commercial irradiation, where D_{max}/D_{min} can reach the value of 2. In the fruit salad in gelatine control samples, the procedures followed during production (low acidity and the 50°C temperature on pouring the liquid gelatine onto the recipes) most likely accounted for the low microbial counts. However, the yeast and mould bioburden may be of concern in relation to diarrhoea production. Taking this into account, irradiation employed at a dose of 2 kGy could be useful, mainly if the product could be stored for periods longer than a week, such as in supermarkets (Table IV).

TABLE III. TOMATO AND CARROT SALAD: MICROBIOLOGICAL RESULTS

Irradiation dose (kGy)	Storage time (days)	Total aerobic mesophiles (CFU/g)	Moulds and yeasts (CFU/g)	Total coliforms/g	Faecal coliforms/g
0	2	1.12×10^6	4×10^3	present	absent
	7	1.60×10^7	8×10^4	present	absent
2	2	50	<50	absent	absent
	7	1×10^2	<50	absent	absent
4	2	<10	<50	absent	absent
	7	<10	<50	absent	absent

TABLE IV. FRUIT SALAD IN GELATINE: MICROBIOLOGICAL RESULTS

Irradiation dose (kGy)	Storage time (days)	Total aerobic mesophiles (CFU/g)	Moulds and yeasts (CFU/g)	Total coliforms/g	Faecal coliforms/g
0	2	2×10^2	1×10^2	absent	absent
	7	5×10^2	4.5×10^2	absent	absent
2	2	<50	<50	absent	absent
	7	<50	<50	absent	absent
4	2	<10	<50	absent	absent
	7	<10	<50	absent	absent

Ice cream control samples met the microbiological requirements of the national legislation (Argentine Alimentary Code) regarding total aerobic mesophiles (200,000 CFU/g for milk ice creams; 100,000 CFU/g for water-based ice creams) but some pathogens were detected. However, it may be that the ice cream was clean when it arrived from the factory and became contaminated in the laboratory during portioning into smaller dishes. However, this was seen as an added benefit considering that the aim of the study was to verify the effectiveness of gamma irradiation in microbial decontamination. The 3 kGy dose was sufficient to attain an acceptable microbiological quality. Using a dose of 6 kGy resulted in counts that were close to those of sterility (Table V).

A similar scenario can be applied to describe what happened with the freshly stuffed pasta. Although there were no national microbiological standards available for food destined for immunosuppressed patients, counts observed in control, and even in 2 kGy, samples were considered excessive to fit a "clean diet" in addition to the presence of pathogens. On the contrary, samples treated with 4 kGy were found to be acceptable (Table VI). The *Staphylococcus* spp. present in irradiated samples was not *S. aureus*, which is the microorganism specified as forbidden in Argentine legislation.

After microwave cooking, no survivors were detected in the control samples. However, use of irradiation treatment justifies considering that the extent of cooking is a variable. It, therefore, seems possible that a microbiologically safe meal in the raw state can be produced with the aid of irradiation treatment thereby avoiding the risk of under-cooking.

TABLE V. ICE CREAMS: MICROBIOLOGICAL RESULTS

Irradiation dose (kGy)	Storage time (days)	Total aerobic mesophiles (CFU/g)	Moulds and yeasts (CFU/g)	Total coliforms/g	*Salmonella* spp./25 g	*Staphylococcus* spp./g
			Vanilla			
0	2	1.6×10^5	2.5×10^3	present	absent	present
	60	9.7×10^4	4.3×10^2	absent	absent	present
3	2	1×10^2	1.3×10^2	absent	absent	absent
	60	1×10^2	20	absent	absent	absent
6	2	30	<10	absent	absent	absent
	60	30	<10	absent	absent	absent
			Raspberry			
0	2	4.7×10^5	9.3×10^4	absent	absent	absent
3	2	2.0×10^2	1.5×10^2	absent	absent	absent
			Peach			
0	2	1.6×10^5	1.3×10^4	present	absent	absent
3	2	1.0×10^3	2.5×10^2	absent	absent	absent

TABLE VI. FRESH RICOTTA STUFFED PASTA (“CANNELNONI”) IN LIQUIFIED FRESH TOMATO SAUCE (RAW): MICROBIOLOGICAL RESULTS

Irradiation dose (kGy)	Storage time (days)	Total aerobic mesophiles (CFU/g)	Moulds and yeasts (CFU/g)	Total coliforms/g	Faecal coliforms/g	*Staphylococcus* spp./g
0	1	4.6×10^6	9×10^2	present	present	present
	6	2.1×10^8	8×10^3	present	present	present
2	1	2.4×10^5	2.6×10^2	present	present	present
	6	1.7×10^5	5.5×10^2	present	present	present
4	1	3.5×10^3	<50	absent	absent	absent
	6	1.1×10^4	50	absent	absent	present

In burgers, neither pouch vacuum loss nor subsequent microbial growth was observed in any sample thereby confirming commercial sterility.

Radappertization, or radiation-sterilisation, is a successful method of food preservation that can be combined with different processes to enhance the overall effect. Such processes include mild heat treatment, vacuum packaging, freezing and the addition of certain additives. For prolonged storage, proteolytic enzymes must be inactivated by heat. Vacuum packaging and freezing prevents nutritional losses and off-flavour development by reducing the production of radiolytic compounds resulting from the application of high-doses. The addition of two salts, sodium chloride and sodium tripolyphosphate, to bind proteins and retain natural juices [20], can improve flavour, texture, juiciness, overall consumer acceptance, and product yield [21].

The purpose of this treatment is to destroy both spoilage microorganisms and food-borne pathogens [22]. In this way, the radappertized products can be stored without refrigeration for long periods, even years, as long as the packaging material retains its integrity. The radappertization process is attractive because there is only a slight temperature rise in the foods during the irradiation treatment. In addition, the advantage of the process is that fresh-like, wholesome, nutritious, disease-free food, even after several years of storage without refrigeration, can be served to the consumer even though he/she may be at a great distance from where the food is produced and processed [23].

When the appropriate dose and temperature conditions are employed, wholly acceptable irradiated meat products can be produced that are both safe and tasty to consume [24].

3.3. Sensory evaluation

Results are shown in Tables VII to IX.

Vegetable salad evaluation was separated in "tomato" and "carrot" since they were scored differently. Although there was no significant difference between control and irradiated tomato samples on the same date of analysis, an irradiation dose of 4 kGy led to an off-aroma and off-flavour described as "pungent" by some panellists.

This may have been due to the tomato variety used and the fact they were grown in the winter season. However, the irradiated tomato salad scored above the acceptability threshold (point 4) at the end of the storage period. Scores were lower at day 2, perhaps because at that stage of storage there was still a fresh control sample available for comparison purposes (Table VII).

Carrot salad was evaluated with slightly higher scores than tomato salad, being considered as acceptable throughout the 7 day storage period. No significant differences were observed due to irradiation (Table VIII). A slight loss in texture and freshness was observed for 4 kGy samples by a number of sensitive panellists although this view was not shared by all of them.

In a second experiment, in which vegetables were sprayed with a calcium chloride solution to reduce softening [25], a preliminary evaluation was carried out by two laboratory staff. Irradiated samples were found to have a better appearance (i.e. they were less wrinkled) than the non-irradiated controls, and their taste was similar to that observed in previous experiments. Control samples were not tasted as a week had elapsed since their preparation and they were not considered microbiologically safe. The calcium chloride addition seemed to have exerted no effect on texture.

The fruit salads treated at doses of 0, 2 and 4 kGy and stored for one week were found to be acceptable by a seven member panel. The only significant differences observed were in colour. Irradiation had destroyed to some extent the artificial red (amaranto) and yellow ("Ocaso" yellow) pigments in the gelatine, which also influenced the judgement on aspect at the 4 kGy dose but not at 2 kGy. Very few panellists reported texture losses in fruits, and a somewhat atypical flavour in banana, especially at the 4 kGy dose. Overall, samples were considered to be acceptable (Table IX).

In a second experiment, both control and irradiated fruit salads prepared with orange juice containing calcium chloride were tasted by two laboratory workers and were found to be unpleasant. The calcium addition made both the control and irradiated salad taste salty, and this combined with the atypical flavour of banana in irradiated samples, made them unacceptable. Fruit texture was slightly improved from previous samples.

In order to prevent this product from tasting salty, when the fruit salads were prepared again, the cut fruits were sprayed with a 2% calcium chloride solution in water, and then dipped into orange juice. Results revealed that the taste was good and not salty.

TABLE VII. TOMATO SALAD: SENSORY EVALUATION

Irradiation dose (kGy)	Day 2 after irradiation		Day 7 after irradiation	
	Average (n = 7)	Standard deviation	Average (n = 7)	Standard deviation
Aroma intensity (carrot and tomato as a whole were evaluated)				
0	1.50	0.84	–	–
2	2.50	1.22	2.86	1.57
4	2.60	1.51	3.14	1.95
Aspect (carrot and tomato as a whole were evaluated)				
0	5.00	0.89	–	–
2	5.29	1.07	5.29	1.11
4	5.14	1.10	5.29	0.76
Colour				
0	3.00	0.89	–	–
2	3.43	1.51	3.72	1.11
4	2.71	1.38	3.71	1.11
Flavour				
0	4.5	1.22	–	–
2	3.43	1.62	4.86	1.07
4	3.71	1.80	4.57	0.79
General acceptability				
0	4.67	1.37	–	–
2	4.14	1.46	4.71	1.50
4	4.00	1.53	4.52	1.27

Scale 1-7 where 7 = highest score. Analysis of variance, Dunnett test, p=95% confidence.

Irradiation destroyed to some extent the added pigments although the colour of irradiated samples containing Punzo or Allura red was stronger than in samples without them. Therefore, when Punzo red was added at a concentration of 200 mg/L and treated with 1.5 kGy, the fruit salad had a strong and pleasant red colour.

Table X shows the results of fruit salad sensory evaluation at the Nutrition School. In this case, the red colour in irradiated gelatine was intense, and general acceptability was good.

Regarding vanilla ice cream, significantly lower values were found in colour, flavour and general acceptability for samples treated with 9 kGy which was evidently too high a dose (Table XI). A greasy, waxy after-taste in 6 and 9 kGy samples was noticeable, while a loss of the milk cream flavour in irradiated samples was observed by more sensitive panellists. This after-taste was sometimes associated with a change from the typical vanilla flavour to a kind of lemon reminiscence, particularly at the higher irradiation doses. A general comment was that individually, most of the vanilla ice creams tasted were acceptable (with the exception of 9 kGy samples) and that differences were noticed only when comparisons were carried out with the control sample.

In a second experiment, both control and irradiated samples were tasted by two laboratory workers and not by a taste panel. They found that for:

– *Chocolate*: the 4 kGy sample presented an unpleasant taste, mostly rancid or burnt-like. Its colour was slightly lighter than the control sample.

- *Raspberry*: the pink colour was lighter in the irradiated sample, and its flavour was slightly weaker (less sweet) than the controls. However, it should be kept in mind that the seeds present in this type of ice cream are not recommended for immunosuppressed patients due to the cellulose content.
- *White chocolate*: the irradiated samples presented a greasy, unpleasant waxy taste, having also lost some sweetness.
- *Peach*: both control and irradiated samples had a similar weak taste and colour.

  Milk jam ("Dulce de Leche") with banana: Irradiated samples presented a pleasant flavour, although not as pleasant as the control samples which were sweeter, and also the irradiated samples left a waxy after-taste. The light-brown colour of the ice cream remained unchanged.
- *Frozen cream with whole cherries*: The whole cherries became lighter in colour after irradiation, and the frozen cream also left a greasy after-taste.
- Apparently, as could be expected from knowledge of radiation chemistry, water-based ice creams would react better to treatment than cream-based ones.

TABLE VIII. CARROT SALAD: SENSORY EVALUATION

Irradiation dose (kGy)	Day 2 after irradiation		Day 7 after irradiation	
	Average (n = 7)	Standard deviation	Average (n = 7)	Standard deviation
Aroma intensity (carrot and tomato were evaluated as a whole)				
0	1.50	0.84	–	–
2	2.50	1.22	2.86	1.57
4	2.60	1.51	3.14	1.95
Aspect				
0	5.17	0.98	–	–
2	5.43	1.13	5.29	1.11
4	5.43	0.79	5.29	0.76
Colour				
0	3.67	1.03	–	–
2	3.71	1.60	4.00	1.16
4	3.57	1.27	4.29	0.76
Flavour				
0	5.0	1.10	–	–
2	4.29	1.11	5.33	0.82
4	4.57	1.13	5.00	0.63
General acceptability				
0	5.00	1.10	–	–
2	4.86	0.90	5.14	0.90
4	4.57	0.53	5.00	0.63

Scale 1-7 where 7 = highest score. Analysis of variance, Dunnett test, p=95% confidence.

TABLE IX. FRUIT SALAD IN GELATINE: SENSORY EVALUATION

Irradiation dose (kGy)	Day 2 after irradiation		Day 7 after irradiation	
	Average (n = 7)	Standard deviation	Average (n = 7)	Standard deviation
		Aroma intensity		
0	4.00	1.73	3.86	1.77
2	3.70	1.60	3.71	1.70
4	4.29	1.11	3.71	1.98
		Aspect		
0	5.86	0.38	5.29	0.76
2	5.86	0.69	5.14	0.90
4	5.71	0.49	4.00#	1.41
		Colour		
0	4.86	0.69	4.57	0.53
2	3.57 [a]	1.13	3.12 [a]	0.64
4	2.57 [a]	0.98	2.57 [a]	1.62
		Flavour		
0	5.57	1.27	4.86	1.46
2	5.50	1.16	5.14	0.69
4	5.24	1.18	5.14	1.07
		General acceptability		
0	5.14	1.22	5.00	1.00
2	5.00	1.15	5.57	0.53
4	4.43	1.13	5.00	1.15

Scale 1-7 where 7 = highest score. Analysis of variance, Dunnett test, p=95% confidence.
[a] Significantly different from non-irradiated control sample.

TABLE X. FRUIT SALAD IN GELATINE: SENSORY EVALUATION

Aroma	5.4 ± 0.8
Aspect	4.9 ± 1.2
Flavour	5.9 ± 0.8
General acceptability	5.7 ± 0.7

(Nutrition School, n = 40)

Some ice cream flavours were chosen for further sensory testing by the two panels as mentioned in Table XII. For peach and raspberry (water-based ice creams) no significant differences were found between control and irradiated samples, and general acceptability was good considering that these products are not usually very tasteful. On the contrary, irradiated cream-based ice creams were evaluated as just acceptable at the Nutrition School. Appreciation of these products may have been influenced by the fact that they were over-frozen (in dry ice).

TABLE XI. VANILLA ICE CREAM: SENSORY EVALUATION

Irradiation dose (kGy)	Day 2 after irradiation		Day 45 after irradiation	
	Average (n = 10)	Standard deviation	Average (n = 10)	Standard deviation
		Aroma intensity		
0	1.20	0.63	1.70	1.06
3	1.00	0	1.90	1.52
6	1.40	0.70	1.60	1.26
9	1.20	0.42	1.90	1.52
		Aspect		
0	5.80	1.23	5.60	1.08
3	6.00	0.94	5.40	1.17
6	5.30	1.34	5.60	0.97
9	5.40	1.26	5.80	1.14
		Colour		
0	4.10	0.99	4.60	0.73
3	3.60	0.97	3.40	1.13
6	3.50	1.18	3.00[a]	1.00
9	2.50 [a]	0.85	2.60[a]	1.01
		Flavour		
0	5.80	0.92	5.30	1.49
3	4.30	1.34	4.70	1.25
6	4.50	1.65	4.20	1.55
4	3.70 [a]	1.49	3.60	1.58
		General acceptability		
0	5.80	1.03	5.00	1.33
3	4.70	1.49	4.60	1.17
6	4.70	1.16	4.20	1.48
9	3.90[a]	1.20	3.60	1.65

Scale 1-7 where 7 = highest score. Analysis of variance, Dunnett test, p=95% confidence.
[a]Significantly different from non-irradiated control sample.

TABLE XII. OTHER ICE CREAMS: SENSORY EVALUATION

	Nutrition school (n = 23).		Ezeiza Atomic Center (n = 11).			
	Milk jam	Vanilla	Peach		Raspberry	
	3 kGy	3 kGy	0 kGy	3 kGy	0 kGy	3 kGy
Aroma	4.8 ± 0.9	3.9 ± 1.6	1.6 ± 0.8	1.8 ± 1.2	1.9 ± 1.1	1.9 ± 0.9
Aspect	5.4 ± 0.9	5.0 ± 0.8	5.1 ± 0.9	5.3 ± 0.7	5.6 ± 0.8	5.2 ± 1.0
Colour	–	–	2.5 ± 1.0	2.6 ± 1.0	4.7 ± 1.1	4.3 ± 1.2
Flavour	3.7 ± 1.5	3.3 ± 1.7	4.9 ± 1.3	4.6 ± 1.4	5.6 ± 1.3	4.9 ± 1.7
Acceptability	4.2 ± 1.4	3.6 ± 1.5	4.7 ± 0.8	4.7 ± 0.8	5.6 ± 1.2	5.1 ± 1.3

Scale 1-7 where 7 = highest score. Analysis of variance, Dunnett test, p=95% confidence.

Though no significant differences were observed between control and irradiated fresh stuffed pasta (“cannelloni”) samples, the latter were generally more preferable in flavour and general acceptability (Table XIII). Colour (red colour of the tomato sauce) was scored slightly higher in irradiated samples than non-irradiated controls on the first day of analysis while at 7 days after irradiation this observation was reversed probably because of phase separation of the sauce. This became more evident as irradiation dose increased. The phase separation could be corrected in the future by adding stabiliser to the sauce. The ricotta filling was found to be more consistent in irradiated samples.

TABLE XIII. FRESH STUFFED PASTA (“CANNELLONI”): SENSORY EVALUATION

Irradiation dose (kGy)	Day 2 after irradiation		Day 7 after irradiation	
	Average (n = 10)	Standard deviation	Average (n = 10)	Standard deviation
	Aroma intensity			
0	2.40	1.26	2.40	1.26
2	2.80	1.40	2.50	1.27
4	2.80	1.23	2.50	1.18
	Aspect			
0	5.00	1.33	5.10	0.99
2	4.80	1.40	4.70	1.42
4	5.10	1.45	4.70	1.25
	Colour (Tomato sauce)			
0	3.70	1.06	3.70	1.25
2	4.20	0.79	3.30	0.68
4	4.10	0.99	3.10	0.74
	Flavour			
0	5.00	1.05	3.80	1.03
2	5.30	0.95	4.80	1.03
4	6.00[a]	0.85	4.70	1.25
	General acceptability			
0	5.30	1.16	4.30	1.42
2	5.60	0.84	4.70	1.25
4	5.70	0.95	4.60	1.35

Scale 1-7 where 7 = highest score. Analysis of variance, Dunnett test, p=95% confidence.
[a]Significantly different from non-irradiated control sample.

Considering that in the sensory evaluation of “cannelloni” previously performed, some panel members had detected a phase separation in the tomato sauce, stabilisers based on xanthan gums were added in order to correct this behaviour. After 7 days storage at 2°C, no phase separation was observed in any sample. In order to determine if the phase separation was caused during microwave cooking, samples were heated for 25 min under the conditions previously described for this product. A slight phase separation was observed in control and irradiated samples without added gum but was not as pronounced as found in the sensory evaluation of the “cannelloni”. However, the stabilising effect of gums was not very noticeable and it was concluded that perhaps a greater concentration should be added to the sauces (i.e. 0.5%). The taste of both control and irradiated samples with and without gum was typical for tomato sauce. FRUTAGEL seemed to be the best gum for stabilising purposes as it also made the sauce look thicker.

TABLE XIV. BURGERS: SENSORY EVALUATION

	Nutrition School (n =40)		Ezeiza Atomic Center (n = 10)	
	Beef	Chicken	Beef	Chicken
Aroma	5.5 ± 0.8	4.6 ± 1.0	3.3 ± 1.4	2.8 ± 1.6
Aspect	5.0 ± 1.0	5.2 ± 1.0	4.9 ± 0.7	4.5 ± 1.2
Softness	–	–	4.8 ± 0.4	4.5 ± 0.5
Juiciness	–	–	4.5 ± 1.3	3.4 ± 1.0
Flavour	5.4 ± 0.8	4.8 ± 0.9	5.0 ± 0.9	4.6 ± 0.7
Acceptability	5.2 ± 0.7	4.7 ± 0.9	4.7 ± 1.0	4.3 ± 1.0

Scale 1-7 where 7 = highest score. Analysis of variance, Dunnett test, p = 95% confidence.

The general acceptability of the beef and chicken burgers was evaluated as good by the taste panel (Table XIV). Beef was found to be more preferable than chicken, although the differences were not significant.

3.4. Chemical analysis

The chemical analyses were not carried out on salads at this stage of the research as according to literature [26] no nutrient losses were expected at the irradiation doses employed. Texture was considered as best evaluated by sensory evaluation. Vitamin losses were not expected to occur to an unacceptable extent [26,27].

3.4.1. Vanilla ice cream

Water content, determined only in control samples so as to assure quality, was determined to be about 60%. Fat analysis was carried out mainly due to the waxy taste observed in high-dose irradiated samples and the results are presented in Tables XV to XVII.

No significant differences of the Student's t test ($p>0.05$) were found between the acid values of control and irradiated samples, indicating that no hydrolysis reactions had affected the triglycerides due to irradiation treatment (Table XV).

Absorbance in the visible region showed three maxima, at 415, 440 and 470 nm, typical of carotenoids, which were mainly attributed to the egg constituent of this ice cream as shown by egg yolk as a standard. Peaks corresponding to milk cream were very small. Irradiation destroyed these pigments to some extent in proportion to the applied doses and the sensory panel also noticed this. The 6 kGy samples showed no peak at the three maxima which was most probably because of the pigments being destroyed during extraction.

Absorbance in the ultraviolet region indicated two zones at 230 and 205 nm. The 230 nm zone is generally attributed to diene conjugated double bonds [28]. Peak shapes and heights were also similar between control and irradiated samples.

Thin layer chromatography showed the same pattern for every ice cream sample (Table XVII). Phospholipids remained at the origin, while more non-polar compounds migrated with different Rfs, according to the sample composition [29]. The standards run along with the samples showed those eggs and milk or milk cream were components of this vanilla ice cream, while margarine was not. This was also evidenced in the visible absorbance results.

TABLE XV. VANILLA ICE CREAM FAT: ACID VALUES

Irradiation dose (kGy)	mg KOH/g fat
0	3.5 ± 0.1
3	2.4 ± 0.04
6	4.5 ± 0.1
9	3.9 ± 0.3

TABLE XVI. VANILLA ICE CREAM FAT: MAXIMUM ABSORBANCE

Fat sample extracted	Wavelength (nm)				
from:	415	440	470	230	205
Ice cream (0 kGy)	0.52	0.63	0.50	1.00	0.44
Ice cream (3 kGy)	0.36	0.44	0.40	0.70	0.50
Ice cream (6 kGy)	No peak	No peak	No peak	1.04	0.53
Ice cream (9 kGy)	0.23	0.28	0.25	1.04	0.68
Egg yolk	1.64	1.90	1.60	1.80	0.53
Milk cream	0.10	0.14	0.16	1.22	0.44
Margarine	–	–	–	0.56	0.50

TABLE XVII. ICE CREAM FAT: THIN LAYER CHROMATOGRAPHY

Spot No.	Diameter (mm)	Rf.	Spot intensity (Visual comparison) (Scale: 1–10)						
			Ice cream (0 kGy)	Ice cream (3 kGy)	Ice cream (6 kGy)	Ice cream (9 kGy)	Egg yolk	Milk cream	Marga-rine
Developing solvent: Petroleum ether (Fraction 60–80°C) – Ethyl ether – Acetic acid, 90–10–1									
1	5	0	5	7	6	6	9	4	2
2	4	0.05	2	1	2	2	3	2	3
3	3	0.08	3	4	4	4	5	3	2
4	3	0.14	–	–	–	–	–	–	3
5	3	0.25	1	1	1	1	2	1	–
6	12	0.35	4	4	4	4	-	5	1
7	20	0.54	5	5	5	5	7	6	7
8	3	0.98	1	1	1	1	3	1	2
Developing solvent: Petroleum ether (Fraction 60–80°C) – Ethyl ether – Acetic acid, 40–60–l									
1	5	0	8	8	8	7	9	1	1
2	2	0.12	1	1	1	1	1	–	–
3	3	0.22	2	2	2	2	–	1	1
4	7	0.76	4	4	4	4	6	1	-
5	6	0.86	1	1	1	1	2	1	1
6	10	0.98	9	9	9	9	9	9	9

In addition, peroxide value determination showed undetectable levels in every case. This parameter, being a measure of rancidity which is important both from the sensory and the nutritional points of view, would have been expected to increase in a fat-containing product after irradiation treatment, especially at the higher doses employed. However, the low temperature during the process would have offered some protection, minimising the secondary effect of irradiation.

The analytical procedures carried out up to this point did not explain the waxy taste noticed by some panellists. However, although the subject merits further research, it is not important in relation to this work since the 3 kGy dose was found to be sufficient for decontamination purposes with samples maintaining an acceptable good sensory quality.

3.4.2. Burgers

3.4.2.1. Vitamin B1 (thiamine) analysis

Results revealed no detectable levels of thiamine (<0.5 μg/g) in any control or irradiated beef or chicken burgers. According to Ref. [29], the natural level of thiamine in some of these products (in the raw state) is 2.3 μg/g for beef muscle, 1 μg/g for beef fillet, 0.9 μg/g for beef rump and 0.7 μg/g for chicken breast with skin. Considering that thiamine is a temperature-sensitive vitamin, its levels in the burgers may have fallen below the detectable limit of the method, both in control and irradiated samples, during enzyme inactivation.

It is generally accepted that there can be some vitamin loss due to high-dose irradiation. Wilson [30] studied the destruction of thiamine, an extremely labile vitamin, the loss of which is the greatest nutritional change in irradiated meats yet recorded. Freezing to –75°C was found to be the most effective method of preventing destruction of this vitamin. According to Urbain [22] the thiamine in processed pork is poorly retained when this product is irradiated with 45 kGy at ambient temperature (2% retention) but that thermal canning also causes significant losses (20% retention). In addition, irradiation at sub-freezing temperatures can improve retention (i.e. pork irradiated with a 45 kGy dose at –80°C retains 85% thiamine). Diehl [31] stated that thiamine is more heat-sensitive than radiation-sensitive while Josephson [32] observed that both beef and pork radiation-sterilised meats retained much more thiamine than canned meats thermally sterilised.

Other authors have also noted how vitamin losses as a result of irradiation are similar to those encountered in the usual heat processing treatments [33], and how the irradiation of food in the frozen state lessens the radiolytic effects [22,34–38]. This happens because freezing alters the rate of reactions of radicals formed by irradiation, and hence the general course of subsequent reactions. According to Taub [20], electrons are constrained by the rigid ice to the regions in which they are formed and would be diverted from reacting with other primary radicals. Merrit [24] stated that although the relationship is not linear, there is an increase in the amount of radiolytic compound formed for a given dose (in this case 45 kGy) as the temperature of irradiation is increased. The lower the temperature the less likely it is for an irradiation flavour to be detected.

4. CONCLUSIONS

Gamma irradiation, in combination with suitable packaging and cold storage, of several ready-to-eat meals, i.e. tomato and carrot salad, fruit salad in gelatine, ice creams, and “cannelloni” (fresh stuffed pasta), has resulted in the production of meals that are microbiologically safe, of acceptable sensory quality, and without changes in their nutritional value. These kinds of meals could make a significant contribution to the diets of immunosuppressed patients, which can often lack fresh produce due to the risk of food-borne diseases.

The irradiation process also offered the possibility of sterile meats of good nutritive and sensory qualities, completely eliminating microbial risk to immunosuppressed patients whose health could be severely damaged if exposed to pathogenic microorganisms.

The experimental work to date should be considered as a screening of possibilities. Further research is needed to improve the results obtained and to broaden the range of foodstuffs available for consumption. Summer season fruits should be tested to evaluate their behaviour when irradiated. Regarding vegetable salads, other combinations could be tried, for example, cheese and vegetables, other tomato varieties, the application of edible coatings along with gamma irradiation. Irradiated water-based ice creams will be also analysed in the near future for their sugar and pigment composition while cream-based ice creams will be analysed for their pigment and fat content.

Other foodstuffs have been pointed out by nutritionists for study. These include pizza, “ravioli” stuffed with chicken and spinach, “empanadas” stuffed with ham and cheese, ground beef or chicken, stuffed tomatoes with boiled potato and carrot salad, stuffed eggs, all of which are nutritive meals, culturally appreciated in Argentina.

A 7 day storage period for the ready meals studied, with the exception of ice cream and burgers, was postulated both to permit a weekly provision to hospital kitchens and also because nutritionists recommend fresher rather than long-life products. However, longer storage times should be tested, not to supply hospital kitchens but perhaps supermarkets, considering that immunosuppressed patients who are clearly recovering and living at home, could also benefit from their availability.

Divulgation of these achievements and also of general knowledge on food irradiation commenced in the year 2000 at the university in Buenos Aires directed particularly to nutritionists. They naturally appeared as the first link in the communication chain, which should be followed considering the delicate subject of immunosuppression, the general lack of knowledge on the usefulness and wholesomeness of irradiated food as well as the practical application of the technology world-wide for treatment of hospital patients requiring clean food. It is the intention to continue offering such seminars and tasting of irradiated food to physicians involved with immunosuppressed patients.

The raddapertized burgers have drawn the attention of the Meat Research and Industrial Technology Center in Buenos Aires and it is intended to combine efforts and resources to develop other radiation sterilised meat formulations.

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DEVELOPMENT OF SHELF-STABLE FOODS FISH PEPES, CHICKEN AND MEAT DISHES THROUGH RADIATION PROCESSING

Z. IRAWATI, M.A. MAHA,[†] N. ANSORI, C.M. NURCAHYA, F. ANAS
Centre for Research and Development of Isotopes and Radiation Technology,
National Nuclear Energy Agency,
Jakarta, Indonesia.

Abstract

Development of safe, shelf-stable foods are necessary to reduce dependence on refrigeration during their storage and distribution. Studies were conducted to develop preparation of shelf-stable meals (fish, chicken and meat dishes) using gamma irradiation sterilisation with a minimum dose of 45 kGy. Goldfish pepes are a very popular dish in Indonesia, particularly in West Java. They are prepared by marinating clean-fish with seasonings, wrapping in banana leaf, then cooking until well done in a pressure cooker for 45–60 min. The cooked fish is then vacuum packed in a plastic pouch, followed by a laminate of polyester/aluminium foil/polyethylene as an outer package. Irradiation was conducted under cryogenic conditions with a minimum dose of 45 kGy and the samples were kept at 28–30°C post-irradiation. It was found that these irradiated fish pepes could be stored for more than one year at room temperature. The sensory quality, pH, moisture, protein and fat content were relatively stable during storage. The free radical content of the bone increased significantly with increasing irradiation dose, but decreased with time. Chicken pepes, opor, semur and curry casserole were also prepared and packed in laminated packaging materials prior to irradiation. Beef meat was used to make rendang, semur and empal. Results showed that the sterile samples vacuum packed in dry laminated bags made of PET 12μ /LDPE adh.2μ /Al-foil 7μ /LDPE adh./LLDPE (C_4) 50μ and comparable laminate pouches of PET 12μ /PE 20μ /Alu-Metalized PET 12μ /adh./LLDPE (C_8) 50μ for chicken dishes only and stored at room temperature (28 ± 2°C) up to 18 and 10 months, respectively, were acceptable by taste panellists. No significant changes in moisture, total protein and fat content, pH, and vitamins were found in the sterile samples during storage. The banana leaf as an inner wrapper packaging material for producing radiation sterilised fish and chicken pepes plays an important role in the prevention of irradiation-odour in these products either during processing or storage.

1. INTRODUCTION

Development of safe, shelf-stable food is necessary to reduce dependence on refrigeration during storage, marketing and distribution of perishable food products, which can be relatively costly for developing countries. Sterile shelf-stable food is urgently needed for hospital patients, in particular, treated in sterile conditions, in addition to various outdoor uses, such as for pilgrimages, hiking, camping, boating and for emergency packs [1].

Pepes is the name of an ethnic dish that is popular in certain parts of Indonesia. Usually, it is made of fish, with Goldfish pepes being very popular in West Java, while in other places, marine fish such as mackerel and anchovies are commonly used in making pepes. Chicken pepes and the chicken casserole dishes, opor, semur and curry, along with the beef meat dishes, rendang, empal and semur, are ethnic dishes also popular in certain parts of Indonesia.

Traditionally, pepes is prepared by mixing fish or chicken with a seasoning or spice mixture, wrapped in banana leaf, and then cooked until well done or the bones become soft. The seasoning used in different places may vary in composition, but usually contains candle-nut or shredded coconut. Basically, the seasonings used for chicken casserole dishes are the same as for pepes, but the preparation is different. An emulsifier, xanthan, and coconut milk are added into each type of chicken casserole dish during processing. With regard to beef dishes, rendang is mostly popular in West Sumatra. Traditionally rendang, empal and semur are prepared by mixing cut-meats with a seasoning or spice mixture, then cooking until well done in coconut milk for rendang and deep frying palm oil

[†] Deceased

for empal. Beef semur is prepared using the same cooking method as for chicken with very slight modifications. The seasonings usually incorporated in the beef dishes are usually chilli, garlic, shallot, and herbs. As the moisture content of all of these products is high, i.e. around 60–70%, they tend to spoil very quickly at room temperature.

Some ethnic dish producers at home and industrial level have inquired about the possibility of using irradiation for extending the shelf-life of these products. A previous study conducted at the Centre of Research indicated that goldfish pepes irradiated at 7.5 kGy can be stored for more than 15 days at room temperature [2]. Irradiation at higher doses had a significant effect on the sensory properties of the fish, since the product was air packed and irradiated at room temperature. Irradiation in a vacuum pack at cryogenic temperature using a sterilisation dose to produce shelf-stable foods has been developed successfully by several investigators [3-7].

The current study was carried out in order to develop the preparation of shelf-stable dishes using ionizing radiation as a sterilisation technique, in order to promote the products to be marketed and distributed widely without refrigeration under tropical conditions.

2. MATERIALS AND METHODS

2.1. Fish pepes

2.1.1. Materials

Live goldfish (*Cyprinus carpio* Linn.) of about 330 g in size, obtained from a freshwater pond near the Centre for Research, were used as the material for pepes. The packaging materials used were banana leaves, low density polyethylene (0.01 mm thick), and aluminium foil laminate of PET/LDPE/Al-foil/LLDPE (0.095 mm thick). A comparative study using another laminate of PET/PE/Alu-metalized PET/adh./LLDPE was carried out. The ingredients needed for seasoning of 1 kg fish were: candle nut (50 g), garlic (10 g). shallot (50 g), turmeric (15 g), ginger root (*Alpina galanga*) (20 g), ginger (20 g), red chilli (30 g), lemon grass (12.5 g), bay leaves (6 pieces), lemon (2 pieces), and salt (27 g). All ingredients except salt were fresh products. This recipe formulation has been optimised in the preliminary study [2].

2.1.2. Sample preparation and treatment

The fish was first eviscerated by removing the intestines, washed with tap water, mixed with lemon juice and salt (juice from one lemon plus 3 g salt/kg fish), kept for 15 min, washed again with tap water, and marinated in seasoning (see Section 2.1.1.). After marinating for 2 h, each fish was wrapped in a banana leaf, steamed in an Inoxpran pressure cooker for 45 or 60 min at 120°C, drained and cooled at room temperature, and stored overnight in a freezer at –13°C. The frozen fish pepes were then individually vacuum packed in LDPE pouches and double packed (vacuum) in Al-foil laminate pouches. The samples were placed in sealed styrofoam boxes (height × width × length = 33.75 × 36.25 × 51.25 cm^3) filled with dry ice and stored overnight prior to being irradiated.

2.1.3. Irradiation treatment

Gamma irradiation was conducted at the IRKA irradiator at the National Nuclear Energy Agency, Pasar Jumat, Jakarta. Cobalt-60 was used as the source of ionizing radiation (capacity of ca. 195 Kci) at a dose rate of 5.2 kGy/h. An FW-60 radiochromic film was used as the calibration dosimeter and red perspex dosimeters were used to determine absorbed dose. Samples were irradiated with a minimum dose of 45 kGy in dry ice as described in Section 2.1.2. resulting in an irradiation temperature of -47°C. The sterilisation dose was determined according to the AAMI (Association for the Advancement of Medical Instrumentation) ISO/DIS 11137.2 method based on the bioburden [8].

2.1.4. Storage conditions

Following completion of irradiation, the experimental samples were stored at room temperature and at 55°C as a comparative observation. Quality evaluation for all dishes was carried out after storage periods of 0, 2 4, 6, 8, 10, 12 and 18 months.

2.1.5. Methods of analysis

Organoleptic attributes (colour, appearance, odour, taste and texture), free radical content of fish bones, moisture content, protein content, fat content, and pH were measured for samples stored at room temperature. Sterility testing was carried out on the samples before and after storage. Observations conducted on samples stored at 55°C included swelling of the package and unacceptable sensory quality. Microbiological assessments were done at each step of the process to determine the microbial load of the fish, tap water and ground seasoning and the processed dishes

2.1.5.1. Microbial assessments and sterility testing

Microbial load was enumerated in Tryptic Soya Agar media after incubation at 30°C for 7 days. Sterility testing of the chicken and beef meat dishes was carried out by putting the sample aseptically into sterile Thyo Glycolate Broth, then incubating at room temperature for up to 21 days. Sterility testing was carried out on samples before and after storage.

2.1.5.2. pH, moisture, protein and fat content

Sample pH was measured using a Microcomputer pH meter LEC 60. Moisture content was determined gravimetrically by drying samples in an oven at 105°C for 1.5 h. Protein content was measured by means of spectrophotometry using the Biuret method. Determination of fat content was done by extraction using petroleum benzene (60–80°C) or 5 h.

2.1.5.3. Sensory analysis

Organoleptic testing was done by 5 to 10 selected panellists using a 5 point hedonic scale. A score of 5 = excellent, 4 = good, 3 = fair, 2 = poor, and 1 = extremely poor.

2.1.5.4. Free radical content of fish bones

The fish bone was first washed with tap water and stored in a freezer at –13°C overnight, freeze-dried for 4 days and ground prior to ESR measurement. The free radical content, expressed in peak area/g, was measured using an ESR RIX JEOL Spectrometer employing the following conditions: Centre field 335.5 mT; sweep width 10.00 mT; sweep time 1.0 min; microwave power 1.00 mW; modulation frequency 9.4350 GHz; modulation width 0.12500 mT; and time constant 0.03 sec.

2.1.6. Results and discussion

Results of microbial assessments on tap water, ground seasoning and the fish after each step of processing before radiation-sterilisation are presented in Table I. It was found that all the materials contained a microbial load in the order of 10^2–10^3 CFU/g and that washing with lemon and salt can reduce microbial contamination. Fish pepes pressure-cooked for 45 min were still found to contain microbes, i.e. spore-forming *Bacillus* in the order of 10^2 CFU/g, which is the reason why this product deteriorates quickly. The microbial load of fish pepes pressure-cooked for 60 min was almost undetectable, but results of sterility testing indicated that the product was not yet sterile.

The pH, moisture, protein and fat content of the radiation-sterilised fish pepes during storage up to one year at room temperature are presented in Table II. All parameters were relatively stable

during storage, indicating that deterioration did not occur. The pH of the non-irradiated control was 6.39%, the moisture content 64.94%, and the fat content 28.64%. Sterile samples stored at 35°C for up to one year were also of good sensory quality, and no swelling was found in the stored samples. Results of sterility testing conducted periodically every month indicated that the samples were sterile.

Organoleptic scores of radiation-sterilised fish pepes stored for up to 18 months at room temperature (28 ± 2°C) are presented in Table III. These data showed that the sensory quality of both products cooked for 45 and 60 min were similar. Differences were found only for the texture of the bones, which were softer in pepes cooked for 60 min, although both were acceptable. The effect of storage time up 18 months was minimal on the sensory quality of the pepes. The pepes cooked for 45 min and irradiated with a dose 45 kGy produced a slight off-odour immediately after irradiation, but the off-odour disappeared upon storage. A comparative study using different packaging materials showed that the pepes stored in the alternative packaging material, Al-Metalized-PET laminate, was acceptable by the taste panellists even after 12 months of storage.

Result of ESR measurements of the free radical content of the bones of the fish pepes are illustrated in Figs 1 and 2. Figure 1 shows that the free radical content was greater in hard bone, i.e. tail bone, than in softer bone, i.e. head bone and backbone. The free radical content decreased with time and after 6 months of storage the concentration measured in irradiated samples was similar to that of the non-irradiated controls, i.e. 1.46×10^5 in head bone, 1.08×10^5 in backbone, and 1.82×10^5 in the tail bone. Figure 2 illustrates the relationship between storage time and free radical content of the backbone of the fish pepes. The difference between fish cooked for 45 and 60 min was relatively small.

TABLE I. MICROBIAL LOAD OF WATER, SEASONING, AND FISH AT EACH STEP OF PROCESSING OF FISH PEPES

Sample	Total plate counts (CFU/g)*
Tap water (not potable)	3.00×10^2
Ground seasoning	3.95×10^3
Fish, after washing with tap water	2.07×10^3
Fish, after washing with lemon + salt followed by tap water	1.40×10^3
Fish, after marinating in seasoning for 2 h	1.12×10^4
Fish pepes after cooking (45 min)	3.57×10^2

* Average of two replicates

TABLE II. RESULTS OF pH, MOISTURE, PROTEIN AND FAT CONTENT MEASUREMENTS* OF RADIATION-STERILISED FISH PEPES STORED FOR 18 MONTHS AT ROOM TEMPERATURE

Duration (months)	pH		Moisture content (%)		Fat content (%)		Protein content (%)	
	45 min	60 min	45 min	60 min	45 min	60 min	45 min	60 min
0	6.31	6.29	61.31	62.02	6.03	27.54	–	–
6	6.08	6.10	60.22	61.90	29.27	27.65	23.03	23.33
12	5.95	6.05	61.19	61.93	27.65	28.95	21.42	20.96
18	6.05	5.95	56.82	59.69	21.78	23.03	18.58	18.96

* Average of three replicates

TABLE III. ORGANOLEPTIC SCORES* OF FISH PEPES COOKED FOR 45 AND 60 MIN THEN STERILISED AT 45–61 kGy AND STORED FOR 18 MONTHS AT ROOM TEMPERATURE

Duration	Appearance		Odour		Taste		Texture	
(months)	45 min	60 min	45 min	60 min	45 min	60 min	45 min	60 min
0	4.5	4.5	4.5	4.5	3.5	4.5	4.5	4.5
2	5.0	5.0	4.5	4.5	4.5	5.0	4.5	4.5
4	5.0	5.0	4.5	4.5	5.0	5.0	4.0	4.5
6	4.5	4.5	3.5	3.5	4.5	4.0	4.5	4.0
8	4.5	4.5	4.0	4.0	4.5	5.0	4.5	4.5
10	4.0	4.0	4.0	4.0	5.0	5.0	4.0	4.0
12	4.0	4.0	4.0	4.0	5.0	5.0	4.0	4.0
18	4.0	4.0	4.0	4.0	5.0	5.0	4.0	4.0

* Average of 10 panellists

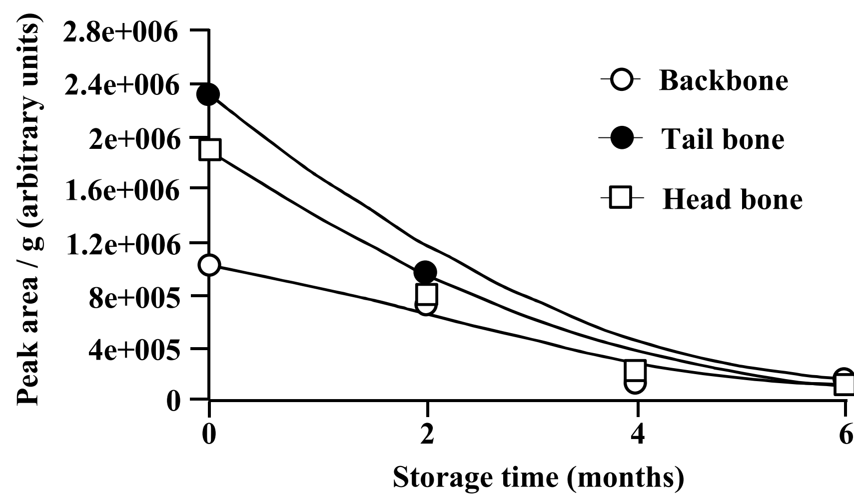

FIG. 1. Relationship between storage time at room temperature and free radical content (peak area/g) in the backbone, tail bone and head bone of fish pepes.

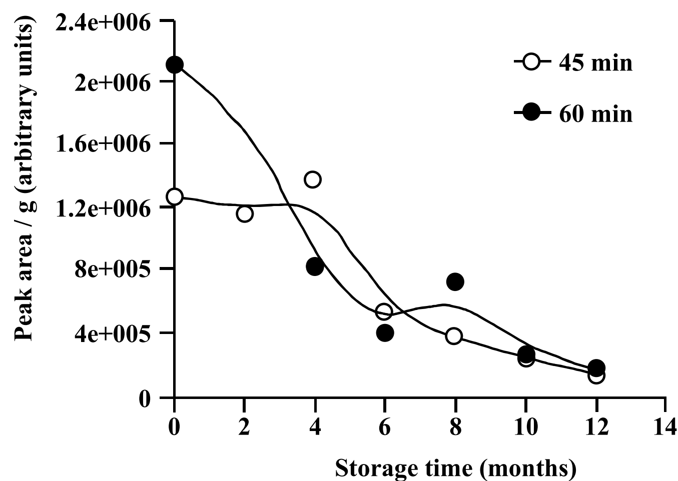

FIG.2. Relationship between storage time at room temperature and free radical content (peak area/g) in the backbone of fish pepes cooked for 45 or 60 min.

2.2. Chicken and beef meat dishes

2.2.1. Materials

Chicken meat with bone purchased from a local market was used for preparation of chicken pepes, opor, semur and curry. The quantity of chicken used for each type of dish was 10 kg or about 120 parts. The packaging materials used were banana leaf as inner-liner (for chicken pepes only) while aluminium foil laminate PET 12 μ/LDPE as adh. 2μ/Al-foil 7μ/LDPE as adh./LLDPE 50μ was used as the outer packaging. For comparison purposes, another type of laminate, PET 12μ/PE 20μ/Alu-Metalized PET 12μ/adh./LLDPE 50μ, was used during the study of chicken dishes only. The pouch size was 21 × 17 cm^2.

Beef meat was purchased from the local market for preparation of rendang, empal and semur. The quantity of meat used for each type of dishes was 20 parts/kg. The packaging material used was an aluminium foil laminate PET 12μ/LDPE as adh.2μ/Al-foil 7μ/LDPE as adh./LLDPE 50μ, as outer pack and the size individual pouch was 21 × 17 cm^2.

The ingredients required for seasoning of the chicken and beef for the different dishes are presented in Tables IV and V, respectively.

TABLE IV. INGREDIENTS REQUIRED FOR SEASONING OF CHICKEN PEPES, OPOR, SEMUR AND CURRY

Ingredients and	Quantity (g/kg chicken)			
Seasonings	Pepes	Opor	Semur	Curry
Alpina galanga	20	20	15	-
Bay leaf	2 pieces	2 pieces	-	-
Candle nut	40	5	2	-
Caraway sed	-	2	1.5	-
Chilli (*rawit*)	10	-	-	-
Cinnamon	-	1 pieces	-	-
Citrus hystrix	2 pieces	-	-	-
Clover	-	1 pieces	-	-
Coconut milk	-	0.5 pieces	0.5 pieces	-
Coriander	-	10	5	-
Fragrant grass leaf	1	2 pieces	2 pieces	-
Garlic	10	10	10	30
Ginger	20	2	20	-
Javanese tamarind	-	0.5	5	-
Lemon	1 pieces	-	-	-
Nutmeg	-	-	-	1 pieces
Palm oil	-	20	-	-
Red chilli	-	-	-	6 pieces
Salt	30	15	15	10
Shallot	50	20	20	50
Sweet soy sauce	-	-	120 cc	-
Tumeric	15	-	-	-
White pepper	-	5	10	-
Xanthan	-	5	5	10

TABLE V. INGREDIENTS REQUIRED FOR SEASONING OF RENDANG, EMPAL AND SEMUR

Ingredients and seasonings	Quantity (g/kg beef meat)		
	Rendang	**Empal**	**Semur**
Red chilli	100	50	-
Chilli (*cabe rawit*)	-	12 pieces	-
Shallot	100	400	100
Garlic	16	150	75
Ground coriander	-	2ts	-
Pepper	-	-	20
Fresh bay leaf	-	4 pieces	-
Fragrant grass leaf	3 pieces	-	-
Turmeric leaf	1 piece	-	-
Lemon leaf	6 pieces	-	-
Nutmeg	-	-	3 pieces
Asam kandis/tamarind	1 piece	-	-
Javanese tamarind	-	2ts	-
Fermented fish	-	2ts	-
Palm sugar	-	2ts	-
Salt	2 sp	3ts	2sp
Soya sauce	-	-	1.5 glass
Palm oil	-	4sp	4 sp
Alpina galanga	35g	150g	-
Ginger	75g	-	-
Coconut oil	1.5 L		

The pressure cooker employed for the experimental work was an Inoxpran with a special sandwich bottom material for heat diffusion pan. A large stainless steel wok was also used for cooking purposes. The vacuum seal machine used was a Multivac Type/Model A-500/12 at pressure under 800 mBar.

2.2.2. Sample preparation and treatment

2.2.2.1. Chicken pepes

Chicken was cut into smaller pieces (80–100 g/piece), washed with tap water, mixed with seasoning and kept for 2 h at 28–30°C. After seasoning (Table IV) the chicken pieces were individually wrapped in banana leaf, two per pack, steamed in a pressure cooker for 1 h at 120°C, drained and cooled at room temperature (28–30°C), and stored in a freezer at –18°C overnight. The frozen chicken pepes was then individually vacuum packed in PET/Al-foil/LLDPE laminate pouches and PET/Metalized-PET/LLDPE laminate pouches and stored in a sealed styrofoam box filled with dry ice overnight prior to being irradiated.

2.2.2.2. Chicken opor

As before, chicken was cut into smaller pieces, washed with tap water and steamed for 0.5 h. All the seasonings (Table IV) for chicken opor, except fragrant grass leaf and *Alpina galanga*, were

finely ground, then cooked in palm oil. When fragrant smells occurred, coconut milk, fragrant grass leaf, and *Citrus hytrix* were added. The chicken pieces and slurry of Javanese tamarind were added to the seasoning-coconut mixtures which was boiled until well cooked after which a slurry of xanthan was added as an emulsifier. The opor was cooled down at room temperature and packaged as described in Section 2.2.1. Each laminate pouch contained two pieces of chicken.

2.2.2.3. Chicken semur

The preparation steps for making semur were the same as for chicken opor, with some different ingredients being used (Table IV). Coconut milk was not added but sweet soya sauce was used as an additional seasoning.

2.2.2.4. Chicken curry

The preparation steps for chicken curry were the same as for chicken opor and semur although some different ingredients were used (Table IV). Coconut milk was added but soya sauce was not used in this recipe.

2.2.2.5. Beef rendang

Beef meat was cut into small cubes (20 cubes/kg), washed with tap water, mixed with seasoning (Table V) and kept for 2 h. After soaking in the seasoning, the mixture was placed in a wok and cooked for 20 min until dry. Coconut milk and *Asam kandis*, a specific tamarind, were added into the mixture that was boiled for another 20 min. After boiling, the cooked beef meat was taken out. The mixture without the meat was boiled for a further 30 min in the wok until the mixture took the form of a brownish slurry. The cooked meat was put back into the wok and fried for another 2 h. The rendang was allowed to cool down at room temperature prior to being vacuum packed in the laminate pouches as described in Section 2.2.1. Two pieces of meat were placed into each pouch and stored in a freezer overnight at –18°C.

2.2.2.6. Empal

Beef meat was cut into slanted shapes, the size being about 1 × 5 × 7 cm/slant, washed with tap water, mixed with coriander and kept for 15 min. The mixture was then cooked with a small quantity of water until tender. All ground seasonings (Table 5), except the bay leaf, were cooked in palm oil. After fragrant smells occurred, the beef meat and coriander mixture were added and cooked until dry. Red chilli and shallot were cut into small pieces and fried with coconut oil. The dried-cooked beef meat was then added into the red chilli and shallot, mixed thoroughly and cooked on a medium stove-fire until well done. The empal was cooled down at room temperature before packing in laminate pouches. Each pouch contained two pieces of beef.

2.2.2.7. Semur

The preparation steps for making semur were similar to chicken semur, but there were some differences in the ingredients used (Table V). Coconut milk was not added but sweet soya sauce was used as an additional seasoning.

2.2.3. Irradiation, storage and methods of analysis

Irradiation and storage treatments along with methods of analysis for the chicken and beef meat dishes have been described in Section 2.1.3. to 2.1.5. In addition, chicken pepes were also analysed for vitamin content. Vitamin B_1 (thiamine) and E (tocopherol) were measured using high performance liquid chromatography (HPLC) employing μ-Bondapack C18 and silica columns.

2.2.4. Results and discussion

Results of microbial assessments on tap water, ground seasonings of chicken and beef meat dishes after each step of processing before and after sterilisation are presented in Tables VI and VII. The data showed that all materials used for various dishes contained microbes in the order of 10^2–10^5 CFU/g. After steaming and cooking for 1 h, the dishes still contained microbes, i.e. spore forming *Bacillus* in order of 10^2 CFU/g. This result indicates that the presence of the bacteria will contaminate the chicken products quickly. Similar results were found for beef which, after washing, contained microbes in the order of 10^2–10^3 CFU/g. Following preparation and cooking the meat contained microbes in the order of 10^2 CFU/g having been reduced by 2 log cycles due to processing. Sterility testing of all irradiated beef meat dishes before and after 12 months storage at room temperature revealed that an irradiation dose of 45 kGy can sterilise these products.

TABLE VI. MICROBIAL LOAD OF TAP WATER, SEASONINGS AND CHICKEN MEAT AT EACH STEP OF PROCESSING

Sample	Total plate count (CFU/g)*	
	aerobic	anaerobic
Tap water (not potable)	3.90×10^2	1.86×10^2
Ground seasoning	2.30×10^3	1.15×10^3
Chicken meat after washing with tap water	2.68×10^3	1.60×10^3
Chicken meat after soaking in seasoning for pepes preparation	3.90×10^4	1.20×10^4
Chicken meat after soaking in seasoning for opor preparation	6.30×10^5	–
Chicken meat after soaking in seasoning semur preparation	2.80×10^5	–
Chicken meat after soaking in seasoning for curry preparation	3.90×10^4	–
Chicken meat after soaking then stored at freezer temp. overnight:		
Chicken pepes	1.95×10^2	1.20×10^2
Chicken opor	1.04×10^2	–
Chicken semur	4.50×10^2	–
Chicken curry	2.10×10^2	–
TPC of irradiated all types of chicken dishes at 45 kGy as final product without storage and sterility test after 18 months of storage at room temperature	0	0

* Average of 3 replicates

The pH, moisture, protein and fat content of the irradiated chicken and beef meat dishes during storage for 18 months at room temperature are presented in Tables VIII and IX. Coconut oil which was added into the mixture during rendang preparation gave a high percentage fat content in the final product. However, results showed that all the parameters measured for the irradiated dishes were relatively stable during storage, indicating that deterioration did not occur.

Results from the analysis of vitamins B_1 and E in irradiated chicken meat and chicken pepes are presented in Table X. It was found that irradiation did not effect the vitamin B_1 content of the samples, but that the vitamin E content increased after meat preparation. This increase was most probably due to the types of seasoning used in making the pepes, which naturally contain antioxidants or vitamins such as vitamin E.

TABLE VII. MICROBIAL LOAD OF TAP WATER, SEASONINGS AND BEEF MEAT DISHES AT EACH STEP OF PROCESSING

Sample	Total plate count (CFU/g)*
Tap water (not potable)	$1.5 \times 10^3 \pm 0.2$
Beef meat after washing with tap water	$1.3 \times 10^3 \pm 0.3$
Rendang	
Ground seasoning/g	$2.3 \times 10^4 \pm 0.8$
Rendang preparation after cooking	$2.0 \times 10^2 \pm 0.7$
Empal	
Ground seasoning/g	$9.7 \times 10^4 \pm 3.0$
Empal preparation after cooking	$7.7 \times 10^2 \pm 5.4$
Semur	
Ground seasoning/g	$16.7 \times 10^3 \pm 2.0$
Semur preparation after cooking	$1.2 \times 10^2 \pm 0.2$
Radiation sterilisation of beef meat dishes at 45 kGy before and after storage	0

* Average of 3 replicates

TABLE VIII.RESULTS OF MOISTURE, PROTEIN AND FAT CONTENT AND pH MEASUREMENTS* OF RADIATION-STERILISED CHICKEN DISHES STORED FOR 18 MONTHS AT ROOM TEMPERATURE

Product	Duration (month)	Moisture content (%)	Protein content (%)	Fat content (%)	pH
Pepes	0	57.39	15.25	31.19	6.25
	6	57.20	15.35	32.25	5.95
	12	56.90	15.16	30.16	5.75
	18	56.40	15.15	29.85	5.25
Opor	0	59.84	17.75	9.07	5.75
	6	59.44	17.50	8.48	5.50
	12	58.60	17.45	8.45	5.00
Semur	0	49.79	17.60	6.49	5.35
	6	49.51	17.45	6.36	5.00
	12	48.47	17.40	6.30	5.00
	18	47.98	17.35	6.25	4.80
Curry	0	60.79	16.85	7.35	5.55
	6	59.84	16.80	7.10	5.25
	12	58.29	16.65	7.05	5.10
	18	57.67	16.50	7.10	4.75

* Average of 3 replicates

TABLE IX. RESULTS OF MOISTURE, PROTEIN AND FAT CONTENT AND pH MEASUREMENTS* OF RADIATION-STERILISED BEEF MEAT DISHES STORED FOR UP TO 18 MONTHS AT ROOM TEMPERATURE

Product	Duration (month)	Moisture content (%)	Protein content (%)	Fatcontent (%)	pH
Rendang	0	59.23	16.35	27.15	6.50
	6	57.20	16.20	27.00	5.70
	12	56.70	16.13	26.85	5.35
	18	55.55	15.93	26.50	5.30
Empal	0	49.75	16.26	11.35	6.30
	6	47.42	16.17	11.15	5.85
	12	45.60	15.85	11.00	5.35
	18	45.30	15.15	10.48	5.25
Semur	0	59.60	17.60	12.18	6.25
	6	58.54	17.45	11.68	6.20
	12	57.35	17.40	11.40	5.95
	18	56.98	17.35	10.70	5.80

* Average of 3 replicates

Table XI shows the organoleptic scores of chicken dishes packed in PET/adh./Al-foil/adh./LLDPE, sterilised at 45 kGy and stored up to 18 months of storage at room temperature. Data obtained showed that all irradiated chicken dishes were still acceptable by the taste panellists for up to 18 months except for chicken opor. The previous study on irradiated chicken pepes found that the pepes directly wrapped with polyethylene were rejected by the panellists because of a "strong irradiation smell" of the product. Chicken pepes without basil leaves showed better results for colour, odour and taste than with basil leaves as an additional seasoning. Therefore, the pepes without basil leaves were more acceptable. The banana leaves give a positive effect on the quality of chicken pepes especially in odour, taste and general appearance during sensory evaluation. The organoleptic score for texture and appearance of chicken opor showed a decrease after 12 months of storage. This was most probably caused by the ingredients used to make the product.

TABLE X. RESULTS OF VITAMIN B_1 (mg/100g), AND VITAMIN E (ng/g) MEASUREMENTS* OF CHICKEN MEAT (A), CHICKEN PEPES BEFORE (B) AND AFTER IRRADIATION (C) AT 45 kGy STORED FOR 12 MONTHS AT ROOM TEMPERATURE

Duration (months)	Vitamin B_1 (mg/100g)			Vitamin E (ng/g)		
	A	B	C	A	B	C
0	4.67	4.67	4.67	0.39	0.64	0.94
12	4.67	4.67	4.67	0.40	0.60	0.90

*Average of 3 replicates

TABLE XI. ORGANOLEPTIC SCORES* OF CHICKEN DISHES: PEPES, OPOR, SEMUR AND CURRY VACUUM PACKED IN LAMINATES OF PET/ADH./AL-FOIL/ADH./LLDPE POUCHES, RESPECTIVELY, THEN STERILISED AT 45 kGy AND STORED FOR UP 18 MONTHS AT ROOM TEMPERATURE

Product	Duration (months)	Appearance	Odour	Taste	Texture
Pepes	0	4.8	4.8	4.8	4.8
	2	4.6	4.4	4.5	4.6
	4	5.0	5.0	5.0	5.0
	6	5.0	5.0	5.0	5.0
	8	4.8	4.8	4.8	4.8
	10	5.0	5.0	5.0	5.0
	12	5.0	5.0	5.0	5.0
	18	4.0	4.0	4.0	4.0
Opor	0	4.4	4.4	4.4	4.8
	2	4.0	4.0	3.5	4.0
	4	4.2	4.2	4.2	4.6
	6	5.0	5.0	5.0	5.0
	8	4.4	4.2	4.3	4.4
	10	4.2	4.2	4.3	4.0
	12	4.6	4.6	4.3	4.6
	18	4.0	4.3	4.0	4.0
Semur	0	4.6	4.4	4.6	4.8
	2	4.3	4.3	4.3	4.3
	4	4.0	3.8	4.6	4.6
	6	5.0	5.0	5.0	5.0
	8	4.2	4.4	4.4	4.4
	10	4.0	4.2	4.3	4.0
	12	4.6	4.6	4.3	4.6
	18	5.0	5.0	5.0	5.0
Curry	0	4.2	4.2	4.2	4.8
	2	4.3	4.1	4.3	4.3
	4	4.6	4.2	4.2	4.6
	6	5.0	5.0	5.0	5.0
	8	4.0	4.2	4.2	4.4
	10	4.2	4.2	4.2	4.4
	12	4.6	4.6	4.3	4.6
	18	4.3	4.2	4.0	4.5

*Average of 10 panellists

Table XII shows an alternative study on using packaging material PET/adh/Alu-Metalized PET/LLDPE for chicken dishes then sterilised at 45 kGy and stored for up to 12 months at room temperature. The results show that chicken dishes packed with this type of packaging material were also acceptable by the panellists. Unfortunately, this type of packaging is not suitable for use as a material for radiation sterilisation shelf-stable foods due to the fact that the metalized material does not possess a strong laminate structure for this purpose.

Table XIII shows the organoleptic score of beef dishes packed separately in PET/adh./Al-foil/adh./LLDPE then sterilised at 45 kGy and stored for up to 18 months of storage at room temperature. Immediately after treatment, the inner part of each rendang meal exhibited a pinkish-red colour. This was probably caused by changes in cell structure within the meat in the frozen state prior to irradiation and during treatment. Data obtained showed that all irradiated beef meat dishes, except for empal, were still acceptable by the taste panellists following storage for 18 months. The empal

meat became more tender after 12 months of storage. The intensity of the brownish colour in rendang decreased after the same storage period. The organoleptic scores for texture and appearance of all beef meat dishes exhibited a decrease after 12 months of storage. This was most likely caused by the long cooking process in making meat dishes, and the high-dose of irradiation that may have affected the tissue integration of beef meat [9].

TABLE XII. ORGANOLEPTIC SCORES* OF CHICKEN DISHES VACUUM PACKED USING PET 12μ/LDPE ADH 2μ/AL-FOIL 7μ/LDPE ADH./LLDPE 50μ (A), AND PET12μ/PE 20μ/ALU-METALIZED PET 12μ/ADH./LLDPE 50μ (B), AND IRRADIATED AT 45 kGy AS A COMPARATIVE STUDY FOR UP TO 12 MONTHS OF STORAGE AT ROOM TEMPERATURE

Product	Duration (months)	Organoleptic properties							
		Appearance		Odour		Taste		Texture	
		A	B	A	B	A	B	A	B
Chicken pepes	0	4.8	4.5	4.8	4.7	4.8	4.6	4.8	4.6
	2	4.6	4.0	4.4	4.6	4.6	4.4	4.6	4.3
	4	5.0	4.8	5.0	4.8	5.0	4.6	5.0	4.8
	6	4.3	4.0	4.6	4.0	4.6	4.3	4.6	4.6
	8	4.8	4.0	4.8	4.5	4.8	4.0	4.8	4.6
	10	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
	12	4.0	4.0	5.0	5.0	5.0	5.0	5.0	5.0
Semur	0	4.9	4.9	4.7	4.4	4.7	4.3	4.7	4.7
	2	4.5	4.0	4.5	4.5	4.5	4.5	4.5	4.5
	4	4.7	4.7	4.5	4.5	4.5	4.5	4.5	4.5
	6	4.7	4.7	4.5	4.5	4.5	4.5	4.5	4.5
	8	4.8	4.8	4.7	4.6	4.6	4.6	4.6	4.6
	10	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
	12	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0

* Average of 10 panellists

The semur showed a better brownish colour after a longer storage time and the taste was improved. This was most likely due to the soya sauce which has a strong influence on the keeping quality and, in particular, the taste of radiation-sterilised semur either prepared from chicken or beef meat.

Generally, for both the chicken and beef meat dishes, the condition of the packaging materials such as vacuum pressure and seal temperature have a strong influence on the quality of the dishes both during processing and storage.

TABLE XIII. ORGANOLEPTIC SCORES* OF THE BEEF MEAT DISHES VACUUM PACKED IN LAMINATES OF PET/ADH./AL-FOIL/ADH./LLDPE POUCHES THEN STERILISED AT 45 kGy AND STORED FOR UP TO 18 MONTHS AT ROOM TEMPERATURE

Product	Duration (months)	Appearance	Odour	Taste	Texture
Rendang	0	5.0	4.8	4.6	5.0
	2	5.0	4.8	4.4	5.0
	4	4.8	4.5	4.2	5.0
	6	4.8	4.5	4.0	4.5
	8	5.0	4.0	4.0	4.5
	10	4.5	4.0	3.8	4.5
	12	4.5	3.8	3.5	4.0
	18	3.9	3.5	3.5	3.5
Empal	0	4.5	4.5	4.5	5.0
	2	4.5	4.5	4.5	5.0
	4	4.0	4.9	5.0	4.0
	6	4.0	4.8	4.5	4.0
	8	4.0	4.5	4.2	3.5
	10	3.2	4.0	3.8	3.5
	12	3.0	3.6	3.0	3.0
	18	2.8	3.2	2.8	2.5
Semur	0	5.0	5.0	4.8	5.0
	2	5.0	5.0	4.8	5.0
	4	5.0	5.0	5.0	5.0
	6	4.8	4.8	4.6	5.0
	8	4.8	4.8	4.6	4.8
	10	4.5	4.6	4.2	4.8
	12	4.0	4.6	4.0	4.6
	18	3.5	4.4	4.0	4.0

*Average of 10 panellists

3. CONCLUSIONS

(a) Fish pepes cooked for 45–60 min in a pressure cooker, vacuum-packed and radiation-sterilised with a minimum dose of 45 kGy in dry ice, can be stored for more than one year at room temperature. The sensory quality, pH, moisture, protein and fat content are relatively stable during storage. It was also found that the free radical content increased significantly due to irradiation, but decreased with time.

(b) Chicken dishes such as pepes, opor, semur and curry along with beef meat dishes such as rendang, empal, and semur, vacuum packed individually in laminates, i.e. PET/Al-foil/LLDPE and PET/adh./Alu-Metalized PET/LLDPE and radiation-sterilised at a minimum dose of 45 kGy in dry ice can be stored for 12 months at room temperature. All objective parameters and sensory quality are relatively stable during storage. The type of seasoning used in making chicken pepes may increase vitamin E content of the final product.

(c) Banana leaf as inner wrapper packaging material for making radiation-sterilised fish and chicken pepes at 45 kGy can improve their sensory quality.

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INOCULATION PACKED STUDIES ON THE SHELF-STABLE FOOD PRODUCTS: I. EFFECTS OF GAMMA IRRADIATION AT 45 kGy ON THE SURVIVAL OF *Clostridium sporogenes* SPORES IN THE FOODS (*Preliminary Results)*

Z.IRAWATI*, L. NATALIA**, N. ANSORI*,
C.M. NURCAHYA*, F.ANAS*, M.SYAFARUDIN**

* Centre for Research and Development of Isotopes and Radiation Technology, National Nuclear Energy Agency, Jakarta, Indonesia
** Research Institute for Veterinary Science, Bogor, Indonesia

Abstract

Inoculation packed studies on Indonesian shelf-stable food products using *Clostridium sporogenes* spores strain CVL 1658/BCC 207 were carried out. The freeze dried bacterial spores were first grown in Robertson's Cooked anaerobic medium (RCMM), and checked for purity on 5% sheep blood agar plate prior to its use.. Multiplication of the spores was done using Duncan and Strong's sporulation medium. . Different type of Indonesian shelf-stable food dishes were inoculated with varying levels of *Clostridium sporogenes* spores, -irradiated with a minimum dose of 45 kGy under cryogenic condition, and stored for 7 days at room temperature. Control, inoculated, and inoculated-irradiated samples were microbiologically examined using different parameters. Calculation of viable spore numbers per-ml suspension was done by most probable number (MPN) while microbial count, isolation and identification of *Clostridium sporogenes* were conducted according to Australian Standard Method. Results showed that a dose of 45 kGy is very effective in eliminating *Clostridium sporogenes* spores inoculated in all food samples.

1. INTRODUCTION

Some traditional Indonesian ready to-eat foods such as fish pepes, chicken dishes, and meat dishes have been successfully developed as safe shelf-stable products using radiation sterilization dose [1]. A minimum radiation dose of 45 kGy, in combination with cryogenic condition during irradiation was selected according to IAEA recommendation of the key steps in the production of shelf-stable foods through irradiation processing [2] and wholesomeness of food irradiated with doses above 10 kGy [3]. The primary concern about microbial safety of irradiated food is that pathogenic bacterial spore formers such as *Clostridium spp.* must be eliminated. *Clostridium sporogenes* was selected as the test organism because of its similarities to *C. botulinum* in radiation resistance. These food products will be packaged in air impermeable pouches and stored at ambient temperature. The food substrate may also affect the survival of the pathogens. Therefore, the purpose of this study was to determine whether a minimum radiation dose of 45 kGy could eliminate *Clostridium sporogenes* spores in different kinds of the shelf-stable Indonesian food products.

2. MATERIALS AND METHODS

2.1. Preparation of *Clostridium sporogenes* spores

Clostridium sporogenes strain CVL 1658/BCC207 obtained from BALIVET Culture Collection of The Research Institute for Veterinary Science was used as test organism. The freezedried bacteria was first grown on Robertson's Cooked Meat Medium (RCMM) and checked for its purity on 5% sheep blood agar plate. The young culture was then obtained by inoculating the single colony of organism into RCMM and incubated for one day. This culture was used as starter for *Clostridium sporogenes* spore preparation. RCMM were always heated in boiling water bath for 10 minutes prior to its use in order to obtain anaerobic condition in the media.

TABLE 1. MICROBIAL EVALUATION OF FISH PEPES INOCULATED WITH *Clostridium sporogenes* SPORES BEFORE AND AFTER IRRADIATION AT MIN. DOSE OF 45 kGy, AND STORED 7 DAYS AT ROOM TEMPERATURE

Sample Code	**Weight of sample (g)**	**Inoculation dosage of *Cl.sporogenes*/g sample**	**Treatment**	***Cl.sporogenes* at:**		**Contaminant* (CFU/g)**
				R RCMM	**B Blood agar (CFU/g)**	
Control	-	none	Unirradiated	n.g.	n.g.	$> 10^6$
Control	-	none	Unirradiated	n.g.	n.g.	$> 10^6$
Irrad.	-	none	Irradiated	n.g.	n.g.	n.g.
Irrad.	-	none	Irradiated	n.g.	n.g.	n.g.
II a.1	222.77	10^4	Irradiated	n.g.	n.g.	n.g.
II a.2	238.27	10^4	Irradiated	n.g.	n.g.	n.g.
IIa.3	210.58	10^4	Irradiated	n.g.	n.g.	n.g.
II b.1	234.30	10^4	Unirradiated	$> 10^6$	$> 10^6$	n.g.
II b.2	264.30	10^4	Unirradiated	$> 10^6$	$> 10^6$	n.g.
III a.1	247.57	10^3	Irradiated	n.g.	n.g.	n.g.
III a.2	237.46	10^3	Irradiated	n.g.	n.g.	n.g.
III a.3	234.34	10^3	Irradiated	n.g.	n.g.	n.g.
III b.1	211.46	10^3	Unirradiated	$> 10^6$	$> 10^6$	n.g.
III b.2	191.64	10^3	Unirradiated	$> 10^6$	$> 10^6$	n.g.
IV a.1	239.08	10^2	Irradiated	n.g.	n.g.	n.g.
IV a.2	257.35	10^2	Irradiated	n.g.	n.g.	n.g.
IV a.3	251.91	10^2	Irradiated	n.g.	n.g.	n.g.
IV b.1	261.43	10^2	Unirradiated	$> 10^6$	$> 10^6$	n.g.
IV b.2	245.10	10^2	Unirradiated	$> 10^6$	$> 10^6$	n.g.
V a.1	273.51	10	Irradiated	n.g.	n.g.	n.g.
V a.2	251.83	10	Irradiated	n.g.	n.g.	n.g.
V a.3	202.20	10	Irradiated	n.g.	n.g.	n.g.
V b.1	265.47	10	Unirradiated	$> 10^6$	$> 10^6$	n.g.
V b.2	379.20	10	Unirradiated	$> 10^6$	$> 10^6$	n.g.

Note: n.g. = no bacterial growth
* = contaminant of bacteria in bacillus form, gram positive

TABLE 2. MICROBIAL EVALUATION OF CHICKEN PEPES INOCULATED WITH *Clostridium sporogenes* SPORES BEFORE AND AFTER IRRADIATION AT MIN. DOSE OF 45 kGy, AND STORED 7 DAYS AT ROOM TEMPERATURE

Sample Code	**Weight of sample (g)**	**Inoculation dosage of *Cl.sporogenes*/g sample**	**Treatment**	***Cl.sporogenes* at:**		**Contaminant (CFU/g)**
				R RCMM	**B Blood agar (CFU/g)**	
Control	106.70	none	Unirradiated	n.g.	n.g.	n.g.
Control	93.00	none	Unirradiated	n.g.	n.g.	n.g.
Control	87.70	none	Unirradiated	n.g.	n.g.	n.g.
Irrad.	-	none	Irradiated	n.g.	n.g.	n.g.
Irrad.	-	none	Irradiated	n.g.	n.g.	n.g.
A a.1	93.10	10^4	Irradiated	n.g.	n.g.	n.g.
A a.2	93.20	10^4	Irradiated	n.g.	n.g.	n.g.
A a.3	113.40	10^4	Irradiated	n.g.	n.g.	n.g.
A b.1	126.60	10^4	Unirradiated	$> 10^6$	$> 10^6$	n.g.
A b.2	126.70	10^4	Unirradiated	$> 10^6$	$> 10^6$	n.g.
A b. 3	140.10	10^4	Unirradiated	$> 10^6$	$> 10^6$	n.g.
B a.1	99.40	10^3	Irradiated	n.g.	n.g.	n.g.
B a.2	113.40	10^3	Irradiated	n.g.	n.g.	n.g.
B a.3	82.70	10^3	Irradiated	n.g.	n.g.	n.g.
B b.1	81.90	10^3	Unirradiated	$> 10^6$	1.7×10^6	n.g.
B b.2	93.20	10^3	Unirradiated	$> 10^6$	4.3×10^6	n.g.
B b.3	87.90	10^3	Unirradiated	$> 10^6$	3.0×10^6	n.g.
C a.1	113.50	10^2	Irradiated	n.g.	n.g.	n.g.
C a.2	117.90	10^2	Irradiated	n.g.	n.g.	n.g.
C a.3	115.00	10^2	Irradiated	n.g.	n.g.	n.g.
C b.1	101.70	10^2	Unirradiated	$> 10^6$	5.0×10^5	n.g.
C b.2	103.30	10^2	Unirradiated	$> 10^6$	2.0×10^5	n.g.
C b.3	102.90	10^2	Unirradiated	$> 10^6$	1.0×10^6	n. n.g.

Sample Code	Weight of sample (g)	Inoculation dosage of *Cl.sporogenes*/g sample	Treatment	*Cl.sporogenes* at:		Contaminant (CFU/g)
				R RCMM	B Blood agar (CFU/g)	
D a.1	112.00	10	Irradiated	n.g.	n.g.	n.g.
D a.2	97.00	10	Irradiated	n.g.	n.g.	n.g.
D a.3	124.00	10	Irradiated	n.g.	n.g.	n.g.
D b.1	86.00	10	Unirradiated	$> 10^6$	1.2×10^6	n.g.
D b.2	117.40	10	Unirradiated	$> 10^6$	5.0×10^5	n.g.
D b.3	108.70	10	Unirradiated	$> 10^6$	4.0×10^6	n.g.

Note: n.g. = no bacterial growth

TABLE 3. MICROBIAL EVALUATION OF CHICKEN OPOR INOCULATED WITH *Clostridium sporogenes* SPORES BEFORE AND AFTER IRRADIATION AT MIN. DOSE OF 45 kGy, AND STORED 7 DAYS AT ROOM TEMPERATURE

Sample Code	**Weight of sample (g)**	**Inoculation dosage of *Cl.sporogenes*/g sample**	**Treament**	***Cl.sporogenes* at:**		**Contaminant (CFU/g)**
				R-RCMM	**B-Blood agar (CFU/g)**	
Control	124.20	none	Unirradiated	n.g.	n.g.	n.g.
Control	90.90	none	Unirradiated	n.g.	n.g.	n.g.
Control	85.00	none	Unirradiated	n.g.	n.g.	n.g.
Irrad.	-	none	Irradiated	n.g.	n.g.	n.g.
Irrad.	-	none	Irradiated	n.g.	n.g.	n.g.
E a.1	71.40	10^4	Irradiated	n.g.	n.g.	n.g.
E a.2	82.80	10^4	Irradiated	n.g.	n.g.	n.g.
E a.3	62.10	10^4	Irradiated	n.g.	n.g.	n.g.
E b.1	74.20	10^4	Unirradiated	$\geq 10^6$	6.0×10^5	n.g.
E b.2	76.20	10^4	Unirradiated	$\geq 10^6$	3.0×10^5	n.g.
E b. 3	84.50	10^4	Unirradiated	$\geq 10^6$	2.0×10^5	n.g.
F a.1	77.50	10^3	Irradiated	n.g.	n.g.	n.g.
F a.2	98.50	10^3	Irradiated	n.g.	n.g.	n.g.
F a.3	77.50	10^3	Irradiated	n.g.	n.g.	n.g.
F b.1	102.30	10^3	Unirradiated	$\geq 10^6$	1.0×10^6	n.g.
F b.2	66.60	10^3	Unirradiated	$\geq 10^6$	1.0×10^6	n.g.
F b.3	117.50	10^3	Unirradiated	$\geq 10^6$	0.9×10^6	n.g.
G a.1	91.70	10^2	Irradiated	n.g.	n.g.	n.g.
G a.2	105.70	10^2	Irradiated	n.g.	n.g.	n.g.
G a.3	101.40	10^2	Irradiated	n.g.	n.g.	n.g.
G b.1	58.70	10^2	Unirradiated	$\geq 10^6$	2.0×10^5	n.g.
G b.2	102.60	10^2	Unirradiated	$\geq 10^6$	1.3×10^5	n.g.
G b.3	73.10	10^2	Unirradiated	$\geq 10^6$	0.4×10^6	n. n.g.

Sample Code	Weight of sample (g)	Inoculation dosage of *Cl.sporogenes*/g sample	Treament	*Cl.sporogenes* at:		Contaminant (CFU/g)
				R-RCMM	B-Blood agar (CFU/g)	
H a.1	94.90	10	Irradiated	n.g.	n.g.	n.g.
H a.2	92.70	10	Irradiated	n.g.	n.g.	n.g.
H a.3	95.80	10	Irradiated	n.g.	n.g.	n.g.
H b.1	79.70	10	Unirradiated	$\geq 10^6$	3.0×10^6	n.g.
H b.2	79.40	10	Unirradiated	$\geq 10^6$	0.8×10^5	n.g.
H b.3	53.50	10	Unirradiated	$\geq 10^6$	0.5×10^6	n.g.

Note: n.g. = no bacterial growth

TABLE 4. MICROBIAL EVALUATION OF CHICKEN SEMUR INOCULATED WITH *Clostridium sporogenes* SPORES BEFORE AND AFTER IRRADIATION AT MIN. DOSE OF 45 kGy, AND STORED 7 DAYS AT ROOM TEMPERATURE

Sample Code	**Weight of sample (g)**	**Inoculation dosage of *Cl.sporogenes*/g sample**	**Treatment**	***Cl.sporogenes* at:**		**Contaminant* (CFU/g)**
				R RCMM	**B Blood agar (CFU/g)**	
Control	99.60	none	Unirradiated	n.g.	n.g.	*Stpcc.sp* (≥ 10^6)
Control	112.30	none	Unirradiated	n.g.	n.g.	*Stpcc.sp* (≥ 10^6)
Control	92.20	none	Unirradiated	n.g.	n.g.	*Stpcc.sp* (≥ 10^6)
Irrad.	58.60	none	Irradiated	-	-	n.g.
Irrad.	94.40	none	Irradiated	-	-	n.g.
Irrad.	83.50	none	Irradiated	-	-	St n.g.
I a.1	93.60	10^4	Irradiated	n.g.	n.g.	n.g.
I a.2	70.50	10^4	Irradiated	n.g.	n.g.	n.g.
I a.3	79.90	10^4	Irradiated	n.g.	n.g.	n.g.
I b.1	75.10	10^4	Unirradiated	≥ 10^6	2.1×10^5	n.g.
I b.2	70.00	10^4	Unirradiated	≥ 10^6	1.5×10^5	n.g.
I b. 3	81.50	10^4	Unirradiated	≥ 10^6	2.3×10^5	n.g.
J a.1	119.10	10^3	Irradiated	n.g.	n.g.	n.g.
J a.2	83.10	10^3	Irradiated	n.g.	n.g.	n.g.
J a.3	101.00	10^3	Irradiated	n.g.	n.g.	n.g.
J b.1	101.30	10^3	Unirradiated	≥ 10^6	2.7×10^6	n.g.
J b.2	102.00	10^3	Unirradiated	≥ 10^6	7.0×10^6	n.g.
J b.3	100.90	10^3	Unirradiated	≥ 10^6	1.9×10^6	n.g.
K a.1	84.60	10^2	Irradiated	n.g.	n.g.	n.g.
K a.2	95.00	10^2	Irradiated	n.g.	n.g.	n.g.
K a.3	92.00	10^2	Irradiated	n.g.	n.g.	n.g.
K b.1	92.80	10^2	Unirradiated	≥ 10^6	7.0×10^5	n.g.
K b.2	110.00	10^2	Unirradiated	≥ 10^6	3.0×10^5	n.g.
K b.3	101.40	10^2	Unirradiated	≥ 10^6	9.0×10^5	n. n.g.

Sample Code	**Weight of sample (g)**	**Inoculation dosage of *Cl.sporogenes*/g sample**	**Treatment**	***Cl.sporogenes* at:**		**Contaminant* (CFU/g)**
				R RCMM	**B Blood agar (CFU/g)**	
L a.1	73.00	10	Irradiated	n.g.	n.g.	n.g.
L a.2	90.00	10	Irradiated	n.g.	n.g.	n.g.
L a.3	82.00	10	Irradiated	n.g.	n.g.	n.g.
L b.1	95.10	10	Unirradiated	$\geq 10^6$	$2.0x\ 10^6$	n.g.
L b.2	83.30	10	Unirradiated	$\geq 10^6$	1.5×10^5	n.g.
L b.3	70.00	10	Unirradiated	$\geq 10^6$	2.5×10^6	n.g.

Note: n.g. = no bacterial growth ; **Stpcc.sp = Staphylococcus spp*.

TABLE 5. MICROBIAL EVALUATION OF CHICKEN CURRY INOCULATED WITH *Clostridium sporogenes* SPORES BEFORE AND AFTER IRRADIATION AT MIN. DOSE OF 45 kGy AND STORED 7 DAYS AT ROOM TEMPERATURE

Sample Code	**Weight of sample (g)**	**Inoculation dosage of *Cl.sporogenes*/g sample**	**Treatment**	***Cl.sporogenes* at:**		**Contaminant* (CFU/g)**
				R RCMM	**B Blood agar (CFU/g)**	
Control	93.10	none	Unirradiated	-	-	*Stpcc.sp* ($\geq 10^6$)
Control	81.80	none	Unirradiated	-	-	*Stpcc.sp* ($\geq 10^6$)
Control	105.40	none	Unirradiated	-	-	*St* *Stpcc.sp* ($\geq 10^6$)
Irrad.	79.90	none	Irradiated	-	-	n.g.
Irrad.	82.70	none	Irradiated	-	-	n.g.
Irrad.	104.10	none	Irradiated	-	-	n.g.
Ma.1	80.00	10^4	Irradiated	n.g.	n.g.	n.g.
Ma.2	82.00	10^4	Irradiated	n.g.	n.g.	n.g.
Ma.3	90.00	10^4	Irradiated	n.g.	n.g.	n.g.
Mb.1	101.10	10^4	Unirradiated	$\geq 10^6$	5.5×10^5	n.g.
Mb.2	113.60	10^4	Unirradiated	$\geq 10^6$	$\geq 10^7$	n.g.
Mb. 3	98.90	10^4	Unirradiated	$\geq 10^6$	$\geq 10^7$	n.g.
Na.1	105.10	10^3	Irradiated	n.g.	n.g.	n.g.
Na.2	90.80	10^3	Irradiated	n.g.	n.g.	n.g.
Na.3	115.10	10^3	Irradiated	n.g.	n.g.	n.g.
Nb.1	109.30	10^3	Unirradiated	$\geq 10^6$	$\geq 10^7$	n.g.
Nb.2	111.40	10^3	Unirradiated	$\geq 10^6$	3.7×10^6	n.g.
Nb.3	89.90	10^3	Unirradiated	$\geq 10^6$	3.5×10^6	n.g.
Oa.1	80.90	10^2	Irradiated	n.g.	n.g.	n.g.
Oa.2	81.20	10^2	Irradiated	n.g.	n.g.	n.g.
O a.3	76.90	10^2	Irradiated	n.g.	n.g.	n.g.
Ob.1	76.00	10^2	Unirradiated	$\geq 10^6$	3.0×10^5	n.g.
O b.2	95.50	10^2	Unirradiated	$\geq 10^6$	3.5×10^5	n.g.

Sample Code	Weight of sample (g)	Inoculation dosage of *Cl.sporogenes*/g sample	Treatment	*Cl.sporogenes* at:		Contaminant* (CFU/g)
				R RCMM	B Blood agar (CFU/g)	
O b.3	105.00	10^2	Unirradiated	$\geq 10^6$	$\geq 10^7$	n. n.g.
P a.1	106.10	10	Irradiated	n.g.	n.g.	n.g.
P a.2	103.80	10	Irradiated	n.g.	n.g.	n.g.
P a.3	99.70	10	Irradiated	n.g.	n.g.	n.g.
P b.1	106.70	10	Unirradiated	$\geq 10^6$	3.2×10^6	n.g.
P b.2	87.60	10	Unirradiated	$\geq 10^6$	9.0×10^5	n.g.
P b.3	108.40	10	Unirradiated	$\geq 10^6$	4.0×10^5	n.g.

Note: n.g. = no bacterial growth **Stpcc.sp = Staphylococcus spp*.

TABLE 6. MICROBIAL EVALUATION OF RENDANG MEAT INOCULATED WITH *Clostridium sporogenes* SPORES BEFORE AND AFTER IRRADIATION AT MIN. DOSE OF 45 kGy, AND STORED 7 DAYS AT ROOM TEMPERATURE

Sample Code	**Weight of sample (g)**	**Inoculation dosage of *Cl.sporogenes*/g sample**	**Treatment**	***Cl.sporogenes* at:**		**Contaminant (CFU/g)**
				R RCMM	**B Blood agar (CFU/g)**	
Control	74.30	none	Unirradiated	-	-	n.g.
Control	90.60	none	Unirradiated	-	-	n.g.
Qa.1	65.40	10^4	Irradiated	n.g.	n.g.	n.g.
Qa.2	76.90	10^4	Irradiated	n.g.	n.g.	n.g.
Qb.1	64.10	10^4	Unirradiated	$\geq 10^4$	2.0×10^3	n.g.
Qb.2	93.20	10^4	Unirradiated	$\geq 10^4$	5.0×10	n.g.
Ra.1	53.50	10^3	Irradiated	n.g.	n.g.	n.g.
Ra.2	88.80	10^3	Irradiated	n.g.	n.g.	n.g.
Rb.1	48.90	10^3	Unirradiated	$\geq 10^3$	2.0×10^3	n.g.
Rb.2	67.40	10^3	Unirradiated	$\geq 10^3$	3.0×10	n.g.
Sa.1	57.90	10^2	Irradiated	n.g.	n.g.	n.g.
Sa.2	71.20	10^2	Irradiated	n.g.	n.g.	n.g.
Sb.1	86.90	10^2	Unirradiated	≥ 10	n.g.	n.g.
Sb.2	80.40	10^2	Unirradiated	≥ 10	n.g.	n.g.
Ta.1	52.50	10	Irradiated	n.g.	n.g.	n.g.
Ta.2	68.40	10	Irradiated	n.g.	n.g.	n.g.
Tb.1	63.10	10	Unirradiated	≥ 10	n.g.	n.g.
Tb.2	63.50	10	Unirradiated	≥ 10	n.g.	n.g.

Note: n.g. = no bacterial growth

TABLE 7. MICROBIAL EVALUATION OF EMPAL MEAT INOCULATED WITH *Clostridium sporogenes* SPORES BEFORE AND AFTER IRRADIATION AT MIN. DOSE OF 45 kGy, AND STORED 7 DAYS AT ROOM TEMPERATURE

Sample Code	**Weight of sample (g)**	**Inoculation dosage of *Cl.sporoge nes*/g sample**	**Treatment**	***Cl.sporogenes* at:**		**Contaminant (CFU/g)**
				R RCMM	**B Blood agar (CFU/g)**	
Control	69.10	None	Unirradiated	-	-	-
Control	73.90	None	Unirradiated	-	-	-
Co Control	73.60	None	Unirradiated	-	-	-
Ua.1	71.70	10^4	Irradiated	n.g.	n.g.	n.g.
Ua.2	68.00	10^4	Irradiated	n.g.	n.g.	n.g.
Ub.1	70.50	10^4	Unirradiated	$\geq 10^3$	7.0×10	n.g.
Ub.2	66.50	10^4	Unirradiated	$\geq 10^3$	4.0×10	n.g.
Vva.1	77.50	10^3	Irradiated	n.g.	n.g.	n.g.
Vva.2	58.40	10^3	Irradiated	n.g.	n.g.	n.g.
VVb.1	78.80	10^3	Unirradiated	$\geq 10^2$	3.0×10	n.g.
VVb.2	82.20	10^3	Unirradiated	$\geq 10^2$	7.0×10	n.g.
Wa.1	71.90	10^2	Irradiated	n.g.	n.g.	n.g.
Wa.2	81.40	10^2	Irradiated	n.g.	n.g.	n.g.
Wb.1	64.40	10^2	Unirradiated	$\geq 10^2$	3.0×10	n.g.
Wb.2	67.90	10^2	Unirradiated	≥ 10	3.0×10	n.g.
Xa.1	43.90	10	Irradiated	n.g.	n.g.	n.g.
Xa.2	70.00	10	Irradiated	n.g.	n.g.	n.g.
Xb.1	66.80	10	Unirradiated	n.g.	n.g.	n.g.
Xb.2	80.30	10	Unirradiated	n.g.	n.g.	n.g.

Note: n.g. = no bacterial growth

TABLE 8. MICROBIAL EVALUATION OF SEMUR MEAT INOCULATED WITH *Clostridium sporogenes* SPORES BEFORE AND AFTER IRRADIATION AT MIN. DOSE OF 45 kGy, AND STORED 7 DAYS AT ROOM TEMPERATURE

Sample Code	**Weight of sample (g)**	**Inoculation dosage of *Cl.sporogenes*/g sample**	**Treatment**	***Cl.sporogenes* at:**		**Contaminant (CFU/g)**
				R RCMM	**B Blood agar (CFU/g)**	
Control	64.50	None	Unirradiated	n.g.	n.g.	n.g.
Control	72.90	None	Unirradiated	n.g.	n.g.	n.g.
Ya.1	67.30	10^4	Irradiated	n.g.	n.g.	n.g.
Ya.2	48.80	10^4	Irradiated	n.g.	n.g.	n.g.
Yb.1	60.70	10^4	Unirradiated	$\geq 10^5$	4.0×10^4	n.g.
Yb.2	94.30	10^4	Unirradiated	$\geq 10^5$	2.0×10^4	n.g.
YYa.1	85.90	10^3	Irradiated	n.g.	n.g.	n.g.
YYa.2	55.90	10^3	Irradiated	n.g.	n.g.	n.g.
YYb.1	49.40	10^3	Unirradiated	$\geq 10^4$	4.0×10^3	n.g.
YYb.2	91.20	10^3	Unirradiated	$\geq 10^4$	8.0×10	n.g.
Za.1	84.60	10^2	Irradiated	n.g.	n.g.	n.g.
Za.2	57.20	10^2	Irradiated	n.g.	n.g.	n.g.
Zb.1	65.90	10^2	Unirradiated	$\geq 10^3$	3.0×10	n.g.
Zb.2	72.00	10^2	Unirradiated	$\geq 10^3$	5.0×10	n.g.
ZZa.1	50.80	10	Irradiated	n.g.	n.g.	n.g.
ZZa.2	48.30	10	Irradiated	n.g.	n.g.	n.g.
ZZb.1	79.00	10	Unirradiated	≥ 10	n.g.	n.g.
ZZb.2	58.60	10	Unirradiated	≥ 10	n.g.	n.g.

Note: n.g. = no bacterial growth

A Duncan and Strong's sporulation medium was used for producing a large number of *Clostridium sporogenes* spores. The active culture in RCMM were inoculated into the sporulation medium which has been freshly steamed and cooled rapidly to 37°C. The volume of inoculum was 5% of the sporulation media. The media was incubated for 3 days at 37°C and checked for bacterial spore formation. One ml sample of sporulating culture was removed after 3 days. This culture sample was smeared on an object glass, heat fixed and stained by Wirtz stain. The spore will be bright green colour and red colour for remainder of organism as observed under microscope.

The culture containing spores were centrifuged at 14000g for 20 min. The supernatant were discarded and the spores were collected. The collected spores were then washed in sterile Phosphate Buffered Saline (PBS) for 2 times. Spores were finally re-suspended in 50% sterile glycerine.

Viable spore numbers per–ml in suspension were determined by most probable number (MPN) method [4], inoculating 4 replicates into RCMM tubes at each decimal dilution level. The inoculated tubes were incubated anaerobically at 37°C for 7 days. Tubes giving evidence of growth were recorded and the spore suspension was stored at 4°C until its used.

2.2. Food products

The survival of *Clostridium sporogenes* spores against radiation exposure in different kind of food substrates, i.e., fish, chicken, and beef dishes were tested.

2.3. *Clostridium sporogenes* inoculated on the food products

Each type of food product was first sterilized by autoclaving prior to inoculation procedure. The colony counts of samples never exceeded 1 × 10^4 cfu/g according to the procedure of Weenk et. al. [5], and Vanderzant and Splittstoesser [6] . The inoculum levels used in this study were: 10^4/g; 10^3/g;10^2/g and 10/g of each food product. An aliquot of a known inoculum ranging from volume of 1 to 2 ml were dispensed on different sides of the food on the basis of weight using a sterile syringe. The inoculated foods were kept at low temperature (± 4°C) and vacuum packaged in a laminated PET/al-foil/LLDPE pouch, then irradiated at the same condition as in the previous method [1].

2.4. Irradiation of the inoculated food products

The purpose of the radiation processing was to eliminate the pathogenic bacteria as well as *Clostridium sporogenes* [7 and 8]. Irradiation process was carried out after inoculation on different food products. .The minimum irradiation dose applied was 45 kGy, under cryogenic condition, and the samples were kept at room temperature in order to allow the growth of the inoculated spores within the products. The evaluation of microbial colony count differences between the irradiated and unirradiated foods would reveal the effect of radiation on the survival of *Clostridium sporogenes* spores.

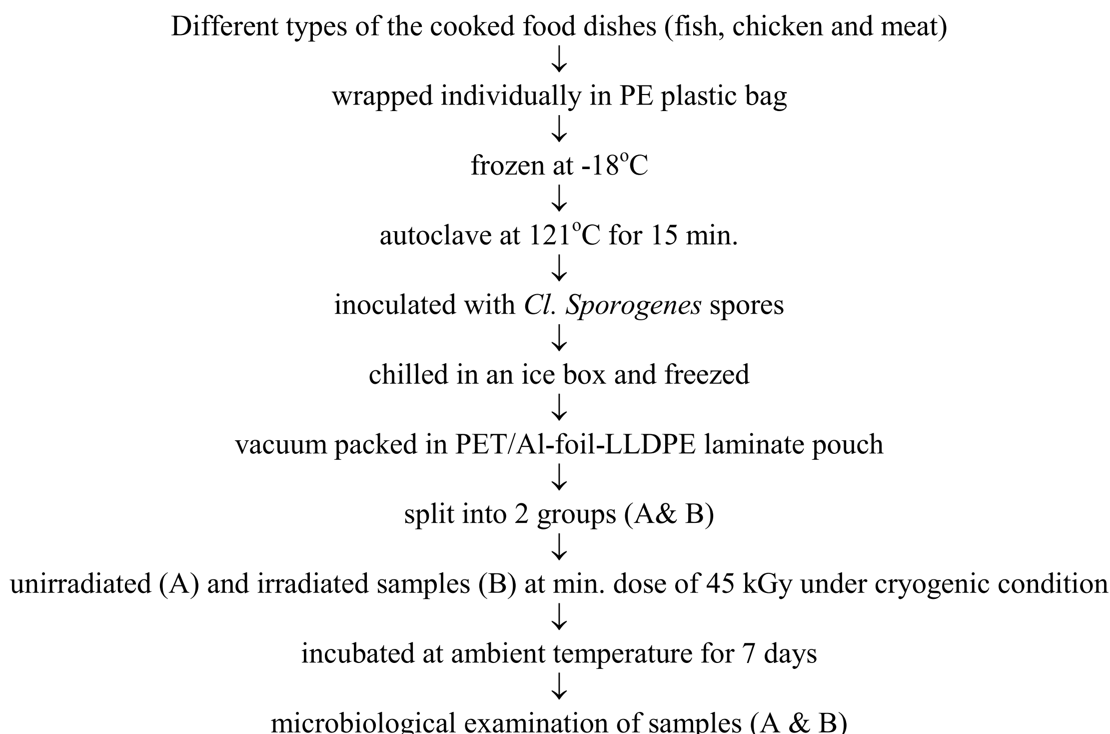
2.5.Microbial count, isolation, and identification of *Clostridium sporogenes* in the irradiated food products

The food samples, both unirradiated and irradiated, were determined microbiologically according to Australian Standard Method [9]. The food samples were transformed into 10% suspension respectively using 0.1% buffered peptone water (BPW). Each 10 g of samples from different preparation of food products was individually ground and homogenized using stomacher 80 for 1 min. Finally the sample was transferred into 10% suspension by adding 90% of BPW. Ten fold serial dilutions of food samples in BPW were made from 10% food suspension into 10^4 dilution. Enrichment culture method using RCMM and 5% sheep blood agar was conditioned under these circumstances. One ml of each sample dilution was inoculated into RCMM tubes and blood agar plates. The survival of clostridia after irradiation were analysed after a 7 day-incubation. Results are

reported as the presence or absence of growth in RCMM tubes and blood agar plates, and the most probable number of *Clostridium sporogenes* in the original sample was estimated.

Isolation and identification steps were conducted as follows: As a short routine procedure, colonies giving typical appearance of *Clostridium sporogenes* on blood agar may be further examined by Gram's stain method and fluorescent antibody technique. More complete list of the properties of *Clostridium sporogenes* can be found in COWAN and STEEL [10] , and LEVETT [11].

The procedure applied in this study can be summarized as follows:



3. RESULTS AND DISCUSSION

The results are presented in Tables 1-8 according to type of food products respectively. It can be seen from the results that contaminating bacteria found in unirradiated fish pepes were *Bacillus sp*. Gram positive, and unirradiated samples such as chicken semur and curry were *Staphylococcus sp.* No bacterial growth were found in all irradiated samples inoculated with *Clostridium sporogenes* spores, while the inoculated-unirradiated samples mostly contained *Clostridium sporogenes* up to 10^6 colonies as determined by using RCMM and blood agar (CFU/g). The results indicated that irradiation at the min. dose of 45 kGy is effective to eliminate *Clostridium sporogenes* spores inoculated in all samples. Some contaminants such as *Bacillus spp*. and *Staphylococcus spp.* were found in some unirradiated inoculated food samples. Bacterial contaminants are present in the air, dust, on the hands and other parts of the body. They are so widely distributed that their presence in or on food is inevitable unless special steps are taken to kill them. Some spoilage organisms were able to grow in unirradiated samples at cold temperature and Staphylococcal enterotoxin has been detected at 10 ^{o}C, but the growth is slow below 4-5°C [12]. Irradiation at the minimum dose of 45 kGy can effectively kill these contaminants. No contaminant were found in unirradiated chicken opor or in meat dishes.

From the results of microbiological examination, it was found that there were some differences in the number of *Clostridium sporogenes* grown on RCM and blood agar (BA). In this case, due to laboratory experience, some of clostridial spores, not vegetative cells, failed to grow in regular surface agar medium such as blood agar, nutrient agar, etc. Therefore, counts of *Clostridium sporogenes* from the food samples on RCM are the presumptive total clostridial vegetative cells. There

are also some advantages of using RCM medium in comparison to blood agar as they are good for recovery of low number of Clostridia, no need to use anaerobic jar, incubation can be prolonged without dehydration of the medium and cultures can be examined daily without disturbing the growth conditions.

In unirradiated samples, *Clostridium sporogenes* were present in large number >10^6 CFU/g of food. Storage of moist foods (such as pepes, curry, opor, and semur) in PET/Al-foil/LLDPE laminate pouch under vacuum condition without means to eliminate moisture, and storage under room temperature will encourage the growth of *Clostridium sporogenes*. In meat dishes, such as rendang and empal or the kind of food with less water content than pepes, *Clostridium sporogenes* were present in fewer numbers (10-10.000 CFU/g of food). The results showed that the water content influenced the growth of bacteria, and the bacteria needed water and nutrients for multiplying. Results from irradiated samples showed that a minimum dose of 45 kGy was able to stop the growth of *Cl. Sporogenes* spores. The bacteriological examinations showed that the irradiation dose is very effective to eliminate *Clostridium sporogenes* spores inoculated in all food samples.

4. CONCLUSION

It can be seen from the preliminary results that irradiation at a minimum dose of 45 kGy under cryogenic condition can eliminate the bacterial contaminants as well as *Clostridium sporogenes, Bacillus spp., and Staphylococcus spp.*. It may be necessary to determine the minimum required dose (MRD) for radiation sterilization of other types of Indonesian foods in order to ensure that irradiation would provide the same margin of safety. Therefore, further research work need to be done is determination of the MRD for each type of the food dishes as it would be processed commercially.

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COMMERCIAL APPLICATION OF HIGH-DOSE IRRADIATION TO PRODUCE SHELF-STABLE MEAT PRODUCTS. PART 1 — EXPERIMENTAL STERILISATION DOSE REQUIRED FOR SHELF-STABLE BEEF CASSEROLE*

I.N. DE BRUYN, B.E. DE VILLIERS
Biogam, Atomic Energy Corporation of South Africa,
Pretoria, South Africa

Abstract

The D_{10} values for several locally isolated and type strains of *Clostridium sporogenes* were determined in phosphate buffer. The D_{10} values obtained compared favourably with those published in the literature for *C. sporogenes* and *Clostridium botulinum*. Three strains representing the least resistant, most resistant and average resistant organisms in any mixed population were selected to be used for inoculation pack studies. Pouches containing a beef casserole were inoculated with a mixed spore suspension and irradiated at –40°C to doses of 10–46 kGy with 4 kGy increments. After irradiation the pouches were incubated at 30°C for six months or until they were swollen. The results obtained indicated an experimental sterilisation dose (ESD) of 26–30 kGy. The calculated minimum radiation dose (MRD) would therefore be in the region of 45 kGy.

1. INTRODUCTION

During processing of radiation sterilized food the chances of a potential botulism outbreak must be taken into consideration. The reasons are that competition is eliminated; the product is cooked, which removes oxygen, and vacuum packed thereby creating an anaerobic atmosphere. Secondly, due to the breakdown of the cellular structure, cellular liquids are released that act as growth substrate for *Clostridium*. In addition, such processed foods are normally stored for extended periods of time thereby increasing the risk of botulism [1]. *Clostridium botulinum* is a highly toxic organism and its spores have the highest resistance to radiation of all spore-forming pathogenic bacteria. [2]

To bring radappertization (radiation-sterilisation) of food in line with the safety requirements of the canning industry it has been suggested that the 12D dose for *C. botulinum* be used [3]. It has been shown that *C. botulinum* is capable of forming toxin in an irradiated meat product without forming sufficient quantities of gas to cause the packaging to “blow” as is common in the food industry when harmless gas forming clostridia survive the heat treatment [4,5]. Viable *C. botulinum* was isolated from bacon irradiated below 20 kGy. The organisms survived in the bacon for at least 8 months without causing visible or toxic spoilage [6]. End-point testing is therefore not an adequate control measure and processing parameters should be determined using an inoculation pack study [3]. During the planning of the inoculation pack study, the envisaged processing parameters should be adhered to strictly as changes in temperature during irradiation [7] as well as the chemical formulation of the meat product [8,9] have an effect on the 12D value.

In addition as irradiation does not destroy *Clostridium* toxin at doses of 45 kGy, which is regarded as the required dose to achieve commercial sterilisation [1,10], raw food of good microbiological quality should be used, good manufacturing practices (GMPs) with adequate record keeping, personal hygiene and sanitation should be employed. Adequate packaging should be used and storage and distribution after processing should be appropriate [11]. If all of the manufacturing requirements are met food irradiated with a 12D dose should provide food with a wide margin of safety that can be stored at non-refrigerated temperatures [12].

During the late 1970’s the then Radiation Technology division at the Atomic Energy Corporation (AEC) commenced with research into the effect of high-dose irradiation of meat products. During the early to mid 1980’s the Department of Health required, firstly, that it be shown that *C. botulinum* was present in South African environments and, secondly if it was present, whether the dose used would be safe enough to ensure that no possible botulism outbreak occurred. Previous

*Research carried out in association with the IAEA under Research Agreement No 9156.

surveys of South African habitats had only yielded three *C. botulinum* type B isolates from soil [13]. During subsequent work one *C. botulinum* type A strain was isolated from sludge in Hartebeespoort dam and four *C. botulinum* type B (proteolytic) and one *C. botulinum* type B (non-proteolytic) strain was isolated from the intestines of a marine fish e.g. hake [14]. The D_{10} values of these locally isolated *C. botulinum* strains were determined (Bekker, unpublished results) and compared with those in the literature as well as with the D_{10} values obtained for locally isolated *Clostridium sporogenes* and *C. sporogenes* type strains. Originally the idea had been to do inoculation pack studies with *C. botulinum*. However for practical purposes it was decided to use *C. sporogenes* as it had the same D_{10} values as *C. botulinum*. The D_{10} values obtained for *C. sporogenes* were also of the same order of magnitude as those published for *C. botulinum* [8] and it was therefore decided that *C. sporogenes* suspensions could be used to determine minimum irradiation doses in meat products.

2. MATERIALS AND METHODS

2.1. Determination of the D_{10} of *C. sporogenes* spores in buffer

2.1.1. Experimental design

Seventeen locally isolated or type strains of *C. sporogenes* were used to determine D_{10} values in buffer and meat products. The organisms were propagated in a medium containing 5% Tryptone (Oxoid), 0.5% Peptone (Biolab) and 0.125% K_2HPO_4 (Merck) at pH of 7.5. Filter sterilised $NaHCO_3$ was added to a final concentration of 0.075%. Spore suspensions were produced using the biphasic method [15] by inoculating the organisms into a 2% $(NH_4)_2SO_4$ overlay with a base medium consisting of 5% Tryptone (Oxoid), 0.5% Peptone (Biolab), 0.125% K_2HPO_4 (Merck), 0.1% Yeast extract (Merck) and 3% agar (Biolab). Incubation was at 30°C for 5 to 6 days. The percentage of germinated spores in the suspension was determined by staining a dry smear of the culture with 0.5% aqueous methylene blue. The stained (germinated cells) were counted using a phase-contrast microscope. After harvesting, the spores were washed three times and then resuspended in 0.067M Sorenson phosphate buffer (0.5675% NaH_2HPO_4, 0.363 % KH_2PO_4, pH 7.0). The spores were stored in the buffer at 2 to 5°C until required.

The spores were enumerated according to the method of Anellis et al. [16] with the exception that Peptone P (Oxoid) was used instead of thiotone (BBL). The PYT medium consisted of 5% Peptone P (Oxoid), 0.5% Tryptone (Oxoid), 0.5% Yeast extract (Merck), 0.05% sodium thioglycolate and 0.75% Agar (Biolab). The pH was adjusted to 7.2 by using 5N NaOH. The enumeration was carried out in triplicate using duplicate series. The long narrow test tubes used ensure an anaerobic environment in the media without additional measures such as an anaerobic glove cabinet or anaerobic flasks. To determine the D_{10} in buffer, 2.5 mL aliquots of a heat shocked (80°C, 10 min) spore suspension was sealed in glass ampoules. The frozen ampoules were placed on ice and irradiated in a Gamma-beam 650 in increments of 2 kGy from 0 to 20 kGy. The dose rate of 14.9 kGy/h was determined by using Fricke dosimeters. Fricke was used as the primary dosimeters and were calibrated against IAEA's alanine dosimeters on a six monthly basis. After irradiation the ampoules were thawed, mixed on a vortex and the survivors determined as before. The contents of each ampoule were inoculated into each of triplicate tubes. The count therefore represented an average of 3 count tubes. In addition, the D_{10} was determined in duplicate over time.

2.1.2. Results

In Fig. 1 it can be seen that the survival curve displays a definite "shoulder" f ± 2 kGy and a "tail" in addition to a straight line exponential decline area. This phenomenon was experienced in all strains tested and is also mentioned for *Clostridium perfringens* [17] and for *C. botulinum* [18]. In Fig. 1 the exponential part of the curve is used for the regression analysis to obtain a straight line while in Fig. 2 the whole curve is used. Visually and statistically the straight line in Fig. 1 provides a better fit but it ignores the shoulder and tail. In Fig. 2 the whole curve is used for the regression analysis and it can be seen that the correlation coefficient (r^2) is lower than that for the line in Fig. 1. Statistically, this is thus a worse fit but biologically it is the correct fit. In Fig. 3 the difference in the slope between

the two lines can be seen. The steeper the slope the lower the calculated D_{10} which provides lower than correct D_{10} values.

Table I lists the D_{10} values obtained when values were calculated from the exponential part of the curve versus the D_{10} obtained when values from the whole curve were used. The correlation coefficient for all regression lines was >0.95. It is noticeable that in all cases higher D_{10} values were calculated when the whole curve was used versus the exponential decline area of the curve.

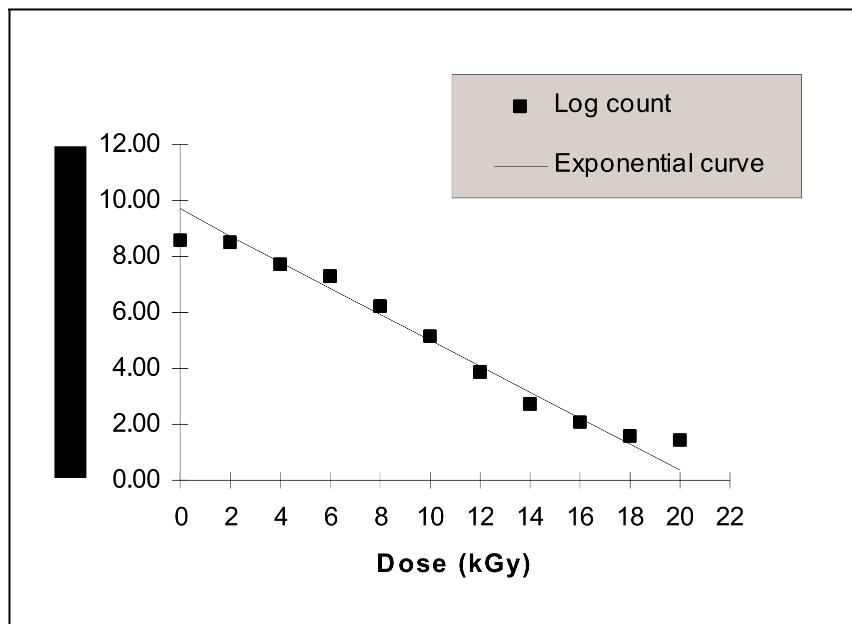

FIG. 1. Survival curve of mixed spore suspension. Actual log counts and the straight line formed by using regression analysis of the exponential decline area is shown. Correlation coefficient (r^2) > 0.98.

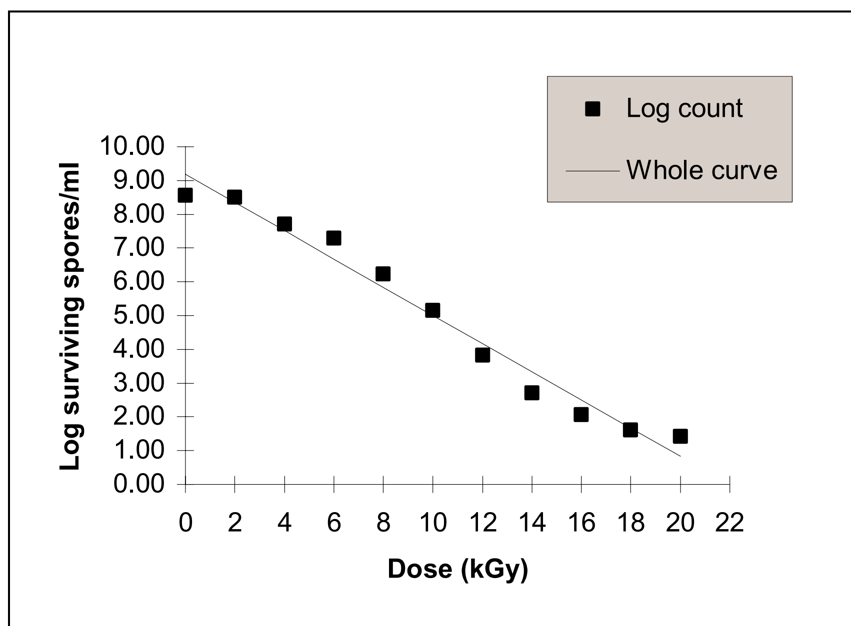

FIG. 2. Survival curve of mixed spore suspension. Actual log counts and the straight line formed by using regression analysis of the whole curve is shown. Correlation coefficient (r^2) > 0.97.

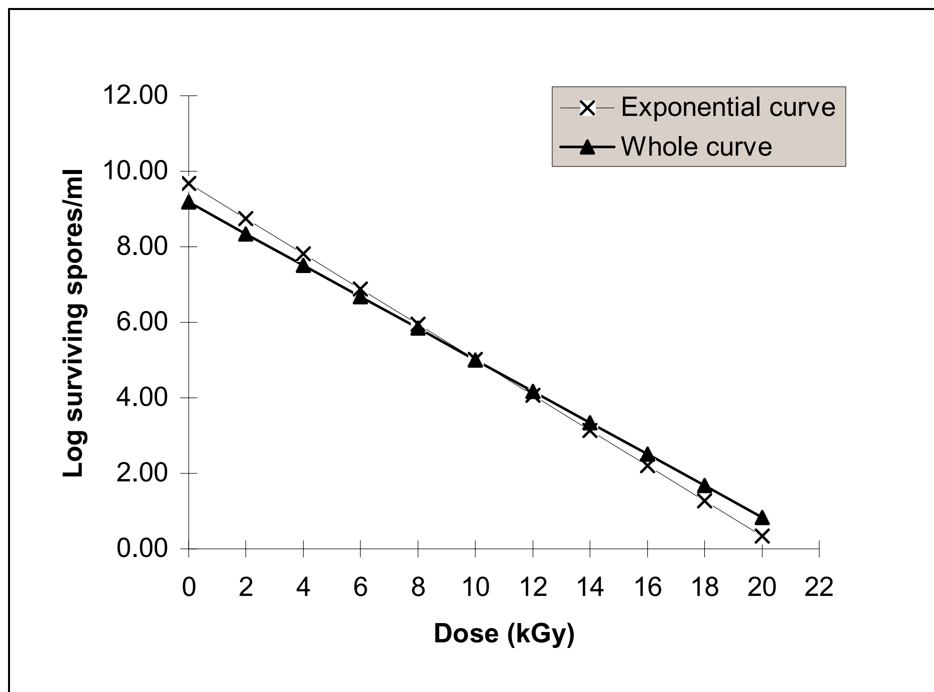

FIG. 3. Survival curve of mixed spore suspension. The straight lines formed by using regression analysis of the exponential decline area and the whole curve is shown.

TABLE I. D_{10} VALUES FOR *C. sporogenes* AS CALCULATED FROM THE EXPONENTIAL DECLINE AREA OF THE GRAPH AS WELL AS FROM THE WHOLE CURVE. CORRELATION COEFFICIENT (r^2) >0.95

Strain	Exponential decline	Whole curve
C. sporogenes Cl 5	2.41	2.91
C. *sporogenes* DSM 795 *	2.29	2.49
C. *sporogenes* DSM 767 *	2.05	2.26
C. *sporogenes* Cl 8	2.20	2.39
C. *sporogenes* Cl 26	2.01	2.21
C. *sporogenes* DSM 1664 *	2.10	2.19
C. *sporogenes* DSM 1446 *	1.58	2.17
C. *sporogenes* Cl 10	1.98	2.13
C. *sporogenes* Cl 3	1.96	2.12
C. *sporogenes* Cl 6	1.88	2.03
C. *sporogenes* ATCC 7955 #	1.82	2.03
C. *sporogenes* Cl 12	1.58	2.01
C. *sporogenes* Cl 1	1.76	2.01
C. *sporogenes* Cl 2	1.70	2.00
C. *sporogenes* DSM 663 *	1.67	1.92
C. *sporogenes* Cl 14	1.35	1.85
C. *sporogenes* Cl 9	1.43	1.76
C sporogenes mixed suspension	2.17	2.39

* — German culture collection
# — American type culture collection
— Locally isolated strains

Three strains were selected to represent the most resistant, least resistant and intermediate strains in a population. These were Cl 5, Cl 9 and DSM 1664, respectively. Cl 5 was the most resistant, Cl 9 the least and DSM 1664 was chosen partly because it represents the intermediate group and partly because it is a type strain. These strains were selected to create a representative spore suspension to use in determining D_{10} values in meat products as well as for the inoculation pack studies.

2.1.3. Conclusions

Unless the shoulder and tail areas are also taken into account when determining D_{10} values the resultant value will be lower than the true value. This is especially critical when minimum irradiation doses are determined for an extremely toxic organism like *C. botulinum.* There is no significant difference between the results obtained for the local strains and that of type strains, therefore, internationally published results for D_{10} values should be similar to those obtained using local strains. This conclusion is borne out by the fact that the results obtained are comparable with published results [8].

2.2. Inoculation pack studies with *C. sporogenes* spores to determine the experimental sterilisation dose (ESD) of pre-cooked, vacuum packaged meat products

2.2.1. Experimental design

The spore suspension was produced and enumerated as before. The three strains mentioned in Section 2.1.2 were used. Separate spore suspensions were produced from the three individual strains. After resuspension, the spores were enumerated and then diluted to produce a suspension of approximately 5×10^8 spores/mL. Equal quantities of the separate suspensions were then combined to form the final suspension. The D_{10} value for the final spore suspension was determined as in Section 2.1.1. and is listed in Table I.

Beef casserole was prepared by browning 6 kg of beef cubes in 150 mL oil, adding 6 L of beef stock, 40 g salt, 6 g pepper and 30 g "pinch of herbs". After allowing the meat to simmer for 1 h, 360 g cake flour was added to thicken the gravy.

A portion weighing 100 g ± 2 g of cooked casserole was packed into four layer laminated flexible pouches. A 1 mL aliquot of a heat shocked (80°C, 10 min) spore suspension containing 10^8 spores/mL was added. Each package was shaken gently to ensure that the spores were spread out evenly in the pouch. The pouches were vacuum sealed, frozen to –40°C and irradiated in a Gamma-beam 650.

The pouches were irradiated from 10–46 kGy at 4 kGy increments. Five pouches were irradiated per dose. The whole sample preparation and radiation cycle was repeated for three weeks and 15 pouches were therefore treated per dose. The pouches were irradiated inside a stainless steel double walled container that was coupled to a Julabo cooling circulating unit. Ethanol at –40 ± 2°C was circulated through the container to ensure that the product was irradiated at –40°C.

The dose rate was an average of 6.74 kGy/h and was determined using Fricke dosimetry. Red perspex type 4034 BT from Harwell Laboratory were used to determine the dose received. The change in absorbance at 640 nm was measured with a Bausch & Lomb Spectronic 1001 spectrophotometer and the dose determined from a calibration graph compiled from the pool irradiator at AEC. Red perspex dosimeters were calibrated against Fricke. During initial experiments the red perspex dosimeters were placed next to the pouches inside the cryogenic unit. The dosimetry results obtained were therefore not reliable due to the effect of the temperature on the perspex and the results of these two series have not been used. In later experiments the irradiation dose was determined using unfrozen "dummy" pouches and a reference dosimeter on the outside of the stainless steel container. During irradiation at –40°C a reference dosimeter was used on the outside of the container to verify the dose received.

After irradiation the samples were kept frozen until all pouches had been irradiated. The samples were allowed to defrost at room temperature and were then incubated at 30 ± 2°C for 6 months. The pouches were examined daily for swelling for the first month and after that on a weekly

basis. All swollen pouches were either tested for survivors immediately or stored at 1 to 3°C until testing could take place. After the six month period, all unswollen samples were stored at 1 to 3°C until testing could take place

The test for survivors was done by aseptically placing 50 g of the pouch contents into 250 mL of gelatine phosphate buffer containing 0.2% gelatine and 0.4% Na_2HPO_4 [16]. After homogenisation in a Stomacher two aliquots of 10 mL of homogenate was pipetted into two separate Schott bottles containing 40 mL PYT medium without agar and with 0.5% glucose and 0.6 mL of 5% $NaHCO_3$ added. One bottle was heat shocked at 80°C for 10 min after which both were incubated at 30°C for 30 days. After 30 days the medium was checked for turbidity and then 1 mL Resazurin solution (Resazurin (BDH) 300 mg, Thionin (Merck) 200 mg, NaHCO3 (Merck) 2 g and distilled water 500 mL was heated until the solids dissolved. After heating, the solution was filtered through Whatman No. 1 filter paper and the resultant solution was diluted to 2 L) was added to determine growth in the medium. A colour change from blue to pinkish-red indicated the lowering of the pH in the media and thereby indicating growth.

2.2.2. Results

All samples that received between 0 and 18 kGy swelled during the first four weeks as can be seen in Table II. None of the samples in series 3 at 22 kGy were swollen while 2 samples in series 1 at 26 kGy were swollen after 2 weeks incubation. Viable *C. sporogenes* cells were isolated from all swollen pouches. At 30 kGy and higher, none of the samples were swollen and survivors were not detected after incubation in PYT medium.

TABLE II. NUMBER OF SWOLLEN POUCHES PER DOSE DURING FIRST FOUR WEEKS OF INCUBATION

Dose (kGy)	Series	Incubation			
		Week 1	Week 2	Week 3	Week 4
0	1	5			
0	2	5			
0	3	5			
10	1	5			
10	2	5			
10	3	5			
14	1	5			
14	2	5			
14	3	5			
18	1	5			
18	2	5			
18	3		2		3
22	1	5			
22	2	5			
22	3				
26	1		2		
26	2				
26	3				
30	1				
30	2				
30	3				

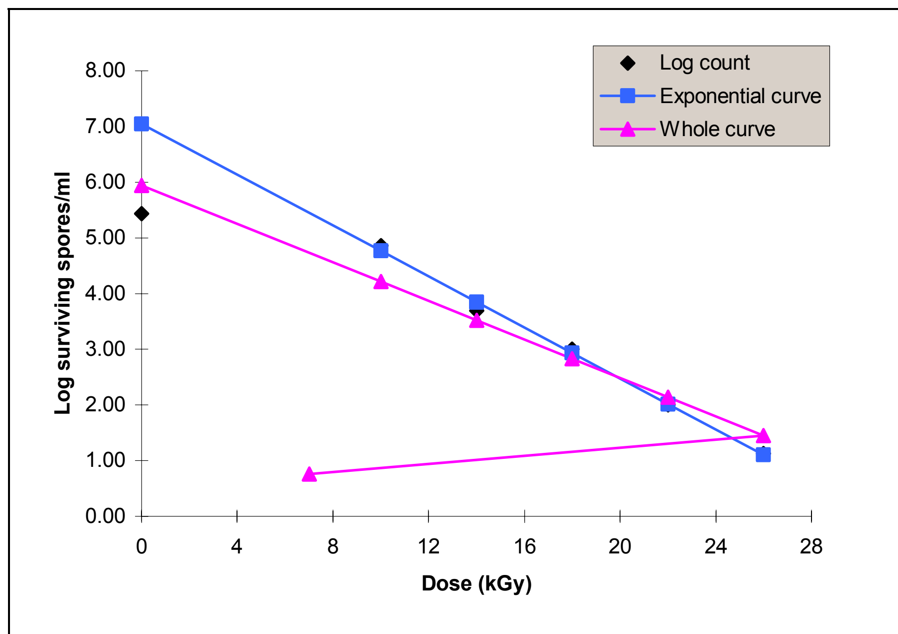

FIG. 4. Survival curve of mixed spore suspension in beef casserole. The straight lines formed by using regression analysis of the exponential decline area and the whole curve is shown. The correlation coefficient (r^2) of the linear decline area of the curve was >0.99 but the correlation coefficient (r^2) for the whole curve was >0.93.

2.2.3. Conclusions

The results obtained during the inoculation pack study compared favourably with published results [12,16] where it was reported that the experimental sterilisation dose (ESD) was 22–26 kGy for enzyme inactivated beef. From these results they calculated statistically that the minimum irradiation dose (MRD) that provided a rigorous margin of safety for enzyme-inactivated beef was 41.2 kGy. It was, therefore, decided that irradiating beef products in the AEC facility at 45 kGy would provide a guaranteed microbiologically safe product.

2.3. Determination of the D_{10} of *C. sporogenes* spores in pre-cooked, vacuum packaged meat products.

2.3.1. Experimental design

During the time that inoculation pack studies were carried out additional inoculated pouches were produced as described previously. These pouches were also irradiated at doses of 10–46 kGy at 4 kGy increments. Three pouches were used per dose. After irradiation the pouches were not incubated but used to determine the D_{10} value. The pouch contents were homogenised in phosphate buffer and serial dilutions were made. Enumeration was carried out as described in Section 2.1.1.

2.3.2. Results

Figure 4 illustrates the results obtained for the experimental decline curve using the beef casserole as substrate. The correlation coefficient (r^2) of the regression analysis to determine the D_{10} value using the linear decline area of the curve was >0.99 but the correlation coefficient (r^2) for the whole curve was >0.93. This curve is therefore not valid probably because no pouches were irradiated at doses between 0 and 10 kGy. Critical data points between 0 and 10 kGy were therefore not available. The correct dose range to use for D_{10} determination in a meat product should therefore be 0 to 30 kGy with 2 or 3 kGy intervals. The D_{10} values of 4.37 (exponential curve) and 5.80 (whole curve) are therefore not valid and can only give an indication of the D_{10} value. The experiment could however not be repeated as the Gamma-beam was not operational.

2.3.3. Conclusions

Dose range should be chosen carefully when an experiment is designed to determine D_{10} values in meat products otherwise the results are not valid. When D_{10} values are published all experimental conditions including substrate and irradiation temperature should be listed. In addition, mention should be made whether the D_{10} value was calculated from the whole curve thereby taking the "shoulder" and tail into account or just the exponential decline area of the curve.

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COMMERCIAL APPLICATION OF HIGH-DOSE IRRADIATION TO PRODUCE SHELF-STABLE MEAT PRODUCTS. PART 2 — PRACTICAL ASPECTS OF MAINTAINING PRODUCT AT TEMPERATURES OF BETWEEN –20°C AND –40°C DURING LARGE SCALE IRRADIATION

I.N DE BRUYN
Biogam, Atomic Energy Corporation of South Africa,
Pretoria, South Africa

Abstract

It has been proven that the temperature in the product has to be maintained between –20°C and –40°C during high-dose irradiation of meat products. This is to ensure that the product is microbiologically safe and of acceptable sensory quality. The way in which the product could be irradiated at this temperature range was investigated in the Package Irradiation Plant at Pelindaba at the Atomic Energy Corporation of South Africa (AEC). It was found that by using 10 kg of dry ice and a polystyrene separator between the ice and the product, it was possible to maintain the temperature within the required range for up to 70 h. These results are facility specific but the paper is intended to point out potential problem areas and parameters that other investigators should take into account when planning high-dose irradiation experiments.

1. INTRODUCTION

During the 1950's to 1970's researchers at the US Army Laboratories at Natick did extensive research on the sterilisation of meat products using ionizing irradiation. They documented the fact that meat irradiated at sub-freezing temperatures is sensorially more acceptable than meat irradiated at higher temperatures [1].

This is probably due to the fact that at lower temperatures the liquid phase is frozen, thereby reducing the amount of free radicals being formed during the process. This would prevent free radical interaction with the flavour compounds in the food products. At ambient temperatures free radical interaction negatively affects the flavour profile of the food. Based on a 9-point hedonic scale, ham that had been irradiated at –80°C and stored for 12 months scored considerably better than the product irradiated at +5°C and stored for the same period of time [2].

Since the bactericidal effect of irradiation is due both to direct hits as well as indirect hits, reducing the amount of indirect hits by freezing the product will also reduce the efficiency of the process to kill bacteria. For this reason several researchers [3–8] investigated the effect of temperature on the effectiveness of irradiation to kill spores of *Clostridium botulinum*.

The most significant result from Table I is the difference in dose required to sterilise a beef product at –30°C versus – 80°C. Reducing the temperature to –80°C requires that the minimum sterilisation dose be almost doubled.

Figure 1 shows the significant difference in D value for *C. botulinum* type 33A as the product temperature changes from –196 to 95°C. It is therefore critical to balance the dose where the product is microbiologically safe and also of acceptable sensory quality. The Natick researchers determined that –30 ± 10°C provides the necessary environment in which these parameters are met. All of their subsequent work was therefore carried out at these temperatures [1].

TABLE I. MINIMUM DOSES OF IONIZING ENERGY FOR STERILISATION OF DIFFERENT FOODS [9]

Product	Irradiation temperature (°C)	ESD[a] (kGy)	MRD (kGy)[b]	
			Range	Mean
Bacon	5 to 25	15–20	26.5–28.7	27.6
Beef	–30 ± 10	22–26	36.4–41.2	38.9
Beef	–80 ± 10	40–45	52.0–61.3	57.0
Ham	5 to 25	20–35	30.0–35.0	32.5
Ham	–30 ± 10	20–23	32.0–38.0	35.0
Codfish Cake	–30 ± 10	25–30	30.4–32.4	31.7
Corned Beef	–30 ± 10	20–25	24.4–25.7	25.1
Pork sausage	–30 ± 10	15–20	23.9–26.5	25.2
Shrimp	–30 ± 10	35–40	19.9–51.2	37.2
Pork	5 to 25	35–40	41.9–49.9	45.6
Pork	–30 ± 10	30–32	43.7–44.8	44.3
Chicken	–30 ± 10	30–35	43.4–46.2	44.8
Chicken[c]	–30 ± 10	40–42	42.7–47.8	43.9
Beef in Brown sauce[d]	–40 ± 2	22–26	36.3–41.2	38.9

[a]Experimental sterilising dose range; [b]Minimum irradiation dose, $D_{10} \times 12$; [c]With 0.75% NaCl and 0.3% sodium tripolyphosphate added; [d]Experimentation done in AEC laboratory [10].

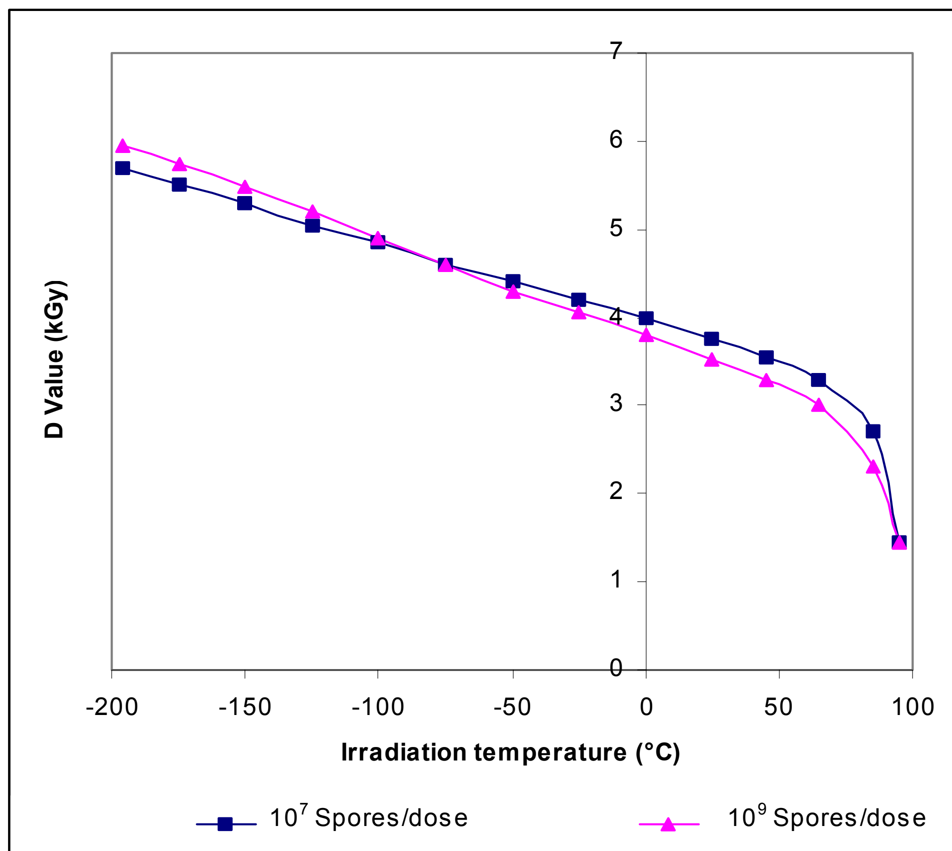

FIG. 1. Statistically computed linear change in D values of spores of C. botulinum type 33A in ground beef with change in irradiation temperature from –196 to 95°C [8].

There are various ways in which the temperature can be maintained during the process. At the Natick Labs this was done by using a specially built Dewar flask with liquid nitrogen as cooling agent [11]. In AEC laboratories, a Julabo circulating unit filled with ethanol/methanol coupled to a double walled stainless steel pot was used to maintain temperatures at –40 ± 2°C during inoculation pack study experiments [10]. However, these systems can only be used for reasonably small quantities for experimental purposes and are not practical for commercial scale operations.

In a commercial facility the product temperature can be maintained by using dry ice with or without mechanical cooling of the irradiation chamber. These options and the way that they are applied will be facility specific, but the basis principles will remain the same. Applying mechanical cooling to the irradiation chamber in the facility at AEC has three main problems:

(a) the water in the storage pool has to be kept above freezing point;
(b) the oil in the pneumatic pushers must be kept fluid; and
(c) the irradiation chamber is fitted with an ozone extractor, which would seriously effect the efficiency of any cooling system.

These problems indicate that mechanical cooling would probably not be a viable option.

In the AEC facility it was, therefore, decided to use dry ice to maintain the temperature of the product between –20°C and –40°C during irradiation. Original irradiation work on radiation-sterilised food was carried out with frozen produce packed into waxed cardboard boxes, three boxes being placed into a polystyrene liner in the irradiation tote. Temperature control was ensured by the addition of 6 kg dry ice to each tote. Due to the cost of the cardboard boxes it was decided to switch to reusable wire baskets (two per tote). Early experiments on temperature profiles were carried out during 1990. These experiments indicated that heat transfer between product and dry ice was greatly enhanced in the case of the wire baskets, and it was decided to use wire baskets in future. With the cardboard boxes the ice was packed directly on top of the cardboard box. However, when the ice was placed directly on top of the product the temperature of the product in the top wire basket dropped down to –58.8°C during initial stages of the irradiation process [12].

Since the irradiation plant is not manned on a 24 h basis, sufficient produce is loaded onto the conveyor system to carry the plant overnight. Product entering the irradiation chamber reaches the source after 14 h, after which it is exposed to irradiation for approximately 40 h. Subsequently, temperature is of no further significance once the product moves away from the source. This 12–14 h lead-time can result in a 70 h run time for the whole radiation process. This time is directly proportional to the dose rate of the facility and can be reduced by increasing the cobalt loading.

It thus became imperative to investigate the temperature profile of the product over an extended period equivalent to a full irradiation cycle to ensure that the minimum temperature does not fall too far below –40°C and that the final temperature does not rise above –20°C.

2. METHODS

The irradiation was carried out in a Nordion JC 6500 tote box irradiator with two levels of passes and four passes per level (two on each side of the source). Two metal baskets filled with product (frozen to –30°C ± 5 °C) were placed in a polystyrene liner inside the irradiation tote box, one on top of the other (Fig. 2). Unless mentioned otherwise the temperature was measured by placing two thermocouples in the centre of the product in each individual basket. The temperature of each thermocouple was read using a RKC Series RE 48 Temperature reader, which can read from –100.0 to +199.9°C. The averages of the temperatures from the top and bottom baskets were calculated.

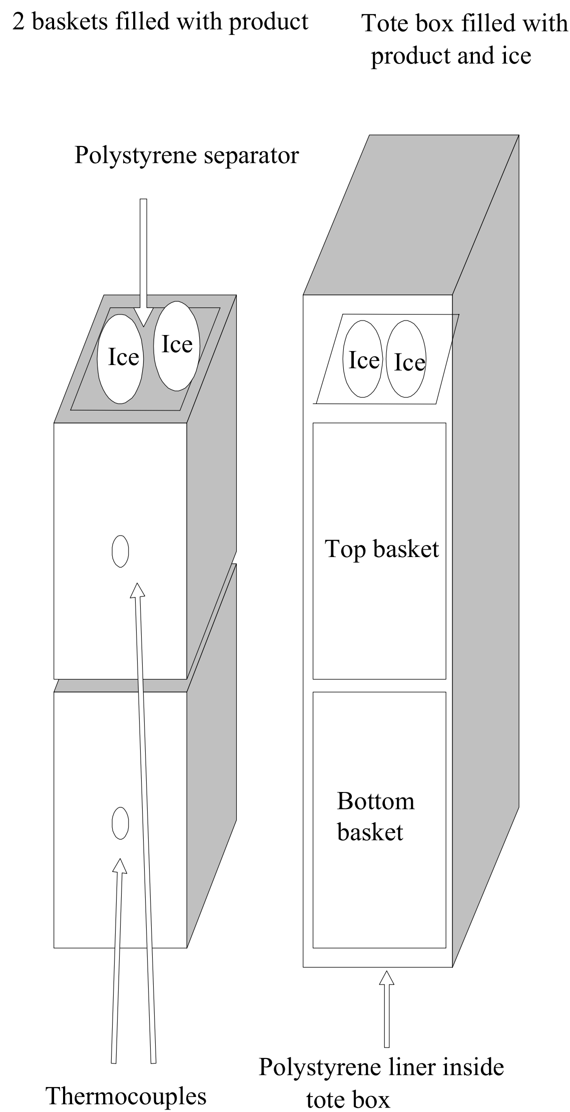

FIG. 2. Experimental layout of tote boxes filled with product and ice.

3. RESULTS

3.1. Determining the effectiveness of a separator

An initial experiment was carried out to confirm the results of the previous work, and to determine whether insulation could be used to prevent the rapid heat transfer between product and dry ice so that the temperature of the product would not drop down to –60°C. The experiment was designed to confirm the quantity of ice required to maintain the temperature and to determine the effectiveness of a separator board. The ice (8 or 10 kg) was therefore placed either directly on top of the product or onto the separator board on top of the product.

Figure 3 shows that using 8 kg of ice was not sufficient to maintain the temperature for the required period of time. Using 10 kg of ice and adding a polystyrene insulation board gave very good results. It can be clearly seen that adding a separator between the dry ice and the product causes the temperature curve to flatten, resulting in a higher minimum temperature and longer time at a temperature of below –20°C. Without the separator the minimum temperature of the product is too low.

3.2. Determining the effect of the size of the ice chunks

The effect of the size of the chunks of ice was determined by placing whole pieces of ice on top of the product in one tote box and in the other the ice was broken into small pieces and scattered on top of the product.

In Fig. 4 it can be seen that it is best to use whole pieces of ice rather than small pieces. The physical size of the blocks of dry ice has a significant effect on the minimum temperature of the product and also the temperature at the end of the required time (note the difference between the slope of the two lines). Larger blocks sublime slower due to the lower surface area per unit mass, resulting in a flatter temperature profile

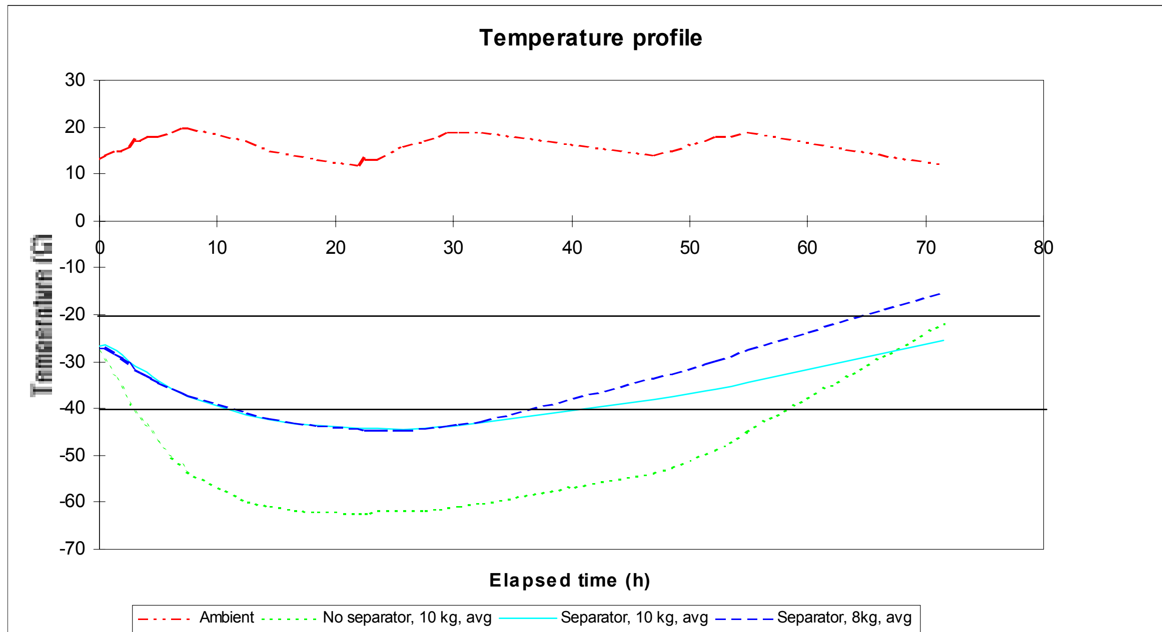

FIG. 3. Effect of separator boards on temperature profile.

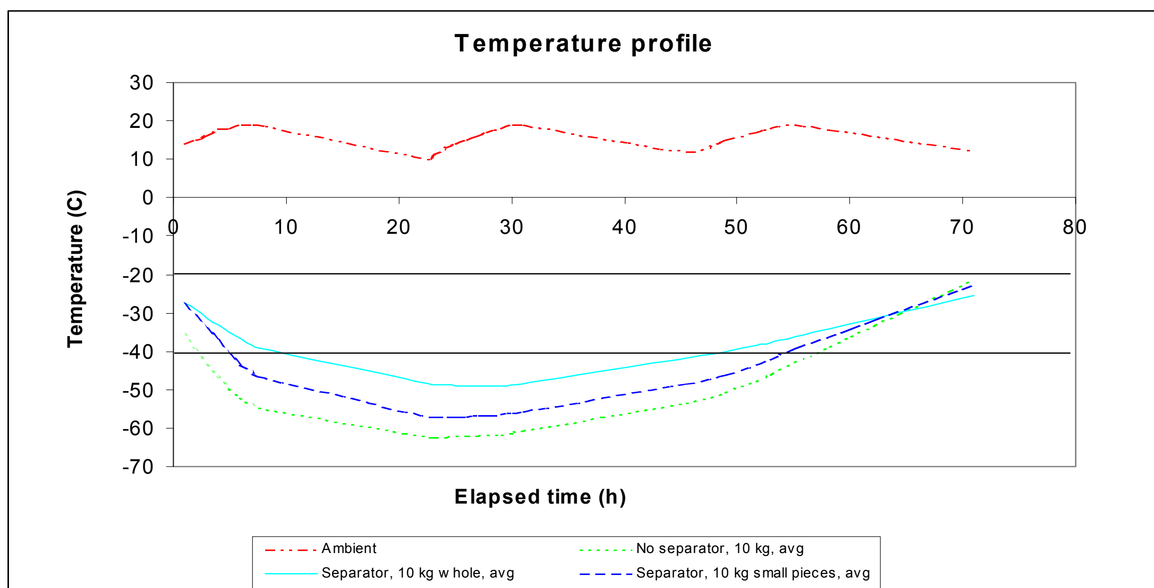

FIG. 4. Determining the effect of the size of the ice chunks.

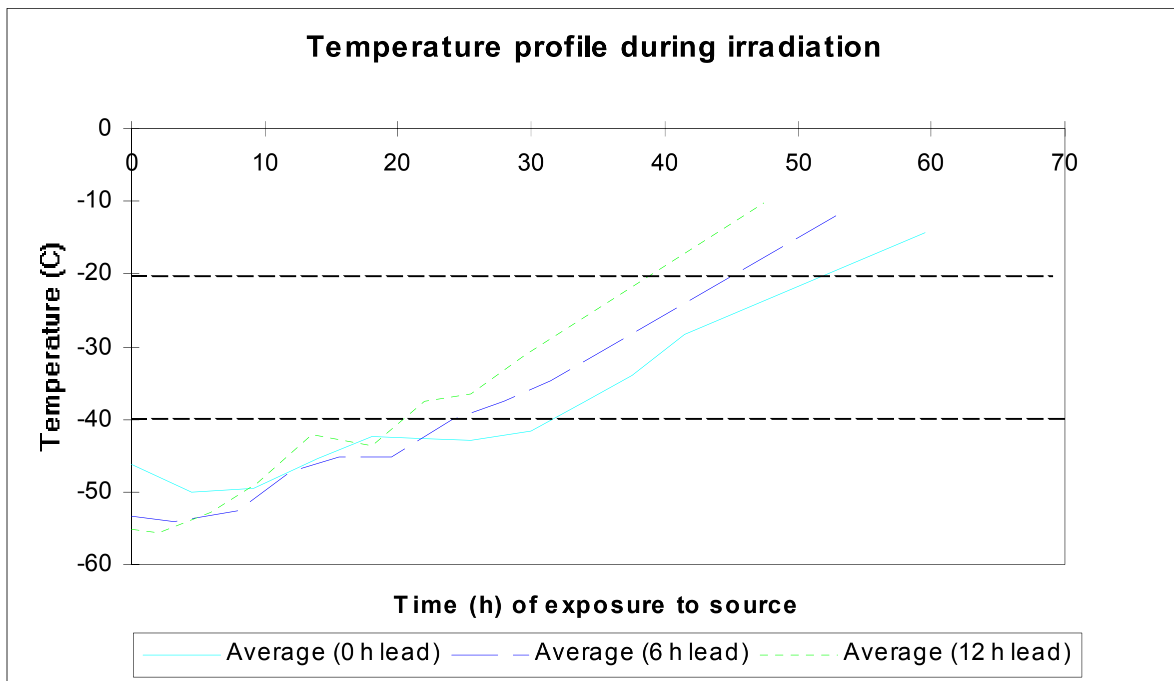

FIG. 5. Temperature profile during irradiation (All totes were packed with separators and 10 kg ice).

3.3. Determining the temperature profile during an irradiation run

These results were extremely promising. With the current cobalt load, it appeared that product temperature could be maintained at acceptable levels during the irradiation process. It was decided, however, to monitor temperature during an actual irradiation run. Figure 5 shows actual exposure of product to irradiation (a period of 40 h). With no lead time (product loaded into the totes and immediately sent into the irradiation chamber), the temperature profile is reasonable. The temperature never drops below –50°C, and is well below –20°C after it leaves the source. With 6 and 12 h lead times, however, temperatures of below –55°C are attained, and in the case of the 12 h lead, product is at –20°C as it leaves the source. It is therefore advisable to load the product just before irradiation so that the temperature of the product does not drop too low during the lead time. According to Fig. 3 the temperature could be maintained for more than 70 h at below –20°C. However from Fig. 5 it would appear that the temperature can only be maintained for 53 h. However, taking into account the 14 h before the product arrives at the source, this time is in actual fact 67 h. The difference between these two results (more than 70 h and 67 h) can probably be ascribed to the physical movement of the tote boxes in the irradiation plant where the box is "shoved" forward during each cycle thereby disturbing the micro-environment inside the tote box and also to the rise in temperature due to the irradiation itself.

3.4. Determining the effect of plastic liners on the temperature profile

During large-scale irradiation of the product it was found that a very small percentage of the pouches were physically damaged during the process. This is probably due to the fact that product is handled in the metal baskets. It was recently decided to investigate the effect on the temperature profile when the product was packed in plastic liners inside the metal baskets.

Polethylene plastic sheets were used to line the bottom and sides of the metal baskets. The pouches were then packed into the baskets in the same way as in the previous experiments. However, the thermocouples were not placed in the middle of the product but between the bottom two layers of product in the bottom basket and between the top two layers of product in the top basket. This was done to try and determine the temperature at the extreme positions in the tote.

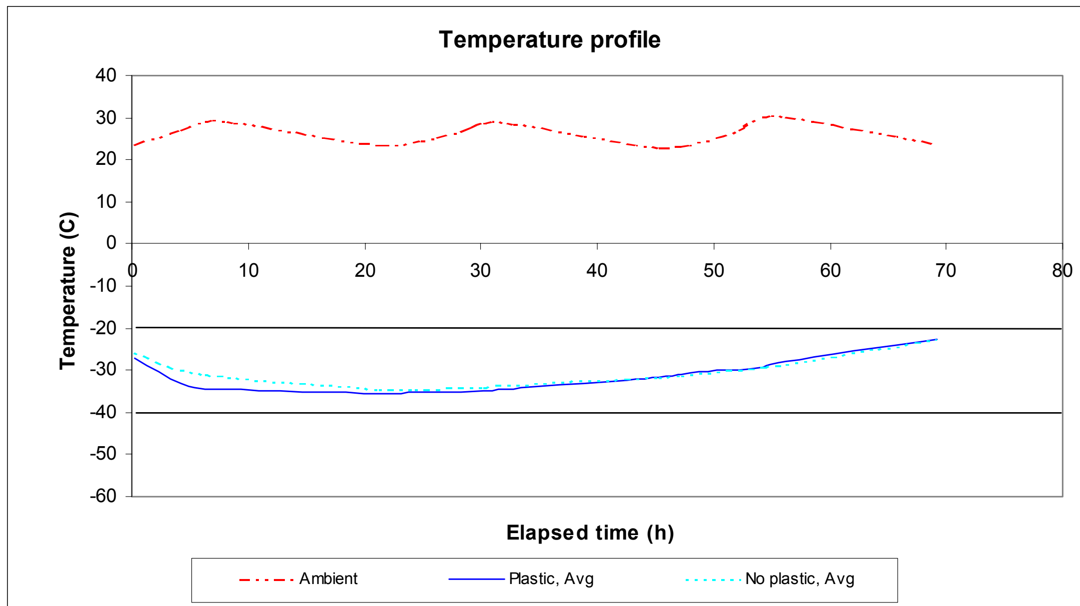

FIG. 6. Effect of plastic liners in baskets, averages depicted (All totes were packed with separators and 10 kg ice).

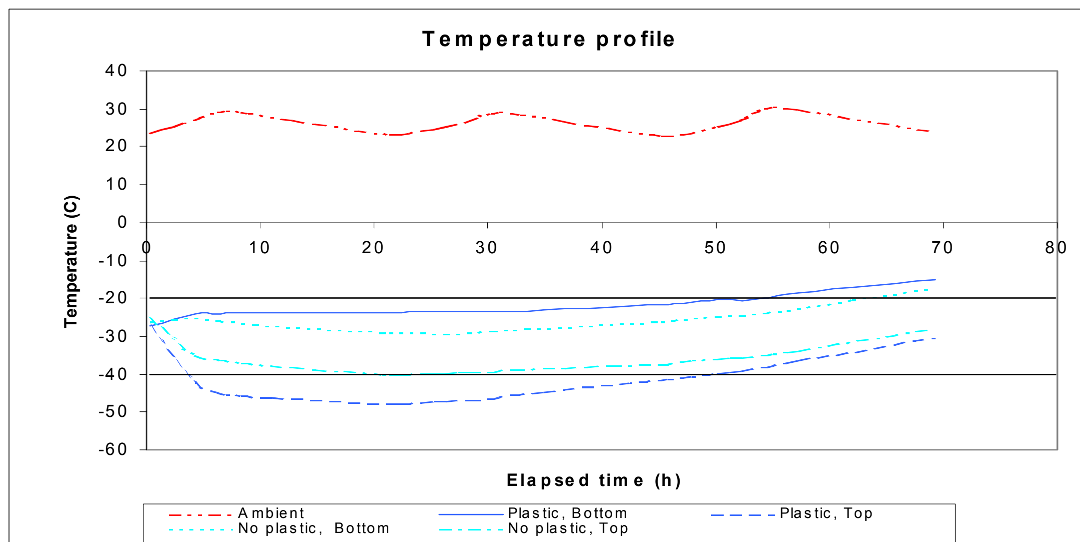

FIG. 7. Effect of plastic liners in baskets, actual results depicted (All totes were packed with separators and 10 kg ice).

In Fig. 6 the average of the readings in the bottom and top baskets of tote was plotted and it would seem as if there is virtually no difference between the tote with the plastic liner and the one without. However in Fig. 7, where the average in the top basket and the average in the bottom basket is plotted individually, it can be seen that there is a significant effect on the temperature profile. In the tote where the plastic was not used, the minimum and maximum temperatures at the top and bottom are much closer with a maximum of 10°C variance. This is in line with the worse case scenario obtained in all previous experiments. However, in the tote where the plastic was used, the variance between the minimum and maximum temperatures at the top and bottom was about 25°C. The minimum in the product was about –48°C and the maximum was barely below –24°C. This wide variance within the tote box is totally unacceptable and is probably due to the fact that the plastic liners affect the free flow of the cold air within the tote and especially down to the bottom basket. It was therefore decided not to use the plastic liners and to treat the product more carefully to prevent damage of the pouches.

4. CONCLUSIONS

The results obtained are facility specific due to the quantities of dry ice required as well as the physical packing of the product in the basket. This would have to be determined for each individual facility. However, it is important to note that the ice must be isolated in such a way that it does not rest directly on top of the product as this leads to a very wide variance between the minimum and maximum temperatures during the irradiation cycle. Again the way in which this is achieved will be facility specific. Furthermore, it is important to use big pieces of ice as this regulates the temperature profile better that when small pieces of ice are used. Lastly, it should be ensured that the free flow of the cold air is not inhibited in any way as this significantly affects the variance between the minimum and maximum at any given time.

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COMMERCIAL APPLICATION OF HIGH-DOSE IRRADIATION TO PRODUCE SHELF-STABLE MEAT PRODUCTS. PART 3 — EFFECT OF POLYPHOSPHATES ON THE TENDERNESS AND SENSORY PROPERTIES OF BEEF SILVERSIDE STERILISED AT 45 kGy

C. ERASMUS, A. MINNAAR, N.N. DERSLEY
CSIR Bio/Chemtek,
National Food Science and Technology Programme,
Pretoria, South Africa

Abstract

The effect of five mixtures of sodium chloride, sodium tripolyphosphate and tetrasodiumpyrophosphate on the texture and sensory properties of irradiated beef *M. biceps femoris* and *M. semitendinosus* muscles sterilised at 45 kGy were evaluated using pH after injection, drip loss measurement, Instron texture measurement and descriptive sensory analysis. The effect of cattle race was also investigated. Three breeds of cattle namely Afrikaner (*Bos indicus*), Hereford (*Bos taurus)* and Simmentaler (*Bos taurus)* were used. Steers were fed and raised in a controlled environment until 18 months of age, followed by slaughtering using the same procedure for each carcass. It was found that cattle breed had a significant influence on irradiated meat tenderness and juiciness. Afrikaner breed meat was the most tender and the most juicy. The Energy to break point measured using a Warner Bratzler Shear cell correlated with the ease of fragmentation (using fingers) as well as Initial Juiciness and Sustained Juiciness. Using polyphosphate levels of 13.2 mmol/kg produced undesirable flavours in the meat. The same tenderness was obtained using levels of 8.2 mmol/kg in the meat without undesirable taste. No differences were found between sodium tripolyphosphate and tetrasodium pyrophosphate treatments. Warmed Over Flavour was a significant factor influencing the quality of the products.

1. INTRODUCTION

Irradiation sterilisation of precooked, hermetically sealed meat provides a shelf-stable, ready-to-eat product that can be stored for long periods of time without refrigeration. High-dose irradiation has a tenderising effect on meat and this effect can be used to make tender roast beef from tougher meat cuts. Irradiation can, however, cause over-tenderising of meat resulting in a mushy texture and excessive degradation of connective tissue, resulting in a friable texture [1, 2]. Although irradiated meat is tender, it is often perceived by consumers to be dry or not juicy, possibly due to the friable texture. Meat texture is, however, also affected by various factors other than irradiation. These factors include breed, muscle fibre characteristics, water holding capacity of the muscle, cooking temperature and cooking method [3–5]. High-dose irradiation causes flavour changes in meat products due to the formation of radiolysis products. The formation of radiolysis products can be reduced greatly by irradiating at temperatures below −20°C [1,6]. Lipid oxidation, which often occurs in uncured cooked irradiated products, also contributes to the flavour changes in irradiated meat products [1,7,8].

2. LITERATURE REVIEW: FACTORS INFLUENCING THE TEXTURE AND FLAVOUR OF COOKED MEAT

2.1. Texture

According to Brady and Hunecke [9], Szczesniak define food texture as “the composite of those properties which arise from structural elements, and the manner in which it registers with the physiological senses”. This definition recognises three essential elements of texture:

- that it is the result of the structure of food
- that it is a composite of several properties
- that it is a sensory quality.

All of these elements will have to be considered in the measurement of food texture.

Meat texture is influenced by the tenderness and the juiciness [4]. It is also affected by various *ante mortem* and *post mortem* factors. Some of the *ante mortem* factors include the species, breed, age and gender of the animal, the anatomical location of the muscle, the muscle fibre characteristics (for example sarcomere length), the connective tissue content of the muscle, the fat content and marbling of the muscle and the water holding capacity of the muscle. *Post mortem* factors include electrical stimulation, chilling temperature, ageing period, cooking temperature and cooking method [3,4,5]. Processing methods such as freezing and irradiation also affects meat texture.

Textural sensations are very complex and as yet it cannot be measured objectively by any one instrument [10,11]. Various tests are thus utilised to measure meat properties:

2.1.1. Tenderness

Tenderness is defined as the resistance to shear or the "hardness" of the meat. It is a sensory characteristic, but mechanical means are commonly used to provide a measure of tenderness. Muscles from different parts of an animal vary in tenderness because of their varying connective tissue content. The tenderness of the same muscle from animals of different ages may also vary greatly [4]. Two structural components namely muscle fibres and connective tissue contributes to meat tenderness [12].

Objective measurements of meat can be measured by many mechanical devices. These include shear devices (such as the Warner-Bratzler shear), biting devices, compression methods, tensile methods, penetration methods, grinding methods and fragmentation methods (e.g. myofibrilar fragmentation index). Structural assessments such as sarcomere length use a description of the fibres to assess tenderness while chemical methods measure chemical changes to provide a means of tenderness assessment, e.g. changes in connective tissue solubility [4]. Both Instron shear force measurements and sarcomere lengths have been associated with meat tenderness – the lower the shear value the more tender the meat [4,13,14,15], while muscles with longer sarcomeres are more tender [16–18]. Myofibrilar fragmentation index is reported to be used as a predictor of cooked beef tenderness [4], but Heinze and Bruggeman [18] did not find a correlation with sensory tenderness.

Connective tissue (collagen and elastin) also contributes to meat tenderness. The cooking of meat changes the texture of the intramuscular connective tissue and its mechanical properties because of the denaturation of collagen. The amount of soluble collagen drastically increases when meat is cooked to a temperature of 70–80°C [4,19]. Collagen content and soluble collagen is determined by isolating the hydroxyproline in the meat and by measuring the hydroxyproline concentration using a colour reaction [17,20–23]. Although collagen content and solubility is usually determined on raw meat, it was also found that collagen content and solubility in cooked samples were not significantly different from the raw samples [17].

2.1.1.1. Measuring Tenderness using the Warner Bratzler shear cell

The tenderness of meat is measured by determining the relationship of stress (kg/mm^2)versus strain (mm/mm) and by producing an Energy curve. The Warner Bratzler cell cuts a selected piece of meat of specific thickness into two pieces perpendicular to the fibres of the meat. The measurements made can consist of the Load (Force in Newton) per mm distance at a specific tempo of cutting the meat. This tempo is determined by the Cross-head speed of the cutting blade in the Warner Bratzler cell and is measured in mm/min. Additional measurements are the energy used to break the meat (Energy at break point) and the load (in Newton) at break point. A typical Instron set-up consists of a load cell, measuring software and a specifically selected shear cell. Shear cells are designed to perform specific tests (e.g. the Kramer cell or the Warner Bratzler cell). During shearing of the food sample by cutting with the Warner Bratzler knife, a graph similar to the one in Fig. 1 is created:

The maximum force experienced when cutting the meat is given by the load at the maximum point. The break point can also be calculated and a break can occur in the following instances:

– If the curve drops off sharply to break, the point is found on the shoulder of the curve, just before the break occurs.

– If the curve is less abrupt, the break point is found where the slope of a line, tangent to the curve, is at a minimum.

The break point is an indication of the first failure of the muscle fibres to resist the pressure from the cutting knife. The break point is shown in Fig. 2.

The area under the curve shown in Fig. 1 gives the energy needed to cut through the piece of meat. The energy can be calculated to the break point. By dividing the Energy value by the volume of the sample under stress, a Toughness value can be determined. However, as the pieces of meat were cut, this volume was not added to the value and the energy values were used as a direct indication of the toughness of the meat. The calculation of the energy value is shown in Fig. 3.

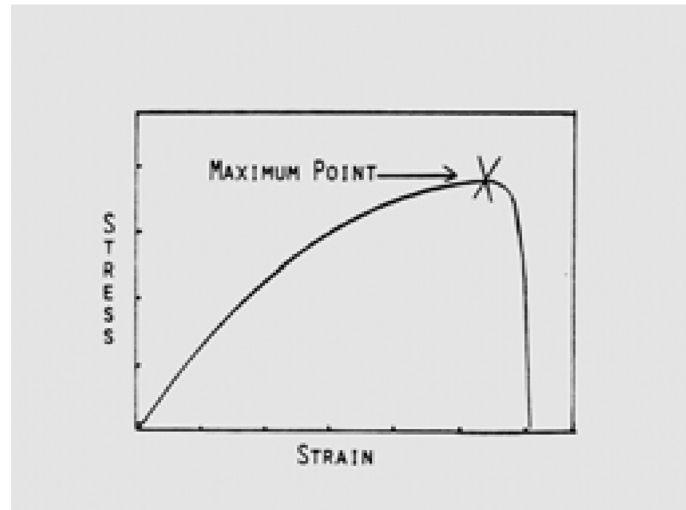

FIG. 1. Typical Stress/Strain relationship when cutting a food product using an Instron Texture measurement system [24].

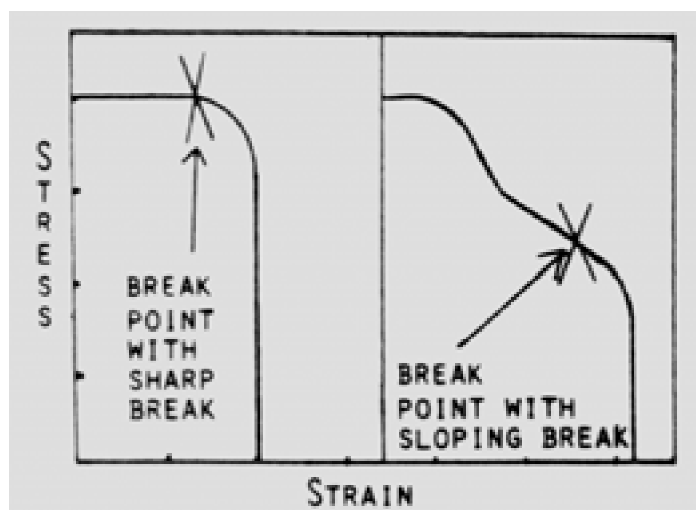

FIG. 2. Example of the determination of the break point in a Stress/Strain curve [24].

2.1.2. Juiciness

Meat juiciness is an important contributor to eating quality and plays a key role in meat texture. Unlike other key aspects of texture, juiciness is a subjective property. The only reliable and consistent measure of juiciness is achieved using sensory methods. The results of studies comparing subjective measurements of juiciness to measuring water holding capacity or to the quantitative or

qualitative measurement of muscle fluid are contradictory. Many researchers found a positive correlation between juiciness and water holding capacity, but just as many found either no correlation or contradictory results. Some researchers reported a relationship in one study, but no relationship in a follow-up study. The relationship between drip loss and juiciness is also conflicting – once again some researchers found a positive correlation, while others did not find any correlation [4]. Zimoch and Gullet [15] found that juiciness assessed early in mastication was positively correlated with tenderness.

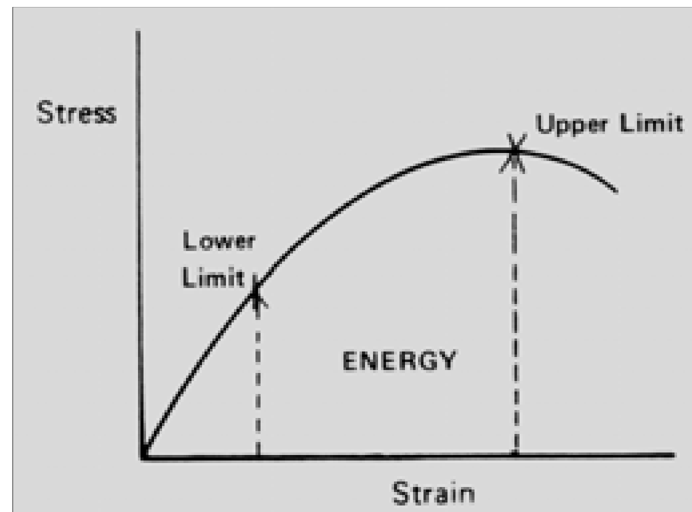

FIG. 3. The calculation of theEnergy to break point [24].

2.1.3. Flavour

Warmed over flavour (WOF) is a stale or rancid flavour described as a cardboard or painty flavour. This flavour rapidly develops in cooked meat upon chilled storage. It is generally accepted that WOF is caused by the autoxidation of membrane phospholipids, but there is also increasing evidence to suggest that the degradation of proteins and other compounds associated with the aroma of freshly cooked meat may also be implicated in WOF, particularly in the disappearance of desirable notes [25,26]. During the oxidation process carbonyl compounds are produced which are strongly associated with the "cardboard" and "painty" off-flavours.

Cooked meat is highly susceptible to lipid oxidation because the cooking process denatures antioxidant components, damages cell structure and exposes membrane lipids to the environment [8]. Various researchers have also indicated that polyphosphates prevent the development of WOF [25,27–29].

2.1.4. Polyphosphates

Polyphosphates are widely used, often in conjunction with salt, to increase water holding and also to reduce cooking losses in the manufacture of products such as cured pork meat, sausages and burger patties [30].

Polyphosphate injection in pork loins increases tenderness and juiciness, but the treatment has an adverse effect on flavour [30]. The polyphosphates promote the depolymerisation of myosin filaments and also weaken the binding of myosin heads to actin, thus promoting the dissociation of actomyosin, allowing limited expansion of the filament lattice. This allows for polyphosphate treated meat to take up and retain more water than untreated meat. The increased tenderness of polyphosphate treated samples can thus be attributed to the weakened muscle structure as well as the water content of the cooked muscles. The polyphosphate treatment can only slightly increase the pH, but it is possible that small differences in pH can be accountable for the significantly altered flavour.

Polyphosphates increase the alkalinity of meat above it's isoelectric point (pH 5.3–5.5), resulting in increased water binding capacity and swelling of the raw meat, and reduced cooking losses in cooked meat [28,29,31]. Polyphosphates have also been found to prevent WOF, the oxidized flavour that develops in cooked meat after a few hours of refrigeration [25,27–29]. Irradiated chicken and pork products with 0.75% salt and 0.3% or 0.5% sodium tripolyphosphate added were significantly superior in terms of texture [13,14,32]. Collagen hydration and solubility also increase with addition of phosphates. Other researchers also found that a 3.5% solution of sodium tripolyphosphate optimises connective tissue hydration whereas 3.5% acidic pyrophosphate optimises collagen solubility.

Shults et al. [31] found that pyrophosphate was more effective than tripolyphosphate in raising pH and increasing the swelling of beef. They also noted that tripolyphosphate is partly converted to pyrophosphate, possibly due to the hydrolytic effect of muscle ATP-ase. Sheard et al. [30] also found the pyrophosphate as the active moiety. Shults and Wierbicky [31] found that tetrasodium pyrophosphate was the most effective food grade phosphate for increasing the water holding capacity of beef muscle. The maximum effect on the reduction of meat shrinkage during cooking was obtained by the addition of 0.5% pyrophosphate or tripolyphosphate along with 1% salt.

2.1.5. Breed

Differences between breeds in terms of muscle and meat quality has been shown to exist, and these may determine the sensory quality of meat. Some researchers determined in their studies on the influence of cattle breed on sensory meat quality during ageing, that texture differed significantly amongst breeds [4]. Pearson and Dutson [4] recorded that meat from *Bos indicus* breeds is usually tougher than that from *Bos taurus* breeds, possibly due to reduced tenderisation during ageing because of the higher stability of the proteinase inhibitor in the breed.

2.1.6. Irradiation

Irradiation is an effective means for sterilising meat to obtain a shelf-stable product. Proteolytic enzymes must be inactivated in order for irradiation sterilised meat to have a long shelf-life. This is achieved by cooking the meat to an internal temperature of at least 70°C [1]. One of the major concerns in irradiating meat is its effect on the generation of off-odours and lipid oxidation [8].

2.1.6.1. Effects of irradiation on texture

As a shelf-stable food, irradiation sterilised meats receive consistently high appraisal for texture, particularly in comparison with the thermally processed items [1]. Various authors [13,14] found that irradiated pork chops and pork rolls were significantly more tender than the non-irradiated products. Exposing the meat to sub-freezing temperatures before and during irradiation treatment may counteract the tenderising effect of irradiation [6]. Irradiation may also lead to excessive degradation of the connective tissue, resulting in a friable texture [1,2]. Collagen is susceptible to breakdown into smaller units especially at doses higher than 50 kGy [2].

2.1.6.2. Effects of irradiation on flavour

Cured meats are less sensitive to oxidative flavour changes than uncured meats. Among uncured meat, whole muscle items (e.g. steaks or chops) are less sensitive to flavour changes than emulsion-type products. This indicates that residual oxygen in the system is at least partially responsible for these flavour changes. Addition of phosphates and a small amount of sodium chloride improve the flavour [1]. Irradiated meat can develop a characteristic odour, which has been described as metallic, sulphide, wet dog or wet grain [8]. Dimethyltrisulfide is the most potent off-odour compound [8] and the changes that occur are distinctly different from those of WOF in oxidized meat. The development of off-flavours, due to the production of radiolysis products in irradiation sterilised

meat can be greatly reduced by excluding oxygen from the product, and by irradiating the products in the frozen state at –20°C to –40°C [1,6].

2.1.6.3. Descriptive analysis

Measurements by a trained sensory panel appear to be very important in meat research, and most of the studies encountered made use of descriptive sensory panels [27, 33]. Several researchers referred to the Cross's [22] method of training in their own research as he described the training and testing of judges for the sensory analysis of meat very thoroughly. His method starts with recruitment of suitable panellists, training them in individual and group sessions and ends with evaluating the performance of the panel.

3. OBJECTIVES OF THE STUDY

The primary objective of this study was to get a better understanding of factors influencing the perceived dryness experienced in irradiated roast beef (specifically Silverside cuts made from *M. biceps femoris and M. semitendinosus* muscles) as manufactured by the Atomic Energy Corporation (AEC) of South Africa as a food product for hikers. As meat cuts are obtained from abattoirs, a selection of meat types are received that differ in terms of age, cattle race, gender and fat content. Due to the many factors that can influence the tenderness of cooked beef roast, it was decided to carry out a test using cattle breed and fed under controlled conditions. Three different races were obtained and only steers were used. The hypotheses tested in this study were that:

– The use of polyphosphates in combination with sodium chloride increases the juiciness of irradiated meat, due to increased water binding.
– Irradiated meat is more tender and has a friable texture due to degradation of the connective tissue during irradiation.
– Meat from different cattle races behaves differently when irradiated.

Secondary objectives of the study were to:

– Determine the effect of different levels of polyphosphates on the sensory properties of irradiated meat in terms of aroma, tenderness, juiciness, fragmentation in the mouth, fragmentation using fingers, and off-flavours.
– Determine the effect of irradiation and the addition of polyphosphates on the texture of the irradiated roast beef in terms of physical characteristics using an Instron texture measurement system.
– Evaluate the effect of the addition of polyphosphates on meat drip loss and pH.

4. MATERIALS AND METHODS

4.1. Treatments

Simmentaler, Hereford and Afrikaner steers of the same age (18 months old) were used for this research. The Simmentaler and Hereford are *Bos taurus* and the Afrikaner, an indigenous South African cattle type is of *Bos indicus* type. The *M. biceps femoris* and *M. semitendinosus* (Silverside) muscles were taken from both sides of each carcass (the rest of the carcass was used for other experimental work). Six carcasses were available per breed, resulting in 12 individual muscles being available for the research. Two muscles of every breed were randomly allocated to each treatment. The muscles were pumped with brine to contain the following additives after pumping:

– 0.7% NaCl (weight/weight) and 0.3% (8.2 mmol/kg) sodium tripolyphosphate (Treatment 1)
– 0.7% NaCl (weight/weight) and 0.5% (13.6 mmol/kg) sodium tripolyphosphate (Treatment 2)

- 0.7% NaCl (weight/weight) and 0.22% (8.2 mmol/kg) tetrasodium pyrophosphate (Treatment 3)
- 0.7% NaCl (weight/weight) and 0.36% (13.6 mmol/kg) tetrasodium pyrophosphate (Treatment 4)
- 0.7% NaCl (weight/weight) (Treatment 5)
- Control (No pumping) (Treatment 6).

It was decided to use similar molarities of the different phosphates, which resulted in different concentrations being used in the meat.

The meat was roasted at 180°C to a core temperature of 80°C in order to inactivate all internal enzymes. The meat was allowed to cool followed by determination of the drip loss (weight of meat before and after roasting). The meat was then cut into 5 mm slices, which were vacuum packed in foil pouches. The pouches were frozen and irradiated in the frozen state (–40°C) with a ^{60}Co gamma source (dose rate of 1.5 kGy/h) until a dose of 45 kGy was obtained. Control samples of each treatment that were not irradiated were kept frozen at –29°C. The flexible pouches consisted of four layers namely Nylon (for flexibility), Aluminium foil (barrier), Polyester (strength) and linear low density polyethylene (LLDPE) (heat seal and food contact material) and were supplied by Kohler flexible packaging, South Africa. The *M. semitendinosus* muscles were used for training of the sensory panel and were not used for other analyses, although the treatments given were exactly the same as with the *M. biceps femoris* muscles.

4.1.1. pH measurements

pH measurements were made on all raw meat samples after pumping. A 50 g aliquot of meat was homogenised and the pH measured with an Orion pH meter.

4.1.2. Drip loss

Drip loss was calculated as the percentage weight loss of each roast based on the weight after trimming and pumping (before roasting) and after roasting.

4.1.3. Texture measurements using Instron

Texture measurements were made on 2.5 cm thick cylinders of samples of cooked meat at the following stages:

- Directly after roasting (at ambient temperature).
- After freezing (–40°C) (the product was thawed and measurements made at ambient temperature).
- After irradiation (the product was incubated for two weeks at 37°C and the measurements were made at ambient temperature).

Ambient temperature in Pretoria was an average of 24.1°C when the measurements were made. The meat samples were cut using a Warner Bratzler shear cell on an Instron model 4301 automated Materials Testing System.

The following calculations were made from the stress/strain relationships:

- Load at maximum (Newtons)
- Energy at break point (joule)

The energy at break point can be regarded as a physical indication of the tenderness of the meat sample. The Warner Bratzler cutting device was preferred to the Kramer shear press for the analysis. Preliminary analysis using the Kramer shear press gave unreliable results because small

fibres interfered with the measurement. It also produced very high forces, which were too high for the Instron load cell to measure. The Warner Bratzler cell is preferred when evaluating the tenderness of intact fibres [4].

4.1.4. Sensory analysis

A trained sensory panel was used to perform generic descriptive analysis on all the samples.

4.1.4.1. Recruitment and screening

Fifteen students who frequently consume beef and who complied with all the criteria set for potential panellists were selected for screening. The candidates took part in four screening sessions. During the screening a total of 10 triangle tests, each consisting of three triangles, were performed (Table I). The treated small muscles *(M. semitendinosus)* were used for screening, and samples were served cold.

TABLE I. TRIANGLE TESTS

Test	Characteristic tested for
1	Differences between irradiated and non-irradiated meat
2	Differences between meat treated with and without salt
3–4	Differences between races
5–7	Differences between meat treated with the different levels of tripolyphosphate
8–10	Differences between meat treated with the different levels of pyrophosphate

A ranking test with salt solutions (0%, 0.1%, 0.2%, 0.5% and 0.7% NaCl in tap water) was also performed. The best 12 candidates were selected to continue with training.

One of the panellists did not complete the training, thus, only 11 panellists went on to the final evaluation.

4.1.4.2. Training

Six training sessions of approximately 2 h each were conducted (Table II). The training procedure was loosely based on the procedure described by Cross et al. [33]. The treated small muscles were used for training. Samples were served at 60°C.

4.1.4.3. Final evaluation

The large muscle (*M. biceps femoris*) samples were used for the final evaluation. Frozen samples were allowed to defrost overnight at 4°C. Visible fat was removed from samples slices before cutting the slices into squares of 1.5 cm × 1.5 cm. Five squares per sample were served to panellists in aluminium foil containers covered with aluminium foil. The samples were heated for 20 min in a preheated oven at 80°C to obtain a sample temperature of 60°C. Samples were served on a preheated sand box, to prevent the sample from cooling. All samples were labelled with random, three digit codes. A problem experienced with the samples was that, within a slice, there were visible differences between different parts of the slice. During training the panellists also commented on big differences within the same sample, e.g. one square of the sample may be rather dry while the next may be quite juicy.

The final evaluation was performed in individual sensory booths, under red lights to prevent colour differences from influencing panellists. Six 1 h sessions was needed for the evaluation. In each session panellists evaluated three sets of samples consisting of four samples each. Evaluation was done by scoring the samples on an 8-point scale for aroma, flavour and texture characteristics (Appendix A). Panellists were allowed a 10 min rest period between sets. Tap water (room temperature) and carrot slices were used to clean the palate before evaluating each sample. Samples from each treatment were evaluated in duplicate (each of the two muscles per treatment was evaluated once).

TABLE II. TRAINING SESSIONS

Session	Training
1	Familiarisation with plain cooked meat and irradiated cooked meat. Language and score sheet development. Identify references for defined flavour descriptors. Decide on best mouth cleanser.
2	Familiarisation with cooked and irradiated cooked meat treated with tripolyphosphate. Language and score sheet development. Identify references for defined flavour descriptors.
3	Familiarisation with cooked and irradiated cooked meat treated with pyrophosphate. Language and score sheet development. Finalise score sheet. Identify references for defined flavour descriptors.
4	Exposure to attribute extremes. Introduction to use of score sheet.
5	Use of score sheet. Evaluate selected samples individually on score sheet, discuss attribute scores in the group, re-evaluate samples.
6	Reproducibility test – the same 4 samples are evaluated in 3 test sessions (as in final evaluation session).

4.1.5. Statistical analysis

ANOVA and MANOVA analysis followed by Duncan's multiple range tests or least significant difference (LSD) tests where applicable were performed on all data generated. Values are considered statistically significantly different when $p<0.05$.

4.1.6. Experimental design

The summarised experimental design is shown on the previous page.

5. RESULTS

5.1. pH measurements

The effect of polyphosphate injection on the raw meat pH before cooking is given in Table III.

5.2. Drip loss

The influence of polyphosphate injection on roast beef drip loss is shown in Table IV. Drip loss was determined as the percentage (%) weight loss (water loss) of the roasts after cooking (oven baked).

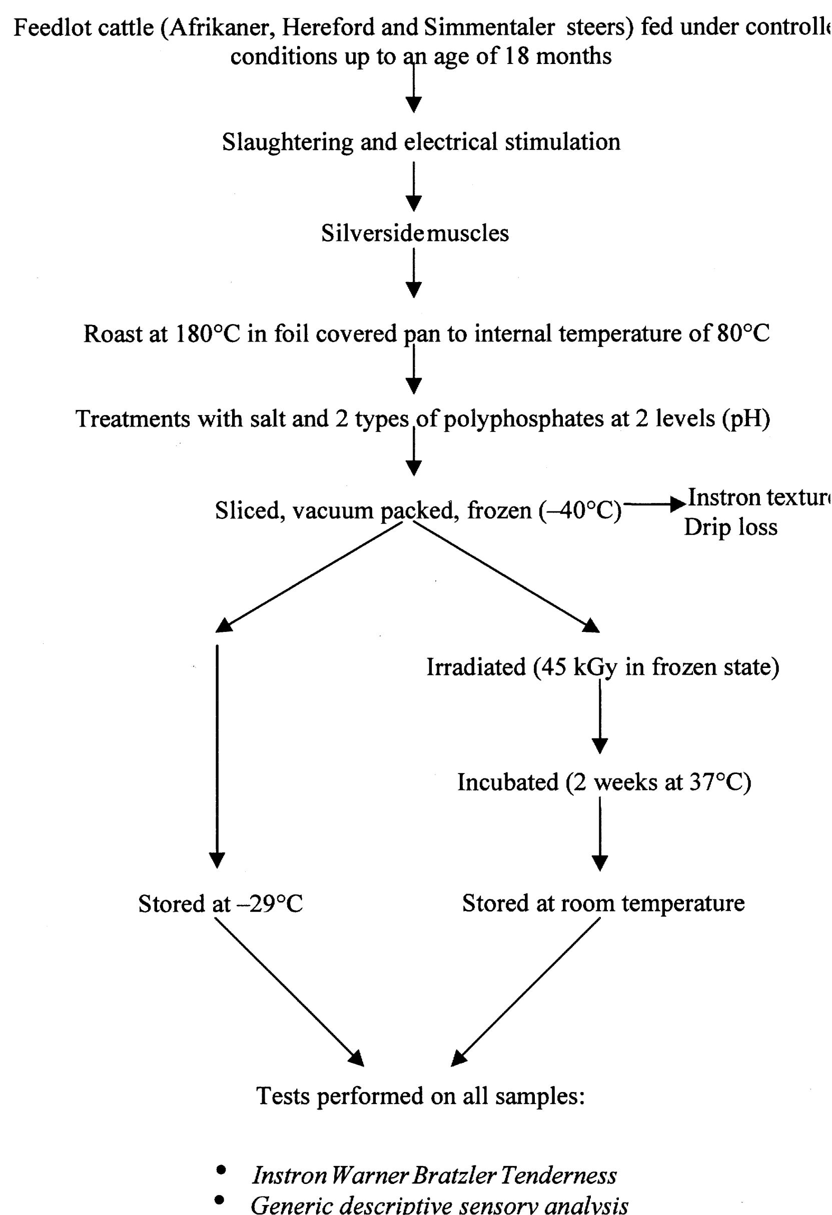

TABLE III. THE EFFECT OF INJECTING ROAST BEEF SILVERSIDE FROM THREE DIFFERENT CATTLE RACES WITH POLYPHOSPHATES ON THE pH OF THE RAW MEAT (LS MEANS OF pH MEASUREMENTS)

	T1*	T2	T3	T4	T5	T6
Afrikaner	6.03[b]**	6.2[b]	5.8[b]	6.1[b]	5.7[a]	5.6[a]
Hereford	6.05[b]	6.1[b]	5.9[b]	6.2[b]	5.6[a]	5.4[a]
Simmentaler	5.9[b]	5.9[b]	5.9[b]	5.9[b]	5.5[a]	5.6[a]

* T1 – T6 = Treatment 1–Treatment 6
**Values with different superscripts in rows and columns are statistically significantly different ($p<0.05$)

TABLE IV. THE EFFECT OF POLYPHOSPHATE INJECTION AND CATTLE RACE ON THE PERCENTAGE DRIP LOSS (LS MEANS) IN COOKED ROASTS (A CORE TEMPERATURE OF 80°C WAS MEASURED FOR EACH ROAST TO INDICATE THE END POINT OF COOKING)

	T1*	T2	T3	T4	T5	T6
Afrikaner	37.4^{a}**	36.7^{a}	38.6^{a}	34.4^{a}	43.7^{c}	37.9^{a}
Hereford	44.5^{c}	40.6^{ab}	38.1^{a}	38.7^{a}	44.6^{c}	39.4^{a}
Simmentaler	37.3^{a}	37.8^{a}	39.8^{a}	37^{a}	41.3^{b}	37.3^{a}

* T1–T6 = Treatment 1–Treatment 6
**Values with different superscripts in rows and columns are statistically significantly different ($p<0.05$)

5.3. Tenderness of the roasts using the Warner Bratzler cell

The influence of freezing and irradiation on meat tenderness is shown in Table V.

The influence of cattle race on the overall tenderness of the meat (all treatments combined) is shown in Table VI.

In Table VII the combined influence of polyphosphate injection on the tenderness of the meat (for all races combined) is shown.

Significant interactions occurred between Race and Polyphosphate injection for both maximum load and Energy to break point. Therefore, a detailed MANOVA analysis was also done for each processing condition.

The results of the effect of race and polyphosphate injection on tenderness in terms of maximum load (Newton) and Energy to break point (Joule) are given in Tables VIII and IX (samples directly after roasting before freezing), Tables X and XI (Samples frozen at –40°C) and the samples after irradiation at 45 kGy).

TABLE V. THE INFLUENCE OF FREEZING AND IRRADIATION ON MEAT TENDERNESS (COMBINED FOR ALL RACES OF CATTLE, LS MEANS OF MAXIMUM LOAD (NEWTON) AND ENERGY TO BREAK POINT (JOULE))

	Before freezing	After freezing	After Irradiation
Maximum Load (N)	92.6^{a}**	91.7^{a}	62.4^{b}
Energy to break Point (Joule)	2019^{a}	1398^{b}	987^{c}

**Values with different superscripts in rows are statistically significantly different ($p<0.05$).

TABLE VI. INFLUENCE OF CATTLE RACE ON THE OVERALL TENDERNESS OF BEEF ROAST (COMBINED RESULTS FOR IRRADIATED AND NON-IRRADIATED PRODUCTS)

	Afrikaner	Hereford	Simmentaler
Maximum Load (N)	76.9^{a}**	83.7^{b}	86.1^{b}
Energy to break Point (Joule)	1370^{a}	1470^{b}	1565^{c}

**Values with different superscripts in rows are statistically significantly different ($p<0.05$)

TABLE VII. INFLUENCE OF POLYPHOSPHATE INJECTION ON THE OVERALL TENDERNESS OF ROAST BEEF (COMBINED RACES)

	T1*	T2	T3	T4	T5	T6
Maximum Load (N)	76.5^{a}**	78.5^{a}	83.2^{a}	83.9	76.9^{a}	94.4^{b}
Energy to break Point (Joule)	1322^{b}	1429^{ab}	1486^{a}	1491^{a}	1433^{ab}	1647^{c}

*T1–T6 = Treatment 1–Treatment 6
**Values with different superscripts in rows and columns are statistically significantly different (p<0.05)

TABLE VIII. THE EFFECT OF CATTLE RACE AND POLYPHOSPHATE INJECTION ON THE MAXIMUM LOAD (NEWTON) OBTAINED WHEN TENDERNESS OF ROAST BEEF (DIRECTLY AFTER COOKING) IS MEASURED USING A WARNER BRATZLER CELL

	T1*	T2	T3	T4	T5	T6
Afrikaner	82^{ab}**	94^{ab}	71^{a}	104^{b}	71	104^{a}
Hereford	76^{a}	84^{a}	96^{ab}	82^{a}	106^{b}	95^{ab}
Simmentaler	91^{ab}	101^{b}	113^{b}	96^{ab}	96^{ab}	102^{b}

*T1–T6 = Treatment 1–Treatment 6
**Values with different superscripts in rows and columns are statistically significantly different (p<0.05)

TABLE IX. THE EFFECT OF CATTLE RACE AND POLYPHOSPHATE INJECTION ON THE ENERGY AT BREAK POINT (JOULE) OBTAINED WHEN TENDERNESS OF ROAST BEEF (DIRECTLY AFTER COOKING) IS MEASURED USING A WARNER BRATZLER CELL

	T1*	T2	T3	T4	T5	T6
Afrikaner	1766^{ab}**	2039^{b}	1562^{a}	2232^{b}	1559^{a}	2193^{b}
Hereford	1543^{a}	1872^{ab}	2075^{b}	1736^{ab}	2219^{bc}	2072^{ab}
Simmentaler	2076^{b}	2206^{b}	2533^{bc}	2059^{b}	2154^{b}	2453^{bc}

*T1–T6 = Treatment 1–Treatment 6
**Values with different superscripts in rows and columns are statistically significantly different (p<0.05)

TABLE X. THE EFFECT OF CATTLE RACE AND POLYPHOSPHATE INJECTION ON THE MAXIMUM LOAD (NEWTON) OBTAINED WHEN TENDERNESS OF ROAST BEEF (AFTER FREEZING AT –40°C) IS MEASURED USING A WARNER BRATZLER CELL

	T1*	T2	T3	T4	T5	T6
Afrikaner	85^{a}**	83^{a}	82^{a}	84^{a}	82^{a}	105^{a}
Hereford	96^{a}	78^{a}	81^{a}	115^{b}	96^{a}	109^{b}
Simmentaler	79^{a}	84^{a}	115^{b}	95^{a}	73^{ac}	108^{b}

*T1–T6 = Treatment 1–Treatment 6
**Values with different superscripts in rows and columns are statistically significantly different (p<0.05)

TABLE XI. THE EFFECT OF CATTLE RACE AND POLYPHOSPHATE INJECTION ON THE ENERGY AT BREAK POINT (JOULE) OBTAINED WHEN TENDERNESS OF ROAST BEEF (AFTER FREEZING AT –40°C) IS MEASURED USING A WARNER BRATZLER CELL

	T1*	T2	T3	T4	T5	T6
Afrikaner	1152^{a}**	1376^{a}	1272^{a}	1329^{a}	1357^{a}	1530^{a}
Hereford	1202^{a}	1229^{a}	1208^{a}	1759^{b}	1536^{a}	1668^{ab}
Simmentaler	1253^{a}	1249^{a}	1624^{b}	1503^{a}	1288^{a}	1631^{ab}

*T1–T6 = Treatment 1–Treatment 6
**Values with different superscripts in rows and columns are statistically significantly different (p<0.05)

TABLE XII. THE EFFECT OF CATTLE RACE AND POLYPHOSPHATE INJECTION ON THE MAXIMUM LOAD (NEWTON) OBTAINED WHEN TENDERNESS OF ROAST BEEF (AFTER FREEZING AT –40°C AND IRRADIATION AT 45 kGy) IS MEASURED USING A WARNER BRATZLER CELL

	T1*	T2	T3	T4	T5	T6
Afrikaner	60^{a}**	61^{a}	53 ^{a}	52^{a}	39^{b}	73^{a}
Hereford	63^{a}	55^{a}	69^{a}	58^{a}	71^{a}	75^{a}
Simmentaler	56^{a}	66^{a}	69^{a}	56^{a}	66^{a}	69^{a}

*T1–T6 = Treatment 1–Treatment 6
**Values with different superscripts in rows and columns are statistically significantly different (p<0.05)

TABLE XIII. THE EFFECT OF CATTLE RACE AND POLYPHOSPHATE INJECTION ON THE ENERGY AT BREAK POINT (JOULE) OBTAINED WHEN TENDERNESS OF ROAST BEEF (AFTER FREEZING AT –40°C AND IRRADIATION AT 45 kGy) IS MEASURED USING A WARNER BRATZLER CELL

	T1*	T2	T3	T4	T5	T6
Afrikaner	1015^{a}**	1010^{a}	886^{ab}	811^{ab}	673^{a}	894^{ab}
Hereford	1000^{ab}	889^{a}	1103^{a}	955^{a}	1155^{a}	1220^{c}
Simmentaler	889^{a}	991^{a}	1114^{b}	1037^{a}	958^{a}	1159^{a}

*T1–T6 = Treatment 1–Treatment 6
**Values with different superscripts in rows and columns are statistically significantly different (p<0.05)

5.4. Descriptive sensory analysis

5.4.1. General ANOVA results (all effects)

5.4.1.1. Differences between duplicates

The only significant difference between the duplicates was for initial juiciness.

5.4.1.2. Breed differences

The breeds differed significantly (p<0.05) in terms of initial juiciness, sustained juiciness and ease of fragmentation (in mouth).

5.4.1.3. Differences between the six basic treatments

The treatments differed significantly ($p<0.05$) in terms of initial juiciness, sustained juiciness and ease of fragmentation (in mouth).

5.4.1.4. Differences between frozen and irradiated samples

The samples differed significantly ($p<0.05$) in terms of roast beef aroma, wet dog aroma, tenderness, juiciness before swallowing, roast beef flavour, wet dog flavour and ease of fragmentation (using fingers).

5.4.2. Sensory analysis of individual products after freezing – MANOVA results for race and treatment (injection with polyphosphates) effects

This discussion refers also to Appendix A for an explanation of each attribute measured. In all referred significant differences, a sample was regarded as significantly different when $p<0.05$ using Duncan's multiple range test with Race and Treatment as the two independent variables. LS means were used for the comparisons. The 6 treatments are those described in Section 4. The summarised results are given in Table XIV.

5.4.3. Sensory analysis of individual products after freezing and irradiation – MANOVA results for race and treatment (injection with polyphosphates) effects

Table XV shows the summarised results for the sensory analysis of the individual products after freezing and treatment with ionizing radiation.

6. DISCUSSION

6.1. pH measurements

No significant differences were found between the pH values of the three control samples (Treatment 6) of the three breeds of cattle. The control pH readings of the raw meat were close to the isoelectric point of beef according to literature [32,33,35]. Pumping with NaCl alone did not change the pH of any race significantly and no significant differences occurred when compared to the untreated control pH. Injection of the muscles with polyphosphates resulted in a significantly increased pH of all samples for all three cattle races. However, the changes only showed minor differences (Treatments 2 and 4 gave slightly higher pH readings due to the higher phosphate concentration), but not to such an extent that statistically significant differences were obtained. All injected samples had statistically significantly higher pH values than the control samples.

6.2. Drip loss

The drip loss (Table IV) was the same for all the control samples (Treatment 6) and the three cattle races showed no significant differences in drip loss of the untreated roasts. The injection of NaCl alone (Treatment 5) significantly increased drip loss in all three races. Treatments with the polyphosphates reduced drip loss in the Afrikaner and Simmentaler races and restored drip loss to the levels of the untreated roasts. The Hereford roasts injected with sodium tripolyphosphate did not show a significant reduction in drip loss which was similar to the salt only injected roasts. Drip loss was reduced, however, when tetrasodium pyrophosphate was used (Treatments 3 and 4). From these results it became apparent that cattle race did have an influence on drip loss (the pH levels of all roasts did not differ significantly from each other) and the most likely explanation is that differences on muscle tissue level may be responsible for the high drip loss in the Hereford roasts. Drip loss is significantly increased by the injection of NaCl and it seems as if the function of the polyphosphates was mainly to restore drip loss to the minimum level, which is the level found in the untreated samples. If drip loss less that the untreated samples needs to be achieved, NaCl cannot be pumped into the roasts. This will, however, make the product unpalatable to most consumers.

TABLE XIV. SENSORY ANALYSIS OF INDIVIDUAL PRODUCTS AFTER FREEZING

Sensory attribute	Significant differences for Race (Yes/No and description)	Significant differences for Treatment (Yes/No and description)
1. Roast beef aroma	No	Yes, Aroma of T2 and T4 reduced.
2. Wet dog aroma	No	No
3. Other aroma	Yes, Hereford meat had a strong aroma after T1, T2 and T3, Afrikaner meat had a strong aroma after T4.	Yes, strong aroma after T2 and T4 for all races.
4. Tenderness	Yes, Afrikaner meat most tender, Hereford the toughest.	Yes, T6 less tender than all others, T2 and T4 were the most tender. No significant differences between T5 and T1 – T4.
5. Initial juiciness	Yes, Afrikaner meat most juicy.	Yes, T6 – most dry. No difference between T5 and T1–T4.
6. Sustained juiciness	Similar to 5.	Similar to 5.
7. Juiciness before swallowing	No	T2 – more juicy, T6 – driest.
8. Ease of fragmentation (mouth)	No	T5 – more soft than T6, T1–T4 softer than T5. No differences among T1–T4.
9. Roast beef flavour	No	T2 and T4 – significantly reduced flavour.
10. Wet dog flavour	No	No
11. Other flavour	No	T2 and T4 – developed onion-like or roast chicken-like flavour.
12. Ease of fragmentation (fingers)	No	No

*T1–T6 = Treatment 1–Treatment 6

TABLE XV. SENSORY ANALYSIS OF INDIVIDUAL PRODUCTS AFTER FREEZING AND IRRADIATION

Sensory attribute	Significant differences for Race (Yes/No and description)	Significant differences for Treatment (Yes/No and description)
1. Roast beef aroma	No	T6 – most intense. T1 – T5 – more bland than frozen products.
2. Wet dog aroma	Aroma was detected, but with no differences between races.	Detected, but no differences between treatments.
3. Other aroma	No, all products were very bland.	No, all products very bland.
4. Tenderness	Yes, Afrikaner – most tender (7.3), Hereford – intermediate (7.0), Simmentaler – toughest (6.9). All products significantly more tender after irradiation.	T5 and T6 – less tender than T1 – T4, no differences among T1–T4.
5. Initial juiciness	Yes, Afrikaner more juicy than others.	T1–T4 – more juicy than T5 and T6. Similar to frozen* samples.
6. Sustained juiciness	Similar to 5.	Similar to 5.
7. Juiciness before swallowing	Yes, Afrikaner more juicy than others.	T1–4 – more juicy than T5 and T6. Irradiated samples similar to frozen products.
8. Ease of fragmentation (mouth)	No	T1–T4 more crumbly than T5 and T6. All irradiated samples – easier to fragment than frozen samples.
9. Roast beef flavour	No	No – all irradiated products less flavour than frozen products.
10. Wet dog flavour	Detected in all, but no differences	Detected, but no difference.
11. Other flavour	No, none found	No, none found.
12. Ease of fragmentation (fingers)	Afrikaner – more fragmented than Hereford, Hereford more than Simmentaler	Irradiated products fragmented easier than frozen products, T1–T4 – more fragmented than T5 and T6. T4 had highest score for ease of fragmentation.

T1–T6 = Treatment 1–Treatment 6

*Frozen–referring to the products in Table XIV

6.3. Tenderness of the roasts using the Warner Bratzler Cell

The combined ANOVA analysis (Table V) showed that both Maximum Load and Energy to break point showed that the beef roasts of all cattle races were significantly more tender after irradiation. Energy to break point also showed a significant decrease after freezing. The Energy to break point reading is more sensitive than the maximum load measurement. This result correlated with the general ANOVA results for sensory evaluation in terms of tenderness and ease of fragmentation (using fingers). The effect of cattle race (Table VI) was significant for all treatments. Again the Energy to break point showed more sensitivity towards tenderness differences than the Maximum Load. This observation correlated with the ease of fragmentation (in the mouth) obtained in the sensory evaluation. The effect of phosphate injection on overall quality is given in Table VII. The control product (T6) with no injection was significantly tougher than all other treatments. The products that were injected with salt only (T5) and no phosphates, were significantly more tender than the untreated samples. They were, however, not significantly less tender than the products containing polyphosphates. Again, the Energy to break point measurements were more sensitive. Separate ANOVA analysis of the treatments for each preparation step (after cooking, after freezing and after irradiation) gave the following results (See Tables VIII–XIII):

- Afrikaner meat was the most tender after cooking, but not significantly different in terms of Maximum Load. The injecting of polyphosphates improved tenderness slightly, but so did the injection with salt only (T5).
- After freezing, significant differences were found between injected samples and the control (T6, not injected). Again the NaCl only injection improved the texture to the same extent as the phosphates.
- After irradiation all products were significantly more tender. Very few significant differences occurred. The Energy at break point showed a significantly tougher control product (T6) for Hereford meat, but not for Afrikaner and Simmentaler meat.
- Before irradiation and after freezing, Afrikaner meat was the most tender. However, after irradiation the opposite scenario occurred. Afrikaner meat was the toughest and Simmentaler was the most tender, but the differences were not significant.

6.4. Descriptive sensory analysis

Referring to the summary of the results in Tables XIV and XV, the following can be highlighted:

- The product samples were homogenous including the repetition of the sensory evaluation – this indicated a well-trained panel.
- There were significant differences between roasts of the different breeds, especially with regards to tenderness. In some cases significant interactions were found between race and injection solution. Race also had an influence on initial juiciness and sustained juiciness. Afrikaner meat was the most juicy in the frozen-only as well as the irradiated products. Race had a significant influence on the ease of fragmentation with the fingers. These results correlated with the Instron Energy to break point measurements. A higher ease of fragmentation with the fingers also correlated with higher scores for juiciness. The Instron Energy to Break point values can, therefore, also be used as an indication of juiciness.
- Wet dog flavour did occur after irradiation. This was very mild and it occurred along with other flavours described as “fruity” or like “roast chicken”. It is suspected that these flavours are actually Warmed Over Flavour (WOF).

– Irradiation caused a significant reduction in flavour intensity in terms of other flavours as well as roast beef flavour and roast beef aroma. Unacceptable Flavours were observed in the polyphosphate injected products T2 and T4. These products contained higher concentrations of polyphosphates than T1 and T3 and this indicated that the levels were too high. This is further supported by the fact that no significant differences in terms of tenderness could be obtained for treatments T1, T2, T3 and T4 after irradiation – indicating that the polyphosphate levels 1 and 3 were sufficient. Treatments 2 and 4 were, however, significantly more tender after freezing. This trend was also observed with the Instron measurements. The control samples (T6) were significantly less tender than the others. No experiment showed any significant difference between sodium tripolyphosphate and tetrasodium pyrophosphate in terms of tenderness.

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Appendix A: Score Sheet

Name: __________________________
Session: _____/_____
Sample code: ______

Aroma intensity

Roast beef aroma
8 Extremely intense
7 Very intense
6 Moderately intense
5 Slightly intense
4 Slightly bland
3 Moderately bland
2 Very bland
1 Extremely bland

Wet dog aroma
8 Extremely intense
7 Very intense
6 Moderately intense
5 Slightly intense
4 Slightly bland
3 Moderately bland
2 Very bland
1 Extremely bland

Other aroma (define):

8 Extremely intense
7 Very intense
6 Moderately intense
5 Slightly intense
4 Slightly bland
3 Moderately bland
2 Very bland
1 Extremely bland

Texture I

Tenderness
8 Extremely tender
7 Very tender
6 Moderately tender
5 Slightly tender
4 Slightly tough
3 Moderately tough
2 Very tough
1 Extremely tough

Texture I (continued)

Initial juiciness
8 Extremely juicy
7 Very juicy
6 Moderately juicy
5 Slightly juicy
4 Slightly dry
3 Moderately dry
2 Very dry
1 Extremely dry

Sustained juiciness
8 Extremely juicy
7 Very juicy
6 Moderately juicy
5 Slightly juicy
4 Slightly dry
3 Moderately dry
2 Very dry
1 Extremely dry

Ease of fragmentation (in mouth)
8 Extremely crumbly
7 Very crumbly
6 Moderately crumbly
5 Slightly crumbly
4 Slightly cohesive
3 Moderately cohesive
2 Very cohesive
1 Extremely cohesive

Juiciness Before Swallowing

8 Extremely juicy
7 Very juicy
6 Moderately juicy
5 Slightly juicy
4 Slightly dry
3 Moderately dry
2 Very dry
1 Extremely dry

Flavour intensity

Roast beef flavour
8 Extremely intense
7 Very intense
6 Moderately intense
5 Slightly intense
4 Slightly bland
3 Moderately bland
2 Very bland
1 Extremely bland

Wet dog flavour
8 Extremely intense
7 Very intense
6 Moderately intense
5 Slightly intense
4 Slightly bland
3 Moderately bland
2 Very bland
1 Extremely bland

Other flavour (define):

8 Extremely intense
7 Very intense
6 Moderately intense
5 Slightly intense
4 Slightly bland
3 Moderately bland
2 Very bland
1 Extremely bland

Texture II

Ease of fragmentation (using fingers)
8 Extremely crumbly
7 Very crumbly
6 Moderately crumbly
5 Slightly crumbly
4 Slightly cohesive
3 Moderately cohesive
2 Very cohesive
1 Extremely cohesive

DEFINITIONS

1. Aroma intensity

- **Roast beef aroma**: The aroma associated with roast beef
- **Wet dog aroma**: The aroma associated with a wet dog or with boiled goat meat

2. Texture

Texture I:

- **Tenderness**: Indication of force required to chew the meat
- **Initial juiciness**: Impression of moisture in mouth after 3 chews
- **Sustained juiciness**: Impression of moisture in mouth after 10 chews
- **Ease of fragmentation (in mouth)**: Indication of how easily the meat fragments in the mouth
- **Juiciness before swallowing**: Impression of moisture in mouth just before the sample is swallowed

Texture II:

- **Ease of fragmentation (using fingers)**: Indication of how easily the meat fragments when pulled apart using fingers

3. Flavour intensity

- **Roast beef flavour**: The flavour associated with roast beef
- **Wet dog flavour**: The flavour associated with the smell of a wet dog or the aroma of boiled goat meat

THE ROLE OF IRRADIATION ON MICROBIOLOGICAL SAFETY AND SHELF-LIFE EXTENSION OF NON-STERILE AND STERILE CONVENIENCE MEAT PRODUCTS STORED AT AMBIENT TEMPERATURES

R. CHANDER, S.P. CHAWLA, S.R. KANATT
Food Technology Division,
Bhabha Atomic Research Centre,
Trombay, Mumbai, India

Abstract

The effect of gamma irradiation in combination with vacuum packaging on the microbiological and sensory quality of Intermediate Moisture (IM) buffalo, chicken or lamb meat cubes, and number of ready-to-cook ethnic meat products stored at ambient temperature (30 ± 2°C) was studied. Irradiation treatment (2.5, 5 and 10 kGy) resulted in a dose-dependent reduction in the total viable count as well as levels of potentially pathogenic *Staphylococcus* spp. and these samples remained microbiologically superior to their non-irradiated counterparts upon extended storage. Irradiation (10 kGy) treatment of vacuum packed IM spiced lamb and chicken cubes resulted in a product in which no viable microorganisms could be detected for 12 months of storage at ambient temperature (30 ± 2°C). Initial analysis of non-irradiated samples showed total viable counts of $>10^6$ CFU/g, *Staphylococcus* counts of $>10^5$ CFU/g and mould counts of 10^3 CFU/g which upon 3 months of storage under similar conditions spoiled showing visible mould growth. Ready-to-use shelf-stable mutton and chicken sheek kababs, mutton and chicken chilli were developed by reducing the water activity either by grilling or by hot air-drying, vacuum packing and irradiation. Microbiological analysis revealed a dose-dependent reduction in the total viable count and in potentially pathogenic *Staphylococcus* spp. upon irradiation treatment (2.5, 5 and 10 kGy). The products subjected to irradiation at 10 kGy showed an absence of viable microorganisms and also had high sensory acceptability up to 9 months at ambient temperature. Amenability of various ready-to-cook and ready-to-eat chicken, mutton and pork products for radappertization using locally available packaging material was evaluated. The sensory quality of all products without gravy was found to be acceptable. However, traditional gravy based products showed changes in the sensory attributes (colour and flavour) leading to lower acceptability of the products.

1. INTRODUCTION

With rapid urbanisation and change in socio-economic status there is an increase in demand for convenience/ready-to-cook/ready-to-eat meat products. A variety of such products such as kababs, tikkas, lollipops, fingers, patties, sausages prepared from mutton, chicken, pork and beef are available in Indian urban markets. Presently, these products are marketed in the frozen state as they have a limited shelf-life at ambient temperature [1]. At present there is only a very small market for these products because of the lack of infrastructure. In a developing country such as India where freezing facilities are inadequate and there is an ever increasing energy crisis, energy intensive food preservation procedures are not affordable. Furthermore, freezing does not eliminate pathogens which thus pose a health hazard [2]. These foods could therefore lead to microbiological risks. Thus, there is a need to develop a technology that can give shelf-stable and microbiologically safe products that will be of great economic and health significance. Development of such a technology will give a boost to the demand for and enhance the distribution of such products to target populations such as defence personnel and consumers at remote places.

Microorganisms play a very important role in determining the shelf-life and safety of these food products. Irradiation treatment of meat and meat products can improve shelf-life and microbiological safety by destruction of spoilage and pathogenic microorganisms and parasites [3].

Investigations were carried out to prepare shelf-stable and microbiologically safe ready-to-serve meat products using a combination of various hurdles such as reduced water activity (a_w), vacuum packaging and gamma irradiation. Intermediate moisture (IM) products in which a_w is reduced by partial dehydration and binding the remaining water, to inhibit growth of microorganisms (bacteria and fungi) can be stored for extended duration under ambient conditions [4]. Shelf-stable meat products that can be stored without refrigeration and require minimal preparation and cooking prior to

consumption are of particular interest to India in view of the inadequate infrastructure of cold-chain and freezing facilities, besides the demand for such convenience products by urban consumers.

Shelf-stable food products with high moisture and fresh-like taste can also be prepared through use of high sterilising doses of gamma irradiation at cryogenic temperatures [5]. However, all food commodities are not amenable to this process because of sensory attributes. Therefore, ethnic meat preparations such as chicken chilli, chicken tikka, chicken kofta, mutton chilli etc. were examined for their amenability to high sterilising doses of gamma irradiation.

2. MATERIALS AND METHODS

IM meat products developed included meat cubes (buffalo, chicken and lamb), spiced meat cubes (chicken and lamb), and convenient ready-to-eat meat products (mutton and chicken sheek kababs, mutton and chicken chilli).

Radappertized meat products evaluated were chicken (chilli, tikka, kofta, lollipop, loaf, sausage, samosa, salami, burger, kabab), mutton (chilli, kabab, samosa), and pork (sausages, salami, luncheon, frankfurter, burgers).

2.1. Preparation of IM meat cubes

Fresh meat (buffalo, chicken and lamb) was procured from a local abattoir, washed with chilled water and the visible fat and ligaments removed. Meat cubes, of about 1 inch in size, were prepared and cooked with infusion broth containing humectants. After cooking, the meat cubes were dehydrated in a hot air drier (60°C). The partially dehydrated product was then vacuum packed in flexible pouches (metalized polyester/polyethylene) which were randomly divided into different lots and subjected to gamma irradiation at doses of 2.5, 5 and 10 kGy. A non-irradiated batch served as controls. The samples were stored at ambient temperature (30 ± 2°C).

Studies were also carried out to develop IM spiced meat cubes. Prior to dehydration the meat cubes were marinated for 18 h at 0–3°C with a marinade consisting of lime juice, ginger-garlic paste, cornflour and a spice mixture and further processed as described above.

2.2. Preparation of shelf-stable mutton sheek kababs

Ready-to-serve IM mutton and chicken sheek kababs, mutton and chicken chilli were prepared by reducing a_w to <0.85 either by grilling for 30 min at 250°C or by hot (60°C) air-drying. After cooling, the product was vacuum packed in multilayered pouches (12μ polyester/12μ metalized polyester/40μ polyethylene) and were subjected to gamma irradiation at doses of 2.5, 5 and 10 kGy in a Package Irradiator. A non-irradiated batch served as controls. Samples were stored at ambient temperature (30 ± 2°C).

2.3. Preparation of radiation sterilized products

Convenience meat products prepared from pork, lamb and chicken meat were procured from a local supplier and steamed for 5–10 min to inactivate the enzymes. After cooling, products were vacuum packed in multilayered flexible pouches, as described in Section 2.2., and frozen in a contact plate freezer. Frozen pouches were transferred into insulated irradiation boxes containing dry ice (solid CO_2) and then irradiated to a minimum absorbed dose of 45 kGy.

2.4. Irradiation

Irradiation treatment of all samples was carried out in a Package Irradiator (Nordion International Inc., Canada) using a ^{60}Co source.

2.5. Analyses

Product samples from both irradiated and non-irradiated lots were examined for quality, immediately after irradiation and subsequently at regular intervals during storage at ambient temperature.

2.5.1. Water activity (a_w) and moisture content measurements

The a_w of the samples was measured using a a_w meter (Acqua Lab). Moisture content was determined by desiccation of the samples to constant weight at 100°C (ca. 18 h).

2.5.2. Microbiological analyses

Samples were analysed for microbiological quality at regular intervals of 2 or 4 weeks. Duplicate samples (10 g) from irradiated and non-irradiated batches were aseptically homogenised for 1 min in a sterile stomacher bag containing 90 mL of sterile saline using a Stomacher 400 Lab Blender (Seward Medical, UK). Appropriate serial dilutions of the homogenate were placed into sterile petri plates in triplicate. Media employed were Plate Count Agar, Baird-Parker Agar, Violet Red Bile Agar, Sulphite Polymyxin Sulphadiazine Agar and Potato Dextrose Agar for determination of total viable counts (TVCs), *Staphylococcus* spp., coliforms, sulphite-reducing *Clostridia* and mould counts, respectively.

2.5.3. Sensory analyses

Sensory evaluation of irradiated and non-irradiated samples was performed by a panel of 6–8 staff members. Panellists were asked to rate samples as "acceptable" or "non-acceptable" on the basis of appearance, odour, flavour and taste (optional).

Radiation sterilized products were stored for a minimum period of two weeks and examined for bulging of the pouches before sensory evaluation.

Sensory attributes such as appearance, flavour, texture and overall acceptability were also assessed on the basis of a 10-point hedonic scale, where 10 corresponded to a product of highest quality and 0 corresponded to a poor quality product. Scores of 6 and above were considered to be acceptable.

Samples were presented to panellists after being subjected to cooking.

3. RESULTS

3.1. IM meat cubes

The a_w of meat cubes prepared by cooking in humectants followed by partial dehydration was 0.85 ± 0.5. Results of microbiological analysis are shown in Table I. The microbiological quality of the product varied from batch to batch and this could be attributed to differences in animal hygiene and extent of handling. Non-irradiated samples had TVCs in the range 10^4–10^6 CFU/g and *Staphylococcus* spp. in the range 10^3–10^5 CFU/g. The immediate effect of irradiation treatment on microbiological quality was a dose-dependent reduction in the TVC as well as in the levels of potentially pathogenic *Staphylococcus* spp. Irradiated samples were microbiologically superior to their non-irradiated counterparts throughout the storage period. The sensory attributes of IM meat cubes were not significantly affected by irradiation treatment. A slight irradiation odour was detected in samples irradiated above 5 kGy which was found to decrease upon storage and also when samples were rehydrated and boiled.

3.2. IM spiced meat cubes

Results of microbiological analyses of IM spiced meat cubes are shown in Table II. Non-irradiated samples showed high bacterial loads (>10^6 CFU/g), high levels of *Staphylococcus* spp. (>10^5 CFU/g) and moulds (10^3 CFU/g). IM spiced meat cubes (lamb and chicken) irradiated at 10 kGy did not show microbial growth in any of the media employed for analysis suggesting the absence of viable microorganisms. After storage at ambient temperature, non-irradiated samples had spoiled within 2–3 months showing visual mould growth whereas irradiated samples were microbiologically safe for 9 months of storage at ambient temperature.

Sensory analysis of spiced IM chicken and lamb meat suggested that marinating with spices resulted in a product with better flavour.

Irradiation treatment of both plain and spiced IM meat cubes improved the colour of the product and this effect was more predominant in chicken followed by lamb meat and least in buffalo meat. However, the texture of these meat cubes was slightly tough which improved substantially when convenient ready-to-eat meat products were prepared.

3.3. IM mutton sheek kababs

Fresh products had a water content in range of 62–65% (Fig. 1) and a_w of 0.97 ± 0.01 (Fig. 2). Grilling treatment reduced the moisture content to 27–32% and a_w to 0.80 ± 0.05. The a_w and moisture content remained constant throughout the storage period (Figs 1 and 2). Fresh mutton sheek kababs had TVCs in the range 10^4–10^5 CFU/g and *Staphylococcus* spp. in the range 10^2–10^4 CFU/g (Table III). One of the batches of fresh kababs tested positive for sulphite-reducing clostridia. After grilling, samples had TVCs in the range 10^2–10^4 CFU/g and *Staphylococcus* spp. in the range 10^1–10^2 CFU/g. Thus grilling resulted in at least a 1 log cycle reduction in bacterial numbers. The immediate effect of irradiation treatment on microbiological quality was a dose-dependent reduction in the TVC as well as levels of potentially pathogenic *Staphylococcus* spp. Irradiated samples were microbiologically superior to their non-irradiated counterparts throughout the storage period. Analysis of vacuum packed ready-to-eat grilled kababs subjected to irradiation treatment (10 kGy) suggested that the product was devoid of viable microorganisms and had a high sensory acceptability for several months of storage at ambient temperature (Fig. 3).

3.4. IM chicken sheek kababs

The moisture content and a_w of fresh chicken sheek kababs were found to be 65–70% and 0.97 ± 0.01, respectively (Figs 4 and 5). On drying, the moisture content was reduced to 15–18% while a_w was 0.65 ± 0.05 and these values did not change significantly during storage. Microbiological analysis revealed that the fresh product had a TVC of 10^3–10^5 CFU/g and *Staphylococcus* spp. of 10^1–10^2 CFU/g) and on drying the initial TVC was 10^4–10^5 CFU/g and *Staphylococcus* spp. in the range 10^2–10^4 CFU/g (Table IV). These studies indicated that drying did not reduce the microbial load of the product as was observed with grilling. A dose-dependent reduction in TVCs as well as levels of potentially pathogenic *Staphylococcus* spp. was observed upon irradiation. Irradiated samples showed lower microbial counts as compared to non-irradiated kababs during storage. Chicken sheek kababs subjected to irradiation treatment (10 kGy) showed no viable microorganisms and had a high sensory acceptability following several months on storage at ambient temperature.

TABLE I. MICROBIOLOGICAL ANALYSES OF INTERMEDIATE MOISTURE (IM) MEAT CUBES

Treatment	Counts (CFU/g)	Storage period (months)					
		0	2	4	6	8	10
Non-irradiated	Total Viable Counts	10^4–10^6	10^5–10^7	10^6–10^8	10^7–10^8	10^7–10^8	NCO
	Staphylococcus spp.	10^3–10^5	10^4–10^5	10^5–10^6	10^5–10^6	10^6	NCO
	Coliforms	ND	ND	ND	ND	ND	NCO
	Sulphite-reducing clostridia	@ND	ND	ND	ND	ND	NCO
Irradiated (2.5 kGy)	Total Viable Counts	10^4–10^5	10^5–10^6	10^5–10^6	10^5–10^6	10^6	10^6
	Staphylococcus spp.	10^3–10^4	10^4–10^5	10^4–10^5	10^4–10^5	10^5	10^5
	Coliforms	ND	ND	ND	ND	ND	ND
	Sulphite-reducing clostridia	ND	ND	ND	ND	ND	ND
Irradiated (5 kGy)	Total Viable Counts	10^2–10^3	10^2–10^4	10^3–10^4	10^3–10^4	10^4–10^5	10^5
	Staphylococcus spp.	$<10^2$	10^2–10^3	10^2–10^3	10^3–10^4	10^4	10^4
	Coliforms	ND	ND	ND	ND	ND	ND
	Sulphite-reducing clostridia	ND	ND	ND	ND	ND	ND
Irradiated (10 kGy)	Total Viable Counts	ND	ND	ND	ND	ND	ND
	Staphylococcus spp.	ND	ND	ND	ND	ND	ND
	Coliforms	ND	ND	ND	ND	ND	ND
	Sulphite-reducing clostridia	ND	ND	ND	ND	ND	ND

Results of 2 batches each of IM of buffalo, chicken and lamb meat cubes
ND = Not detected by methods employed
NCO = Analyses not carried out
@ = Tested positive in one batch each of IM chicken and lamb meat.

TABLE II. MICROBIOLOGICAL ANALYSES OF SPICED INTERMEDIATE MOISTURE (IM) MEAT CUBES

Treatment	Counts (CFU/g)	Storage period (months)				
		0	3	6	9	12
Non-irradiated	Total Viable Counts	10^4–10^6	10^5–10^7	NCO	NCO	NCO
	Staphylococcus spp.	10^3–10^5	10^4–10^5	NCO	NCO	NCO
	Coliforms	ND	ND	NCO	NCO	NCO
	Moulds	10^4–10^5	V/NCO	NCO	NCO	NCO
	Sulphite-reducing clostridia	ND	ND	ND	ND	ND
Irradiated (10 kGy)	Total Viable Counts	ND	ND	ND	ND	ND
	Staphylococcus spp.	ND	ND	ND	ND	ND
	Coliforms	ND	ND	ND	ND	ND
	Moulds	ND	ND	ND	ND	ND
	Sulphite-reducing clostridia	ND	ND	ND	ND	ND

Results of 2 batches each of IM Spiced Chicken and Lamb meat cubes
ND = Not detected by methods employed
NCO = Analysis not carried out as sample was spoiled
V = Visual mould growth seen.

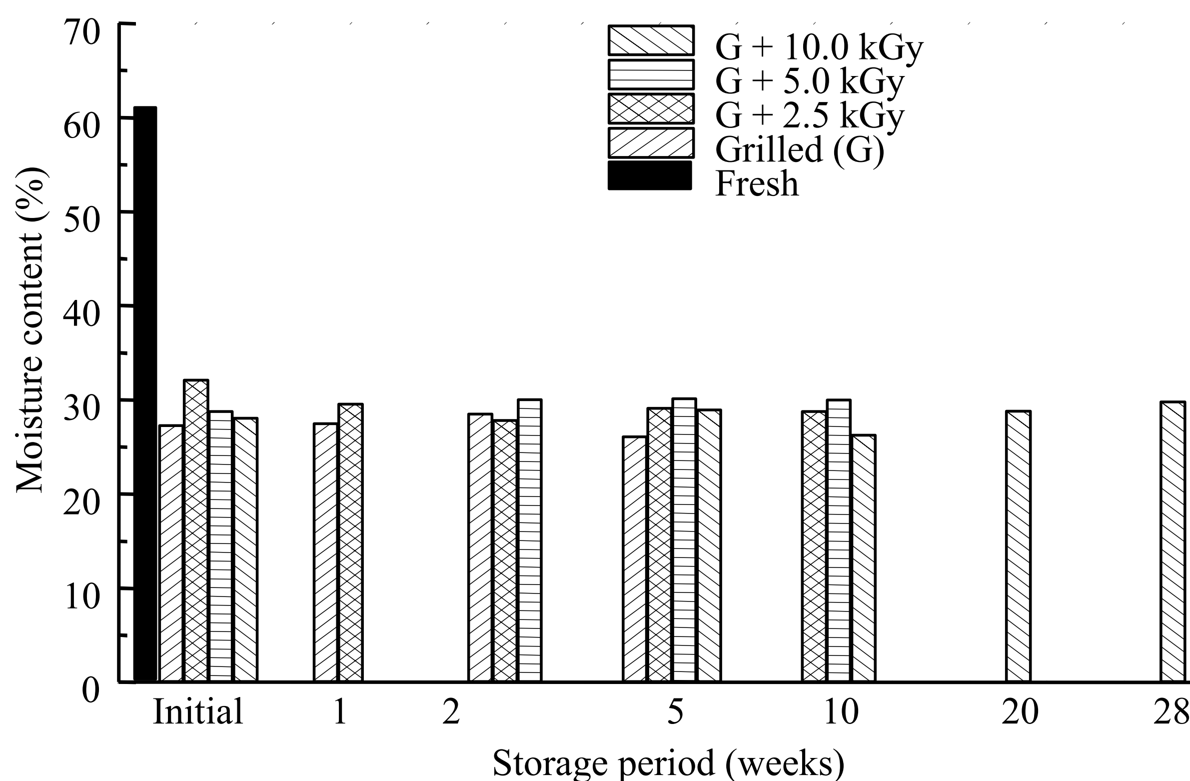

FIG. 1. Moisture content of mutton sheek kabab during storage at ambient temperature. Each data point represents mean moisture content of 6 samples.

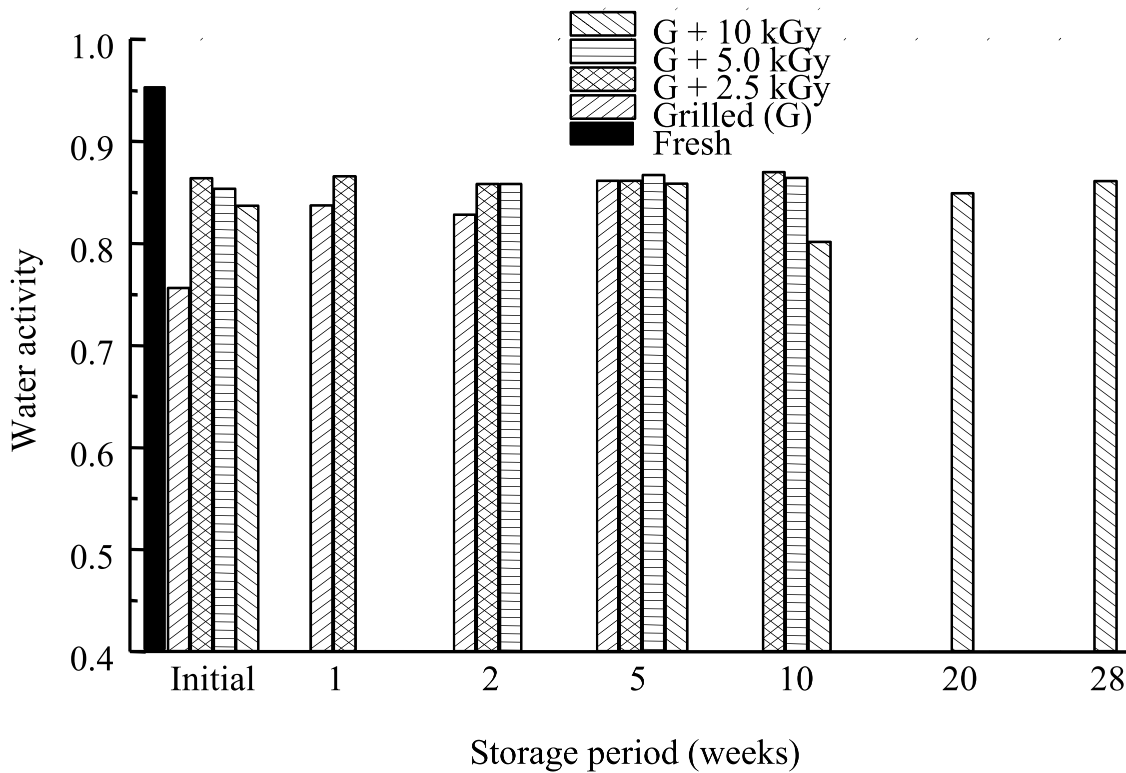

FIG. 2. Water activity (a_w) of mutton sheek kabab during storage at ambient temperature. Each data point represents mean a_w of 6 samples.

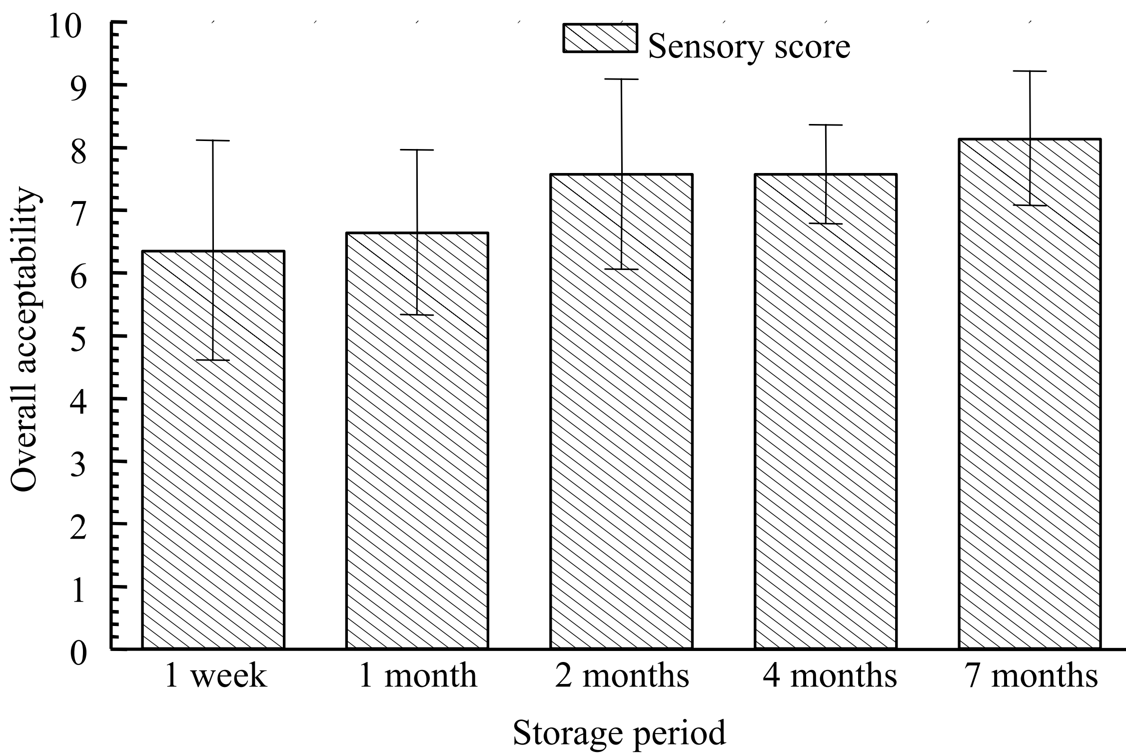

FIG. 3. Sensory evaluation of irradiated (10 kGy) IM Mutton sheek kabab during storage at ambient temperature..

TABLE III. MICROBIOLOGICAL ANALYSES OF INTERMEDIATE MOISTURE (IM) MUTTON SHEEK KABABS

Treatment	Counts (CFU/g)	Storage Period (weeks)				
		0	2	4	6	12
Fresh	Total Viable Counts	10^4–10^5	NCO	NCO	NCO	NCO
	Staphylococcus spp.	10^2–10^4	NCO	NCO	NCO	NCO
	Coliforms	ND	NCO	NCO	NCO	NCO
	Sulphite-reducing clostridia	@	NCO	NCO	NCO	NCO
Non-irradiated (Grilled)	Total Viable Counts	10^2–10^4	10^3–10^5	10^4–10^6	10^5–10^7	NCO
	Staphylococcus spp.	10^1–10^2	10^2–10^3	10^3–10^4	10^4–10^6	NCO
	Coliforms	ND	ND	ND	ND	NCO
	Sulphite-reducing clostridia	ND	ND	ND	ND	NCO
Irradiated (2.5 kGy)	Total Viable Counts	10^1–10^2	10^1–10^2	10^2–10^3	10^2–10^3	10^3
	Staphylococcus spp.	≥ 10	10^1–10^2	10^1–10^2	10^1–10^2	10^2
	Coliforms	ND	ND	ND	ND	ND
	Sulphite-reducing clostridia	ND	ND	ND	ND	ND
Irradiated (5 kGy)	Total Viable Counts	ND	ND	10^1–10^2	10^1–10^2	10^1–10^2
	Staphylococcus spp.	≤ 10	≤ 10	≤ 10	10^1–10^2	10^1–10^2
	Coliforms	ND	ND	ND	ND	ND
	Sulphite-reducing clostridia	ND	ND	ND	ND	ND
Irradiated (10 kGy)	Total Viable Counts	ND	ND	ND	ND	ND
	Staphylococcus spp.	ND	ND	ND	ND	ND
	Coliforms	ND	ND	ND	ND	ND
	Sulphite-reducing clostridia	ND	ND	ND	ND	ND

Results of 3 independent experiments on different batches of IM mutton sheek kababs; @ = Tested positive in 1 batch; ND = Not detected by methods employed; NCO = Analyses not carried out; 10 kGy irradiated did not show any viable organism up to 9 months of storage.

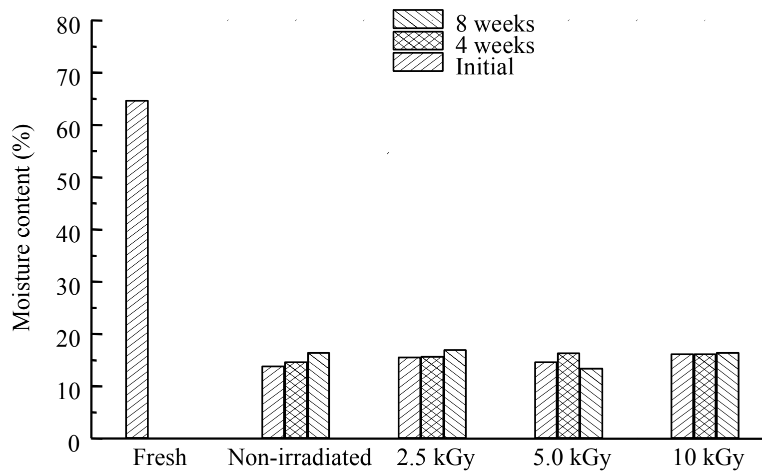

FIG. 4. Moisture content of chicken sheek kabab during storage at ambient temperature. Each data point represents the mean moisture content of 6 samples.

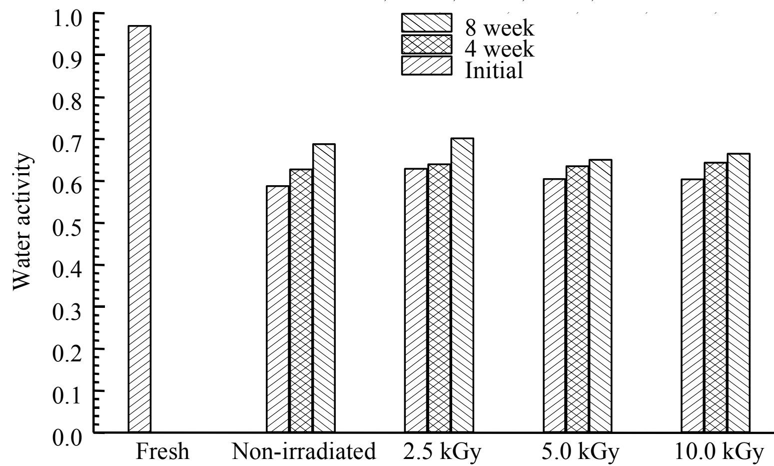

FIG. 5. Water activity (a_w) of chicken sheek kabab during storage at ambient temperature. Each data point represents the mean a_w of 6 samples.

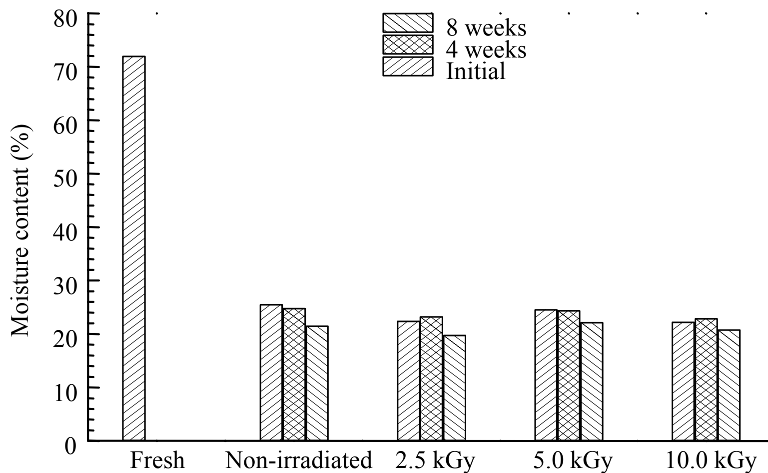

FIG. 6. Moisture content of chicken chilli during storage at ambient temperature. Each data point represents the mean moisture content of 6 samples.

TABLE IV. MICROBIOLOGICAL ANALYSES OF INTERMEDIATE MOISTURE (IM) CHICKEN SHEEK KABABS

Treatment	Counts (CFU/g)	Storage period (weeks)		
		0	4	8
Fresh	Total Viable Counts	10^3–10^5	NCO	NCO
	Staphylococcus spp.	10^1–10^2	NCO	NCO
	Coliforms	ND	NCO	NCO
	Sulphite-reducing clostridia	ND	NCO	NCO
Non-irradiated (Dried)	Total Viable Counts	10^4–10^5	10^4–10^6	10^4–10^6
	Staphylococcus spp.	10^2–10^3	10^2–10^4	10^2–10^3
	Coliforms	ND	ND	ND
	Sulphite-reducing clostridia	ND	ND	ND
Irradiated (2.5 kGy)	Total Viable Counts	10^3–10^4	10^3–10^4	10^3–10^4
	Staphylococcus spp.	10^2–10^3	10^2–10^3	10^1–10^2
	Coliforms	ND	ND	ND
	Sulphite-reducing clostridia	ND	ND	ND
Irradiated (5.0 kGy)	Total Viable Counts	10^1–10^2	10^2–10^3	10^1–10^3
	Staphylococcus spp.	ND	10^1–10^2	ND
	Coliforms	ND	ND	ND
	Sulphite-reducing clostridia	ND	ND	ND
Irradiated (10 kGy)	Total Viable Counts	ND	ND	ND
	Staphylococcus spp.	ND	ND	ND
	Coliforms	ND	ND	ND
	Sulphite-reducing clostridia	ND	ND	ND

Results of 2 independent experiments on different batches of IM Chicken sheek kababs; ND = Not detected by methods employed; NCO = Analyses not carried out; Storage studies are in progress.

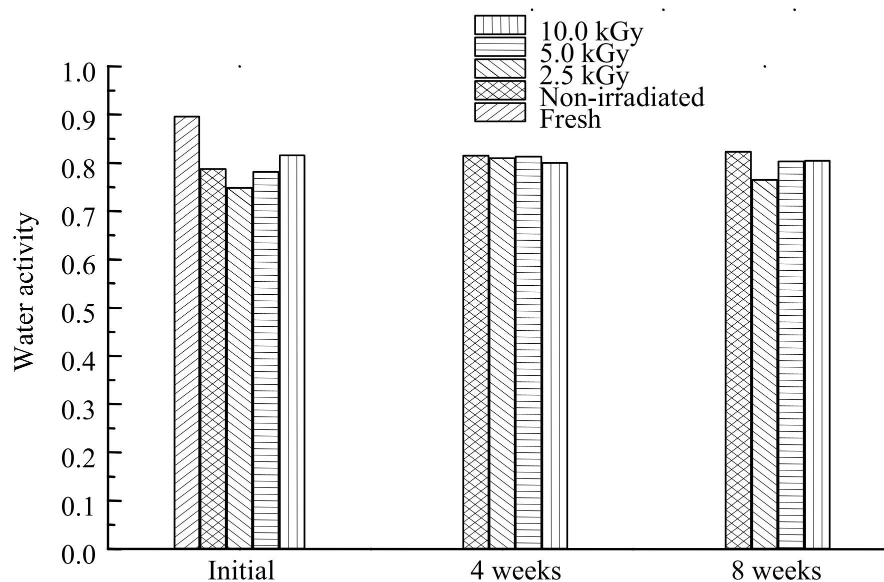

FIG. 7. Water activity (a_w) of chicken chilli during storage at ambient temperature. Each data point represents the mean a_w of 6 samples.

TABLE V. MICROBIOLOGICAL ANALYSES OF INTERMEDIATED MOISTURE (IM) CHICKEN CHILLI

Treatment	Counts (CFU/g)	Storage period (weeks)		
		0	4	8
Fresh	Total Viable Counts	10^3–10^5	NCO	NCO
	Staphylococcus spp.	10^1–10^2	NCO	NCO
	Coliforms	ND	NCO	NCO
	Sulphite-reducing clostridia	ND	NCO	NCO
Non-irradiated (Grilled)	Total Viable Counts	10^4–10^5	10^4–10^6	10^6–10^7
	Staphylococcus spp.	10^2–10^3	10^2–10^4	10^5–10^6
	Coliforms	ND	ND	ND
	Sulphite-reducing clostridia	ND	ND	ND
Irradiated (2.5 kGy)	Total Viable Counts	10^3–10^4	10^3–10^4	10^3–10^5
	Staphylococcus spp.	10^2–10^3	10^2–10^3	10^3–10^4
	Coliforms	ND	ND	ND
	Sulphite-reducing clostridia	ND	ND	ND
Irradiated (5.0 kGy)	Total Viable Counts	10^1–10^2	10^2–10^3	10^1–10^2
	Staphylococcus spp.	ND	10^1–10^2	10^1
	Coliforms	ND	ND	ND
	Sulphite-reducing clostridia	ND	ND	ND
Irradiated (10 kGy)	Total Viable Counts	ND	ND	ND
	Staphylococcus spp.	ND	ND	ND
	Coliforms	ND	ND	ND
	Sulphite-reducing clostridia	ND	ND	ND

Results of 2 independent experiments on different batches of chicken chilli; ND = Not detected by methods employed; NCO = Analyses not carried out; Storage studies are in progress.

3.5. IM chicken chilli

Data on the moisture content and a_w of IM chicken chilli are presented in Figs 6 and 7, respectively. The moisture content was 23–26% and a_w 0.75 ± 0.05 both of which remained constant during storage. The microbiological analysis data of the samples is presented in Table V. Fresh products had TVCs in the range 10^3–10^5 CFU/g while *Staphylococcus* counts were in the range 10^1–10^2 CFU/g. The TVC of IM products was in the range 10^4–10^5 CFU/g and *Staphylococcus* spp. were 10^2–10^3 CFU/g. Irradiation treatment resulted in a dose-dependent reduction in the TVC as well as the levels of potentially pathogenic *Staphylococcus* spp. Irradiated samples were found to show lower microbial counts compared to non-irradiated samples throughout the storage period. No viable microorganisms were detected in samples subjected to irradiation treatment (10 kGy) and the sensory quality was acceptable for several months of storage at ambient temperature.

3.6. Sterile chicken products

A number of ready-to-fry chicken products available in Indian markets were steamed for 5−10 min to inactivate autolytic enzymes and subjected to sterilising doses of gamma irradiation. Microbiological analysis of radappertized products did not show microbial growth in any of the media employed for analysis suggesting the absence of viable organisms. Sensory evaluation of these products is presented in Table VI.

TABLE VI. SENSORY ANALYSES OF VARIOUS READY-TO-EAT CHICKEN PRODUCTS SUBJECTED TO HIGH-DOSE RADIATION PROCESSING

Product	Appearance	Flavour	Texture	Overall acceptability
Chicken chilli	7.17 ± 0.60	7.50 ± 0.60	7.70 ± 0.82	7.50 ± 0.60
Chicken kofta	7.71 ± 0.98	7.57 ± 0.98	7.57 ± 0.98	7.86 ± 0.90
Chicken loaf	8.14 ± 1.21	7.85 ± 0.69	7.71 ± 1.11	7.86 ± 0.90
Chicken salami	7.57 ± 0.98	7.29 ± 1.25	7.57 ± 1.27	7.57 ± 1.27
Chicken burger	7.57 ± 0.82	7.25 ± 0.76	7.50 ± 0.54	7.42 ± 0.66
Chicken lollipop	7.60± 1.34	7.00 ± 1.22	6.70 ± 1.10	6.95 ± 1.18
Chicken tikka	7.40 ± 1.14	6.60 ± 1.14	6.10 ± 1.24	6.60 ± 1.14
Chicken sausage (plain)	7.88 ± 0.64	7.25 ± 1.03	7.63 ± 0.52	7.44 ± 1.12
Chicken sausage (big one)	7.63 ± 0.74	7.12 ± 0.83	7.75 ± 0.71	7.63 ± 0.69
Chicken sausage (mint)	6.88 ± 0.99	7.00 ± 0.54	6.38 ± 0.74	6.63 ± 0.74
Chicken sausage (spicy)	8.25 ± 0.71	7.94 ± 0.26	8.13 ± 0.64	8.13 ± 0.69
Chicken burger	7.17 ± 0.60	7.50 ± 0.60	7.70 ± 0.82	7.50 ± 0.60

Sensory analysis was carried out by 8 panellists. Attributes such as appearance, flavour, texture and overall acceptability were assessed on the basis of a 10-point scale, where 10 corresponds to product of highest quality and 0 corresponds to poor quality of product.

TABLE VII SENSORY ANALYSES OF VARIOUS READY-TO-EAT MUTTON AND PORK PRODUCTS SUBJECTED TO HIGH-DOSE RADIATION PROCESSING

Product	Appearance	Flavour	Texture	Overall acceptability
Mutton chilli	7.75 ± 1.28	7.00 ± 1.06	7.17 ± 1.06	7.38 ± 1.06
Mutton shammi kabab	6.80 ± 0.45	6.10 ± 1.75	5.60 ± 1.64	6.10 ± 1.02
Pork salami	7.60 ± 0.89	7.60 ± 0.55	7.40 ± 0.55	7.60 ± 0.55
Hamburger	7.33 ± 0.82	6.83 ± 0.98	6.42 ± 0.92	6.75 ± 0.88
Pork cocktail sausage	7.17 ± 0.41	6.16 ± 0.98	6.83 ± 0.75	6.33 ± 0.81
Luncheon meat	7.60 ± 0.55	7.40 ± 0.90	7.60 ± 0.55	7.60 ± 0.55

Sensory analysis was carried out by 8 panellists. Attributes such as appearance, flavour, texture and overall acceptability were assessed on the basis of a 10-point scale, where 10 corresponds to product of highest quality and 0 corresponds to poor quality of product.

The mean values of appearance, flavour, texture and overall acceptability shown in Table VI indicated that all these products were acceptable. One of the popular products, chicken samosa with starch wrapping, lost its shape and was mushy, therefore, could not be fried and evaluated for sensory quality. Traditional gravy based preparations such as chicken masala showed changes in sensory attributes leading to unacceptability of the product.

3.7. Sterile mutton and pork products

A variety of ready-to-cook or -eat mutton and pork products were also examined for their amenability to sterilising doses (45 kGy) of gamma irradiation at cryogenic temperatures and the results are shown in Table VII. Initial sensory analysis of radiation sterilized products showed that all products were acceptable. Mutton samosa with starch wrap lost shape and was mushy thus could not be fried. Gravy based mutton preparations showed changes in the sensory attributes (colour and flavour) and were not acceptable.

Other than the above mentioned meat products, traditional Indian ethnic gravy preparations like panner tikka masala (milk based), channa masala (legume based) were also investigated for preservation through high-dose irradiation processing. There were time dependent changes in the sensory attributes (colour and flavour) leading to poor acceptability of the products. The sensory quality of another most popular and common dish, pulao (seasoned rice) with no gravy, was unacceptable as it was also mushy.

4. DISCUSSION

A number of safe, shelf-stable meat and meat products have been developed using a combination of hurdles such as reduced a_w, vacuum packing in multilayered pouches and irradiation treatment (10 kGy). Results from this experimental work have clearly established the safety and stability of these products as none of the microorganisms could be detected during storage at ambient temperature for several months. As a_w of these products was less than 0.85, there would be no risk due to *Clostridium botulinum* [6] or enterotoxin from *Staphylococcus* spp. [7]. Under normal circumstances, a_w of 0.85 or less is sufficient to prevent bacterial spoilage [8–10]. Furthermore, drying of the meat products involved heating (grilling/hot air) which inactivates autolytic enzymes; thus, there is no enzymatic deterioration during extended storage at ambient temperatures. The enzyme inactivation assumes significance when the meat products are to be stored at ambient temperatures for an extended period [11]. Vacuum packing of these products limits chemical deterioration during storage [12] and will also result in the inhibition of growth of aerobic microorganisms and has been shown to improve the keeping quality of preserved foods. A dose-dependent reduction in bacterial counts was observed upon irradiation and experimental results showed a reduction in non-detectable levels at 10 kGy, therefore, resulting in these meat products being stable, safe to eat and of acceptable sensory quality. The use of irradiation as a means of eliminating pathogenic microorganisms in food of animal origin has been well documented [13,14].

In addition to IM meat products, these studies have established the use of high-doses of gamma irradiation for the preparation of various sterile ethnic meat products. There is a pressing need to provide a sterile diet with good organoleptic qualities to immunosuppressed patients [15]. A number of tasty, fresh like radappertized chicken, mutton and pork products have been prepared and these should be market-tested by a target patient population. Studies also revealed that the sensory qualities of gravy products are impaired when subjected to high-doses of gamma irradiation. This may be due to the presence of edible starch used in these foods as a thickening agent. Depolymerisation of starch is well documented [16,17].

5. CONCLUSIONS

A number of meat and meat products have been developed which are safe to eat and can be stored at ambient temperatures for several months using a combination of hurdles such as heat, reduced a_w, vacuum packing and gamma irradiation. These meat products have no detectable microorganisms and are a benefit to immunosuppressed patients, defence personnel etc. This technology will not only save energy but will also boost the demand for and enhance the distribution of such products within the country and also facilitate export possibilities.

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IMPACT OF IRRADIATION ON THE QUALITY OF PROCESSED MEAT AND FISHERY PRODUCTS

A. NOOMHORM, P. VONGSAWASDI, C. INPRASIT, J. YAMPRAYOON,
P. SIRISOONTARALAK, M.E.A. INGLES, A. ADULPICHIT
School of Environment, Resource and Development,
Asian Institute of Technology,
Pathumthani, Thailand

Abstract

Gamma irradiation in combination with packaging method was investigated with a view to extending the shelf-life of processed meat and fishery products. The effect on product quality and film properties was also determined. With a combination of irradiation with packaging, shelf-life of sliced ham and smoked sausages could be extended from 1 and 6 weeks to more than two months, while the shelf-life of semi-dried pork was more than 8 months. However, irradiation caused semi-dried pork to become susceptible to rancidity and also some fluctuations in the colour characteristics. It resulted in a higher drip loss in sliced ham but not in smoked sausages. Semi-dried pork treated with non- and irradiated spice was microbiologically safe after five months of storage with modified atmosphere packaging (MAP). A darker colour of product was observed. From sensory testing, no irradiation flavour, oxidation flavour or taint was detected by taste panellists. Both vacuum and MAP methods had a synergistic effect on the extension of product shelf life. They had different beneficial effects on product quality. With regard to film packaging, tensile and tear strength and elongation decreased after the storage period. Transmission rates for gas and water vapour decreased with irradiation dose. Elongation decreased after irradiation. However, the small changes in film properties confirmed that irradiation with a low-dose did not effect the packaging material. For semi-dried shrimp, irradiation at 2 and 4 kGy could extend the shelf-life to 35 and 49 days of storage compared to 10 days for control samples. During storage, thiobarbituric acid (TBA) values, total volatile nitrogen (TVN) values and hardness increased. Also, the redness of semi-dried shrimp decreased as time progressed due to oxidation of astaxanthin.

1. INTRODUCTION

Meat and fishery processing is one critical industry where quite a number of steps are involved and have been developed as new types of products are formulated with the aim of improving palatability and varying the forms to conform to consumer demands. Such products are possible carriers of food-borne diseases as they contain a high protein content and are most conducive for the proliferation of spoilage bacteria. As they undergo many different steps in the processing line, microbial contamination is expected to occur thereby decreasing shelf-life and limiting the supply. A preservation method effective in ensuring the availability of food as well as its safety for consumption would greatly assist in the provision of a continuous food supply which is safe for the escalating world population.

There are various types of processed meat and fishery products preserved with various techniques such as sausage, ham, semi-dried meat, dried meat, dried shrimp, dried fish and cured fish. The shelf-life of these products is extended due to the low moisture content, addition of salt or sugar and chemical preservatives. However, due to the harmful effect of chemical preservatives attempts are now being made to produce pathogen-free food products and with extended shelf-life without adding any chemical substances. An alternative is to decontaminate meat and meat products in a final processing step, and one method being studied to do this is irradiation.

Within the regulations of many countries, irradiation (1-10 kGy) is being applied for food products. Low-dose irradiation will not kill the entire microbial population. Hence, with preferable conditions for food products, the remaining microbes can proliferate and cause spoilage. To enhance the efficiency of irradiation in extending product shelf-life, a combination of irradiation and other preservation techniques is required. For maintaining meat and meat products with a good shelf-life, plastic pouches of relatively low oxygen permeability are widely used with various packaging systems such as modified atmosphere packaging (MAP) and vacuum packaging. In any packaging system, the environment created facilitates the inhibition of enzymatic and microbial activity, which slows down

the spoilage process. In addition to regulating the entry of gases from the outside environment and the egress of gases inside the pouches, packaging materials can protect products against contamination from the outside environment. Taking into account the benefits of such packaging systems, it is possible to combine these techniques with irradiation.

As stated above, irradiation can reduce the initial load of microorganisms to a preferred level and the growth of remaining microorganisms can be inhibited with the environmental conditions created in a packaging system. Consequently, it is possible to extend the shelf-life of meat and meat products as well as maintaining the good quality of foods. In addition, the combination of irradiation and packaging technologies may also improve product palatability.

Besides meat products, fishery products make up a high percentage of Thailand's food market. Dried shrimp is one of the popular products that is not only consumed domestically but is also exported. However, with a high variation in water activity (a_w) (0.6–0.85), dried shrimp is susceptible to pathogen growth and can be very harmful especially in the ready-to-eat form and stored at ambient temperature. Irradiation could possibility improve product palatability while at the same time accomplishing microbial safety.

Therefore, the aim of these studies was to investigate the use of irradiation in combination with packaging to increase the shelf-life of fresh meat and meat products and to evaluate the changes in product quality and performance of packaging materials during storage. Another purpose was to investigate the application of irradiation to extend the shelf-life of fishery products stored at ambient temperature.

2. REVIEW

2.1. Meat and fishery products

Meat is a highly perishable food material presenting a very conducive environment for the proliferation of many microbes. The popular meat products widely consumed in Thailand are cured meats. Sliced and cooked vacuum packaged cured meats generally include cooked hams, corned beef, emulsion-type sausages, and luncheon meats. In order to preserve the colour of cured meat, oxygen should be excluded which is the main basis for using impermeable materials for packaging of cooked cured meats. The addition of curing agents, spice and antioxidants followed by thermal processing and/or smoking contribute to the slowing down of deterioration. Meat product spoilage normally results from post-processing contamination. The most frequently observed types of bacterial spoilage in vacuum packed, sliced cooked cured meats are; sweet/sour odour caused by lactobacilli, *Leuconostocs* and streptococci, cheesy odour caused by *Brochothrix thermophacta*, and a sulphide odour caused by *Vibrios* and *Enterobacteriaceae* [1].

Semi-dried pork is a processed meat that is popular in Thailand. A drying technique is applied to this product whereby the pork is dried and grilled to give quite a tough texture. Sugar, salt and spice are added to improve flavour. In addition to salt and sugar as natural preservatives, a chemical preservative, Benzoate, is commercially used to extend shelf-life. Products are in the ready-to-eat form and do not require refrigeration. As the product has a moisture content of 18–23%, a sugar content of 20% and 4% lipid, discoloration from browning and oxidation, rancidity and bacterial contamination can occur easily. Normally, shelf-life is between 3–4 weeks and up to two months with vacuum packaging.

Like meat products, many preservation methods are applied to preserve fresh marine foods and consequently there are many fishery products such as dried shrimp, dried fish, cured fish, etc. Among these products, dried shrimp makes up a high percentage of the market. The production of dried shrimp is composed of boiling and drying. The boiling process is required to eliminate microorganisms, i.e. *Bacillus* spp. and *Pseudomonas* spp. During boiling, salt is added but no information is available about the exact concentration of the salt solution used. In practice, more salt is

added into water for the first batch to be boiled while for the subsequent batches less salt and water are added. Usually, the same water will be used for boiling all the shrimps to be processed in one day [2]. Besides the effect on product taste, the salt content is related to the growth of microorganisms and shelf-life of the product. The guideline issued by the US Food and Drug Administration (FDA) [3] for fish salting states that the recommended salt content should be 10%. The dried shrimp product is in ready-to-eat form and stored at ambient temperature. Low water activity (a_w) makes dried food shelf-stable. Usually shrimp is dried until a_w meets the specification at 0.85. A a_w of 0.85 or below will prevent the growth and toxin production of pathogens such as *Staphylococcus aureus* and *Clostridium botulinum*. The US FDA [3] recommended that the maximum a_w value of fishery products should be 0.85. However, due to improper cooking and inadequate drying, the a_w of the finished product varies. Dried shrimp can be considered as dried if the a_w is 0.6–0.7 and semi-dried if a_w is 0.7–0.85. With the high variation in a_w (0.6–0.85) of the finished product, it is susceptible to pathogen growth and toxin formation due to improper cooking and inadequate drying.

2.2. Shelf-life extension of meat products using irradiation

Numerous studies have been made on the effectiveness of irradiation in eliminating spoilage bacteria and pathogens in fresh and processed meat [4]. For processed meat, some effects of salt (NaCl), sucrose and water content on effectiveness of irradiation have been investigated. The results indicated that the survival of food-borne pathogens on irradiated meats with reduced water content or increased NaCl levels may be greater than expected [4]. Besides microbial inhibition, property changes of food and food products are other aspects to be considered after irradiation. Many research studies have shown that a significant reduction of microbiological population was achieved with the application of irradiation (<10 kGy) but effects on the different chemical and sensory characteristics of meat occurred. The effect of irradiation on meat lipids, peroxide value, iodine value, and malonaldehyde (2-thiobarbituric acid (TBA) values) formation were determined. Results showed that such irradiation doses (<10 kGy), there was no significant change in meat lipids [5]. However, when oxidative rancidity was evaluated, TBA values and carbonyl content for irradiated samples of ground chicken meat were higher than for non-irradiated samples.

Colour and flavour are important sensory characteristics of meat foods. Many investigators have studied the effects of gamma irradiation on the sensory and microbiological properties of meat products. Paul et al. [6] reported that freshly ground mutton irradiated at 2.5 kGy had better colour, odour, and microbiological acceptability than non-irradiated samples or those irradiated at 1 kGy. When the effects of 5 and 10 kGy irradiation on the colour and oxidative stability of meats treated with nitrite or a nitrite-free curing system were investigated, it was found that irradiation had no detrimental effects on the colour or flavour of either cured samples [7]. Fu et al. [8] report consistently that Hunter Lab values and visual evaluation scores showed no colour difference between control beefsteaks and those irradiated at 1.5 kGy. However, an increase in redness of pork colour due to irradiation has been observed [9–11].

2.3. Shelf-life extension of meat products using packaging techniques

Vacuum packaging, which is the exclusion of gases from packages, can greatly increase the shelf-life of freshly packaged meat [12]. Aside from shelf-life extension, vacuum packaging is very effective in retaining the pink colour of meat products and prevents the development of mould growth, delays the proliferation of aerobic spoilage bacteria, slows down the oxidation of lipids [13] and maintains the sensory attributes of products. With the appropriate packaging material, i.e. those with less oxygen (O_2) permeability, reduction of undesirable bacterial growth is facilitated [14] and shelf-life of fresh red meat can be extended to 21–28 days [15]. With this type of film the carbon dioxide (CO_2) produced from bacterial metabolism will be accumulated and further enhance microbial inhibition. Some bacteria are unaffected by CO_2 gas, particularly lactic acid bacteria [16] explaining the dominance of these bacteria in most stored meat products. The only disadvantage of vacuum packaging to meat processing is the high weight losses in packaged products as compared to MAP [17].

For MAP, the containment of food under a gaseous environment other than the outside conditions (air) to inhibit normal respiratory activities has been found to be a possible alternative to vacuum packaging. Young et al. [15] reported that MAP inhibits enzymatic spoilage hence extending the shelf-life of meat. Utilising CO_2, nitrogen (N_2) or O_2 gases or a combination of these, microbiological population is retarded. In mixtures with a high O_2 concentration, meat myoglobin is readily oxygenated thereby producing the desired bright red colour commonly preferred by the consumer [18]. Carbon dioxide, on the other hand, has been reported to possess inhibitory characteristics against spoilage bacteria [19]. With 5% O_2, balanced CO_2 and good refrigeration, meat can be stored up to 14–17 days and 10–12 days if O_2 concentration is increased to 65–80% [15]. The mixture found to be the most beneficial for meat products was 20% CO_2 and 80% N_2 [20]. The shelf-life extension of meat was attributed to the bacterial inhibition capacity of CO_2, which is enhanced with decreasing temperature [21,22].

2.4. Shelf-life extension of meat products using a combination of irradiation and packaging

A major concern in irradiating meat, however, is its effect on meat quality, mainly because of free radical reactions and possible off-odour generation during irradiation. Because most muscle cells (75%) are composed of water and surrounded by lipid bilayers, irradiating meat may produce hydroxyl radicals leading to lipid oxidation. Besides lipid oxidation, which results in off-odours and off-flavours, colour is another important characteristic contributing to the acceptance of irradiated meat products.

Packaging under vacuum and at low temperatures have been suggested as ways to reduce the number of oxygen radicals formed during irradiation. Ahn et al. [23] studied the effects of vacuum packaging and irradiation on lipid oxidation and volatiles in pork patties. They found that packaging and storage conditions of raw meat after irradiation were important factors in lipid oxidation of cooked meat. Irradiation of vacuum packaged pork resulted in redder products that were stable over time. The product exhibited significant surface discoloration at 4.5 kGy which decreased as dose was increased [24]. It has been consistently reported that irradiated meat samples stored under vacuum were darker and more red than samples stored under air [25]. From sensory evaluation, Mattison et al. [26] reported that vacuum packaged pork loins irradiated at 1 kGy exhibited minimal sensory changes with no detectable differences between treated and control samples after 14 days. There was also no difference between irradiated and control samples for overall acceptance, meatiness, freshness, tenderness, or juiciness in cooked pork chops (2.5 kGy, vacuum) [27].

2.5. Effect of irradiation on packaging materials

The barrier properties of films contribute to the control of entry and egress of gases in the packages. Depending on the composition of the films, they vary in physical and mechanical characteristics. Films are responsible for controlling the rate of transmission of moisture vapour to prevent condensation problems caused by the accumulation of supersaturated vapour inside the packages. Mass transfers involving food packaging interactions have been recognised as causing scalping of aroma and migration of packaging components resulting in off-flavours, off-odours or toxicological concerns. Irradiation could provoke changes in the kinetics of these interactions [28].

The stability of a multilayered plastic for the application of irradiation was evaluated with regard to inertness to maintain food quality [29]. It showed that irradiation of a Nylon/PVDC/EVA barrier film caused changes in mass transfer of the packaging material. Sensory evaluation of irradiated water indicated development of off-odours and taints, even at 1 kGy. Oxygen permeability was also affected by irradiation. However, results indicated that the material would lose its high-barrier properties if irradiation was applied to pouches at a very high-dose (25 kGy). Versanyi [30] reported no significant differences in gas permeability of low density polyethylene (LDPE) irradiated at doses up to 8 kGy. The tensile strength of LDPE films irradiated at 0–8 kGy was reduced but no significant change in elongation at break was observed.

2.6. Shelf-life extension of fishery products using irradiation

Spoilage organisms are different for various fishery products [31]. *Pseudomonas* is the principal contaminant of white-flesh fish and shrimp. The maximum dose without detectable sensory change for fresh shrimp, sea-fish and fresh-water fish is 2, 1–4 and 1–3 kGy that extend their shelf-life to 5–14, 18–38 and 13–20 days, respectively. Type-E *C. botulinum* is associated with marine and fresh-water foods under certain conditions. This pathogen is of special concern because of its ability to grow and produce toxin at temperatures as low as 3.5°C. Holding marine and fresh-water foods for extended periods could represent a hazard from this organism if the temperature was not below 3.5°C. Some degrees of protection are afforded by the cooking process, which ordinarily is sufficient to inactivate any botulinum toxin that may have formed.

Shrimp (*Crangon vulgaris*) cooked in sea-water and irradiated with 1–2 kGy has been maintained with satisfactory quality under refrigeration for 20–24 days, a period approximately double that without irradiation [31]. Dried fish may be subject to damage by insects. Doses of 0.15–0.5 kGy are usually effective in controlling insect infestation of dried and/or smoked fish. In a recent study, Piccini et al. [32] investigated the effect of irradiation on the oxidative state and sensory scores of different fish samples. While the non-irradiated control samples reached maximum TBA values after 12 days, irradiated samples attained a maximum TBA value after 24 days cold storage. There was a delay in reaching the malonaldehyde peak maxima in the irradiated samples.

3. MATERIALS AND METHODS

Experiments were conducted on meat and fishery products. For meat products, irradiation was combined with packaging for fresh meat, sliced ham, smoked sausage and semi-dried pork. For fishery products, the study of extending shelf-life of cooked fish sausage and semi-dried shrimp stored at ambient temperature was performed.

3.1. Meat products

3.1.1. Smoked sausage

Smoked sausages were purchased, from CP Interfood (Thailand) Co., Ltd, Bangkok, on the day of processing and packed in normal packaging with LDPE pouches for control samples, vacuum packaging in nylon polyethylene ionomer resin (NPI) plastic pouches at 90 psi vacuum level and MAP in laminated films of polyvinylidene chloride-coated nylon and polyethylene (PVNP). All samples for vacuum packaging were vacuum-sealed in a Model LAPACK 450 while those for MAP were flushed with 20% CO_2 and 80% N_2 at 50% gas flushing capacity (Supervac by Packmart Co., Ltd, Thailand) before sealing at 86°C. Sausage samples were irradiated at the Thai Irradiation Centre (TIC), Pathumthani, Thailand, using a ^{60}Co Gammacell 220 facility at a dose rate of 0.22 kGy/min. Sausage samples were treated with 0, 2 and 4 kGy, stored in chill room (5 ± 2°C) and sampled every week for quality evaluation. The qualities assessed were microbial levels, texture, pH, moisture and fat content, colour, drip loss and sensory attributes. The performances of packaging films in terms of thickness, tear and tensile strengths and gas and water vapour permeability were evaluated at the start and end of the storage period.

3.1.2. Sliced ham

Sliced ham was purchased from CP Interfood (Thailand) Co., Ltd, Bangkok and had the same recipe as ham sold in the market. A 100 g weight was packed into flexible pouches of NPI at 90 psi vacuum level. The packaging materials were 3-sided sealed flexible pouches with an inside dimension of 255 × 125 mm. The control samples were packed in LDPE pouches. The temperature of the ham was maintained at 2 ± 2°C during transport to the laboratory. Samples were irradiated with doses of 0, 2, 4 or 6 kGy and then stored in a chill room at 2 ± 2°C for quality determination.

3.1.3. Semi-dried pork

Semi-dried pork was prepared by S. Khonkaen Co., Ltd, Bangkok. Ham butts were frozen overnight at –10 to –20°C and then sliced into small pieces of approximate sizes of 40 mm × 60 mm × 2.0–2.5 mm. The samples were mixed with sugar and seasonings and kept in a chill room for not more than 5 days for marinating. These samples were ready for drying at 80–100°C for 1.5 h and roasting for not more than 10 min at 200°C. Weights of 100 g semi-dried pork were packed using NPI with controls being packed in LDPE pouches.

The irradiation experiment for semi-dried pork was conducted in two parts. Firstly, to investigate the shelf-life elongation of the product, the commercially prepared semi-dried pork with chemical preservative addition was used. After vacuum-packaging, samples were irradiated at 0, 2, 4 or 6 kGy and then stored at ambient condition for every week of quality evaluation. Secondly, to improve product palatability by increasing the moisture content of the samples and to study the effect of spice addition, semi-dried pork prepared using the same procedure with no chemical preservatives was used. The moisture content of the finished products was varied to three levels expected to be approximately 16, 20 and 25%. Spice used as the main ingredient was irradiated at 4 kGy before adding to the products. All samples were packed in PVNP pouches under modified atmosphere conditions with 20% CO_2 and 80% N_2. Then semi-dried pork samples with irradiated and non-irradiated spice were irradiated at 6 kGy and stored at ambient temperature. Each month, irradiated product quality was evaluated and compared with controls. In this study, the tear strength, tensile strength and elongation of PVNP film were the properties evaluated to determine packaging performance during storage.

3.2. Fishery products

3.2.1. Semi-dried shrimp

Sea water shrimps (*Penaeus* spp.) were washed, boiled in a salt solution, dried, the shells removed and packed in plastic bag. The final moisture content of semi-dried shrimp samples was around 35–37%, which was higher than the dried shrimp commercially sold in market (20–25%). The shrimp were irradiated at 0, 2 or 4 kGy and kept under ambient conditions for quality analysis. The quality indices evaluated were a_w, moisture content, pH, texture properties, and colour. The shelf-life was determined chemically by total volatile nitrogen (TVN) and TBA value and microbiologically by total plate counts (TPCs), yeast and mould counts, and the presence of *E. coli*, *Staphylococcus* spp. and Salmonella spp. Sensory evaluation was conducted using 10 trained panellists.

3.3. Product quality determination

3.3.1. pH levels

A 10 g aliquot of sample was blended for 1 min with 90 mL distilled water using a high speed Waring blender. The slurry was measured by a pH meter at 25 ± 2°C within 2 min.

3.3.2. Water activity (a_w)

The samples were evaluated for a_w by a a_w thermoconstanter.

3.3.3. Moisture and fat content

The moisture and fat content of the meat samples were determined using CEM methods (AOAC, 1984). Fat extraction was carried out using a Soxtec System HT2 (1045 Extraction Unit, Soxtec, Sweden) using 70 mL hexane as the extraction solvent at 120°C. Fat removal was carried out over 5 h, after which samples were dried and weighed.

3.3.4. Drip loss

Drip loss, which is the change in the weight of meat samples attributed to loss of water, was determined by weighing meat samples at the start and the end of storage to the nearest 0.001 g. The difference in the weights was used to calculate the percentage weight loss.

3.3.5. Lipid oxidation

TBA values were used as an index of lipid oxidation of meat products. A 10 g sample of meat was blended, transferred into a Kjeldahl flask, washed with distilled water and the pH adjusted to 3.5. The sample was then distilled until 50 mL of distillate was collected. A aliquot of 5 mL of distillate was mixed with 5 mL of TBA reagent before immersing in a boiling water bath for 35 min. The mixture was then cooled in tap water for 10 min. The absorption density was measured at a wavelength of 538 nm by a UV spectronic 3000. The TBA number was calculated as mg malonaldehyde/g sample.

3.3.6. Colour

The colour of samples was determined by a Juki Colorimeter. A standard plate of L = 98.26, a = –0.06 and b = –0.29 was used to calibrate the equipment. The data were reported as 'L' (lightness-darkness), 'a' (redness-greenness), and 'b' (yellowness-blueness).

3.3.7. Texture

For processed meat, samples were cut and placed in a Kramer Shear Cell and analysed using an Instron Universal Testing Machine operated at various conditions. For semi-dried pork, a cross-head speed and load cell of 100 mm/min and 1 kg, respectively, was used. For other meat products, the cross-head speed, chart speed and maximum load set were 50 mm/min, 200 mm/min and 500 kg, respectively.

3.3.8. Microbial count

Microbiological counts of samples were carried out by decimal solution techniques followed by standard spread plate count. Analysis was done according to the Bergy's Manual of Determinative Bacteriology (8th Edition, 1984). The media used for each type of bacteria determination were as follows:

Total bacterial count	Nutrient agar
Yeast and fungal count	Potato dextrose agar
E. coli	Most Probable Number (MPN method)
Salmonella spp.	Brilliant green agar
Staphylococcus spp.	Mannitol salt agar

3.3.9. Sensory evaluation

Ten semi-trained panellists aged between 28–40 years were used to assess the colour, texture, flavour, juiciness and overall acceptability of the meat products. The scores were correlated with the closeness to the natural properties of products. The colour ranged from dark red-brown (maximum score) to dark green or black (minimum score). The trait flavour intensity was varied from meaty and spicy flavour to spoilage flavour such as rancid, sour, bitter, etc. Tenderness and texture scores ranged from tender to tough and firm to mushy, respectively.

3.4. Packaging property test

3.4.1. Thickness

The thickness of the material was computed based on an average of 10 readings to the nearest 0.0025 mm from three representative samples using a Model 549 ME Micrometer.

3.4.2. Tear and tensile strength

Tear strength of the film was obtained from the average readings of five representative samples cut from machine direction and traverse direction using a Dumb Bell specimen cutter following procedures outlined by ASTM D1922 (1993). Tearing resistance of films were determined using the Elmendorf-type tear tester. Determination for the tensile strength was facilitated using five 1.5 cm width strips using a Twin Blade cutter. Both machine and traverse directions were properly represented (ASTM D882, 1991) to obtain accurate data.

3.4.3. Gas and water vapour permeability

The permeability of the packaging materials regulates the movement of gases and water vapour across the package. Employing the procedures specified by ASTM D1434 (1982), film samples were cut in 10.4 cm diameter and conditioned at 25°C for 2 days. Films were then fitted and wax-sealed to a dish containing calcium hydroxide and placed in a desiccator with water. The changes in weight of the dish were monitored every 6 h and used to compute the water vapour transmission rate for the film being tested. Film samples cut in 14.0 cm diameter, conditioned at the same conditions as above, were set in a permeability cell with O_2 and N_2 gases flowing at 108 mL/min in the upper and the lower chambers, respectively. The variations in gas concentration at the lower chamber as shown in the different curves from a gas chromatograph (GC 14B Shimadzu Corp., Kyoto, Japan) represented the permeability of the films to O_2 gas.

3.5. Statistical analysis

All the data gathered were analysed using Analyses of Variance (ANOVA) using Statistical Packages for Social Sciences (SPSS, 1994). Least square means were determined and differences in the means were further computed using Duncan's mean range test (DMRT) method.

4. RESULTS

4.1. Shelf-life extension of processed meat by irradiation

From microbial count results (Table I and Table II) of smoked sausages, sliced ham and semi-dried pork, distinct shelf-life extensions were observed as an effect of packaging method. Vacuum packaging was effective in extending the shelf-life of sliced ham from 5 days for controls to 4 weeks using NPI. The storage-life of smoked sausages was doubled and the storability of semi-dried pork was prolonged for up to 2 months. For the MAP, bacterial growth was hindered and shelf-life extended to a period of time similar with vacuum packaging although it was one week longer for the smoked sausages. At the initial stage, the microbial population present in semi-dried pork was recorded at 495 CFU/g but as time progressed, the initial count became less detectable due to the preservative effects of the chemical present in the processed meat which is mostly sugar causing osmotic pressure in microbial cells and decreasing the a_w of products. Irradiation was able to reduce the microbial population and increase the shelf-life considerably of processed meat. From the initial bacterial count of $\log_{10}$ 3.5 and 3.2 CFU/g present in smoked sausages, a reduction of 28.46% and 68.79% was observed upon the application of 2 kGy in vacuum packaged and MAP samples, respectively. A decrease in bacterial density of 71.12% was found in vacuum packaged samples when treated with a 4 kGy dose (Table I). These remarkable reductions resulted in smoked sausages having a shelf-life longer than the 2 week scheduled storage period.

Sliced ham, on the other hand, had a bacterial count of 1.8×10^4 CFU/g at the beginning and increased to 4.6×10^6 CFU/g on the fourth week of storage, after which a maximum bacterial density of $\log_{10}$ 7 CFU/g was reached making the food item unsuitable for human consumption. However, irradiation was very effective in the reduction of microbes (Table II). A notable decrease was observed to the point when the TPC was below the 30 CFU/g limit, particularly for those samples treated with 6 kGy. For the semi-dried pork, the TPC showed a considerable decrease in the population. There was no observed bacterial activity in the non-irradiated samples during the storage period although a few colonies were counted at the start (495 CFU/g). This was attributed to the bactericidal reaction of the chemicals, particularly the sugar present in the meat product. Moreover, those products treated with irradiation showed no noticeable microbial activity. If there was any microbial activity the TPC was beyond the minimum limit to be detectable.

The moisture contents of sliced ham, semi-dried pork and smoked sausage were in the range 68.9–74.84%, 15.39–18.33% and 58.39–63.32%, respectively. Moreover, the fat contents of the processed meat were also found to change in the same manner as the moisture content. Fluctuations were observed but variations were within a small range. There was a slight increase in their fat contents due to the sensitivity of lipid during irradiation. Also, sausages packed in LDPE, both in vacuum and modified atmosphere, produced the highest increase as compared with the other treatments.

When the pH level of processed meat was considered, the initial readings of the sliced ham showed a range between 6.56–6.62. These readings decreased slightly during the storage period. Irradiation did not produce dramatic changes in the pH readings. Smoked sausages had pH levels higher than the other samples at the start of the experiment. Samples packed in LDPE pouches exhibited a faster rate of decline as compared to samples in NPI and PVNP. Although all sausages followed the same trend, irradiated samples had slower rates of decrease than others did, but the difference was not significant. These trends showed the ineffectiveness of the low-dose irradiation in the inhibition of enzymatic activity, regardless of packaging method and material. For the non-irradiated samples, the decrease was attributed to the microbial activity, specifically by lactobacilli, which increased the acidity of food items as a result of their metabolism.

TABLE I. EFFECT OF IRRADIATION DOSE AND PACKAGING ON MICROBIAL POPULATION OF SMOKED SAUSAGE SAMPLES STORED AT CHILL CONDITION

Storage time (weeks)	Microbial population (Log CFU/g)							
	Cont. 1	Vac-0	Vac-2	Vac-4	Cont. 2	MAP-0	MAP-2	MAP-4
0	3.5682	3.4624	2.4771	1.0000	3.1461	3.2041	1.0000	1.0000
1	4.6901	3.7559	2.7160	1.4771	3.8692	3.3010	1.3010	1.3010
2	4.8388	4.3979	3.2041	1.7782	4.9191	4.0792	2.2041	1.0000
3	5.2304	4.6532	3.2532	2.3979	5.2553	4.5798	2.7782	1.0000
4	5.8633	4.7160	3.5441	2.4771	5.9777	4.7782	3.0414	1.3010
5	6.6335	5.5979	4.5139	2.6532	6.5911	5.3802	3.6721	1.6021
6	7.3451	5.8624	5.3010	2.7160	7.2947	5.9243	4.3802	2.4314
7	–	5.9121	5.5051	2.6021	–	5.9956	4.6902	2.3979
8	–	6.2928	5.5315	3.0792	–	6.5315	5.0414	2.4914

Cont. 1 = control for vacuum (LDPE vacuum packed with no irradiation); Vac-0 = vacuum packaging with no irradiation; Vac-1 = vacuum packaging with irradiation at 1 kGy; Vac-2 = vacuum packaging with irradiation at 2 kGy; Vac-4 = vacuum packaging with irradiation at 4 kGy; Cont. 2 = control for MAP (LDPE MAP packed with no irradiation); MAP-0 = modified atmosphere packaging with no irradiation; MAP-1 = modified atmosphere with irradiation at 1 kGy; MAP-2 = modified atmosphere with irradiation at 2 kGy; MAP-4 = modified atmosphere with irradiation at 4 kGy.

TABLE II. EFFECT OF IRRADIATION DOSE AND PACKAGING ON MICROBIAL POPULATION OF SLICED HAM SAMPLES STORED AT CHILL CONDITIONS AND SEMI-DRIED PORK STORED AT AMBIENT CONDITIONS

Storage time (weeks)	Microbial population (Log CFU/g)							
	Irradiation dose of sliced ham (kGy)				Irradiation dose of semi-dried pork (kGy)			
	0	2	4	6	0	2	4	6
0	6.6484	3.2934	ND	ND	ND	ND	ND	ND
1	7.3766	3.6857	ND	ND	ND	ND	ND	ND
2	7.5105	3.8633	ND	ND	ND	ND	ND	ND
3	7.5563	4.3702	3.3021	ND	ND	ND	ND	ND
4	7.5670	5.3010	3.9031	ND	ND	ND	ND	ND
6	–	7.0434	4.9085	ND	ND	ND	ND	ND
8	–	7.5888	5.1021	ND	ND	ND	ND	ND

ND = Not detected.

When the pH level of processed meat was considered, the initial readings of the sliced ham showed a range between 6.56–6.62. These readings decreased slightly during the storage period. Irradiation did not produce dramatic changes in the pH readings. Smoked sausages had pH levels higher than the other samples at the start of the experiment. Samples packed in LDPE pouches exhibited a faster rate of decline as compared to samples in NPI and PVNP. Although all sausages followed the same trend of decreasing, irradiated samples had slower rates than others, but the difference was not significant. These trends showed the ineffectiveness of the low-dose irradiation in the inhibition of enzymatic activity, regardless of packaging method and material. For the non-irradiated samples, the decrease was attributed to the microbial activity, specifically by lactobacilli which increased the acidity of food items as a result of their metabolism.

For drip loss, between vacuum and modified atmosphere packaging, the former resulted in a higher loss in processed meat. Vacuum packed smoked sausages resulted in a significant loss but those in LDPE pouches exhibited the greatest loss at 2.87%. The application of irradiation lessened the loss of water. The higher the dose, the lower the decrease in the drip loss after the storage period. Furthermore, for drip loss in sliced ham, untreated samples exhibited a greater loss during the second week of storage than those treated with 2 kGy and then proceeded to decline with time. Samples exposed to 4 and 6 kGy had their highest loss during week 4 of storage being 6.2 mL/100 g and 7.0 mL/100 g, respectively. Higher weight loss was observed in samples exposed to higher irradiation doses, in contrast to smoked sausages.

For colour and texture of processed meats, smoked sausages exhibited varying colour attributes although those in LDPE pouches both in vacuum and modified atmosphere had lesser degrees of redness (‘a’ values) as compared with the other treatments. Packaging in vacuum produced a more stable colour quality than MAP while application of irradiation with 2 and 4 kGy created some fluctuations in the intensity of the different colour attributes. In the modified atmosphere packages, exposure to 2 kGy produced sausages with high L* values making the colour lighter than other samples. This may be due to the higher fat content of the 2 kGy treated samples which interacted with tissues in the meat products. Ho et al. [35] reported that heme pigments are strong oxidation catalysts if brought into contact with lipids, which consequently produces discoloration. As stated before, hardness is affected by the different processing methods and fat content in meat. Due to small changes in the fat content and the composition of comminuted meat of smoked sausages, the values of hardness were relatively lower.

The level of rancidity in the meat samples is expected to rise as a result of oxidation, particularly in food items with a high fat content. Semi-dried pork is one of the meat products with a considerably high fat content. Based on the results obtained (Tables III), TBA values for sliced ham were higher than those of semi-dried pork. Reports have shown that irradiation treatment may cause an increase in TBA values and any increase is an indication of the proneness of the product to rancidity. In this experiment, an increase in TBA values was observed, but the sensory panel did not notice any significant signs of rancidity except during the later part of storage.

From sensory evaluation of colour, texture, odour and overall acceptability of various processed meats, no significant differences were observed in the changes of the quality attributes of the semi-dried pork and sliced ham samples. Evaluation scores were basically the same and both samples were acceptable to consumers. This phenomenon suggested that

TABLE III. EFFECT OF IRRADIATION DOSE AND PACKAGING ON LIPID OXIDATION OF SLICED HAM STORED AT CHILL CONDITIONS AND SEMI-DRIED PORK STORED AT AMBIENT CONDITIONS

Storage time (weeks)	Irradiation dose of sliced ham (kGy)				Storage time (months)	Irradiation dose of semi-dried pork (kGy)			
	TBA values					TBA values			
	0	2	4	6		0	2	4	6
0	0.54	0.89	0.91	0.95	0	0.35	0.29	0.30	0.44
1	0.63	0.84	0.91	0.89	1	0.50	0.47	0.35	0.53
2	0.56	0.92	0.99	1.13	2	0.89	0.79	0.77	0.62
3	0.60	0.83	0.84	0.95	4	1.01	1.31	1.37	1.84
4	0.69	0.89	0.95	0.87	6	3.12	2.92	2.42	4.69
6	0.65	1.05	0.87	0.94	8	3.74	5.27	4.73	4.19
8	0.67	0.81	0.84	0.88					

consumers would accept a wide range of differences for processed meat products according to their preferences. For the smoked sausages, vacuum-packed samples were more stable than those in MAP pouches in terms of colour attributes. Irradiated samples, although there were slight changes in the colour characteristics, were preferred by most of the taste panel members, particularly during the later part of the storage period.

4.2. Effect of spice on shelf-life extension of semi-dried pork by irradiation

Semi-dried pork is produced through addition of sugar, salt and spices to improve favour followed by drying and grilling. This product is normally packed in sealed plastic bags. With these characteristics, it can be kept for 2 months under ambient condition. As from the previous study (Section 4.1), the quality of semi-dried pork irradiated at 2–6 kGy and packed in vacuum were still acceptable by taste panellists after 8 months of storage under ambient conditions. Shelf-life was extended since the product had a low moisture content (16%) and sugar was added. There was a possibility to increase moisture content of semi-dried pork to more than 16% and up to 25%, which would make the product more palatable. Samples were prepared as mentioned previously. Physical, chemical and sensory qualities of irradiated semi-dried pork with irradiated spice (irsp-ir), irradiated semi-dried pork with non-irradiated spice (nirsp-ir), non-irradiated semi-dried pork with irradiated spice (irsp-nir) and non-irradiated semi-dried pork with non-irradiated spice (nirsp-nir) were determined during a 5 month storage period.

4.2.1. Water activity (a_w) and moisture content

The a_w of the semi-dried pork ranged from 0.63–0.76 being varied by its moisture content. From its a_w, semi-dried pork can be classified as an intermediate moisture (IM) food the a_w of which is in the range of 0.6–0.9. Therefore, semi-dried pork is susceptible to spoilage from halophilic bacteria (minimum a_w for growth being 0.75), osmophilic yeast (a_w 0.6) and xerophilic moulds (a_w 0.6). The products with a higher moisture content (23.02 ± 0.37 and 24.65 ± 0.53%) are susceptible to three types of microorganisms because their a_w is above 0.75 which is the minimum a_w these microorganisms require to grow. Semi-dried pork with a moisture content of 18.40 ± 0.39% is safer because of its lower a_w. It is susceptible to only osmophilic yeasts and xerophilic moulds whose minimum a_w for growth is 0.60.

During 5 months of storage at ambient temperature, the a_w of irradiated semi-dried pork was lowered (Table IV). Comparing the a_w of semi-dried pork at the beginning of and after 5 months of storage, the reduction in moisture content was about 7, 12 and 10% for 18.40 ± 0.39, 23.02 ± 0.37 and 24.65 ± 0.53%, respectively. These reductions can be explained by the fact that dried or wet foods may take up or give up moisture to the surrounding environment depending upon whether the surrounding air is drier or more moist. In this case, in storage, the surrounding air may have lower relative humidity (RH), therefore, the surface of the food would be dried and cause the reduction of a_w.

The reduction of a_w of semi-dried pork during storage should be considered in regard to the microbiological safety of the products. The rate of growth of bacteria is greater than that of yeasts and moulds. Hence with foods of high a_w, bacteria will outgrow the fungi and cause spoilage, while at a_w values that restrict or prevent bacterial growth, the fungi grow and become dominant. Products that contain high salt concentrations (low a_w) will be spoiled by halotolerant or halophilic bacteria. After storage, semi-dried pork had a a_w below 0.75 which is generally that of dried foods. A safe a_w level for storage is usually considered to be 0.70 or less. Since most microorganisms will not grow below this a_w level, if semi-dried pork is reduced to this level, it could be storable at room temperature without irradiation treatment.

During 5 months of storage, there was a small decrease in moisture content ranging from 2–4%. Usually the moisture reduction during storage is associated with moisture loss or purge loss of products. The moisture reduction of all samples seemed to be unaffected by the application of irradiation. As from previous studies, of the two packaging methods, MAP was better for water retention than vacuum packaging and application of irradiation helped in minimising the purge losses in fresh and processed meat [34]. This is caused by the presence of N_2 as it has the capacity to maintain the water holding capacity of meat tissues upon its interaction with them [17]. The use of packaging atmosphere containing both N_2 and CO_2 might be effective for simultaneously minimising weight losses during storage and for inhibiting the growth of microbes. Besides the packaging method, packaging film (PVNP) was also responsible in preventing gas and water permeability that resulted in retention of moisture content of semi-dried pork during storage.

4.2.2. pH levels

After slaughter, the normal pH of meat carcass is between 6.4–7.0 [35]. For semi-dried pork, pH was quite lower (6.02–6.08) than fresh meat due to its processing method and ingredients. Usually meat has undergone glycolysis resulting in a decline in pH due to the conversion of glycogen to lactic acid by enzymatic reactions. However, for semi-dried pork, there was no observed significant variance among product treatments, which meant that the application of irradiation had no effect on the pH values. For all moisture levels, the pH of irsp-ir, nirsp-ir, irsp-nir and nirsp-nir was around of 5.75–6.30 during 5 months of storage. There was no change in the acidity of semi-dried pork and this was most likely due to its low moisture content and high temperature during grilling resulting in the inhibition of enzymatic and microbial activities which were the main causes of low pH in meat products.

TABLE IV. CHANGES IN WATER ACTIVITY OF SEMI-DRIED PORK WITH DIFFERENT PRODUCT MOISTURE CONTENTS AND IRRADIATION TREATMENTS DURING 5 MONTHS OF STORAGE UNDER AMBIENT CONDITIONS

	Month	Values											
		Irsp-ir			Nirsp-ir			Irsp-nir			Nirsp-nir		
		18.40 ± 0.39%	23.02± 0.37%	24.65± 0.53%	18.40 ± 0.39%	23.02± 0.37%	24.65± 0.53%	18.40 ± 0.39%	23.02± 0.37%	24.65± 0.53%	18.40 ± 0.39%	23.02± 0.37%	24.65± 0.53%
a_w	0	0.634	0.758	0.764	0.634	0.764	0.764	0.634	0.758	0.764	0.634	0.758	0.764
	4	0.610	0.680	0.690	0.600	0.690	0.700	0.590	0.680	0.690	0.600	0.690	0.690
	5	0.590	0.670	0.690	0.590	0.670	0.680	0.580	0.660	0.680	0.580	0.660	0.680

TABLE V. EFFECT OF SPICE IRRADIATION AND PRODUCT MOISTURE CONTENT ON MICROBIAL POPULATION OF SEMI-DRIED PORK STORED AT AMBIENT CONDITIONS FOR UP TO 5 MONTHS

	Month	Values											
		Irsp-ir			Nirsp-ir			Irsp-nir			Nirsp-nir		
		18.40 ± 0.39%	23.02 ± 0.37%	24.65 ± 0.53%	18.40 ± 0.39%	23.02 ± 0.37%	24.65 ± 0.53%	18.40 ± 0.39%	23.02 ± 0.37%	24.65± 0.53%	18.40 ± 0.39%	23.02 ± 0.37%	24.65 ± 53 %
TPC	0	ND	ND	ND	ND	50 ± 0	30 ± 0	50 ± 0	90 ± 14	190 ± 14	75 ± 7	120 ± 0	245 ± 7
	1	10 ± 0	5 ± 7	20 ± 14	ND	10 ± 0	10 ± 0	160 ± 0	180 ± 0	105 ± 21	125 ± 21	155 ± 7	250 ± 7
	2	ND	10 ± 0	20 ± 0	ND	10 ± 0	15 ± 7	50 ± 17	170 ± 0	230 ± 28	160 ± 28	205 ± 7	300 ± 0
	3	ND	ND	10 ± 0	ND	ND	ND	45 ± 7	100 ± 0	245 ± 7	225 ± 21	250 ± 14	450 ± 70
	4	15 ± 7	15 ± 7	25 ± 7	ND	ND	10 ± 0	10 ± 0	335 ± 35	305 ± 7	25 ± 7	150 ± 14	705 ± 35
	5	10 ± 0	13 ± 12	10 ± 0	ND	ND	ND	53 ± 9	243 ± 9	383 ± 12	16 ± 4	350 ± 24	465 ± 12
Yeasts and moulds	0	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	1	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	2	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	3	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	4	10 ± 0	20 ± 0	10 ± 0	15 ± 7	10 ± 0	30 ± 0	0 ± 0	25 ± 7	15 ± 7	25 ± 7	25 ± 7	25 ± 7
	5	5 ± 0	20 ± 0	10 ± 0	10 ± 8	35 ± 5	0 ± 0	0 ± 0	20 ± 8	23 ± 4	26 ± 4	26 ± 4	13 ± 4

4.2.3. Microbiological quality

The experimental work showed that irradiation treatment had an effect on the total bacterial count. Irsp-ir and nirsp-ir products exposed to irradiation after packing rarely showed microbial activity while the initial count of irsp-nir product is less detectable than that of nirsp-nir products. These findings indicated that one source of microbial contamination was spice. Irradiating the spice was, therefore, an approach to reduce the initial microbial load. For the nirsp-ir product, although non-irradiated spice was used in the process, irradiation was applied afterwards thus reducing bacterial activity of products from around 50–245 CFU/g to 0–50 CFU/g.

Moisture content seemed to affect microbial activity. As shown in Table V, the TPC of irsp-nir product at moisture contents of 18.40 ± 0.39%, 23.02 ± 0.37% and 24.65 ± 0.53% were 50, 90 and 190 CFU/g, respectively, and those of nirsp-nir products at moisture contents of 18.40 ± 0.39%, 23.02 ± 0.37% and 24.65 ± 0.53% were 75, 120 and 245 CFU/g, respectively. The greater the moisture content of the product, the greater the amount of colonies detected. This was because there was more available water as solvent and medium for microbial growth. Although moisture content seemed to affect microbial activity, irradiation could greatly reduce microbial load in all semi-dried pork products at the beginning and thus maintain their microbial safety during 5 months of storage. At all moisture levels, TPCs of nirsp-nir and irsp-nir products were rarely detected while they increased slightly during storage. However, the TPC did not exceed $\log_{10}$ 7.0 CFU/g which was the value used for determining the termination of storage for the food products. This meant that a low moisture content contributed to the prevention of microbial activities in both products until the end of storage. Microbial populations could be found in irsp-ir and nirsp-ir products during storage but the activities were less than 50 CFU/g. This may have resulted from the germination or growth of microbial spores which were more resistant to irradiation than vegetative cells [36].

Considering the effect of spice irradiation, at all moisture levels of non-irradiated semi-dried pork, the microbial population in irsp-nir products was lower than in nirsp-nir products during storage. This meant that before being added into the semi-dried pork products, spice treated with irradiation had a lower microbial load and thus shelf-life of products could be extended. However, the effect of spice irradiation was barely noticeable when irradiation was applied afterwards to semi-dried pork products. During storage, due to changes in product characteristics, i.e. a_w, pH, and storage conditions as oxygen was present in the packaging, the microbial growth pattern was altered. At the fourth month of storage, yeasts and moulds became predominant. The reduction of a_w of semi-dried pork to around 0.58–0.69 facilitated growth of microorganisms as well as osmophilic yeasts and xerophilic moulds, which have a minimum a_w for growth at 0.60. In addition, pH was slightly reduced from 6.01–6.08 to 5.75–5.87 at the end of storage.

The pH has an effect on microorganisms. From these results, the application of irradiation (6 kGy) reduced the bacterial population remarkably; hence prolonging the shelf-life of treated semi-dried pork to 5 months or more. In addition, the use of irradiation has been suggested as a method for eliminating or reducing contamination of foods by bacterial pathogens. Since 50 to 70% of the bacterial cell mass is water, it absorbs much of the irradiation making the microorganisms susceptible to inactivation.

4.2.4. Lipid oxidation

The level of rancidity in the irradiated meat samples was expected to rise as a result of oxidation. However, it was found that there was no difference between irradiated and non-irradiated treatments (Table VI), i.e. the TBA values of both irsp and nirsp-ir products were less than 0.2 being no different from those of irsp-nir and nirsp-nir products at all moisture levels. These results showed that irradiation had no significant effect on meat lipids. During 5 months of storage, TBA values remained relatively unchanged. This was because all products were packed under modified atmosphere conditions (N_2 80%, CO_2 20%) and the packaging film used was a good barrier for O_2 and

water vapour. These products also had a low moisture level (18–25%), therefore, the oxidation reaction caused by O_2 and water were limited.

A major concern of treating meat with ionizing radiation is its effect on meat quality mainly because of free radical reactions and possible off-odour generation during irradiation. Ionizing radiation generates hydroxyl radicals in aqueous or oil emulsion systems. Because most muscle cells (75%) are composed of water and surrounded by lipid bilayers, irradiating meat may produce hydroxyl radicals leading to lipid oxidation. Any development of lipid oxidation in irradiated raw or cooked meat would be influenced by packaging, storage and other processing conditions before and after irradiation. Murano et al. [25] also reported no difference in lipid oxidation within the first week of storage, regardless of packaging atmosphere, between irradiated (2 kGy) and non-irradiated ground beef patties. However, after week one, higher TBA values were observed in samples irradiated and stored in air, as well as in those irradiated under vacuum but stored in air.

4.2.5. Texture

The products from all treatments maintained their tenderness after storing for 5 months. Irsp-ir, nirsp-ir, irsp-nir and nirsp-nir products had hardness values of 2.56–3.05, 1.86–2.73 and 1.67–2.46 kN for the product treatments at 18.40 ± 0.39%, 23.02 ± 0.37% and 24.65 ± 0.53% moisture content, respectively. Hardness of semi-dried pork samples with all irradiation treatments was quite constant during 5 months of storage, especially with medium and high moisture content level. This was due to the fact that the film used in this study is water-vapour resistant, therefore, the products could maintain their moisture and hence their tenderness. However, there were some fluctuating values of hardness, which may be evaluated from different parts of meat muscle such as lean meat or fat tissue. The previous findings on tenderness of irradiated meat have been varied. Hashim et al. [37] reported that cooked irradiated chicken (1.66–2.86 kGy) was more tender while Luchsinger et al. [10] found no difference between irradiated and control samples for overall acceptance, meatiness, freshness, tenderness, or juiciness in cooked pork chop (2.5 kGy under vacuum packaging).

4.2.6. Colour

The difference in the treatments resulted in the same trend for the colour attributes. At the beginning of the experiment, irsp-ir, nirsp-ir, irsp-nir and nirsp-nir products at all moisture levels gave colour parameters, i.e. L, a and b values, in the range of 55.56–57.54, 0.89–2.24 and 0.22–1.69, respectively, which meant that irradiation treatment had no effect on product colour. Fu et al. [8] also reported that Hunter Lab values and visual evaluation scores showed no colour difference between control and irradiated (1.5 kGy) beefsteaks.

TABLE VI. EFFECT OF SPICE IRRADIATION AND PRODUCT MOISTURE CONTENT ON LIPID OXIDATION OF SEMI-DRIED PORK STORED AT AMBIENT CONDITIONS FOR UP TO 5 MONTHS

Month	TBA Values (mg malonaldehyde/ g sample)											
	Irsp-ir			Nirsp-ir			Irsp-nir			Nirsp-nir		
	18.40 ± 0.39%	23.02± 0.37%	24.65± 0.53%	18.40 ± 0.39%	23.02± 0.37%	24.65± 0.53%	18.40 ± 0.39%	23.02± 0.37%	24.65± 0.53%	18.40 ± 0.39%	23.02± 0.37%	24.65± 0.53%
0	0.09	0.09	0.09	0.13	0.09	0.10	0.07	0.10	0.10	0.15	0.14	0.16
1	0.11	0.15	0.15	0.12	0.15	0.19	0.11	0.14	0.06	0.13	0.14	0.10
2	0.06	0.11	0.07	0.08	0.09	0.10	0.07	0.09	0.07	0.05	0.07	0.08
3	0.07	0.08	0.09	0.09	0.08	0.08	0.07	0.09	0.07	0.08	0.09	0.10
4	0.06	0.04	0.06	0.06	0.06	0.05	0.05	0.06	0.04	0.04	0.04	0.05
5	0.07	0.09	0.08	0.09	0.10	0.09	0.07	0.05	0.05	0.04	0.04	0.06

An America Meat Institute Foundation (AMIF) [38] study also reported that the colour of aged chilled beefsteaks was not affected by irradiation.

In contrast, an increase in the redness of pork colour due to irradiation has been reported [9]. For processed meat such as semi-dried pork, the unchanged colour values after irradiation were observed due to its original colour and ingredients added. The original colour was brown and did not distinctly exhibit redness. However, the colour attributes changed as the storage time increased. The L parameter decreased to 25.45−29.52 while a and b parameters increased to 4.93−8.1 and 3.12−7.86, respectively. During 5 months of storage, the L attribute decreased slightly until the second month and then remained relatively unchanged through the rest of storage period. The same trend was observed for a and b values.

4.2.7. Sensory attributes

Results of sensory evaluation are presented in Table VII. The panel members found that the qualities of all product treatments at the beginning were comparable to those after 5 month storage. The product colour ranged from yellow brown for all treatments at 23.02 ± 0.37% and 24.65 ± 0.53% moisture content to dark red brown for all treatment at 18.40 ± 0.39% moisture content. However, both shades were the natural colour of semi-dried pork and were acceptable by consumers. All products provided good flavour and taste resulting from the ingredients and raw materials. No irradiation flavour, oxidation flavour or taint were detected. The product texture was firm and the panellists seemed to prefer the samples prepared at 18.40 ± 0.39% moisture content which is close to semi-dried pork commercially sold. This may due to the fact that panellists got used to the dry texture of traditional samples and these three levels of moisture content were too close to distinguish their texture. During storage, almost all of the scores for all irradiated treatments and sensory attributes of semi-dried pork were slightly reduced. The fluctuation of scores may be due to non-uniformity of samples and variation of the judgement of the panellists. A remarkable decrease was observed for tenderness, especially in irradiated semi-dried pork with a low moisture content. Considering other attributes such as colour, flavour, texture and overall acceptability, irradiated semi-dried pork exhibited minimal sensory changes with no detectable differences between treated and control samples after 5 months of storage.

4.3. Effect of irradiation on film properties

In the study of shelf-life extension of smoked sausages, the properties of the films used were tested according to their tear and tensile strengths, thickness and gas and water vapour transmission rates (Table VIII). The NPI film used for vacuum packaging and PVNP for MAP were irradiated at 0, 2 or 4 kGy with smoked sausage samples. The variations in film properties were used to relate the quality changes observed in the processed meats.

Initial properties of films were found to change after the storage period. Although different packaging materials were used for different packaging methods, tests showed distinct variations in the initial and final properties of each film. Elongation and tear strengths varied with direction: longer elongation was observed for samples cut in the machine direction (MD) than in the cross direction (CD). Tensile strengths, on the other hand, had a reverse relationship. Permeability, expressed in transmission rates, varied with the type of packaging materials. Among the films used, NPI exhibited the least gas permeability with a gas transmission rate of 5.72 m^3/m^2/day, followed by LDPE with 6.07 m^3/m^2/day and PVNP with 6.67 m^3/m^2/day.

TABLE VII. EFFECT OF SPICE IRRADIATION AND PRODUCT MOISTURE CONTENT ON THE SENSORY PROPERTIES OF SEMI-DRIED PORK STORED AT AMBIENT CONDITIONS FOR UP TO 5 MONTHS

	Month	Scores											
		Irsp-ir			Nirsp-ir			Irsp-nir			Nirsp-nir		
		18.40 ± 0.39%	23.02 ± 0.37%	24.65 ± 0.53%	18.40 ± 0.39%	23.02 ± 0.37%	24.65 ± 0.53%	18.40 ± 0.39%	23.02 ± 0.37%	24.65 ± 0.53%	18.40 ± 0.39%	23.02 ± 0.37%	24.65 ± 0.53%
Colour	0	4.4 ± 0.5	3.7 ± 0.5	3.6 ± 1.4	4.9 ± 0.3	3.6 ± 1.3	4.4 ± 0.7	4.9 ± 0.3	4.9 ± 0.3	4.7 ± 0.7	4.9 ± 0.3	4.8 ± 0.6	4.2 ± 1.2
	2	3.8 ± 1.0	4.3 ± 0.8	4.2 ± 0.8	4.2 ± 0.8	4.1 ± 0.9	4.3 ± 0.7	4.2 ± 0.9	4.5 ± 0.8	4.7 ± 0.5	4.6 ± 0.5	4.1 ± 0.7	4.4 ± 0.7
	4	3.8 ± 1.0	4.3 ± 0.8	4.2 ± 0.8	4.2 ± 0.5	4.1 ± 0.8	4.3 ± 0.7	4.1 ± 0.9	4.5 ± 0.8	4.7 ± 0.5	4.6 ± 0.5	4.1 ± 0.7	4.4 ± 0.7
	5	4.1 ± 0.6	3.7 ± 0.5	3.6 ± 0.7	4.0 ± 0.8	3.6 ± 0.7	3.5 ± 0.5	4.5 ± 0.5	4.6 ± 0.7	3.6 ± 0.8	4.0 ± 0.8	4.1 ± 0.7	4.1 ± 0.9
Flavour	0	4.4 ± 0.7	4.4 ± 0.7	4.4 ± 0.5	4.4 ± 0.5	4.4 ± 0.9	4.4 ± 0.7	4.3 ± 0.5	4.7 ± 0.7	4.7 ± 0.7	4.6 ± 0.7	4.8 ± 0.6	4.3 ± 0.7
	2	3.7 ± 0.8	4.3 ± 0.5	4.5 ± 0.5	4.5 ± 0.7	4.4 ± 0.5	4.1± 0.9	4.1 ± 0.7	4.3 ± 0.5	4.3 ± 0.5	4.2 ± 0.6	4.3 ± 0.9	3.9 ± 0.6
	4	3.7 ± 0.8	4.3 ± 0.5	4.5 ± 0.5	4.5 ± 0.7	4.4 ± 0.5	4.1 ± 0.9	4.0 ± 0.7	4.3 ± 0.5	4.3 ± 0.5	4.2 ± 0.6	4.3 ± 0.9	3.9 ± 0.6
	5	4.6 ± 0.7	4.0 ± 0.7	4.1 ± 0.9	4.3 ± 0.5	4.2 ± 0.6	4.0 ± 0.9	4.5 ± 0.5	4.5 ± 0.7	4.5 ± 0.5	4.5 ± 0.5	4.0 ± 0.5	4.1 ± 0.9
Tenderness	0	4.3 ± 0.5	4.2 ± 0.9	4.0 ± 1.5	3.6 ± 0.7	3.9 ± 1.2	3.4 ± 1.4	3.0 ± 1.1	4.3 ± 0.7	4.7 ± 0.7	2.8 ± 1.3	4.6 ± 0.7	3.0 ± 1.2
	2	3.1 ± 1.6	4.2 ± 0.9	4.4 ± 0.9	4.0 ± 1.1	3.5 ± 1.2	4.5 ± 0.7	3.1 ± 1.3	3.6 ± 0.9	4.1 ± 1.1	3.7 ± 0.9	4.3 ± 0.9	4.2 ± 0.9
	4	3.1 ± 1.6	3.7 ± 0.8	4.4 ± 0.9	3.5 ± 1.2	4.0 ± 1.1	4.5 ± 0.7	3.0 ± 1.3	3.6 ± 0.9	4.1 ± 1.1	3.7 ± 0.9	4.3 ± 0.9	4.2 ± 0.9
	5	3.2 ± 1.2	3.8 ± 1.1	4.2 ± 1.0	2.5 ± 1.4	3.7 ± 1.3	4.2 ± 0.9	2.9 ± 0.9	4.8 ± 0.2	4.3 ± 0.9	3.4 ± 1.3	3.4 ± 1.5	4.0 ± 0.9
Texture	0	4.2 ± 0.8	4.3 ± 0.9	3.6 ± 1.2	4.4 ± 0.5	4.3 ± 0.7	4.0 ± 0.9	4.6 ± 0.7	4.4 ± 0.7	4.2 ± 0.6	4.3 ± 0.9	4.2 ± 0.9	3.8 ± 1.3
	2	3.7 ± 0.9	4.2 ± 0.9	4.0 ± 1.2	4.4 ± 0.8	4.2 ± 0.8	3.8 ± 0.8	4.1 ± 0.6	4.3 ± 0.8	4.1 ± 0.9	4.4 ± 0.5	4.2 ± 0.8	3.9 ± 0.9
	4	3.7 ± 0.9	4.2 ± 0.9	4.3 ± 1.1	4.4 ± 0.8	4.2 ± 0.8	3.8 ± 0.8	4.1 ± 0.6	4.3 ± 0.8	4.1 ± 0.9	4.4 ± 0.5	4.2 ± 0.8	3.9 ± 0.9
	5	4.1 ± 0.9	3.7 ± 0.8	3.7 ± 0.8	3.9 ± 0.3	3.7 ± 0.9	3.4 ± 0.8	4.2 ± 0.9	4.2 ± 0.8	3.9 ± 0.7	4.4 ± 0.7	4.4 ± 0.5	3.9 ± 0.9
Acceptability	0	4.3 ± 0.5	4.3 ± 0.7	3.9 ± 1.2	3.9 ± 0.9	4.2 ± 0.9	4.2 ± 0.9	4.4 ± 0.7	4.6 ± 0.7	4.8 ± 0.6	3.8 ± 1.0	4.4 ± 0.9	4.1 ± 0.9
	2	3.6 ± 0.9	4.1 ± 0.6	4.1 ± 0.7	4.2 ± 1.0	4.0 ± 0.5	4.1 ± 0.6	3.8 ± 0.6	4.4 ± 0.8	4.3 ± 0.8	4.2 ± 0.6	4.4 ± 0.7	4.0 ± 0.7
	4	3.6 ± 0.9	4.1 ± 0.6	4.1 ± 0.7	4.2 ± 1.0	4.0 ± 0.5	4.1 ± 0.6	3.8 ± 0.7	4.4 ± 0.8	4.3 ± 0.8	4.2 ± 0.6	4.4 ± 0.7	4.0 ± 0.7
	5	4.1 ± 0.9	4.2 ± 0.8	4.3 ± 0.9	3.9 ± 0.9	4.0 ± 0.7	3.5 ± 0.8	4.2 ± 0.4	4.9 ± 0.3	4.5 ± 0.7	4.1 ± 0.9	3.9 ± 0.6	4.1 ± 0.9

TABLE VIII. EFFECT OF IRRADIATION ON FILM PROPERTIES USED FOR SMOKED SAUSAGE PRODUCT STORED IN CHILLED CONDITIONS

Film Properties	Control (LDPE)		Vacuum packaging				Control (LDPE)		MAP			
	Initial Cond'n	Final Cond'n	Initial Cond'n	Final Condition			Initial Cond'n	Final Cond'n	Initial Cond'n	Final Condition		
				0 kGy	2 kGy	4 kGy				0 kGy	2 kGy	4 kGy
Thickness, mm	0.0530	0.061	0.076	0.075	0.075	0.076	0.053	0.056	0.089	0.089	0.09	0.09
Tensile Strength, kg/m^2												
cross direction	235.76	204.70	561.88	504.55	508.75	508.15	235.76	207.88	420.79	397.23	388.70	396.90
machine direction	206.29	183.97	423.53	390.39	369.26	369.43	206.29	181.03	459.51	432.70	398.50	405.10
Elongation, mm												
cross direction	313.33	383.33	37.43	31.67	33.33	30.54	313.33	275.00	36.67	40.00	40.00	36.67
machine direction	182.96	220.00	63.33	65.00	51.67	55.00	182.96	163.33	38.33	33.33	35.00	31.67
Tear resistance, gm												
cross direction	980.50	950.48	32.00	30.50	32.00	31.88	980.50	960.00	72.00	82.32	82.00	95.36
machine direction	856.25	830.00	37.42	33.34	32.38	34.86	856.25	842.88	78.88	86.64	83.36	86.32
Transmission rates												
water vapour, g/m^2/day	4.320	11.040	19.680	23.040	12.480	2.880	4.320	7.200	5.280	5.760	4.92	3.84
oxygen, m^3/m^2/day	6.065	7.140	5.722	7.565	5.602	9.849	6.065	7.189	6.666	7.477	5.14	4.74

These differences were influenced by the various properties of films laminated together to form these packaging materials, such as nylon which is stable at high temperatures and is reported to be impermeable to gas. Polyethylene film is soft and flexible but water impermeable [39]. Whereas, ionomer resin is heat stable and chemically inert, nylon with polyethylene is highly resistant to grease, oil, water vapour and gas.

Although the properties differed initially, at the end of the storage period when films were exposed to low temperatures and high humidity, all underwent changes. Tensile strengths were reduced as well as elongation and tear strengths. Transmission rates increased due to the exposure of films to high humidity conditions [40,41]. The application of irradiation reduced the film permeability. The higher the irradiation dose, the larger the decrease in film performance. These results showed that the energy produced during the irradiation process can really effect film performance although some studies reported that irradiation had no effect on packaging materials at doses below 20 kGy [42]. When semi-dried pork packed in NPI vacuum pouches was irradiated at 6 kGy, the changes of film properties in terms of tear, tensile strength and elongation during 5 months of storage at ambient condition were shown and are presented in Table IX. Initial properties of films were found to change after irradiation with 6 kGy. Elongation and tear strengths varied with direction: longer elongation was observed with samples cut in machine direction (MD) than in cross direction (CD). Tensile strengths, on the other hand, exhibited a reverse relationship. Tear and tensile strength of film were decreased after irradiation from 34.0 to 33.6 gf and 389.5 to 368.5 kgf/cm^2, respectively. Elongation, on the other hand, had a reverse relationship. It was found to increase from 86.6 to 93.95% after irradiation.

During storage films treated with irradiation showed greater property changes, especially in elongation, compared to films without irradiation. However, there was no striking changes in film properties during storage. Due to the small changes of film properties shown in these result, it can be assumed that the energy produced during irradiation did not affect film performance which was comparable with the work of Desrosier [42] who reported that irradiation has no effect on packaging materials at doses below 20 kGy.

4.4. Shelf-life extension of semi-dried shrimp by irradiation

For better palatability, semi-dried shrimp was prepared with a higher moisture content than commercial products by reducing drying time. The final moisture content of the semi-dried shrimp was around 36% compared to the 20–25% of dried shrimp sold in markets. The increase of moisture content would make semi-dried shrimp more susceptible to spoilage. When a_w was considered, semi-dried shrimp was classified as IM food due to the range of a_w around 0.6617–0.6650. During storage for 7 weeks, the moisture content and a_w of products were almost constant because foods did not take up or give up moisture to the surrounding environment due to the protection afforded by the packaging materials.

Within this range of a_w, semi-dried shrimp was prone to spoilage by yeasts and moulds whose optimum a_w is 0.60. A TPC and mould count of dried shrimp of not more than 5 and 2.301 $\log_{10}$ CFU/g is used as the shelf-life index. As can be observed in Table X for the fungal count, control sample were spoiled around 3 weeks of storage while the shelf-life of 2 and 4 kGy irradiated samples were 5 weeks and more than 7 weeks of storage, respectively.

TABLE IX. EFFECT OF IRRADIATION ON FILM PROPERTIES USED SEMI-DRIED PORK PRODUCT PACKED IN NPI VACUUM POUCHES AND STORED IN CHILLED CONDITIONS

Film properties	Non-irradiated sample at storage time (months)						Irradiated sample at storage time (months)					
	0	1	2	3	4	5	0	1	2	3	4	5
Tear strength (gf)												
machine direction	34.0	33.0	41.0	34.0	30.6	41.6	35.2	37.0	38.0	34.0	40.0	41.6
cross direction	34.0	32.0	35.0	31.4	36.0	39.2	32.0	32.0	33.0	31.0	34.0	38.8
Average	34.0	32.5	38.0	32.7	33.3	40.4	33.6	34.5	35.5	32.5	37.0	40.2
Tensile strength (kgf/cm^2)												
machine direction	381	398	379	393	372	371	375	359	386	368	346	379
cross direction	398	373	380	395	371	355	362	378	365	375	365	376
Average	389.5	385.5	379.5	394	371.5	363	368.5	368.5	375.5	371.5	355.5	377.5
Elongation (%)												
machine direction	86.7	93.3	89.2	89.0	82.7	90.0	90.4	80.4	88.9	87.9	72.0	88.0
cross direction	86.5	70.8	75.4	78.5	74.7	71.0	97.5	77.9	67.7	73.5	72.7	76.0
Average	86.6	82.05	82.3	83.75	78.7	80.5	93.95	79.15	78.3	80.7	72.35	82.0

TABLE X. EFFECT OF IRRADIATION ON MICROBIAL POPULATION OF SEMI-DRIED SHRIMP DURING STORAGE AT AMBIENT CONDITIONS

Types	Storage time (days)	Microbial population (Log_{10} CFU/g)		
		Control	2 kGy	4 kGy
TPC	1	3.1271	1.8451	1.7782
	4	2.6232	1.7404	1.3010
	7	3.1790	1.4771	1.1761
	10	2.6675	1.8129	1.3979
	14	2.4065	1.3979	<1
	21	2.5052	1.1761	<1
	35	2.4914	1.1761	<1
	49	2.3802	1.1761	<1
Yeasts and moulds	1	2.000	<1	<1
	7	2.176	1.699	1.699
	14	2.061	1.699	<1
	21	2.176	1.699	<1
	35	3.544	2.176	2
	49	3.550	2.699	2
E. coli	1	Neg.	Neg.	Neg.
Staphylococcus spp.	4	Neg.	Neg.	Neg.
Salmonella spp.	7	Neg.	Neg.	Neg.

Neg. = negative results

TABLE XI. EFFECT OF IRRADIATION ON SENSORY QUALITY OF SEMI-DRIED SHRIMP DURING STORAGE AT AMBIENT CONDITIONS

Storage time (days)	Overall acceptability		
	Control	2 kGy	4 kGy
1	7.917	7.750	7.500
4	8.083	8.083	7.750
7	6.250	7.833	7.833
10	5.250	7.583	7.667
14	3.417	7.333	7.500
21	2.333	7.083	7.667
28	–	6.250	7.083
35	–	5.167	6.917
42	–	3.583	6.333
49	-	–	5.583

However, sensory qualities should be considered when shelf-life is determined. From Table XI, although at the beginning the sensory score of control samples was higher than irradiated samples, with the progress of storage, the sensory qualities deteriorated faster in the control than 2 kGy and 4 kGy irradiated samples. Consequently, panellists rejected the control product after 10 days of storage. This was due to an acceptability score of 5.250, which was neither representative of the like nor dislike trend (scores 5–6). On the other hand, the 2 and 4 kGy irradiated samples were accepted before 35 and 49 days of storage with acceptability scores of 5.167 and 5.583 respectively.

When chemical changes of TVN, which was an index of freshness, and TBA, which showed the degree of lipid oxidation, were considered, both values were found to increase during storage (Table XII). The oxidation occurred due to the presence of oxygen in the packages. With normal packaging lipid oxidation could not be prevented and from the data of Ahn et al. [23] the oxygen exposure of products after cooking has more influence on lipid oxidation than packaging, irradiation, or storage condition. For irradiation effect, the irradiated samples showed higher TBA values than the non-irradiated samples. Lipid oxidation may be dose-dependent as can be seen from the higher TBA values for the 4 kGy irradiated samples than samples given 2 kGy. These results correlated well with the findings of Katusin-Razem et al. [43].

TABLE XII. EFFECT OF IRRADIATION ON CHEMICAL CHANGES OF SEMI-DRIED SHRIMP DURING STORAGE AT AMBIENT CONDITIONS

	Storage time (days)	Irradiation dose (kGy)		
		0	2	4
pH	1	7.88	7.91	7.90
	4	7.98	8.00	8.04
	7	7.91	7.88	7.91
	10	7.89	7.92	7.93
	14	8.18	8.13	8.18
	21	8.00	7.95	7.96
	35	–	7.78	7.85
	49	–	7.77	7.76
TVN	1	43.380	38.843	38.053
	4	50.216	48.117	49.094
	7	44.439	54.292	54.254
	10	43.873	48.694	53.371
	14	48.932	47.637	59.283
	21	54.289	58.504	59.427
	35	–	55.886	54.255
	49			
TBA	1	0.810	0.953	0.820
	4	0.765	0.872	0.899
	7	1.138	1.128	1.242
	10	1.790	1.659	1.397
	14	1.824	1.690	1.425
	21	1.352	2.004	2.113
	35	-	2.892	3.023
	49	-	3.149	3.404

Texture and colour qualities influenced consumer acceptability. From Table XIII, hardness of semi-dried shrimp treated with or without increased during storage from 3152.67–5394.23, 3104.69–5311.20 and 3323.01–5403.29 for control, 2 and 4 kGy irradiated products, respectively. The hardness of muscle food may be due to loss of water to the storage environment. With regard to colour (Table XIII), lightness of semi-dried shrimp (‘L’ value) showed a small decrease during storage but it was not significantly changed. A reduction of redness (‘a’ value) of all samples during storage was observed. The changes of redness were higher in control samples compared with irradiated products. Astaxanthin is the major pigment subjected to oxidation in dried shrimp [44].

TABLE XIII. EFFECT OF IRRADIATION ON TEXTURE AND COLOUR OF SEMI-DRIED SHRIMP DURING STORAGE AT AMBIENT CONDITIONS

	Storage time (days)	Irradiation dose (kGy)		
		0	2	4
Texture (g force)	1	3152.67	3104.69	3323.01
	4	3361.93	3297.38	3324.45
	7	3515.66	3345.38	3605.39
	14	4024.08	3631.10	3804.78
	21	4023.56	4207.36	4428.54
	35	5394.23	5875.15	5791.32
	49	-	5311.20	5403.29
Colour				
‘L’	1	43.39	42.64	42.99
	4	44.72	43.69	44.47
	7	44.82	45.88	43.44
	14	4201	42.40	42.67
	21	42.54	40.95	41.69
	35	–	41.21	42.40
‘a’	1	14.18	12.91	12.52
	4	10.21	10.64	11.62
	7	11.05	9.86	9.87
	14	10.18	9.47	8.73
	21	8.46	ND	ND
	35	–	7.73	8.72
‘b’	1	15.15	14.37	14.00
	4	13.85	13.88	14.03
	7	15.31	15.16	14.63
	14	13.82	13.89	14.59
	21	14.08	ND	ND
	35	–	15.14	14.00

ND = not determined, — = sample was already spoiled

5. CONCLUSIONS

Using irradiation combined with packaging extended the shelf-life of smoked sausages stored at 5° C from 6 weeks to more than 2 months with a minimum dose of 2 kGy. The shelf-life of sliced ham stored at 2° C increased to 6 weeks and more than 2 months using doses of 2 and 4 kGy, respectively compared to one week for controls. Semi-dried pork was relatively stable with a shelf-life of more than 8 months. It was found that the higher the irradiation dose, the longer the shelf-life. However, irradiation caused semi-dried pork to become susceptible to rancidity and also some fluctuations in colour characteristics. It also resulted in a higher drip loss in sliced ham but not in smoked sausages.

When semi-dried pork with a higher moisture content and added spice were irradiated, samples given all treatments were microbiologically safe and of good quality after 5 months of storage with MAP. Irradiation of spice was used as an approach to reduce the initial load of final products. Irradiation did not affect product tenderness and colour. However, the darker trend of colour was observed during storage. Products exhibited increased redness until 2 months of storage. Samples showed no rancidity with all treatments. From sensory testing, no irradiation flavour, oxidation flavour

or taint was detected by taste panellists. Taste panel members seemed to prefer the samples with 18% moisture content because they became accustomed to the dry and tough texture recognised as semi-dried pork. Therefore, the semi-dried pork products with high moisture content (>16–18%) should be promoted as new kinds of meat products. Regarding film packaging, due to the high humidity conditions in storage, the properties of films, i.e. tensile and tear strengths, decreased after the storage period. Irradiation was able to decrease the tensile strength of NPI while fluctuations were observed in PVNP films. Transmission rates for gas and water vapour through NPI and PVNP decreased with irradiation dose. Elongation decreased after irradiation. However, the small changes in film properties confirmed that irradiation with low-doses does not effect the packaging material.

For semi-dried shrimp, irradiation at 2 and 4 kGy could inhibit microbiological spoilage from 3 weeks to 5 weeks and more than 7 weeks, respectively. With regard to sensory scores, the 2 and 4 kGy samples were acceptable before 35 and 49 days of storage compared to 10 days for control samples. During storage, TBA and TVN values increased. Due to the loss of water to the environment, the hardness of semi-dried shrimp increased. The redness of semi-dried shrimp decreased as storage time progressed due to oxidation of astaxanthin. However, the changes were less significant in irradiated samples.

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A STUDY ON THE USE OF IRRADIATION IN COMBINATION WITH VACUUM PACKAGING TO PRODUCE NON-STERILE SHELF-STABLE FOOD, SEMI-DRIED MEAT, SEAFOOD AND VEGETABLES

X. LUO, S. CUI, Y. LI, Y. LI, T. JIANG
Department of Microbiology and Mycology,
Institute of Food Safety Control and Inspection, Ministry of Health,
Beijing, China

Abstract

This study was conducted in order to determine the minimum irradiation doses required to inactivate all spoilage microorganisms, non-spore and spore pathogenic microorganisms in shelf-stable foods, semi-dried pork, fish, bean curd and pickle which have been vacuum packaged. The water activity (a_w) of semi-dried food products ranged between 0.930–0.940 for pork, 0.852–0.895 for fish, 0.918–0.934 for bean curd and 0.908–0.915 for pickle. The semi-dried food products were inoculated with 40, 400 and 400 cells/g of *Staphylococcus aureus, Bacillus cereus* and *Salmonella enteritidis*, respectively. The irradiation doses used in the study were 0, 1, 2, 2.5, 5, 7.5, 10 and 20 kGy. Results showed that the minimum irradiation dose required to inactivate all spoilage microorganisms in semi-dried pork, fish and bean curd was 10 kGy, while all spoilage microorganisms in semi-dried pickle could be inactivated at a dose of 15 kGy. *S. enteritidis* in semi-dried fish, bean curd and pickle could be eliminated at a dose of 2.5 kGy and the minimum irradiation dose required to inactivate this pathogen in pork was 5 kGy. *S. aureus* in semi-dried pork, fish and pickle was inactivated using a dose of 2 kGy while 2.5 kGy could inactivate this pathogen in semi-dried bean curd. *B. cereus* was more resistant to irradiation. A dose of 5 kGy was required to inactivate this pathogen in semi-dried pork and pickle while 7.5 kGy was necessary for fish and bean curd.

1. INTRODUCTION

The contamination of foods, especially those of animal origin, with pathogens is a significant health problem and an important cause of human suffering in China [1-4]. According to a report made in 1999 by the Department of Legislation and Inspection, Ministry of Health, China, food poisoning outbreaks occurred from January to October with *Salmonella* spp., *Vibrio parahaemolyticus*, *Staphylococcus* spp., *Proteus*, pathogenic *Colibacillus*, including spore formers such as *Bacillus cereus* and *Clostridium botulinum*, being the major food poisoning pathogens [5]. Shelf-stable and ready-to-eat foods have become very popular in China in recent years due to their convenience. These kinds of foods can be stored and marketed without refrigeration requiring minimum preparation and cooking prior to consumption. They are of particular interest to China in view of the inadequate infrastructure in cold chain and freezing facilities. There can be significant savings made in energy costs when foods are stored and marketed at ambient temperature. However, such food products can often be contaminated with potentially pathogenic microorganisms during processing/handling resulting in a health hazard to consumers. Development of a technology that can ensure the safety and maintain the quality of ready-to-eat food products under ambient storage conditions will be of great economic and health significance. The application of irradiation technology in combination with vacuum packaging is one of the most effective approaches to ensure the safety of ready-to-eat foods.

2. MATERIALS AND METHODS

2.1. Source and preparation of samples

Shelf-stable and ready-to-eat foods, including semi-dried pork, fish (approximately 50 g each), bean curd (0.5 cm × 5 cm × 5 cm) and pickle were purchased from the local market. Semi-dried pork was cut into slices of approximately 1 cm × 3 cm.

2.2. Inoculation and packaging procedures

S. enteritidis No.50041, *S. aureus* No.ATCC 6538 and *B. cereus* No.44001 were obtained from the Institute of Biological Products and Pharmaceutical Inspection, Ministry of Health, China.

These strains were cultured in Nutrient Agar at 36°C for 48 h. The cultures were washed and diluted with sterile saline to yield a suspension of ca. 10^5 CFU/ml for *S. enteritidis,* 10^4 CFU/ml for *B. cereus* and 10^3 CFU/ml for *S. aureus.* A 1 mL aliquot of each suspension was inoculated into 25 g samples so that they contained 40, 400 and 4000 cells/g for each strain, respectively. The food samples were then vacuum packed in flexible pouches (polyester/polyethylene) which were divided into different lots.

2.3. Water activity (a_w)measurement

The a_w of the samples was measured using a WHMW water activity meter (made in Tianjin, China).

2.4. Irradiation treatment

Treated food samples were subjected to gamma irradiation at doses of 1, 2, 2.5, 5, 7.5, 10, 15 and 20 kGy using a ^{60}Co source at the Beijing Radiation Application Center. Non-irradiated batches served as controls.

2.5. Microbiological analyses

Duplicate samples (25 g) of irradiated as well as non-irradiated batches of foodstuffs were aseptically homogenised for 1 min in a sterile container containing 225 mL sterile normal saline using a mechanical blender (Made in Jiangsu, China). Appropriate serial dilutions of the homogenate were placed in sterile culture dishes in triplicate. Media employed were Plate Count Agar, SS Agar, DHL (deoxycholate hydrogen sulfide lactose) agar, MYP (mannitol-egg yolk-polymyxin) agar for determination of total plate count (TPC), S. *enteritidis, S. aureus* and *B. cereus*, respectively.

3. RESULTS AND DISCUSSION

The a_w of the food products ranged between 0.930–0.940 (0.935 ± 0.007) for semi-dried pork, 0.8522–0.895 (0.876 ± 0.032) for fish, 0.918–0.934 (0.928 ± 0.01) for bean curd, and 0.908–0.915 (0.911 ± 0.07) for pickle. The TPCs of the semi-dried food samples are shown in Table I. The TPC for the pork samples ranged between 0–3.210^6 CFU/g. It was found that TPCs were highest (3.210^6 CFU/g) for pork samples which were not irradiated, relatively lower (1.010–2.610^3 CFU/g) for some of the samples irradiated with 2, 2.5 or 5 kGy and absent from pork samples irradiated with 10, 15 or 20 kGy. It is important to note that TPCs were highest (1.610^5 CFU/g) for fish samples which were not irradiated, relatively lower (2.010^2–3.010^2 CFU/g) for some of the fish samples irradiated with 1 or 2 kGy and absent for samples irradiated with 2.5, 5, 10 or 20 kGy. This was most likely due to the lower a_w. TPCs for non-irradiated bean curd samples were highest (2.310^6 CFU/g). The minimum irradiation dose required to inactivate all spoilage microorganisms in bean curd samples was 10 kGy, whilst 15 kGy was required for pickles.

The combined effect of irradiation and vacuum packaging on pathogenic microorganisms in semi-dried pork is shown in Table II. *S. enteritidis* is very sensitive to irradiation with a dose of 1 kGy eliminating this pathogen in fish and pickle and 2 kGy being required for bean curd. While *S. enteritidis* in pork is rather resistant to irradiation, the minimum radiation dose required to inactivate the pathogen is 5 kGy

S. aureus in semi-dried food samples proved to be the most sensitive pathogen to irradiation treatment. This pathogen can be inactivated at a dose of 2 kGy in pork, fish and pickle, and a dose of 2.5 kGy can eliminate this pathogen from bean curd. The combined effects of irradiation and vacuum packaging on *S. aureus* are shown in Table III.

B. cereus in semi-dried food samples was found to be quite resistant to irradiation. The minimum dose required to inactivate this microorganism in pork and pickle was 5 kGy, while a dose of 7.5 kGy was required for inactivation in fish and bean curd. Results are presented in Table IV.

TABLE I. COMBINED EFFECTS OF IRRADIATION AND VACUUM PACKAGING ON TOTAL PLATE COUNTS (TPCs) IN SEMI-DRIED FOOD PRODUCTS

Irradiation dose	Semi-dried food products (CFU/g)			
(kGy)	Pork	Fish	Bean curd	Pickle
0	3.210^{6}	1.610^{5}	$2.3\ 10^{6}$	2.510^{4}
1	8.010^{4}	3.010^{2}	2.510^{5}	8.010^{4}
2	2.610^{3}	2.010^{2}	8.210^{3}	4.210^{4}
2.5	4.010^{2}	ND	4.610^{3}	2.010^{4}
5	1.010	ND	1.410	1.310^{2}
10	ND	ND	ND	3.010
15	ND	ND	ND	ND
20	ND	ND	ND	ND

ND = Not detected

TABLE II. COMBINED EFFECTS OF IRRADIATION AND VACUUM PACKAGING ON *S. enteritidis* IN SEMI-DRIED FOOD PRODUCTS

Irradiation dose	Semi-dried food products			
(kGy)	Pork	Fish	Bean curd	Pickle
0	+	+	+	+
1	+	–	+	–
2	+	–	–	–
2.5	+	–	–	–
5	-	–	–	–
7.5	NT	NT	NT	NT
10	–	–	–	–

+ = Positive; – = Negative; NT = Not tested

TABLE III. COMBINED EFFECTS OF IRRADIATION AND VACUUM PACKAGING ON *S. aureus* IN SEMI-DRIED FOOD PRODUCTS

Irradiation dose	Semi-dried food products			
(kGy)	Pork	Fish	Bean curd	Pickle
0	+	+	+	+
1	+	+	+	–
2	–	–	+	–
2.5	–	–	–	–
5	–	–	–	–
7.5	NT	NT	NT	NT
10	–	–	–	–

+ = Positive; – = Negative; NT = Not tested

TABLE IV. COMBINED EFFECTS OF IRRADIATION AND VACUUM PACKAGING ON *B. cereus*

Irradiation dose	Semi-dried food products			
(kGy)	Pork	Fish	Bean curd	Pickle
0	+	+	+	+
1	+	+	+	+
2	+	+	+	+
2.5	+	+	+	+
5	–	+	+	–
7.5	–	–	–	–
10	–	–	–	–

+ = Positive; – = Negative

4. CONCLUSION

Based on the results described above, it can be concluded that a combination of a 2.5 kGy irradiation dose and vacuum packaging can inactivate all spoilage microorganisms in semi-dried fish. A dose of 10 kGy may inactivate the spoilage microorganisms in pork and bean curd samples, and 15 kGy will inactivate them in pickle. Experimental work where semi-dried food samples artificially contaminated with *S. enteritidis, S. aureus* and *B. cereus* were treated with a combination of irradiation and vacuum packaging showed that S. *enteritidis* and *S. aureus* in semi-dried food samples were rather sensitive to irradiation. The minimum dose required to inactivate *S. enteritidis* in fish and pickle was 1 kGy while a dose of 2 kGy could inactivate *S. enteritidis* in bean curd and *S. aureus* in pork, fish and pickle. *S. enteritidis* in pork is inactivated at a dose of 5 kGy while a dose of 2.5 kGy can eliminate *S. aureus* in bean curd. A dose of 5 kGy can eliminate *B. cereus* in semi-dried pork and pickle, with a dose 7.5 kGy being required for fish and bean curd.

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IRRADIATION OF RAW PORTUGUESE SAUSAGES AND MEAT BASED READY-TO-EAT MEALS

M.J. TRIGO*, M.E. ANDRADE**, M.G. VELOSO***, A. BARRETO***

* National Institute of Agricultural Research, National Agronomic Station, Department of Technology for Agricultural Products, Oeiras, Portugal.
** Nuclear and Technological Institute, Sacavém, Portugal.
*** Technical University of Lisbon, Lisbon, Portugal.

Abstract

Portuguese "chorizo", traditionally processed, contains a high load of a mixed microbial population, including some food-borne pathogenic bacteria. However, commercially manufactured "chorizo" undergoes a technological process based on hot smoking, which eliminates most microbial flora present. In such conditions it is not justifiable to use irradiation. On the contrary, home-made "chorizo" is smoked at low temperatures and produced on a small scale in small premises spread over the country. In this case, irradiation is justifiable and economically feasible if the chorizo is collectively sent to irradiation centres for treatment. The use of irradiated meals is justified for preservation of their natural organoleptic properties when full botulinum cooking is not affordable. In irradiated "chorizo", a total aerobic count was dominant, faecal streptococci were low in number while coliforms, *E. coli* and *Staphylococcus aureus* were absent from the control samples. Faecal streptococci showed more sensitivity to irradiation than aerobic microflora which can be explained by the presence of endospore-forming bacteria. Faecal streptococci survived an irradiation dose of 2 kGy dose but not 4 kGy. An 8 kGy dose was not sufficient to destroy all the spores of sulphite-reducing clostridia present. The D_{10} determined for faecal streptococci was 1.25 kGy. In ready-to-eat meals, the microbial contamination of control dishes of "stewed veal with onions and carrots" cooked in the laboratory was lower than in samples of commercially refrigerated meat based meals. The reduction of microbial flora was found to be proportional to irradiation dose. A reduction of 2 logs in the total aerobic microflora was achieved with a 4 kGy dose. Approximately 10^7 vegetative and viable spores of *Clostridium sporogenes* per gram were suspended in "stewed veal with onions and carrots" meal and irradiated at –30°C. The irradiation D_{10} value for vegetative and spore formers was 4.67 kGy. The D_{10} value for viable spores of the same microorganism was 5.58 kGy.

1. INTRODUCTION

"Chorizo" is a Portuguese raw hot smoked sausage made exclusively from pork, lean meat and fat. A high content of a mixed microbial population, including some food-borne pathogenic bacteria, could be present in home-made "chorizo" when traditionally processed. Commercially manufactured "chorizo" produced on a large scale undergoes a technological process based on hot smoking which eliminates most microbial flora present.

Safe, shelf-stable meals are necessary for some special consumer groups such as humanitarian/rescue missions (immunosuppressed patients, military combat meals). In many cases lethal heat treatment (botulinum cook) cannot be used to sterilise/decontaminate these meals. In such situations, radiation sterilization could provide a convenient method to ensure a high level of safety.

The meals studied in this experimental work were selected after meetings organised with a food service hospital assisting immunosuppressed patients and a quartermaster military service. The microbiological flora of the meals was collected and identified. Appropriate packaging materials were selected and procured in the local market.

The minimum absorbed radiation dose to sterilize the experimental meals in order to obtain safe, shelf-stable foods, was calculated by the killing effect on *Clostridium sporogenes.*

2. OBJECTIVES

The aim of this work was to ascertain the lethal effect of gamma irradiation on specific pathogens and opportunistic bacteria found in raw semi-moist sausages ("chorizo") and in mild

cooked meat based meals in order to provide appetising and safe, shelf-stable food for special purposes.

The food products selected for this study were traditional Portuguese “chorizo” and a ready-to-eat meat based meal with vegetables.

3. MATERIAL AND METHODS

3.1. Irradiation

The irradiation was carried out in a ^{60}Co plant at the Sacavém campus of the Nuclear and Technological Institute. The dose rate employed was 13 kGy/h.

Each sample was composed of two “chorizos” packed in plastic bags sealed under vacuum and irradiated at room temperature with doses of 2, 4, 6, 8 and 10 kGy. Eight samples of “chorizo” were used at each dose.

Each meal sample, composed of 100 g of stewed veal with onions and carrots, was packed in 4 layer laminated flexible pouches (Nylon/Foil/Polyester/LLDPE). Each pouch was sealed under vacuum. The irradiation was carried out in CO_2 dry ice, at temperatures between –0ºC and –40ºC, with absorbed doses of 2, 4, 6, 8 and 10 kGy. Ten samples of the meal were used for each dose.

The artificially contaminated meal sample was composed of 20 g of stewed veal with onions and carrots. Each sample was packed in laminated flexible pouches as described above. Irradiation was carried out in CO_2 dry ice, at temperatures between –30ºC and –40ºC, with absorbed doses of 2.5, 7.5, and 5 to 50 kGy at intervals of 5 kGy. Ten samples of the meal were used for each dose.

3.2. Microbiological analysis

Ten grams of “chorizo” or 20 g of the meal were taken, homogenised with 90 mL or 180 mL of 0.1% Triptone Saline Solution in a Stomacher for 2 min. Serial 10 fold dilutions in the same solution were made and the following determinations performed:

- Total aerobic count, in Tryptone Glucose Extract Agar (Oxoid). Incubation at 30 ± 2°C for 72 ± 2 h.
- Most probable number (MPN) of coliform bacteria. Cultured in Lactose Broth (single and double strength) incubated for 24 h or 48 ± 2 h at 30 ± 2°C. Sub-cultures of all positive tubes (showing culture and gas production) were made into Brilliant Green Bile (BGB) broth (2%) (Merck) and incubated for 24 h or 48 ± 2h at 30 ± 2°C. All positive tubes (showing culture and gas production) were entered into MPN tables for 2 tubes.
- MPN of *Escherischia coli*: by duplicate sub-cultures of each positive BGB tube in 1% Peptone Water and BGB tubes, incubated for 24 ± 2 h at 44.5 ± 0.5°C and testing for both indol and gas production.
- Faecal streptococci counts on Kanamycin Aesculin Azide Agar (Difco) incubated at 37 ± 2° C for 24 or 48 ± 2 h.
- *Enterobacteriaceae* counts on Violet Red Bile Glucose Agar (Oxoid) for 24 or 48 ± 2 h at 37 ± 2°C.
- *Staphylococcus aureus* counts on Baird-Parker Agar (BPA) with Tellurite (Difco) for 48 h at 37 ± 2°C.
- *S. aureus* detection on Chapman (single and double strength) incubated for 24 h at 37 ± 2°C. From the cultures obtained in Chapman, inoculation on BPA with Tellurite (Difco) and incubation for 48 h at 37 ± 2 °C.

– *Clostridium perfringens* spore counts (vegetative cells killed by heat treatment at 80 ± 1°C for 10 min) in Sulphite Polymyxin Sulfadiazine Agar (Merck) for 24 ± 2 h at 44.5 ± 0.5°C.

All counts were expressed by the logarithm of the number of colony forming units per gram (log_{10} CFU/g).

3.3. Inoculated pack studies

3.3.1. Test organism

The test organism used was *C. sporogenes* (Cl 28–6/7/78) obtained from the National Institute of Industrial Engineer and Technology, Lisbon, Portugal.

3.3.2. Preparation of microorganism suspension

In order to obtain vegetative and spore forms, a suspension of *C. sporogenes* was prepared on Reinforced Clostridial Medium (RCM; Oxoid). The inoculated RCM bottles were incubated at 37ºC under anaerobic conditions (12% CO_2 and 88.2% N_2) for 7 days. The extent of sporulation was determined by phase contrast microscopy.

The former suspension was centrifuged four times at 5000 rpm for 20 min. Between each centrifugation, the supernatant fluid was discarded and pellets resuspended in sterile distilled water. The final stock suspension contained a concentration of ca. 10^9 vegetative and spore formers/mL; the concentration of spore formers was ca. 10^5/mL.

3.3.3. Inoculated pack studies

The cooked meal was inoculated with appropriate dilutions of *C. sporogenes* in order to obtain a concentration of ca. 10^7 vegetative and spore formers/g. The concentration of spores was ca. 10^5/g.

3.3.4. Microbiological testing

The artificially contaminated meal portions (20 g) were homogenised for 2 min in a Stomacher with 180 mL of 0.1% Triptone Saline solution. A series of 10 fold dilutions in the same solution were performed to estimate *C. sporogenes* viable cell counts. To estimate the spore numbers, the dilutions were heat treated at 80 ± 1°C for 10 min in order to kill vegetative cells.

The dilutions, heated and not heated, were plated in Reinforced Clostridial Agar (RCA; Oxoid). The plates were incubated at 37ºC, under anaerobic conditions (%CO_2 and %N_2) for 3 days.

4. RESULTS AND DISCUSSION

4.1. Portuguese "chorizo"

Twenty one samples of Portuguese "chorizo" were analysed in order to determine its physico-chemical composition. Moisture, carbohydrate, protein, fat and ash content along with determination of water activity (a_w) and pH were performed. Average values are presented in Table I.

The microbial content of the irradiated "chorizo" samples (Table II) was lower than the samples of "chorizo" analysed in the first part of this work (Table III). Total aerobic microorganisms were dominant; faecal streptococci were in low numbers, coliforms, *E. coli* and *S. aureus* were absent in the control samples (0 kGy). This is due to the heat treatment that the "chorizos" were submitted to during processing (hot-smoking).

TABLE I. AVERAGE CHEMICAL COMPOSITION, pH AND WATER ACTIVITY (a_w)

Water %	Carbohydrate %	Protein %	Fat %	Ash %	a_w	pH
34.00	1.50	16.00	43.20	5.30	0.89	6.0

TABLE II. EFFECT OF IRRADIATION ON THE BACTERIAL POPULATION OF "CHORIZO"

Irradiation dose (kGy)	Total aerobic count (log_{10} CFU/g)	Faecal streptococci (log_{10} CFU/g)	Coliforms (log_{10} MPN/g)	*E. coli* (log_{10} MPN/g)	*S. aureus* (log_{10} CFU/g)	Spores of sulphite-reducing clostridia (log_{10} CFU/g)
0	5.18	3.20	<0	<0	<0	<0
2	3.42	1.20	<0	<0	<0	0.23
4	2.16	<0	<0	<0	<0	<0
6	1.98	<0	<0	<0	<0	0.04
8	1.68	<0	<0	<0	<0	0.04
10	1.62	<0	<0	<0	<0	<0

TABLE III. MICROBIAL ANALYSIS OF 21 SAMPLES OF PORTUGUESE "CHORIZO"

Total aerobic count (log_{10} CFU/g)	*Enterococci* (log_{10} CFU/g)	Coliforms/g	*E. coli*/g	*S. aureus/g*	*C. perfringens* /g	*Salmonella in 25 g*
6.25	4.30	85%<1 5%<10>1 5%<10^2>10 5%<10^3>10^2	100%<1	95%<1 5%<10>1	57%<1 33%<10>1 10%<10^2>10	100% <1

The lethal effect on total aerobic microflora was proportional to the irradiation dose (Fig. 1). However, a "tailed" curve was observed for the highest doses.

Faecal streptococci were more sensitive to irradiation than the total aerobic count which can be explained by the presence in "chorizo" of endospore-forming bacteria resistant to the irradiation. The D_{10} value calculated for faecal streptococci was 1.25 kGy. The 8 kGy dose was not sufficient to destroy the spores of sulphite-reducing clostridia when present in "chorizo" (Table II).

4.2. Meat based meals

The results obtained by standardised food microbiology methods for 90 refrigerated samples of several dishes of meat and vegetables from catering companies are presented in Figs 2 to 6.

Salmonellae were absent in all 25 g samples. Beef based meals samples were more heavily contaminated with coliforms, *E. coli*, *S. aureus* and *C. perfringens* than the other meals. Pork, lamb and veal samples were the least contaminated.

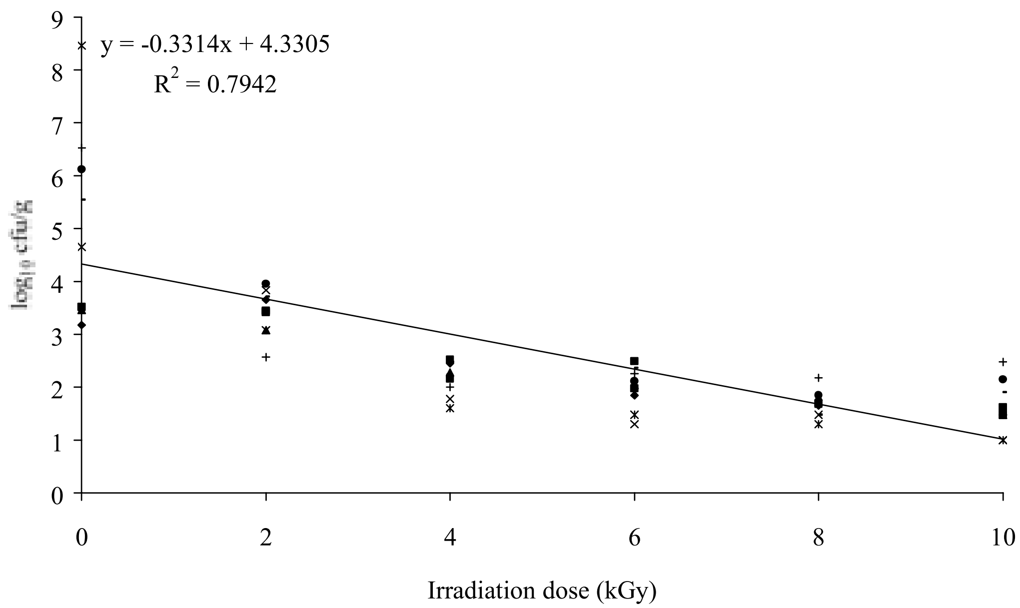

FIG.1. Radiation effect on total aerobic microflora in "chorizo".

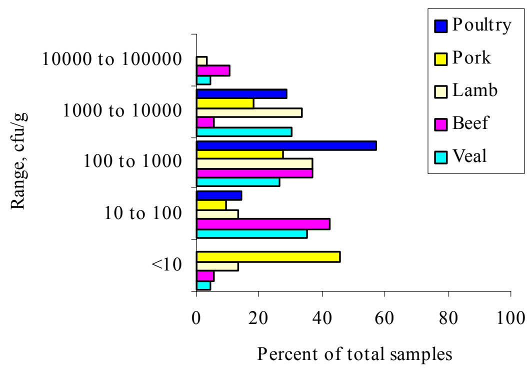

FIG. 2. Distribution (percentage of samples) of coliform bacteria in meat based meals sample.

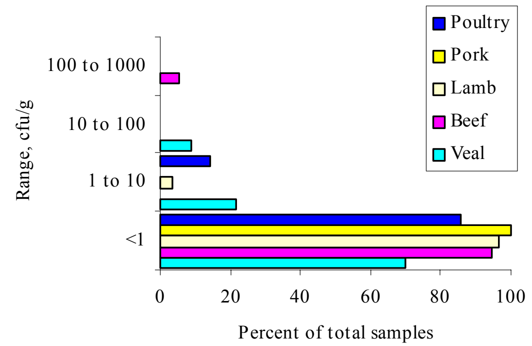

FIG. 3. Distribution (percentage of samples) of E. coli in meat based meals samples.

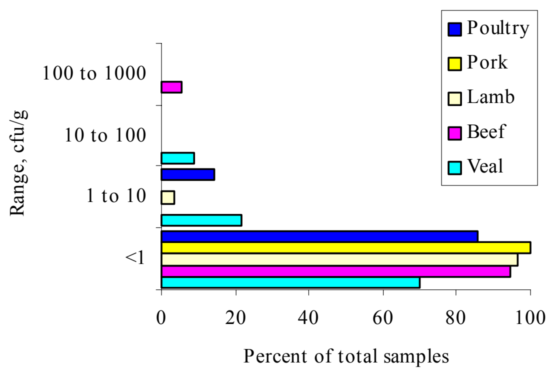

FIG. 4. Distribution (percentage of samples) of S. aureus in meat based meals samples.

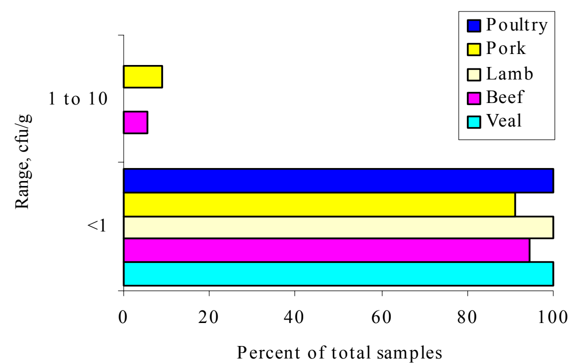

FIG. 5. Distribution (percentage of samples) of C. perfringens in meat based meals samples.

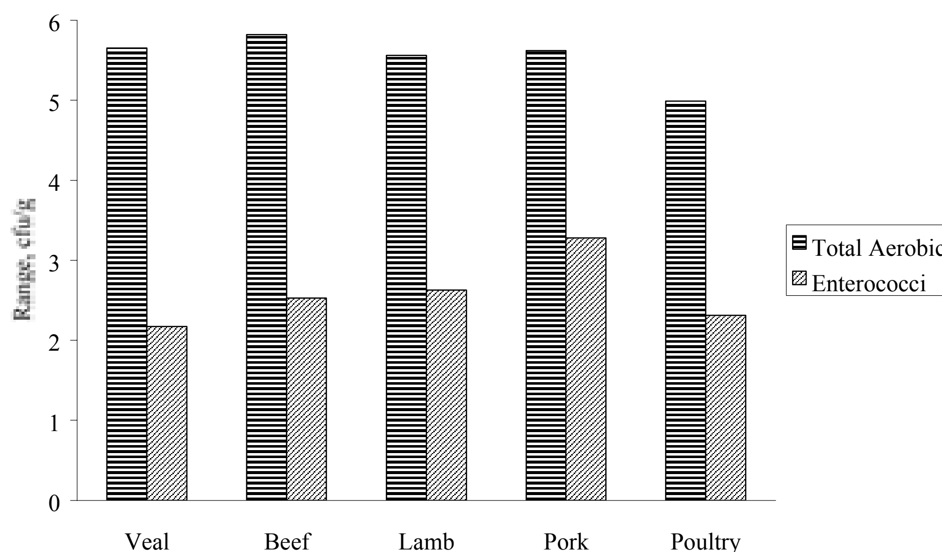

FIG. 6. Total aerobic and Enterococci average in meat based meal samples.

TABLE IV. EFFECT OF IRRADIATION ON THE BACTERIAL POPULATION OF "STEWED VEAL WITH ONIONS AND CARROTS" MEAL

Irradiation dose (kGy)	Total aerobic count (log_{10} CFU/g)	Faecal streptococci average (log_{10} CFU/g)	Coliforms (log_{10} MPN/g)	*E. coli* (log_{10} MPN/g)	*S. aureus* (log_{10} CFU/g)	Spores of sulphite-reducing clostridia (log_{10} CFU/g)	*Enterobacteriaceae* (log_{10} CFU/g)
0	3.56	2.04	<0	<0	<0	<0	2.00
2	1.96	<1	<0	<0	<0	<0	<1
4	1.21	<1	<0	<0	<0	<0	<1
6	1.10	<1	<0	<0	<0	<0	<1
8	1.15	<1	<0	<0	<0	<0	<1
10	<1	<1	<0	<0	<0	<0	<1

The microbial contamination of control samples of stewed veal with onions and carrots (Table IV) was lower than for the samples of refrigerated meat based meals. This result can be explained by the level of hygiene and the quick chilling used in the processing of the stewed veal in the laboratory.

Aerobic bacteria were dominant. Faecal streptococci and *Enterobacteriaceae* were present in low numbers while coliforms, *E. coli*, *S. aureus* and spores of sulphite-reducing clostridia were absent in all the unirradiated control samples.

The lethal effect of irradiation on total aerobic microflora was proportional to the dose (Fig. 7). A dose of 2 kGy induced a reduction of 2 logs of the faecal streptococci and *Enterobacteriaceae* in stewed veal meal.

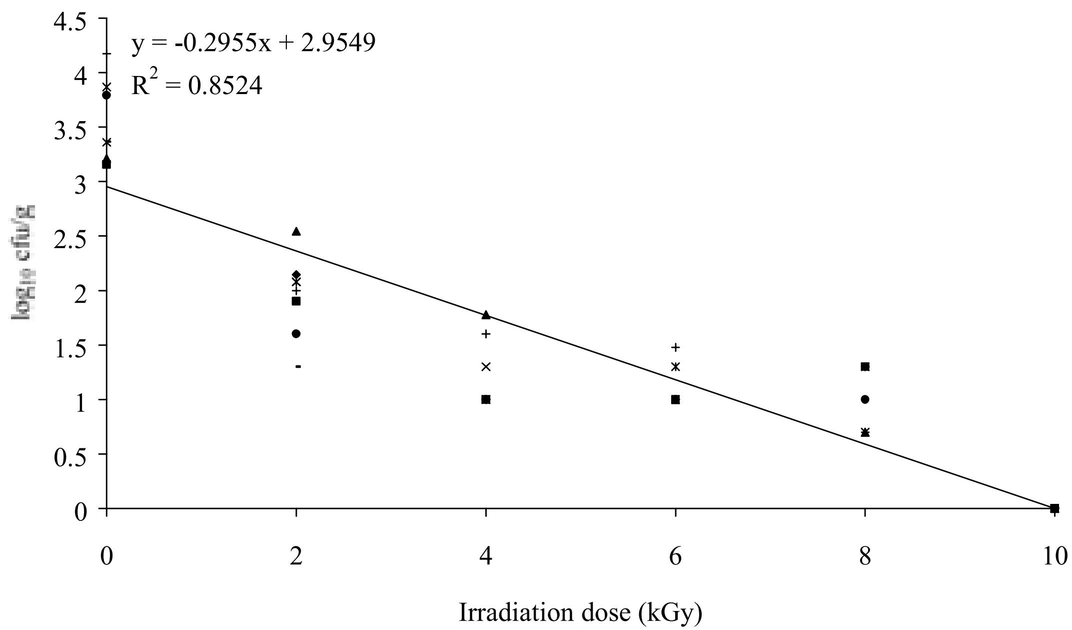

FIG. 7. Effect of irradiation on total aerobic microflora of "stewed veal with onions and carrots" meal.

4.3. Radiation-resistance of test microorganisms

The data shows that the log of surviving vegetative bacteria and spores decreases linearly with increasing doses of gamma irradiation applied (Figs 8 and 9). The effect of gamma irradiation on the spores in artificially contaminated cooked meal is shown in Fig. 8. The D_{10} value for *C. sporogenes* spores obtained was 5.58 kGy. This value is similar to data (4.0–6.8 kGy) obtained for *C. botulinum* in roast products at –29ºC and in ground beef at –196°C [1,2].

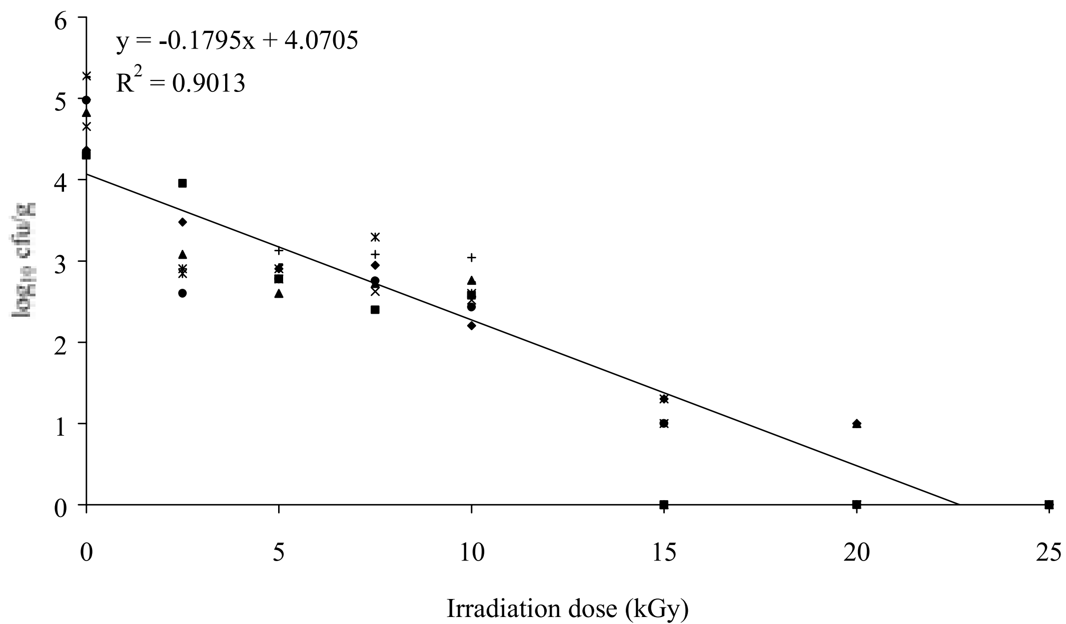

FIG 8. Effect of irradiation on C. sporogenes spores inoculated in "stewed veal with onions and carrots" meal.

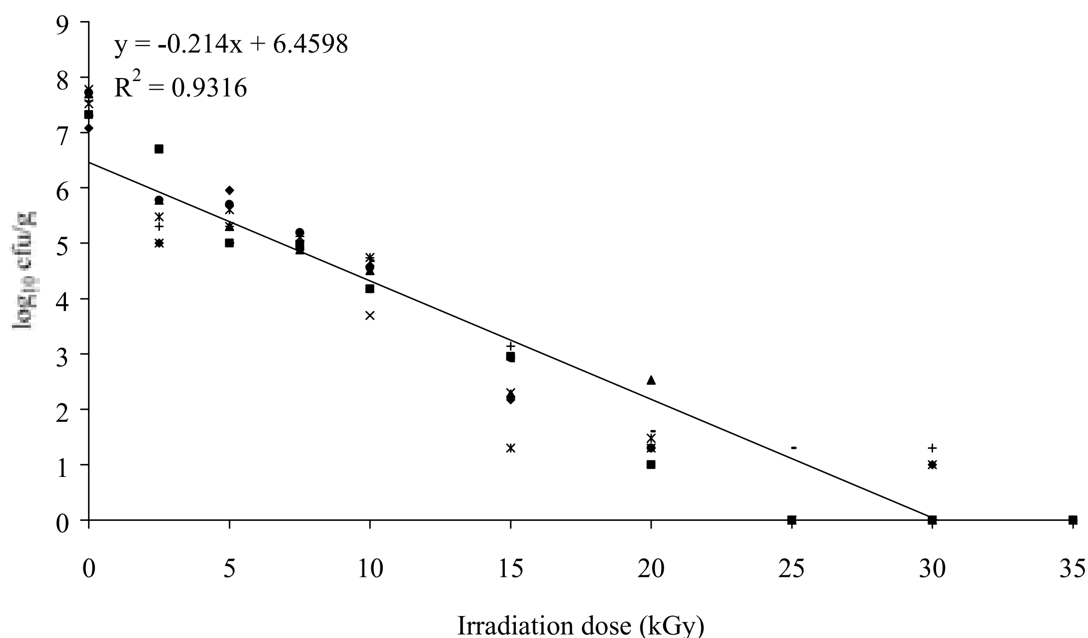

FIG. 9. Effect of irradiation on vegetative and spore forms of C. sporogenes inoculated in "stewed veal with onions and carrots" meal.

If the 12-D concept is applied, then a D_{10} = 5.58 kGy for –30ºC in "stewed beef" yields a dose requirement of approximately 66.9 kGy for commercially safe radiation sterilisation of stewed beef at –30ºC. In spite of this 12-D dose being high and beyond the values normally used to sterilise meals [3–10], the D_{10} value was similar to that obtained by Shamsuzzaman and Lucht [11] in the fat of beef, pork and chicken. However, there is no comparative research on the radio-resistance of *C. sporogenes* and *C. botulinum* to allow extrapolation of these results so as to calculate 12-D dose for *C. botulinum*.

The D_{10} value for vegetative and spore formers was 4.67 kGy, which was less than that found for spores alone (5.58 kGy).

5. CONCLUSIONS

The total aerobic microflora present in "chorizo" were less sensitive to irradiation than faecal streptococci. An irradiation dose of 8 kGy was not sufficient to destroy the spores of sulphite-reducing clostridia. The D_{10} determined for faecal streptococci present in "chorizo" was 1.25 kGy. This D_{10} and the response of aerobic microflora to irradiation suggest the use of an absorbed dose of 3 kGy to radio-pasteurise this product. It can, therefore, be concluded that ionising radiation is a good method to pasteurise commercially produced semi-moist raw sausages and will be economically feasible if the production line is suitably modified.

The microbial contamination of the samples of unirradiated "stewed veal with onions and carrots" was lower than for the samples of refrigerated meat based meals. The reduction of microbial flora is proportional to the irradiation dose. A dose of 2 kGy resulted in a 2 log reduction of faecal streptococci and *Enterobacteriaceae*. A 2 log reduction in the total aerobic microflora was achieved using a dose of 4 kGy.

The D_{10} value for vegetative and spores forms of *C. sporogenes* in the artificially contaminated cooked meals was 4.67 kGy. The D_{10} value for viable spores of the same microorganism in the same substrate was 5.58 kGy.

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OPTIMISING PROCESSING CONDITIONS FOR IRRADIATED CURED FISH

J. NKETSIA-TABIRI, A. ADU-GYAMFI, K.G. MONTFORD,
C.M. GBEDEMAH, S. SEFA-DEDEH
Department of Food Science and Radiation Processing,
Ghana Atomic Energy Commission,
Legon, Ghana

Abstract

Cured fish products were investigated with the view to developing and/or improving shelf-stability and safety of ready-to-eat varieties through irradiation processing. The products were ready-to-eat smoked sardines (*Sardinella* spp.) and a marinated fish (*Diplodus puntazzo*) product. The moisture content of the smoked sardines ranged between 11.3–24.9%, salt content 0.85–1.3% d.b., fat content 4.6–24.9%, and water activity 0.62–0.92. The total viable count was $\log_{10}$ 6.74–8.96 CFU/g fish. Microbes associated with the smoked sardines included *Staphylococcus* spp. (but not *S. aureus*), *Enterobacter sakazaki*, *Klebsiella pneumonae ozaenae* and *Bacillus* spp. Fungi identified were *Penicillium* spp., *Absidia* spp., *Aspergillus restrictus*, *Aspergillus versicolor*, *Aspergillus achraeceous*, *Aspergillus niger*, *Aspergillus terreus*, *Aspergillus wentii*, *Aspergillus penicilloides*, mucor and yeasts. Radiation sensitivity of *Klebsiella pneumonae ozaenae* (D_{10} (peptone) = 0.33 kGy) was determined. Although irradiation (7–11 kGy) controlled microbial activity in some of the smoked sardines during a 12 week storage period, others had unacceptable colour, flavour and texture. The ready-to-eat marinated fish had a moisture content of 41.9–58.6%, salt content of 2.1–5.3% d.b., fat content of 6.2–26.9% d.b., free fatty acids content of 7.0–20.2%, peroxide value of 24.3–77.1 mEq/kg fat, acidity 77.9–121.5% and water activity of 0.913–0.944. The total viable count was $\log_{10}$ 2.6–7.2 CFU/g. Irradiation at levels of 8–10 kGy substantially reduced the coliform count and extended the shelf-life and overall acceptability of the product by 3 days.

1. INTRODUCTION

Curing processes such as salting, fermentation and drying, including smoking, contribute immensely to the preservation and distribution of fish in most developing countries. The methods used are simple and have developed over the years according to local traditions. In Ghana, over 60% of the total fish landed is cured before being consumed [1]. Cured fish products are relatively more stable than fresh fish under ambient tropical conditions although considerable losses are suffered due to insects, bacteria and moulds [1,2]. Much of the loss has been attributed to inherent characteristics of traditional curing technologies. For example, smoked fish that is stored by the traditional method requires resmoking at regular intervals to control microbial and insect proliferation. This practice, however, renders the final product excessively dry and susceptible to fragmentation with negative effects on sensory attributes. For processes involving open sun-drying, the fish is susceptible to infestation by air-borne microbes and insects.

Salting can reduce available moisture in the product and control microbial activity but salt-tolerant bacteria, *Halobacterium* and *Halococcus,* inherent to solar salt [3], persist on the fish and cause discoloration. Resmoking and redrying in the sun as measures for further reducing product moisture content and controlling microbial and insect activity are temporary and ineffective. These measures can be replaced or complemented with additional hurdles such as acidification and irradiation treatment.

2. OBJECTIVES

The objectives of the research were to:

(a) determine the microbiological profile of traditionally smoked sardines;

(b) isolate, identify and determine D_{10} values for the organisms in order to establish the most effective irradiation dose for the preservation of smoked sardines;

(c) develop a ready-to-eat marinated fish product in combination with irradiation to ensure safety and shelf-stability;

(d) determine the water activity (a_w) of ready-to-eat smoked sardines and marinated fish; and

(e) evaluate the efficacy of retail and bulk packaging of smoke-dried sardines during storage under ambient conditions.

3. MATERIALS AND METHODS

3.1. Production of unsalted ready-to-eat smoked sardines

3.1.1. Preparation of smoked sardines

A traditional processor was engaged to smoke the fish. The sardines (*Sardinella* spp.) were brought from the seaside and smoked as they were, without scaling, gutting or brining. Smoking was carried out using the Chorker smoker which involves less direct handling of the fish during the smoking process. The smoking temperature was controlled by manipulating the burning firewood. The fish was smoked for 2 or 4 days; the former having a higher moisture content and hence was softer (soft-smoked) than the latter (smoke-dried). Traditional fish smoking is a hot-smoking process which cooks the fish, thus, the products are in a ready-to-eat form. After sampling for initial microbiological and physico-chemical analysis, the smoked sardines were retail packaged in polyethylene pouches. Each pack contained 8 smoked sardines and had a total weight between 260–300 g.

3.1.2. Irradiation and storage of soft-smoked sardines

The packaged soft-smoked sardines were irradiated (0–7.0 kGy) and stored under ambient conditions. The samples were visually examined for mould growth during storage.

3.1.3. Retail and bulk-packaging studies using smoke-dried sardines

Six retail pouches of the smoke-dried sardines were packed into paper cartons (33 × 20 × 14 cm). Eight such cartons were prepared after which they were sealed with packing tape. Twelve of the cartons containing the smoked sardines were irradiated (7–11 kGy) while the remaining six served as non-irradiated controls. The control and the irradiated smoked sardines were stored under ambient conditions. After 12 weeks of storage, the paper cartons were opened and the retail packs analysed for moisture content, free fatty acids and, peroxide value. Aerobic plate count (APCs), coliform counts, and mould/yeast counts were also carried out.

3.2. Production of ready-to-eat marinated fish

3.2.1. Preparation of marinated fish

In the preparation of ready-to-eat marinated fish *Diplodus puntazzo* was used because sardines were not in season. A preliminary study was conducted to determine how salting and acidification could most effectively be used to develop a moist shelf-stable fish product. The fish was bought in the frozen state and thawed. After scaling and filleting, the fish was washed, cleaned and treated as follows:

(a) brined for 30 min in a solution containing 7% sodium chloride solution; or
(b) marinated in a solution containing 7% sodium chloride and 2% acetic acid.

Both sets of treated fish fillets were cooked in a thermostatically controlled oven: the cycle used was 30 min at 50–60°C and then 2 h at 90–110°C.

The ready-to-eat fish fillets were allowed to cool and subjected to sensory evaluation to determine consumer acceptability. The majority of the taste panellists preferred the marinated samples because of their mild astringent taste. The samples had a shelf-life of 3 days under ambient conditions (31–36°C; 68–84% relative humidity (RH)). Based on these observations further studies focused on the cooked marinated fish with the view to investigating optimum marinating conditions and also the contribution that irradiation can make towards the production of an acceptable non-sterile shelf-stable product.

3.2.2. Studies on effects of marinating time, irradiation dose and storage time on fish quality

Using a central composite rotatable design (CCRD) for k=3 [4], 18 treatment combinations of marinating time, 0–30 min; irradiation dose, 0–10 kGy; storage time, 1–7 days were studied. Eighteen batches (2 kg each) of the fish were used. The definition of the variables are shown in Table I. After processing, the fish was cooled. Six pieces of cooked marinated fish fillets were put in polyethylene pouches (0.002 inch gauge) and heat-sealed. The samples were stored under ambient conditions (31–36°C; 68–84% RH). The quality indices measured were moisture content, salt content, fat content, a_w, acidity, free fatty acids, peroxide value, APCs, coliform counts, mould counts and overall acceptability scores.

3.3. Packaging and irradiation of fish

All the fish samples were packaged and irradiated with the doses specified under the appropriate sections. Irradiation was carried out using a ^{60}Co source.

TABLE I. DEFINITION OF INDEPENDENT VARIABLES AND LEVELS FOR CCRD K=3

Independent variable	Code	Levels				
		–1.682	–1	0	+1	+1.682
Marinating time (min)	X_1	0	6	15	24	30
Irradiation dose (kGy)	X_2	0	2	5	8	10
Storage time (days)	X_3	1	2	4	6	7

3.4. Storage conditions

When investigating the effect of packaging material on the long-term storage of smoke-dried sardines, the storage room was first sprayed with 0.01% diazinon E.C. and the floor later mopped with detergent before the packaged control and irradiated fish were arranged on different shelves in the same room. The ambient temperature fluctuated between 28–31°C and RH was between 63–89%

3.5. Sensory tests

Fish samples were evaluated for overall acceptability (taste, appearance and texture) by panellists using a 10 point scoring scale. In some instances, panellists were asked to provide descriptive words for specific sensory indices.

3.6. Physical and chemical analyses

Moisture was determined by the oven-drying method [5]. Salt content was determined by the precipitation of salt as silver chloride [5] while fat content, free fatty acids and peroxide values were determined on the extracted fat [5].

3.7. Microbiological examination

APCs were determined on nutrient agar and incubated at 37°C for 24 h. Coliforms were determined on Violet Red Bile Agar (VRBA) at 37°C for 24 h or 48 h. Yeast and moulds were enumerated on Potato Dextrose Agar (PDA) after incubation at 21°C for 3days. Standardised methods were used according to Ref. [6].

3.8. Determination of radiation-sensitivity of microbes

A standard procedure [7] was used with peptone water as the medium for irradiation. Regression analysis of the data was performed.

3.9. Statistical analysis of data

Data was subjected to analysis of variance, regression analysis and multiple range tests as and when considered appropriate. Significance of differences were determined at $p \leq 0.05$.

4. RESULTS AND DISCUSSION

4.1. Quality of the soft-smoked sardines

The moisture content of the soft-smoked sardines (*Sardinella* spp.) varied widely (Table II). This is a major problem with the traditional method used for smoking and stems largely from the difficulty in controlling the smoking temperature. For unsalted fish that has not been treated in any other way to reduce the availability of water, using moisture content as a quality index is largely a reflection of product a_w. In batches where the fish are directly in contact with each other, as in traditional practice, the shelf-life of the batch is limited by the fish with the highest a_w. The APC was $\log_{10}$ 4.8–7.5 CFU/g and mould count $\log_{10}$ 4.2–6.6 CFU/g. There was no growth of coliforms on the VBRA after 48 h.

TABLE II. QUALITY INDICES OF FRESHLY SMOKED SARDINES

Quality indices	
% Moisture content	23.4–33.5%
% Salt content (d.b.)	0.7–0.9%
Aerobic plate count ($\log_{10}$ CFU/g)	4.8–7.5
Mould count ($\log_{10}$ CFU/g)	4.2–6.6
Coliforms ($\log_{10}$ CFU/g)	no growth on VRBA/48 h
Water activity (a_w)	0.923–0.986
Sensory description	Soft, moist, juicy, mild smoky flavour/aroma

Due to the high microbial count, soft-smoked sardines generally have a short shelf-life of approximately 3 days (Table III). Shelf-life under ambient conditions was doubled when the fish was irradiated with 2 kGy. The high moisture content of soft-smoked sardines was reflected by the sensory description provided by consumers. There is a demand for both soft-smoked and smoke-dried fish, however, the choice for a particular type is dependent on how this would be utilised. The soft-smoked fish is generally susceptible to disintegration if incorporated into a meal which requires a long cooking time. In general, soft-smoked fish is generally consumed directly with hot pepper; they do, however, have the tendency to disintegrate when incorporated into light-consistency soups which undergo vigorous boiling. In view of the fact that soft-smoked fish may be consumed directly, it is important that they are microbiologically safe. Local marketing techniques also contribute to post-processing contamination of

the fish before they are consumed. Generally, smoked fish is not retail packaged as they are exposed to enable potential buyers to feel the texture before a purchase is made. Thus, there is a need to introduce packaged smoked fish to consumers since without effective packaging the application of irradiation for shelf-life extension and microbiological safety of smoked fish cannot be realised.

4.2. Quality of smoke-dried fish

4.2.1. Effects of packaging and irradiation of quality during storage

Three types of packaging material were investigated to determine the most appropriate type for storage studies. Hot-smoked sardines are smoke-dried for long-term storage and this is usually done when a bumper harvest is made and the fish attract a low price. The conversion of soft-smoked fish to smoke-dried forms usually take long periods of gentle resmoking whilst the fish is under ambient storage in the traditional ovens.

TABLE III. EFFECT OF IRRADIATION DOSE ON THE APPEARANCE OF MOULDS DURING AMBIENT STORAGE

Irradiation dose (kGy)	Appearance of moulds
0	3 days after smoking
2	6 days after irradiation
4.5	7 days after irradiation
7	7 days after irradiation

TABLE IV. EFFECT OF IRRADIATION DOSE, PACKAGING TYPE AND STORAGE TIME ON THE MOISTURE CONTENT, FREE FATTY ACIDS AND PEROXIDE VALUE OF SMOKED FISH

	% Moisture				% Free Fatty Acids				Peroxide value (mEq/kg fat)			
Dose (kGy)	0		1		0		1		0		1	
Storage time (weeks)	0	5	0	5	0	5	0	5	0	5	0	5
Paper	11.8	16.1	14.1	15.7	10.3	13.3	12.2	13.9	67.4	49.7	49.9	44.5
sd ±	0.5	0.3	3.1	1.2	3.2	1.1	0.1	3.1	5.4	16.7	1.3	14.2
Polyethylene	11.2	13.1	12.2	17.0	12.1	11.4	13.0	14.8	53.2	53.6	43.3	71.1
sd ±	0.9	1.5	2.1	3.4	1.3	1.4	2.4	3.0	18.0	23.1	19.0	30.7
Paper/ Polyethylene	17.7	14.9	12.9	15.2	19.9	11.6	9.7	9.7	61.7	36.2	42.7	53.0
sd ±	6.5	1.2	2.7	2.5	5.2	3.2	2.8	0.4	21.2	12.8	14.0	19.1
Traditional storage method	13.6	12.1			13.9	9.1			60.8	43.0		
sd ±	4.5	0.4			4.7	4.7			7.2	25.7		

The smoke-dried sardines had an initial moisture content of 10.3–24%, a_w of 0.786–0.883, crude protein of 72.1–86.6% d.b., crude lipid of 4.6–24.7%, a free fatty acid value of 7.0–25% and peroxide values of 21.9–83.3 mEq/kg fat. The wide variation in the lipid content was not unexpected because the lipid content of fatty fish is linked to its feeding and spawning cycle [8]. The wide variation in moisture content of the fish is a reflection of the difficulty encountered in controlling the smoking temperature. Variations in moisture, free fatty acids and peroxides in irradiated and non-irradiated smoke-dried sardines at the beginning and after 5 weeks storage are shown in Table IV.

The smoked sardines stored in the traditional oven were drier than those stored in polyethylene. This was due to the regular resmoking during storage to control microbial and insect spoilage. The excessive dryness of smoke-dried fish stored in the traditional oven makes them brittle and highly susceptible to fragmentation resulting in economic losses. Effective packaging coupled with an effective irradiation dose could replace resmoking and result in products that are not brittle thereby improving economic returns after storage.

After 5 weeks of storage, multifactorial analysis of variance tests indicated that the type of packaging and irradiation dose employed had no significant effect on the moisture content of the smoke-dried sardines. The storage time did, however, have a significant effect on the moisture content as that of the sardines increased as storage progressed. Analysis of the data further indicated that the moisture content of the sardines stored in the traditional ovens was significantly lower than the non-irradiated sardines packaged in polyethylene. The observed variations in the free fatty acids and peroxide values were not significant.

No live insects were found in any of the packaged smoke-dried sardines. Eleven cast skins of Dermestes larvae were, however, found in the control polyethylene/paper which had round holes. Neither live larvae nor adults of this species, which is a known storage pest of fish, were detected. Fungi were present on the sardines in the control polyethylene, irradiated polyethylene and irradiated polyethylene/paper packages.

TABLE V. SENSORY DESCRIPTION OF IRRADIATED (7–11 kGy) SMOKED SARDINES STORED FOR 12 WEEKS

	Fresh smoke-dried sardine	Irradiated smoke-dried sardines After 12 weeks storage
Texture	firm, dry	dry, spongy
Appearance	grey, brown	brown or with red tinge
Aroma	mild smoky aroma	mild smoky, mild rancid, strong rancid aroma
Water activity (a_w)	0.786–0.883	0.764–0.857 (details in Table IV)

4.2.2. Quality of retail packaged irradiated smoke-dried fish during storage

The a_w values for fresh smoke-dried sardines ranged between 0.616–0.981. Descriptive words provided by consumers to describe the sensory quality of fresh smoke-dried sardines are listed in Table V. After ambient storage for 12 weeks, all the sardines in the control retail packs were mouldy. Although, in some cases, not all the sardines within the pack were mouldy, any pack which contained a mouldy sardine was rejected since by the direct contact which prevailed between the sardines within a pack, the other sardines would eventually become mouldy. In the case of the irradiated samples, the proportion of mouldy retail packs per carton of six packs ranged from 0–66.7% (Table VI). The a_w profile of irradiated sardines in retail packs which had no visible growth of microbes after the 12 week ambient storage ranged between 0.764–0.85 (Table VII). This might be the safe range for long-term ambient storage of irradiated smoke-dried sardines. The problem of rancidity (Table V) requires further investigation into the use of natural or chemical antioxidants. Alternatively, sardines which are to be smoke-dried for long-term storage must be limited to those harvested early in the bumper season since those harvested late in the season are more fatty.

TABLE VI. QUALITY OF IRRADIATED SMOKE-DRIED SARDINES AFTER 12 WEEKS AMBIENT STORAGE (6 RETAIL PACKS PER CODED CARTON)

Code	210	208	205	215	212	204	214	206	201	202	211	207
A	4	3	3	3	4	5	2	2	4	4	2	6
B	2	3	3	3	2	1	4	4	2	2	4	0

A = the number of retail packs with no mould growth on the sardines; B = mouldy retail packs.

TABLE VII. WATER ACTIVITY OF EDIBLE, IRRADIATED SMOKE-DRIED SARDINES AFTER 12 WEEKS STORAGE

Sample numbers	Water Activity (a_w) units						
1–7	0.831	0.779	0.796	0.840	0.772	0.790	0.784
8–14	0.819	0.808	0.800	0.816	0.786	0.797	0.805
15–21	0.835	0.828	0.839	0.818	0.811	0.828	0.829
22–28	0.789	0.844	0.825	0.832	0.829	0.774	0.794
29–35	0.817	0.843	0.816	0.854	0.802	0.813	0.809
36–42	0.811	0.821	0.833	0.832	0.803	0.819	0.803
43–49	0.857	0.812	0.764	0.814	0.823	0.828	0.793

TABLE VIII. MICROORGANISMS ASSOCIATED WITH NON-IRRADIATED AND IRRADIATED SMOKE-DRIED SARDINES

Irradiated smoke-dried sardines after 12 weeks storage (no visible microbial spoilage)	
Aerobic plate count (log_{10} CFU/g)	log_{10} 4.1–4.8
Mould count (log_{10} CFU/g)	log_{10} 2.5–2.7
Coliforms (log_{10} CFU/g)	log_{10} 3.7–3.9
Microbes on irradiated smoked sardines after 12 weeks storage	
Bacteria	**Fungi**
	Penicillum spp.
Staphylococcus spp. (not S. aureus)	*Absidia spp.*
Enterobacter sakazaki	*Aspergillus achraeceous*
Klebsiella pneumonae ozaenae	*Aspergillus niger*
Bacillus spp.	*Aspergillus terreus*
Aspergillus restrictus	*Aspergillus wentii*
Absidia spp.	Aspergillus penicilloides
	Mucor and Yeasts

Microbes associated with the control and irradiated smoke-dried sardines after 12 weeks storage included *Staphylococcus* spp. (but not *S. aureus*), *Enterobacter sakazaki*, *Klebsiella pneumonae ozaenae* and *Bacillus* spp. Others included *Penicillium* spp., *Absidia* spp., *Aspergillus restrictus*, *Aspergillus versicolor*, *Aspergillus achraeceous*, *Aspergillus niger*, *Aspergillus terreus*, *Aspergillus wentii*, *Aspergillus penicilloides*, mucor/yeasts (Table VIII).

4.2.3. Radiation-sensitivity of Klebsiella pneumonae ozaenae

The D_{10} value (in peptone water) for *Klebsiella pneumonae ozaenae* was calculated from the following regression equation which was developed from the counts obtained. The coefficient of determination of 0.86 was highly significant ($p<0.01$).

$$Y = 6.7584 – 3.2146 X \quad R^2 = 0.86$$

where Y is log_{10} of survivors and X is dose in kGy

$$D_{10} = 1/slope = 1/3.2146 = 0.32 \text{ kGy}$$

TABLE IX. MODELS DEVELOPED FOR MOISTURE CONTENT, AEROBIC PLATE COUNT (APC) AND ACCEPTABILITY SCORE

Coefficients	% Moisture	Aerobic Plate Count ($\log_{10}$)	Acceptability score
Constant	49.576	2.912	5.854
X_1	-0.642	0	0
X_2	3.131	0	0
X_3	0	0.714	0
X_1^2	0.016	0	0
X_2^2	–0.234	0	0.054
X_3^2	0	0	0.075
X_1X_2	0	0	0
X_1X_3	0	0	0
X_2X_3	–0.512	–0.181	–0.120
R^2	0.46	0.22	0.51

With peptone water as the irradiation medium, the D_{10} value of 0.32 kGy was higher than 0.22–0.24 kGy reported [9] for the organism on paper disc.

4.3. Effects of marinating time, irradiation dose and storage time on quality of marinated fish

4.3.1. Moisture content

The moisture content of the ready-to-eat marinated fish samples was between 41.9–58.6%, a_w was 0.913–0.944, salt content 2.1–5.3% and acidity 89.8–121.5%. Prediction equations could not be developed for a_w, salt and acidity; an implication that within the limits of the experiment, these indices were not significantly affected by the marinating time, irradiation dose and storage time. The model in Table IX suggested a negative linear relationship between moisture and marinating time; thus, the longer the fish were marinated, the lower the moisture content. The model also suggested a significant positive relationship between irradiation dose and moisture content. The model also suggested a significant interaction between irradiation dose and storage time. The effects of the components of the marinate, salt and acetic acid and irradiation dose on the moisture content of the fish product might indirectly be attributed to the effects on microbial activity. Salt and acid do cause protein denaturation and consequent loss of their water holding capacity resulting in a relatively lower moisture content in the marinated fish products [10].

4.3.2. Fat content, free fatty acid and peroxide value

The fat content of the marinated fish was between 6.2–26.9, free fatty acid content 7.0–20.2% and peroxide value between 24.3–77.1 mEq/kg fat. Within the limits of experiment, the free fatty acids and peroxide values of the fish samples did not seem to be significantly influenced by the marinating time, irradiation dose and storage time. The wide variation in the fat content of the marinated fish products reflect variations in the spawning and eating behaviour of the fish [8]. Free fatty acids and peroxides have an impact on the flavour of foods [11,12].

4.3.3. Aerobic plate count (APC), coliform count, yeast and mould count

The APC for the marinated fish samples ranged between 0 to $\log_{10}$ 6.5 CFU/g fish, the coliform count was 0–300 CFU/g with the yeast and mould count being 0–380 CFU/g. Prediction equations could not be developed for the coliform or yeast and mould counts. It is, however, important to note that the highest coliform count (300 CFU/g) was on samples which were not irradiated,

relatively lower (50–100 CFU/g) on some of the samples irradiated with 2 or 5 kGy and absent on samples irradiated with 8 or 10 kGy. Mould and yeast growth occurred on some of the fish samples irradiated with 2, 5 or 8 kGy but were, however, absent from the fish sample treated with 10 kGy. The equation developed for the APC is shown in Table IX. The model suggested that irradiation dose had a significant inverse effect on the APC, that is, the load decreased as the dose increased. However, within the limits of the experiment, the marinating time and storage time had no significant effect on microbial load and probably a greater change in the product acidity was necessary to have an impact on microbial activity. Although increasing product acidity could suppress the growth of spoilage microbes and ultimately contribute to shelf-life extension, the limit of acidity is determined by that which would be acceptable to consumers. The product acidity could not be increased because the taste of the marinated fish was considered too sour; this observation reflected consumer attitude towards new products [10]. Further studies need to be carried out to find a suitable combination of the components of the marinade that would control microbial activity without adverse effect on product taste.

4.3.4. Sensory evaluation and overall acceptability

Scores for overall acceptability ranged between 5.6–8.9, thus, each of the combination treatments resulted in products that were highly acceptable. From the model coefficients presented in Table IX, the overall acceptability was influenced by dose and storage time. The model also suggested interaction between storage time and dose; thus, the effect of the storage time on the acceptability appeared to be dependent on the irradiation dose. Based on the model, contours generated (not shown) indicated that within the limits of the experiment, the predicted lowest acceptability score of 5.86 occurred at a combination treatment of 1 kGy and 1 week storage time. Predicted acceptability scores increased with dose but decreased with storage time.

5. CONCLUSIONS

Treatment of smoked sardines with 7–11 kGy extended product shelf-life although this trend was dependent on the product water activity. Smoke-dried sardines having a water activity between 0.764 and 0.857 are likely to be shelf-stable for some 12 weeks when irradiated. Treatment of marinated dried fish (a_w 0.913–0. 944) with a maximum dose of 10 kGy extended shelf-life from 3 to 7 days.

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PRODUCTION AND APPLICATION OF EDIBLE FILMS AND COATING IN RELATION TO RADIATION PRESERVATION OF CONVENIENCE FOODS

M. LACROIX*, B. OUATTARA*, C. LE TIEN* ***, S.F. SABATO***,
R. YESAF****, M. RESSOUANY*, C. VACHON*, G. D'APRANO*****,
P. ISPAS-SZABO**, D. ST-GELAIS******, M.A. MATEESCU**,
G. DELMAS-PATTERSON**

* Canadian Irradiation Centre (CIC) – Research Center in Microbiology and Biotechnology, Laval, Quebec), Canada

** Chemistry-Biochemistry department, Université du Québec à Montréal, Montreal, Quebec, Canada

*** Departamento de Aplicações Nucleares, São Paulo, SP, Brazil

**** Centre de dévelopement des techniques nucléaires, Alger, Algeria

***** Sabex Inc., Boucherville, Quebec), Canada

****** Food Research and Development Center, Agriculture and Agri-food Canada, Saint-Hyacinthe, Quebec, Canada

Abstract

The mechanical properties of cross-linked edible films based on calcium caseinate, whey and soya proteins were investigated. Cross-linking of the proteins was carried out using thermal and radiation treatments. The formation of bityrosine was found to increase with the irradiation dose for all formulations. Gamma irradiation combined with thermal treatment improved significantly the puncture strength for all types of films. Transmission electron microscopy showed that the mechanical characteristics of cross-linked films are closely related to their microstructures. Irradiated formulations showed also significant improvements of the barrier properties, namely water vapour permeability. Size-exclusion chromatography performed on the cross-linked proteins showed that gamma irradiation increased the molecular weight of calcium caseinate while little change was observed for the whey proteins. For cross-linked proteins, the molecular weight distribution was $\geq 2 \times 10^6$ kDa. Structural analysis of biofilms was also investigated. No major alterations of the structural conformation of the proteins were observed by FTIR for biofilms obtained after heat treatment, while gamma irradiation induced some modifications in the protein structure. Cross-linking by gamma irradiation seems to modify to a certain extent the conformation of proteins which will adopt more ordered and more stable structures, as suggested by X ray diffraction analysis. A loss of protein-water interaction was observed on cross-linked protein by isothermal calorimetry. Biodegradability evaluation on cross-linked films showed that the net bacterial degradation was 86% and 36% for films irradiated at 4 and 64 kGy, respectively, confirming that cross-links produced by gamma irradiation slowed the biodegradation of the material. The combined effect of gamma irradiation and antimicrobial coating on the shelf-life of pre-cooked shrimp (*Penaeus* spp.) and oven-ready all dressed pizza was also investigated. Antimicrobial coatings were obtained by incorporating various concentrations of thyme oil and trans-cinnamaldehyde in protein solutions prepared from soy and whey protein isolates. Coated shrimps and pizza were stored at 4°C under aerobic conditions and evaluated periodically for total aerobic plate counts (APCs) and *Pseudomonas putida*. Sensory evaluation for appearance, odour, and taste was also performed. Results showed a significant ($p \leq 0.05$) synergistic effect of gamma irradiation and coating in reducing the APCs and *P. putida* with at least 12 days shelf-life extension for shrimps and over 18 days for pizza. Without irradiation, the inhibitory effect of the coating solutions was closely related to the presence of thyme oil and trans-cinnamaldehyde. No detrimental effect of gamma irradiation on sensory parameters (appearance, odour, and taste) was observed.

1. INTRODUCTION

The last decade has seen considerable interest in the development of protein-based biodegradable edible films and coatings due to their application in the food industry, as substitutes for traditional plastic films [1]. Many publications have revealed their effectiveness in preventing quality changes in processed food and thus enhancing product shelf-life by serving as selective barriers to moisture transfer, oxygen uptake, loss of volatile flavours and aromas, as well as lipid oxidation [1,2]. Currently, a new concept is been developed in which selected antimicrobial compounds can be incorporated into packaging films or coatings in order to maintain high concentrations of preservatives on the surface of foods for longer storage times [3].

However, protein films exhibit poor water vapour barrier properties due to the hydrophilic nature of their amino acid groups [2,4]. Recent studies have concentrated on improving the structural properties of protein solutions and mechanical or barrier properties of resulting films [5-7]. For instances, the cross-linking of proteins by means of chemical, enzymatic or physical treatments was reported to improve the permeability, as well as the mechanical properties. These methods are thought to induce the formation of hydrogen, electrostatic, and covalent bonds [8]. For example, improvements in protein functionality by cross-linking soybean 11S and whey protein isolate using guinea pig liver transglutaminase has been reported by Yildirim et al. [9]. However, high production costs and limited availability of transglutaminase have limited its potential use in food systems. Electrostatic complexes between proteins and acidic polysaccharides, such as alginate, pectate and carboxymethyl cellulose, are also interesting mechanisms. However, these complexes are very unstable due to their sensitivity to pH changes [10,11].

The recent approval of meat irradiation by the Food and Drug Administration [12] has made consumers more confident in the technology and attracted the interest of industries concerned with food quality, food safety, and packaging technology. Gamma irradiation was recently reported to increase the cohesive strength of the protein by the formation of cross-links [13]. Indeed, the irradiation of aqueous protein solutions generates hydroxyl radicals (•OH) that produce stable compounds [14]. The gamma irradiation method presents more conveniences: it is a well known process for the sterilisation of foods [15] and it is less expensive than using enzymes. It is also known that the application of irradiation is an effective process to assure food safety. The recent review of Lacroix and Ouattara [16] indicates that more than 26 countries throughout the world are now using irradiation on a commercial scale. According to the microbiological hurdle concept [17] gamma irradiation technology can be used to develop edible films and coatings for food preservation. The present study focused on the use of gamma irradiation to develop edible films and coatings for applications in food preservation.

2. MATERIAL AND METHODS

2.1. Reagents

Calcium caseinate (Alanate 380, 91.8% w/w protein) was provided by New Zealand Milk Product Inc. (CA, USA). Whey protein isolate (WPI) was lyophilised and dried for 3 h in a vacuum oven at 80°C (Model 19 Laboratory oven, Precision Scientific Inc., Chicago, IL) from the solution purified at the Food Research and Development Centre (St-Hyacinthe, Québec, Canada). SUPRO 500E soy protein isolate (SPI) was provided by Dupont Campbell Protein Technologies (St-Louis, MO, USA). Polyethylene glycol (PEG, M.W= 8000) and carboxymethyl cellulose sodium salt (CMC, low viscosity) were obtained from Sigma Chemicals (St-Louis, MO, USA). Glycerol (99.5%, reagent grade), D-sorbitol pure and D-mannitol (U.S.P.) were purchased from American Chemicals Ltd Calcium chloride ($CaCl_2$, laboratory reagent) was obtained from BDH Chemicals (Canada). All products were used as received without further purification.

2.2. Preparation of films and coatings

Calcium caseinate, WPI and SPI were solubilised in distilled water, under stirring, at 80°C to obtain a total protein concentration of 5% (w/v) in the film forming solution. The pH was adjusted at 8.5, 0.25% (w/v) CMC was added. After complete solubilisation, 2.5% (w/v) of glycerol or desired amounts of PEG (0.5% w/w), sorbitol or mannitol (2.5% w/w) were added. Irradiation of the solutions was carried out at the Canadian Irradiation Centre (CIC) at dose levels of 0, 8,16, 32, 64, 96 or 128 kGy using a ^{60}Co source UC-15A (MDS-Nordion International Inc., Kanata, Ontario, Canada) at a mean dose rate of 31.24 kGy/h. Films were then cast by pipetting 5 mL of the solution onto smooth rimmed 8.5 cm (i.d.) polymethacrylate (Plexiglas) plates which were sitting on a levelled surface. Solutions were spread evenly and allowed to dry overnight at room temperature (20 ± 2°C) in a climatic chamber (45–50% relative humidity (RH)). Dried films could be peeled intact from the casting surface. Coating solutions were prepared following the same procedure, although the solutions

were used directly instead of being cast. Antimicrobial coatings were obtained by incorporation of selected essential oils or organic acids into the solutions. Shrimps were coated with different types of coating solutions: a base solution (without antimicrobial additives), the base solution plus 0.9% of a mixture of essential oils (EO–0.9), and the base solution plus 1.8% of the mixture of essential oils (EO–1.8). The coating solution for pizzas consisted of a calcium caseinate based solution (Longevita, BioEnvelop Technology, Inc.).

2.3. Fluorescence measurements

The formation of bityrosine was measured using a Spectrophotometer 2070 (Varian, CA, USA) according to the method previously reported by Davies [18].

2.4. Mechanical properties of films

Puncture tests were carried out using a Stevens LFRA Texture Analyzer Model TA/1000 (NY, USA) as described previously by Gontard et al. [19]. Films were equilibrated for 48 h in a desiccator containing a saturated NaBr solution ensuring a 56% RH atmosphere. A cylindrical probe (2 mm diameter) was moved perpendicularly at the film surface at a constant speed (1 mm/sec) until it passed through the film. Strength values at the puncture point were used to determine hardness and deformation capacity of the film. In order to avoid any thickness variation, the puncture strength values were divided by the thickness of the film.

2.5. Size-exclusion chromatography

Size-exclusion chromatography was performed using a Varian Vista 5500 HPLC coupled with a Varian Auto Sampler model 9090. Detection of the protein solution was carried out using a standard UV detector set at 280 nm. Two Supelco Progel TSK PWH and GMPW columns followed by two Waters Hydrogel columns (2000 and 500) were used for the molecular weight determination of the cross-linked proteins. The molecular weight calibration curve was established using a series of protein molecular weight markers (Sigma, MW-GF-1000, USA) ranging from 2×10^3 kDa to 29 kDa. All soluble protein solutions (0.5% w/v) were filtered on Whatman 0.45 μm filters (Whatman International Ltd, Maidstone, UK) prior to injection.

2.6. Isothermal calorimetry

These measurements were obtained with a calorimeter C80 (Setaram, France) in an isothermal mode (heats of swelling). A known mass of dried sample (30 mg) was introduced in a home-made thin glass bulb and sealed under vacuum. After thermal equilibrium, the bulb was broken by pushing gently from the top of the calorimeter a stem going through the stopper of the cell. Due to the vacuum in the bulb, water fills the entire glass bulb and interacts with the sample. The value of $\Delta H_{\text{experimental}}$ after integration of the heat flow change is the sum of three contributions:

$$\Delta H_{\text{experimental}} = \Delta H_{\text{interaction}} + \Delta H_{\text{glass-breaking}} + \Delta H_{\text{vaporization}}$$

The two last terms can be measured by blank experiments. By subtracting their value (about –200 to –150 mJ) from $\Delta H_{\text{experimental}}$, the $\Delta H_{\text{interaction}}$ is obtained.

2.7. FTIR Spectroscopic analysis

FTIR spectra were recorded using a BOMEM Hartman & Braun (Bomem, Inc., Québec, Canada) equipped with DTGS detector (Deuterated triglycine sulfate). Spectra were analysed using the BOMEM GRAMS software (Ver. 1.51). The biofilms were placed in the BOMEM cell for scanning spectral region (4000–500 cm^{-1}) and 50 scans were recorded with a 1 cm^{-1} resolution. The second derivatives of spectra that narrow the broad Amide I band related to the different protein chain conformations, were equally analysed [20].

2.8. X ray diffraction

The diffraction pattern of protein films was recorded by a Siemens D-5000 diffractometer with cobalt cathode operating in reflectance mode at wavelength λ = 1.79019 Å (30000 Volts and 16 mA).

2.9. Water vapour permeability

Water vapour permeability (WVP) of films was determined gravimetrically using a modified ASTM 15.09:E96 (1983) procedure. The films were sealed with silicone sealant High Vacuum Grease Dow Corning (Midland, MI, USA) in a glass permeation cell containing phosphorous pentoxide (0% RH, 0 mmHg water vapour pressure). The permeation cell was placed in a desiccator maintained at 100% RH (17.54 mmHg water vapour pressure, at 20°C) with distilled water. The water vapour transferred through the film and absorbed by the desiccant was determined from the weight gain of the cell. The permeability values were calculated as described by Gontard et al. [19] using the following equation:

$$WVP = (W \cdot X)/A \cdot T \cdot (P1 - P2)$$

where W is the weight gain of the cups (g), T is the time (days), X is the film thickness (mm), A is the exposed area of the film (m^2) and P2–P1 is the water vapour pressure differential across the film (32.23 mmHg for 100% RH and 9.82 mmHg for 56% RH).

2.10. Insolubility measurements

The average dry weight of the films was determined on seven films by drying them in an oven at 45°C until constant weight was achieved (6 or 7 days). Seven more films were dropped in 100 mL of boiling water for 30 min. The flasks were removed from the heat and the films remained in the water for another 24 h. Then, the solid films were removed and dried in the oven as previously described. Results are calculated using the following equation:

$$[\text{Dry Weight (solid residues)/ Dry Weight (untreated film)}] \times 100$$

2.11. Transmission electron microscopy (TEM)

Dry films were first immersed in a solution of 2.5% glutaraldehyde in cadodylate buffer, washed and post-fixed in 1.3% osmium tetroxide in collidine buffer. Samples were then dehydrated in acetone (25, 50, 75, 95 and 100%) before embedding in a SPURR resin. Polymerisation of the resin proceeded at 60°C for 24 h. Sections were made with an ultramicrotome (LKB 2128 Ultrotome) using a diamond knife and transferred on Formvar-carbon coated grids. Sections were stained 20 min with uranyl acetate (5% in 50% ethanol) and 5 min with lead citrate. Grids were observed with a Hitachi 7100 transmission electron microscope operated at an accelerating voltage of 75 keV.

2.12. Resistance to microbial degradation

This experiment was conducted to evaluate the degradation of the films by *Pseudomonas aeruginosa* (ATCC 15442). An overnight culture of the bacterial strain was first washed three times in sterile saline solution (NaCl, 0.9%, w/v) to remove all traces of the culture media. The bacterial cells were then resuspended in flasks containing 300 mL of sterile stock solution described in ASTM D5209 (10^7 cells/ml, final concentration) in the presence of films under study. In order to accurately measure the bacterial growth due to the degradation of the films, three solutions were compared: one stock solution inoculated with *P. aeruginosa* without film and two stock solutions inoculated with *P. aeruginosa* and containing various formulations of unirradiated or irradiated films. All flasks were incubated at 37 ± 1°C with shaking (130 rpm) for 60 days and samples were taken periodically for soluble N analysis using a Leco FP-428 combustion oven apparatus (Leco Corporation, St. Joseph, MI, 49085-2396

USA). Values of nitrogen produced in media without bacteria were subtracted from those obtained in solutions inoculated with *P. aeruginosa* in order to obtain the net soluble N released by bacteria.

2.13. Antimicrobial and shelf-life tests

Peeled shrimp (*Penaeus* spp.) samples were purchased chilled at a local grocery store (IGA, Laval, Quebec, Canada) and transported to CIC in a thermal container. Upon arrival (within 20 min of purchase), samples were defrosted overnight at 4°C prior to application of the coating solutions. All dressed refrigerated pizzas were purchased from Sorrento Inc. (Chicoutimi, Quebec) and used within 24 h of manufacture.

Shrimp samples were randomly assigned into six treatment lots consisting of one control (uncoated) and three lots treated with the following coating solutions; Base, EO–0.9, EO–1.8. For each coated lot, approximately 200 shrimp were immersed for 5 min in 500 mL of the coating solution with gentle swirling using a sterile glass rod to ensure complete contact of the shrimp with the coating solution. Shrimp were removed and allowed to drain for 5 min on a pre-sterilised metal net under a biological containment hood. After draining off any excess coating solution, samples were placed in sterile Petri plates (8.1 cm i.d.) (approximately 15 shrimp/plate). Plates containing uncoated and coated shrimp were divided into two groups. One group was irradiated with a dose of 3 kGy using a UC15 under water calibrator (MDS Nordion, Kanata, Ontario, Canada). The second group served as unirradiated controls. Pizza samples were first assigned into two treatment groups (uncoated and coated with Longevita). For each group, three sub-groups were constituted and irradiated at 0, 1, and 2 kGy. Both shrimp and pizzas were stored at 4°C for 21 days, and duplicate samples were taken periodically for aerobic plate counts (APCs).

In a separate experiment, the effect of gamma irradiation and coating was evaluated on artificially contaminated shrimp with *P. putida* isolated from refrigerated beef at the Food Research and Development Center (St-Hyacinthe, Quebec, Canada). Samples were prepared following the procedure described above, but shrimp were first dipped in sterilised saline water (0.9%, w/v) containing approximately 10^5 colony forming units (CFU)/ml of *P. putida*. The mean level of bacterial contamination obtained at day one was approximately 2 $\log_{10}$ cells/g of shrimp before irradiation.

2.14. Microbial analysis

Approximately 10 g of shrimp or pizza were weighed and homogenised in sterile peptone water (0.1%) using a Lab-blender 400 stomacher (Laboratory Equipment, London, UK). From this mixture, serial dilutions were prepared and appropriate ones were spread plated on sterile Petri plates containing plate count agar (Difco Laboratories, Detroit, MI, USA) for the numeration of total APCs. Plate were incubated at 35 ± 1°C for 48 h. The numeration of *P. putida* was done on Brain Infusion Agar (BIA, Difco Laboratories, Detroit, MI, USA) following the same procedure. Experiments were done in duplicate and three samples were analysed at each sampling time. The limit of acceptability was calculated based on the onset of shrimp and pizzas spoilage that was considered to be 10^7 and 10^6, respectively.

2.15. Sensory evaluation

Samples were given three-digit codes and assessed by a panel of eleven untrained members (students and employees of INRS-Institut Armand-Frappier, Laval, Quebec, Canada) for colour, taste and general appearance on a nine-point hedonic scale [21]. On this hedonic scale, 1 represented attributes most disliked and 9 represented attributes most liked.

2.16. Statistical analysis

All the data were subjected to analysis of variance (Anova procedure, SPSS Inc., Chicago, IL) for determination of sample and combined effects of various factors including types of formulation, irradiation treatment, types and concentrations of antimicrobials incorporated into coating

solutions. Comparisons between means were performed through Duncan multiple range test, Least Significant Difference (LSD) test, or Student's *t* test. Differences between means were considered significant when $p \leq 0.05$.

3. RESULTS

3.1. Physico-chemical characteristics and mechanical properties of films

3.1.1. Formation of bityrosine

Irradiation of the protein solutions resulted in the formation of bityrosine as suggested by the fluorescence analysis (Fig. 1). Bityrosine is a covalently bound phenol, produced by reaction of two tyrosyl radicals or a tyrosyl radical plus a tyrosine molecule [22]. It can be seen that the fluorescence signal of bityrosine increased with the irradiation dose resulting in a higher number of cross-links between tyrosine units. Results showed that the bityrosine fluorescence continues to increase with irradiation doses suggesting, at first glance, an increase in the number of cross-links. For the basic formulation, the bityrosine signal was 20 times more intense at 128 kGy than 0 kGy.

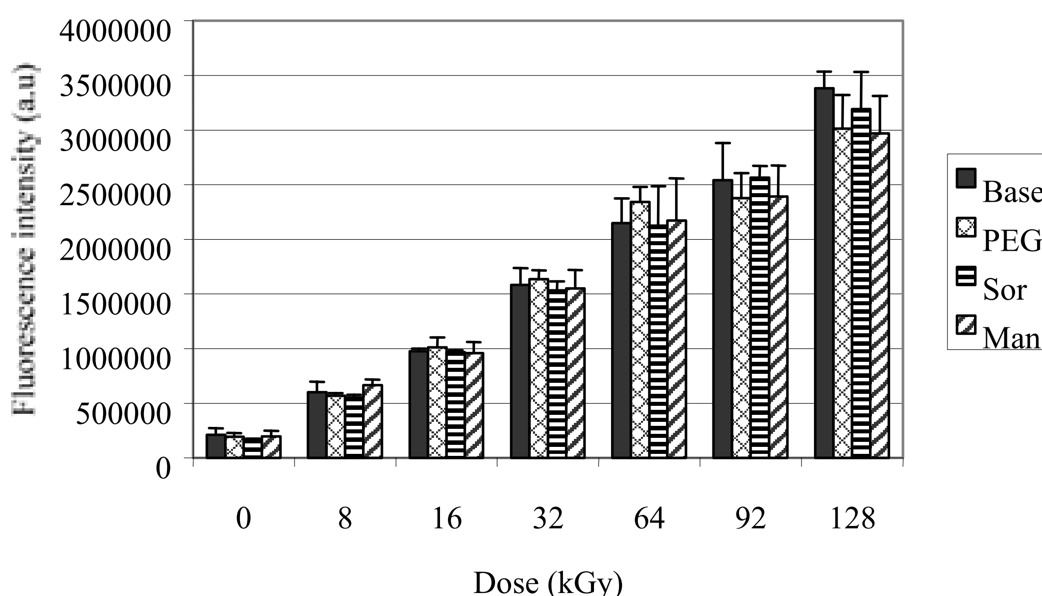

FIG. 1. Formation of bityrosine in calcium caseinate solution as a function of irradiation dose (PEG = Polyethyleneglycol; Sor = sorbitol; Man = Manitol).

3.1.2. Puncture strength

Figure 2 shows the puncture strength variations of films cast from solutions containing soya, WPI, calcium caseinate proteins, soya-WPI, and casein-WPI proteins (5% w/w total protein solution). For instance, a protein ratio of 50–50 corresponds to 2.5% of each protein. The figure shows the results obtained for solutions that were unirradiated or irradiated at 32 kGy. It can be seen that gamma irradiation significantly increased ($p \leq 0.05$) the mechanical properties of the films by inducing cross-links between protein chains for all formulations. For instance, for films based only on calcium caseinate, gamma irradiation increased the puncture strength by more than 35% (78 to 106 N/mm). For WPI, gamma irradiation increased the puncture strength by 3% (39–40 N/mm). For the films made from an equal protein ratio (50–50), the increase of puncture strength was 20% (84–100 N/mm) for caseinate-WPI. Results also showed that gamma irradiation resulted in a significant increase in puncture strength of 7% (49–52 N/mm) and 21% (33–41 N/mm) respectively for soya and soya-whey system.

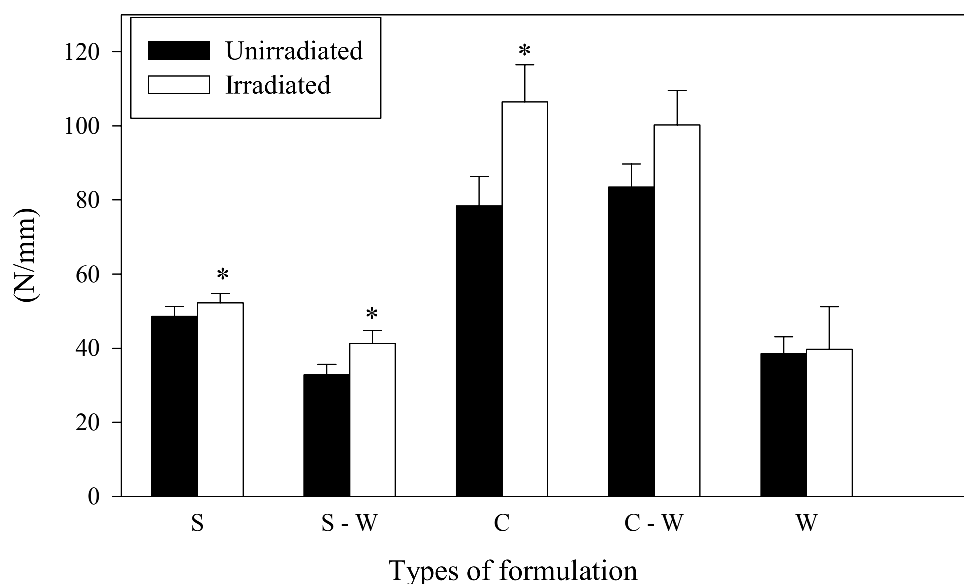

*FIG. 2. Effect of gamma irradiation at 32 kGy on puncture strength of protein-based edible films (S = Soy protein isolate; W = Whey protein isolate; C = Calcium caseinate; * = Significant difference compared to corresponding unirradiated films).*

3.1.3. Size-exclusion chromatography

Figure 3 shows the elution curves obtained for native calcium caseinate, heated and irradiated. No molecular weight changes occurred when the protein was heated at 90°C for 20 min. However, when the protein was submitted to gamma irradiation at a dose of 32 kGy, cross-linking occurred and the molecular weight distribution peak shifted to higher molecular weights by more than 60-fold ($\geq 2 \times 10^6$ daltons). Previous works had demonstrated that gamma irradiation induced the formation of bityrosine [13,23,24].

Figure 4 shows the elution curves obtained for whey proteins, heated and irradiated. It can be seen that gamma irradiation induced very little molecular weight changes in the protein. SPI showed two main molecular weight peaks, in the range of ca. 60 kDa and ca. 2000 kDa (Fig. 5). When gamma irradiation was combined with heating, further cross-links were generated, as suggested by the size exclusion chromatography elution patterns, by more than 15-fold.

The irradiation of the soya system at 32 kGy induced a decrease of the 60 kDa peak and an increase of a peak at ca. 200–2000 kDa. It is clear that the combination of gamma irradiation with thermal treatment generates a much more important protein aggregation, since further cross-links are formed: bityrosine in addition to disulfide bonds.

3.1.4. Barrier properties

Table I presents the WVP for the formulations investigated, expressed in g·mm/m^2·24h·mmHg. Values of WVP for unirradiated protein were 3.16, 3.44 and 2.08 for soy, caseinate, and caseinate-whey proteins, respectively. The contribution of the irradiation treatment was found to be significant only in caseinate and soya proteins formulations. The WVP of irradiated soya and caseinate proteins were 2.03 and 2.59, respectively, corresponding to a decrease of 36% for soya and 25% for caseinate proteins. This behaviour could be explained by the increase of protein-protein interactions resulting from the formation of bityrosine, viz cross-links, which results in a decrease of the diffusivity of the permeant [25]. Results showed also, that the addition of whey did not have a significant impact on the barrier properties of the biofilms.

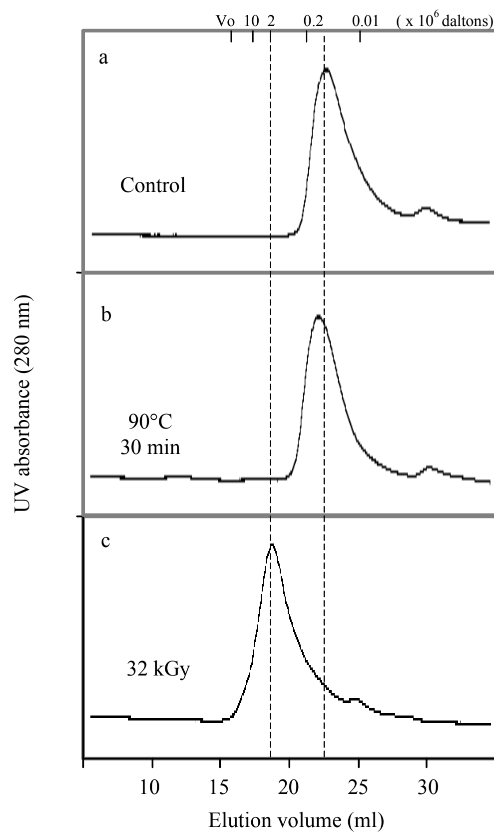

FIG. 3. Size Exclusion Chromatography. Elution curves for calcium caseinate (Alanate 380):(a) native; (b) heated at 90℃ for 30 min; and (c) irradiated at 32 kGy.

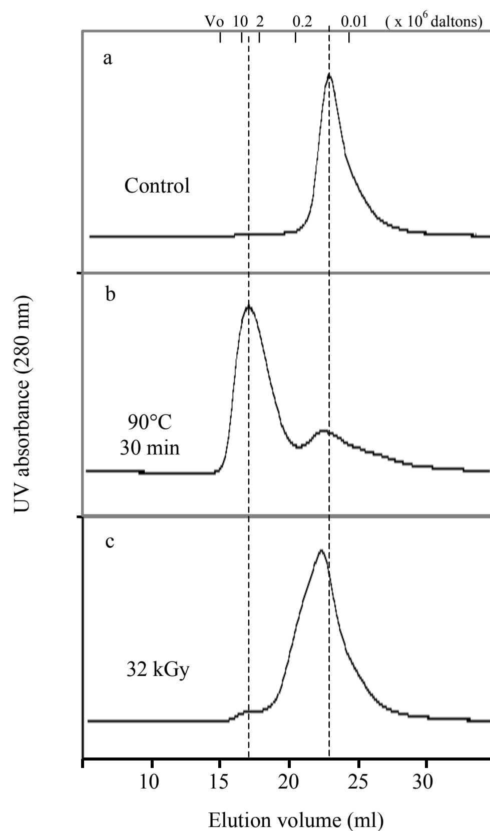

FIG. 4. Size Exclusion Chromatography. Elution curves for commercial whey proteins (WPC): (a) native; (b) heated at 90℃ for 30 min; and (c) irradiated at 32 kGy.

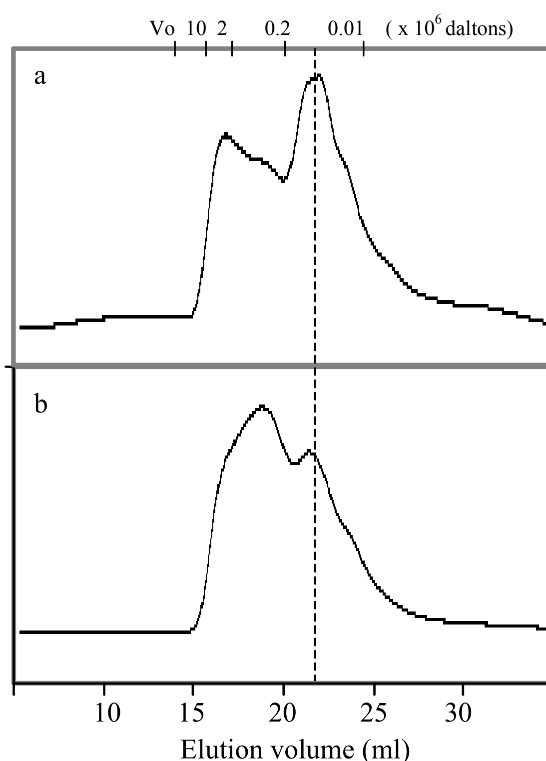

FIG. 5. Size Exclusion Chromatography. Elution profile of SPI (a) heated and (b) heated in combination to irradiation at 32 kGy.

3.1.5. Isothermal calorimetry

Table II shows the values of the $\Delta H_{interaction}$ with water at 30°C of the unirradiated and irradiated caseinate, caseinate/whey and whey proteins. The high negative values of ΔH (–64 to –65 J/g) for the caseinate/whey and whey samples reflect the hydrophilic character of the non-treated films. The negative values of the ΔH are associated with the formation of hydrogen bonds between water and proteins at the moment of immersion. The effect of treatments is probably related to the formation of hydrogen bonds between the chains of the protein. Consequently, the OH– or other polar groups are no longer available for interacting with water. The diminution of $\Delta H_{interaction}$ due to the treatment from –65 to –10 J/g for irradiated whey proteins, from –28 to –19 J/g for irradiated caseinate and from –64 to –56 J/g for caseinate/whey proteins reflects the loss of film-water interaction. These results reinforce data obtained by the solubility tests.

TABLE I. EFFECT OF GAMMA IRRADIATION ON THE WATER VAPOUR PERMEABILITY (WVP) OF EDIBLE PROTEIN BASED FILMS[1]

Formulation	Unirradiated	Irradiated
	g·mm/m²·d·mmHg	g·mm/m²·d·mmHg
Caseinate	3.44 ± 0.19	2.59 ± 0.59*
Caseinate-WPI	2.08 ± 0.51	1.66 ± 0.53
WPI	2.85 ± 0.19	1.56 ± 0.17*
SPI	3.16 ± 0.39	2.03 ± 0.28*
SPI–WPI	2.68 ± 0.29	2.95 ± 0.31

[1]Mean values of irradiated samples marked with asterisks are significantly different ($p \leq 0.05$) from corresponding unirradiated films.
WPI = Whey protein isolate
SPI = Soy protein isolate

TABLE II. RESULT OF ISOTHERMAL CALORIMETRY SHOWING THE EFFECT OF GAMMA IRRADIATION ON ΔH INTERACTION PROTEIN-BASED FILMS WITH WATER

Formulation	Unirradiated (J/g)	Irradiated (J/g)
Calcium caseinate	–27.95 ± 0.75	–18.57 ± 1.02
Calcium caseinate/WPI	–64.21 ± 3.15	–56.15 ± 1.60
WPI*	–65.00 ± 5.10	–10.12 ± 2.15

*Formulation based on entrapment technology
WPI = Whey protein isolate

3.1.6. FT-IR analysis

FT-IR spectra obtained from the films (control, heating and gamma irradiation) were analysed in two interesting spectral regions: 3600–3000 cm^{-1} and 1700–1600 cm^{-1} (amide I). For the spectral region 3600–3000 cm^{-1}, a strong band was observed at 3293 cm^{-1} (Figs 6 and 7) almost due to NH stretch of proteins. Although there are few studies on proteins concerning this spectral region, Bandekar [26] noted that the band of NH stretch mode is generally at 3254 cm^{-1}. There is therefore a displacement of the band that could be due, in the case of this work, to the presence of other components in the biofilm formulation, especially glycerol and cellulose with large amounts of hydroxyl groups. No major differences were observed for heated and gamma irradiated films but all differed to the control films for which a larger band (3300–3600 cm^{-1}) was observed. This phenomenon of enlargement, in the untreated films, could be related to the unbounded or free HO– groups vibration. These free HO– groups could be ascribed mainly to water retained in the control film.

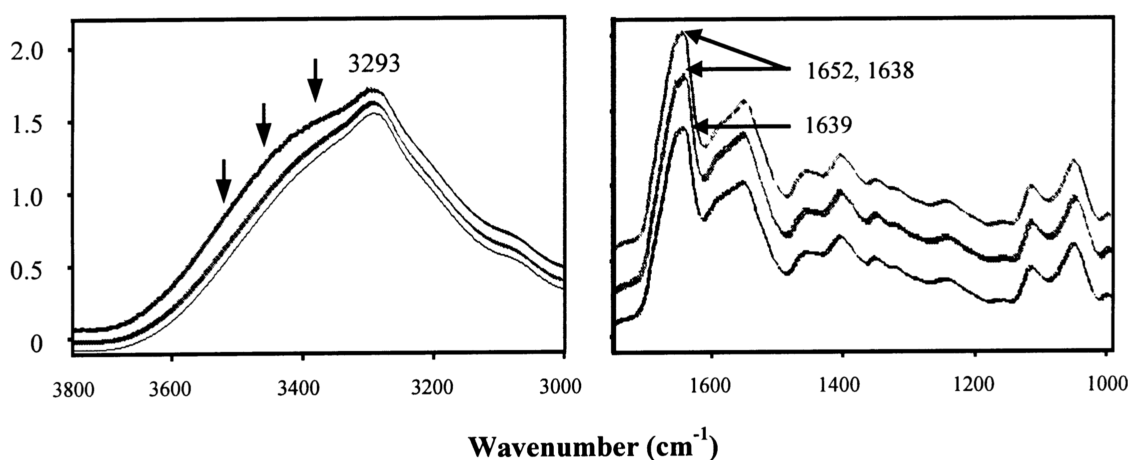

FIG. 6. FT-IR spectra of whey protein film. (a) Spectral regions 3600–3000 cm^{-1}, and (b) spectral regions 1700–1000 cm^{-1} (upper curve: control film; middle curve: heated film; lower curve: irradiated film).

An explanation could be related to the protein cross-linking. It is supposed also when cross-linked, these groups are more involved in hydrogen association and are less susceptible to hydration.

As reported by several studies, whey proteins contain, in the majority, α-lactalbumin and β-lactoglobulin [27,28]. These proteins are organised generally in α-helix (small fraction) and β-sheet structures. Another important fraction consists of random conformations that can be considered as 'unordered structure'. In the amide I, several bands at 1655–1635 cm^{-1} spectral region were observed [26]. For the control and heated films, two strong bands were noted at 1653 cm^{-1} and 1638 cm^{-1}. These

bands most likely result from α-helix and β-sheet conformation, respectively. However, the band at 1653 cm^{-1} was not found for films processed by gamma irradiation. Consequently, these results suggest that the gamma irradiation can cross-link proteins and, at the same time, lead to an alteration of the conformation of proteins while the heating does not. These changes could be related to a tendency of the proteins to adopt a more stable structure after cross-linking.

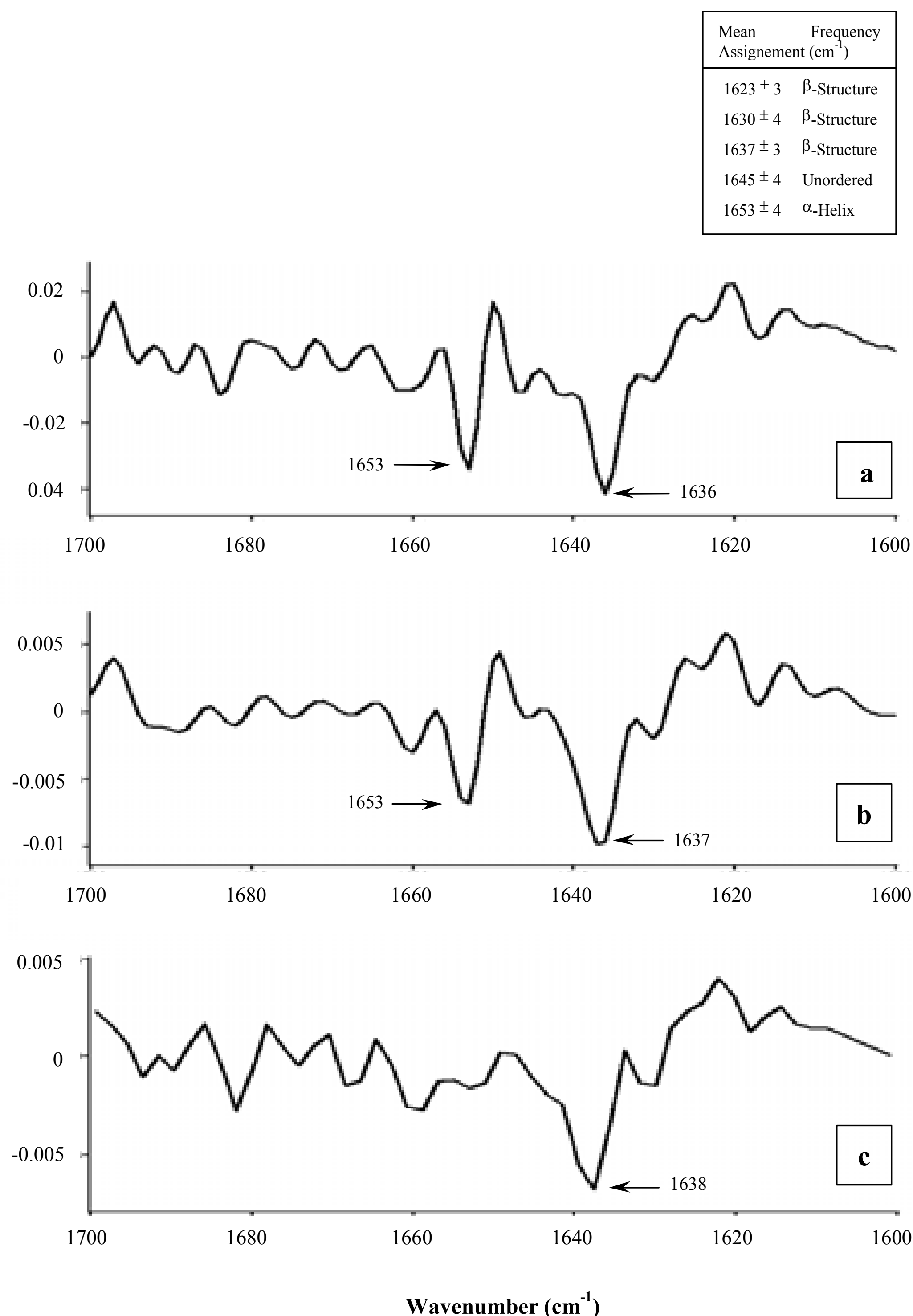

FIG. 7. Second derivative of the FT-IR spectra of whey protein films: (a) control film; (b) heated film; and (c) irradiated film. Assignment of main frequencies (insert) is based on spectral data from 17 proteins (including β-lactoglobulin and α-lactalbumin) according to Byler and Susy [20].

3.1.7. X ray diffraction

Results for the diffractogram are presented in Fig. 8. Increasing the gamma irradiation dose from 48 to 64 kGy, resulted in a change of the X ray diffraction profiles showing that the sharp angle of films increases as a function of the degree of cross-linking. The gamma irradiation induced chains cross-linking and a new structure, more ordered and more stable. The same results were obtained (data not shown) when films were submitted to different degrees of relative humidity (0, 56 and 100% RH) suggesting that the order of these films is independent of moisture.

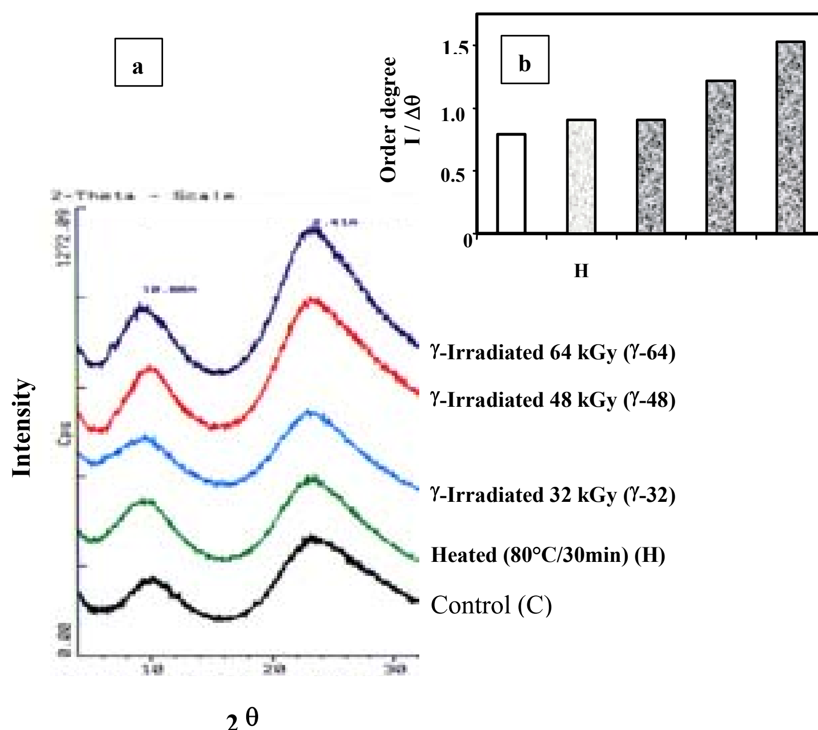

FIG. 8. X ray diffraction of whey protein films (a) and ordered degree (I/Δθ, where I is the band intensity and Δθ is the half-peak maxima) for different treatments (b).

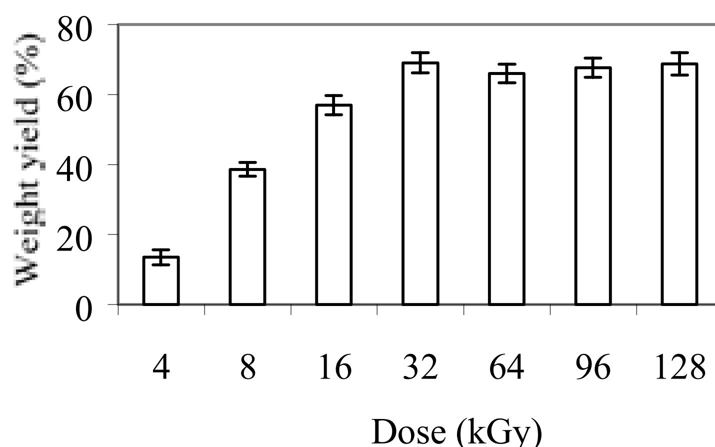

FIG. 9. Fraction of insoluble matter of calcium caseinate films as a function of the irradiation dose. Results are expressed as the percentage in solid yield after soaking the films 24 h in water.

3.1.8. Insolubility

Figure 9 shows the results obtained for calcium caseinate films irradiated at different doses. It can be seen that the proportion of the insoluble fraction increases with the irradiation dose up to 32 kGy where 70% of the film remains insoluble after 24 h. Similar results are obtained for cross-linked soya and whey proteins (data not shown). These results are also supported by the size exclusion chromatography results (Figs 3 and 4) which suggest that a maximum cross-linking density was obtained near 32 kGy. The size exclusion chromatography results combined with the insolubility measurements indicate that the irradiation of protein solutions led to the formation of an insoluble fraction of high molecular weight.

3.1.9. Microstructure observations

The cross-sections of the films were observed using transmission electron microscopy (TEM). Figure 10 shows the micrographs obtained for cross-sections of films made from calcium caseinate. The micrographs show that the structure of these films is highly porous. However, the microstructure of the films cast from irradiated solutions (Fig. 10b) is clearly more dense than the films cast from unirradiated solutions (Fig. 10a). Cross-links, which are present in the irradiated films, increase the molecular proximity of the protein chains. This increased molecular proximity as well as the additional molecular bonds directly influence the macroscopic characteristic of the films in terms of mechanical strength and water-resistance. The topography of the films varies from a porous structure to a more granular one. Similar correlations between microstructure and mechanical strength were observed in films based on whey protein. However, the general structure of films containing WPI was generally more dense and more homogeneous (Fig. 11).

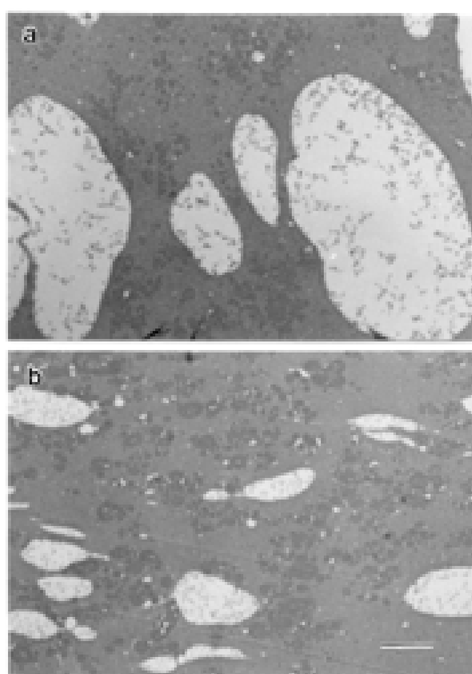

FIG. 10. Cross-sections of (a) unirradiated, or(b) irradiated (32 kGy) calcium caseinate films (9 mm bar = 3 μm).

3.1.10. Soluble nitrogen analysis

Figure 12 shows the percentage of nitrogen from calcium caseinate films converted to soluble N content as a function of time for 4 kGy and 64 kGy films. When the films irradiated at 4 kGy were incubated with the bacteria, a significant increase ($p \leq 0.05$) of soluble N content was noted

on day 3 and reached a value of 0.63% and remained higher than 0.5% until day 50. When the 64 kGy films were incubated in presence of the bacteria, the percentage of nitrogen converted to soluble N was lower than 0.10% until day 50. The overall increase for the 64 kGy films over the entire experimental period was much less than for the 4 kGy films. On day 60, the net bacterial degradation was 86% and 36%, respectively, for 4 and 64 kGy films, confirming that cross-links produced by gamma irradiation slowed the biodegradation of the material.

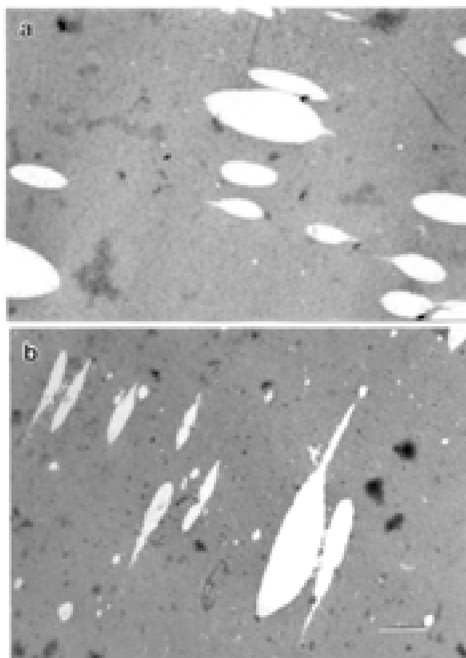

FIG. 11. Cross sections of WPI-calcium caseinate films: (a) heated at 90℃ for 30 min, and (b) heated at 90℃ for 30 min and irradiated at 32 kGy (9 mm bar = 3 μm).

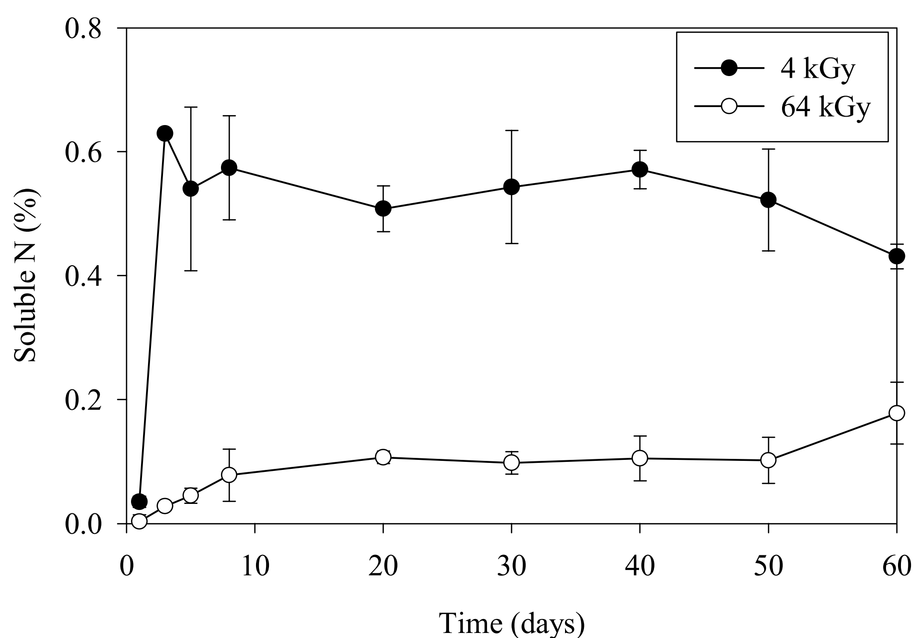

FIG. 12. Percentage of nitrogen from calcium caseinate films converted to soluble N (% ± S.D) by P. aeruginosa in standard stock solutions.

3.2. Antimicrobial tests

3.2.1. Shrimps

Counts of bacterial population in unirradiated and irradiated shrimp are shown in Fig. 13. In unirradiated samples APCs increased significantly during storage. The inhibitory effect observed was closely related to the concentration of the mixture of essential oils in the coating solutions (Fig. 13a). The patterns of bacterial growth in irradiated samples were quite different to those observed in unirradiated samples (Fig. 13b). The irradiation process resulted in significant ($p \leq 0.05$) increase of lag periods before initiation of bacterial growth.

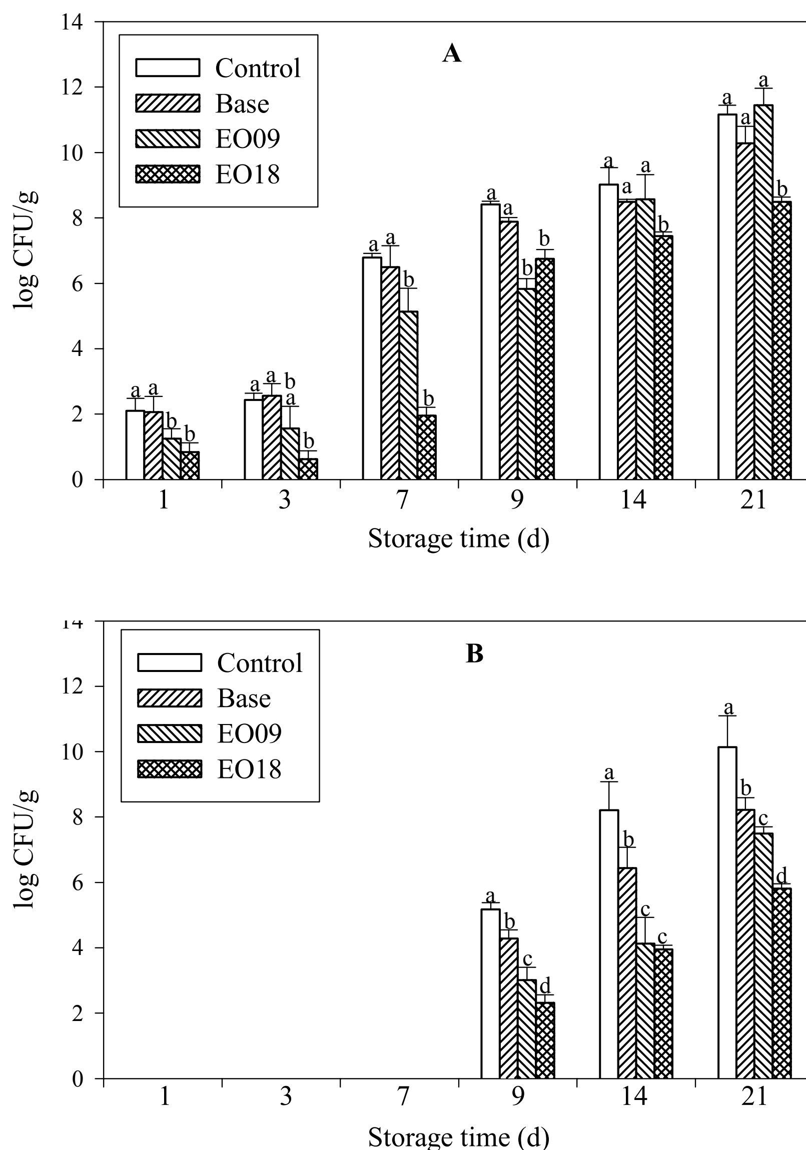

FIG. 13. Counts of bacterial population (APCs) in unirradiated (a) and irradiated (b) shrimp during storage at 4°C.

For both uncoated and coated samples, no viable colony forming units were detected during the first 7 days of storage. Furthermore, at all the sampling days, total APCs in irradiated samples were significantly ($p \leq 0.05$) lower than corresponding unirradiated samples. Similarly, growth of *P. putida* increased significantly to reach maximum values of 10.76 to 12.24 CFU/g after 21 days of storage (Fig. 14). With irradiation, initiation of bacterial growth occurred only after 7 days, and total counts

remained significantly ($p \leq 0.05$) lower than in unirradiated samples over all the experimental period (21 days). A synergistic inhibitory effect was also observed when irradiation was combined edible antimicrobial coating.

Based on the onset of shrimp spoilage, established at 10^7 bacteria/g, the shelf-life periods of unirradiated and irradiated shrimp were estimated (Fig. 15). Data indicated that without irradiation, the limit of acceptability was reached after 9 days for uncoated and samples coated with the base solution, and 14 days for those coated with EO–0.9 and EO–1.8. With irradiation, the limits of acceptability ranged from 14 to more than 21 days, corresponding to a shelf-life extension of 5 to more than 12 days.

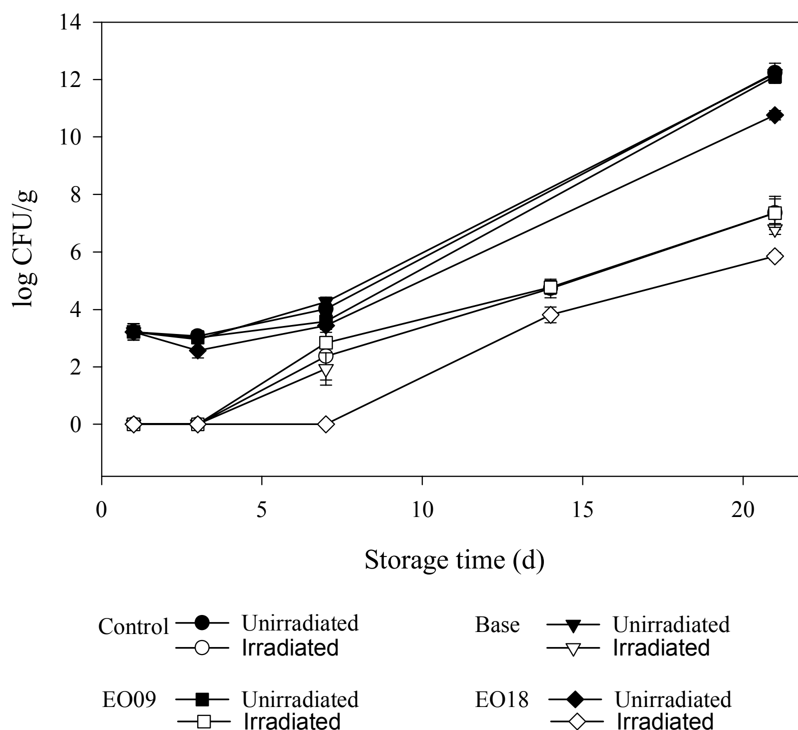

FIG. 14. Effect of gamma irradiation and antimicrobial coating on the growth of P. putida.

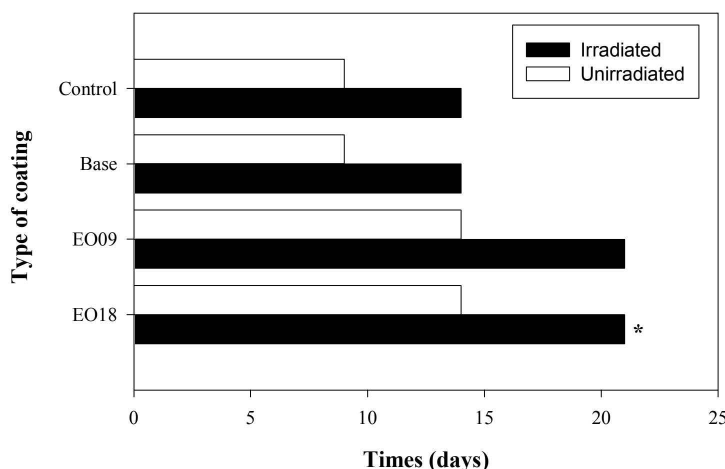

*FIG. 15. Shelf-life extension of precooked shrimp as affected by gamma irradiation and antimicrobial coating. Results are expressed in terms of time to reach the onset spoilage (10^7 bacteria/g). * The onset of spoilage was not reached at the end of the experimental period (21 days).*

3.2.2. Pizzas

The effect of irradiation alone and irradiation combined with antimicrobial coating on the shelf-life of pizza samples is presented in Fig. 16. The level of contamination before irradiation and coating treatments was 4.3 log CFU/g. Gamma irradiation alone produced 2 to 3.5 log unit reduction of APCs depending on the dose (Fig. 16a). Furthermore, growth rates during storage were significantly reduced ($p<0.05$). Shelf-life periods obtained were 3 days for unirradiated samples compared to 12 and 14 days for samples irradiated at 1 and 2 kGy, respectively. Combining irradiation with antimicrobial coating resulted in a synergistic inhibitory effect (Fig. 16b). Indeed, the shelf-life periods were extended to 21 days for coated sample irradiated at 1 kGy and more than 21 days for those irradiated at 2 kGy.

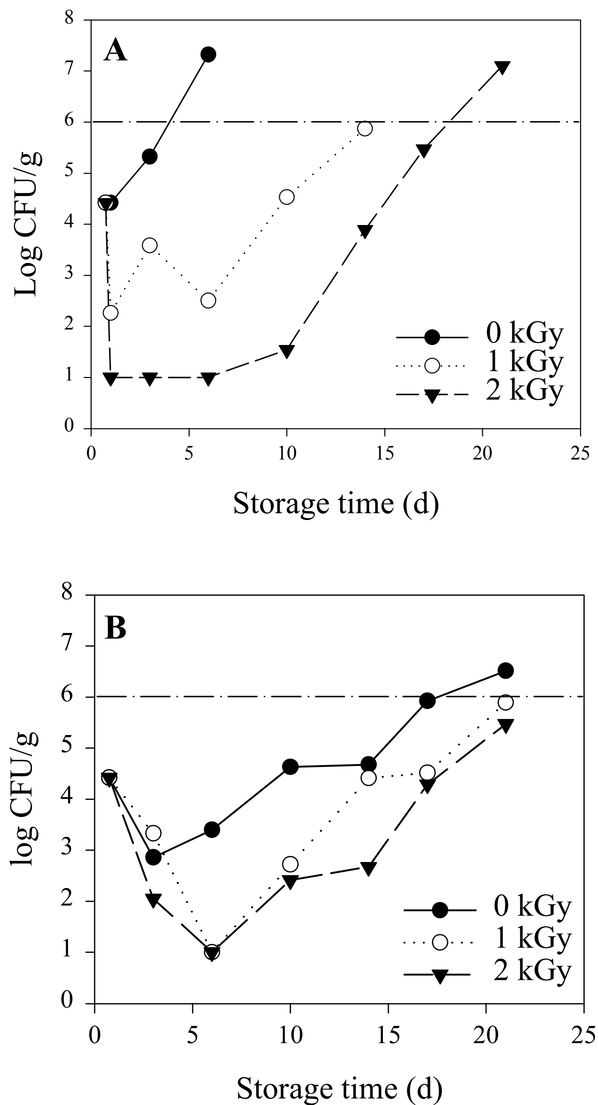

FIG. 16. Shelf-life extension of ready-to-eat pizzas as affected by gamma irradiation and edible coating during storage at 4°C.

Experimental results showed that a combination of gamma irradiation and antimicrobial coating enhanced the microbiological quality and shelf-life of peeled shrimp and refrigerated pizzas. This effect was characterised by a longer lag period, lower growth rates, and therefore significant shelf-life extension in irradiated samples. The primary mechanism of microbial inhibition by ionizing radiation is by the breakage of chemical bonds within the DNA molecules or alteration of membrane permeability and other cellular functions [29,30]. This may facilitate the contact between antimicrobial

molecules with cell membranes, and increase their inhibitory effects. Several previous reports on the combination of gamma irradiation and other treatments suggested that microorganisms which survive irradiation treatment, will probably be more sensitive to environmental conditions (temperature, pH, nutrients, inhibitors, etc.) than untreated ones [31,32,16]. These observations are also supported by the report of Mahrour et al. [33] who combined marinating in natural plant extracts with gamma irradiation, and obtained a significant reduction in the irradiation dose required to control pathognenic *Salmonella* on fresh poultry. Also, incorporation of ascorbyl palmitate (200 ppm) in ground beef prior to irradiation at 1.5 kGy resulted in a 3-log unit additional reduction of total aerobic and lactic acid bacteria counts [34].

Immobilising antimicrobials in coating solutions is a very advantageous technology for food preservation. The resulting biofilms or coatings provide more inhibitory effects against spoilage and pathogenic bacteria by lowering the diffusion processes and maintaining high concentrations of the active molecules on the food surfaces [35,36]. Siragusa and Dickson [37] immobilised various organic acids into a calcium alginate gel and obtained a greater reduction in the growth of *Listeria monocytogenes* on lean beef muscle than when the organic solutions were directly applied by dipping. Similarly, a chitosan-based biofilm containing acetic acid, propionic acid, trans-cinnamaldehyde, or lauric acid has been found to produce a significant extension of the shelf-life of cooked meat products by inhibiting the growth of total *Enterobacteriaceae* and *Serratia liquefaciens* [38]. The mechanism by which microorganisms are inhibited by phenolic compounds present in essential oils involves a sensitisation of the phospholipid bilayer of cell membrane, causing an increase of permeability and leakage of vital intracellular constituents [39,40], or impairment of bacterial enzyme systems [41]. Other compounds acts by inhibiting the amino acid decarboxylase in target bacteria [41].

The enhancement of the effectiveness of the antimicrobial coatings by gamma irradiation can also be attributed to the cross-linking effects of ionising radiation on protein molecules. As previously reported by Brault et al. [13], irradiation of aqueous protein solutions generates hydroxyl radicals which react with aromatic residues to form covalent bonds. That observation was further confirmed by size-exclusion chromatographic analysis showing an increase of high molecular weight protein in protein-based film forming solutions subjected to various irradiation doses [24]. It can be hypothesized that an increase of the molecular weight of proteins in the carrier matrix will reduce the diffusion and lead to a higher concentration of the active compounds in the coating for longer storage time. The hypothesis of reduction of diffusion can also be explained in terms of structural modification of protein molecules that limit the transport of active compounds present in essential oils through the network. This is consistent with published data on the diffusion characteristics of active compounds incorporated into polymer solutions [38,42].

3.3. Sensory evaluation

From variance analysis relative to sensory evaluation of shrimp, none of the sensory parameters (appearance, odour or taste) was significantly affected by gamma irradiation. Mean values on the hedonic scale ranged from 6.40 to 6.70 for unirradiated samples and 6.45 to 6.73 for irradiated shrimp (Table III). These results support those of Giroux and Lacroix [43] who found that low-dose irradiation can be used to extend the shelf-life of food products without having detrimental effects on biochemical and nutritional characteristics.

In general, unacceptable odours or flavours result from lipid oxidation by free radicals generated by high irradiation doses [44]. Coating did not effect the appearance of shrimps but significantly reduced ($p \leq 0.05$) acceptability for odour and taste. When essential oils were added to the base solution, acceptability for taste and odour decreased significantly ($p \leq 0.05$) to reach 4.38–4.50 and 4.67–4.86, respectively. The low score obtained in sensory testing for odour and taste can be related to the intrinsic sensory characteristics of thyme oil and trans-cinnamaldehyde.

TABLE III. EFFECT OF COATING AND GAMMA IRRADIATION ON THE SENSORY PARAMETERS OF SHRIMP: APPEARANCE, ODOUR, AND TASTE[1, 2]

	Sensory parameters					
	Appearance		Odour		Taste	
	Unirradiated	Irradiated	Unirradiated	Irradiated	Unirradiated	Irradiated
Control	6.56±2.30	6.45±1.37	7.20±1.93[a]	6.91±1.92[a]	7.30±1.34[a]	7.70±1.49[a]
Base	6.40±2.07	6.55±1.57	6.89±1.45[a]	6.55±1.75[a]	6.78±1.20[a]	6.82±1.99[a]
EO–0.9	6.70±2.16	6.73±1.49	6.25±1.49[ab]	4.33±1.66[b]	4.13±2.23[b]	5.00±2.24[ab]
EO–1.8	6.40±2.22	6.64±1.57	4.86±1.86[b]	4.67±1.73[b]	4.50±1.77[b]	4.38±1.92[b]

[1]Means within a column bearing the same letter are not significantly different (p>0.05) as determined by Least Significant Difference test.
[2]No significant difference (p>0.05) was found between irradiated and unirradiated samples as determined by Student's *t* test

4. CONCLUSIONS

This experimental work clearly showed the effect of the irradiation dose in combination with different plasticizers on the mechanical properties of cross-linked calcium caseinate films. This was explained in terms of salt bridges and electrostatic bonds that could reduce molecular distances. Plasticizers generally decreased puncture strength values. Sorbitol had the greatest plasticizing effect and significantly increased the distance to puncture of the films for all irradiation doses while mannitol decreased distance to puncture. Furthermore, the insolubility results demonstrate that it is selective enough to produce films containing a high ratio of insoluble matter. Combination of irradiation and thermal treatments on the films based on soya, calcium caseinate and whey proteins resulted in an increase in the puncture strength of the corresponding films. The observation of the films' microstructure by transmission electron microscopy revealed that all films were characterised by a highly porous structure. However, pore size distribution varied depending on the protein ratio which might be correlated in part, with the mechanical behaviour of these films. Structural analysis of biofilms were equally investigated to explain, at least in part, the behaviour of the whey proteins biofilms, particularly the biofilms obtained by gamma irradiation which exhibit better properties. However, the gamma irradiation responsible for the cross-linking (via formation of bityrosine bridges) moderately affected the protein structure. Modification of protein conformation could be a result of this treatment inducing modified structures which were more ordered and stable.

The present studies dealt also with the control of bacterial growth of pre-cooked shrimp and oven-ready all dressed pizza using gamma irradiation combined with edible antimicrobial coatings. This technology showed significant potential for inhibiting total aerobic counts and *P. putida*, and as a result, the microbial shelf life was extended by 5 days for shrimps and by 10–16 days for pizza. The use of gamma irradiation in combination with a protein-based coating containing essential oils extended the shelf-life by 11 days for shrimps and by over 18 days for pizza. Although phenolic compounds present in essential oils are known to possess scavenging properties for free radicals, the concentration used in the coating solutions was found to affect odour and taste. Therefore, further work is necessary to optimise the hurdle antimicrobial effect provided by essential oil components.

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PACKAGING QUALITY ASSURANCE GUIDANCE MANUAL MODEL FOR SAFE, SHELF-STABLE, READY-TO-EAT FOOD THROUGH HIGH-DOSE IRRADIATION

Y. HARUVY*, L. DESCHENES**
* Soreq Nuclear Research Center, Yavne, Israel
** Food R&D Center, Agriculture and Agrifood, St-Hyacinthe, Quebec, Canada

Abstract

Packaging Quality Assurance, mandatory for safe, shelf-stable and ready-to-eat food through high-dose irradiation, should be based on a detailed Guidance Manual. The manual described hereafter lists the complete set of guidance elements required for the quality assurance system of food packaging for high-dose irradiated food.

1. INTRODUCTION

1.1. Purpose and scope

The Quality Assurance (QA) Guidance Manual Model describes the guidelines for establishing a quality assurance system, policies, procedures and facilities, for radiation-processed food packaging. It aims to provide producers of this packaging with a good understanding of the QA System needed in order to supply quality safe food-products to their customers. These guidelines need to be adapted by each manufacturer to a specific QA Manual fitting their product and factory. The guidelines should further be specified in the form of procedures and protocols.

1.2. General concepts

The objective of the QA System described hereafter is to ensure that supplied shelf-stable packaged food products meet the detailed safety, quality and contractual requirements. This objective is in agreement with the requirements of current good manufacturing practice (GMP) and ISO 9001 standards.

Foremost, the packaging manufacturer should establish a Product Assurance System responsible for carrying out activities to maintain and improve quality, safety and reliability of the targeted packaging products. This objective can be achieved by systematic monitoring of product design, manufacturing, production, and customer service. Nevertheless, the responsibility for quality and reliability rests foremost with the management and all the workers involved.

The quality efforts must start at the very early stages of the product life-cycle, as from agreement with customers on the requirements and the pre-design. The QA team should support all the stages of the development and production processes, and revise the QA Manual as needed.

The Packaging Assurance Policy aims at supporting the development, manufacturing, marketing and servicing of the packaging, so they conform to applicable GMP standards, food-safety regulations, and customer specifications, at a reasonable cost. The companies responsible for the food packaging and food sterilisation operations should implement similar policies, in order to ensure the chain of quality and reliability all the way to the market.

2. PACKAGING: CRITICAL FUNCTIONS AND RELATED ISSUES

For the safety of packaging for shelf-stable ready-to-eat foods [1], the long term reliability of the packaging integrity and non-toxicity needs to be certified in relation to the commonplace packaging fabrication and sealing techniques, as well as in relation to the especially applied irradiation techniques. This combination brings forth a set of issues that need to be addressed and resolved. The

solutions identified for these issues should be eventually reflected in the producer's standards and procedures. These issues are:

- *Extractable Migrants:* Molecules of low molecular weight and high diffusivity that can migrate from the packaging polymer(s) that are in contact with the food. These extractables may be residual from the polymerization, additives to the polymer, or degradation products from the mechanical, thermal or radiation processing. For already approved food-packaging materials, it is only necessary to focus on molecules either formed or released due to the irradiation. The quantity of migrants can be determined by well-accepted protocols, before and after irradiation. The toxicity of the various migrants is more difficult to assess.

- *Packaging Integrity:* Particular assessment for the packaging walls (e.g. puncturing), sealing areas, and intra-laminate adhesion is required. Double packaging may provide extended protection of the food-contacting layer and may also eliminate the need to use laminates. In the sealing areas, welding appears to be much safer than gluing. The durability of seals needs to be tested with respect to the combined effect of mechanical loads, heat, and radiation. Well-established food packaging materials, with documented testing and market experience (including sealing and lamination) should be preferred. Some of these are already radiation-tested for other purposes and thus, only the combined effect needs to be tested.

- *Packaging Permeability and Swellability:* Extremely low permeability and swellability of the food packaging polymers is required for their long-term reliability as oxygen and water barriers. Permeability and swellability need to be tested before and after irradiation as part of the screening process of polymers.

- *Packaging Additives:* A wide variety of additives are commonly present in polymer films, typically kept proprietary. In some cases, additives are employed *ad hoc* and are not even documented. Of particular interest are aromatic antioxidants having a toxic potential.

- *The Food-Contacting Layer:* In multilayered structures that "likely satisfy the demands of radiation processing prepackaged food" [2], the layer in contact with the food should be the one most strictly tested as to the formation and migration of potentially toxic compounds.

3. PACKAGING CRITICAL PARTS AND CHECKLISTS

Packaging integrity and safety are crucial for the safety and quality of shelf-stable food. Therefore, a systematic assessment of integrity-related safety is required, to eliminate any defects that may theoretically exist and impair the packaging integrity and safety. This is especially important for radiation-sterilised shelf-stable ready-to-eat food, which is expected to be safe for several years of storage. There are three primary parts in the packaging:

- Packaging walls, typically made of flat films.

- Packaging sealing areas, typically made by gluing, welding or both.

- Packaging intra-laminate adhesion, if applicable, typically made by gluing or welding.

- *Packaging walls:* Durability of flat-wall areas is practically the same as that of the specific polymer employed. Puncturing durability, the crucial factor for prolonged food safety, must be required and tested. Puncturing, in this respect, relates to accidental damage upon shipping, handling and storage, including insect-penetration.
  Double packaging may provide the solution for extended protection of the food-contacting layer and hence an overall extended reliability of the packaging. Double or triple packaging may even eliminate the need to use laminates as the food-contacting layer.

- *Packaging sealing areas:* A greater potential for integrity failures exists in the sealing areas. Welding seems to be much safer than gluing for sealing the packaging, especially after loading of the food into the packaging. Nevertheless, the durability of welded areas needs to be thoroughly tested in respect to the combined effects of mechanical loads, heat and radiation, since damage aggravation may occur by synergistic effect of these factors.

- *Packaging intra-laminate adhesion:* A significant potential for integrity failures is delamination. In particular, radiation-induced delamination typical of adhesion-laminates produced with adhesives that are non radiation-stable. Welding-lamination seems to be much safer than gluing-lamination. Lamination durability needs to be thoroughly tested in respect to the combined effects of mechanical loads, heat and radiation, in view of possible synergistic effects.

- *Radiation-approved polymers:* By employing radiation-tested polymers and their sealing procedures, and validating their durability at doses exceeding the designed application dose, the high-dose packaging dilemma is practically shifted back to the arena of regular commercial food packaging. Thus, well-established food packaging materials and sealing techniques may be adopted and, hence, their safety approved by relying on their well-documented testing and market experience in all non-radiation issues.

  An example of this approach is the highly radiation-durable PET polymer. This polymer is a well-established material for high quality food packaging. A suggested adaptation of this polymer for the purposes of this work, via an all-PET laminate, is depicted in Fig. 1.

  The following explicit polymer-quality issues are of particular importance:

- *Molecular weight distribution:* A certificate of this data, for the raw food-contacting polymer, is very important to assure a controlled level of migrants. It should be obtained from the original producer of the resin(s) and provided to the QA system with the final product.

FIG 1. Suggested laminate ensemble for high-dose radiation-sterilised food-packaging.

- *Additives, antioxidants in particular:* A certificate of this data, for the raw polyethylene, is very important to assure a controlled level of migrants and their toxicity. It should be obtained from the producer of the LLDPE resin(s) and producer of the film (if additional additives were introduced) and provided to the QA system with the product.

- *Crystallinity:* A certificate of this data, for the food contacting film, is important to assure a reproducible and reliable durability to radiation. It should be obtained from the producer of the film(s) and provided to the QA system with the product. FTIR charts, typically recorded as part of the quality assurance of the producer, may support the certificate of crystallinity.

– *Orientation:* A certificate of this data, for the food contacting film, is important to assure a reproducible and reliable durability to radiation. Non-oriented films are preferred, in view of their higher radiation durability. The strength of the packaging should be obtained by biaxially oriented external layers that are not in contact with the food.

– *Adhesive(s):* A certificate of the chemical constitution data, for the adhesives and solvents, is very important to assure a controlled level of migrants and their toxicity. Trade names and codes are unacceptable. The data should be obtained from the producer of the adhesives and provided to the QA expert with the final product.

– *Radiation durability testing:* Needs to be carried out per packaging product type, per producer, once in a couple of years (e.g. 5 or so). If the results are satisfactory and well documented, and all later batches of the product carry the certificates mentioned above, comprising data comparable to that of the batch tested, the radiation durability of the product is most likely to be acceptable. The radiation durability should test the packaging dose-damage profile to a dose well above the dose intended for sterilisation.

– *Migrants testing:* Needs to be carried out per packaging product-type, per producer, once in a couple of years (e.g. 5 or so). If the results are satisfactory and well documented, and all later batches of the product carry the certificates mentioned above, comprising data comparable to that of the batch tested, the migrants level in the packaging is most likely to be acceptable. Of special importance is testing of the radiation effect on migrants quantity and nature.

TABLE I. QA CHECK-LIST FOR HIGH-DOSE-DURABLE PACKAGING POLYMERS

QA Factors		Data		Durability Assessment	
Group	Factor	Full/Part/N/A	Reliability	Theoretical level	Practical level
Polymer	Family				
	Producer				
	Specific brand				
	Additives				
	MW distribution				
	Linearity				
	Crystallinity				
	Extractables				
History	Processing				
	Thermal				
	Mechanical				
	Irradiation				
	Recycling				
Irradiation	Dose				
	Dose-Rate				
	Atmosphere				
	Gamma or E.Beam				
Food	Type/Make				
	Atmosphere/pH				
	Absorption (g/g)				
	Swelling (cm/cm)				
Durability	RADIATION				
	Tear (before/after)				
	Puncture (-”-)				
	Abrasion (-”-)				

4. APPLICABLE DOCUMENTS

4.1. Regulatory documents

– CGMP
– US FDA requirements

4.2. International quality documents

– *ISO/IEC Guide 25:* General requirements for the competence of calibration and testing laboratories.
– *ISO 9001:* Quality system Model for quality assurance in design, development, production, installation and servicing.

4.3. Manufacturer's documents

– *Product-Specific Procedures:* Raw materials analysis; Extractives testing; Lamination; Lamination Testing; Packaging Filling; Deaeration; Sealing; Sealing Testing; Product Inspection.
– *General Product Documents:* Policies; General Production; Product Handling, Packaging, Storage and Shipment; Customer Instructions.

5. PACKAGING QUALITY ASSURANCE-ORGANISATION AND PROCEDURES

5.1. Organisation

The first document in any QA manual should describe the structure of the organisation, and the assimilation of the QA functions in this organisation, as illustrated in Fig. 2 below.

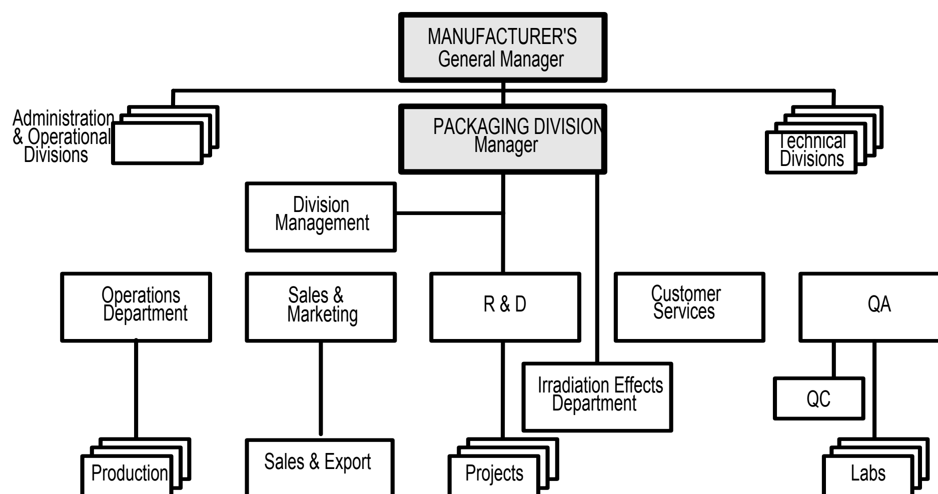

FIG 2. QA function in the organisation.

Packaging QA manager should be reporting to the Director of QA, who reports directly to the General Manager. The Director of QA should have the authority to verify that approved planning, manufacturing and quality control procedures are strictly followed and that product performance and quality conform to specifications.

5.2. QA procedures

QA procedures must be management directives, which address specific quality elements and thus serve to identify the course of action required to implement the Quality Policy.

Food Packaging Standards should define levels of acceptance for product parameters. Such standards may be referenced from separate documents and regulations. Food Packaging Standards used in manufacturing greatly affect the quality of products, whereas Testing and Inspection can only detect failures *post-factum*.

Packaging standards for high-dose irradiated shelf-stable foods should be designed to provide the technical, manufacturing and inspection personnel with parameters and criteria for ensuring the required quality, safety and radiation durability of radiation-sterilised food packaging products produced by the manufacturer.

Standard Operation Procedures (SOP's) should define in detail materials, equipment, operations, methods for their implementation, production processes and parameters, and inspection procedures. All these are required in order to accomplish qualified chemical, mechanical, biological and irradiation processes. SOP's should also be subject to periodic review for relevancy, adequacy and correctness. An example of such a procedure is shown in the Appendix 1.

Test Procedures, e.g. mechanical, chemical, biological barrier properties, should be used for the inspection of the packaging materials and products of the manufacturer.

The entire set of QA Procedures, Food Packaging Standards, Process Specifications and Chemical and Biological Test Procedures should be collected in a comprehensive set of controlled documents with a controlled distribution. Additional procedures having a direct relationship to the quality function should also be included, for reference.

6. INITIAL QUALITY PLANNING

Initial Quality Planning should precede the production. Its main objective is to identify unusual processes or requirements that may call for special actions in order to achieve timely implementation of inspection and testing in compatibility with affected manufacturing operations.

Quality Control Plans should be individually generated for specific programs or product lines, when required. These plans should address applicable elements of the quality operating procedures and include the specific quality requirements of the contract.

Work Instructions are destined to translate product requirements into clear and documented step-by-step instructions for the production operator. Format and scope of the instructions have to be adapted to the personnel involved in their execution. Typically, the work instructions take the form of a key document called the Method Sheet or the Route Card.

Route Cards may supplement production procedures and provide detailed information such as description of the operations, their sequence, product's routing, methods employed, inspection points, and tooling. Reference can be made to existing specifications, standards, etc.

Statistical analysis must be utilised to assess the quality performance and identify process obstacles. Sampling plans should provide the protection levels required by the food-safety practice and regulations.

7. CONFIGURATION MANAGEMENT (CM)

7.1. Configuration Management (CM) Principle

The CM principle comprises three elements:

(1) The CM guidelines should be defined in the appropriate manufacturer's QA Procedure.
(2) Each food-packaging material/product developed or produced should be approved by the applicable authority, e.g. Ministry of Health or Agriculture.

(3) Any change in an approved material/product must receive a new approval. Request for approval of a change should contain details on the material/product, the requested change, the reasons, effects and implications, etc.

7.2. Production standards and change control

A Document Control system should be implemented to ensure that only the latest issues of relevant documents are utilised by the purchasing, production and inspection functions. Reports reflecting the latest revision of international standards should be issued by a central source. Obsolete documents must be collected and disposed of to prevent their use.

Change Initiations should be documented on a specific form, then reviewed and approved on the same form. The form should comprise the entire data on the affected products and documents, the reason for change, the specific changes requested, and the initiator data. It should also comprise a section of change-review and recommendations for modification, approval or disapproval, and referees data. It should further comprise a section of decision and effectivity by date or batch number.

7.3. Updating of Standard Operation Procedures (SOPs)

SOP's subjected to changes should be updated when an approved change is made. The revision status should be marked in the appropriate area in the cover page of the SOP. Safety of Changes is a crucial parameter whenever changes are made regarding the production of shelf-stable food or its packaging. Therefore, each of such changes must be reviewed and approved also by the Chief Food-Safety Officer of the food packaging manufacturer.

8. PURCHASE CONTROL

8.1. Supplier selection, control, inspection and rating

Selection of qualified sources should be done on the basis of three primary factors:

(1) The supplier's past performance for the type of goods or service involved.
(2) Evaluation survey of supplier's facility and quality system.
(3) Certification of the Supplier's quality system and by an accredited body.

Control and continued monitoring of supplier's quality should be maintained by means of reviewing his: Quality Program; Quality of supplied products; and Periodic Audits.

Inspection of purchasing source is carried out by the manufacturer's personnel at the supplier's premises. Such inspection may be required when receiving inspection at the manufacturer's premises is impractical. Rating of suppliers should be carried out on the basis of their quality performance. This data is effective in source selection and corrective actions.

8.2. Purchase documentation review

Procurement of materials, processes, services or items that are utilised in delivered products, should be reviewed and controlled. The Purchase Order needs to be prepared in response to an approved Purchase Requisition that specifies the requested items to be purchased. It is the task of Quality Assurance to review and approve Purchase Orders.

9. INSPECTION AND TESTING

9.1. Principles of inspection

The inspection program should be established upon the principles of Food Safety and Food Packaging Safety and Reliability [1,3]. It should specify the parameters to be inspected for product

performance and safety, the analyses needed, and the criteria to identify defects in the product performance or safety.

The sampling plan should be set primarily on grounds of the basic food safety guidelines, and updated during the planning stages. It should be based on the GMP and adapted per the inspection method and the criticality of the inspected parameter.

Inspection Equipment must have a level of accuracy at least four times better than the tolerance of the measured parameter.

9.2. Inspection plan and status

An inspection plan which specifies the inspection points and the methods at each point should be prepared as part of the Quality Control Plan. It should provide a co-ordinated sequence of inspection and test operations at successive steps of production, to ensure a compliant product with an efficient application of facilities and personnel.

Inspection status of every item must be identifiable, by the analysis records or by route cards or inspection tags attached to the product. An inspection stamp and inspector's signature should indicate completion of the inspected stage of product, its compliance with the SOP and food-safety requirements, and thus the acceptability of the item. Products awaiting inspection must be segregated from those approved for use. Non-conforming products awaiting disposition must be segregated separately, in quarantine storage.

Inspection documentation should specify all the inspection stamps in use, and identify the inspector carrying each stamp at each time interval.

9.3. Receiving inspection

All goods, purchased for use in radiation-sterilised shelf-stable food-packaging products, should be inspected by Receiving Inspection, verifying that the goods received, the documentation provided, the analysis data attached, the inspections and testing documentation all comply with what was required in the purchase documentation.

9.4. Process inspections

Process inspection relates to a three-fold inspection that comprises Set-Up Inspection, In-Line Inspection, and the Process Inspection itself.

Set-up inspection and approval is required for machine-dominated operations, before production commences. Such inspections may include verification samples initially produced by the machine, to determine variance. Set-up stability should be monitored by the process control.

In-line Inspection is an integral part of the manufacturing cycle of a given product. Its scope and design within the process are discussed in Section 9.1.

Process Inspection is needed wherever inspection of the processed materials is impractical. Instead, acceptance of the product may be accomplished through inspection of the process. Product samples may be inspected to verify the effectiveness of the process control.

Environmental Analysis should consist of sampling clean areas, products and operators, at the beginning and at the end of production processes. Control of environmental and airborne bacterial load in the production line should comply with regulatory, GMP and contractual requirements, as well as in-house specifications.

9.5. Final inspections

Final Inspections relate to a three-fold inspection: upon product completion, upon product storage in the storeroom, and prior to shipping to customers.

Final Inspection of the product includes inspection of a completed material or product for sale, checking their compliance with the relevant specifications and food-safety standards. This activity is supplementary to the previously performed inspections.

Storeroom Inspection should monitor proper tagging, segregation and storage of parts, products and materials. The tags should include item identifications, lot numbers, inspection status, and expiration dates where applicable.

Shipping Inspection should monitor the products, prior to shipping to customers, for lack of damage, attachment of all the required documents; and proper packaging and tagging.

10. CALIBRATION

A documented calibration system should be employed, to ensure continued accuracy and proper application of measuring and test equipment, in compliance with ISO/ICE GUIDE 25.

Traceability of calibration of all the measurement and testing equipment should be to the nearest National Laboratory of Metrology. Such equipment should bear a label carrying the calibration date, expiration date, and calibration performer’s signature. Standards, used as media for accepting deliverable materials, should be periodically tested by a certified laboratory.

Initial Acceptance of new test or measurement equipment should be followed by tagging with an identification number, recording in a logbook, issuing a record card, assignment of use procedure, and verification of its calibration. A calibration interval should be established according to the manufacturer's recommendations.

Calibration Recall of each instrument on due date should allow for maintenance and recalibration. Inactive equipment must be labelled and segregated.

11. CONTROL OF NON-CONFORMING MATERIALS AND PRODUCTS

11.1. Control

Control system for non-conforming items should be established to ascertain their identification and prevention of use, as well as initiate and document prompt and effective corrective actions. Each non-conforming item should be reviewed by the QA personnel in order to assign to it one of the following dispositions:

– *Scrap*: Products that are obviously unfit for use, rework or repair, to be disposed of.
– *Returns*: Purchased items that are found non-conforming, to be returned to the supplier.
– *Rework*: Non-conforming products that can and should be reworked to full conformance.
– *Material Review Board (MRB)*: When all the other dispositions seem inappropriate.

11.2. Material Review Board (MRB)

The MRB, appointed by the management, typically comprises the QA Manager; representatives of production and inspection departments; the chief technologist; and advisers invited as needed. Its optional dispositions assigned to non-conforming items are:

– *Use-as-is*: for minor non-conformance that does not affect the intended use or safety.
– *Rework*: for a non-conformance that can be reworked and tested to full compliance.

– *Repair*: for a non-conforming item that can be repaired and tested to an acceptable compliance. The MRB authorises and documents the repair procedures.
– *Scrap*: where repair or rework of the product is impractical or uneconomical.
– *Recall*: where a non-conformance in a product is found after its shipping to customers.
– *Special dispositions*: for a non-conforming item to be used for internal experiments.

12. REPORTING AND DATA ANALYSIS

The quality level reporting and a statistical analysis system should be established, designated to detect, record, analyse and correct all conditions adverse to quality. Reports and analyses should be prepared by the QA Manager and submitted to the management for review. They should include: Incoming inspection results; Process inspection reports; Statistical process analyses; and Final inspection results.

"Quality Costs" are also reported to the management, to enable it to assess and improve the effectiveness of the quality program. Of particular importance are Failure Costs, which include the direct costs of scrap, rework, repair or recall, and the indirect costs of reputation loss.

13. AUDIT, CORRECTIVE AND PREVENTIVE ACTIONS

Quality Auditing assesses the effectiveness of the quality program and the need for corrective actions. It should examine the degree to which policies and procedures affecting product quality are followed. It should be pre-scheduled, use an established checklist of audit criteria, and cover subjects such as materials, processes, work instructions, inspection, tooling and standards, records, and material handling. The quality audits should be carried out by the QA Manager, Chief technologist, and Chief Food-Safety Officer, and reported to the Food-Packaging Division manager for review and initiation of corrective actions.

Corrective and preventive actions aim at the detection and elimination of actual or potential obstacles to quality in a timely and orderly manner. They should extend to operations such as purchasing, manufacturing, inspection, shipping, and handling customer complaints. They should be verified for effectiveness and reported periodically for management review.

Corrective action should be taken as a result of: Product non-conformance hinting at a systematic fault; Technical investigation of repetitive non-conformance; Quality audits; Quality level reports; and Customer complaints.

Preventive action should be taken as a result of: Quality audits; Analysis of customer complaints; Review of SOPs, Analysis of quality cost reports

The result of corrective and preventive actions can be: Changes of processes, instructions, inspection and testing, or personnel training; Changes or repairs in tooling, machinery or materials; Notification to vendors of a need for changes in their product.

14. QA RECORDS

QA Records are objective evidence of appropriate quality system. These records should comprise documentation of: Raw materials; Production; Inspection, Analysis and testing; Process and operator certifications; Quality audits; Failures; Customer complaints; MRB; Corrective and preventive actions; Calibrations; Product conformance and release; Quality levels; Quality costs; Management reviews. The QA records should be complete, reliable, easily accessible, and retained for at least 7 years.

15. TRAINING, PERSONNEL DEVELOPMENT AND CERTIFICATION

Training and Personnel Development: The manufacturer should maintain an active training and instruction program for its employees, including QA training. Employees should be continually made aware through bulletins and notices of new know-how and technical data received by the manufacturer.

Personnel Certification: Personnel performing processes and operations that require special skills must be trained and certified to the level required by the process specification. Certifications should be issued and documented for specified periods of time, and renewed following a performance review by the QA manager.

16. QA FACILITIES

The manufacturer's Product Assurance activities need to be supported by laboratories furnished with modern equipment and operated by experienced personnel. All QA facilities should preferably be certified to ISO/ICE GUIDE 25. The laboratories for food packaging should be capable of: Polymer characterisation; Mechanical testing; Barrier testing; Migrants testing; Biological burden testing; Calibration.

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APPENDIX 1

Document Type:	Quality Procedure	**Applicability:**	Packaging Polymers for Irradiated-Food	
Document No.:	LDYH-001	**Revision: A**	**Original Issue**	Unclassified
Document File:	pc4324 for publication.doc	**Page 256 of 272**	**Date:** 20-7-1999	**Copy N/A of __**
Document Title:	**Selection of Packaging Polymers for Foods to be Irradiated**			

Prepared By:	**Louise Deschenes and Yair Haruvy**			**Date:** 15-3-2000
APPROVAL	Louise Deschenes			**Date:** 12-7-2000
	YAIR HARUVY		yh	**Date:** 12-7-2000
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R e v i s i o n s

Rev. Ltr.	Date	Description	ECO No.	Pages	Signature
A	15-3-2000	Original Procedure Document			yh

Replacing Document No.: N/A	**Revision:**	**Date:**

1. General

Packaging of food to be irradiated for extended shelf-life requires careful selection of the packaging materials. Particular care is required in view of the wide variety, available in the market, of polymer families, types, brands, additives used, treatments, and so on. Only methodical and comprehensive assessment of the objectives vs. the properties of candidate materials, followed by comprehensive and reliable testing of selected ones, can assure optimal as well as cost-effective fabrication packaging fully complying with the product requirements.

2. Objective

The objective of this protocol is to establish a procedure for proper selection and quality assessment of packaging materials for foods to be prepackaged and irradiated. This procedure comprises four steps:

(i) Conceptual selection of packaging type and design;
(ii) Selection and assessment of candidate materials;
(iii) Design and assessment of the processes of packaging fabrication;
(iv) Design and assessment of the food packaging process prior to the irradiation.

The packaging selection procedure should be carried out in accordance with the objectives of the food irradiation process. There exist two different objectives for such irradiation: (a) to pasteurise the food, namely, to lower bioburden (bacteria, fungi, insects, etc.), aiming at improved safety and extended shelf-life (a few days to several weeks); or (b) to sterilise pre-cooked foods, and eliminate all living organisms, aiming at a shelf-life of several years.

Packaging for radiation-sterilised foods should be strictly impermeable, to eliminate both dehydration and rancidity development. Packaging for non-sterile irradiated foods must ensure sufficient in-permeation of oxygen, or anti-bacterial agents (see Section 5.2), to inhibit growth of anaerobic pathogens, *Clostridium botulinum* in particular.

The underlying principle of this protocol is to require comprehensive assessment of suitability, durability and quality of each material and process involved in the packaging. This principle calls for minimising the variety of materials and processes to be utilised for packaging of the foods to be irradiated and, hence, cost-effective production of high-quality safe packaging.

3. Applicable Documents

3.1. Standard Guide for Packaging Materials for Foods to be Irradiated, ASTM F-1640-95; and references therein.

3.2. International Consultative Group on Food Irradiation (ICGFI) Database on Approved Packaging Materials, http://www.iaea.org/icgfi.

3.3. Y. HARUVY, "Packaging Considerations", Chapter 7 in Joint FAO/IAEA/WHO Report **890**, High Dose Irradiation: Wholesomeness of Food Irradiated With Doses Above 10 kGy, Geneva (1999); and references therein.

3.4. Test methods references, listed in Section 5.2.

4. Applicable Terminology

4.1. **Packaging** – Ensemble of materials employed for containing and sealing the food prior to irradiation.

4.2. **Laminate** – Ensemble of flat films permanently attached together, by means of adhesive or welding, or co-extrusion.

4.2. **Sealing Area** – Area of packaging film or laminate that is joined by means of adhesive or welding, to form the packaging container prior to filling with food, or seal the packaging with food inside.

5. Parameters to be Assessed and Applicable Test Methods

5.1. General

The parameters listed in Table I should to be properly assessed for each and every material in the packaging, films and adhesives alike. The data compilation and assessment may require extensive studies in addition to packaging development and fabrication efforts. Therefore, minimising the number of materials and packaging schemes used may result in cost-effective optimisation of product needs and packaging quality.

TABLE I. QUALITY ASSURANCE CHECK-LIST OF CANDIDATE POLYMERS

QA Factors		Data		Durability Assessment	
Group	**Factor**	**Full/Part/N /A**	**Reliability**	**Theoretical level**	**Practical level**
Polymer	Family				
	Producer				
	Specific brand				
	Additives				
	MW distribution				
	Linearity				
	Crystallinity				
	Extractables				
History	Processing				
	Thermal				
	Mechanical				
	Irradiation				
	Recycling				
Irradiation	Dose				
	Dose-Rate				
	Atmosphere				
	Gamma or E-beam				
Food	Type/Make				
	Atmosphere/pH				
	Absorption (g/g)				
	Swelling (cm/cm)				
Durability	RADIATION				
	Tear (before/after)				
	Puncture (-”-)				
	Abrasion (-”-)				

For each parameter to be tested, several standard test methods are suggested. They can be chosen on a laboratory device availability basis. Additional test-procedures, commonly in use at specific institutes, can be employed as well if properly documented and certified. Nevertheless, it is important that the actual methods of testing should be selected according to the polymeric nature and the form of the material, e.g. flexible or rigid, thick or thin films, mono- or multi- layered, etc.

Post-irradiation ageing effects can be ignored if polymers are selected, that have radiation damage threshold well above (e.g. ×100) the dose intended for food treatment. Otherwise, polymers’ ageing needs to be tested under real temperature-time conditions, or proper accelerated ageing procedure.

5.2. Thickness testing

ASTM D4802–94	Standard Specification for Poly(Methyl Methacrylate) Acrylic Plastic Sheet
ASTM D2103–92	Standard Specification for Polyethylene Film and Sheeting, Section 7.9
ASTM D1593–92	Standard Specification for Nonrigid Vinyl Chloride Plastic Sheeting
ASTM D4801–95	Standard Specification for Polyethylene Sheeting in Thickness of 0.25 mm (0.010 in.) and Greater
ASTM E252-84	Standard Test Method for Thickness of Thin Foil and Film by Weighing
ASTM D645/D645M–97	Standard Test Method for Thickness of Paper and Paperboard
ASTM D4635–95	Standard Specification for Polyethylene Films Made from Low–Density Polyethylene for General Use and Packaging Applications
ASTM D3981–95	Standard Specification for Polyethylene Films Made from Medium–Density Polyethylene for General Use and Packaging Applications
ASTM D2673–94a	Standard Specification for Oriented Polypropylene Film
ASTM D5047–95	Standard Specification for Polyethylene Terephthalate Film and Sheeting
ISO 4591:1992	Plastics – Film and sheeting – Determination of average thickness of a sample, and average thickness and yield of a roll, by gravimetric techniques (gravimetric thickness)
ISO 4593:1993	Plastics – Film and sheeting – Determination of thickness by mechanical scanning

5.3. Permeability testing

Gas transmission rates and permeability through polymer films may strongly depend on swelling by humidity as well as other vapours. Therefore, it is highly recommended to test the permeability characteristics of candidate materials under conditions as close as possible to those of the intended use. In particular, relative humidity level, as well as presence of other vapours, when applicable.

ASTM D3985–95	Standard Test Method for Oxygen Gas Transmission Rate Through Plastic Film and Sheeting Using a Coulometric Sensor
ASTM F1927–98	Standard Test Method for Determination of Oxygen Gas Transmission Rate, Permeability and Permeance at Controlled Relative Humidity Through Barrier Materials Using a Coulometric Detector
ASTM F372–94	Standard Test Method for Water Vapor Transmission Rate of Flexible Barrier Materials Using an Infrared Detection
ASTM F1249–90	Standard Test Method for Water Vapor Transmission Rate Through Plastic Film and Sheeting Using a Modulated Infrared Sensor
ASTM E96–95	Standard Test Methods for Water Vapor Transmission of Materials Technique
ASTM D3079–94	Standard Test Method for Water Vapor Transmission of Flexible Heat–Sealed Packages for Dry Products
ASTM D895–94	Standard Test Method for Water Vapor Permeability of Packages
ISO/DIS 15105–1	Plastics –Film and sheeting – Determination of gas transmission rate – Part 1: Differential–pressure method
ISO/DIS 15105–2	Plastics – Film and sheeting – Determination of gas transmission rate – Part 2: Equal-pressure method
ISO/DIS 15106–1	Plastics – Film and sheeting – Determination of water vapour transmission rate – Part 1: Humidity detection sensor method
ISO/DIS 15106–2	Plastics – Film and sheeting – Determination of water vapour transmission rate – Part 2: Infrared detection sensor method
ISO/DIS 15106–3	Plastics – Film and sheeting – Determination of water vapour transmission rate – Part 3: Electrolytic detection sensor method

5.4. Tensile properties

ASTM D638–98	Standard Test Method for Tensile Properties of Plastics
ASTM D882–97	Standard Test Method for Tensile Properties of Thin Plastic Sheeting
ASTM D828–97	Standard Test Method for Tensile Properties of Paper and Paperboard Using Constant–Rate–of–Elongation Apparatus
ISO 527–3:1995	Plastics – Determination of tensile properties – Part 3: Test conditions for films and sheets
ISO 6383–1:1983	Plastics – Film and sheeting – Determination of tear resistance – Part 1: Trouser tear method
ISO 6383–2:1983	Plastics – Film and sheeting – Determination of tear resistance – Part 2: Elmendorf method

5.5. Lamination strength

ASTM F521–83(1997)	Standard Test Methods for Bond Integrity of Transparent Laminates
ASTM E1220–92	Standard Test Method for Visible Liquid Penetrant Examination Using the Solvent–Removable Process

5.6. Sealing strength

ASTM F88–99	Standard Test Method for Seal Strength of Flexible Barrier Materials

5.7. Sensory Acceptability

ASTM E1870–98	Standard Test Method for Odor and Taste Transfer from Polymeric Packaging Film
ASTM E619–84	Standard Practice for Evaluating Foreign Odors in Paper Packaging
ASTM E460–88	Standard Practice for Determining Effect of Packaging on Food and Beverage Products during Storage
AFNOR XP V 09–009	Septembre 1995 – Analyse sensorielle – Methodes d'evaluation des composes etrangers transmis par les emballages aux denrees alimentaires (V 09–009)

5.8. Migration of indirect additives

ISO 177:1988	Plastics – Determination of migration of plasticizers
ISO/DIS 15033	Plastics – Determination of caprolactam and its cyclic and linear oligomers by HPLC
ASTM D4526–96	Standard Practice for Determination of Volatiles in Polymers by Static Headspace Gas Chromatography
ASTM D4754–98	Standard Test Method for Two–Sided Liquid Extraction of Plastic Materials Using FDA Migration Cell
ASTM D5227–95	Standard Test Method for Measurement of Hexane Extractable Content of Polyolefins
ASTM D5524–94	Standard Test Method for Determination of Phenolic Antioxidants in High Density Polyethylene Using Liquid Chromatography
Please Note:	Additional test methods must be applied, when required by legislation, in the specific countries where the food is intended to be distributed.

5.9. Additional recommended testing

ASTM F1929–98	Standard Test Method for Detecting Seal Leaks in Porous Medical Packaging by Dye Penetration
ASTM F1884–98	Standard Test Method for Determining Residual Solvents in Packaging Materials

ASTM D4649–95	Standard Guide for Selection of Stretch Wrap Films
ASTM E1697-95	Standard Test Method for Unipolar Magnitude Estimation of Sensory Attributes
ASTM D1292–86(1995)e1	Standard Test Method for Odor in Water
ISO 8570:1991	Plastics – Film and sheeting – Determination of cold–crack temperature

5.10. General analytical references

Materials and articles in contact with foodstuffs: Guide for examination of plastic food contact materials, 1997, van Battum, D. and van Lierop, J.B.H., 110 p, CEN TC 194/SC1/WG2 document N118.

Guide to the Expression of Uncertainty in Measurement, 1st Ed., 1995, Intl. Org. for Standardisation, 101 p., CHF 88,50, ISBN 92–67–10188–9.

When reporting the result of a measurement of a physical quantity, some quantitative indication of the result has to be given to assess its reliability and to allow comparisons to be made. The Guide to the expression of uncertainty in measurement establishes general rules for evaluating and expressing uncertainty in measurement that can be followed at many levels of accuracy and in many fields.

The Role of Reference Materials in Achieving Quality in Analytical Chemistry. 1997, Intl. Org. for Standardization, 12 p., free, ISBN 92–67–10255–9.

This document deals with the application of reference materials and is written for those involved in the daily practice of analytical chemistry.

5.11. References for screening of indirect additives

This part is the most costly, compared to usual testing of polymer films.

A Quick Methods to Control Compliance of Plastic Materials with Food Packaging Regulations. Food Additives and Contaminants, 1997, Feigenbaum, A., Bouquant, J., Hamdani, M., Metois, P., Riquet, A.M. and Scholler, D., **"Food Additives and Contaminants"**, 4(6-7), pp.571-582.

6. Methodology

6.1. Conceptual selection of packaging type and design

This is carried out in view of the irradiation objective, and the type and structure of food to be irradiated. This selection is schematically described in the following flow-chart.

6.1.1. Selection of dose range that is applicable to the objective product: pasteurised or sterilised food;
6.1.2. Selection, in the case of the food to be pasteurised, of either passive or active packaging, to maintain controlled oxygen permeability through the packaging, or anti-bacterial agents in the packaging, respectively;
6.1.3. Selection, in the case of the food to be sterilised, of either passive or active packaging, to maintain total impermeability of the packaging, or oxygen scavenging agents in the packaging, respectively;
6.1.4. General design of the packaging: construction and layering;

6.2. Selection and assessment of candidate materials should be assisted with data-compilation, employing the regulation list in Appendix 2 and the Check-list (Table I; Section 5.1), and comprise the following steps:

6.2.1. Selection of dose and dose-margins applicable to the product;

6.2.2. Screening of polymer types approved for use at the selected dose (including process margins) in the country of production and/or use;

6.2.3. Screening of polymer types, from the above, that meet the goals permeation characteristics: Note: Polymer type selection does not guarantee that all brands comply with the general data relating to the general type;

6.2.4. Screening of polymer types, from the above, which provide radiation durability at the selected dose (including process margins): Note: polymer type selection does not guarantee that all brands comply with the general data relating to the general type;

6.2.5. Selection of polymer brands from the above that provide:

a) High molecular weight ($>10^5$ Daltons) and narrow distribution ($2\sigma < 0.5$ in log MW curve);

b) High crystallinity (applicable to PE);

c) Low content of migrants;

d) Adequate tear and puncture strength of films;

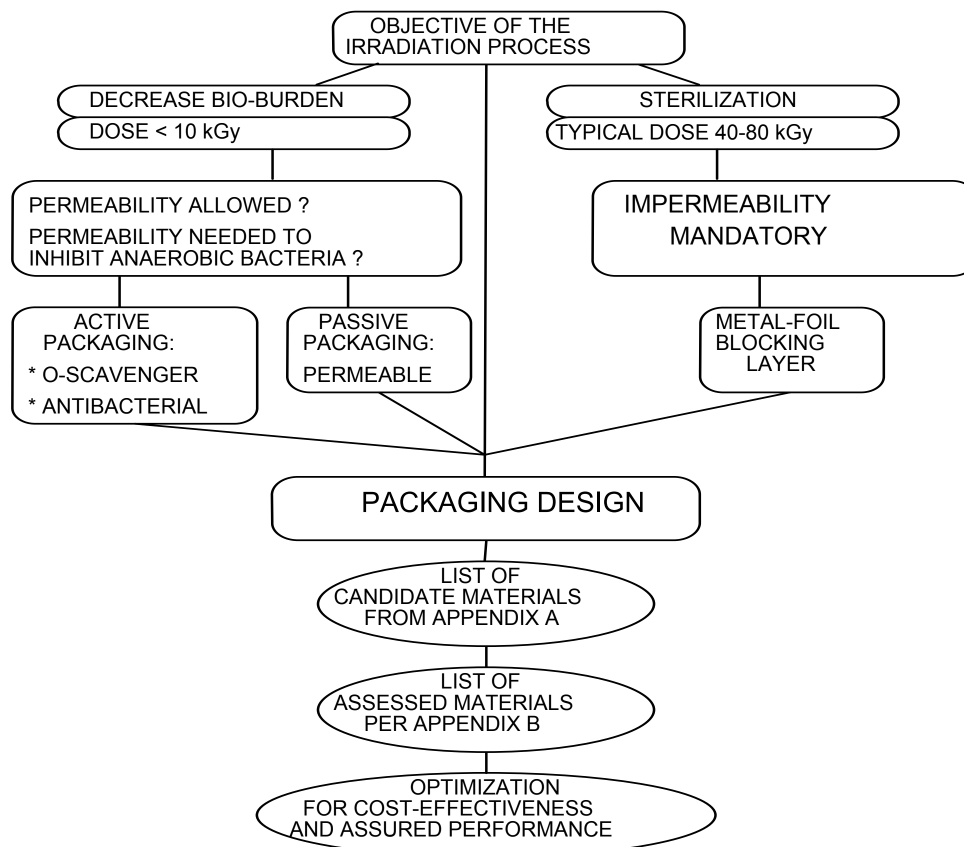
e) Adequate lamination strength;

f) Adequate sealing strength adhesion or welding).

6.2.6. Screening of indirect additives, adhesives and materials related to active packaging, e.g. antimicrobial agents or oxygen scavengers, where applicable.

6.3. Design and assessment of the packaging fabrication processes is typically based on existing techniques, machines, and regularly used raw materials. Hence, process-wise, only adaptation of such a process to the above selected raw materials is necessary. However, upgrading of the manufacturing practices to GMP rules is mandatory in order to comply with the safety and reliability stipulations on packaging for food to be irradiated (c.f. 2.3).

6.4. Design and assessment of the food packaging process prior to the irradiation is an integral part of the task, aiming at reliable fault-free processes of packaging and sealing of the food to be irradiated. Adequate advance planning of these stages is therefore a must.



7. Documentation, Testing and Records

7.1. All the decisions about objectives of the irradiation process, design, material and process selection must be properly documented for future reference.

7.2. All crucial properties of the packaging must be listed, as well as the required testing, performance, and allowed tolerance margins.

7.3 All testing of the packaging must be carried according to approved protocols, using properly calibrated instruments.

7.4. All the testing results must be recorded and documented in the product files, in accordance with the test protocols. The product files must carry proper identification of the product type and batch. These files should also contain reference to the raw materials identification, manufacturers' data, and relevant testing.

7.5. The Compliance of Conformance (COC) certificate of the product should contain the list of available traceability of product data.

8. Responsibility

8.1. The product engineer is responsible for defining and documenting the objective of the irradiation process.

8.2. The packaging engineer is responsible for defining and documenting the packaging requirements, components, applicable fabrication processes, and list of applicable test procedures, required performance, and allowed tolerance margins.

8.3. The irradiation engineer is responsible for defining and documenting the irradiation requirements, parameters, test procedures, required performance, and allowed margins.

8.4. The product manager is responsible for QA guidance and systematic monitoring of all quality aspects and records mentioned above.

Appendix 2

FOOD PACKAGING MATERIALS AUTHORIZED FOR IRRADIATING PREPACKAGED FOOD [UPDATED BY ICGFI SECRETARIAT TO SEPT. 1997 (A)]

No.	Packaging Material	Max. Dose (kGy)	Country (b)	Date
1	Cardboard	10; 35	UK; POL	1991 (c)
2	Cryovac E Bag	10	USA; CAN	1999
3	Cryovac Super L Bag	10	USA; CAN	1999
4	Polyethylene coextruded Polyvinylacetate	30	USA; CAN	1988
5	Poly-ethylene-co-vinylacetate	30	USA	1989
6	Fiberboard	10	IND	1997
7	Fiberboard, wax coated (boxes)	10	USA; CAN	1989
8	Glassine Paper	10	USA	1975
9	Glass	10	IND	1997
10	Hessian Sacks	10	UK	1991 (b)
11	Kraft Paper	0.5	USA	1975
12	Nitrocellulose-coated-cellophane	10	USA; IND	1975
13	Nylon 11	10	USA; IND	1975
14	Nylon 6	60	USA; IND	1975
15	Paper	10; 35	UK; POL	1991 (c)
16	Paper-coated or laminated with wax or PE	10; 35	IND; POL	1990
17	Paper-laminated with aluminum foil	35	POL	1990
18	Polyamide film or PA coextruded w. PE	35	POL	1990
19	Polyester-metallized-Polyethylene laminate	35	POL	1990
20	Polyester-Polyethylene laminate	35	POL	1990
21	Polyethylene film (various densities)	60; 35; 10	USA; POL; IND	1975
22	Polyethylene-paper-aluminum laminate	35	POL	1990
23	Polyethylene-terephthatate film	60	USA	1975
24	Polyolefin (LD, as middle or sealant layer)		CAN	1989
25	Polyolefin (HD, as external layer)		CAN	1989
26	Polyolefin film	10	USA	1975
27	Polypropylene sacks	10; 35	UK; POL	1990 (c)
28	Polypropylene-metallized	35	POL	1990
29	Polystyrene film	10	USA; IND	1975
30	Polystyrene foam trays (Styron 685 D)	10	CAN; IND	1989
31	Rubber hydrochloride film	10	USA; IND	1975
32	Steel, tin plated or enamel lined	10	IND	1997
33	Vegetable parchment	60; 10	USA; IND	1975
34	Vinylchloride-co-vinylacetate film	60; 10	USA; IND	1975
35	Vinylidenechloride coated cellophane	10	USA	1975
36	Vinylchloride-co- vinylidenechloride film	10	USA; IND	1975
	Wood	35; 10	POL; IND	1990
	Viscose	35	POL	1990

(a) For updated data see http://www.iaea.org/icgfi
(b) Approvals: USA – 1975; Canada (CAN) – 1989; Poland (POL) – 35 kGy, 1986-1990; India (IND) – 10 kGy 1996–1997; Earliest date of approval is cited.
(c) For dry herbs

LIST OF PARTICIPANTS

Chander, R.	Bhabha Atomic Research Center, Food Technology Division, Trombay, Bombay 400 085, India Fax: 0092-225505151 or 92-22-551 96 13 E-Mail: ftecdiv@magnum.barctl.ernet.in
Deschenes, L.	Food Research and Development Center, Food Preservation Technologies Section, Agriculture Canada, 3600 Cassavant Blvd West, Saint Hyacinthe, Quebec, Canada J2S 8E3 Fax. 001-450 77 38 461 email: deschenesl@em.agr.ca
Erasmus, C.	CSIR – Bio/Chemtek, National Food Science & Technology Programme, P.O.Box 395, Pretoria, South Africa Fax: 27 12 841 2386 E-Mail: CErasmus@csir.co.za
De Bruyn, I.N.	CSIR – Bio/Chemtek, National Food Science & Technology Programme, P.O.Box 395, Pretoria, South Africa Fax: 27 12 841 2386
Farkas, J.	Szent Istvan University, Faculty of Food Science, Dept. of Refrigeration & Livestock Production Technology, 1118 Budapest Menesi ut 45, Budapest, Hungary Fax: 003611664635 E-Mail: cs.farkas@hoya.kec.hu
Haruvy, Y.	Division of Applied Radiation Technology, Soreq Nuclear Research Center, Israel Atomic Energy Commission, Yavne 81800, Israel Fax: 0097289434403 E-Mail: haruvy@ndc.soreq.gov.il
Lacroix, M.	Canadian Irradiation Center (CIC), Research Center in Microbiology-Biotechnology, INRS - Institut Armand - Frappier, 535 Blvd. Cartier, 320 Succ. L.D.R., Laval, Québec, Canada H7V 1B7 Tel: 005146872223 Fax:0015146875792
Loaharanu, P.	Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, International Atomic Energy Agency, P.O.Box 100, Wagramer Strasse 5, A-1400 Vienna, Austria Tel: 2060 21638 Fax: 20607 E-Mail: P.Loaharanu@iaea.org
Luo Xueyun.	Institute of Food Safety Control and Inspection, Ministry of Nanli Health, 7 Panjia Yuan, Beijing 100021, China Tel: 67711813, 15 Fax: 00861067711813

Narvaiz, P.	Comision Nacional de Energia Atomica, Gerencia de Area Radioisotopos y Radiaciones, (1842) Agencia Minipost-Centro Atomica Ezelza, Buenos Aires, Argentina Fax: 541 480 0615 E-mail: narvaiz@cnea.edu.ar
Noomhorm, A.	Asian Institute of Technology, Km. 41 Phaholyothin Hwy, Klongluang, Pathumthani 10120,Processing Technology Program, G.P.O. Box 2754, Klongluang, Bangkok 12120, Thailand Tel: 524 5476 Fax: 006625246200; E-Mail: athapol@ait.ac.th
Patterson, M.F.	Agriculture and Food Science Centre, Department of Agriculture & Rural Development, The Queen's University of Belfast, Newforge Lane, Belfast BT9 5PX, Northern Ireland, United Kingdom Tel: 00441232 255316 Fax: 00441232668376
Stewart, E.M.	Agriculture and Food Science Centre, Department of Agriculture & Rural Development, The Queen's University of Belfast, Newforge Lane, Belfast BT9 5PX, Northern Ireland, United Kingdom Tel: 00441232 255316 Fax: 00441232668376
Tabiri N. J.	Biotechnology and Nuclear Agriculture Research Institute, Ghana Atomic Energy Commission, P.O.Box 80, Legon-Accra, Ghana Tel: 23321400310 Fax: 0023321400807 E-Mail: bnargaec@ghana.com or nnri@idngh.com
Thayer, D.W.	Food Safety Research Unit, US Department of Agriculture, Agricultural Research Service, North Atlantic Area, Eastern Regional Research Center, Wyndmoor, PA 19038, United States of America Tel: 2152336582 Fax: 0012152336406 E-Mail: DTHAYER@ARSERRC.GOV
Trigo, M.J.	DTPA, Instituto Nacional de Investigação Agraria, Quinta do Marques, 2780 Oeiras, Portugal Tel: (01) 4422249 Fax: 003514413208 E-Mail: meandrad@itn1.itn.pt
Zubaidah Irawati, K.	Research and Development of Isotopes and Radiation Technology, National Atomic Energy Agency, Jalan Cinere Pasar Jumat Kotak Pos 7002 JKSKL, Jakarta 12070, Indonesia Tel: 7690709 Fax: 0062217691607